The invention relates to novel conjugates of a binder or a derivative thereof with one or more molecules of an active component, wherein the active component is a CDK9 kinase inhibitor, which is conjugated to the binder via a linker Z as defined herein, and methods for their preparation, their use for the treatment and/or prophylaxis of disorders, in particular of hyper-proliferative disorders.
**Introduction and state of the art**

The invention relates to novel conjugates of a binder or a derivative thereof with one or more molecules of an active component, wherein the active component is a CDK9 kinase inhibitor, which is conjugated to the binder via a linker Z as described and defined herein, and methods for their preparation, their use for the treatment and/or prophylaxis of disorders, in particular of hyper-proliferative disorders.

The family of cyclin-dependent kinase (CDK) proteins consists of members that are key regulators of the cell division cycle (cell cycle CDK's), that are involved in regulation of gene transcription (transcriptional CDK's), and of members with other functions. CDKs require for activation the association with a regulatory cyclin subunit. The cell cycle CDKs CDK1/cyclin B, CDK2/cyclin A, CDK2/cyclinE, CDK4/cyclinD, and CDK6/cyclinD get activated in a sequential order to drive a cell into and through the cell division cycle. The transcriptional CDKs CDK9/cyclin T and CDK7/cyclin H regulate the activity of RNApolymerase II via phosphorylation of the carboxy-terminal domain (CTD). Positive transcription factor b (P-TEFb) is a heterodimer of CDK9 and one of four cyclin partners, cyclin T1, cyclin K, cyclin T2a or T2b.

Whereas CDK9 (NCBI GenBank Gene ID 1025) is exclusively involved in transcriptional regulation, CDK7 in addition participates in cell cycle regulation as CDK-activating kinase (CAK).

Transcription of genes by RNA polymerase II is initiated by assembly of the pre-initiation complex at the promoter region and phosphorylation of Ser 5 and Ser 7 of the CTD by CDK7/cyclin H. For a major fraction of genes RNA polymerase II stops mRNA transcription after it moved 20-40 nucleotides along the DNA template. This promoter-proximal pausing of RNA polymerase II is mediated by negative elongation factors and is recognized as a major control mechanism to regulate expression of rapidly induced genes in response to a variety of stimuli (Cho et al., Cell Cycle 9, 1697, 2010). P-TEFb is crucially involved in overcoming promoter-proximal pausing of RNA polymerase II and transition into a productive elongation state by phosphorylation of Ser 2 of the CTD as well as by phosphorylation and inactivation of negative elongation factors.

Activity of PTEFb itself is regulated by several mechanisms. About half of cellular PTEFb exists in an inactive complex with 7SK small nuclear RNA (7SK snRNA), La-related protein 7 (LARP7/PIP7S) and hexamethylene bis-acetamide inducible proteins 1/2 (HEXIM1/2, He et al., Mol Cell 29, 588, 2008). The remaining half of PTEFb exists in an active complex containing the bromodomain protein Brd4 (Yang et al., Mol Cell 19, 535, 2005). Brd4 recruits PTEFb through interaction with acetylated histones to
chromatin areas primed for gene transcription. Through alternately interacting with its positive and negative regulators, PTEFb is maintained in a functional equilibrium: PTEFb bound to the 7SK snRNA complex represents a reservoir from which active PTEFb can be released on demand of cellular transcription and cell proliferation (Zhou & Yik, Microbiol Mol Biol Rev 70, 646, 2006). Furthermore, the activity of PTEFb is regulated by posttranslational modifications including phosphorylation/de-phosphorylation, ubiquitination, and acetylation (reviewed in Cho et al., Cell Cycle 9, 1697, 2010).

Deregulated activity of CDK9 kinase activity of the PTEFb heterodimer is associated with a variety of human pathological settings such as hyper-proliferative diseases (e.g. cancer), virally induced infectious diseases or cardiovascular diseases:

Cancer is regarded as a hyper-proliferative disorder mediated by a disbalance of proliferation and cell death (apoptosis). High levels of anti-apoptotic Bcl-2-family proteins are found in various human tumors and account for prolonged survival of tumor cells and therapy resistance. Inhibition of PTEFb kinase activity was shown to reduce transcriptional activity of RNA polymerase II leading to a decline of short-lived anti-apoptotic proteins, especially Mcl-1 and XIAP, reinstalling the ability of tumor cells to undergo apoptosis. A number of other proteins associated with the transformed tumor phenotype (such as Myc, NF-kB responsive gene transcripts, mitotic kinases) are either short-lived proteins or are encoded by short-lived transcripts which are sensitive to reduced RNA polymerase II activity mediated by PTEFb inhibition (reviewed in Wang & Fischer, Trends Pharmacol Sci 29, 302, 2008).

Many viruses rely on the transcriptional machinery of the host cell for the transcription of their own genome. In case of HIV-1, RNA polymerase II gets recruited to the promoter region within the viral LTR's. The viral transcription activator (Tat) protein binds to nascent viral transcripts and overcomes promoter-proximal RNA polymerase II pausing by recruitment of PTEFb which in turn promotes transcriptional elongation. Furthermore, the Tat protein increases the fraction of active PTEFb by replacement of the PTEFb inhibitory proteins HEXIM1/2 within the 7SK snRNA complex. Recent data have shown that inhibition of the kinase activity of PTEFb is sufficient to block HIV-1 replication at kinase inhibitor concentrations that are not cytotoxic to the host cells (reviewed in Wang & Fischer, Trends Pharmacol Sci 29, 302, 2008). Similarly, recruitment of PTEFb by viral proteins has been reported for other viruses such as B-cell cancer-associated Epstein-Barr virus, where the nuclear antigen EBNA2 protein interacts with PTEFb (Bark-Jones et al., Oncogene, 25, 1775, 2006), and the human T-lymphotropic virus type 1 (HTLV-1), where the transcriptional activator Tax recruits PTEFb (Zhou et al., J Virol. 80, 4781, 2006).

Cardiac hypertrophy, the heart's adaptive response to mechanical overload and pressure (hemodynamic stress e.g. hypertension, myocardial infarction), can lead, on a long term, to heart failure and death.
Cardiac hypertrophy was shown to be associated with increased transcriptional activity and RNA polymerase II CTD phosphorylation in cardiac muscle cells. PTEFb was found to be activated by dissociation from the inactive 7SK snRNA/HEXIM1/2 complex. These findings suggest pharmacological inhibition of PTEFb kinase activity as a therapeutic approach to treat cardiac hypertrophy (reviewed in Dey et al., Cell Cycle 6, 1856, 2007).

In summary, multiple lines of evidence suggest that selective inhibition of the CDK9 kinase activity of the PTEFb heterodimer (= CDK9 and one of four cyclin partners, cyclin T1, cyclin K, cyclin T2a or T2b) represents an innovative approach for the treatment of diseases such as cancer, viral diseases, and/or diseases of the heart. CDK9 belongs to a family of at least 13 closely related kinases of which the subgroup of the cell cycle CDKs fulfills multiple roles in regulation of cell proliferation. Thus, co-inhibition of cell cycle CDKs (e.g. CDK1/cyclin B, CDK2/cyclin A, CDK2/cyclinE, CDK4/cyclinD, CDK6/cyclinD) and of CDK9, is expected to impact normal proliferating tissues such as intestinal mucosa, lymphatic and hematopoietic organs, and reproductive organs. To maximize the therapeutic margin of CDK9 kinase inhibitors, molecules with high selectivity towards CDK9 are required.

CDK inhibitors in general as well as CDK9 inhibitors are described in a number of different publications: WO2008 129070 and WO2008 129071 both describe 2,4 disubstituted aminopyrimidines as CDK inhibitors in general. It is also asserted that some of these compounds may act as selective CDK9 inhibitors (WO2008 129070) and as CDK5 inhibitors (WO2008129071), respectively, but no specific CDK9 IC50 (WO2008 129070) or CDK5 IC50 (WO2008 129071) data is presented.

WO2008 129080 discloses 4,6 disubstituted aminopyrimidines and demonstrates that these compounds show an inhibitory effect on the protein kinase activity of various protein kinases, such as CDK1, CDK2, CDK4, CDK5, CDK6 and CDK9, with a preference for CDK9 inhibition (example 80).

WO2005026129 discloses 4,6 disubstituted aminopyrimidines and demonstrates that these compounds show an inhibitory effect on the protein kinase activity of various protein kinases, in particular CDK2, CDK4, and CDK9.

WO 2009118567 discloses pyrimidine and [1,3,5]triazine derivatives as protein kinase inhibitors, in particular CDK2, CDK7 and CDK9.

WO2011 1116951 discloses substituted triazine derivatives as selective CDK9 inhibitors.

WO20121 17048 discloses disubstituted triazine derivatives as selective CDK9 inhibitors.

WO20121 17059 discloses disubstituted pyridine derivatives as selective CDK9 inhibitors.
WO2012143399 discloses substituted 4-aryl-N-phenyl-1,3,5-triazin-2-amines as selective CDK9 inhibitors.

EP1218360 Bl, which corresponds to US2004116388A1, US7074789B2 and WO2001025220A1, describes triazine derivatives as kinase inhibitors, but does not disclose potent or selective CDK9 inhibitors.

WO2008079933 discloses aminopyridine and aminopyrimidine derivatives and their use as CDK1, CDK2, CDK3, CDK4, CDK5, CDK6, CDK7, CDK8 or CDK9 inhibitors.

WO2011026917 discloses carboxamides derived from substituted 4-phenylpyridine-2-amines as inhibitors of CDK9.

WO2012066065 discloses phenyl-heteroaryl amines as inhibitors of CDK9. A selectivity towards CDK9 over other CDK isoforms is preferred, however disclosure of CDK-inhibition data is confined to CDK 9. Within the group attached to C4 of the pyrimidine core, alkoxy phenyls can be regarded as encompassed, but there is no suggestion for a specific substitution pattern characterised by a fluoro atom attached to C5 of the pyrimidine ring, and an aniline at C2 of the pyrimidine, featuring a substituted sulfonyl-methylene group in meta position. Compounds shown in the examples typically feature a substituted cycloalkyl group as R^1 but no phenyl.

WO2012066070 discloses 3-(aminoaryl)-pyridine compounds as inhibitors of CDK9. The biaryl core mandatorily consists of two heteroaromatic rings.

WO2012101062 discloses substituted bi-heteroaryl compounds featuring a 2-aminopyridine core as inhibitors of CDK9. The biaryl core mandatorily consists of two heteroaromatic rings.

WO2012101063 discloses carboxamides derived from substituted 4-(heteroaryl)-pyridine-2-amines as inhibitors of CDK9.

WO 2012101064 discloses N-acyl pyrimidine biaryl compounds as inhibitors of CDK9.

WO 2012101065 discloses pyrimidine biaryl compounds as inhibitors of CDK9. The biaryl core mandatorily consists of two heteroaromatic rings.
WO 2012101066 discloses pyrimidine biaryl compounds as inhibitors of CDK9. Substitution $R^1$ of the amino group attached to the heteroaromatic core is confined to non-aromatic groups but does not cover substituted phenyls. Furthermore, the biaryl core mandatorily consists of two heteroaromatic rings.

WO 201077171 discloses 4,6-disubstituted aminopyrimidine derivatives as inhibitors of CDK9.

WO 2014031937 discloses 4,6-disubstituted aminopyrimidine derivatives as inhibitors of CDK9.

WO 2013037896 discloses disubstituted 5-fluoropyrimidines as selective inhibitors of CDK9.

WO 2013037894 discloses disubstituted 5-fluoropyrimidine derivatives containing a sulfoximine group as selective inhibitors of CDK9.

Wang et al. (Chemistry & Biology 17, 1111-1121, 2010) describe 2-anilino-4-(thiazol-5-yl)pyrimidine transcriptional CDK inhibitors, which show anticancer activity in animal models.

WO 2014060376 discloses substituted 4-(ortho)-fluorophenyl-5-fluoropyrimidin-2-yl amine derivatives containing a sulfone group as selective inhibitors of CDK9.

WO 2014060375 discloses substituted 5-fluoro-N-(pyridin-2-yl)pyridin-2-amine derivatives containing a sulfone group as selective inhibitors of CDK9.

WO 2014060493 discloses substituted N-(pyridin-2-yl)pyrimidin-4-amine derivatives containing a sulfone group as selective inhibitors of CDK9.

WO 2014076028 discloses substituted 4-(ortho)-fluorophenyl-5-fluoropyrimidin-2-yl amine derivatives containing a sulfoximine group as selective inhibitors of CDK9.

WO 2014076091 discloses substituted 5-fluoro-N-(pyridin-2-yl)pyridin-2-amine derivatives containing a sulfoximine group as selective inhibitors of CDK9.

WO 2015001021 discloses 5-fluoro-N-(pyridin-2-yl)pyridin-2-amine derivatives containing a sulfoximine group as selective inhibitors of CDK9.
WO 2015136028 discloses 5-fluoro-N-(pyridin-2-yl)pyridin-2-amine derivatives containing a sulfone group as selective inhibitors of CDK9.

WO2004009562 discloses substituted triazine kinase inhibitors. For selected compounds CDK1 and CDK4 test data, but no CDK9 data is presented.

WO2004072063 describes heteroaryl (pyrimidine, triazine) substituted pyrroles as inhibitors of protein kinases such as ERK2, GSK3, PKA or CDK2.

WO2010009155 discloses triazine and pyrimidine derivatives as inhibitors of histone deacetylase and/or cyclin dependent kinases (CDKs). For selected compounds CDK2 test data is described.

WO2003037346 (corresponding to US7618968B2, US7291616B2, US2008064700A1, US2003153570A1) relates to aryl triazines and uses thereof, including to inhibit lysophosphatidic acid acyltransferase beta (LPAAT-beta) activity and/or proliferation of cells such as tumor cells.

WO2005037800 discloses sulfoximine substituted anilino-pyrimidines as inhibitors of VEGFR and CDK kinases, in particular VEGFR2, CDK1 and CDK2, having no aromatic ring directly bonded to the pyrimidine ring and having the sulfoximine group directly bonded to the aniline group. No CDK9 data are disclosed.

WO2008025556 describes carbamoyl sulfoximides having a pyrimidine core, which are useful as kinase inhibitors. No CDK9 data is presented. No molecules are exemplified, which possess a fluoropyrimidine core.

WO2002066481 describes pyrimidine derivatives as cyclin dependent kinase inhibitors. CDK9 is not mentioned and no CDK9 data is presented.

WO2008109943 concerns phenyl aminopyrimidine compounds and their use as kinase inhibitors, in particular as JAK2 kinase inhibitors. The specific examples mainly focus on compounds having a pyrimidine core.

WO2009032861 describes substituted pyrimidinyl amines as JNK kinase inhibitors. The specific examples mainly focus on compounds having a pyrimidine core.

WO201 1046970 concerns amino-pyrimidine compounds as inhibitors of TBK1 and/or IKK epsilon. The specific examples mainly focus on compounds having a pyrimidine core.
WO2012142329 concerns amino-pyrimidine compounds as inhibitors of TBK1 and/or IKK epsilon.

WO2012139499 discloses urea substituted anilino-pyrimidines as inhibitors of various protein kinases.

WO2014106762 discloses 4-pyrimidinylamino-benzenesulfonamide derivatives as inhibitors of polo-like kinase-1.

Macrocyclic compounds have been described as therapeutically useful substances, in particular of various protein kinases including cyclin dependent kinases. However, the documents listed below do not disclose specific compounds as inhibitors of CDK9.

WO 2007147574 discloses sulfonamido-macrocycles as inhibitors of Tie2 showing selectivity over CDK2 and Aurora kinase C, *inter alia* for the treatment of diseases accompanied with dysregulated vascular growth.

WO 2007147575 discloses further sulfonamido-macrocycles as inhibitors of Tie2 and KDR showing selectivity over CDK2 and Plkl, *inter alia* for the treatment of diseases accompanied with dysregulated vascular growth.

WO 2006066957 / EP 1674470 discloses further sulfonamido-macrocycles as inhibitors of Tie2 showing low cytotoxicity, *inter alia* for the treatment of diseases accompanied with dysregulated vascular growth.

WO 2006066956 / EP 1674469 discloses further sulfonamido-macrocycles as inhibitors of Tie2 showing low cytotoxicity, *inter alia* for the treatment of diseases accompanied with dysregulated vascular growth.

WO 2004026881 / DE 10239042 discloses macrocyclic pyrimidine derivatives as inhibitors of cyclin dependent kinases, in particular CDK1 and CDK2, as well as VEGF-R, *inter alia* for the treatment of cancer. The compounds of the present invention differ from those disclosed in WO 2004026881 in featuring a mandatory biaromatic portion within the macrocyclic ring system. Furthermore, none of the example compounds disclosed in WO 2004026881 features a group -CFh-A-R1, in which A and R1 are as defined for the compounds of the formula (I) of the present invention, attached to one of the two aromatic portions of the macrocyclic ring system.

WO 2007079982 / EP 1803723 discloses macrocyclic benzenacyclononaphanes as inhibitors of multiple protein kinases, *e.g.* Aurora kinases A and C, CDK1, CDK2 and c-Kit, *inter alia* for the treatment of cancer. The compounds of the present invention differ from those disclosed in WO 2007079982 in
featuring a mandatory biaromatic portion within the macrocyclic ring system. Furthermore, the compounds of the present invention do not feature a group \(-S(=0)(N=\text{R}^3)\text{R}^1\) directly attached to the phenylene portion of the macrocyclic ring system as disclosed in WO 2007079982.

WO 2006106895 / EP 1710246 discloses sulfoximine-macrocycle compounds as inhibitors of Tie2 showing low cytotoxicity, *inter alia* for the treatment of diseases accompanied with dysregulated vascular growth.

WO 2012009309 discloses macrocyclic compounds fused to benzene and pyridine rings for the reduction of beta-amyloid production.

WO 2009132202 discloses macrocyclic compounds as inhibitors of JAK 1, 2 and 3, TYK2 and ALK and their use in the treatment of JAK/ALK-associated diseases, including inflammatory and autoimmune disease as well as cancer.

ChemMedChem 2007, 2(1), 63-77 describes macrocyclic aminopyrimidines as multitarget CDK and VEGF-R inhibitors with potent antiproliferative activity. The compounds of the present invention differ from those disclosed in said journal publication in featuring a mandatory biaromatic portion within the macrocyclic ring system. Furthermore, none of the compounds disclosed in ChemMedChem 2007, 2(1), 63-77 features a group \(-\text{CH}_2\text{-A-R}^1\) in which \text{A} and \text{R}^1 are as defined for the compounds of the formula (I) of the present invention, attached to one of the two aromatic portions of the macrocyclic ring system.

Cancer diseases are the consequence of uncontrolled cell growth of the most diverse tissues. In many cases, the new cells penetrate into existing tissue (invasive growth), or they metastase into remote organs.

Cancer diseases occur in the most diverse organs and often have tissue-specific courses of the disease. The term cancer as a generic term therefore describes a large group of defined diseases of various organs, tissue and cell types.

Tumours in early stages can possibly be removed by surgical and radiotherapy measures. Metastasized tumours as a rule can only be treated palliatively by chemotherapeutics. The aim here is to achieve the optimum combination of an improvement in the quality of life and prolonging of life.

Conjugates of binder proteins with one or more active compound molecules are known, in particular in the form of antibody drug conjugates (ADCs) in which an internalising antibody directed against a tumour-associated antigen is covalently attached via a linker to a cytotoxic agent. Following introduction of the ADCs into the tumour cell and subsequent dissociation of the conjugate, either the cytotoxic agent itself or a cytotoxic metabolite formed therefrom is released within the tumour cell and can unfold its action therein directly and selectively. In this manner, in contrast to conventional chemotherapy, damage

It is an object of the present invention to provide substances which, after administration at a relatively low concentration, unfold apoptopic action and may therefore be of benefit for cancer therapy.

A particular object of the invention is to provide binder drug conjugates of CDK9 kinase inhibitors, which show an improved anti-proliferative activity in tumor cell lines, such as NCI-H292, compared to the compounds known from prior art.

**Summary of the invention**

Against this background it is an object of the present invention to provide substances which, after administration at a relatively low concentration, unfold apoptotic action and may therefore be of benefit for cancer therapy.

To achieve this object, the invention provides conjugates of a binder or derivatives thereof with one or more active compound molecules, the active compound molecule being a CDK9 inhibitor attached to the binder via a linker L. The binder is preferably a binder protein or peptide, particularly preferably a human, humanized or chimeric monoclonal antibody or an antigen-binding fragment thereof, in particular an anti-TWEAKR antibody or an antigen-binding fragment thereof or an anti-EGFR antibody or an antigen-binding fragment thereof. Particular preference is given to an anti-TWEAKR antibody which binds specifically to amino acid D in position 47 (D47) of TWEAKR (SEQ ID NO: 169), in particular the anti-TWEAKR antibody TPP-2090, or the anti-EGFR antibodies cetuximab, TPP-4030, TPP-5653, or nimotuzumab.

The inventors have found a number of ways to attach the binder to the CDK9 inhibitor in order to achieve the object mentioned above.

According to the invention, the CDK9 inhibitor may have the structure (I) below:
1 represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2-, -S(=0)(=NR 5)-;

5 L represents a C2-C6-alkylene group,

wherein said group is optionally substituted with

(i) one substituent selected from hydroxy, C2-C3-alkenyl, C2-C3-alkynyl, C3-C4-cycloalkyl, hydroxy-Ci-Cs-alkyl, -(CH2)NR'R7, and/or

(ii) one or two or three substituents, identically or differently, selected from halogen and Ci-Cs-alkyl,

with the proviso that a C2-alkylene group is not substituted with a hydroxy group,

or wherein

one carbon atom of said C2-C6-alkylene group forms a three- or four-membered ring together with a bivalent group to which it is attached, wherein said bivalent group is selected from -CH2CH2-, -CH2CH2CH2-, -CH2OCH2-;

10 X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

15 R1 represents -Z-#l or a group selected from Ci-C6-alkyl-, C3-C6-alkenyl, C3-C6-alkynyl, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, phenyl-Ci-C3-alkyl- and heteroaryl-Ci-C3-alkyl-, wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, Ci-C6-alkyl-, halo-Ci-C3-alkyl-, Ci-C6-alkoxy-, Ci-C3-fluoroalkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, -OP(=0)(OH)¾ -C(=0)OH, -C(=0)NH₂;
R represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-;

R\textsuperscript{3}, R\textsuperscript{4} represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-;

R\textsuperscript{5} represents -Z-#l or a group selected from a hydrogen atom, cyano, -C(=0)R, -C(=0)OR, -S(=0)R, -C(=0)NR, -C(=0)C\textsubscript{3}-C\textsubscript{7}-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl,

wherein said Ci-C6-alkyl, C\textsubscript{3}-C\textsubscript{7}-cycloalkyl-, heterocyclyl-, phenyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH\textsubscript{2}, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, C1-C3-fluoroalkoxy-;

R\textsuperscript{6}, R\textsuperscript{7} represent, independently from each other, a group selected from a hydrogen atom, Ci-C6-alkyl-, C\textsubscript{3}-C\textsubscript{7}-cycloalkyl-, heterocyclyl-, phenyl, benzyl and heteroaryl,

wherein said Ci-C6-alkyl-, C\textsubscript{3}-C\textsubscript{7}-cycloalkyl-, heterocyclyl-, phenyl, benzyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH\textsubscript{2}, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-, or

R\textsuperscript{6} and R\textsuperscript{7}, together with the nitrogen atom they are attached to, form a cyclic amine;

R\textsuperscript{8} represents a group selected from Ci-C6-alkyl-, halo-Ci-C3-alkyl-, C\textsubscript{3}-C\textsubscript{7}-cycloalkyl-, heterocyclyl-, phenyl, benzyl and heteroaryl,

wherein said group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH\textsubscript{2}, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-,

wherein one of the substituents R\textsuperscript{1} or R\textsuperscript{5} is Z-#l,

wherein Z stands for the linker and # 1 for the bond to the binder;

or the salts, solvates or salts of solvates thereof.
According to the invention, the CDK9 inhibitor may be attached to the binder via a linker by substitution of one of the substituents R\(^1\) or R\(^5\).

The CDK9 inhibitor which is attached to the binder (or the CDK9 inhibitors, since frequently more than one CDK9 inhibitor is attached to the binder), is preferably a compound of the formula (la) below:

\[
\begin{align*}
\text{A} & \text{ represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2- and -S(=0)(=NR)}^5; \\
\text{X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;} \\
\text{R}^1 & \text{ represents -Z-#l or a group selected from Ci-C6-alkyl-, C3-C6-alkenyl, C3-C6-alkynyl, C3-C7-cycloalkyl-, heterocycl1-, phenyl, heteroaryl, phenyl-Ci-C3-alkyl- and heteroaryl-Ci-C3-alkyl-,} \\
& \text{wherein said group is optionally substituted with one or two or three substituents, identically or differently,} \\
& \text{selected from the group consisting of hydroxy, cyano, halogen, Ci-C6-alkyl-, halo-Ci-C3-alkyl-, Ci-C6-alkoxy-, Ci-C3-fluoroalkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-,} \\
& \text{cyclic amines, -OP(=0)(OH)\text{2-}, -C(=0)OH, -C(=0)NH\text{2-};} \\
\text{R}^5 & \text{ represents -Z-#l or a group selected from a hydrogen atom, cyano, -C(=O)R}^5, -C(=O)OR}^5, -S(=O)2R}^8, -C(=0)NR\text{2}^5, \text{Ci-C6-alkyl-, C3-C7-cycloalkyl-, heterocycl1-, phenyl, heteroaryl, wherein said Ci-C6-alkyl,} \\
& \text{C3-C7-cycloalkyl-, heterocycl1-, phenyl or heteroaryl group is optionally substituted with one, two or three} \\
& \text{substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, C1-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-,} \\
& \text{cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-;} \\
& \text{wherein one of the substituents R}^1 \text{ or R}^5 \text{ is Z-#l,}
\end{align*}
\]
wherein Z stands for the linker and #1 for the bond to the binder; or the salts, solvates or salts of solvates thereof.

The conjugates according to the invention can have chemically labile linkers, enzymatically labile linkers or stable linkers. Particular preference is given to stable linkers and linkers which can be cleaved by cathepsin.

The invention furthermore provides processes for preparing the conjugates according to the invention, and also precursors and intermediates for the preparation.

The preparation of the conjugates according to the invention regularly comprises the following steps:

(i) Preparation of a linker precursor which optionally carries protective groups and has a reactive group which is capable of coupling to the binder;

(ii) Conjugation of the linker precursor to the derivative, which optionally carries protective groups, of a low-molecular weight CDK9 inhibitor (preferably a CDK9 inhibitor having the structure I, and particularly preferably of formula (la)), giving a reactive CDK9 inhibitor/linker conjugate which optionally carries protective groups;

(iii) Removal of any protective groups present in the CDK9 inhibitor/linker conjugate and

(iv) Conjugation of the binder to the CDK9 inhibitor/linker conjugate, giving the binder/CDK9 inhibitor conjugate according to the invention.

Attachment of the reactive group may also take place after the construction of an optionally protected CDK9 inhibitor/linker precursor conjugate.

Depending on the linker, succinimide-linked ADCs may, after conjugation, be converted according to Scheme A into the open-chain succinamides, which have an advantageous stability profile.

As illustrated above, conjugation of the linker precursor to a low-molecular weight CDK9 inhibitor may take place by substitution of \( R^1 \) or \( R^3 \) in formula (I), or \( R^1 \) or \( R^3 \) in formula (la) with the linker. In the synthesis steps prior to the conjugation, any functional groups present may also be present in protected form. Prior to the conjugation step, these protective groups are removed by known methods of peptide chemistry. Conjugation can take place chemically by various routes, as shown in an exemplary manner in Schemes 8 to 26 in the examples. In particular, it is optionally possible to modify the low-molecular weight CDK9 inhibitor for conjugation to the linker, for example by introduction of protective groups or leaving groups to facilitate substitution.
Instead of -Z-#l, it is also possible for the group -Z-#3 to be present in the compound, where Z represents the linker and #3 represents the reactive group for binding to the binder or the derivative thereof. Compounds comprising -Z-#3 are reactive compounds which react with the binder or the derivative thereof. #3 is preferably a group which reacts with an amino or thiol group with formation of a covalent bond, preferably with the cysteine residue in a protein. The cysteine residue in a protein may of course be present naturally in the protein, may be introduced by biochemical methods or, preferably, may be generated by prior reduction of disulphides of the binder.

The invention furthermore provides CDK9 inhibitor-linker-intermediates which are compounds of general formula (I)

$$\text{(I)}$$

wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)₂ and -S(=0)(=NR₅);  
X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;  
R¹ represents -Z or a group selected from C₁-C₆-alkyl-, C₃-C₆-alkenyl, C₃-C₆-alkynyl, C₃-C₇-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, phenyl-C₃-C₆-alkyl- and heteroaryl-C₃-C₆-alkyl-,  
wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, C₁-C₆-alkyl-, halo-C₃-C₆-alkyl-, C₃-C₆-alkoxy-, C₃-C₆-fluoroalkoxy-, -N₃₄ alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, -OP(=0)(OH)¾ -C(=0)OH, -C(=0)NH₂;
R\textsuperscript{3}, R\textsuperscript{4} represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-;

R\textsuperscript{5} represents -Z or a group selected from a hydrogen atom, cyano, -C(=O)R\textsuperscript{8}, -C(=O)OR\textsuperscript{8}, -S(=O)\textsubscript{2}R\textsuperscript{8}, -C(=O)NR\textsuperscript{7}, Ci-C6-alkyl-, C\textsubscript{3}-C\textsubscript{7} -cycloalkyl-, heterocycl-\textsubscript{1}, phenyl, heteroaryl, wherein said Ci-C6-alkyl-, C\textsubscript{3}-C\textsubscript{7} -cycloalkyl-, heterocycl-\textsubscript{1}, phenyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, C1-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-;

R\textsuperscript{6}, R\textsuperscript{7} represent, independently from each other, a group selected from a hydrogen atom, Ci-C6-alkyl-, C\textsubscript{3}-C\textsubscript{7} -cycloalkyl-, heterocycl-\textsubscript{1}, phenyl, benzyl and heteroaryl, wherein said Ci-C6-alkyl-, C\textsubscript{3}-C\textsubscript{7} -cycloalkyl-, heterocycl-\textsubscript{1}, phenyl, benzyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-, or

R\textsuperscript{8} represents a group selected from Ci-C6-alkyl-, halo-Ci-C3-alkyl-, C\textsubscript{3}-C\textsubscript{7} -cycloalkyl-, heterocycl-\textsubscript{1}, phenyl, benzyl and heteroaryl, wherein said group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-,

wherein one of the substituents R\textsuperscript{1} or R\textsuperscript{5} is Z,

wherein Z stands for the linker;
or the enantiomers, diastereomers, salts, solvates or salts of solvates thereof.

**Detailed description of the invention**

The invention provides conjugates of a binder or derivative thereof with one or more active compound molecules, the active compound molecule being a CDK9 kinase inhibitor (CDK9 inhibitor) attached to the binder via a linker Z.

The conjugate according to the invention can be represented by the general formula
where $AB$ represents the binder, preferably an antibody, $Z$ represents the linker, $MC$ represents the CDK9 inhibitor, and $n$ represents a number from 1 to 50, preferably from 1.2 to 20 and particularly preferably from 2 to 8. Here, $n$ is a mean of the number of CDK9 inhibitor/linker conjugates per BINDER. CDK9 preferably has the formula (I) shown above. The binder is preferably a binder peptide or protein such as, for example, an antibody. Furthermore, the linker is preferably attached to different amino acids of the binder peptide or protein or derivative thereof. Particular preference is given to binding to different cysteine or lysine residues of the binder, even more preferable is binding to different cysteine residues of the binder.

Binders which can be used according to the invention, CDK9 inhibitors which can be used according to the invention and linkers which can be used according to the invention which can be used in combination without any limitation are described below. In particular, the binders represented in each case as preferred or particularly preferred can be employed in combination with the CDK9 inhibitors represented in each case as preferred or particularly preferred, optionally in combination with the linkers represented in each case as preferred or particularly preferred.

**CDK9 kinase inhibitors**

Despite the fact that various inhibitors of CDKs are known, there remains a need for selective CDK9 inhibitors to be used for the treatment of diseases such as hyper-proliferative diseases, viral diseases, and/or diseases of the heart, which offer one or more advantages over the compounds known from prior art, such as:

- improved activity and/or efficacy, allowing e.g. a dose reduction
- beneficial kinase selectivity profile according to the respective therapeutic need
- improved side effect profile, such as fewer undesired side effects, lower intensity of side effects, or reduced (cyto)toxicity
- improved physicochemical properties, such as solubility in water, body fluids, and aqueous formulations, e.g. for intravenous administration
- improved pharmacokinetic properties, allowing e.g. for dose reduction or an easier dosing scheme
- improved duration of action, e.g. by improved pharmacokinetics and/or improved target residence time
- easier drug substance manufacturing e.g. by shorter synthetic routes or easier purification.
The CDK9 inhibitors used in the binder drug conjugates according to the invention preferably show an improved anti-proliferative activity in tumor cell lines, such as HeLa, HeLa-MaTu-ADR, NCI-H460, DU145, Caco-2, B16F10, A2780 or MOLM-13, compared to the compounds known from prior art.

Further, the CDK9 inhibitors used in the binder drug conjugates according to the invention preferably show an increased potency to inhibit CDK9 activity (demonstrated by a lower IC50 value for CDK9/Cyclin T1) compared to the compounds known from prior art.

Further, the CDK9 inhibitors used in the binder drug conjugates according to the invention preferably show an increased potency to inhibit CDK9 activity at high ATP concentrations compared to the compounds known from prior art.

Further, the CDK9 inhibitors used in the binder drug conjugates according to the invention preferably show an increased target residence time compared to the compounds known from prior art.

Further, the CDK9 inhibitors used in the binder drug conjugates according to the invention preferably show an improved pharmacokinetic profile, e.g. a higher metabolic stability and/or a longer terminal half-life upon administration in vivo.

Further, the CDK9 inhibitors used in the binder drug conjugates according to the invention preferably show an improved duration of action, e.g. by improved pharmacokinetics and/or improved target residence time.

Further, the CDK9 inhibitors used in the binder drug conjugates according to the invention preferably show an improved CaCo-2 permeability and/or an improved CaCo-2 efflux ratio, compared to the compounds known from prior art.

Further, the CDK9 inhibitors used in the binder drug conjugates according to the invention preferably show an improved aqueous solubility compared to the compounds known from prior art.

Further, the CDK9 inhibitors used in the binder drug conjugates according to the invention preferably show, compared to the compounds known from prior art, an increased selectivity for CDK9/Cyclin T1 as compared to CDK2/Cyclin E.

Further, the CDK9 inhibitors used in the binder drug conjugates according to the invention preferably show, compared to the compounds known from prior art, an improved anti-proliferative activity in tumor cell lines, such as HeLa, HeLa-MaTu-ADR, NCI-H460, DU145, Caco-2, B16F10, A2780 or MOLM-13, and/or which show an increased potency to inhibit CDK9 activity (demonstrated by a lower IC50 value for
CDK9/Cyclin T1), and/or which show an increased potency to inhibit CDK9 activity at high ATP concentrations, and/or which show an improved pharmacokinetic profile, e.g. a higher metabolic stability and/or a longer terminal half-life upon administration \textit{in vivo}, and/or which show an increased target residence time compared to the compounds known from prior art.

Further, the CDK9 inhibitors used in the binder drug conjugates according to the invention preferably show, compared to the compounds known from prior art, an improved CaCo-2 permeability and/or an improved CaCo-2 efflux ratio, and/or which show an improved aqueous solubility, and/or show an increased selectivity for CDK9/Cyclin T1 as compared to CDK2/Cyclin E.

According to the present invention, the CDK9 inhibitors are described by the following structure:

![Chemical Structure](image)

wherein

- **A** represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2-, -S(=0)(=NR 5)-;

- **L** represents a C2-C6-alkylene group, wherein said group is optionally substituted with
  - (iii) one substituent selected from hydroxy, C2-C3-alkenyl, C2-C3-alkynyl, C3-C4-cycloalkyl, hydroxy-Ci-C3-alkyl, -(CH2)NR8R7, and/or
  - (iv) one or two or three substituents, identically or differently, selected from halogen and Ci-C3-alkyl,

with the proviso that a C2-alkylene group is not substituted with a hydroxy group,
or wherein
one carbon atom of said C2-C6-alkylene group forms a three- or four-membered ring together with a bivalent group to which it is attached, wherein said bivalent group is selected from -CH2CH2-, -CH2CH2CH2-, -CH2OCH2-;

5. X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R represents -Z-#1 or a group selected from Cl-C6-alkyl-, C3-C6-alkenyl, C3-C6-alkynyl, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, phenyl-Ci-C3-alkyl- and heteroaryl-Ci-C3-alkyl-, wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, Cl-C6-alkyl-, halo-Ci-C3-alkyl-, Cl-C6-alkoxy-, Cl-C3-fluoroalkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, -OP(=0)(OH)₃, -C(=0)OH, -C(=0)NH₃;

10. R represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C3-alkyl-, Cl-Ci-C3-alkoxy-, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-;

15. R, R4 represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C3-alkyl-, Cl-C3-alkoxy-, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-;

20. R represents -Z-#1 or a group selected from a hydrogen atom, cyano, -C(=0)R, -C(=0)OR, -S(=0)₂, -C(=0)NR₃, Cl-C6-alkyl-, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, wherein said Ci-C6-alkyl, C3-C7-cycloalkyl, heterocyclyl-, phenyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, Cl-C3-alkyl-, Cl-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Cl-C3-fluoroalkoxy-;

25. R, R7 represent, independently from each other, a group selected from a hydrogen atom, Ci-C6-alkyl-, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, benzyl and heteroaryl, wherein said Ci-C6-alkyl, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, benzyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Cl-C3-alkyl-, Cl-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-, or
R⁶ and R⁷, together with the nitrogen atom they are attached to, form a cyclic amine;

R⁸ represents a group selected from Ci-C6-alkyl-, halo-Ci-C3-alkyl-, C₃-C₇-cycloalkyl-, heterocycl-, phenyl, benzyl and heteroaryl,

wherein said group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH₂, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-,

wherein one of the substituents R¹ or R⁵ is Z-#₁,

wherein Z stands for the linker and #₁ for the bond to the binder;

or the salts, solvates or salts of solvates thereof.

CDK9 inhibitors or precursors thereof used for the synthesis of the conjugate according to the invention are the compounds of the formula (I) and the salts, solvates and solvates of the salts thereof, the compounds of the hereinafter recited formula which are encompassed by formula (I) and the salts, solvates and solvates of the salts thereof, and the compounds which are encompassed by formula (I) and are mentioned hereinafter as exemplary embodiments and the salts, solvates and solvates of the salts thereof, where the compounds which are encompassed by formula (I) and are mentioned hereinafter are not already salts, solvates and solvates of the salts.

The CDK9 inhibitors or precursors thereof of formula (I) may, depending on their structure, exist in stereoisomeric forms (enantiomers, diastereomers). The invention therefore relates to the enantiomers or diastereomers and respective mixtures thereof. The stereoisomERICally pure constituents can be isolated in a known manner from such mixtures of enantiomers and/or diastereomers.

If the compounds according to the invention can be in tautomeric forms, the present invention encompasses all tautomeric forms.

Further, the CDK9 inhibitors or precursors thereof can exist in free form, e.g. as a free base, or as a free acid, or as a zwitterion, or can exist in the form of a salt. Said salt may be any salt, either an organic or inorganic addition salt, particularly any physiologically acceptable organic or inorganic addition salt, customarily used in pharmacy.

Salts which are preferred for the purposes of the present invention are physiologically acceptable salts of the compounds according to the invention. However, salts which are not suitable for pharmaceutical applications
per se, but which, for example, can be used for the isolation or purification of the compounds according to the invention, are also comprised.

The term "physiologically acceptable salt" refers to a relatively non-toxic, inorganic or organic acid addition salt of a compound of the present invention, for example, see S. M. Berge, et al. "Pharmaceutical Salts," J. Pharm Sci. 1977, 66, 1-19.

Physiologically acceptable salts of the compounds according to the invention encompass acid addition salts of mineral acids, carboxylic acids and sulfonic acids, for example salts of hydrochloric acid, hydrobromic acid, hydroiodic, sulfuric acid, bisulfuric acid, phosphoric acid, nitric acid or with an organic acid, such as formic, acetic, acetoacetic, pyruvic, trifluoroacetic, propionic, butyric, hexanoic, heptanoic, undecanoic, lauric, benzoic, salicylic, 2-(4-hydroxybenzoyl)-benzoic, camphoric, cinnamon, cyclopentanepropionic, digluconic, 3-hydroxy-2-naphthoic, nicotinic, pamoic, pectinic, persulfuric, 3-phenylpropionic, picric, pivalic, 2-hydroxyethanesulfonate, itaconic, sulfamic, trifluoromethanesulfonic, dodecylsulfuric, ethansulfonic, benzenesulfonic, para-toluene sulfonic, methansulfonic, 2-naphthalenesulfonic, naphthalenedisulfonic, camphorsulfonic acid, citric, tartaric, stearic, lactic, oxalic, malonic, succinic, malic, adipic, alginic, maleic, fumaric, D-gluconic, mandelic, ascorbic, glucoheptanoic, glycerophosphoric, aspartic, sulfosalicylic, hemisulfuric, or thiocyanic acid, for example.

Physiologically acceptable salts of the compounds according to the invention also comprise salts of conventional bases, such as, by way of example and by preference, alkali metal salts (for example sodium and potassium salts), alkaline earth metal salts (for example calcium and magnesium salts) and ammonium salts derived from ammonia or organic amines with 1 to 16 C atoms, such as, by way of example and by preference, ethylamine, diethylamine, triethylamine, ethyldiisopropylamine, monoethanolamine, diethanolamine, triethanolamine, dicyclohexylamine, dimethylaminoethanol, procaine, dibenzylamine, N-methylmorpholine, arginine, lysine, ethylenediamine, N-methylpiperidine, N-methylglucamine, dimethylglucamine, ethylglucamine, 1,6-hexadiamine, glucosamine, sarcosine, serinol, tris(hydroxymethyl)aminomethane, aminopropanediol, Sovak base, and 1-amino-2,3,4-butane triol. Additionally, the compounds according to the invention may form salts with a quarternary ammonium ion obtainable e.g. by quarternisation of a basic nitrogen containing group with agents such as lower alkylhalides such as methyl-, ethyl-, propyl-, and butylchlorides, -bromides and -iodides; dialkylsulfates such as dimethyl-, diethyl-, dibutyl- and diamylsulfates, long chain halides such as decyl-, lauryl-, myristyl- and stearylchlorides, -bromides and -iodides, aralkyl halides such as benzyl- and phenethyl bromides and others. Examples of suitable quarternary ammonium ions are tetramethylammonium, tetraethylammonium, tetra(«-propyl)ammonium, tetra (n-butyl)ammonium, or N-benzy l-N-N-trimethyl lammonium.
The present invention includes all possible salts of the compounds of the present invention as single salts, or as any mixture of said salts, in any ratio.

Solvates is the term used for the purposes of the invention for those forms of the CDK9 inhibitors or precursors thereof which form a complex with solvent molecules by coordination in the solid or liquid state. Hydrates are a special form of solvates in which the coordination takes place with water. Hydrates are preferred as solvates within the scope of the present invention.

The invention also includes all suitable isotopic variations of a CDK9 inhibitor or precursors thereof. An isotopic variation is defined as one in which at least one atom is replaced by an atom having the same atomic number but an atomic mass different from the atomic mass usually or predominantly found in nature. Examples of isotopes that can be incorporated into a compound of the invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, chlorine, bromine and iodine, such as $^2$H (deuterium), $^3$H (tritium), $^{11}$C, $^{14}$C, $^{15}$N, $^{17}$O, $^{18}$O, $^{32}$P, $^{33}$P, $^{34}$S, $^{35}$S, $^{36}$S, $^{37}$Cl, $^{38}$Br, $^{82}$I, $^{123}$I, $^{124}$I, $^{125}$I and $^{129}$I, respectively. Certain isotopic variations of a compound of the invention, for example, those in which one or more radioactive isotopes such as $^3$H or $^{14}$C are incorporated, are useful in drug and/or substrate tissue distribution studies. Tritiated and carbon-14, i.e., $^{14}$C, isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with isotopes such as deuterium may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased in vivo half-life or reduced dosage requirements and hence may be preferred in some circumstances. Isotopic variations of a compound of the invention can generally be prepared by conventional procedures known by a person skilled in the art such as by the illustrative methods or by the preparations described in the examples hereafter using appropriate isotopic variations of suitable reagents.

Furthermore, the present invention includes all possible crystalline forms, or polymorphs, of the CDK9 inhibitors or precursors thereof, either as single polymorphs, or as a mixture of more than one polymorphs, in any ratio.

Accordingly, the present invention includes all possible salts, polymorphs, metabolites, hydrates, solvates, prodrugs (e.g.: esters) thereof, and diastereoisomeric forms of the the CDK9 inhibitors or precursors thereof as single salt, polymorph, metabolite, hydrate, solvate, prodrug (e.g.: esters) thereof, or diastereoisomeric form, or as mixture of more than one salt, polymorph, metabolite, hydrate, solvate, prodrug (e.g.: esters) thereof, or diastereoisomeric form in any ratio.

For the purpose of the present invention the term metabolite is understood as the product of the (e.g., enzymatic or chemical) cleavage of the conjugate of a binder or a derivative thereof according to the
present invention. Accordingly, the metabolite will comprise a molecule of an active component, wherein
the active component is a CDK9 kinase inhibitor. In one embodiment, if the conjugate according to the
invention comprises a stable linker, the metabolite of the CDK9 inhibitor as such comprises an amino
acid residue, preferably a cysteine or a lysine residue. In another embodiment, if the conjugate according
to the invention comprises an instable linker, the metabolite of the CDK9 inhibitor as such is possibly
connected with only part of the linker moiety, i.e. the metabolite of the CDK9 inhibitor as such does not
comprise a cysteine and/or a lysine residue which originally belonged to the binder protein or peptide.

For the purposes of the present invention, the substituents have the following meaning, unless otherwise
specified:

The term "halogen", "halogen atom" or "halo" represents fluorine, chlorine, bromine and iodine,
particularly bromine, chlorine or fluorine, preferably chlorine or fluorine, more preferably fluorine.

The term "alkyl" represents a linear or branched alkyl group having the number of carbon atoms
specifically indicated, e.g. C1-C10one, two, three, four, five, six, seven, eight, nine or ten carbon atoms,
e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, teri-butyl, pentyl, isopentyl, hexyl,
heptyl, octyl, nonyl, decyl, 2-methylbutyl, 1-methylbutyl, 1-ethylpropyl, 1,2-dimethylpropyl, neo-
pentyl, 1,1-dimethylpropyl, 4-methylpentyl, 3-methylpentyl, 2-methylpentyl, 1-methylpentyl, 2-
ethylbutyl, 1-ethylbutyl, 3,3-dimethylbutyl, 2,2-dimethylbutyl, 1,1-dimethylbutyl, 2,3-dimethylbutyl, 1,3-
dimethylbutyl, or 1,2-dimethylbutyl. If the number of carbon atoms is not specifically indicated the term
"alkyl" represents a linear or branched alkyl group having, as a rule, 1 to 9, particularly 1 to 6, preferably
1 to 4 carbon atoms. Particularly, the alkyl group has 1, 2, 3, 4, 5 or 6 carbon atoms ("C1-C6-alkyl"), e.g.
methyl, ethyl, n-propyl-, isopropyl, n-butyl, teri-butyl, pentyl, isopentyl, hexyl, 2-methylbutyl, 1-
methylbutyl, 1-ethylpropyl, 1,2-dimethylpropyl, neo-pentyl, 1,1-dimethylpropyl, 4-methylpentyl, 3-
methylpentyl, 2-methylpentyl, 1-methylpentyl, 2-ethylbutyl, 1-ethylbutyl, 3,3-dimethylbutyl, 2,2-
dimethylbutyl, 1,1-dimethylbutyl, 2,3-dimethylbutyl, 1,3-dimethylbutyl, or 1,2-
dimethylbutyl. Preferably, the alkyl group has 1, 2 or 3 carbon atoms ("C1-C3-alkyl"), methyl, ethyl, n-
propyl or isopropyl.

The term "C2-C6-alkylene" is to be understood as preferably meaning a linear, bivalent and saturated
hydrocarbon group having 2 to 6, particularly 2, 3 or 4 carbon atoms, as in "C2-C4-alkylene" e.g. ethylene,
^3-propylene, n-butylene, n-pentylene, or n-hexylene, preferably ^3-propylene or n-butylene.

The term "C2-C6-alkenyl" is to be understood as preferably meaning a linear or branched, monovalent
hydrocarbon group, which contains one double bond, and which has 2, 3, 4, 5 or 6 carbon atoms ("C2-C6-
alkenyl”). Particularly, said alkenyl group is a C2-C3-alkenyl, C3-C6-alkenyl or C3-C7-alkenyl group. Said alkenyl group is, for example, a vinyl, allyl, (E)-2-methylvinyl, (Z)-2-methylvinyl or isopropenyl group.

The term "C2-C6-alkynyl" is to be understood as preferably meaning a linear or branched, monovalent hydrocarbon group which contains one triple bond, and which contains 2, 3, 4, 5 or 6 carbon atoms. Particularly, said alkynyl group is a C2-C3-alkynyl, C3-C6-alkynyl or C3-C7-alkynyl group. Said C2-C3-alkynyl group is, for example, an ethynyl, prop-1-ynyl or prop-2-ynyl group.

The term "C3-C7-cycloalkyl" is to be understood as preferably meaning a saturated or partially unsaturated, monovalent, monocyclic hydrocarbon ring which contains 3, 4, 5, 6 or 7 carbon atoms. Said C3-C7-cycloalkyl group is for example, a monocyclic hydrocarbon ring, e.g. a cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl group. Said cycloalkyl ring is non-aromatic but can optionally contain one or more double bonds e.g. cycloalkenyl, such as a cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl or cycloheptenyl group, wherein the bond between said ring with the rest of the molecule may be to any carbon atom of said ring, be it saturated or unsaturated. Particularly, said cycloalkyl group is a C4-C6-cycloalkyl, a Cs-Cs-cycloalkyl or a cyclohexyl group.

The term "C3-Cs-cycloalkyl" is to be understood as preferably meaning a saturated, monovalent, monocyclic hydrocarbon ring which contains 3, 4 or 5 carbon atoms. In particular said C3-Cs-cycloalkyl group is a monocyclic hydrocarbon ring such as a cyclopropyl, cyclobutyl or cyclopentyl group. Preferably said "C3-Cs-cycloalkyl" group is a cyclopropyl group.

The term "C3-Cs-cycloalkyl" is to be understood as preferably meaning a saturated, monovalent, monocyclic hydrocarbon ring which contains 3 or 4 carbon atoms. In particular, said C3-Cs-cycloalkyl group is a monocyclic hydrocarbon ring such as a cyclopropyl or cyclobutyl group.

The term "heterocyclyl" is to be understood as meaning a saturated or partially unsaturated, monovalent, mono- or bicyclic hydrocarbon ring which contains 3, 4, 5, 6, 7, 8 or 9 carbon atoms and further containing 1, 2 or 3 heteroatom-containing groups selected from oxygen, sulfur, nitrogen. Particularly, the term "heterocyclyl" is to be understood as meaning a "4- to 10-membered heterocyclic ring".

The term "a 4- to 10-membered heterocyclic ring" is to be understood as meaning a saturated or partially unsaturated, monovalent, mono- or bicyclic hydrocarbon ring which contains 3, 4, 5, 6, 7, 8 or 9 carbon atoms, and further containing 1, 2 or 3 heteroatom-containing groups selected from oxygen, sulfur, nitrogen.
A C₃-C₉-heterocyclyl is to be understood as meaning a heterocyclyl which contains at least 3, 4, 5, 6, 7, 8 or 9 carbon atoms and additionally at least one heteroatom as ring atoms. Accordingly in case of one heteroatom the ring is 4- to 10-membered, in case of two heteroatoms the ring is 5- to 11-membered and in case of three heteroatoms the ring is 6- to 12-membered.

Said heterocyclic ring is for example, a monocyclic heterocyclic ring such as an oxetanyl, azetidinyl, tetrahydrofuranyl, pyrrolidinyl, 1,3-dioxolanyl, imidazolidinyl, pyrazolidinyl, oxazolidinyl, isoazolidinyl, 1,4-dioxanyl, pyrrolinyl, tetrahydropyranyl, piperidinyl, morpholinyl, 1,3-dithianyl, thiomorpholinyl, piperezinyl, or chinuclidinyl group. Optionally, said heterocyclic ring can contain one or more double bonds, e.g. 4H-pyranyl, 2H-pyranyl, 2,5-dihydro-1H-pyrrolyl, 1,3-dioxolyl, 4H-1,3,4-thiadiazinyl, 2,5-dihydrofuranyl, 2,3-dihydrofuranyl, 2,5-dihydrothienyl, 2,3-dihydrothienyl, 4,5-dihydrooxazolyl, 4,5-dihydroisoaxazolyl, or 4H-1,4-thiazinyl group, or, it may be benzo fused.

Particularly, a C₃-C₇-heterocyclyl is to be understood as meaning a heterocyclyl which contains at least 3, 4, 5, 6, or 7 carbon atoms and additionally at least one heteroatom as ring atoms. Accordingly in case of one heteroatom the ring is 4- to 8-membered, in case of two heteroatoms the ring is 5- to 9-membered and in case of three heteroatoms the ring is 6- to 10-membered.

Particularly, a C₃-C₆-heterocyclyl is to be understood as meaning a heterocyclyl which contains at least 3, 4, 5 or 6 carbon atoms and additionally at least one heteroatom as ring atoms. Accordingly in case of one heteroatom the ring is 4- to 7-membered, in case of two heteroatoms the ring is 5- to 8-membered and in case of three heteroatoms the ring is 6- to 9-membered.

Particularly, the term "heterocyclyl" is to be understood as being a heterocyclic ring which contains 3, 4 or 5 carbon atoms, and 1, 2 or 3 of the above-mentioned heteroatom-containing groups (a "4- to 8-membered heterocyclic ring"), more particularly said ring can contain 4 or 5 carbon atoms, and 1, 2 or 3 of the above-mentioned heteroatom-containing groups (a "5- to 8-membered heterocyclic ring"), more particularly said heterocyclic ring is a "6-membered heterocyclic ring", which is to be understood as containing 4 carbon atoms and 2 of the above-mentioned heteroatom-containing groups or 5 carbon atoms and one of the above-mentioned heteroatom-containing groups, preferably 4 carbon atoms and 2 of the above-mentioned heteroatom-containing groups.

The term "C₆-alkoxy-" is to be understood as preferably meaning a linear or branched, saturated, monovalent, hydrocarbon group of formula -O-alkyl, in which the term "alkyl" is defined supra, e.g. a methoxy, ethoxy, n-propoxy, 2₆O-propoxy, n-butoxy, 2₆O-butoxy, teri-butoxy, sec-butoxy, pentyloxy, iso-pentyloxy, n-hexyloxy group, or an isomer thereof. Particularly, the "C₆-alkoxy-" group is a "C₁-C₄-
alkoxy-", a "Ci-C3-alkoxy-", a methoxy, ethoxy, or propoxy group, preferably a methoxy, ethoxy or propoxy group. Further preferred is a "Ci-C2-alkoxy-" group, particularly a methoxy or ethoxy group.

The term "Ci-C3-fluoroalkoxy-" is to be understood as preferably meaning a linear or branched, saturated, monovalent, Ci-C3-alkoxy- group, as defined supra, in which one or more of the hydrogen atoms is replaced, identically or differently, by one or more fluoro atoms. Said Ci-C3-fluoroalkoxy- group is, for example a 1,1-difluoromethoxy-, a 1,1,1-trifluoromethoxy-, a 2-fluoroethoxy-, a 3-fluoropropoxy-, a 2,2,2-trifluoroethoxy-, a 3,3,3-trifluoropropoxy-, particularly a "Ci-C2-fluoroalkoxy-" group.

The term "alkylamino-" is to be understood as preferably meaning an alkylamino group with one linear or branched alkyl group as defined supra. (Ci-C3)-alkylamino- for example means a monoalkylamino group with 1, 2 oder 3 carbon atoms, (Ci-C6)-alkylamino- with 1, 2, 3, 4, 5 or 6 carbon atoms. The term "alkylamino-" comprises for example methylamino-, ethylamino-, n-propylamino-, iso-propylamino-, tert.-butylamino-, n-pentylamino- or n-hexylamino-.

The term "dialkylamino-" is to be understood as preferably meaning an alkylamino group having two linear or branched alkyl groups as defined supra, which are independent from each other. (Ci-C3)-dialkylamino- for example represents a dialkylamino group with two alkyl groups each of them having 1 to 3 carbon atoms per alkyl group. The term "dialkylamino-" comprises for example: N,N-dimethylamino-, N,N-diethylamino-, N-ethyl-N-methy lamino-, N-methyl-N-propylamino-, N-wo-propyl-N-n-propylamino-, N-tert-butyl-N-methylamino-, N-ethyl-N-n-pentylamino- and N-n-hexyl-N-methylamino-.

The term "cyclic amine" is to be understood as preferably meaning a cyclic amine group. Preferably, a cyclic amine means a saturated, monocyclic group with 4 to 10, preferably 4 to 7 ring atoms of which at least one ring atom is a nitrogen atom. Suitable cyclic amines are especially azetidine, pyrrolidine, piperidine, piperazine, 1-methylpiperazine, morpholine, thiomorpholine, which could be optionally substituted by one or two methyl groups.

The term "halo-Ci-C3-alkyl-", or, used synonymously, "Ci-C3-haloalkyl-", is to be understood as preferably meaning a linear or branched, saturated, monovalent hydrocarbon group in which the term "Ci-C3-alkyl" is defined supra, and in which one or more hydrogen atoms is replaced by a halogen atom, identically or differently, i.e. one halogen atom being independent from another. Preferably, a halo-Ci-C3-alkyl- group is a fluoro-Ci-C3-alkyl- or a fluoro-Ci-C2-alkyl- group, such as for example -CF3, -CHF2, -CH2F, -CF2CF3, or -CH2CF3, more preferably it is -CF3.
The term "hydroxy-Ci-C3-alkyl-", is to be understood as preferably meaning a linear or branched, saturated, monovalent hydrocarbon group in which the term "Ci-C3-alkyl" is defined supra, and in which one or more hydrogen atoms is replaced by hydroxy group, preferably not more than one hydrogen atom per carbon atom being replaced by a hydroxy group. Particularly, a hydroxy-Ci-C3-alkyl- group is, for example,

\[
\text{-CH}_2\text{OH, -CH}_2\text{-CH}_2\text{OH, -C(H)OH-CH}_2\text{OH, -CH}_2\text{-CH}_2\text{-CH}_2\text{OH.}
\]

The term "phenyl-Ci-C3-alkyl-" is to be understood as preferably meaning a phenyl group, in which one of the hydrogen atoms is replaced by a Ci-C3-alkyl group, as defined supra, which links the phenyl-Ci-C3-alkyl- group to the rest of the molecule. Particularly, the "phenyl-Ci-C3-alkyl-" is a phenyl-Ci-C2-alkyl-, preferably it is a benzyl- group.

The term "heteroaryl" is to be understood as preferably meaning a monovalent, aromatic ring system having 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 ring atoms (a "5- to 14-membered heteroaryl" group), particularly 5 (a "5-membered heteroaryl") or 6 (a "6-membered heteroaryl") or 9 (a "9-membered heteroaryl") or 10 ring atoms (a "10-membered heteroaryl"), and which contains at least one heteroatom which may be identical or different, said heteroatom being such as oxygen, nitrogen or sulfur, and can be monocyclic, bicyclic, or tricyclic, and in addition in each case can be benzo-condensed. Particularly, heteroaryl is selected from thienyl, furanyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, tetrazolyl etc., and benzo derivatives thereof, such as, for example, benzothienyl, benzothiazolyl, benzoxazolyl, benzisoxazolyl, benzimidazolyl, benzotriazolyl, indazolyl, indolyl, isoindolyl, etc.; or pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, etc., and benzo derivatives thereof, such as, for example, quinolinyl, quinazolinyl, isoquinolinyl, etc.; or azocinyl, indolizinyl, purinyl, etc., and benzo derivatives thereof; or cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, naphthyridinyl, pteridinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, xanthenyl, or oxepinyl, etc. Preferably, heteroaryl is selected from monocyclic heteroaryl, 5-membered heteroaryl or 6-membered heteroaryl.

The term "5-membered heteroaryl" is understood as preferably meaning a monovalent, aromatic ring system having 5 ring atoms and which contains at least one heteroatom which may be identical or different, said heteroatom being such as oxygen, nitrogen or sulfur. Particularly, "5-membered heteroaryl" is selected from thienyl, furanyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, triazolyl, thiadiazolyl, tetrazolyl.

The term "6-membered heteroaryl" is understood as preferably meaning a monovalent, aromatic ring system having 6 ring atoms and which contains at least one heteroatom which may be identical or different, said heteroatom being such as oxygen, nitrogen or sulfur. Particularly, "6-membered heteroaryl" is selected from pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl.
The term "heteroaryl-C1-C3-alkyl-" is to be understood as preferably meaning a heteroaryl, a 5-membered heteroaryl or a 6-membered heteroaryl group, each as defined supra, in which one of the hydrogen atoms is replaced by a C1-C3-alkyl group, as defined supra, which links the heteroaryl-C1-C3-alkyl- group to the rest of the molecule. Particularly, the "heteroaryl-C1-C3-alkyl-" is a heteroaryl-C1-C2-alkyl-, a pyridinyl-C1-C3-alkyl-, a pyridinylmethyl-, a pyridinylethyl-, a pyridinylpropyl-, a pyrimidinyl-C1-C3-alkyl-, a pyrimidinylmethyl-, a pyrimidinylethyl-, a pyrimidinylpropyl-, preferably a pyridinylmethyl- or a pyridinylethyl- or a pyrimidinylethyl- or a pyrimidinylpropyl- group.

As used herein, the term "leaving group" refers to an atom or a group of atoms that is displaced in a chemical reaction as stable species taking with it the bonding electrons. Preferably, a leaving group is selected from the group comprising: halo, in particular chloro, bromo or iodo, methanesulfonyloxy, p-toluensulfonyloxy, trifluoromethanesulfonyloxy, nonafluorobutanesulfonyloxy, (4-bromobenzene)sulfonyloxy, (4-nitro-benzene)sulfonyloxy, (2-nitro-benzene)-sulfonyloxy, (4-isopropylbenzene)sulfonyloxy, (2,4,6-tri-isopropyl-benzene)-sulfonyloxy, (2,4,6-trimethyl-benzene)sulfonyloxy, (4-tertbutyl-benzene)sulfonyloxy, benzenesulfonyloxy, and (4-methoxy-benzene)sulfonyloxy.

As used herein, the term "C1-C3-alkylbenzene" refers to a partially aromatic hydrocarbon consisting of a benzene ring which is substituted by one or two C1-C3-alkyl groups, as defined supra. Particularly, "C1-C3-alkylbenzene" is toluene, ethylbenzene, cumene, n-propylbenzene, ortho-xylene, meta-xylene or para-xylene. Preferably, "C1-C3-alkylbenzene" is toluene.

As used herein, the term "carboxamide based solvent" refers to lower aliphatic carboxamides of the formula G-C2-alkyl-C(=0)-N(C1-C2-alkyl)2, or lower cyclic aliphatic carboxamides of the formula

\[ \text{G} \quad \text{N} \quad \text{C}_1\text{-C}_2\text{-alkyl} \]

in which G represents -CH2-, -CH2-CH2- or -CH2-CH2-CH2-. Particularly, "carboxamide based solvent" is \(N,N\)-dimethylformamide, \(N\)-dimethylacetamide or \(N\)-methylpyrrolidin-2-one. Preferably, "carboxamide based solvent" is \(N\)-methyl-pyrrolidin-2-one.

The term "C1-C10", as used throughout this text, e.g. in the context of the definition of "C1-C10-alkyl" is to be understood as meaning an alkyl group having a finite number of carbon atoms of 1 to 10, i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbon atoms. It is to be understood further that said term "C1-C10" is to be interpreted as any sub-range comprised therein, e.g. C1-C10, C1-C9, Ci-Cs, C1-C7, Ci-Ce Ci-Cs, C1-C4, Ci-
Similarly, as used herein, the term "Ci-Ce", as used throughout this text, e.g. in the context of the definition of "Ci-C6-alkyl", "Ci-C6-alkoxy" is to be understood as meaning an alkyl group having a finite number of carbon atoms of 1 to 6, i.e. 1, 2, 3, 4, 5 or 6 carbon atoms. It is to be understood further that said term "Ci-Ce" is to be interpreted as any sub-range comprised therein, e.g. Ci-Ce Ci-Cs, C1-C4, C1-C3, C1-C2, C2-C6, C2-C5, C2-C4, C2-C3, C3-C6, C3-C5, C3-C4, C4-C6, C4-C5, C5-C6.

Similarly, as used herein, the term "C1-C4", as used throughout this text, e.g. in the context of the definition of "Ci-C4-alkyl", "Ci-C4-alkoxy" is to be understood as meaning an alkyl group having a finite number of carbon atoms of 1 to 4, i.e. 1, 2, 3 or 4 carbon atoms. It is to be understood further that said term "C1-C4" is to be interpreted as any sub-range comprised therein, e.g. C1-C4, C1-C3, C1-C2, C2-C4, C2-C3, C3-C4.

Similarly, as used herein, the term "C1-C3", as used throughout this text, e.g. in the context of the definition of "Ci-C3-alkyl", "Ci-C3-alkoxy" or "Ci-C3-fluoroalkoxy" is to be understood as meaning an alkyl group having a finite number of carbon atoms of 1 to 3, i.e. 1, 2 or 3 carbon atoms. It is to be understood further that said term "C1-C3" is to be interpreted as any sub-range comprised therein, e.g. Ci-C3, C1-C2, C2-C3.

Further, as used herein, the term "C3-C6" as used throughout this text, e.g. in the context of the definition of "C3-C6-cycloalkyl", is to be understood as meaning a cycloalkyl group having a finite number of carbon atoms of 3 to 6, i.e. 3, 4, 5 or 6 carbon atoms. It is to be understood further that said term "C3-C6" is to be interpreted as any sub-range comprised therein, e.g. C3-C6, C3-C5, C3-C4, C4-C6, C4-C5, C5-C6.

Further, as used herein, the term "C3-C7" as used throughout this text, e.g. in the context of the definition of "C3-C7-cycloalkyl", is to be understood as meaning a cycloalkyl group having a finite number of carbon atoms of 3 to 7, i.e. 3, 4, 5, 6 or 7 carbon atoms, particularly 3, 4, 5 or 6 carbon atoms. It is to be understood further that said term "C3-C7" is to be interpreted as any sub-range comprised therein, e.g. C3-C7, C3-C6, C3-C5, C3-C4, C4-C7, C4-C6, C4-C5, C5-C7, C5-C6, C6-C7.

A symbol / at a bond denotes the linkage site in the molecule.

As used herein, the term "one or more times", e.g. in the definition of the substituents of the compounds of the general formulae of the present invention, is understood as meaning one, two, three, four or five
times, particularly one, two, three or four times, more particularly one, two or three times, even more particularly one or two times.

Where the plural form of the word compounds, salts, hydrates, solvates and the like, is used herein, this is taken to mean also a single compound, salt, isomer, hydrate, solvate or the like.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of \(-S-, -S(=0)-, -S(=0)2-, -S(=0)(=NR^5)-;\)

L represents a C2-C6-alkylene group,

wherein said group is optionally substituted with one substituent selected from hydroxy, C\(_2\)-C\(_3\)-alkenyl, C\(_2\)-C\(_3\)-alkynyl, C\(_3\)-C\(_4\)-cycloalkyl, hydroxy-C\(_3\)-C\(_6\)-alkyl, -(CH\(_2\))NR\(_R^7\),

and optionally with one or two or three additional substituents, identically or differently, selected from halogen and C\(_3\)-alkyl,

with the proviso that a C2-alkylene group is not substituted with a hydroxy group,

or wherein

one carbon atom of said C2-C6-alkylene group forms a three- or four-membered ring together with a bivalent group to which it is attached, wherein said bivalent group is selected from -CH2CH2-, -CH2CH2CH2-, -CH2OCH2-;

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R\(^1\) represents \(-Z-#l, a group selected from \(-C(=0)OH, -C(=0)NH\_2, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, -OP(=0)(OH)\_2, -C(=0)OH, -C(=0)NH_2;\)

R\(^2\) represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, C\(_3\)-alkyl-, C\(_3\)-alkoxy-, halo-C\(_3\)-alkyl-, C\(_3\)-fluoroalkoxy-;
R³, R⁴ represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C₃-alkyl-, Ci-C₃-alkoxy-, halo-Ci-C₃-alkyl-, Ci-C₃-fluoroalkoxy-;

R⁵ represents -Z-#₁, a group selected from a hydrogen atom, cyano, -C(=0)R⁸, -C(=0)OR⁸, -S(=0)-R⁸, -C(=0)NR⁷, Ci-C₅-C₇-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, wherein said Ci-C₆-alkyl-, Ci-C₃-C₇-cycloalkyl-, heterocyclyl-, phenyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, Ci-C₃-alkyl-, Ci-C₃-alkoxy-, -NH₂, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C₃-alkyl-, Ci-C₃-fluoroalkoxy-;

R⁶, R⁷ represent, independently from each other, a group selected from a hydrogen atom, Ci-C₆-alkyl-, Ci-C₃-C₇-cycloalkyl-, heterocyclyl-, phenyl, benzyl and heteroaryl, wherein said Ci-C₆-alkyl-, Ci-C₃-C₇-cycloalkyl-, heterocyclyl-, phenyl, benzyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C₃-alkyl-, Ci-C₃-alkoxy-, -NH₂, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C₃-alkyl-, Ci-C₃-fluoroalkoxy-, or

R⁸ and R⁷, together with the nitrogen atom they are attached to, form a cyclic amine;

R⁸ represents a group selected from Ci-C₆-alkyl-, halo-Ci-C₃-alkyl-, Ci-C₃-C₇-cycloalkyl-, heterocyclyl-, phenyl, benzyl and heteroaryl, wherein said group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C₃-alkyl-, Ci-C₃-alkoxy-, -NH₂, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C₃-alkyl-, Ci-C₃-fluoroalkoxy-, wherein one of the substituents R¹ or R₅ is Z-#₁,

wherein Z stands for the linker and #₁ for the bond to the binder;

or the salts, solvates or salts of solvates thereof.

In another embodiment of the present invention the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)₂-, -S(=0)(=NR₅)-;
L represents a C₂-C₄-alkylene group,
wherein said group is optionally substituted with
(i) one substituent selected from hydroxy, C₃-C₄-cycloalkyl, hydroxy-Ci-C₃-alkyl,
-\((\text{CH}_2)\text{NR}^6\text{R}^7\), and/or
(ii) one or two or three additional substituents, identically or differently, selected from halogen
and Ci-C₃-alkyl,
with the proviso that a C₂-alkylene group is not substituted with a hydroxy group,

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y
represents N;

R¹ represents -Z#1 or a group selected from Ci-C₆-alkyl-, Cs-Cs-cycloalkyl-, phenyl and phenyl-Ci-
C₃-alkyl-,
wherein said group is optionally substituted with one or two or three substituents, identically or
differently, selected from the group consisting of hydroxy, cyano, halogen, Ci-C₃-alkyl-, fluoro-Ci-
C₂-alkyl-, Ci-C₃-alkoxy-, Ci-C₂-fluoroalkoxy-, -NH₂, alkylamino-, dialkylamino-, cyclic amines, -
\(\text{OP}(=0)(\text{OH})_2\), \(-\text{C}(=0)\text{OH}, -\text{C}(=0)\text{NH}_2\);

R² represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom,
cyano, Ci-C₂-alkyl-, Ci-C₂-alkoxy-, fluoro-C i-C₂-alkyl-, Ci-C₂-fluoroalkoxy-;

R³, R⁴ represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a
chloro atom, a bromo atom, cyano Ci-C₂-alkyl-, Ci-C₂-alkoxy-, fluoro-C i-C₂-alkyl-, Ci-C₂-fluoroalkoxy-;

R⁵ represents -Z#1 or a group selected from a hydrogen atom, cyano, -\(\text{C}(=0)\text{R}^5\), -\(\text{C}(=0)\text{OR}^5\),
-\(\text{S}(=0)\text{R}^8\), -\(\text{C}(=0)\text{NR}^6\text{R}^7\), Ci-Ce-alkyl-, C₃-C₅-cycloalkyl-, phenyl,
wherein said Ci-C₆-alkyl, Cs-Cs-cycloalkyl- or phenyl group is optionally substituted with one, two
or three substituents, identically or differently, selected from the group consisting of halogen,
hydroxy, cyano, Ci-C₃-alkyl-, Ci-C₃-alkoxy-, -NH₂, alkylamino-, dialkylamino-, cyclic amines,
fluoro-C i-C₂-alkyl-, Ci-C₂-fluoroalkoxy-;

R⁶, R⁷ represent, independently from each other, a group selected from a hydrogen atom, Ci-C₆-alkyl-,
c₃-c₅ -cycloalkyl-, phenyl and benzyl,
wherein said Ci-C₆-alkyl-, C₃-C₅-cycloalkyl-, phenyl or benzyl group is optionally substituted with
one, two or three substituents, identically or differently, selected from the group consisting of
halogen, hydroxy, Ci-C₃-alkyl-, Ci-C₃-alkoxy-, -NH₂, alkylamino-, dialkylamino-, cyclic amines,
fluoro-C i-C₂-alkyl-, Ci-C₂-fluoroalkoxy-, or
R^6 and R^7, together with the nitrogen atom they are attached to, form a cyclic amine;

R^8 represents a group selected from Ci-C6-alkyl-, fluoro-Ci-C3-alkyl-, Cs-Cs-cycloalkyl-, phenyl and benzyl,

wherein said group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines, fluoro-Ci-C2-alkyl-, Ci-C2-fluoroalkoxy-,

wherein one of the substituents R^1 or R^5 is Z-#l,

wherein Z stands for the linker and #1 for the bond to the binder;

or the salts, solvates or salts of solvates thereof.

In another embodiment of the present invention the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2-, -S(=0)(=NR^5)-;

L represents a C2-C4-alkylene group,

wherein said group is optionally substituted with one substituent selected from hydroxy, Ci-C4-cycloalkyl, hydroxy-Ci-C3-alkyl, -(CH^2)NR^7,

and optionally with one or two or three additional substituents, identically or differently, selected from halogen and Ci-C3-alkyl,

with the proviso that a C2-alkylene group is not substituted with a hydroxy group,

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R^1 represents -Z#l or a group selected from Ci-C6-alkyl-, Cs-Cs-cycloalkyl-, phenyl and phenyl-Ci-C3-alkyl-,

wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, Ci-C3-alkyl-, fluoro-Ci-C2-alkyl-, Ci-C3-alkoxy-, Ci-C2-fluoroalkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines, -OP(=0)(OH)\_2, -C(=0)OH, -C(=0)NH\_2;

R^2 represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C2-alkyl-, Ci-C2-alkoxy-, fluoro-Ci-C2-alkyl-, Ci-C2-fluoroalkoxy-;
R^3, R^4 represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C2-alkyl-, Ci-C2-alkoxy-, fluoro-Ci-C2-alkyl-, C1-C2-fluoroalkoxy-;

R^5 represents -Z#l or a group selected from a hydrogen atom, cyano, -C(=0)R, -C(=0)OR, -S(=0)R, -C(=0)NRR, C3-C5-cycloalkyl-, phenyl,

wherein said Ci-C6-alkyl, Cs-Cs-cycloalkyl- or phenyl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines, fluoro-Ci-C2-alkyl-, Ci-C2-fluoroalkoxy-;

R^6, R^7 represent, independently from each other, a group selected from a hydrogen atom, Ci-C6-alkyl-, C3-C5-cycloalkyl-, phenyl and benzyl,

wherein said Ci-C6-alkyl-, Cs-Cs-cycloalkyl-, phenyl or benzyl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines, fluoro-Ci-C2-alkyl-, Ci-C2-fluoroalkoxy- or

R^6 and R^7, together with the nitrogen atom they are attached to, form a cyclic amine;

R^8 represents a group selected from Ci-C6-alkyl-, fluoro-Ci-C3-alkyl-, Cs-Cs-cycloalkyl-, phenyl and benzyl,

wherein said group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines, fluoro-Ci-C2-alkyl-, Ci-C2-fluoroalkoxy-,

wherein one of the substituents R^1 or R^5 is Z#l,

wherein Z stands for the linker and #1 for the bond to the binder;

or the salts, solvates or salts of solvates thereof.

In a preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2-, -S(=0)(=NR)^5-;

L represents a C2-C4-alkylene group,

wherein said group is optionally substituted with

(i) one substituent selected from C3-C4-cycloalkyl and hydroxymethyl-, and/or
(ii) one or two additional substituents, identically or differently, selected from Cl-C2-alkyl,

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R^1 represents -Z^#1 or a group selected from C_1-C_4-alkyl-, C_3-C_5-cycloalkyl- and phenyl,

wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, Cl-C2-alkyl-, Cl-C_2-alkoxy-, -NH_2, -C(=0)OH;

R^2 represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, cyano, methyl, methoxy-, trifluoromethyl-, trifluoromethoxy-;

R^3 represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, cyano, methyl, methoxy-, trifluoromethyl-, trifluoromethoxy-;

R^4 represents a hydrogen atom or a fluoro atom;

R^5 represents -Z^#1 or a group selected from a hydrogen atom, cyano, -C(=0)NR^6R^7, -C(=0)R^8, -C(=0)OR^8, -S(=0)R^8, Cl-C_4-alkyl-,

wherein said C_1-C_4-alkyl- group is optionally substituted with one substituent selected from the group consisting of halogen, hydroxy, cyano, Cl-C_3-alkoxy-, -NH_2, alkylamino-, dialkylamino-, cyclic amines;

R^6, R^7 represent, independently from each other, a group selected from a hydrogen atom, Cl-C_4-alkyl- and Cl-C_3-C_5-cycloalkyl-,

wherein said Cl-C_4-alkyl- or C_3-C_5-cycloalkyl- group is optionally substituted with one or two substituents, identically or differently, selected from the group consisting of hydroxy, Cl-C2-alkyl-, Cl-C_2-alkoxy-, -NH_2, alkylamino-, dialkylamino-, cyclic amines, or

R^6 and R^7, together with the nitrogen atom they are attached to, form a cyclic amine;

R^8 represents a group selected from Cl-C_6-alkyl-, fluoro-Cl-C_3-alkyl-, C_3-C_5-cycloalkyl- and phenyl,

wherein said group is optionally substituted with one substituent selected from the group consisting of halogen, hydroxy, Cl-C2-alkyl-, Cl-C_2-alkoxy-, -NH_2,

wherein one of the substituents R^1 or R^5 is Z^#1,

wherein Z stands for the linker and #1 for the bond to the binder;

or the salts, solvates or salts of solvates thereof.
In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2-, -S(=0)(=NR 5)-;

5

L represents a C2-C4-alkylene group,

wherein said group is optionally substituted with one substituent selected from C3-C4-cycloalkyl and hydroxymethyl-, and optionally with one or two additional substituents, identically or differently, selected from C1-C2-alkyl,

10

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

15 R1 represents -Z#l or a group selected from C1-C4-alkyl-, C3-Cs-cycloalkyl- and phenyl,

wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, Ci-C2-alkyl-, C1-C2-alkoxy-, -NH2;

20 R2 represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, cyano, methyl, methoxy-, trifluoromethyl-, trifluoromethoxy-;

R3 represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, cyano, methyl, methoxy-, trifluoromethyl-, trifluoromethoxy-;

25 R4 represents a hydrogen atom or a fluoro atom;

R5 represents -Z#l or a group selected from a hydrogen atom, cyano, -C(=0)NR 7, -C(=0)R 8, -S(=0)2R 8, Ci-C4-alkyl-, wherein said C1-C4-alkyl- group is optionally substituted with one substituent selected from the group consisting of halogen, hydroxy, cyano, G-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines;

30 R6, R7 represent, independently from each other, a group selected from a hydrogen atom, G-C4-alkyl- and C3-C5-cycloalkyl-, wherein said G-C4-alkyl- or C3-C5 -cycloalkyl- group is optionally substituted with one or two substituents, identically or differently, selected from the group consisting of hydroxy, G-C2-alkyl-, G-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines, or

35 R6 and R7, together with the nitrogen atom they are attached to, form a cyclic amine;
R^8 represents a group selected from Ci-C6-alkyl-, fluoro-Ci-C3-alkyl-, Cs-Cs-cycloalkyl- and phenyl, wherein said group is optionally substituted with one substituent selected from the group consisting of halogen, hydroxy, Ci-C2-alkyl-, Ci-C2-alkoxy-, -NH2, wherein one of the substituents R^1 or R^5 is Z-#l, wherein Z stands for the linker and #1 for the bond to the binder; or the salts, solvates or salts of solvates thereof.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2-, -S(=0)(=NR^5)-;

L represents a C2-C4-alkylene group;

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R^1 represents -Z#1 or a group selected from C1-C4-alkyl-, wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, Ci-C2-alkoxy-, -NH2, -C(=0)OH;

R^2 represents a hydrogen atom;

R^3 represents a group selected from a hydrogen atom, a fluoro atom;

R^4 represents a hydrogen atom;

R^5 represents -Z#1 or a group selected from a hydrogen atom, cyano, -C(=0)NR^7, -C(=0)R^8, -C(=0)OR^8, -S(=0)R^8, Ci-C4-alkyl-, R^6, R^7 represent, independently from each other, a group selected from a hydrogen atom, C1-C4-alkyl- and c3-c5 cycloalkyl-, or R^6 and R^7, together with the nitrogen atom they are attached to, form a cyclic amine;

R^8 represents a group selected from C1-C6 -alkyl-, fluoro-Ci-C3 -alkyl-, C3-Cs-cycloalkyl- and phenyl, wherein said group is optionally substituted with one substituent selected from the group consisting of halogen, hydroxy, Ci-C2-alkyl-, Ci-C2-alkoxy-, -NH2.
wherein one of the substituents $R^1$ or $R^5$ is $Z\#l$,
wherein $Z$ stands for the linker and $\# l$ for the bond to the binder;

or the salts, solvates or salts of solvates thereof.

5

In a particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein

$A$ represents a bivalent group selected from the group consisting of $-S(=0)2-$, $-S(=0)(=NR^5)-$;

$L$ represents a C2-C4-alkylene group;

$X, Y$ represent CH or N with the proviso that one of $X$ and $Y$ represents CH and one of $X$ and $Y$ represents N;

$R^1$ represents $-Z\#l$ or a group selected from C1-C4-alkyl-,

wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, C1-C2-alkoxy-, $-N\frac{3}{4}$ -C(=0)OH;

$R^2$ represents a hydrogen atom;

$R^3$ represents a group selected from a hydrogen atom, a fluoro atom;

$R^4$ represents a hydrogen atom;

$R^5$ represents $-Z\#l$ or a group selected from a hydrogen atom, cyano, $-C(=0)NR^7$, $-C(=0)R^8$, $-C(=0)OR^8$, $-S(=0)2R^6$, C1-C4-alkyl-,

$R^6, R^7$ represent, independently from each other, a group selected from a hydrogen atom, C1-C4-alkyl- and C3-C5-cycloalkyl-, or

$R^8$ represents a group selected from C1-C6-alkyl-, fluoro-C1-C3-alkyl-, C3-Cs-cycloalkyl- and phenyl,

wherein said group is optionally substituted with one substituent selected from the group consisting of halogen, hydroxy, C1-C2-alkyl-, C1-C2-alkoxy-, $-N\frac{3}{4}$ ,

wherein one of the substituents $R^1$ or $R^5$ is $Z\#l$,

wherein $Z$ stands for the linker and $\# l$ for the bond to the binder;

or the salts, solvates or salts of solvates thereof.
In a particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of \(-S=02-,\)
\(-S(=0)(=NR^5)\)-;

L represents a \(C_3-C_4\)-alkylene group;

\(X, Y\) represent CH or N with the proviso that one of \(X\) and \(Y\) represents CH and one of \(X\) and \(Y\) represents N;

\(R^1\) represents \(-Z#1\) or a methyl- group;

\(R^2\) represents a hydrogen atom;

\(R^3\) represents a group selected from a hydrogen atom, a fluoro atom;

\(R^4\) represents a hydrogen atom;

\(R^5\) represents \(-Z#1\) or a group selected from a hydrogen atom, \(-C(=0)NR^7, -C(=0)R^8,\)
\(-C(=0)OR^8, -S(=0)\)\(^6\), \(-C_1-C_2\)-alkyl-,

\(R^6, R^7\) represent, independently from each other, a group selected from a hydrogen atom, \(-C_1-C_2\)-alkyl-

\(R^8\) represents a \(-C_1-C_2\)-alkyl- group,

wherein one of the substituents \(R^1\) or \(R^5\) is \(-Z#1\),

wherein \(Z\) stands for the linker and \(#1\) for the bond to the binder;

or the salts, solvates or salts of solvates thereof.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of \(-S-, -S(=0)-, -S(=0)2-,\)
\(-S(=0)(=NR^5)\)-;

L represents a \(-CH_2CH_2CH_2-\) group;

\(X, Y\) represent CH or N with the proviso that one of \(X\) and \(Y\) represents CH and one of \(X\) and \(Y\) represents N;

\(R^1\) represents \(-Z#1\) or a group selected from \(-C_1-C_2\)-alkyl-,
wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, Ci-C2-alkoxy-, -NH2, -C(=0)OH;

R² represents a hydrogen atom;

R³ represents a group selected from a hydrogen atom, a fluoro atom;

R⁴ represents a hydrogen atom;

R⁵ represents -Z#l or a group selected from a hydrogen atom, cyano, -C(=0)NR⁷, -C(=0)R⁸, -C(=0)OR⁸, -S(=0)₂R⁵, Ci-C₄-alkyl,

R⁶, R⁷ represent, independently from each other, a group selected from a hydrogen atom, C₁-C₄-alkyl- and C₃-C₅-cycloalkyl-, or

R⁶ and R⁷, together with the nitrogen atom they are attached to, form a cyclic amine;

R⁸ represents a group selected from Ci-C₆-alkyl-, fluoro-Ci-C₃-alkyl-, C₃-Cs-cycloalkyl- and phenyl, wherein said group is optionally substituted with one substituent selected from the group consisting of halogen, hydroxy, Ci-C2-alkyl-, Ci-C2-alkoxy-, -NH2,

wherein one of the substituents R¹ or R⁵ is Z-#l,

wherein Z stands for the linker and # 1 for the bond to the binder;

or the salts, solvates or salts of solvates thereof.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of -S(=0)₂-, -S(=0)(=NR⁵)-;

L represents a -CH₂CH₂CH₂- group;

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R¹ represents -Z#l or a methyl- group;

R² represents a hydrogen atom;

R³ represents a group selected from a hydrogen atom, a fluoro atom
R^4 represents a hydrogen atom;

R^5 represents -Z#l or a group selected from a hydrogen atom, -C(=0)NR \(^6\)R^7, -C(=0)R \(^8\), -C(=0)OR \(^8\), -S(=0)\(^2\)R^8, Ci-C\(_4\)-alkyl-.

5 R^6, R^7 represent, independently from each other, a group selected from a hydrogen atom, Ci-C\(_2\)-alkyl-;

R^8 represents a Ci-C\(_2\)-alkyl- group, wherein one of the substituents R^1 or R^5 is Z-#l,

10 wherein Z stands for the linker and #1 for the bond to the binder;

or the salts, solvates or salts of solvates thereof.

15 In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2-, -S(=0)(=NR \(^5\))-;

20 L represents a C\(_2\)C\(_4\)-alkylene group;

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

25 R^1 represents -Z#l or a C\(_1\)-C\(_2\)-alkyl- group;

R^2 represents a hydrogen atom;

R^3 represents a group selected from a hydrogen atom, a fluoro atom;

30 R^4 represents a hydrogen atom;

R^5 represents -Z#l or a group selected from a hydrogen atom, cyano, -C(=0)NR \(^6\)R^7, -C(=0)R \(^8\), -C(=0)OR \(^8\), -S(=0)\(^2\)R^8, Ci-C\(_4\)-alkyl-,

35 R^6, R^7 represent, independently from each other, a group selected from a hydrogen atom, C\(_1\)-C\(_4\)-alkyl- and C\(_3\)-C\(_5\)-cycloalkyl-, or

R^6 and R^7, together with the nitrogen atom they are attached to, form a cyclic amine;

40 R^8 represents a group selected from Ci-C\(_6\)-alkyl-, fluoro-Ci-C\(_3\)-alkyl-, Cs-Cs-cycloalkyl- and phenyl, wherein said group is optionally substituted with one substituent selected from the group consisting of hydroxy, Ci-C\(_2\)-alkyl-, Ci-C\(_2\)-alkoxy-,

wherein one of the substituents R^1 or R^5 is Z-#l,
wherein Z stands for the linker and #1 for the bond to the binder;

or the salts, solvates or salts of solvates thereof.

5 In a particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of \(-S(=0)2-, -S(=0)(=NR^5)-\);

10 L represents a \(-\text{CH}_2\text{CH}_2\text{CH}_2-\) group;

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

15 \(R^1\) represents \(-Z\#1\) or a methyl- group;

\(R^2\) represents a hydrogen atom;

\(R^3\) represents a fluoro atom;

\(R^4\) represents a hydrogen atom;

\(R^5\) represents \(-Z\#1\) or a group selected from a hydrogen atom, \(-C(=0)NR^7, -C(=0)R^8, -C(=0)OR^8, -S(=0)\#R^8, \text{methyl}-\);

25 \(R^6\) represents an ethyl- group;

\(R^7\) represents a hydrogen atom;

\(R^8\) represents a \(\text{C}_2\text{-C}_4\)-alkyl- group,

wherein one of the substituents \(R^1\) or \(R^5\) is \(Z\#1\),

wherein Z stands for the linker and #1 for the bond to the binder;

or the salts, solvates or salts of solvates thereof.

35 In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of \(-S-, -S(=0)-, -S(=0)(=NR^5)-\);

40 L represents a \(\text{C}_2\text{-C}_4\)-alkylene group,

wherein said group is optionally substituted with one or two methyl groups;

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;
R¹ represents \(-Z\#l\) or a \(C_1\)-\(C_4\)-alkyl- group,
wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, a fluoro atom, \(Ci\)-\(C_2\)-alkoxy-, -NH₂;

5 R² represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, cyano, methyl, methoxy-, trifluoromethyl-, trifluoromethoxy-;

R³ represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, cyano, methyl, methoxy-, trifluoromethyl-, trifluoromethoxy-;

10 R⁴ represents a hydrogen atom or a fluoro atom;

R⁵ represents \(-Z\#l\) or a group selected from a hydrogen atom, cyano, -\((=0)NR\) R⁷;

15 R⁶, R⁷ represent, independently from each other, a group selected from a hydrogen atom and \(Ci\)-\(C_4\)-alkyl-,
wherein said \(Ci\)-\(C_4\)-alkyl- group is optionally substituted with one substituent selected from the group consisting of hydroxy, \(Ci\)-\(C_2\)-alkoxy-, -Nth, alkylamino-, dialkylamino-, cyclic amines,

20 wherein one of the substituents R¹ or R⁵ is \(-Z\#l\),
wherein Z stands for the linker and # 1 for the bond to the binder;

or the salts, solvates or salts of solvates thereof.

25 In a particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein
A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)(=NR) ⁵;
L represents a \(c_2\)-\(c_4\)-alkylene group;

30 X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R¹ represents \(-Z\#l\) or a \(Ci\)-\(Ci\)-alkyl- group;

R² represents a hydrogen atom or a fluoro atom;

35 R³ represents a hydrogen atom or a fluoro atom;

R⁴ represents a hydrogen atom;

R⁵ represents \(-Z\#l\) or a group selected from a hydrogen atom, -\((=0)NR\) R⁷;

R⁶, R⁷ represent, independently from each other, a group selected from a hydrogen atom and \(Ci\)-\(C_2\)-alkyl-,
wherein one of the substituents R¹ or R⁵ is \(-Z\#l\),

40 wherein Z stands for the linker and # 1 for the bond to the binder;
or the salts, solvates or salts of solvates thereof.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)(=NR 5);  
L represents a C₃-C₄-alkylene group;  
X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;  
R¹ represents -Z#l or a methyl group;  
R² represents a hydrogen atom;  
R³ represents a fluoro atom;  
R⁴ represents a hydrogen atom;  
R⁵ represents -Z#l or a hydrogen atom;  
wherein one of the substituents R¹ or R⁵ is Z-#l,  
wherein Z stands for the linker and # 1 for the bond to the binder;  
or the salts, solvates or salts of solvates thereof.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I), wherein

A represents a bivalent group selected from the group consisting of -S(=0)2-, -S(=0)(=NR 5);  
L represents a C₃-C₄-alkylene group;  
X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;  
R¹ represents -Z#l or a methyl group;  
R² represents a hydrogen atom;  
R³ represents a fluoro atom;  
R⁴ represents a hydrogen atom;  
R⁵ represents -Z#l or a hydrogen atom;  
wherein one of the substituents R¹ or R⁵ is Z-#l,  
wherein Z stands for the linker and # 1 for the bond to the binder;  
or the salts, solvates or salts of solvates thereof.
In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2-, -S(=0)(=NR 5)-.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which A represents a bivalent group -S-.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which A represents a bivalent group selected from the group consisting of -S(=0)2-, -S(=0)(=NR 5)-.

In a preferred embodiment of the invention, the CDK9 inhibitors are compounds of formula (I) in which A represents a bivalent group selected from the group consisting of -S(=0)2-, -S(=0)(=NR 5)-.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which A represents a bivalent group -S(=0)2-.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which A represents a bivalent group -S(=0)(=NR 5)-.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which L represents a C2-C6-alkylene group, wherein said group is optionally substituted with one substituent selected from hydroxy, C2-C3-alkenyl, C2-C3-alkynyl, C3-C4-cycloalkyl, hydroxy-Ci-C3-alkyl, -(CH2)NR R7, and optionally with one or two or three additional substituents, identically or differently, selected from halogen and Ci-C3-alkyl, with the proviso that a C2-alkylene group is not substituted with a hydroxy group, or wherein one carbon atom of said C2-C6-alkylene group forms a three- or four-membered ring together with a bivalent group to which it is attached, wherein said bivalent group is selected from -CH2CH2-, -CH2CH2CH2-, -CH2OCH2-.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which L represents a C2-C6-alkylene group, wherein said group is optionally substituted with

(i) one substituent selected from hydroxy, C2-C3-alkenyl, C2-C3-alkynyl, C3-C4-cycloalkyl, hydroxy-Ci-C3-alkyl, -(CH2)NR R7, and/or
(ii) one or two or three substituents, identically or differently, selected from halogen and C1-C3-alkyl,

with the proviso that a C2-alkylene group is not substituted with a hydroxy group,

or wherein

5 one carbon atom of said C2-C6-alkylene group forms a three- or four-membered ring together with a bivalent group to which it is attached, wherein said bivalent group is selected from -CH2CH2-, -CH2CH2CH2-, -CH2OCH2-.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which L represents a C2-C4-alkylene group,

wherein said group is optionally substituted with one substituent selected from hydroxy, C3-C4-cycloalkyl, hydroxy-Ci-Cs-alkyl, -(CH2)NR7R7, and optionally with one or two or three additional substituents, identically or differently, selected from halogen and Ci-C3-alkyl,

15 with the proviso that a C2-alkylene group is not substituted with a hydroxy group.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which L represents a C2-C4-alkylene group,

wherein said group is optionally substituted with

20 (i) one substituent selected from hydroxy, C3-C4-cycloalkyl, hydroxy-Ci-C3-alkyl, -(CH2)NR7R7, and/or

(ii) one or two or three additional substituents, identically or differently, selected from halogen and Ci-C3-alkyl,

with the proviso that a C2-alkylene group is not substituted with a hydroxy group.

In a preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which L represents a C2-C4-alkylene group,

wherein said group is optionally substituted with one substituent selected from C3-C4-cycloalkyl and hydroxymethyl-, and optionally with one or two additional substituents, identically or differently, selected from Ci-C2-alkyl.

30 In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which L represents a C2-C4-alkylene group,
wherein said group is optionally substituted with

(i) one substituent selected from C_{3}-C_{4} cycloalkyl and hydroxymethyl-, and/or

(ii) one or two additional substituents, identically or differently, selected from C_{1}-C_{6}-alkyl.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which L represents a C_{2}-C_{4}-alkylene group, wherein said group is optionally substituted with one or two methyl groups.

In a particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which L represents a C_{2}-C_{4}-alkylene group.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which L represents a C_{3}-C_{4}-alkylene group.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which L represents a group -CH_{2}CH_{2}CH_{2}- or -CH_{2}CH_{2}CH_{2}CH_{2}-. In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which L represents a group -CH_{2}CH_{2}CH_{2}-.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which L represents a group -CH_{2}CH_{2}CH_{2}CH_{2}-. In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^{1} represents -Z#l or a group selected from C_{i}-C_{j}-alkyl-, C_{3}-C_{6}-alkenyl, C_{3}-C_{6}-alkynyl, C_{3}-C_{7}-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, phenyl-C_{i}-C_{j}-alkyl- and heteroaryl-C_{i}-C_{j}-alkyl-, wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, C_{i}-C_{j}-alkyl-, halo-C_{i}-C_{j}-alkyl-, C_{i}-C_{j}-alkoxy-, C_{i}-C_{j}-fluoroalkoxy-, -NH_{2}, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, -OP(=0)(OH)_{3}, -C(=0)OH, -C(=0)NH_{2};

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^{1} represents -Z#l or a group selected from C_{3}-C_{4}-alkyl-, C_{3}-C_{6}-cycloalkyl-, phenyl and phenyl-C_{i}-C_{j}-alkyl-,
wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, Ci-C3 -alkyl-, fluoro-Ci-C2-alkyl-, Ci-C3-alkoxy-, Ci-C2 -fluoroalkoxy-, -NH2, alkylamo-, dialkylamino-, cyclic amines, -OP(=0)(OH)2, -C(=0)OH, -C(=0)NH₂.

In a preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R¹ represents -Z₁ or a group selected from C₁-C₈-alkyl-, C₃-Cs-cycloalkyl- and phenyl, wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, Ci-C2 -alkyl-, Ci-C2 -alkoxy-, -NH2, -C(=0)OH.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R¹ represents -Z₁ or a Ci-C₈-alkyl- group,

wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, a fluoro atom, Ci-C2 -alkoxy-, -NH2.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R¹ represents -Z₁ or a Ci-C₈-alkyl- group,

wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, a fluoro atom, Ci-C2 -alkoxy-, -NH₂, -C(=0)OH.

In a particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R¹ represents -Z₁ or a Ci-C₈-alkyl- group.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R¹ represents -Z₁ or a Ci-C₃ -alkyl- group.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R¹ represents -Z₁ or a Ci-C₂ -alkyl- group.
In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R\(^1\) represents -Z\(#\)I or an ethyl group.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R\(^1\) represents -Z\(#\)I or a methyl group.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R\(^1\) represents -Z\(#\).
In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \( R^1 \) represents a methyl group, and \( R^2 \) represents a hydrogen atom.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \( R^2 \) represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \( R^2 \) represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C2-alkyl-, Ci-C2-alkoxy-, fluoro-Ci-C2-alkyl-, Ci-C2-fluoroalkoxy-.

In a preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \( R^2 \) represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, cyano, methyl, methoxy-, trifluoromethyl-, trifluoromethoxy-.

In a particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \( R^2 \) represents a hydrogen atom or a fluoro atom.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \( R^2 \) represents a fluoro atom.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \( R^2 \) represents a hydrogen atom.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \( R^2 \) represents a hydrogen atom, \( R^3 \) represents a fluoro atom, and \( R^4 \) represents a hydrogen atom.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \( R^1 \) represents a methyl group, \( R^2 \) represents a hydrogen atom, \( R^3 \) represents a fluoro atom, and \( R^4 \) represents a hydrogen atom.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \( R^3 \) and \( R^4 \) represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, halo-Ci-C3-alkyl-, C1-C3-fluoroalkoxy-. 
In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^3 and R^4 represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano -alkyl-, cyano -alkoxy-, fluoro-Ci-C2-alkyl-, ci-C2-fluoroalkoxy-.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^3 represents a fluoro atom, and in which R^4 represents a hydrogen atom.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^3 and R^4 represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a chloro atom, cyano, methyl, methoxy-, trifluoromethyl-, trifluoromethoxy-.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^3 and R^4 represent, independently from each other, a hydrogen atom or a fluoro atom.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^3 represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C3 -alkyl-, Ci-C3 -alkoxy-, halo-Ci-C3-alkyl-, Ci-C3 -fluoroalkoxy-, and in which R^4 represents a hydrogen atom or a fluoro atom.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^3 represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano Ci-C2 -alkyl-, Ci-C2 -alkoxy-, fluoro-Ci-C2-alkyl-, Ci-C2 -fluoroalkoxy-, and in which R^4 represents a hydrogen atom or a fluoro atom.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^3 represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano Ci-C2 -alkyl-, Ci-C2 -alkoxy-, fluoro-Ci-C2-alkyl-, Ci-C2 -fluoroalkoxy-, and in which R^4 represents a hydrogen atom.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^3 represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, cyano, methyl, methoxy-, trifluoromethyl-, trifluoromethoxy-, and in which R^4 represents a hydrogen atom.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^3 represents a hydrogen atom or a fluoro atom, and in which R^4 represents a hydrogen atom.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^3 represents a fluoro atom, and in which R^4 represents a hydrogen atom.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^3 represents a hydrogen atom or a fluoro atom, and in which R^4 represents a hydrogen atom.
In a preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R₃ represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, cyano, methyl, methoxy-, trifluoromethyl-, trifluoromethoxy-.

In a particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R₃ represents a hydrogen atom or a fluoro atom.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R₃ represents a fluoro atom.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R₃ represents a hydrogen atom.

In a preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R₄ represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, cyano, methyl, methoxy-, trifluoromethyl-, trifluoromethoxy-.

In a particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R₄ represents a hydrogen atom or a fluoro atom.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R₄ represents a fluoro atom.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R₄ represents a hydrogen atom.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R₅ represents -Z#1 or a group selected from a hydrogen atom, cyano, -C(=0)R, -C(=0)OR, -S(=0)₂Rₘ, -C(=0)NR, Ci-C₆-alkyl-, C₃-C₇-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, wherein said Ci-C₆-alkyl, C₃-C₇-cycloalkyl-, heterocyclyl-, phenyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, Ci-C₃-alkyl-, Ci-C₃-alkoxy-, -Nₖth, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C₃-alkyl-, Ci-C₃-fluoroalkoxy-;

In another embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R₅ represents -Z#1 or a group selected from a hydrogen atom, cyano, -C(=0)R, -C(=0)OR, -S(=0)₂Rₘ, -C(=0)NR, Ci-C₆-alkyl-, C₃-C₇-cycloalkyl-, phenyl,
wherein said Ci-C6-alkyl, Cs-Cs-cycloalkyl- or phenyl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines, fluoro-Ci-C2-alkyl-, Ci-C2-fluoroalkoxy-.

5 In a preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R5 represents -Z#1 or a group selected from a hydrogen atom, cyano, -C(=0)NR 7 R 7 , -C(=0)R 8 , -S(=0)2R 8 , R 5 represents -Z#1 or a group selected from a hydrogen atom, cyano, -C(=0)NR 7 R 7 , -C(=0)R 8 , -S(=0)2R 8 , Ci-C 4-alkyl-, wherein said Ci-C 4-alkyl- group is optionally substituted with one substituent selected from the group consisting of halogen, hydroxy, cyano, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines.

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R5 represents -Z#1 or a group selected from a hydrogen atom, cyano, -C(=0)NR 7 R 7 , -C(=0)R 8 , -S(=0)2R 8 , Ci-C 4-alkyl-, wherein said Ci-C 4-alkyl- group is optionally substituted with one substituent selected from the group consisting of halogen, hydroxy, cyano, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines.

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R5 represents -Z#1 or a group selected from a hydrogen atom, cyano, -C(=0)NR 6R 7 , -C(=0)OR 8 , -S(=0)2R 8 , Ci-C 4-alkyl-.

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R5 represents -Z#1 or a group selected from a hydrogen atom, -C(=0)NR 6R 7 , -C(=0)R 8 , -C(=0)OR 8 , -S(=0)2R 8 , Ci-C 4-alkyl-.

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R5 represents -Z#1 or a group selected from a hydrogen atom, -C(=0)NR 6R 7 , -C(=0)R 8 , -C(=0)OR 8 , -S(=0)2R 8 , methyl-.

In a particularly preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R5 represents -Z#1 or a group selected from a hydrogen atom, -C(=0)NR 6R 7 , -C(=0)R 8 , -C(=0)OR 8 , -S(=0)2R 8 , Ci-C 4-alkyl-.

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R5 represents -Z#1 or a group selected from a hydrogen atom, -C(=0)NR 6R 7 , -C(=0)R 8 , -C(=0)OR 8 , -S(=0)2R 8 .
In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which \( R^5 \) represents -Z=O\(^2\) or a group selected from a hydrogen atom, -C(=0)R\(^8\), -C(=0)OR\(^8\), -S(=0)\(_2\)R\(^8\).

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which \( R^5 \) represents -Z=O\(^2\) or a group selected from a hydrogen atom, -C(=0)NR\(^7\), -C(=0)R\(^8\), -C(=0)OR\(^8\), -S(=0)\(_2\)R\(^8\).

In a particularly preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which \( R^5 \) represents -Z=O\(^2\) or a group selected from -C(=0)NR\(^7\), -C(=0)R\(^8\), -C(=0)OR\(^8\), -S(=0)\(_2\)R\(^8\), methyl-.

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which \( R^5 \) represents -Z=O\(^2\) or a group selected from -C(=0)NR\(^7\), -C(=0)R\(^8\), -C(=0)OR\(^8\), -S(=0)\(_2\)R\(^8\).

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which \( R^5 \) represents -Z=O\(^2\) or a group selected from -C(=0)R\(^8\), -C(=0)OR\(^8\), -S(=0)\(_2\)R\(^8\).

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which \( R^5 \) represents -Z=O\(^2\) or a group selected from -C(=0)NR\(^7\), -C(=0)R\(^8\), -C(=0)OR\(^8\).

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which \( R^5 \) represents -Z=O\(^2\) or a group selected from -C(=0)NR\(^7\), -C(=0)R\(^8\), -S(=0)\(_2\)R\(^8\).

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which \( R^5 \) represents -Z=O\(^2\) or a group selected from -C(=0)R\(^8\), -S(=0)\(_2\)R\(^8\).

In a particularly preferred embodiment of the present invention the CDK9 inhibitors are compounds of
formula (I) in which R⁵ represents –Z#1.

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R⁵ represents a -C(=0)OR group.

5 In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R⁵ represents a -C(=0)R group.

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R⁵ represents a -S(O)₂R group.

10 In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R⁵ represents a C₁-C₄-alkyl group.

15 In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R⁵ represents a methyl group.

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R⁵ represents a group selected from a hydrogen atom, cyano, -C(=0)NR group.

20 In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R⁵ represents a group selected from a hydrogen atom, -C(=0)NR group.

In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R⁵ represents a cyano group.

25 In another preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R⁵ represents a -C(=0)NR group.

30 In a particularly preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R⁵ represents a hydrogen atom.

In another particularly preferred embodiment of the present invention the CDK9 inhibitors are compounds of formula (I) in which R³ represents a fluoro atom, R⁴ represents a hydrogen atom, and R⁵ represents a hydrogen atom.

35 In another particularly preferred embodiment of the present invention the CDK9 inhibitors are
compounds of formula (I) in which R³ represents a fluoro atom, R⁴ represents a hydrogen atom, and R⁵ represents -Z#l.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R¹ represents a methyl group, R³ represents a fluoro atom, R⁴ represents a hydrogen atom, and R⁵ represents -Z#l.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R¹ represents a methyl group, R² represents a hydrogen atom, R³ represents a fluoro atom, R⁴ represents a hydrogen atom, and R⁵ represents -Z#l.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R¹ represents a methyl group and R⁵ represents -Z#l.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁶ and R⁷ represent, independently from each other, a group selected from a hydrogen atom, Ci-C6-alkyl-, C⁵-C⁷ -cycloalkyl-, heterocycl-, phenyl, benzyl and heteroaryl, wherein said Ci-C6-alkyl-, C⁵-C⁷ -cycloalkyl-, heterocycl-, phenyl, benzyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C³-alkyl-, Ci-C³-alkoxy-, -NH₂, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C³-alkyl-, Ci-C³-fluoroalkoxy-, or R⁶ and R⁷, together with the nitrogen atom they are attached to, form a cyclic amine.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁶ and R⁷ represent, independently from each other, a group selected from a hydrogen atom, Ci-C6-alkyl-, C⁵-C⁷ -cycloalkyl-, phenyl and benzyl, wherein said Ci-C6-alkyl-, C⁵-C⁷ -cycloalkyl-, phenyl or benzyl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C⁵-alkyl-, Ci-C³-alkoxy-, -NH₂, alkylamino-, dialkylamino-, cyclic amines, fluoro-Ci-C₂-alkyl-, C¹-C²-fluoroalkoxy-, or R⁶ and R⁷, together with the nitrogen atom they are attached to, form a cyclic amine.

In another embodiment of the invention, the CDK9 inhibitors are compounds of formula (I) in which R⁶ represents a group selected from a hydrogen atom, Ci-C6-alkyl-, C⁵-C⁷ -cycloalkyl-, phenyl and benzyl, wherein said Ci-C6-alkyl-, C⁵-C⁷ -cycloalkyl-, phenyl or benzyl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-
c_{3}-alkyl-, \textit{Ci}-C_{3} -alkoxy-, -NH_{2}, alkylamino-, dialkylamino-, cyclic amines, fluoro-C_{1}-C_{2}-alkyl-, C_{1}-C_{2}-fluoroalkoxy-, and in which \textit{R} \textsuperscript{7} represents a hydrogen atom or a C_{1}-C_{3} alkyl- group, or \textit{R} \textsuperscript{6} and \textit{R} \textsuperscript{7}, together with the nitrogen atom they are attached to, form a cyclic amine.

5 In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \textit{R} \textsuperscript{6} represents a group selected from a hydrogen atom, Ci-C_{6} -alkyl- and phenyl, wherein said Ci-C_{6} -alkyl- or phenyl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C_{3} -alkyl-, C_{1}-C_{3}-alkoxy-, dialkylamino-, and in which \textit{R} \textsuperscript{7} represents a hydrogen atom or a C_{1}-C_{3} alkyl- group, or

10 \textit{R} \textsuperscript{6} and \textit{R} \textsuperscript{7}, together with the nitrogen atom they are attached to, form a cyclic amine.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \textit{R} \textsuperscript{6} represents a group selected from a hydrogen atom, Ci-C_{6} -alkyl- and phenyl, wherein said Ci-C_{6} -alkyl- or phenyl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C_{3} -alkyl-, C_{1}-C_{3}-alkoxy-, dialkylamino-, and in which \textit{R} \textsuperscript{7} represents a hydrogen atom or a C_{1}-C_{3} alkyl- group.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \textit{R} \textsuperscript{6} and \textit{R} \textsuperscript{7}, together with the nitrogen atom they are attached to, form a cyclic amine.

20 In a preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \textit{R} \textsuperscript{6} and \textit{R} \textsuperscript{7} represent, independently from each other, a group selected from a hydrogen atom, Ci-C_{4}- alkyl- and C_{3}-C_{5} -cycloalkyl-, wherein said Ci-C_{4} -alkyl- or C_{3}-C_{5}-cycloalkyl- group is optionally substituted with one or two substituents, identically or differently, selected from the group consisting of hydroxy, Ci-C_{2} -alkyl-, Ci-C_{2} -alkoxy-, -NH_{2}, alkylamino-, dialkylamino-, cyclic amines, or \textit{R} \textsuperscript{6} and \textit{R} \textsuperscript{7}, together with the nitrogen atom they are attached to, form a cyclic amine.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which \textit{R} \textsuperscript{6} represents a group selected from a hydrogen atom, Ci-C_{4}- alkyl- and C_{3}-C_{5}-cycloalkyl-, wherein said Ci-C_{4} -alkyl- or C_{3}-C_{5}-cycloalkyl- group is optionally substituted with one or two substituents, identically or differently, selected from the group consisting of hydroxy, Ci-C_{2} -alkyl-, Ci-C_{2} -alkoxy-, -NH_{2}, alkylamino-, dialkylamino-, cyclic amines, and in which \textit{R} \textsuperscript{7} represents a hydrogen atom or a C_{1}-C_{3} alkyl- group, or \textit{R} \textsuperscript{6} and \textit{R} \textsuperscript{7}, together with the nitrogen atom they are attached to, form a cyclic amine.
In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁶ represents a group selected from a hydrogen atom, C₁-C₄-alkyl- and C₃-C₅-cycloalkyl-,
wherein said C₁-C₄-alkyl- or Cs-Cs-cycloalkyl- group is optionally substituted with one or two substituents, identically or differently, selected from the group consisting of hydroxy, Ci-C₂-alkyl-, Ci-C₂-alkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines, and in which R⁷ represents a hydrogen atom or a C₁-C₃-alkyl- group.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁶ and R⁷ represent, independently from each other, a group selected from a hydrogen atom and Ci-C₄-alkyl-,
wherein said C₁-C₃-alkyl- group is optionally substituted with one substituent selected from the group consisting of hydroxy, Ci-C₂-alkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁶ represents a group selected from a hydrogen atom and Ci-C₄-alkyl-,
wherein said C₁-C₃-alkyl- group is optionally substituted with one substituent selected from the group consisting of hydroxy, Ci-C₂-alkoxy-, -NH₂, alkylamino-, dialkylamino-, cyclic amines, and in which R⁷ represents a hydrogen atom or a C₁-C₃-alkyl- group.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁶ and R⁷ represent, independently from each other, a group selected from a hydrogen atom, Ci-C₃-alkyl- and C₃-Cs-cycloalkyl-, or R⁶ and R⁷, together with the nitrogen atom they are attached to, form a cyclic amine

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁶ and R⁷ represent, independently from each other, a group selected from a hydrogen atom, Ci-C₃-alkyl- and C₃-Cs-cycloalkyl-.

In a particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁶ and R⁷ represent, independently from each other, a group selected from a hydrogen atom and Ci-C₂-alkyl-.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁶ represents a group selected from a hydrogen atom and Ci-C₂-alkyl-, and in which R⁷ represents a hydrogen atom.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are
compounds of formula (I) in which R^6 represents a group selected from a hydrogen atom and Ci-C_2-alkyl.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^7 represents a hydrogen atom.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^6 represents a Ci-C_2-alkyl group, and in which R^7 represents a hydrogen atom.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^6 represents a Ci-C_2-alkyl group.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^6 represents an ethyl group, and in which R^7 represents a hydrogen atom.

In another particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^6 represents an ethyl group.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^8 represents a group selected from Ci-C_6-alkyl-, halo-Ci-C_3-alkyl-, C_3-C_7-cycloalkyl-, heterocyclyl-, phenyl, benzy1 and heteroaryl, wherein said group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C_3-alkyl-, Ci-C_3-alkoxy-, -Nth, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C_3-alkyl-, C_1-C_3-fluoroalkoxy-.

In another embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^8 represents a group selected from Ci-C_6-alkyl-, fluoro-Ci-C_3-alkyl-, C_3-C_5-cycloalkyl-, phenyl and benzyl, wherein said group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C_3-alkyl-, Ci-C_3-alkoxy-, -Nth, alkylamino-, dialkylamino-, cyclic amines, fluoro-Ci-C_2-alkyl-, Ci-C_2-fluoroalkoxy-.

In a preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R^8 represents a group selected from Ci-C_6-alkyl-, fluoro-Ci-C_3-alkyl-, C_3-C_5-cycloalkyl- and phenyl, wherein said group is optionally substituted with one substituent selected from the group consisting of
halogen, hydroxy, Ci-C2-alkyl-, Ci-C2-alkoxy-, -N¾.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁸ represents a group selected from C₁-C₄-alkyl-, fluoro-Ci-C₃-alkyl-.

5 In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁸ represents a C₁-C₄-alkyl- group.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁸ represents a methyl- group.

10 In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁸ represents an ethyl- group.

In another preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁸ represents a fluoro-Ci-C₃-alkyl- group.

20 In a particularly preferred embodiment of the present invention, the CDK9 inhibitors are compounds of formula (I) in which R⁸ represents a trifluoromethyl- group.

It is to be understood that the present invention relates to any sub-combination within any embodiment of the present invention of compounds of formula (I), supra.

More particularly still, the present invention covers compounds of formula (I) which are disclosed in the Example section of this text, infra.

30 Very specially preferred are combinations of two or more of the abovementioned preferred embodiments.

A particulary preferred embodiment are the CDK9 inhibitors compounds of formula (la) below:
wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2- and -S(=0)(=NR 5)-;

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R 1 represents -Z-#l or a group selected from Ci-C6-alkyl-, C3-C6-alkenyl, C3-C6-alkynyl, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, phenyl-Ci-C3-alkyl- and heteroaryl-Ci-C3-alkyl-, wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, Ci-C6-alkyl-, halo-Ci-C3-alkyl-, Ci-Ce-alkoxy-, Ci-C3-fluoroalkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, -OP(=0)(OH)¾ -C(=0)OH, -C(=0)NH 2;

R 5 represents -Z-#l or a group selected from a hydrogen atom, cyano, -C(=O)R 8, -C(=O)OR 8, -S(=O)2R 8, -C(=O)NR 5R 7, Ci-C6-alkyl-, C3-C7-cycloalkyl-, heterocycl1-, phenyl, heteroaryl, wherein said Ci-Ce-alkyl, C3-C7-cycloalkyl-, heterocycl1-, phenyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-;

wherein one of the substituents R 1 or R 5 is Z-#l,

wherein Z stands for the linker and # 1 for the bond to the binder;

or the salts, solvates or salts of solvates thereof.

In particular, the CDK9 inhibitor is a compound of the formula (la), wherein X represents N and Y represents CH.

Most preferably, the CDK9 inhibitor which is attached to the binder is a compound of the formula (la), wherein
A represents a -S(=0)2-;

R1 represents -Z-#l;

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N; and

wherein Z stands for the linker and # 1 for the bond to the binder;
or the salts, solvates or salts of solvates thereof,

or wherein

A represents -S(=0)(=NR 5)-;

R1 represents a methyl group;

R5 represents -Z-#l;

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N; and

wherein Z stands for the linker and # 1 for the bond to the binder;
or the salts, solvates or salts of solvates thereof,

Even more preferably
the CDK9 inhibitor which is attached to the binder is a compound of the formula (1a), wherein

A represents a -S(=0)2-;

R1 represents -Z-#l;

X represents N and Y represents CH; and

wherein Z stands for the linker and # 1 for the bond to the binder;
or the salts, solvates or salts of solvates thereof,

or wherein

A represents -S(=0)(=NR 5)-;

R1 represents a methyl group;

R5 represents -Z-#l;

X represents N and Y represents CH; and

wherein Z stands for the linker and # 1 for the bond to the binder;
or the salts, solvates or salts of solvates thereof.
The literature discloses various options for covalently coupling (conjugating) organic molecules to binders such as, for example antibodies (see, for example, K. Lang and J. W. Chin. Chem. Rev. 2014, 114, 4764-4806, M. Rashidian et al. Bioconjugate Chem. 2013, 24, 1277-1294). Preference according to the invention is given to conjugation of the CDK9 inhibitors to an antibody via one or more sulphur atoms of cysteine residues of the antibody which are either already present as free thiols or generated by reduction of disulphide bridges, and/or via one or more NH groups of lysine residues of the antibody. However, it is also possible to attach the CDK9 inhibitor to the antibody via tyrosine residues, via glutamine residues, via residues of unnatural amino acids, via free carboxyl groups or via sugar residues of the antibody. For coupling, use is made of linkers. Linkers can be categorized into the group of the linkers which can be cleaved in vivo and the group of the linkers which are stable in vivo (see L. Ducry and B. Stump, Bioconjugate Chem. 2011, 5-13 (2010)). The linkers which can be cleaved in vivo have a group which can be cleaved in vivo, where, in turn, a distinction may be made between groups which are chemically cleavable in vivo and groups which are enzymatically cleavable in vivo. "Chemically cleavable in vivo" and "enzymatically cleavable in vivo" means that the linkers or groups are stable in circulation and are cleaved only at or in the target cell by the chemically or enzymatically different environment therein (lower pH; elevated glutathione concentration; presence of lysosomal enzymes such as cathepsin or plasmin, or glycosidases such as, for example, B-glucuronidases), thus releasing the low-molecular weight CDK9 inhibitor or a derivative thereof. Groups which can be cleaved chemically in vivo are in particular disulphide, hydrazone, acetal and aminal; groups which can be cleaved enzymatically in vivo are in particular the 2-8-oligopeptide group, especially a dipeptide group or glycoside. Peptide cleavage sites are disclosed in Bioconjugate Chem. 2002, 13, 855-869, and Bioorganic & Medicinal Chemistry Letters 8 (1998) 3341-3346 and also Bioconjugate Chem. 1998, 9, 618-626. These include, for example, valine-alanine, valine-lysine, valine-citrulline, alanine-lysine and phenylalanine-lysine (optionally with additional amide group).

Linkers which are stable in vivo are distinguished by a high stability (less than 5% metabolites after 24 hours in plasma) and do not have the chemically or enzymatically in vivo cleavable groups mentioned above.

The linker -Z- preferably has one of the basic structures (i) to (iv) below:

(i) $\text{§-(CH}_2\text{)}^n\text{-(CO)}_m\text{-SGI}-\text{L1-}\text{L2-§}$
(ii) $\text{§-(CH}_2\text{)}^n\text{-(CO)}_m\text{-L1-SG}-\text{L1-}\text{L2-§}$
(iii) $\text{§-(CH}_2\text{)}^n\text{-(CO)}_m\text{-L1-L2-§§}$
(iv) $\text{§-(CH}_2\text{)}^n\text{-(CO)}_m\text{-L1-SG-L1-§§}$
where § represents the attachment point to the active component; §§ represents the attachment point to the binder; m is 0 or 1; v is 0, 1 or 2; SG is a (chemically or enzymatically) in vivo cleavable group (in particular disulphide, hydrazone, acetal and aminal; or a 2-8-oligopeptide group which can be cleaved by cathepsin or plasmin), SGI is an oligopeptide group or preferably a dipeptide group, LI independently of one another represent in vivo stable organic groups, and L2 represents a coupling group to the binder or a single bond. Here, coupling is preferably to a cysteine residue or a lysine residue of the binder. Alternatively, coupling can be to a tyrosine residue, glutamine residue or to an unnatural amino acid of the binder. The unnatural amino acids may contain, for example, aldehyde or keto groups (such as, for example, formylglycine) or azide or alkyne groups (see Lan & Chin, Cellular Incorporation of Unnatural Amino Acids and Bioorthogonal Labeling of Proteins, Chem.Rev. 2014, 114, 4764-4806).

Particular preference according to the invention is given to the basic linker structure (iii), in particular when the binder is an anti-TWEAKR antibody or an anti-EGFR antibody. Via metabolization, the administration of a conjugate according to the invention having a basic linker structure (iii) and coupling of the linker to a cysteine or lysine residue of the binder protein or peptide leads to cysteine or lysine derivatives of the formulae below:

\[
-L_1-L_2-\text{NH-}(\text{CH}_2)_4-\text{NH}_2, \quad -L_1-L_2-\text{S-CH}_2-\text{NH}_2
\]

where LI is in each case attached to the low-molecular weight CDK9 inhibitor, i.e. a compound of formula (I).

Preference according to the invention is also given to the basic linker structures (ii) and (iv), in particular when group LI has one of the following structures:

(a) -NH-(CH\text{\textsubscript{2}}O-4-(CHCH\text{\textsubscript{2}})O-4-CHY\text{\textsubscript{5}}-CO-Y\text{\textsuperscript{7}}, where Y\text{\textsubscript{5}} represents H or NHY\text{\textsubscript{6}}, where Y\text{\textsubscript{6}} represents H or -COCH\text{\textsubscript{3}}, and Y\text{\textsuperscript{7}} represents a single bond or -NH -(CH\text{\textsubscript{2}}O-4 -CHNH\text{\textsubscript{2}}CO-, so that after cleavage the corresponding structure -NH-(CH\text{\textsubscript{2}}O-4-(CHCH\text{\textsubscript{2}})O-4-CHY\text{\textsubscript{5}}-COOH or -NH-(CH\text{\textsubscript{2}}O-4-(CHCH\text{\textsubscript{2}})O-4-CHY\text{\textsubscript{5}}-CO-NH -(CH\text{\textsubscript{2}}O-4 -CHNH\text{\textsubscript{2}}COOH is obtained.

(b) -CH\text{\textsubscript{2}}S\text{\textsubscript{x}}-(CH\text{\textsubscript{2}}O-4-CHY\text{\textsubscript{5}}-CO-, where x is 0 or 1, and Y\text{\textsubscript{5}} represents H or NHY\text{\textsubscript{6}}, where Y\text{\textsubscript{6}} represents H or -COCH\text{\textsubscript{3}}, such that after cleavage the corresponding structure -CH\text{\textsubscript{2}}S\text{\textsubscript{x}}-(CH\text{\textsubscript{2}}O-4-CHY\text{\textsubscript{5}}-COOH is obtained.

This embodiment is preferred when LI is attached in each case to the low-molecular weight CDK9 inhibitor of formula (I). The binder is preferably an anti-TWEAKR antibody or an anti-EGFR antibody.
If the linker is attached to a cysteine side chain or a cysteine residue, L2 is preferably derived from a group which reacts with the sulphhydryl group of the cysteine. These include haloacetylts, maleimides, aziridines, acryloyls, arylating compounds, vinylsulphones, pyridyl disulphides, TNB thiols and disulphide-reducing agents. These groups generally react in an electrophilic manner with the sulphhydryl bond, forming a sulphide (e.g. thioether) or disulphide bridge. Preference is given to stable sulphide bridges. L2 is preferably

\[
\begin{align*}
&\text{R}^{22} \text{ represents COOH, COOR, COR, CONHR, CONR}_2 \text{ (where R in each case represents C}^{1-3}\text{-alkyl), CONH}2, \text{ preferably COOH.}
\end{align*}
\]
Particularly preferred for L2 is:

\[
\text{\Formula{A\text{lo}rA2}}
\]

where \#1 denotes the point of attachment to the sulphur atom of the binder, \#2 denotes the point of attachment to the active compound, \(x\) represents 1 or 2, and \(R^{22}\) represents COOH, COOR, COR, CONHR (where \(R\) in each case represents \(Ci-3\)-alkyl), CONH2, preferably COOH. It is preferred when \(x=1\) and \(R^{22}\) represents COOH.

In a conjugate according to the invention or in a mixture of the conjugates according to the invention, the bonds to a cysteine or lysine residue of the binder are present, to an extent of preferably more than 80%, particularly preferably more than 90% (in each case based on the total number of bonds of the linker to the binder), particularly preferably as one of the two structures of the formula A1 or A2. Here, the structures of the formula A1 or A2 are generally present together, preferably in a ratio of from 60:40 to 40:60, based on the number of bonds to the binder. The remaining bonds are then present as the structure

\[
\text{\Formula{A1}}
\]

In the basic structure (i):

If \(R^{1}\) is the linker \(Z\), \(v\) is preferably 2 and \(m\) is preferably 0.
If R^5 is the linker Z, v is preferably 0 and m is preferably 0.

In the basic structure (ii) to (iv):

If R^1 is the linker Z, v is preferably 2 and m is preferably 0.

If R^5 is the linker Z, v is preferably 0 and m is preferably 1.

According to the invention, L1 is preferably represented by the formula

\[ \text{G1} \cdot (\text{R}^{10})^{n} \cdot (\text{Gl})_{o} \cdot \text{G2} \]

where

R^10 represents H, NH₂ or C₁₋₅-alkyl;

G1 represents -NHCO-, -CONH- or \( \text{N} \_ \text{CO} \) \( \text{N} \_ \text{CO} \); (R^10 is preferably not NH₂, if G1 represents

\( \text{NH}_2 \) or \( \text{N}-\text{CO} \)); n is 0 or 1;

0 is 0 or 1; and

G2 represents a straight-chain or branched hydrocarbon chain which has 1 to 100 carbon atoms from arylene groups and/or straight-chain and/or branched and/or cyclic alkyne groups and which may be interrupted or more than once by one or more of the groups -0-, -S-, -SO-, SO₂, -NR-, -NR\_CO-, -C(NH)NR\_-, CONR\_-, -NR\_NR\_-, -SO₂NR\_NR\_-, -CONR\_R\_ (where R\_ represents H, phenyl, C₁-C₁₀-alkyl, C₂-C₅-alkenyl or C₂-C₅-alkynyl, each of which may be substituted by NHCONH₂, -COOH, -OH, -NH₂, NH-CNNH₂, sulphonamide, sulphone, sulphoxide or sulphonic acid), -CO-, -CR\_N=O- (where R\_ represents H, C₁-C₃-alkyl or phenyl) and/or a 3- to 10-membered aromatic or non-aromatic heterocycle having up to 4 heteroatoms selected from the group consisting of N, O and S, -SO- or -SO₂- (preferably

\( \text{N} \_ \text{CO} \) \( \text{N} \_ \text{CO} \)), where the hydrocarbon chain including any side chains may be substituted by -NHCONH₂, -COOH, -OH, -NH₂, NH-CNNH₂, sulphonamide, sulphone, sulphoxide or sulphonic acid.

G2 represents a straight-chain or branched hydrocarbon chain having 1 to 100 carbon atoms from arylene groups and/or straight-chain and/or branched and/or cyclic alkyne groups and which may be interrupted once or more than once by one or more of the groups -0-, -S-, -SO-, SO₂, -NH-, -CO-, -NHCO-, -CONH-, -NMe-, -NHNH-, -SO₂NHNH-, -CONHNH- and a 5- to 10-membered aromatic or non-aromatic
heterocycle having up to 4 heteroatoms selected from the group consisting of N, O and S, or -SO-
(N N CO), where the side chains, if present, may be substituted by -NHCONH2, -COOH, -OH, -NH2, NH-CNNH2, sulphonamide, sulphone, sulphoxide or sulphonic acid.

G2 preferably represents a straight-chain or branched hydrocarbon chain having 1 to 100 carbon atoms from arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups and which may be interrupted once or more than once by one or more of the groups -O-, -S-, -SO-, -NH-, -CO-, -NHCO-, -CONH-, -NMe-, -NHNH-, -SO2NHNH-, -CONHNH-, -CR=x=N-O- (where R=x represents H, C1-C3-alkyl or phenyl) and a 3- to 10-membered, for example 5- to 10-membered, aromatic or non-aromatic heterocycle having up to 4 heteroatoms selected from the group consisting of N, O and S, -SO- or -SO2-(N N CO), where the hydrocarbon chain including the side chains, if present, may be substituted by -NHCONH2, -COOH, -OH, -NH2, NH-CN-NH2, sulphonamide, sulphone, sulphoxide or sulphonic acid.

Further interrupting groups in G2 are preferably

where Rx represents H, C1-C3-alkyl or phenyl.

Here, #1 is the bond to the CDK9 inhibitor and #2 is the bond to the coupling group to the binder (e.g. L2).

A straight-chain or branched hydrocarbon chain of arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups generally comprises a α,ω-divalent alkyl radical having the respective number of carbon atoms stated. Examples which may be mentioned as being preferred are: methylene, ethane-1,2-diy1 (1,2-ethylene), propane-1,3-diy1 (1,3-propylene), butane-1,4-diy1 (1,4-butylene), pentane-1,5-diy1 (1,5-pentylene), hexane-1,6-diy1 (1,6-hexylene), heptane-1,7-diy1 (1,7-hexylene), octane-1,8-diy1
(1,8-octylene), nonane-1,9-diyl (1,9-nonylene), decane-1,10-diyl (1,10-decylene). However, the alkylene groups in the hydrocarbon chain may also be branched, i.e. one or more hydrogen atoms of the straight-chain alkylene groups mentioned above may optionally be substituted by Ci-io-alkyl groups, thus forming side chains. The hydrocarbon chain may furthermore contain cyclic alkylene groups (cycloalkanediyl), for example 1,4-cyclohexanediyl or 1,3-cyclopentanediyl. These cyclic groups may be unsaturated. In particular, aromatic groups (arylene groups), for example phenylene, may be present in the hydrocarbon group. In turn, in the cyclic alkylene groups and the arylene groups, too, one or more hydrogen atoms may optionally be substituted by Ci-io-alkyl groups. In this way, an optionally branched hydrocarbon chain is formed. This hydrocarbon chain has a total of 0 to 100 carbon atoms, preferably 1 to 50, particularly preferably 2 to 25 carbon atoms.

The side chains, if present, may be substituted by -NHCONH₂, -COOH, -OH, -NH₂, NH-CNNH₂, sulphonamide, sulphone, sulphotoxide or sulphonic acid.

The hydrocarbon chain may be interrupted once or more than once by one or more of the groups -O-, -S-, -SO-, SO₂, -NH-, -CO-, -NHCO-, -CONH-, -NMe-, -NHNH-, -SO₂NHNH₂, -CONHNH₂ and a 5- to 10-membered aromatic or non-aromatic heterocycle having up to 4 heteroatoms selected from the group consisting of N, O and S, -SO- or -SO₂- (preferably \[
\begin{array}{c}
N \\
\vdots \\
CO
\end{array}
\]).

Further interrupting groups in G2 are preferably
Preferably, the linker corresponds to the formula below:

$$-(\text{CH}_2)^v-(\text{CO})^m-\text{L}_1-\text{L}_2-\text{§}$$

where

- $v$ is 0, 1 or 2
- $m$ is 0 or 1;

§ represents the bond to the active compound molecule and
§§ represents the bond to the binder peptide or protein, and L1 and L2 have the meaning given above.

Particularly preferred embodiments of L1 are described below.

Particularly preferably, L1 has the formula -NR11B-, where R11 represents H;
B represents -[(CH2)x(X4)y]w-(CH2)z-,
where:
- w = 0 to 20;
- x = 0 to 5;
- y = 0 or 1;
- z = 0 to 5; and
- X4 represents -0-, -CONH- or -NHCO-.

If R1 is the linker Z, linkers Z which are preferred in accordance with the invention have the formula below:

![Diagram](attachment:image.png)

where:
- #3 represents the bond to the active compound molecule,
- #4 represents the bond to the binder peptide or protein,
- R11 represents H or N¾;
- B represents -[(CH2)x(X4)y]w-(CH2)z-,
where:
- w = 0 to 20;
- x = 0 to 5;
- y = 0 or 1;
- z = 1 to 5; and
- X4 represents -0-, -CONH-, -NHCO- or

Further linkers Z which are preferred in accordance with the invention, when R1 is the linker Z, have the formula below:
where

#3 represents the bond to the active compound molecule,

#4 represents the bond to the binder peptide or protein,

$R_{11}^1$ represents H or NH;

B represents $-(\text{CH}_2)_x-(\text{X}_y)^w-(\text{CH}_2)_z-$,

$w = 0$ to 20;

$x = 0$ to 5;

$y = 0$ or 1;

$z = 1$ to 5; and

$X_y^4$ represents -0-, -CONH-, -NHCO- or

Preferable linker substructures are as follows below, where #1 represents the (generally thioether) linkage to the binder peptide or protein and #2 the point of attachment to the modified active compound molecule:
Preference in accordance with the invention is furthermore given to the linkers below: In a conjugate according to the invention or in a mixture of the conjugates according to the invention, the bonds to a cysteine or lysine residue of the binder are present, to an extent of preferably more than 80%, particularly preferably more than 90% (in each case based on the total number of bonds of the linker to the binder), particularly preferably as one of the two structures of the formula A3 or A4:

Formula A3

Formula A4
where

\#^1 \quad \text{denotes the point of attachment to the sulphur atom of the binder,}

\#^2 \quad \text{denotes the point of attachment to group LI, and}

R^{2^2} \text{ represents COOH, COOR, COR, CONHR (where R in each case represents Cl-3-alkyl). CONH}_2,

preferably COOH.

Here, the structures of the formula A3 or A4 are generally present together, preferably in a ratio of from 60:40 to 40:60, based on the number of bonds to the binder. The remaining bonds are then present as the structure

\[
\text{Other linkers } -Z- \text{ attached to a cysteine side chain, lysine side chain, cysteine residue, or lysine residue have the formula below:}
\]

\[
\begin{align*}
\&-\left(\text{CH}_2\text{CH}_2\text{O}\right)_p-\left(\text{CH}_2\text{H}_2\right)_m-S(O)_nL_3\end{align*}
\]

where

\§ \text{ represents the bond to the active compound molecule and}

\§§ \text{ represents the bond to the binder peptide or protein,}

m is 0, 1, 2 or 3;

n is 0, 1 or 2;

p is 0 to 20; and

L3 represents
where

\( o \) is 0 or 1;
and

\( G_3 \) represents a straight-chain or branched hydrocarbon chain having 1 to 100 carbon atoms from arylene groups and/or straight-chain and/or cyclic alkylene groups and which may be interrupted once or more than once by one or more of the groups -O-, -S-, -SO-, S0₂, -NH-, -CO-, -NHCO-, -CONH-, -NMe-, -NHNH-, -SO₂NH-, -CONHNH- and a 3- to 10-membered (preferably 5- to 10-membered) aromatic or non-aromatic heterocycle having up to 4 heteroatoms selected from the group consisting of N, O and S, -SO- or SO₂ (preferably

\[
\begin{array}{c}
\text{N} \\
\text{N} \\
\text{CO}
\end{array}
\]

), where the side chains, if present, may be substituted by -NHCONH₂, -COOH, -OH, -NH₂, NH-CNNH₂, sulphonamide, sulphone, sulphoxide or sulphonic acid.

In the formula above, preferably

m is 0, 1 or 2;
p is 0;
n is 0;
and \( L_3 \) represents

where

\( o \) is 0 or 1; and

\( G_3 \) represents -(CH₂)\( s \)(CH₂)\( t \)(CO)\( u \)(CH₂)\( v \)(CH₂)\( w \), where

\( s, t, v \) and \( w \) each independently of one another are from 0 to 20 and \( u \) is 0 or 1.

Preferred groups \( L_1 \) in the formula \( §-(CH₂)\( v \)-(CO)m-L1-L2-§§ \) above are those below, where \( r \) in each case independently of one another represents a number from 0 to 20, preferably from 0 to 15, particularly preferably from 1 to 20, especially preferably from 2 to 10. It is understood that the groups \( L_1 \) below are read from left to right, meaning that the left-hand symbol \( / \) in the Table below denotes the linkage site to \( §-(CH₂)\( v \)-(CO)m- \) and the right-hand symbol \( / \) in the Table below denotes the linkage site to \( -L2-§§. \)
Further Examples of a linker moiety L1 are given in Table A-I and A-II below. The table furthermore states the preferred value for m, this is whether there is a carbonyl group in front of L1 or not (cf. §-(CH2)v-(CO)m-L1-L2-§§). If the preferred value for m is 1, v (i.e. the number of CF₃ groups in front of L1 not (cf. §-(CH2)v-(CO)m-L1-L2-§§)) is preferably 0. It is understood that the groups L1 below are read from left to right, meaning that the left-hand symbol ‘’ in the Tables below denotes the linkage site to §-(CH2)v-(CO)m- and the right-hand symbol ‘’ in the Table below denotes the linkage site to -L2-§§.

Table A-I

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Further examples of a linker moiety LI are given in Table A-III. The table furthermore states with which group L2 these examples of LI are preferably combined and also the preferred coupling point (R₁ or R₅), and the preferred value for m, this is whether there is a carbonyl group in front of LI or not (cf. §-(0¾) ν-(CO)m-LI-L2-§§). The preferred value for ν in these examples is 0, this is whether there is a CH₄ group in front of LI or not (cf. §-(CH₂)ν-(CO)m-LI-L2-§§). The first column furthermore states the example numbers for the cetuximab ADCs in which the linkers in question are used, but which likewise apply in each row for ADCs with other antibodies. If L2 is a succinimide or derived therefrom, this imide may also be fully or partially in the form of the hydrolysed open-chain succinamide, as described above. Depending on LI, this hydrolysis to open-chain succinamides may be more or less pronounced or not present at all. It is understood that the groups LI below are read from left to right, meaning that the left-hand symbol / in the Table below denotes the linkage site to §-(CH₂)ν-(CO)m- and the right-hand symbol \ in the Table below denotes the linkage site to -L2-§§.

Table A-III

<table>
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<th>L2</th>
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<td>![Image]</td>
</tr>
<tr>
<td>8A</td>
<td>R²</td>
<td>1</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>9A</td>
<td>R²</td>
<td>1</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>No.</td>
<td>$R^3$</td>
<td>$n$</td>
<td>Structure 1</td>
<td>Structure 2</td>
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<tr>
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<td><img src="image16.png" alt="Structure 2" /></td>
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</table>
Further examples of a linker moiety $L_I$ are given below, wherein $L_I$ represents $-(\text{CH}_2)_q-L'$, wherein $q$ is 0 or 1.

Examples of a linker moiety $L'$ are given in Tables A-IV and A-V below. The table furthermore states with which group $L_2$ these examples of $L_I$ are preferably combined. The preferred value for $v$ in these examples is 0, this is whether there is a $\text{CH}_2$ group in front of $L_I$ or not (cf. §-(CH2)$_v$-(CO)$_m$-$L_I$-$L_2$-§§), and the preferred value for $m$ is 0, this is whether there is a carbonyl group in front of $L_I$ or not (cf. §-(CH2)$_v$-(CO)$_m$-$L_I$-$L_2$-§§). The first column furthermore states the example numbers for the cetuximab ADCs in which the linkers in question are used, but which likewise apply in each row for ADCs with other antibodies. If $L_2$ is a succinimide or derived therefrom, this imide may also be fully or partially in the form of the hydrolysed open-chain succinamide, as described above. Depending on $L_I$, this hydrolysis to open-chain succinamides may be more or less pronounced or not present at all. It is understood that the groups $L_I$ below are read from left to right, meaning that the left-hand symbol ‘‘ in the Table below denotes the linkage site to §-(CH2)$_v$-(CO)$_m$- and the right-hand symbol ‘‘ in the Table below denotes the linkage site to -L$_2$-§§.
<table>
<thead>
<tr>
<th>L'</th>
<th>L2</th>
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<tbody>
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<tr>
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<td><img src="image4" alt="Chemical Structure 4" /></td>
</tr>
<tr>
<td><img src="image5" alt="Chemical Structure 5" /></td>
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<tr>
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<td><img src="image19" alt="Chemical Structure 19" /></td>
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Table A-IV
Table A-V

<table>
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<tr>
<td><img src="image5" alt="Structure 5" /></td>
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<td><img src="image7" alt="Structure 7" /></td>
<td><img src="image8" alt="Structure 8" /></td>
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<tr>
<td>Note</td>
<td>Structure</td>
</tr>
<tr>
<td>------</td>
<td>-----------</td>
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<tr>
<td>***</td>
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<tr>
<td></td>
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<td>and</td>
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</table>

See note ***
<table>
<thead>
<tr>
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<th>Structure 2</th>
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<tbody>
<tr>
<td><img src="image1" alt="Structure 1" /></td>
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</table>

and

See note ***

<table>
<thead>
<tr>
<th>Structure 3</th>
<th>Structure 4</th>
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<tbody>
<tr>
<td><img src="image3" alt="Structure 3" /></td>
<td><img src="image4" alt="Structure 4" /></td>
</tr>
</tbody>
</table>
and

See note ***
With particular preference, the linkers \( L_1 \) given in these rows are attached to a linker \( L_2 \) selected from:

![Formula A5](image)

Formula A5

and/or

![Formula A6](image)

Formula A6

where \( #^1 \) denotes the point of attachment to the sulphur atom of the binder, \( #^2 \) denotes the point of attachment to group \( L_1 \), \( R_{22} \) preferably represents COOH. In a conjugate according to the invention or in a mixture of the conjugates according to the invention, the bonds to a cysteine or lysine residue of the binder are present, to an extent of preferably more than 80\%, particularly preferably more than 90\% (in each case based on the total number of bonds of the linker to the binder), particularly preferably as one of the two structures of the formula A5 or A6. Here, the structures of the formula A5 or A6 are generally present together, preferably in a ratio of from 60:40 to 40:60, based on the number of bonds to the binder. The remaining bonds are then present as the structure

![Formula](image)

When this structure \( L_2 \) is present, there may simultaneously be a structure \( L_2 \) of the formula below:

![Formula](image)
Further examples of LI are given in Table B, in which this group is highlighted in a box.

Examples of conjugates having corresponding linkers have the following structures, where A, R¹ to R⁴, L, X, Y and LI have the meanings given above, AB represents an antibody attached via a cysteine or a lysine residue and n is a number from 1 to 10. With particular preference, AB is a human, humanized or chimeric monoclonal antibody or an antigen-binding fragment thereof, in particular an anti-TWEAKR antibody or an antigen-binding fragment thereof or an anti-EGFR antibody or an antigen-binding fragment thereof. Particular preference is given to an anti-TWEAKR antibody which binds specifically to amino acid D in position 47 (D47) of TWEAKR (SEQ ID NO: 169), in particular the anti-TWEAKR antibody TPP-2090, or the anti-EGFR antibodies cetuximab, TPP-4030, TPP-5653, or nimotuzumab.
Preferred examples of conjugates having corresponding linkers have the following structures, where R\textsuperscript{1} to R\textsuperscript{4}, L, X, Y and L\textsubscript{I} has the meanings given above, AB represents an antibody attached via a cysteine residue or a lysine residue and n is a number from 1 to 10. With particular preference, AB is a human, humanized or chimeric monoclonal antibody or an antigen-binding fragment thereof, in particular an anti-TWEAKR antibody or an antigen-binding fragment thereof or an anti-EGFR antibody or an antigen-binding fragment thereof. Particular preference is given to an anti-TWEAKR antibody which binds specifically to amino acid D in position 47 (D47) of TWEAKR (SEQ ID NO: 169), in particular the anti-TWEAKR antibody TPP-2090, or the anti-EGFR antibodies cetuximab, TPP-4030, TPP-5653, or nimotuzumab.
More preferred examples of conjugates having corresponding linkers have the following structures, where X, Y and L1 has the meanings given above, AB represents an antibody attached via a cysteine residue or a lysine residue and n is a number from 1 to 10. With particular preference, AB is a human, humanized or chimeric monoclonal antibody or an antigen-binding fragment thereof, in particular an anti-TWEAKR antibody or an antigen-binding fragment thereof or an anti-EGFR antibody or an antigen-binding fragment thereof. Particular preference is given to an anti-TWEAKR antibody which binds specifically to amino acid D in position 47 (D47) of TWEAKR (SEQ ID NO: 169), in particular the anti-TWEAK antibody TPP-2090, or the anti-EGFR antibodies cetuximab, TPP-4030, TPP-5653, or nimotuzumab.
Preference according to the invention is furthermore given to the basic structure (i), where SGI represents a group which can be cleaved by cathepsin and L1 and L2 have the meanings given above. Particular preference is given to the following groups if R1 is a linker Z:

- **-NH-Val-Ala-CONH-** (hereby cleavage of the amide bond at the C-terminal amide of alanine)
- **-NH-Val-Lys-CONH-** (cleavage of the amide bond at the C-terminal amide of lysine)
- **-NH-Val-Cit-CONH-** (cleavage of the amide bond at the C-terminal amide of citrulline)
- **-NH-Phe-Lys-CONH** (cleavage of the amide bond at the C-terminal amide of lysine)
- **-NH-Ala-Lys-CONH-** (cleavage of the amide bond at the C-terminal amide of lysine)
- **-NH-Ala-Cit-CONH-** (cleavage of the amide bond at the C-terminal amide of citrulline)

Particular preference is given to the following groups if R5 is a linker Z:

- **-Val-Ala-CONH-** (hereby cleavage of the amide bond at the C-terminal amide of alanine)
- **-Val-Lys-CONH-** (cleavage of the amide bond at the C-terminal amide of lysine)
- **-Val-Cit-CONH-** (cleavage of the amide bond at the C-terminal amide of citrulline)
- **-Phe-Lys-CONH** (cleavage of the amide bond at the C-terminal amide of lysine)
- **-Ala-Lys-CONH**- (cleavage of the amide bond at the C-terminal amide of lysine)
- **-Ala-Cit-CONH-** (cleavage of the amide bond at the C-terminal amide of citrulline)

If R1 is the linker Z in basic structure (i), v is preferably 2, m is preferably 0, and SGI is particularly preferably

![Diagram](image-url)
X represents H or a Ci-io-alkyl group which may optionally be substituted by -NHCONH₂, -COOH, -OH, NH₂, -NH-CNNH₂ or sulphonic acid.

5

If \( R^5 \) is the linker \( Z \) in basic structure (i), \( v \) is preferably 0, \( m \) is preferably 0 and \( SG \) is particularly preferably

10

X represents H or a Ci-io-alkyl group which may optionally be substituted by -NHCONH₂, -COOH, -OH, NH₂, -NH-CNNH₂ or sulphonic acid.

15

Preference according to the invention is furthermore given to the basic structure (ii) or (iv), where \( SG \) represents a group which can be cleaved by cathepsin and \( L1 \) and \( L2 \) have the meanings given above. Particular preference is given to the following groups:

- Val-Ala-CONH- (hereby cleavage of the amide bond at the C-terminal amide of alanine)
- NH-Val-Lys-CONH- (cleavage of the amide bond at the C-terminal amide of lysine)
- NH-Val-Cit-CONH- (cleavage of the amide bond at the C-terminal amide of citrulline)
- NH-Phe-Lys-CONH (cleavage of the amide bond at the C-terminal amide of lysine)
- NH-Ala-Lys-CONH- (cleavage of the amide bond at the C-terminal amide of lysine)
- NH-Ala-Cit-CONH- (cleavage of the amide bond at the C-terminal amide of citrulline)
In basic structure (ii) and (iv) SG is particularly preferably

\[ \begin{align*}
\text{O} & \quad \text{N} \\
\text{H} & \quad \text{CH}_2 \text{X} \quad \text{O} \\
\text{N} & \quad \text{H}
\end{align*} \]

or

\[ \begin{align*}
\text{O} & \quad \text{N} \\
\text{H} & \quad \text{CH}_2 \text{X} \quad \text{O} \\
\text{N} & \quad \text{H}
\end{align*} \]

or

\[ \begin{align*}
\text{O} & \quad \text{H} \quad \text{CH}_3 \\
\text{N} & \quad \text{N} \\
\text{H} & \quad \text{CH}_2 \text{X} \quad \text{O} \\
\text{N} & \quad \text{H}
\end{align*} \]

X represents H or a Ci-io-alkyl group which may optionally be substituted by -NHCONH2, -COOH, -OH, NH$_2$, -NH-CNNH2 or sulphonic acid.

Table B below gives examples of a linker moiety -SGI -LI- or -L1-SG-L1-, where SGI and SG are groups which can be cleaved by cathepsin. Table B furthermore states with which group L2 these examples of -SGI -LI- and -L1-SG-L1- are preferably combined, and also the preferred coupling point (R$^1$ or R$^5$) and the preferred value for m, thus whether there is a carbonyl group in front of LI or not (cf. §-(CH2)$^v$-(CO)m-LI-L2-§§). These linkers are preferably coupled to a cysteine residue. The first column furthermore states the example numbers, given in an exemplary manner for cetuximab ADCs, in which corresponding linkers are used. They apply in the same manner to the corresponding ADCs with other antibodies. The LI group is highlighted in a box. However, these groups LI can be replaced by one of the groups LI given for formula §-(CH2)$^v$-(CO)m-LI-L2-§§ above. If L2 is a succinamide or derived therefrom, this amide may also be fully or partially in the form of the hydrolysed open-chain succinamide, as described above.
### Table B

<table>
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<th>Ex.</th>
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<th>v</th>
<th>m</th>
<th>-SG1-L1- or -L1-SG-L1-</th>
<th>L2</th>
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</tr>
<tr>
<td>4A</td>
<td>R²</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5A</td>
<td>R²</td>
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</tr>
<tr>
<td>6A</td>
<td>R²</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>19A</td>
<td>R²</td>
<td>2</td>
<td>0</td>
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</table>
Examples of conjugates having basic structure (i) have the following structure, where R1, R2, R3, R4, X, Y, SGI and L1 have the meanings given above, AB represents an antibody attached via a cysteine residue or a lysine residue and n is a number from 1 to 10. Particularly preferably, AB is an anti-TWEAKR antibody, in particular an anti-TWEAKR antibody which binds specifically to amino acid D in position 47 (D47) of TWEAKR (SEQ ID NO: 169), in particular the anti-TWEAKR antibody TPP-2090, or an anti-EGFR antibodies, in particular cetuximab, TPP-4030, TPP-5653 or nimotuzumab.
Preferable examples of conjugates having basic structure (i) have the following structure, where R1, R2, R3, R4, L, X, Y, SGI and L1 have the meanings given above, AB represents an antibody attached via a cysteine residue or a lysine residue and n is a number from 1 to 10. Particularly preferably, AB is an anti-TWEAKR antibody, in particular an anti-TWEAKR antibody which binds specifically to amino acid D in position 47 (D47) of TWEAKR (SEQ ID NO: 169), in particular the anti-TWEAKR antibody TPP-2090, or an anti-EGFR antibodies, in particular cetuximab, TPP-4030, TPP-5653 or nimotuzumab.

CDK9 inhibitor - linker-intermediates and preparation of the conjugates

The conjugates according to the invention are prepared by initially providing the low-molecular weight CDK9 inhibitor with a linker. The intermediate obtained in this manner is then reacted with the binder (preferably antibody).

The CDK9 inhibitor-linker-intermediates are compounds of general formula (Γ)
wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2- and -S(=0)(=NR 5)-;

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R1 represents -Z or a group selected from Ci-C6-alkyl-, C3-C6-alkenyl, C3-C6-alkynyl, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, phenyl-Ci-C3-alkyl- and heteroaryl-Ci-C3-alkyl-,

wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, Ci-C6-alkyl-, halo-Ci-C3-alkyl-, Ci-Clalkoxy-, Ci-C3-fluoroalkoxy-, N3 alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, -OP(=O)(OH)3/4 -C(=0)OH, -C(=0)NH2;

R3, R4 represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-;

R3 represents -Z or a group selected from a hydrogen atom, cyano, -C(=O)R5, -C(=O)OR5, -S(=O)2R5, -C(=0)NR 7R7, Ci-C6-alkyl-, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, wherein said Ci-Ce-alkyl, C3-C7-cycloalkyl-, heterocyclyl-, phenyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-.
R₆, R₇ represent, independently from each other, a group selected from a hydrogen atom, \textit{Ci-C₆-alkyl-}, \textit{C₃-C₇-cycloalkyl-}, \textit{heterocyclyl-}, phenyl, benzyl and heteroaryl, wherein said \textit{Ci-C₆-alkyl-}, \textit{C₃-C₇-cycloalkyl-}, \textit{heterocyclyl-}, phenyl, benzyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, \textit{Ci-C₃-alkyl-}, \textit{Ci-C₃-alkoxy-}, -Nth, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-\textit{Ci-C₃-alkyl-}, \textit{Ci-C₃-fluoroalkoxy-}, or

R₆ and R₇, together with the nitrogen atom they are attached to, form a cyclic amine;

R₈ represents a group selected from \textit{Ci-C₆-alkyl-}, \textit{halo-Ci-C₃-alkyl-}, \textit{C₃-C₇-cycloalkyl-}, \textit{heterocyclyl-}, \textit{phenyl}, \textit{benzyl} and \textit{heteroaryl}, wherein said group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, \textit{Ci-C₃-alkyl-}, \textit{Ci-C₃-alkoxy-}, -Nth, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-\textit{Ci-C₃-alkyl-}, \textit{Ci-C₃-fluoroalkoxy-},

wherein one of the substituents R¹ or R₅ is Z,

wherein Z stands for the linker;

or the enantiomers, diastereomers, salts, solvates or salts of solvates thereof.

The linker -Z- represents one of the following general structures (v) to (viii):

\begin{align*}
  \text{(i)} & \quad \&-(\text{CH}_2)_v-(\text{CO})_m\text{SGI}-\text{LI} -\text{L3} \\
  \text{(ii)} & \quad \&-(\text{CH}_2)_v-(\text{CO})_m\text{LI}-\text{SG}-\text{LI} -\text{L3} \\
  \text{(iii)} & \quad \&-(\text{CH}_2)_v-(\text{CO})_m\text{LI}-\text{L3} \\
  \text{(iv)} & \quad \&-(\text{CH}_2)_v-(\text{CO})_m\text{LI}-\text{SG}-\text{L3}
\end{align*}

wherein

\begin{itemize}
  \item v is 0, 1 or 2
  \item m is 0 or 1,
  \item \& represents the attachment point to the active component;
  \item SG and SGI represent the same \textit{in vivo} cleavable group as described above, LI represent independently of each other \textit{in vivo} non-cleavable organic groups as described above, and
  \item L₃ preferably represents
\end{itemize}
wherein

#\(^1\) represents the attachment site to the group LI.

5

Preferred embodiments for the compounds of formula (I) correspond to the preferred binder conjugates described above with the exception that the binding moiety -L2-§§ is replaced with -L3.

10 Preferably, for coupling to a cysteine residue, one of the compounds below is reacted with the cysteine-containing binder such as an antibody, which is optionally partially reduced for this purpose:
where $R_1, R_2, R_3, R_4, A, L, X$ and $Y$ have the same meaning as in formula (I), SGI and L1 have the same meaning as described above.
More preferably, for coupling to a cysteine residue, one of the compounds below is reacted with the cysteine-containing binder such as an antibody, which is optionally partially reduced for this purpose:

\[
\begin{align*}
\text{HN} & \quad \text{O} & \quad \text{L} & \quad \text{R}^2 & \quad \text{SG1} & \quad \text{S} & \quad \text{N} & \quad \text{SG1} & \quad \text{L1} & \quad \text{O} & \quad \text{F} & \quad \text{R}^4 \\
\text{HN} & \quad \text{O} & \quad \text{L} & \quad \text{R}^2 & \quad \text{SG1} & \quad \text{S} & \quad \text{N} & \quad \text{SG1} & \quad \text{L1} & \quad \text{O} & \quad \text{F} & \quad \text{R}^4 \\
\end{align*}
\]

where \( R^1, R^2, R^3, R^4, A, L, X \) and \( Y \) have the same meaning as in formula (I). SGI and LI have the same meaning as described above.

Most preferably, for coupling to a cysteine residue, one of the compounds below is reacted with the cysteine-containing binder such as an antibody, which is optionally partially reduced for this purpose:
where X and Y have the same meaning as in formula (I), SGI and L1 have the same meaning as
described above.

The compound may be employed, for example, in the form of its trifluoroacetic acid salt. For the reaction
with the binder such as, for example, the antibody, the compound is preferably used in a 2- to 12-fold
molar excess with respect to the binder.

For an intermediate coupling to a cysteine residue, the reactions can be illustrated as follows:
The other intermediates and other antibodies can be reacted correspondingly.

In accordance with the invention, this gives preferably the following conjugates:
Depending on the linker, succinimide-linked ADCs may, after conjugation, be converted into the open-chain succinamides, which have an advantageous stability profile.

**Scheme A**

This reaction (ring opening) can be carried out at pH 7.5 to 9, preferably at pH 8, at a temperature of from 25°C to 37°C, for example by stirring. The preferred stirring time is 8 to 30 hours.

In the above formulae, $R^1, R^2, R^3, R^4, A, L, X$ and $Y$ have the same meaning as in formula (I). SGI and $L_1$ have the same meaning as described above. $AB$ is an antibody coupled via a cysteine residue or a lysine residue. With particular preference, $AB$ and $AB_2$ are anti-TWEAKR antibodies, in particular antibodies which bind specifically to amino acid D in position 47 (D47) of TWEAKR (SEQ ID NO: 169), in particular the anti-TWEAKR antibody TPP-2090.
Binders

In the broadest sense, the term "binder" is understood to mean a molecule which binds to a target molecule present at a certain target cell population to be addressed by the binder/active compound conjugate. The term binder is to be understood in its broadest meaning and also comprises, for example, lectins, proteins capable of binding to certain sugar chains, and phospholipid-binding proteins. Such binders include, for example, high-molecular weight proteins (binding proteins), polypeptides or peptides (binding peptides), non-peptidic (e.g. aptamers (US5,270,163) review by Keefe AD., et al., Nat. Rev. Drug Discov. 2010; 9:537-550), or vitamins) and all other cell-binding molecules or substances. Binding proteins are, for example, antibodies and antibody fragments or antibody mimetics such as, for example, affibodies, adnectins, anticalins, DARPinS, avimers, nanobodies (review by Gebauer M. et al., Curr. Opinion in Chem. Biol. 2009; 13:245-255; Nuttall S.D. et al., Curr. Opinion in Pharmacology 2008; 8:608-617). Binding peptides are, for example, ligands of a ligand/receptor pair such as, for example, VEGF of the ligand/receptor pair VEGF/KDR, such as transferrin of the ligand/receptor pair transferrin/transferrin receptor or cytokine/cytokine receptor, such as TNFalpha of the ligand/receptor pair TNFalpha/TNFalpha receptor.

The literature also discloses various options of covalent coupling (conjugation) of organic molecules to antibodies. Preference according to the invention is given to the conjugation of the toxophores to the antibody via one or more sulphur atoms of cysteine residues of the antibody and/or via one or more NH groups of lysine residues of the antibody. However, it is also possible to bind the toxophore to the antibody via free carboxyl groups or via sugar residues of the antibody.

A "target molecule" in the broadest sense is understood to mean a molecule which is present in the target cell population and which may be a protein (for example a receptor of a growth factor) or a non-peptidic molecule (for example a sugar or phospholipid). It is preferably a receptor or an antigen.

The term "extracellular" target molecule describes a target molecule, attached to the cell, which is located at the outside of a cell, or the part of a target molecule which is located at the outside of a cell, i.e. a binder may bind on an intact cell to its extracellular target molecule. An extracellular target molecule may be anchored in the cell membrane or be a component of the cell membrane. The person skilled in the art is aware of methods for identifying extracellular target molecules. For proteins, this may be by determining the transmembrane domain(s) and the orientation of the protein in the membrane. These data are usually deposited in protein databases (e.g. SwissProt).

The term "cancer target molecule" describes a target molecule which is more abundantly present on one or more cancer cell species than on non-cancer cells of the same tissue type. Preferably, the cancer target molecule is selectively present on one or more cancer cell species compared with non-cancer cells of the same tissue type, where selectively describes an at least two-fold enrichment on cancer cells compared to
non-cancer cells of the same tissue type (a "selective cancer target molecule"). The use of cancer target
molecules allows the selective therapy of cancer cells using the conjugates according to the invention.

The binder can be attached to the linker via a bond. Attachment of the binder can be via a heteroatom of
the binder. Heteroatoms according to the invention of the binder which can be used for attachment are
sulphur (in one embodiment via a sulphhydryl group of the binder), oxygen (according to the invention
by means of a carboxyl or hydroxyl group of the binder) and nitrogen (in one embodiment via a primary
or secondary amine group or amide group of the binder). These heteroatoms may be present in the natural
binder or are introduced by chemical methods or methods of molecular biology. According to the
invention, the attachment of the binder to the toxophore has only a minor effect on the binding activity of
the binder with respect to the target molecule. In a preferred embodiment, the attachment has no effect on
the binding activity of the binder with respect to the target molecule.

In accordance with the present invention, the term "antibody" is to be understood in its broadest meaning
and comprises immunoglobulin molecules, for example intact or modified monoclonal antibodies,
polyclonal antibodies or multispecific antibodies (e.g. bispecific antibodies). An immunoglobulin
molecule preferably comprises a molecule having four polypeptide chains, two heavy chains (H chains)
and two light chains (L chains) which are typically linked by disulphide bridges. Each heavy chain
comprises a variable domain of the heavy chain (abbreviated VH) and a constant domain of the heavy
chain. The constant domain of the heavy chain may, for example, comprise three domains CH1, CH2 and
CH3. Each light chain comprises a variable domain (abbreviated VL) and a constant domain. The
constant domain of the light chain comprises a domain (abbreviated CL). The VH and VL domains may
be subdivided further into regions having hypervariability, also referred to as complementarity
determining regions (abbreviated CDR) and regions having low sequence variability (framework region,
abbreviated FR). Typically, each VH and VL region is composed of three CDRs and up to four FRs. For
example from the amino terminus to the carboxy terminus in the following order: FR1, CDR1, FR2,
CDR2, FR3, CDR3, FR4. An antibody may be obtained from any suitable species, e.g. rabbit, llama,
camel, mouse or rat. In one embodiment, the antibody is of human or murine origin. An antibody may, for
example, be human, humanized or chimeric.

The term "monoclonal" antibody refers to antibodies obtained from a population of substantially
homogeneous antibodies, i.e. individual antibodies of the population are identical except for naturally
occurring mutations, of which there may be a small number. Monoclonal antibodies recognize a single
antigenic binding site with high specificity. The term monoclonal antibody does not refer to a particular
preparation process.

The term "intact" antibody refers to antibodies comprising both an antigen-binding domain and the
constant domain of the light and heavy chain. The constant domain may be a naturally occurring domain
or a variant thereof having a number of modified amino acid positions.
The term "modified intact" antibody refers to intact antibodies fused via their amino terminus or carboxy terminus by means of a covalent bond (e.g. a peptide bond) with a further polypeptide or protein not originating from an antibody. Furthermore, antibodies may be modified such that, at defined positions, reactive cysteines are introduced to facilitate coupling to a toxophore (see Junutula et al. Nat Biotechnol. 2008 Aug;26(8):925-32).

The term "human" antibody refers to antibodies which can be obtained from a human or which are synthetic human antibodies. A "synthetic" human antibody is an antibody which is partially or entirely obtainable in silico from synthetic sequences based on the analysis of human antibody sequences. A human antibody can be encoded, for example, by a nucleic acid isolated from a library of antibody sequences of human origin. An example of such an antibody can be found in Soderlind et al., Nature Biotech. 2000, 18:853-856.

The term "humanized" or "chimeric" antibody describes antibodies consisting of a non-human and a human portion of the sequence. In these antibodies, part of the sequences of the human immunoglobulin (recipient) are replaced by sequence portions of a non-human immunoglobulin (donor). In many cases, the donor is a murine immunoglobulin. In the case of humanized antibodies, amino acids of the CDR of the recipient are replaced by amino acids of the donor. Sometimes, amino acids of the framework, too, are replaced by corresponding amino acids of the donor. In some cases the humanized antibody contains amino acids present neither in the recipient nor in the donor, which were introduced during the optimization of the antibody. In the case of chimeric antibodies, the variable domains of the donor immunoglobulin are fused with the constant regions of a human antibody.

The term complementarity determining region (CDR) as used herein refers to those amino acids of a variable antibody domain which are required for binding to the antigen. Typically, each variable region has three CDR regions referred to as CDR1, CDR2 and CDR3. Each CDR region may embrace amino acids according to the definition of Kabat and/or amino acids of a hypervariable loop defined according to Chotia. The definition according to Kabat comprises, for example, the region from about amino acid position 24 - 34 (CDR1), 50 - 56 (CDR2) and 89 - 97 (CDR3) of the variable light chain and 31 - 35 (CDR1), 50 - 65 (CDR2) and 95 - 102 (CDR3) of the variable heavy chain (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The definition according to Chotia comprises, for example, the region from about amino acid position 26 - 32 (CDR1), 50 - 52 (CDR2) and 91 -96 (CDR3) of the variable light chain and 26 - 32 (CDR1), 53 - 55 (CDR2) and 96 - 101 (CDR3) of the variable heavy chain (Chothia and Lesk; J Mol Biol 196: 901-917 (1987)). In some cases, a CDR may comprise amino acids from a CDR region defined according to Kabat and Chotia.

Depending on the amino acid sequence of the constant domain of the heavy chain, antibodies may be categorized into different classes. There are five main classes of intact antibodies: IgA, IgD, IgE, IgG and
IgM, and several of these can be divided into further subclasses. (Isotypes), e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The constant domains of the heavy chain, which correspond to the different classes, are referred to as [alpha/α], [delta/δ], [epsilon/ε], [gamma/γ] and [mu/μ]. Both the three-dimensional structure and the subunit structure of antibodies are known.

The term "functional fragment" or "antigen-binding antibody fragment" of an antibody/immunoglobulin is defined as a fragment of an antibody/immunoglobulin (e.g. the variable domains of an IgG) which still comprise the antigen binding domains of the antibody/immunoglobulin. The "antigen binding domain" of an antibody typically comprises one or more hypervariable regions of an antibody, for example the CDR, CDR2 and/or CDR3 region. However, the "framework" or "skeleton" region of an antibody may also play a role during binding of the antibody to the antigen. The framework region forms the skeleton of the CDRs. Preferably, the antigen binding domain comprises at least amino acids 4 to 103 of the variable light chain and amino acids 5 to 109 of the variable heavy chain, more preferably amino acids 3 to 107 of the variable light chain and 4 to 111 of the variable heavy chain, particularly preferably the complete variable light and heavy chains, i.e. amino acids 1 - 109 of the VL and 1 to 113 of the VH (numbering according to WO97/08320).

"Functional fragments" or "antigen-binding antibody fragments" of the invention encompass, non-conclusively, Fab, Fab', F(ab')2 and Fv fragments, diabodies, Single Domain Antibodies (DAbs), linear antibodies, individual chains of antibodies (single-chain Fv, abbreviated to scFv); and multispecific antibodies, such as bi and tri-specific antibodies, for example, formed from antibody fragments C. A. K Borrebaeck, editor (1995) Antibody Engineering (Breakthroughs in Molecular Biology), Oxford University Press; R. Kontermann & S. Duebel, editors (2001) Antibody Engineering (Springer Laboratory Manual), Springer Verlag. Antibodies other than "multispecific" or "multifunctional" antibodies are those having identical binding sites. Multispecific antibodies may be specific for different epitopes of an antigen or may be specific for epitopes of more than one antigen (see, for example WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., 1991, J. Immunol. 147:60 69; U. S. Pat. Nos. 4,474,893; 4,714,681 ; 4,925,648; 5,573,920; 5,601,819; or Kostelny et al., 1992, J. Immunol. 148: 1547 1553). An F(ab')2 or Fab molecule may be constructed such that the number of intermolecular disulphide interactions occurring between the Chi and the CL domains can be reduced or else completely prevented.

"Epitopes" refer to protein determinants capable of binding specifically to an immunoglobulin or T cell receptors. Epitopic determinants usually consist of chemically active surface groups of molecules such as amino acids or sugar side chains or combinations thereof, and usually have specific 3-dimensional structural properties and also specific charge properties.

"Functional fragments" or "antigen-binding antibody fragments" may be fused with another polypeptide or protein, not originating from an antibody, via the amino terminus or carboxyl terminus thereof, by
means of a covalent bond (e.g. a peptide linkage). Furthermore, antibodies and antigen-binding fragments may be modified by introducing reactive cysteines at defined locations, in order to facilitate coupling to a toxophore (see Junutula et al. Nat Biotechnol. 2008 Aug; 26(8):925-32).

Polyclonal antibodies can be prepared by methods known to a person of ordinary skill in the art. Monoclonal antibodies may be prepared by methods known to a person of ordinary skill in the art (Kohler and Milstein, Nature, 256, 495-497, 1975). Human and humanized monoclonal antibodies may be prepared by methods known to a person of ordinary skill in the art (Olsson et al., Meth Enzymol. 92, 3-16 or Cabilly et al. US 4,816,567 or Boss et al. US 4,816,397).

A person of ordinary skill in the art is aware of diverse methods for preparing human antibodies and fragments thereof, such as, for example, by means of transgenic mice (N Lonberg and D Huszar, Int Rev Immunol. 1995; 13(l):65-93) or Phage Display Technologien (Clackson et al., Nature. 1991 Aug 15;352(6336):624-8). Antibodies of the invention may be obtained from recombinant antibody libraries consisting for example of the amino acid sequences of a multiplicity of antibodies compiled from a large number of healthy volunteers. Antibodies may also be produced by means of known recombinant DNA technologies. The nucleic acid sequence of an antibody can be obtained by routine sequencing or is available from publically accessible databases.

An "isolated" antibody or binder has been purified to remove other constituents of the cell. Contaminating constituents of a cell which may interfere with a diagnostic or therapeutic use are, for example, enzymes, hormones, or other peptidic or non-peptidic constituents of a cell. A preferred antibody or binder is one which has been purified to an extent of more than 95% by weight, relative to the antibody or binder (determined for example by Lowry method, UV-Vis spectroscopy or by SDS capillary gel electrophoresis). Moreover an antibody which has been purified to such an extent that it is possible to determine at least 15 amino acids of the amino terminus or of an internal amino acid sequence, or which has been purified to homogeneity, the homogeneity being determined by SDS-PAGE under reducing or non-reducing conditions (detection may be determined by means of Coomassie Blau staining or preferably by silver coloration). However, an antibody is normally prepared by one or more purification steps.

The term "specific binding" or "binds specifically" refers to an antibody or binder which binds to a predetermined antigen/target molecule. Specific binding of an antibody or binder typically describes an antibody or binder having an affinity of at least 10⁻⁹ M (as Kd value; i.e. preferably those with smaller Kd values than 10⁻⁷ M), with the antibody or binder having an at least two times higher affinity for the predetermined antigen/target molecule than for a non-specific antigen/target molecule (e.g. bovine serum albumin, or casein) which is not the predetermined antigen/target molecule or a closely related antigen/target molecule. The antibodies preferably have an affinity of at least 10⁻⁷ M (as Kd value; in other words preferably those with smaller Kd values than 10⁻⁷ M), preferably of at least 10⁻⁸ M, more
preferably in the range from $10^{-6}$ M to $10^{-11}$ M. The Kd values may be determined, for example, by means of surface plasmon resonance spectroscopy.

The antibody-drug conjugates of the invention likewise exhibit affinities in these ranges. The affinity is preferably not substantially affected by the conjugation of the drugs (in general, the affinity is reduced by less than one order of magnitude, in other words, for example, at most from $10^{-8}$ M to $10^{-7}$ M).

The antibodies used in accordance with the invention are also notable preferably for a high selectivity. A high selectivity exists when the antibody of the invention exhibits an affinity for the target protein which is better by a factor of at least 2, preferably by a factor of 5 or more preferably by a factor of 10, than for an independent other antigen, e.g. human serum albumin (the affinity may be determined, for example, by means of surface plasmon resonance spectroscopy).

Furthermore, the antibodies of the invention that are used are preferably cross-reactive. In order to be able to facilitate and better interpret preclinical studies, for example toxicological or activity studies (e.g. in xenograft mice), it is advantageous if the antibody used in accordance with the invention not only binds the human target protein but also binds the species target protein in the species used for the studies. In one embodiment the antibody used in accordance with the invention, in addition to the human target protein, is cross-reactive to the target protein of at least one further species. For toxicological and activity studies it is preferred to use species of the families of rodents, dogs and non-human primates. Preferred rodent species are mouse and rat. Preferred non-human primates are rhesus monkeys, chimpanzees and long-tailed macaques.

In one embodiment the antibody used in accordance with the invention, in addition to the human target protein, is cross-reactive to the target protein of at least one further species selected from the group of species consisting of mouse, rat and long-tailed macaque (Macaca fascicularis). Especially preferred are antibodies used in accordance with the invention which in addition to the human target protein are at least cross-reactive to the mouse target protein. Preference is given to cross-reactive antibodies whose affinity for the target protein of the further non-human species differs by a factor of not more than 50, more particularly by a factor of not more than ten, from the affinity for the human target protein.

**Antibodies directed against a cancer target molecule**

The target molecule towards which the binder, for example an antibody or an antigen-binding fragment thereof, is directed is preferably a cancer target molecule. The term "cancer target molecule" describes a target molecule which is more abundantly present on one or more cancer cell species than on non-cancer cells of the same tissue type. Preferably, the cancer target molecule is selectively present on one or more cancer cell species compared with non-cancer cells of the same tissue type, where selectively describes an at least two-fold enrichment on cancer cells compared to non-cancer cells of the same tissue type (a
"selective cancer target molecule"). The use of cancer target molecules allows the selective therapy of cancer cells using the conjugates according to the invention.

Antibodies which are specific against an antigen, for example cancer cell antigen, can be prepared by a person of ordinary skill in the art by means of methods with which he or she is familiar (such as recombinant expression, for example) or may be acquired commercially (as for example from Merck KGaA, Germany). Examples of known commercially available antibodies in cancer therapy are Erbitux® (cetuximab, Merck KGaA), Avastin® (bevacizumab, Roche) and Herceptin® (trastuzumab, Genentech). Trastuzumab is a recombinant humanized monoclonal antibody of the IgGlkappa type which in a cell-based assay (Kd = 5 nM) binds the extracellular domains of the human epidermal growth receptor with high affinity. The antibody is produced recombinantly in CHO cells.

In a preferred embodiment, the target molecule is a selective cancer target molecule.

In a particularly preferred embodiment, the target molecule is a protein.

In one embodiment, the target molecule is an extracellular target molecule. In a preferred embodiment, the extracellular target molecule is a protein.

Cancer target molecules are known to those skilled in the art. Examples of these are listed below.

Examples of cancer target molecules are:

(1) EGF receptor (NCBI reference sequence NP_005219.2), SEQ ID NO: 213 (1210 amino acids):

>gi|29725609|ref|NP_005219.2| EGF receptor precursor [Homo sapiens]
MRPSGTAGAALLALLALCPASRALEEKVCOGTNSKLTLONGTFEDHFSLROMFNNCEWLG
NLEITYVORYNDSLKTOEAVAGYVLALNTVERIPLENLQIIRGNYENSYALAVLSNYDAN
KTLGKELPRMNLOEILIHAVGVRFSNPALCNVESIQWDRIVSSDFSLSNMSMDFONHLGSCOKCDP
SCPNGSCWGAGENOKLTAKCAOOSGCRGKPSDDCCHNOCAAGCTGPDRESDCLVCKFRD
EATCKDTCPLMLNYPTTTYQMDVNPENGKYSFGATCVKKCPRNYVTDHGSCVRACGADSYYEM
EEDGVKRCCKKCMEGPRKVCNGIGIGEFKDSLShSNTNJKHFKNCTSISGLHLIPVAFRGDSFTHTP
PLDPOELDILKTVKEITGFLIOAWPENRTDLHAFENLEIIRGRTKOHGOFSLAWSLNIITSLGLRS
LKEISDGVDIISNGNKNLYANTINWKKLFSGOKTIISNRGENSCKATGOVCHALCSPEGCWG
PEPRDCVSCRNVSRGRECVDKCNLLEGEPEFVERSECIOCHPECLPOAMNITCGRPHDNCIOC
AHYIDPHCVKTCPAVMGNNTLVVKYAD AGHVCHLCHPNCYTGYCGBPGLGCPTNGKPIS
IATGMVGALLLLLLALGIGFMRIRRHRIVKRTLRLLQERELVEPLPSGEAPNQALLRILKETE
FKKIKVLSGSGAFTVYKGLWPEGEVKVIPVAIKREATSPKANEELDEAYVMSVNDPHVCR
LLGICLSTVQULTQLMPGCLLLDYREHDKNIGSQQLYLWCQIAGMNYLDERRVHRDLA
RRVLVKTQPVHKTIDFGLAKLLGAAEKEYAHEGGKVPKWMALELSILHRYTHQSDVWSYGVT
WELMTGSKPYDGPASEISILEGKRLPPQICIDTVYMIMVCKWMIDASRPFKRELIEFSKM
ARDPQRYLQIDGHMPLHPSDNSFYRALMEDEMDMDWADDYLIPOQGFFSPSTSRTPLLS
SLSATSNSTVACIDRNGLQSCPIDEKDSLFRYSSDPTGALTEDSIDTFLPVEYTNQVSPKPRAG
SVQNPVYHNQPLNAPSRDPHYQDPSHTAVNGPELYNVTQPTCVNSTFDSPAHWAQKGHSQISL
DNPDYQQDFPPKEAKPNGIATAGSTENAEYLVRAPQSSEFIGA

The extracellular domain is marked by underlining.
(2) mesothelin (SwissProt reference Q13421-3), SEQ ID NO: 214 (622 amino acids):

>sp|Q13421-3|MSLN_HUMAN isoform 2 of mesothelin OS=Homo sapiens GN=MSLN MALPTARPLLGLSGCTPALGSLLFLFSLGWVPQSRTLAGEGQEAAPLGDVLANPNIISS LSPRQLLGFCAEVSGLSTERVRELAVAQAOKNKVLSTEQLRCLAHRLSEPPEDLDPL

where mesothelin is encoded by amino acids 296-598. Amino acids 37-286 are coding for the megakaryocyte-potentiating factor. Mesothelin is anchored in the cell membrane via a GPI anchor and is localized extracellularly.

(3) carboanhydrase IX (SwissProt reference Q16790), SEQ ID NO: 215 (459 amino acids):

>sp|Q16790|CAH9_HUMAN carbonic anhydrase 9 OS=Homo sapiens GN=CA9 PE=1 SV=2 MAPLCPSPWLPLPAAPGTLVOLLSSLVLLLVHPVHP ORLPRMQEDESLPGLGGSSGEDDPL

The extracellular domain is marked by underlining.

(4) C4.4a (NCBI reference sequence NP_055215.2; synonym LYPD3), SEQ ID NO: 216 (346 amino acids):

>gi|93004088|ref|NP_055215.2| Iy6/PLAUR domain-containing protein 3-precursor [Homo sapiens]

The mature extracellular domain is marked by underlining.

(5) CD52 (NCBI reference sequence NP_001794.2 ), SEQ ID NO: 217

>gi|68342030|ref|NP_001794.2| CAMPATH-1 antigen-precursor [Homo sapiens]

The mature extracellular domain is marked by underlining.

(6) Her2 (NCBI reference sequence NP_004439.2), SEQ ID NO: 218
>gi|54792096|ref|NP_004439.2| receptor tyrosine-protein kinase erbB-2 isoform a [Homo sapiens]
MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQWQGNLELT
YLPNATSFLSQEVQGYVLHANHQVRQVPLRLRIVGTTQLFEDYALAVDNGDPLNNTTP
VTGASPGLRLERQLRSITEILKGGVLRILQRNPCLCYQYDITLWKKDIFHKNQLALTLDTRNASCHP
CSPMCKGSRCGESDCQSLSRTVCAAGGCAKRGKPLTDCHECQAAGCTGPKHSCLACLHL
FNHSIGELHCPALVTYNTTFSEPENPGRFTGFCSVTAZPYNLSTDVGSTCLVCHLNPHE
TAEQTGRCEKSCPKCARYCGLMEHRLREVRAVTSSANIEFAGCKKIFGSFLAEPSDFGDPAS
NTAPLQEQPQVFETLIEEIGYLIASWDPSLPLSVFQNLQVRIRGLHKNGAYSTLQLGIGSWL
GLRSLRELGSHLGNHNDLVPHTVQDFRNPQHLALLHTANPDECVEGGLACHQLCAR
GHCCGEPGTQVCNCSQELRGQECEVECRVLQLQPREYVNNRHARHCPHEPCQPNQSHTCFGPE
ADQCVACHYKDPFFCVARCSVPGVKPLDLSYMPIKFDEGAEQCPICPINHTSCVDDLDGGCPCA
EQRASPLTISASAWGILLLVGFWGIFLKRQKIRYMTTRLLQETLEVEPLTPGMPNQA
QMRKIELERLKVVLGSAGFTVYNGWPDGNEVKIPVAKVLRENTSKANEILDEAYVA
GMGSVPVSRLGLTSTVQLVTLMPYGFHCDDLEHRRGQDQLNNMLNWCMQAKGMYSLED
VRLVHRDLAARNVLVKSNHKNTDLGLLLDIDETYHADDGKVPIKMALESLRRRTFO
SDVWSYTGVVMETLMGFQIGAPREILDLLEKERLPLQQCOPTCIDTVYMIVMKCMISSCERP
RFRELVESFSLMSMRDPQRFQWIQNEDGLPSPLDTSTFYRSLDDDDMLVDLVAEELYVPLQGGFC
PDPAGGGMVVHRHRSSHSTRGGDGLTGLLEEPSEEAPRSAPLSEAGSGDVFDDGLGMGA
KGLQSLPHTDSPLQRXEPETDYGVAUTLCTSPQEEVQPVDRPFPQPSGPLPAARP
AGATLERPRTLSPGKNWVDVFQFGAAGENPEYLTQPGGAAPQHPHPAFSPADNYLYWDO
DPPEGAPPSTFKGTPAENPEYLGLDVLP

(7) CD20 (NCBI reference sequence _NP_068769.2), SEQ ID NO: 219

>gi|23110987|ref|NP_068769.2| B-lymphocyte antigen CD20 [Homo sapiens]
MTTPRNSVNTFPAEMPKGIPAMQSGPKPLFRMMSSLGVPQTSFFMRESKTGLAVGQIMNGLFHIA
LGLLMIPAGIYAPICVTVWPLWGGIMYIISGSLLAATEKNSKRCLVKGKIMNMSLFALASG
MILSMDILNIKSHFLKMFSLNFIRAHTPYNIYCEPANNPSTQCYSIQLSLGLSVMILV
AFFQELVIAIGNEENWKTSCRPSKNISSLSEKKEQITEIEEKEEWLGTETSSQPQNEDEIIPI
QEEEEEETNFPEPQPQQEESPIENDSSP

(8) the lymphocyte activation antigen CD30 (SwissProt ID P28908), SEQ ID NO: 220

>gi|68348711|ref|NP_001234.2| tumor necrosis factor receptor superfamily member 8 isoform 1-
precursor [Homo sapiens]
MRVLLAHGLLFLGALRAFPQDRFEDTCNGSNPSHYYDVAVRCCYRCPMGFLFTPQCPQPRPT
DCRKQCQEPDYIYLDEARDCTACTVCSRDLLVEKTPCAWNSSRVECRPGMFCSTSAVNSCARCF
FHSVCVPMGKGFTQGAQNTCPCEAPSGQFAPSGAPNSCEPSGGTIPQAKPVTGPSATSSASTM
PVRGGTRLAQEAASKLTLAPDPSVVGPSDDPLGLSTCPQPEGSDCRAKQCPDEYYLDEAERC
TACVSCSRDLLVEKTPCRPGMICATSAATNRSARCPVSPCSECPAICAEVTQPQDAEKED
TTFEAPPQLPTQDCNPETPENAGAPSTSEQVLLDSQASKTLPIPTSAPAVLSSTKPVLDAGPV
FWVILVWLGASALCHRRACRKRIRQKHLHCYPVQTSQPCLKELVDSRRSTQSLRSGAV
TEPVAEERGLMSQPLMETCSVGAAYLESLPLQDSAPPPSPRPDLEPVSTEHTNKKIEIYI
MKADTVVELTVKAELPEGRHLAGPAEPELEEEEADHTHPQETYETEPLGLSCDVMSVEEEEK
EDPLPTAASGK

(9) the lymphocyte adhesion molecule CC22 (SwissProt ID P20273), SEQ ID NO: 221

>gi|157168355|ref|NP_001762.2| B-cell receptor CD22 isoform 1-precursor [Homo sapiens]
MHLLGFPWLLLVLYESLAFSDSSKWVFEEPEIYAWEGACVWIPCTYRALDGDLESILFH
NKNTSKFDGRTLYESKDGKPSEQKVRQFGLDKNKNCTLSIHYPVHLNSDGQLGRMESSTK
WMERIHLVSERPFPFPQPQILPEIEQSESQVTLTCLLNFSYQGPIQLQWLEGVPMQAATTVST
TIKSVFTRSELKFPSPQWSHSHGKIVTCQLQDADGKFLSNDTVLNVKHTPKEIKVTPSDAVREG
DSVTMTCEVSSSNPEYTTVSWLKDGTSLKQNFTFNLREWTKDSGKYCCQVSNVDGPRSEE
VFLLVQYAPESTVQLHASPVEGQVFLECMSNLAPLTNYTHNGKEMQGRFKEKVHIPIL
PHWAGTYSCEAVNLGTTQGPAGELDQYPPKVTVQNPMPIREGDTVTLSNCYNSNSPST
RYEWPQHGWAEWPLGVNVTIACAcAANCNWCSAWPSVALNYQAPDRVVRKIK
PLSEIHSNSVSLQCFSSSHPEFQFWEKNGRLKEQSLNFDSIDPEADGYSVWNNNSGQT
ASKAWTLEVLVAPRRLRVSMSPGDQVMEGKSATLCESDANPPSHYTWDWNNQLPSYHQS
KLRLEPVQVKQHAYCQWGTNIVSVKRPSTLSTTVYSSPETIRGRAVGLSAILAILCIGLK
LQQRRWKRTSQSQQLDQNSSQFFVRNKKVRRAPLSLGHYCNPM.MEDMGISTRRTLRFPMN
IPRTGDAESEMQRPPDCDVTYSLAHKRVQVDYENVIPDFDEGGHEYSELIQFGVGERPFAQ
ENVYDVIILKH

(10) the myloid cell surface antigen CD33 (SwissProt ID P20138), SEQ ID NO: 222

>gi|3097998|ref|NP_001763.3| myeloid cell surface antigen CD33 isoform 1-precursor [Homo sapiens]
MLPLLLPLWAGALAMDNPFWLVQVQRESVTQVEQLCVLVPCTFFHIPYYPYDKNSPVHGYWFRE
GAISRSVPATNLQDEVQETQGFRLLGDPSRRNCSLISVDAARRDNGSYYFMRGRTSKYS
YKSPOLSVHTDLTHRPJLPGTLEPGTNSVCSWAVECQGTPPIPSLASSAALGRTTHSS
VLIITPRQDHTGNTLTCQVFKAAGAVTTERTIQLNVTYVPQNPPTGFPGDGSGQKQETRAWHG
AIGGAGVTALLALCLLCFLIVKTHRKLAAARTAVGRNANDTHPTTGSASPKHQKKSLSHGPETETSSC
SGAAPTVMDEELHYASLNFHGMNPSKTSTSEYERVTQ

(11) the transmembrane glycoprotein NMB (SwissProt ID Q14956), SEQ ID NO: 223

>gi|52694752|ref|NP_001005340.1| transmembrane glycoprotein NMB isoform a-precursor [Homo sapiens]
MECLYYFLGFLLAARLPLDAAKRFHDVLGNERPSAYMREHNQLNGWSSDENDWNEKLYPVW
KRGRDMRKWSNWKGGRVQAVALSDSPALVGSNIKFAVNLIFPRCQKEADANGINIVKNC REAG
LSADPVVYNWTAWSEDSDEGTGQSHHNFDPGKPPHPGWRRWNVFIYFHTLQGFYQKL
GRCSVRVSNTAVNLQPLMEVTVYRRHGRAYVIPAQVKDVYWTIDQPIFVFTMFQKDRNS
SDETFLKDLPMFVDLHIDPSHLNYSTINYKYSGFDNTGLVSTNHTVNYHTLVNGLTSLNLTV
KAAAPGCPPPSSSPKTPSLLTALKYSDNSTPGAGDNPLELSRPDENCQINRYGHFQATITI
VEGILEVINQMTDMLMVPWEPSSLLFVTQGQEPSTECHTPGESTITEQNTVCSPVDVEMEC
LTVVRRTFNQGTYCTNLTDGDLSTLSTLISVPRDPSAPLMANASLISVGCLAIFVTISLL
VYKKHKEYNPIENSPGN WRSKGLSVFLNRAKAVFFPGNQEKEQPDLKLNNKQEQKVS

(12) the adhesion molecule CD56 (SwissProt ID P13591), SEQ ID NO: 224

>gi|94420689|ref|NP_000606.3| neural cell adhesion molecule 1 isoform 1 [Homo sapiens]
MLQTKDLWTLFLGTAGLTVLQDIVPSQGEISVGEKFFLCQVAGDASKDKISWSFSPNEKLTZN
QQRISVVVWDDSSSTLTYIANIDAGIYKICWTEGDGESEATVNVKIFQKLMFKNAPTPQER
EGEDAVIVCDV5SSLPTIIWKHKGRDVLKLDKDRFIVLSNYNQIGRIKTEDGTYRCEGRIARL
GEINFQDIQFVNPQIPPTQAQRNVINANLQGSVLVCLDAEGFPEMTSWKQGEQIEEQEDDE
KYIFSDDSSLQLTIIKVDKEIDAEIAQNEGAQDTHLKVFAKPKITYVENAAMELEEQVT
LTECESGDPIPSITWTRSTTRNSIEEKTDLGMRWSHAYSSLNLKQSIYTDAGEYICTASINTIQ
DSQSMYLEVQAPPKVYGONW

40 QVNTCIEVFAYPSATISWFRDQQLPSNSSNYIKNTPSAYSLEYVTDFSENDFGYNCTAVNRIG
QESLEFLFDQATPSPSSIDQVEPSYSTAVQVFDEBEGATGVPILKYAAWRAVGEEVWHSKWD
AYEASMEGIVTGILPETYTVLRNLNGKLGIEAIASEFKTQPVQGEPASKLEGMGD
NSIKVNLKQDDGGSPHRVLYRALSSEWKEPLPSGHDVMLKSLDWNAYEYVWAENQ
QGKSKAHAHFVFRTAASQPTAIAPANSGSPSTSLTGAIVGILIVFVLLLVWDITCYFLNKGLMFCIA
VNLCGKAGPGAKGKDEEKAASDSKEPKEVIRTEEERTPHNDGKHTEPNETTPLTEPEK
GPEAVKPECQETETPKPAPAEVKTVPNDATQKENESKA
(13) the surface molecule CD70 (SwissProt ID P32970), SEQ ID NO: 225

>gi|4507605|ref|NP_00 1243.1| CD70 antigen [Homo sapiens]
MPEEGSCGVSRpRYPGCVLPvAAPLVLAVAGVGLYICLWCIQRFAQAQQQLPLSLGWDVAILQNLH
TGPQQDPRLYWQGGPALGRSFLHGPELDKQGLRHRDGQYVHIQVTLAICSSTTASRRHPTTLA
VGIQCPASRISLLRLSFLHQGCTIASQLTPLARGDITCLNTLMTGTLPSRNRTDETFGQVWVRP

(14) the surface molecule CD74 (SwissProt ID P04233), SEQ ID NO: 226

>gi|10835071|ref|NP_004346.1| HLA class II histocompatibility antigen gamma chain isoform b [Homo sapiens]
MHRRRSRCREDQKPVMDDQRLSNNEQPLMLGRPGAPESKCSRGALTYTGFSILVTLLAGQ
ATTAYFLYQQGRLDNLTVSNCILQLENLRMLKPKPVPKVMRATMLQMQLPMGALPQGG
MQNAKTYGKMTEDHVMLHLQNDPALKVYPPLKGSIPENLRHLKNTMTIDWKFQSVWMMH
LLEFMSRSHELQKPTDAPPKESLELEDPSGQLVTQDLGVPM

(15) the B-lymphocyte antigen CD19 (SwissProt ID P15391), SEQ ID NO: 227

>gi|296010921|ref|NP_00171569.1| B-lymphocyte antigen CD19 isoform 1-precursor [Homo sapiens]
MPPRLLFFLLFLTPMEVYPEPLWKEEEDNVQLCQLKGTSDGPQQTQILTWRSERPLKPFKLQLS
GLPLGHIHPRLAIWLFIFNSVSQMQGGFYLCQPGPSEKAWPGWTVNVEGSQELFRNVNSDL
GGLGCGLKNRSSEGPSSPGKLMSPKLYYVAKDRPEIWEGEPCLPRDSLQRSLSQDLMTAPGS
TLWLSGYPDSVRPLSWTHVHGPKSLSLLEKLDGRPRAMDWVMETGQLLYPRATAQDG
KYCYCHRNLMTSFIHLETARPVWLHWWLRTGKVCSAVTLYLIFCLCGLVILHQQRALVLR
KRKRMDTPRRTFKVTPPGPQPNYQGNYNLPLSTPTSGLGRALQRAWAAGLGTAPSYGPSSDV
QADALGSRSSPVGPEEEEGGEYDDSDSEEFSEDSEYENDNLSQQLQDQSGYENPEDELPGE
DEDSFSNAEYSYNEDELTQVARTMDFLSPHGSAWDPRESATSLAGSQYEDMRGBLYAAPQL
RSIRGQPGLNVHEEDADSYENMDNPDGPDPAWGGGRMGTWSTR

(16) the surface protein mucin-1 (SwissProt ID P15941), SEQ ID NO: 228

>gi|65301117|ref|NP_002447.4| mucin-1 isoform 1-precursor [Homo sapiens]
MTPGTPQSPFLLLTLTLVTWTGSGHASSTPGPEKETATSATQRSSVSSTKALSTGTGVSFFLSFH
SNLQFQNSSLSPDDSTPYQQEQLQRDISEMFQLKQGGFLGLSNKFRPGGSGTWQLTAFREGTNHV
DVEQFQNYKTEAASRYNLTVSLVTSSPQFSAQGAVGPGWIALLLVCLVLAGLYLIA
LAVCQCRKKNYQQLDIFPARDDTYPMSEYPTYTHGTRYVPPSSPSTDSPYKVSAGNGSSLYT
NPVAAATSANL

(17) the surface protein CD138 (SwissProt ID P18827), SEQ ID NO: 229

>gi|29568086|ref|NP_002988.3| syndeacan-1 isoform [Homo sapiens]
MRRAALWICLALALSLQPMALPQIVATNLPPEDQDNGGDSRFTSGALGAPRQITSPT
WKDTQQLTALTSPETGLEATAASTSLPAPGAPKEVAVLPEVEPGTAREQATPRRETQT
LPTTHQASTTTATATCQPATSHHRMQGHPHETSTPAGPSQADLHPTHTEDGSGAPATAEAD
GASSQLPASQEGSEQDFTETFSGENTAWAVEPDRNQPSVPSQGATGSGQLLDRKEVLLGAVIA
GGLVGLIFAVCLVGFMLYYRMKKEGYSLEEPKQANGAYQKPTKQEEFYA

(18) the integrin alphaV (Genbank Accession No.: NP_002201.1), SEQ ID NO: 230

>gi|4504763|ref|NP_002201.1| integrin alpha-V isoform 1-precursor [Homo sapiens]
(19) the teratocarcinoma-derived growth factor 1 protein TDGF1 (Genbank Accession No.: NP_003203.1), SEQ ID NO: 231

>gi|4507425|ref|NP_003203.1| teratocarcinoma-derived growth factor 1 isoform 1-precursor [Homo sapiens]
MDCRKMARFSYSVIWIMAIISKVFELGLVALGHLQEFARPSRYGLAFRDRDSIWPQEEPAIRPRSSQ

(20) the prostate-specific membrane antigen PSMA (Swiss Prot ID: Q04609), SEQ ID NO: 232

>gi|4758398|ref|NP_004467.1| glutamate carboxypeptidase 2 isoform 1 [Homo sapiens]
MWNLLHETDSSAVTPGALAGGFLGLAGHLQEFARPSRYGLAFRDRDSIWPQEEPAIRPRSSQ

(21) the tyrosine protein kinase EPHA2 (Swiss Prot ID: P29317), SEQ ID NO: 233

>gi|3296731|ref|NP_004422.1| ephrin type-A receptor 2 precursor [Homo sapiens]
MELQARACFALLWGCALAALAAQGKEWLLDLFAAGGELGHLQEFARPSRYGLAFRDRDSIWPQEEPAIRPRSSQ

(20) the prostate-specific membrane antigen PSMA (Swiss Prot ID: Q04609), SEQ ID NO: 232

>gi|4758398|ref|NP_004467.1| glutamate carboxypeptidase 2 isoform 1 [Homo sapiens]
MWNLLHETDSSAVTPGALAGGFLGLAGHLQEFARPSRYGLAFRDRDSIWPQEEPAIRPRSSQ

(21) the tyrosine protein kinase EPHA2 (Swiss Prot ID: P29317), SEQ ID NO: 233

>gi|3296731|ref|NP_004422.1| ephrin type-A receptor 2 precursor [Homo sapiens]
MELQARACFALLWGCALAALAAQGKEWLLDLFAAGGELGHLQEFARPSRYGLAFRDRDSIWPQEEPAIRPRSSQ
(22) the surface protein SLC44A4 (Genbank Accession No: NP_001171515), SEQ ID NO: 234

>gi|295849282|ref|NP_001171515.1| choline transporter-like protein 4 isoform2 [Homo sapiens]

MGGKQRDEDEGRAYGPVYDPSPFRGPIKNSRCEDVICCVLFLLLILGYTWIGAVAWLYGDPRQVL YPRNSTGAYCGMGENKDPYLLYNFIISLSSIENISVAENGLQCPTQVTSQLQEPCSFLLPS APALEGCRFVTNTPPAPLPGTDNTQIQGISGLIDSNLARDISVKIFSEDQASWSWYVILVALGVAL VLSLLFILLRLVAPGLVLVLILGVLVLAYGIYYCWEEYRVLRDKGASISQLGFITNLSAYQSVQETWLAALVLAILEALLMLFLFRQIRIAAILAKSEASKAVGQMSTMVFPIVFLVLLLIAAY WAMTALEYLATSQPPQYYVLWASNISSPGCEKPVPNSTCNPTAHLYVSNSCPGLMCVFQYSSKGLI QRSVFNLYQYYGLLFWTLNWVLLALGQCVLAGAFASYFYWAHKPDIPFTFLISAFIRTLRYHTG SLaFGALILTLVQIARVILEYIDHLGRGVPNVARCMCCFKCCCLCWCLEKFKELRNYMIAIY GKNFCVSAKNAFMLLRNIVVRVLDKVTDLLLFFGKLLWGVGLSFFFSGRIPGLGKDFK SPHLNYWWLPMTSILGAYVASGSFSVFGMCVDTLFLCFLDELDNERNGSLDRPYMSKSSLKILG KKNAPPDNKRRKK

(23) the surface protein BMPR1B (SwissProt: 000238)

(24) the transport protein SLC7A5 (SwissProt: Q01650)

(25) the epithelial prostate antigen STEAP1 (SwissProt: Q9UHE8)

(26) the ovarian carcinoma antigen MUC16 (SwissProt: Q8WXI7)

(27) the transport protein SLC34A2 (SwissProt: 095436)

(28) the surface protein SEMA5b (SwissProt: Q9P283)

(29) the surface protein LYPD1 (SwissProt: Q8N2G4)

(30) the endothelin receptor type B EDNRB (SwissProt: P24530)

(31) the ring finger protein NRF4 (SwissProt: Q68DV7)

(32) the prostate carcinoma-associated protein STEAP2 (SwissProt: Q8NFT2)

(33) the cation channel TRPM4 (SwissProt: Q8TD43)

(34) the complement receptor CD21 (SwissProt: P20023)
(35) the B-cell antigen receptor complex-associated protein CD79b (SwissProt: P40259)

(36) the cell adhesion antigen CEACAM6 (SwissProt: P40199)

(37) the dipeptidase DPEP1 (SwissProt: P16444)

(38) the interleukin receptor IL20Ralpha (SwissProt: Q9UHF4)

(39) the proteoglycan BCAN (SwissProt: Q96GW7)

(40) the ephrin receptor EPHB2 (SwissProt: P29323)

(41) the prostate stem cell-associated protein PSCA (Genbank Accession No: NP_005663.2 )

(42) the surface protein LHFPL3 (SwissProt: Q86UP9)

(43) the receptor protein TNFRSF13C (SwissProt: Q96RJ3)

(44) the B-cell antigen receptor complex-associated protein CD79a (SwissProt: P11912)

(45) the receptor protein CXCR5 (SwissProt: P32302)

(46) the ion channel P2X5 (SwissProt: Q93086)

(47) the lymphocyte antigen CD180 (SwissProt: Q99467)

(48) the receptor protein FCRL1 (SwissProt: Q96LA6)

(49) the receptor protein FCRL5 (SwissProt: Q96RD9)

(50) the MHC class II molecule la antigen HLA-DOB (Genbank Accession No: NP_002111.1)

(51) the T-cell protein VTCN1 (SwissProt: Q7Z7D3)

(52) TWEAKR (SEQ ID NO: 169 (protein); SEQ ID NO: 170 (DNA).

(53) the lymphocyte antigen CD37 (Swiss Prot: P11049)

(54) the FGF receptor 2; FGFR2 (Gene ID: 2263; official symbol: FGFR2). The FGFR2 receptor occurs in different splice variants (alpha, beta, Il1b, IIIc). All splice variants may act as target molecule.

(55) the transmembrane glycoprotein B7H3 (CD276; Gene ID: 80381).

(56) the B cell receptor BAFFR (CD268; Gene ID: 115650)
(57) the receptor protein ROR 1 (Gene ID: 4919)

(58) the surface receptor IL3RA (CD123; Gene ID: 3561)

(59) the CXC chemokine receptor CXCR5 (CD185; Gene ID 643)

(60) the receptor protein syncytin (Gene ID 30816)

In a preferred subject matter of the invention, the cancer target molecule is selected from the group consisting of the cancer target molecules (1) - (60), in particular (1), (6) and (52).

In a further particularly preferred subject matter of the invention, the binder binds to an extracellular cancer target molecule which is selected from the group consisting of the cancer target molecules (1) - (60), in particular (1), (6) and (52).

In a further particularly preferred subject matter of the invention, the binder binds specifically to an extracellular cancer target molecule which is selected from the group consisting of the cancer target molecules (1) - (60), in particular (1), (6) and (52). In a preferred embodiment the binder is, after binding to its extracellular target molecule on the target cell, internalized by the target cell as a result of the binding. This causes the binder/active compound conjugate, which may be an immunoconjugate or an ADC, to be taken up by the target cell. The binder is then processed, preferably intracellularly, with preference lysosomally.

In one embodiment the binder is a binding protein. In a preferred embodiment the binder is an antibody, an antigen-binding antibody fragment, a multispecific antibody or an antibody mimic.

Preferred antibody mimetics are affibodies, adnectins, anticalins, DARPin, avimers, or nanobodies. Preferred multispecific antibodies are bispecific and trispecific antibodies.

In a preferred embodiment the binder is an antibody or an antigen-binding antibody fragment, more preferably an isolated antibody or an isolated antigen-binding antibody fragment.

Preferred antigen-binding antibody fragments are Fab, Fab’, F(ab’)2 and Fv fragments, diabodies, DAbs, linear antibodies and scFv. Particularly preferred are Fab, diabodies and scFv.

In a particularly preferred embodiment the binder is an antibody. Particularly preferred are monoclonal antibodies or antigen-binding antibody fragments thereof. Further particularly preferred are human, humanized or chimeric antibodies or antigen-binding antibody fragments thereof.
Antibodies or antigen-binding antibody fragments which bind cancer target molecules may be prepared by a person of ordinary skill in the art using known processes, such as, for example, chemical synthesis or recombinant expression. Binders for cancer target molecules may be acquired commercially or may be prepared by a person of ordinary skill in the art using known processes, such as, for example, chemical synthesis or recombinant expression. Further processes for preparing antibodies or antigen-binding antibody fragments are described in WO 2007/070538 (see page 22 "Antibodies"). The person skilled in the art knows how processes such as phage display libraries (e.g. Morphosys HuCAL Gold) can be compiled and used for discovering antibodies or antigen-binding antibody fragments (see WO 2007/070538, page 24 ff and AK Example 1 on page 70, AK Example 2 on page 72). Further processes for preparing antibodies that use DNA libraries from B cells are described for example on page 26 (WO 2007/070538). Processes for humanizing antibodies are described on page 30-32 of WO2007070538 and in detail in Queen, et al., Pros. Natl. Acad. Sci. USA 86:10029-10033, 1989 or in WO 90/0786. Furthermore, processes for the recombinant expression of proteins in general and of antibodies in particular are known to the person skilled in the art (see, for example, in Berger and Kimmel (Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol. 152, Academic Press, Inc.); Sambrook, et al., (Molecular Cloning: A Laboratory Manual, (Second Edition, Cold Spring Harbor Laboratory Press; Cold Spring Harbor, N.Y.; 1989) Vol. 1-3); Current Protocols in Molecular Biology, (F. M. Ausabel et al. [Eds.], Current Protocols, Green Publishing Associates, Inc. / John Wiley & Sons, Inc.); Harlow et al., (Monoclonal Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1988, Paul [Ed.]); Fundamental Immunology, (Lippincott Williams & Wilkins (1998)); and Harlow, et al., (Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1998)). The person skilled in the art knows the corresponding vectors, promoters and signal peptides which are necessary for the expression of a protein/antibody. Commonplace processes are also described in WO 2007/070538 on pages 41-45. Processes for preparing an IgGl antibody are described for example in WO 2007/070538 in Example 6 on page 74 ff. Processes which allow the determination of the internalization of an antibody after binding to its antigen are known to the skilled person and are described for example in WO 2007/070538 on page 80. The person skilled in the art is able to use the processes described in WO 2007/070538 that have been used for preparing carboanhydrase IX (Mn) antibodies in analogy for the preparation of antibodies with different target molecule specificity.

**anti-EGFR antibodies**

Examples of antibodies which bind the cancer target molecules EGFR are cetuximab (INN number 7906), *panitumumab* (INN number 8499), *nimotuzumab* (INN number 8545), "TPP-4030", and "TPP-5653". Cetuximab (Drug Bank Accession Number DB00002) is a chimeric anti-EGFR1 antibody which is produced in SP2/0 mouse myeloma cells and is sold by ImClone Systems Inc/Merck KgaA/Bristol-Myers Squibb Co. Cetuximab is indicated for the treatment of metastasizing, EGFR expressing, colorectal carcinoma with wild type K-Ras gene. It has an affinity of 10^{-9}M.
Cetuximab Light Chain (kappa), SEQ ID NO: 235:

```
VELTQSPVILSVSPGERVSFSCRASQGSIHNWIYQQRTNGSPRLLIKYASEISIGIPSRSFGSGST
DFTLSINSVESDIADYYCQNNWPTTFAGTKEKRTAVAPSIFIPPSDEQLKSGTASWC
LNNFYPREAKVQWVDNALQSGNSQESVETQDSDSTLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC
```

Cetuximab Heavy Chain, SEQ ID NO: 236:

```
DILLTQSPVILSVSPGERVSFSCRASQGSIHNWIYQQRTNGSPRLLIKYASEISIGIPSRSFGSGST
DFTLSINSVESDIADYYCQNNWPTTFAGTKEKRTAVAPSIFIPPSDEQLKSGTASWC
LNNFYPREAKVQWVDNALQSGNSQESVETQDSDSTLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC
```

Panitumumab (INN number 8499) (Drug Bank Accession Number DB01269) is a recombinant monoclonal human IgG2 antibody which binds specifically to the human EGF receptor 1 and is sold by Abgenix/Amgen. Panitumumab originates from the immunization of transgenic mice (XenoMouse). These mice are capable of producing human immunoglobulin (light and heavy chains). A specific B-cell clone was selected which produces antibodies against EGFR, and this clone was immortalized with CHO cells (Chinese hamster ovary cells). These cells are now used for the production of a 100% human antibody. Panitumumab is indicated for the treatment of EGFR-expressing, metastasizing colorectal carcinoma, which is resistant to chemotherapeutic treatment with fluoropyrimidine, oxaliplatin and irinotecan. It has an affinity of $10^{-11}$ M.

Sequence:

Panitumumab Light Chain (kappa), SEQ ID NO: 237:

```
DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNYWYQQPGBKPKLLEHADSNLETGVPSRFSGS
GSGTDFTTSSSLQPEDATYFCQHFDHLPLAFGGGTKEIKRTVAPSVFIPPSDEQLKSGTASV
VCLLNNFYPREAKVQWVDNALQSGNSQESVETQDSDSTLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC
```

Panitumumab Heavy Chain, SEQ ID NO: 238:

```
QVQLQESGPGLVKPSETLSLCTVTSGGSVSSGDYYWTWIRQSPBKGEWIGHIYYSQNTYNPSL
KSRLLTISIDTSKQTFLQKLSSVTADAIYCYCRDVRGTADFWGGGTQGQTMVTSSASTKPGSVPFLA
PCRSRTSESTAAALGCLUVDKYFPEPVTWNSGALTSGVHTFPAVLQSGSLSSWTVPSNFGT
QTYTCDHHSNKTVKDTVRKKCCECPPAPPAGPSVFLFPKPDKTLMSRTVEPTCVW
DVSHEDPEGQFNYFDVEVHNAKTPKREEQFNSFRWSVLTHQWDWLNGKEYKCKVSNK
GLPAPIEKTIKSKGQPREPQYVTLPSSREEMTKQVSLTCLVKGFPSDIA韦ESNGQPENNY
KTTPMLDSGSFLYSLKTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLLSPG
```
Nimotuzumab (INN number 8545) (EP 00586002, EP 00712863) is a humanized monoclonal IgGl antibody which binds specifically to the human EGF receptor 1 and is sold by YM BioSciences Inc. (Mississauga, Canada). It is produced in non-secreting NSO cells (mammalian cell line). Nimotuzumab is approved for the treatment of head-and-neck tumours, highly malignant astrocytoma and glioblastoma multiforms (not in EU and US) and pancreatic carcinoma (Orphan drug, EMA). It has an affinity of $10^{-8}$ M.

Nimotuzumab Light Chain, SEQ ID NO: 239:

DIQMTQSPSSLSASVGDRVTITCRSSQNIVHSNGNTYLDWYQQTPGKAPKLLYKVSNPVFSGVPS
RFSGSGSSTTQFENYQHVPWFGQGKLQTRTVAAPSVFIFPSDEQLKS
GTASWCLNNFYPREAVKQVWKDNALEQNSPQSEVTEQDSKDYSLKADYEKHK
VYACEVTHQGSLSPVTQSFNRGEC

Nimotuzumab Heavy Chain, SEQ ID NO: 240:

QVQLQQSAGAEVKPGSSVKVSCKASGYTFTNYYIYWVRQAPGQGLEWIGGINPTSGGSNFNEKF
KTRVVITADESSSTAYMELSSLRSEDATAFVFCTRQGLWFDSDGRGDFWQGQTTVTVSSASTKGP
SVFPLAPPSKSTSGTAAALCGCLVKDYFEPVTVSWNSGALTSGVHTFPAVLQSGLYSLSSWTVP
PSSSLGTQTYICNVHKNSPNTKVDKKEPVSCKDHTTCTCPPAPELLGGPSVFLFPPKPDTCMGIS
RTPEVTVCDVSDHEPFEFKVNYGDGEVHNAKTPREEQYNSTYRWSVTLHQLDNLGKEYKCKVSNKALPAIKETSKUAKQGPREPQYTVLPPSRDELTKNSLVTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPVLSDKSFLKMGLTVKDGSQVGGNFSCSVHEALHNHYTQKSLSLPG

TPP-4030

VH (SEQ ID NO: 241):

QVQLKQSGPGLVQPSQSLSITCTSVGFSLNTNIGVHWVRQSPGKGLELWLGVIWSSGGNTDYNTPFT
SRLSINKDNSKSVFFKMNSLQ SNDTALYCCARALTADYEFAWQGQGTLVTVSA
VL (SEQ ID NO: 242):

DILLTQSPVLSVPGERVVSFCRASQSGTNIHWYQQRTNGSPRLIKYASESISGIPSRSFSGSGT
DFTLSINSVESEDIADYYCQQNNNWPTTFGAGKLELK

TPP-5653

VH (SEQ ID NO: 243):

QVQLQESGPGLVKKSETLSLTCSTGVSSISNYGVHWIRQPPGKGLEWIGVIWSGGNTDYNTPFTS
RVTISVDTSKSVSLKLSSTVTAADTVYYCARALTYDYEFAYWQGQGTLVTVSA
Further embodiments of EGFR antibodies are as follows:

- Zalutumumab / 2F8 / HuMax-EGFr, from Genmab A/S (WO 02/100348, WO 2004/056847, INN number 8605)
- Matuzumab / anti-EGFR MAb, Merck KGaA / anti-EGFR MAb, Takeda / EMD 72000 / EMD-6200 / EMD-72000 and EMD-55900 / MAb 425 / monoclonal antibody 425, from Merck KGaA / Takeda (WO 92/15683, INN number 8103 (Matuzumab))
- ISU-101, from Isu Abxis Inc (ISU Chemical Co Ltd) / Scancell (WO 2008/004834-A1)
- SYM-004 (consists of two chimeric IgGl antibodies (992 and 1024)), from Symphogen A/S (WO 2010/022736-A2)
- MRI-1 /MRI-IKDEL, from IVAX Corp (Teva Pharmaceutical Industries Ltd) (Duke University), (patent: WO2001/062931-A2)
- Antibody against the deletion mutant, EGFRvIII, from Amgen/Abgenix (WO 2005/010151, US 7,628,986)
- SC-100, from Scancell Ltd (WO 01/088138-A1)
In a preferred embodiment, the anti-EGFR antibodies are selected from the group consisting of cetuximab, panitumumab, nimotuzumab, zalutumumab, necitumumab, matuzumab, RG-716, GT-MAB 5.2-GEX, ISU-101, ABT-806, SYM-004, MRI-1, SC-100, MDX-447, DXL-1218, "TPP-4030", and "TPP-5653".

In a particularly preferred embodiment the anti-EGFR antibodies are selected from the group consisting of cetuximab, panitumumab, nimotuzumab, matuzumab, "TPP-4030", and "TPP-5653".

The person skilled in the art knows of processes which can be used to prepare further antibodies, from the CDR regions of the abovementioned antibodies by means of sequence variations, these further antibodies having a similar or better affinity and/or specificity for the target molecule.

In a further embodiment, the anti-EGFR antibodies or antigen-binding antibody fragments are selected from the group consisting of antibodies or antigen-binding antibody fragments comprising the three CDR regions of the light chain and the three CDR regions of the heavy chain of one of the following antibodies: cetuximab, panitumumab, nimotuzumab, zalutumumab, necitumumab, matuzumab, RG-716, GT-MAB 5.2-GEX, ISU-101, ABT-806, SYM-004, MRI-1, SC-100, MDX-447, DXL-1218, "TPP-4030", and "TPP-5653".

In a further embodiment, the anti-EGFR antibodies or antigen-binding antibody fragments are selected from the group consisting of antibodies or antigen-binding antibody fragments comprising three CDR regions of the light chain and the three CDR regions of the heavy chain of one of the following antibodies: cetuximab, panitumumab, nimotuzumab, matuzumab, "TPP-4030", and "TPP-5653". By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

**anti-Carboanhydrase IX antibodies**

Examples of antibodies which bind the cancer target molecule carboanhydrase IX are described in WO 2007/070538-A2 (e.g. Claims 1 - 16).

**anti-C4.4a antibodies:**

According to the invention, use may be made of C4.4a antibodies.

Examples of C4.4a antibodies and antigen-binding fragments are described in WO 2012/143499 A2. By reference, all antibodies of WO 2012/143499 A2 are hereby incorporated into the description of the present invention, and they can be used in the present invention. The sequences of the antibodies are given in Table 1 of WO 2012/143499 A2, where each row shows the respective CDR amino acid sequences of the variable light chain or the variable heavy chain of the antibody listed in column 1.

In one embodiment, the anti-C4.4a antibodies or antigen-binding antibody fragments thereof are, after binding to a cell expressing C4.4a, internalized by the cell.

In a further embodiment, the anti-C4.4a antibodies or antigen-binding antibody fragments comprise at least one, two or three CDR amino acid sequences of an antibody listed in Table 1 of WO 2012/143499 A2 or Table 2 of WO 2012/143499 A2. Preferred embodiments of such antibodies are likewise listed in WO 2012/143499 A2 and incorporated herein by reference.

**anti-HER2 antibodies:**

An example of an antibody binding to the cancer target molecule Her2 is trastuzumab (Genentech). Trastuzumab is a humanized antibody used *inter alia* for the treatment of breast cancer.

Further examples of antibodies binding to HER2 are, in addition to trastuzumab (INN 7637, CAS No.: RN: 180288-69-1) and Pertuzumab (CAS No.: 380610-27-5), the antibodies disclosed in WO 2009/123894-A2, WO 200/8140603-A2 or in WO 2011/044368-A2. An example of an anti-HER2 conjugate is trastuzumab-emtansine (INN-No. 9295). By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

**anti-CD20 antibodies:**

An example of an antibody binding to the cancer target molecule CD20 is rituximab (Genentech). Rituximab (CAS Number: 174722-31-7) is a chimeric antibody used for the treatment of non-Hodgkin
lymphoma. By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

*anti-CD52 antibodies:*

An example of an antibody binding to the cancer target molecule CD52 is alemtuzumab (Genzyme). Alemtuzumab (CAS Number: 216503-57-0) is a humanized antibody used for the treatment of chronic lymphocytic leukaemia. By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

*anti-Mesothelin antibodies:*

Examples of anti-mesothelin antibodies are described, for example, in WO 2009/068204. By reference, all antibodies described in WO 2009/068204 are hereby incorporated into the present description, such that these antibodies can be used in the context of the invention disclosed herein.

The anti-mesothelin antibodies used in accordance with the invention are also notable preferably for an invariant binding to mesothelin. Invariant binding is characterized, for example, in that the antibody used in accordance with the invention binds to an epitope of mesothelin which cannot be masked by a further extracellular protein. Such a further extracellular protein is, for example, the protein ovarian cancer antigen 125 (CA125). Antibodies which are used with preference are characterized in that their binding to mesothelin is not blocked by CA125.

*anti-CD30 antibodies*

Examples of antibodies which bind the cancer target molecule CD30 and can be used for the treatment of cancer, for example Hodgkin lymphoma, are brentuximab, iratumumab and antibodies disclosed in WO 2008/092117, WO 2008/036688 or WO 2006/089232. An example of an anti-CD30 conjugate is brentuximab vedotin (INN No. 9144). By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

*anti-CD22 antibodies*

Examples of antibodies which bind the cancer target molecule CD22 and can be used for the treatment of cancer, for example lymphoma, are inotuzumab and epratuzumab. Examples of anti-CD22 conjugates are inotuzumab ozagamycin (INN No. 8574) or anti-CD22-MMAE and anti-CD22-MC-MMAE (CAS RN: 139504-50-0 and 474645-27-7, respectively). By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

*anti-CD33 antibodies*

Examples of antibodies which bind the cancer target molecule CD33 and can be used for the treatment of cancer, for example leukaemia, are gemtuzumab and lintuzumab (INN 7580). An example of an anti-
CD33 conjugate is gemtuzumab-ozagamycin. By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

**anti-NMB antibodies**

An example of an antibody which binds the cancer target molecule NMB and can be used for the treatment of cancer, for example melanoma or breast cancer, is glembatumumab (INN 9199). An example of an anti-NMB conjugate is glembatumumab vedotin (CAS RN: 474645-27-7). By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

**Anti-CD56 antibodies**

An example of an antibody which binds the cancer target molecule CD56 and can be used for the treatment of cancer, for example multiple myeloma, small-cell lung carcinoma, MCC or ovarian carcinoma is lorvotuzumab. An example of an anti-CD56 conjugate is lorvotuzumab mertansine (CAS RN: 139504-50-0). By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

**anti-CD70 antibodies**

Examples of antibodies which bind the cancer target molecule CD70 and can be used for the treatment of cancer, for example non-Hodgkin lymphoma or renal cell cancer, are disclosed in WO 2007/038637-A2 and WO 2008/070593-A2. An example of an anti-CD70 conjugate is SGN-75 (CD70 MMAF). By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

**anti-CD74 antibodies**

An example of an antibody which binds the cancer target molecule CD74 and can be used for the treatment of cancer, for example multiple myeloma, is milatuzumab. An example of an anti-CD74 conjugate is milatuzumab-doxorubicin (CAS RN: 23214-92-8). By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

**anti-CD19 antibodies**

An example of an antibody which binds the cancer target molecule CD19 and can be used for the treatment of cancer, for example non-Hodgkin lymphoma, is disclosed in WO 2008/031056-A2. Further antibodies and examples of an anti-CD19 conjugate (SAR3419) are disclosed in WO 2008/047242-A2. By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.
anti-Mucin antibodies

Examples of antibodies which bind the cancer target molecule mucin-1 and can be used for the treatment of cancer, for example non-Hodgkin lymphoma, are clivatuzumab and the antibodies disclosed in WO 2003/106495-A2, WO 2008/028686-A2. Examples of anti-mucin conjugates are disclosed in WO 2005/009369-A2. By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

anti-CD138 antibodies

Examples of antibodies which bind the cancer target molecule CD138 and conjugates thereof, which can be used for the treatment of cancer, for example multiple myeloma, are disclosed in WO 2009/080829-A1, WO 2009/080830-A1. By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

anti-Integrin-alphaV antibodies

Examples of antibodies which bind the cancer target molecule integrin alphaV and can be used for the treatment of cancer, for example melanoma, sarcoma or carcinoma, are intetumumab (CAS RN: 725735-28-4), abciximab (CAS RN: 143653-53-6), etaracizumab (CAS RN: 892553-42-3) and the antibodies disclosed in US 7,465,449, EP 719859-A1, WO 2002/012501-A1 and WO2006/062779-A2. Examples of anti-integrin AlphaV conjugates are intetumumab-DM4 and other ADCs disclosed in WO 2007/024536-A2. By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

anti-TDGF1 antibodies

Examples of antibodies which bind the cancer target molecule TDGF1 and can be used for the treatment of cancer are the antibodies disclosed in WO 02/077033-A1, US 7,318,924, WO 2003/083041-A2 and WO 2002/088170-A2. Examples of anti-TDGF1 conjugates are disclosed in WO 2002/088170-A2. By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

anti-PSMA antibodies

Examples of antibodies which bind the cancer target molecule PSMA and can be used for the treatment of cancer, for example prostate carcinoma, are the antibodies disclosed in WO 97/35616-A1, WO 99/47554-A1, WO 01/009192-A1 and WO2003/034903. Examples of anti-PSMA conjugates are disclosed in WO 2009/026274-A1 and WO 2007/002222. By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

anti-EPHA2 antibodies

Examples of antibodies which bind the cancer target molecule EPHA2 and can be used for preparing a conjugate and for the treatment of cancer are disclosed in WO 2004/091375-A2. By reference, these
antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

*anti-SLC44A4 antibodies*

Examples of antibodies which bind the cancer target molecule SLC44A4 and can be used for preparing a conjugate and for the treatment of cancer, for example pancreas or prostate carcinoma, are disclosed in WO2009/033094-A2 and US2009/0175796-A1. By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

*anti-HLA-DOB antibodies*

An example of an antibody binding to the cancer target molecule HLA-DOB is the antibody Lym-1 (CAS RN: 301344-99-0) which can be used for the treatment of cancer, for example non-Hodgkin lymphoma. Examples of anti-HLA-DOB conjugates are disclosed, for example, in WO 2005/081711-A2. By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

*anti-VTCN1 antibodies*

Examples of antibodies which bind the cancer target molecule VTCN1 and can be used for preparing a conjugate and for the treatment of cancer, for example ovarian carcinoma, pancreas, lung or breast cancer, are disclosed in WO 2006/074418-A2. By reference, these antibodies and antigen-binding fragments thereof are incorporated herein, and they can be used in the context of the present invention.

*anti-FGFR2 antibodies*

According to the invention, use may be made of anti-FGFR2 antibodies.

Examples of anti-FGFR2 antibodies and antigen-binding fragments are described in WO2013076186. By reference, all antibodies of WO2013076186 are hereby incorporated into the description of the present invention, and they can be used in the present invention. The sequences of the antibodies are shown in Table 9 and Table 10 of WO2013076186. Preference is given to antibodies, antigen-binding fragments and variants of the antibodies derived from the antibodies referred to as M048-D01 and M047-D08. Preferred anti-FGFR2 bind to the various splice variants known of FGFR2.

In one embodiment, the anti-FGFR2 antibodies or antigen-binding antibody fragments thereof are, after binding to a cell expressing FGFR2, internalized by the cell.

In a further embodiment, the anti-FGFR2 antibodies or antigen-binding antibody fragments comprise at least one, two or three CDR amino acid sequences of an antibody listed in Table 9 or Table 10 of WO2013076186. Preferred embodiments of such antibodies are likewise listed in WO2013076186 and incorporated herein by reference.
Anti-TWEAKR antibodies

In a preferred embodiment, when an anti-TWEAKR antibody or an antigen-binding fragment thereof is used in the processes according to the present invention, this antibody or fragment is selected from those described below. In addition, antibodies which bind to TWEAKR are known to the person skilled in the art, see, for example, WO2009/020933(A2) or WO2009140177 (A2).

The invention relates in particular to conjugates with antibodies or antigen-binding antibody fragments thereof or variants thereof which lead to strong activation of the TWEAKR (SEQ ID NO: 169 (protein); SEQ ID NO: 170 (DNA)), resulting in a strong induction of apoptosis in various cancer cells overexpressing TWEAKR.

The agonistic activity of TWEAKR with regard to the induction of apoptosis and inhibition of the proliferation of the anti-TWEAKR antibodies already described (e.g. PDL-192) is limited and does not reach the efficacy of the endogenous ligand TWEAK. This lack of agonistic activity is not based on reduced affinity, since these antibodies bind at the TWEAKR with affinities which, compared to the endogenous ligand TWEAK, are in a similar range (Michaelson JS et al, MAbs. 2011 Jul-Aug;3(4):362-75; Culp PA et al, Clin Cancer Res. 2010 Jan 15;16(2):497-508), and even antibodies having a higher binding affinity do not necessarily display a more effective signalling activity (Culp PA, et al, Clin Cancer Res. 2010 Jan 15;16(2):497-508). In addition, it has been shown that the antitumour activity of the antibodies already described depends on the Fc effector function, and it was shown that ADCC plays an important role for the in-vivo efficacy in mouse models.

Generation of the anti-TWEAKR antibodies

The anti-TWEAKR antibodies were generated, for example, by screening of a phage display library for recombinant human TWEAKR. Particularly the antibody TPP-2090 is an important example. Generation and sequence of this antibody are disclosed in WO2014/198817A1. The antibody TPP2090 was used for the working examples described below in the experimental section. In addition, antibodies which bind to TWEAKR are known to the person skilled in the art; see, for example, WO2009/020933(A2) or WO2009140177 (A2).

Antibodies of the invention can furthermore be obtained by methods known in the art such as antibody phage display screening (see, for example, Hoet RM et al., Nat Biotechnol 2005;23(3):344-8), the well-established hybridoma technology (see, for example, Kohler and Milstein Nature. 1975 Aug 7;256(5517):495-7) or immunization of mice, *inter alia* immunization of hMAb mice (e.g. VeloclImmune mouse®).
Particular embodiments of anti-TWEAKR antibodies

One embodiment of the invention is the provision of antibodies or antigen-binding antibody fragments thereof or variants thereof showing strong induction of caspase 3/7 in one or more TWEAKR-expressing cell lines. In a preferred embodiment, the one or more TWEAKR-expressing cell line(s) is/are present in the group consisting of WiDr, A253, NCI-H322, HT29 and 786-O. "Induction of caspase 3/7" can be measured by customary methods known in the art, including those described herein. In one embodiment, the "induction of caspase 3/7" is determined in accordance with the present invention using the activity determination with caspase 3/7 solution (Promega, #G8093) and reading the luminescence on a VICTOR V (Perkin Elmer). At the end of the incubation time, the caspase 3/7 activity was determined and the induction factor of caspase 3/7 was determined in comparison to untreated cells. An antibody is said to show "strong induction" of caspase 3/7 when the induction factor is greater than 1.2, preferably greater than 1.5, even more preferably greater than 1.8, even more preferably greater than 2.1, even more preferably greater than 2.5. What is provided are anti-TWEAKR antibodies leading to stronger induction of caspase 3/7 in HT29 cells compared to agonistic antibodies already described [e.g. PDL-192(TPP-1104), P4A8(TPP-1324), 136.1(TPP-2194)] and also compared to 300 ng/ml recombinant human TWEAK. This strong activity of inducing caspase 3/7 in cancer cells was also observed in WiDr, A253, NCI-H322 and 786-0 cells where in most experiments the antibodies of the invention examined induced higher factors of change compared to the reference antibodies [PDL-192(TPP-1104), P4A8(TPP-1324)] and to 300 ng/ml TWEAK. Some antibodies of the invention bind to the TWEAKR only with moderate affinity (>10 nM) which is clearly less than the affinity of the endogenous ligand TWEAK, and also less compared to other known agonistic antibodies. This property offers further possible advantages such as, for example, potentially deeper penetration into the tumour.

In this regard, one embodiment of the invention is the provision of antibodies or antigen-binding antibody fragments thereof binding specifically to a TWEAKR at a novel epitope characterized by selective binding to aspartate (D) at position 47 (D47) of TWEAKR (SEQ ID NO:169; see also Figure 1). The dependencies identified for certain TWEAKR amino acids for antibody interaction correlate with the agonistic activity determined for these antibodies. The native ligand TWEAK shows an effective activation of the TWEAKR and binds depending on leucine 46 in the cysteine-rich domain of TWEAKR (Pellegrini et al, FEBS 280:1818-1829). P4A8 displays a very low agonistic activity and interacts at least partially with domains outside of the cysteine-rich domain of TWEAKR. PDL-192 displays a moderate agonistic activity and binds depending on R56 to the cysteine-rich domain, but opposite the TWEAK ligand site. Antibodies of the present invention (e.g. TPP-2090) bind depending on D47, and TWEAK binds depending on L46. Thus, TWEAK binds to a similar but different binding site (Figure 7). Accordingly, the antibodies of the present invention displaying strong agonistic activity bind to a novel epitope (D47-dependent) for antibodies associated with very high agonistic activity.
The amino acid at position 47 (D47) of TWEAKR (SEQ ID NO: 169) is considered to be critical for binding of the antibodies according to the invention, which means that the antibody binds specifically to the D at position 47 (D47) of TWEAKR (SEQ ID NO: 169) when the antibody loses more than 20%, alternatively more than 30%, alternatively more than 40%, alternatively more than 50%, alternatively more than 60%, alternatively more than 70%, alternatively more than 80%, alternatively more than 90%, alternatively 100% of its ELISA signal by modification of this residue into alanine, as described in AK-Example 2 and Figure 6. Alternatively, an antibody binds specifically to the D at position 47 (D47) of TWEAKR (SEQ ID NO: 169) when the antibody loses more than 20%, alternatively more than 30%, alternatively more than 40%, alternatively more than 50%, alternatively more than 60%, alternatively more than 70%, alternatively more than 80%, alternatively more than 90%, alternatively 100% of its ELISA signal for TPP-2614 compared to TPP-2203. Preferably, an antibody binds specifically to the D at position 47 (D47) of TWEAKR (SEQ ID NO: 169) when the antibody loses more than 80% of its ELISA signal for TPP-2614 compared to TPP-2203.

In the present application, reference is made to the following preferred antibodies of the invention, as shown in the table and sequence listing below: "TPP-2090", "TPP-2149", "TPP-2093", "TPP-2148", "TPP-2084", "TPP-2077", "TPP-1538", "TPP-883", "TPP-1854", "TPP-1853", "TPP-1857", "TPP-1858".
### Table: Protein sequences of the antibodies:

<table>
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<tr>
<th></th>
<th>IgG1 light chain</th>
<th>IgG1 heavy chain</th>
<th>L-CDR1</th>
<th>L-CDR2</th>
<th>L-CDR3</th>
<th>H-CDR1</th>
<th>H-CDR2</th>
<th>H-CDR3</th>
<th>VL protein</th>
<th>VH protein</th>
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<tbody>
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<td><strong>Anti-TWEAKR antibodies according to the invention:</strong></td>
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<td><strong>Comparative antibodies:</strong></td>
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TPP-2090 is an antibody which comprises a region of the heavy chain corresponding to SEQ ID NO: 2 and a region of the light chain corresponding to SEQ ID NO: 1.

TPP-2149 is an antibody which comprises a region of the heavy chain corresponding to SEQ ID NO: 12 and a region of the light chain corresponding to SEQ ID NO: 11.

TPP-2093 is an antibody which comprises a region of the heavy chain corresponding to SEQ ID NO: 22 and a region of the light chain corresponding to SEQ ID NO: 21.

TPP-2148 is an antibody which comprises a region of the heavy chain corresponding to SEQ ID NO: 32 and a region of the light chain corresponding to SEQ ID NO: 31.

TPP-2084 is an antibody which comprises a region of the heavy chain corresponding to SEQ ID NO: 42 and a region of the light chain corresponding to SEQ ID NO: 41.

TPP-2077 is an antibody which comprises a region of the heavy chain corresponding to SEQ ID NO: 52 and a region of the light chain corresponding to SEQ ID NO: 51.

TPP-1538 is an antibody which comprises a region of the heavy chain corresponding to SEQ ID NO: 62 and a region of the light chain corresponding to SEQ ID NO: 61.

TPP-883 is an antibody which comprises a region of the heavy chain corresponding to SEQ ID NO: 72 and a region of the light chain corresponding to SEQ ID NO: 71.

TPP-1854 is an antibody which comprises a region of the heavy chain corresponding to SEQ ID NO: 82 and a region of the light chain corresponding to SEQ ID NO: 81.

TPP-1853 is an antibody which comprises a region of the heavy chain corresponding to SEQ ID NO: 92 and a region of the light chain corresponding to SEQ ID NO: 91.

TPP-1857 is an antibody which comprises a region of the heavy chain corresponding to SEQ ID NO: 102 and a region of the light chain corresponding to SEQ ID NO: 101.

TPP-1858 is an antibody which comprises a region of the heavy chain corresponding to SEQ ID NO: 112 and a region of the light chain corresponding to SEQ ID NO: 111.

TPP-2090 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 10 and a variable region of the light chain corresponding to SEQ ID NO: 9.

TPP-2149 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 20 and a variable region of the light chain corresponding to SEQ ID NO: 19.

TPP-2093 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 30 and a variable region of the light chain corresponding to SEQ ID NO: 29.

TPP-2148 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 40 and a variable region of the light chain corresponding to SEQ ID NO: 39.

TPP-2084 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 50 and a variable region of the light chain corresponding to SEQ ID NO: 49.
TPP-2077 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 60 and a variable region of the light chain corresponding to SEQ ID NO: 59.

TPP-1538 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 70 and a variable region of the light chain corresponding to SEQ ID NO: 69.

TPP-883 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 80 and a variable region of the light chain corresponding to SEQ ID NO: 79.

TPP-1854 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 90 and a variable region of the light chain corresponding to SEQ ID NO: 89.

TPP-1853 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 100 and a variable region of the light chain corresponding to SEQ ID NO: 99.

TPP-1857 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 110 and a variable region of the light chain corresponding to SEQ ID NO: 109.

TPP-1858 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 120 and a variable region of the light chain corresponding to SEQ ID NO: 119.

TPP-4030 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 241 and a variable region of the light chain corresponding to SEQ ID NO: 242.

TPP-5653 is an antibody which comprises a variable region of the heavy chain corresponding to SEQ ID NO: 243 and a variable region of the light chain corresponding to SEQ ID NO: 244.

TPP-2305

SEQ ID NO: 245 (polypeptide):

EQAPGTAPCSRGSWSADLDKCMDCASCRAPPHSDFCLGCAAAPPAPFRLLWP

Table: DNA sequences of antibodies according to the invention

<table>
<thead>
<tr>
<th>Antibody</th>
<th>SEQ ID NO:</th>
<th>177</th>
<th>178</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPP-2090</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPP-2149</td>
<td></td>
<td>179</td>
<td>180</td>
</tr>
<tr>
<td>TPP-2093</td>
<td></td>
<td>181</td>
<td>182</td>
</tr>
<tr>
<td>TPP-2148</td>
<td></td>
<td>183</td>
<td>184</td>
</tr>
</tbody>
</table>
Preferred embodiments of the anti-TWEAKR antibody are those below:

1. An anti-TWEAKR antibody or an antigen-binding fragment thereof which binds specifically to the D at position 47 (D47) of the TWEAKR (SEQ ID NO:169).

2. The antibody or an antigen-binding fragment thereof according to embodiment 1 where the antibody is an agonistic antibody.

3. The antibody or an antigen-binding fragment thereof according to embodiment 1 or 2 which comprises:

   a variable heavy chain comprising:
(a) a CDR1 of the heavy chain encoded by an amino acid sequence comprising the formula PYPMX (SEQ ID NO: 171), where X is I or M;

(b) a CDR2 of the heavy chain encoded by an amino acid sequence comprising the formula YISPSGGXTHYADSVKG (SEQ ID NO: 172), where X is S or K; and

(c) a CDR3 of the heavy chain encoded by an amino acid sequence comprising the formula GGDTYFDYFDY (SEQ ID NO: 173);

and a variable light chain comprising:

(a) a CDR1 of the light chain encoded by an amino acid sequence comprising the formula RASQSISXYLN (SEQ ID NO: 174), where X is G or S;

(b) a CDR2 of the light chain encoded by an amino acid sequence comprising the formula XASSLQS (SEQ ID NO: 175), where X is Q, A or N; and

(c) a CDR3 of the light chain encoded by an amino acid sequence comprising the formula QQSYYXXPXIT (SEQ ID NO: 176), where X at position 5 is T or S, X at position 6 is T or S and X at position 8 is G or F.

4. The antibody or an antigen-binding fragment thereof according to any of the preceding embodiments, comprising:

a. a variable heavy chain comprising the variable CDR1 sequence of the heavy chain, as shown in SEQ ID NO: 6, the variable CDR2 sequence of the heavy chain, as shown in SEQ ID NO: 7 and the variable CDR3 sequence of the heavy chain, as shown in SEQ ID NO: 8, and also

a variable light chain comprising the variable CDR1 sequence of the light chain shown in SEQ ID NO: 3, the variable CDR2 sequence of the light chain shown in SEQ ID NO: 4 and the variable CDR3 sequence of the light chain shown in SEQ ID NO: 5 or

b. a variable heavy chain comprising the variable CDR1 sequence of the heavy chain, as shown in SEQ ID NO: 16, the variable CDR2 sequence of the heavy chain, as shown in SEQ ID NO: 17, the variable CDR3 sequence of the heavy chain, as shown in SEQ ID NO: 18, and also

a variable light chain comprising the variable CDR1 sequence of the light chain shown in SEQ ID NO: 13, the variable CDR2 sequence of the light chain shown in SEQ ID NO: 14 and the variable CDR3 sequence of the light chain shown in SEQ ID NO: 15 or

c. a variable heavy chain comprising the variable CDR1 sequence of the heavy chain, as shown in SEQ ID NO: 26, the variable CDR2 sequence of the heavy chain, as shown in SEQ ID NO: 27, the variable CDR3 sequence of the heavy chain, as shown in SEQ ID NO:28, and also

a variable light chain comprising the variable CDR1 sequence of the light chain shown in SEQ ID NO: 23, the variable CDR2 sequence of the light chain shown in SEQ ID NO: 24 and the variable CDR3 sequence of the light chain shown in SEQ ID NO:25 or
d. a variable heavy chain comprising the variable CDR1 sequence of the heavy chain, as shown in SEQ ID NO: 36, the variable CDR2 sequence of the heavy chain, as shown in SEQ ID NO: 37, the variable CDR3 sequence of the heavy chain, as shown in SEQ ID NO: 38, and also

a variable light chain comprising the variable CDR1 sequence of the light chain shown in SEQ ID NO: 33, the variable CDR2 sequence of the light chain shown in SEQ ID NO: 34 and the variable CDR3 sequence of the light chain shown in SEQ ID NO: 35 or

e. a variable heavy chain comprising the variable CDR1 sequence of the heavy chain, as shown in SEQ ID NO: 46, the variable CDR2 sequence of the heavy chain, as shown in SEQ ID NO: 47, the variable CDR3 sequence of the heavy chain, as shown in SEQ ID NO: 48, and also

a variable light chain comprising the variable CDR1 sequence of the light chain shown in SEQ ID NO: 43, the variable CDR2 sequence of the light chain shown in SEQ ID NO: 44 and the variable CDR3 sequence of the light chain shown in SEQ ID NO: 45 or

f. a variable heavy chain comprising the variable CDR1 sequence of the heavy chain, as shown in SEQ ID NO: 56, the variable CDR2 sequence of the heavy chain, as shown in SEQ ID NO: 57, the variable CDR3 sequence of the heavy chain, as shown in SEQ ID NO: 58, and also

a variable light chain comprising the variable CDR1 sequence of the light chain shown in SEQ ID NO: 53, the variable CDR2 sequence of the light chain shown in SEQ ID NO: 54 and the variable CDR3 sequence of the light chain shown in SEQ ID NO: 55 or

g. a variable heavy chain comprising the variable CDR1 sequence of the heavy chain, as shown in SEQ ID NO: 66, the variable CDR2 sequence of the heavy chain, as shown in SEQ ID NO: 67, the variable CDR3 sequence of the heavy chain, as shown in SEQ ID NO: 68, and also

a variable light chain comprising the variable CDR1 sequence of the light chain shown in SEQ ID NO: 63, the variable CDR2 sequence of the light chain shown in SEQ ID NO: 64 and the variable CDR3 sequence of the light chain shown in SEQ ID NO: 65 or

h. a variable heavy chain comprising the variable CDR1 sequence of the heavy chain, as shown in SEQ ID NO: 76, the variable CDR2 sequence of the heavy chain, as shown in SEQ ID NO: 77, the variable CDR3 sequence of the heavy chain, as shown in SEQ ID NO: 78, and also

a variable light chain comprising the variable CDR1 sequence of the light chain shown in SEQ ID NO: 73, the variable CDR2 sequence of the light chain shown in SEQ ID NO: 74 and the variable CDR3 sequence of the light chain shown in SEQ ID NO: 75 or

i. a variable heavy chain comprising the variable CDR1 sequence of the heavy chain, as shown in SEQ ID NO: 86, the variable CDR2 sequence of the heavy chain, as shown in SEQ ID NO: 87, the variable CDR3 sequence of the heavy chain, as shown in SEQ ID NO: 88, and also

a variable light chain comprising the variable CDR1 sequence of the light chain shown in SEQ ID NO: 83, the variable CDR2 sequence of the light chain shown in SEQ ID NO: 84 and the variable CDR3 sequence of the light chain shown in SEQ ID NO: 85 or
j. a variable heavy chain comprising the variable CDR1 sequence of the heavy chain, as shown in SEQ ID NO: 96, the variable CDR2 sequence of the heavy chain, as shown in SEQ ID NO: 97, the variable CDR3 sequence of the heavy chain, as shown in SEQ ID NO: 98, and also
a variable light chain comprising the variable CDR1 sequence of the light chain shown in SEQ ID NO: 93, the variable CDR2 sequence of the light chain shown in SEQ ID NO: 94 and the variable CDR3 sequence of the light chain shown in SEQ ID NO: 95 or

k. a variable heavy chain comprising the variable CDR1 sequence of the heavy chain, as shown in SEQ ID NO: 106, the variable CDR2 sequence of the heavy chain, as shown in SEQ ID NO: 107, the variable CDR3 sequence of the heavy chain, as shown in SEQ ID NO: 108, and also
a variable light chain comprising the variable CDR1 sequence of the light chain shown in SEQ ID NO: 103, the variable CDR2 sequence of the light chain shown in SEQ ID NO: 104 and the variable CDR3 sequence of the light chain shown in SEQ ID NO: 105 or

l. a variable heavy chain comprising the variable CDR1 sequence of the heavy chain, as shown in SEQ ID NO: 116, the variable CDR2 sequence of the heavy chain, as shown in SEQ ID NO: 117, the variable CDR3 sequence of the heavy chain, as shown in SEQ ID NO: 118, and also
a variable light chain comprising the variable CDR1 sequence of the light chain shown in SEQ ID NO: 113, the variable CDR2 sequence of the light chain shown in SEQ ID NO: 114 and the variable CDR3 sequence of the light chain shown in SEQ ID NO: 115.

5. The antibody or the antigen-binding fragment thereof according to any of the preceding embodiments, comprising:

a. a variable sequence of the heavy chain, as shown in SEQ ID NO: 10, and also a variable sequence of the light chain, as shown in SEQ ID NO: 9, or

b. a variable sequence of the heavy chain, as shown in SEQ ID NO: 20, and also a variable sequence of the light chain, as shown in SEQ ID NO: 19, or

c. a variable sequence of the heavy chain, as shown in SEQ ID NO: 30, and also a variable sequence of the light chain, as shown in SEQ ID NO: 29, or

d. a variable sequence of the heavy chain, as shown in SEQ ID NO: 40, and also a variable sequence of the light chain, as shown in SEQ ID NO: 39, or

e. a variable sequence of the heavy chain, as shown in SEQ ID NO: 50, and also a variable sequence of the light chain, as shown in SEQ ID NO: 49, or

f. a variable sequence of the heavy chain, as shown in SEQ ID NO: 60, and also a variable sequence of the light chain, as shown in SEQ ID NO: 59, or

g. a variable sequence of the heavy chain, as shown in SEQ ID NO: 70, and also a variable sequence of the light chain, as shown in SEQ ID NO: 69, or
h. a variable sequence of the heavy chain, as shown in SEQ ID NO:80, and also a variable sequence of the light chain, as shown in SEQ ID NO:79, or

i. a variable sequence of the heavy chain, as shown in SEQ ID NO:90, and also a variable sequence of the light chain, as shown in SEQ ID NO:89, or

j. a variable sequence of the heavy chain, as shown in SEQ ID NO:100, and also a variable sequence of the light chain, as shown in SEQ ID NO:99, or

k. a variable sequence of the heavy chain, as shown in SEQ ID NO:110, and also a variable sequence of the light chain, as shown in SEQ ID NO:109, or

l. a variable sequence of the heavy chain, as shown in SEQ ID NO:120, and also a variable sequence of the light chain, as shown in SEQ ID NO:119.

6. The antibody according to any of the preceding embodiments which is an IgG antibody.

7. The antibody according to any of the preceding embodiments, comprising:

a. a sequence of the heavy chain, as shown in SEQ ID NO:2, and also a sequence of the light chain, as shown in SEQ ID NO:1, or

b. a sequence of the heavy chain, as shown in SEQ ID NO:12, and also a sequence of the light chain, as shown in SEQ ID NO:11, or

c. a sequence of the heavy chain, as shown in SEQ ID NO:22, and also a sequence of the light chain, as shown in SEQ ID NO:21, or

d. a sequence of the heavy chain, as shown in SEQ ID NO:32, and also a sequence of the light chain, as shown in SEQ ID NO:31, or

e. a sequence of the heavy chain, as shown in SEQ ID NO:42, and also a sequence of the light chain, as shown in SEQ ID NO:41, or

f. a sequence of the heavy chain, as shown in SEQ ID NO:52, and also a sequence of the light chain, as shown in SEQ ID NO:51, or

g. a sequence of the heavy chain, as shown in SEQ ID NO:62, and also a sequence of the light chain, as shown in SEQ ID NO:61, or

h. a sequence of the heavy chain, as shown in SEQ ID NO:72, and also a sequence of the light chain, as shown in SEQ ID NO:71, or

i. a sequence of the heavy chain, as shown in SEQ ID NO:82, and also a sequence of the light chain, as shown in SEQ ID NO:81, or

j. a sequence of the heavy chain, as shown in SEQ ID NO:92, and also a sequence of the light chain, as shown in SEQ ID NO:91, or

k. a sequence of the heavy chain, as shown in SEQ ID NO:102, and also a sequence of the light chain, as shown in SEQ ID NO:101, or

l. a sequence of the heavy chain, as shown in SEQ ID NO:112, and also a sequence of the light chain, as shown in SEQ ID NO:111.

8. The antigen-binding fragment according to any of the preceding embodiments or an antigen-binding fragment of an antibody according to any of the preceding embodiments which is an scFv, Fab, Fab’ fragment or a F(ab’)2 fragment.

9. The antibody or the antigen-binding fragment according to any of the preceding embodiments which is a monoclonal antibody or an antigen-binding fragment thereof.
10. The antibody or the antigen-binding fragment according to any of the preceding embodiments, which is a human, humanized or chimeric antibody or an antigen-binding fragment.

Particular preference is given to the anti-TWEAKR antibody TPP-2090.

5

Therapeutic use

The ADCs according to the invention show a valuable pharmacological and pharmacokinetic spectrum of action which could not have been predicted.

10

They are therefore suitable for use as medicaments for the treatment and/or prophylaxis of disorders in humans and animals.

Within the scope of the present invention, the term “treatment” includes prophylaxis.

15

The hyper-proliferative diseases, for the treatment of which the compounds according to the invention may be employed, include in particular the group of cancer and tumour diseases. In the context of the present invention, these are understood to mean especially the following diseases, but without any limitation thereto: mammary carcinomas and mammary tumours (mammary carcinomas including ductal and lobular forms, also in situ), tumours of the respiratory tract (small-cell and non-small cell carcinoma, bronchial carcinoma, bronchial adenoma, pleuropulmonary blastoma, and mesothelioma), cerebral tumours (e.g. of the brain stem and of the hypothalamus, astrocytoma, ependymoma, glioblastoma, glioma, medulloblastoma, meningioma and neuro-ectodermal and pineal tumours), tumours of the digestive organs (carcinomas of the oesophagus, stomach, gall bladder, small intestine, large intestine, rectum and anal carcinomas), liver tumours (inter alia hepatocellular carcinoma, cholangiocarcinoma and mixed hepatocellular cholangiocarcinoma), tumours of the head and neck region (larynx, hypopharynx, nasopharynx, oropharynx, lips and oral cavity carcinomas, oral melanomas), skin tumours (basaloidomas, spinaliomatas, squamous cell carcinomas, Kaposi’s sarcoma, malignant melanoma, non-melanomatous skin cancer, Merkel cell skin cancer, mast cell tumours), tumours of soft tissue (inter alia soft tissue sarcomas, osteosarcomas, malignant fibrous histiocytomas, chondrosarcomas, fibrosarcomas, hemangiosarcomas, leiomyosarcomas, liposarcomas, lymphosarcomas and rhabdomyosarcomas), tumours of the eyes (inter alia intraocular melanoma and retinoblastoma), tumours of the endocrine and exocrine glands (e.g. of the thyroid and parathyroid glands, pancreas and salivary gland carcinomas, adenocarcinomas), tumours of the urinary tract (tumours of the bladder, penis, kidney, renal pelvis and ureter) and tumours of the reproductive organs (carcinomas of the endometrium, cervix, ovary, vagina, vulva and uterus in women and carcinomas of the prostate and testes in men). These also include proliferative blood diseases of the blood, the lymph system and the spinal cord, in solid form and as circulating cells, such as leukaemias, lymphomas and myeloproliferative diseases, for example acute myeloid, acute lymphoblastic, chronic
lymphocytic, chronic myelogenous and hairy cell leukaemia, and AIDS-correlated lymphomas, Hodgkin's lymphomas, non-Hodgkin's lymphomas, cutaneous T cell lymphomas, Burkitt's lymphomas and lymphomas in the central nervous system.

A preferred subject matter of the present invention is the use of the ADCs according to the invention for the treatment and/or prophylaxis of lung carcinomas, especially non-small cell lung carcinomas; liver tumors or colon tumors, particularly lung carcinoma, especially non-small cell lung carcinomas. These well-characterized diseases in humans can also occur with a comparable aetiology in other mammals and can likewise be treated there with the compounds of the present invention.

The treatment of the cancer diseases mentioned above with the compounds according to the invention comprises both a treatment of the solid tumors and a treatment of metastasizing or circulating forms thereof.

In the context of this invention, the term "treatment" or "treat" is used in the conventional sense and means attending to, caring for and nursing a patient with the aim of combating, reducing, attenuating or alleviating a disease or health abnormality, and improving the living conditions impaired by this disease, as, for example, in the event of a cancer.

The present invention thus further provides for the use of the compounds according to the invention for the treatment and/or prevention of disorders, in particular the disorders mentioned above.

The present invention further provides for the use of the compounds according to the invention for producing a medicament for the treatment and/or prevention of disorders, in particular the disorders mentioned above.

The present invention further provides for the use of the compounds according to the invention in a method for treatment and/or prevention of disorders, in particular the disorders mentioned above.

The present invention further provides a method for treatment and/or prevention of disorders, in particular the disorders mentioned above, using an effective amount of at least one of the compounds according to the invention.

A further subject matter of the present invention is the use of the ADCs according to the invention as a medicament.

A further subject matter of the present invention is the use of the ADCs according to the invention for the treatment and/or prophylaxis of disorders, in particular of the disorders mentioned above.

A preferred subject matter of the present invention is the use of the ADCs according to the invention for
the treatment and/or prophylaxis of lung carcinomas, especially non-small cell lung carcinomas; liver
tumors or colon tumors, particularly lung carcinomas, especially non-small cell lung carcinomas. .

A further subject matter of the present invention are the ADCs according to the invention for the use as a
medicament.

A further subject matter of the present invention are the ADCs according to the invention for the
treatment and/or prophylaxis of the disorders mentioned above.

A preferred subject matter of the present invention are the ADCs according to the invention for the
treatment and/or prophylaxis of lung carcinomas, especially non-small cell lung carcinomas, liver tumors
or colon tumors, particularly lung carcinomas, especially non-small cell lung carcinomas..

A further subject matter of the present invention are the ADCs according to the invention for the use in a
method for the treatment and/or prophylaxis of the disorders mentioned above.

A preferred subject matter of the present invention are the ADCs according to the invention for the use in
a method of treatment and/or prophylaxis of lung carcinomas, especially non-small cell lung carcinomas,
liver tumors or colon tumors, particularly lung carcinomas, especially non-small cell lung carcinomas..

A further subject matter of the present invention is the use of the ADCs according to the invention in the
manufacture of a medicament for the treatment and/or prophylaxis of disorders, in particular the disorders
mentioned above.

A preferred subject matter of the present invention is the use of the ADCs according to the invention in the
manufacture of a medicament for the treatment and/or prophylaxis of lung carcinomas, especially
non-small cell lung carcinomas, liver tumors or colon tumors, particularly lung carcinomas, especially
non-small cell lung carcinomas..

A further subject matter of the present invention is a method for the treatment and/or prophylaxis of
disorders, in particular the disorders mentioned above, using an effective amount of the ADCs according
to the invention.

A preferred subject matter of the present invention is a method for the treatment and/or prophylaxis of
lung carcinomas, especially non-small cell lung carcinomas; liver tumors or colon tumors, particularly
lung carcinoma, especially non-small cell lung carcinomas..
Another aspect of the present invention relates to pharmaceutical combinations comprising a compound according to the invention in combination with at least one or more further active ingredients.

As used herein the term "pharmaceutical combination" refers to a combination of at least one conjugate according to the invention as active ingredient together with at least one other active ingredient with or without further ingredients, carrier, diluents and/or solvents.

Another aspect of the present invention relates to pharmaceutical compositions comprising a compound according to the invention in combination with an inert, nontoxic, pharmaceutically suitable adjuvant.

As used herein the term "pharmaceutical composition" refers to a galenic formulation of at least one pharmaceutically active agent together with at least one further ingredient, carrier, diluent and/or solvent.

Another aspect of the present invention relates to the use of the pharmaceutical combinations and/or the pharmaceutical compositions according to the invention for the treatment and/or prophylaxis of disorders, in particular of the disorders mentioned above.

Another aspect of the present invention relates to the use of the pharmaceutical combinations and/or the pharmaceutical compositions according to the invention for the treatment and/or prophylaxis of lung carcinomas, especially non-small cell lung carcinomas; liver tumors or colon tumors, particularly lung carcinoma, especially non-small cell lung carcinomas.

Another aspect of the present invention relates to pharmaceutical combinations and/or the pharmaceutical compositions according to the invention for the treatment and/or prophylaxis of disorders, in particular of the disorders mentioned above.

Furthermore, the ADCs according to the invention may be utilized, as such or in compositions, in research and diagnostics, or as analytical reference standards, and the like, which are well known in the art.

The compounds according to the invention can be used alone or, if required, in combination with one or more other pharmacologically active substances, provided that this combination does not lead to
undesirable and unacceptable side effects. The present invention furthermore therefore provides medicaments containing at least one of the compounds according to the invention and one or more further active compounds, in particular for treatment and/or prevention of the abovementioned disorders.

For example, the compounds of the present invention can be combined with known anti-hyper-proliferative, cytostatic or cytotoxic substances for the treatment of cancer diseases. Examples of suitable combination active compounds include:

R763, raloxifene, raltitrexed, ranimustine, razoxane, refametinib, regorafenib, risedronic acid, rituximab, romidepsin, romiplostim, roninciclib, ruoxolitinib, saragamostim, sipuleucel-T, sizofiran, sobuzoxane, sodium glycididazole, SNS-314, sorafenib, streptozocin, sunitinib, talaporfin, tamibarotene, tamoxifen, tasonermin, teceleukin, tegafur, tegafur + gimeracil + oteracil, temoporfin, temozolomide, temsirolimus, teniposide, testosterone, tetrofosmin, thalidomide, thiotepa, thymalfasin, TKM-PLK1, tioguanine, tocilizumab, topotecan, toremifene, tositumomab, tozasertib, trabectedin, trametinib, trastuzumab, trastuzumab emtansine, treosulfan, tretinoin, trilostane, trofosfamide, tryptophan, ubenimex, valrubicin, vandetanib, vapreotide, vemurafenib, vinblastine, vincristine, vindesine, vinflunine, vinorelbine, volasertib, vorinostat, vorozol, XL228, yttrium-90 glass microbeads, zinostatin, zinostatin-stimalamer, zoledronic acid, zorubicin.

In addition, the compounds of the present invention can be combined, for example, with binders which, by way of example, can bind to the following targets: OX-40, CD137/4-1BB, DR3, ID01/ID02, LAG-3, CD40.

In addition, the compounds according to the invention can also be used in combination with radiotherapy and/or surgical intervention.

Generally, the following aims can be pursued with the combination of compounds of the present invention with other cytostatically or cytotoxically active agents:

- improved efficacy in slowing the growth of a tumour, in reducing its size or even in the complete elimination thereof, compared with treatment with an individual active compound;
- improved efficacy in slowing the growth of a tumour, in reducing its size or even in the complete elimination thereof, compared with treatment with the antibody itself;
- the possibility of using the chemotherapeutics used in a lower dosage than in the case of monotherapy;
- the possibility of a more tolerable therapy with fewer side effects compared with individual administration;
- the possibility of treatment of a broader spectrum of tumour diseases;
- the achievement of a higher rate of response to the therapy;
- a longer survival time of the patient compared with present-day standard therapy.

In addition, the compounds according to the invention can also be used in combination with radiotherapy and/or surgical intervention.
The present invention further provides medicaments which comprise at least one compound according to the invention, typically together with one or more inert, nontoxic, pharmaceutically suitable excipients, and the use thereof for the aforementioned purposes.

The compounds according to the invention can act systemically and/or locally. For this purpose, they can be administered in a suitable manner, for example parenterally, possibly inhalatively or as implants or stents.

The compounds according to the invention can be administered in suitable administration forms for these administration routes.

Parenteral administration can bypass an absorption step (for example intravenously, intraarterially, intracardially, intraspinally or intralumbally) or include an absorption (for example intramuscularly, subcutaneously, intracutaneously, percutaneously or intraperitoneally). Administration forms suitable for parenteral administration include preparations for injection and infusion in the form of solutions, suspensions, emulsions or lyophilizates. Preference is given to parenteral administration, especially intravenous administration.

It is preferable that the conjugate of the present invention shows a significantly higher efficacy in inhibiting the growth of a tumour e.g. by reducing its size, compared with the treatment with the antibody itself when measured in those tumor cells, especially in lung carcinoma cells, preferably NCI-H292 cells by MTT assay.

In context of the present invention, the IC50 value with respect to CDK9 at high ATP concentrations can be determined by the methods described in the method section below. Preferably, it is determined according to Method C-3 ("CDK9/CycT1 high ATP kinase assay") as described in the Materials and Method section C-3 below.

The present invention furthermore provides medicaments comprising at least one compound according to the invention, usually together with one or more inert, nontoxic, pharmaceutically suitable adjuvants, and their use for the purposes mentioned above.

When the compounds of the present invention are administered as pharmaceuticals, to humans or animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1% to 99.5% (more preferably 0.5% to 90%) of active ingredient in combination with one or more inert, nontoxic, pharmaceutically suitable adjuvants.

Regardless of the route of administration selected, the ADCs according to the invention and/or the
pharmaceutical composition of the present invention are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels and time course of administration of the active ingredients in the pharmaceutical compositions of the invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient without being toxic to the patient.

Examples

The examples which follow illustrate the invention. The invention is not restricted to the examples.

Unless stated otherwise, the percentages in the tests and examples which follow are percentages by weight; parts are parts by weight. Solvent ratios, dilution ratios and concentration data for the liquid/liquid solutions are in each case based on volume.

Abbreviations:

Ab  antibody
abs. absolute
Ac  acetyl
ATCC  American Tissue Type Collection
ACN  acetonitrile
ADC  antibody drug conjugate
aq. aqueous (solution)
ATP  adenosine triphosphate
Boc  tert-butoxycarbonyl
br. broad (¾ NMR signal)
cat. catalytic
CI  chemical ionization (in MS)
CDK  cyclin-dependent kinase
cone. concentrated
Cyc  cyclin
d  day(s)
d  doublet (¾ NMR signal)
DAD  diode array detector
DAR  drug/antibody ratio
DCI  direct chemical ionization (MS)
DCM  dichloromethane
DCE  1,2-dichloroethane
d.e.  diastereomeric excess
DMAP  4-NN -dimethylaminopyridine
DME  1,2-dimethoxyethane
DMEM  Dulbecco’s Modified Eagle Medium (standardized nutrient medium for cell culture)
DMF  N N -dimethylformamide
DMSO  dimethyl sulfoxide
DPBS, D-PBS, PBS  Dulbecco’s phosphate-buffered salt solution
PBS = DPBS = D-PBS, pH 7.4, from Sigma, No D8537

composition:

0.2 g KCl
0.2 g KH2PO4 (anhyd)
8.0 g NaCl
1.15 g Na2HPO4 (anhyd)
made up ad 1 l with H2O

DRC  dose-response curve
DTT  DL-dithiothreitol
EC50  half maximal effective concentration
EDC, EDCI  N’-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride
EDTA  ethylenediaminetetraacetic acid
EGFR  epidermal growth factor receptor
EI  electron impact ionization (MS)
ELISA  enzyme-linked immunosorbent assay
eq.  equivalent(s)
ESI  electro-spray ionization (MS)
ESI-MicroTofQ  ESI-MicroTofq (name of the mass spectrometer with Tof = time of flight and Q = quadrupol)
Et  ethyl
FACS  fluorescence-activated cell sorting
FCS  fetal calf serum
FITC  fluorescein isothiocyanate
Fmoc  (9H-fluoren-9-ylmethoxy)carbonyl
GuHCl  guanidinium hydrochloride
GTP  guanosine-5’-triphosphate
h   hour(s)
HATU 0-(7-azabenzotriazol-1-yl)-NNN',N'-tetramethyluronium hexafluorophosphate
HEPES 4-(2-hydroxyethyl)piperazine-1-ethanesulphonic acid
HOAc acetic acid
HOAt 1-hydroxy-7-azabenzotriazole
HOBt 1-hydroxy-1H-benzotriazole hydrate
HOSu N-hydroxysuccinimide
HPLC high-pressure high-performance liquid chromatography
IC_{50} half-maximal inhibitory concentration
IgG immunoglobulin G
i.v. intravenously, administration into the vein
LC/MS, LC-MS liquid chromatography-coupled mass spectroscopy
m multiplet (¾ NMR signal)
mAb monoclonal antibody
MeCN acetonitrile
MeOH methanol
min minute(s)
MS mass spectroscopy
MTBE methyl tert-butyl ether
MTP microtiter plate
MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
m/z mass-to-charge ratio (MS)
NCI-H292 human mucoepidermoid lung carcinoma cells
NHS N-hydroxysuccinimide
Ni-NTA nickel-nitrilotriacetic acid
NMP N-methyl-2-pyrrolidinone
NMR nuclear magnetic resonance spectrometry
of th. of theory (chemical yield)
Pd/C palladium on activated carbon
PEG Polyethylene glycol
P-gp P-gycoprotein, a transporter protein
PNGaseF enzyme for cleaving sugar
P-TEFb positive transcription factor b
q quartet (¾ NMR signal)
quant. quantitative (yield)
rac racemic
Rf  | TLC retention factor
RP  | reverse phase (HPLC)
RPMI 1640 | Roswell Park Memorial Institute (RPMI) 1640 medium
rt, RT | room temperature
Rt | retention time (HPLC)
s | singlet (¹H NMR signal)
sat. | saturated (solution)
SEC | size-exclusion chromatography
SFC | supercritical fluid chromatography
t | triplet (¾ NMR signal)
iBu | tert-butyl
TBS | tert-butyldimethylsilyl
TBDPS | tert-butyldiphenylsilyl
TCEP | tris(2-carboxyethyl)phosphin
tert | tertiary
TFA | trifluoroacetic acid
THF | tetrahydrofuran
TIC | total ion chromatogram
TIPS | triisopropylsilyl
TLC | thin layer chromatography
TR-FRET | time-resolved fluorescence energy transfer
Tris | tris(hydroxymethyl)aminomethane
UV | ultraviolet
v/v | ratio by volume (of a solution)
Z | benzyloxy carbonyl
Syntheses of compounds

The syntheses of the macrocyclic compounds of formula (I) according to the present invention are preferably carried out according to the general synthetic sequences as shown in Schemes la, lb, lc, 2a, 3a, 3b, 3c, 4 and 5.

In addition to said routes described below, also other routes may be used to synthesise the target compounds, in accordance with common general knowledge of a person skilled in the art of organic synthesis. The order of transformations exemplified in the following Schemes is therefore not intended to be limiting, and suitable synthesis steps from various schemes can be combined to form additional synthesis sequences. In addition, interconversion of any of the substituents R₁, R₂, R₃, R₄ and/or R₅ can be achieved before and/or after the exemplified transformations. These modifications can be such as the introduction of protective groups, cleavage of protective groups, reduction or oxidation of functional groups, halogenation, metallation, metal catalysed coupling reactions, substitution or other reactions known to a person skilled in the art. These transformations include those which introduce a functionality allowing for further interconversion of substituents. Appropriate protective groups and their introduction and cleavage are well-known to a person skilled in the art (see for example T.W. Greene and P.G.M. Wuts in Protective Groups in Organic Synthesis, 4th edition, Wiley 2006). Specific examples are described in the subsequent paragraphs. Further, it is possible that two or more successive steps may be performed without work-up being performed between said steps, e.g. a "one-pot" reaction, as it is well-known to a person skilled in the art.

The geometry of the sulfoximine moiety renders some of the compounds of the general formula (I) chiral. Separation of racemic sulfoximines into their enantiomers can be achieved by methods known to the person skilled in the art, preferably by means of preparative HPLC on chiral stationary phase.

The syntheses of the pyridine derivatives of formulae (8), (9), (10), (11) and (12), all of them constituting subsets of the general formula (I) according to the present invention, are preferably carried out according to the general synthetic sequences as shown in Schemes la, 1b and lc.
Schemes 1a, 1b and 1c, wherein $R_1$, $R_2$, $R_3$, $R_4$ and $R_5$ are as defined for the compound of general formula (I) according to the present invention, outline the preparation of pyridine-based macrocyclic compounds of formulae (8), (9), (10), (11) and (12), from 2-chloro-5-fluoro-4-iodopyridine (1; CAS# 884494-49-9). Said starting material (1) is reacted with a boronic acid derivative of formula (2), in which $R_3$ and $R_4$ are as defined for the compound of general formula (I), to give a compound of formula (3). The boronic
acid derivative (2) may be a boronic acid (R = -H) or an ester of the boronic acid, e.g. its isopropyl ester (R = -CH(CH3)2), preferably an ester derived from pinacol in which the boronic acid intermediate forms a 2-aryl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (R-R = -C(=CH2)2-C(=CH2)2-).

Said coupling reaction is catalyzed by palladium catalysts, e.g. by Pd(0) catalysts such as tetrakis(triphenylphosphine)palladium(0) [Pd(PPh3)4], tris(dibenzylideneacetone)di-palladium(0) [Pd2(dba)3], or by Pd(II) catalysts such as dichlorobis(triphenylphosphine)-palladium(II) [Pd(PPh3)2Cl2], palladium(II) acetate and triphenylphosphine or by [1,1’-bis(diphenylphosphino)ferrocene]palladium dichloride.

The reaction is preferably carried out in a mixture of a solvent such as 1,2-dimethoxyethane, dioxane, DMF, DME, THF, or isopropanol with water and in the presence of a base such as potassium carbonate, sodium bicarbonate or potassium phosphate.


The reaction is performed at temperatures ranging from room temperature (i.e. approx. 20°C) to the boiling point of the respective solvent. Further on, the reaction can be performed at temperatures above the boiling point using pressure tubes and a microwave oven. The reaction is preferably completed after 1 to 36 hours of reaction time.

In the second step, a compound of formula (3) is converted to a compound of formula (4). This reaction can be carried out by a Palladium-catalyzed C-N cross-coupling reaction (for a review on C-N cross coupling reactions see for example: a) L. Jiang, S.L. Buchwald in ‘Metal-Catalyzed Cross-Coupling Reactions’, 2nd ed.: A. de Meijere, F. Diederich, Eds.: Wiley-VCH: Weinheim, Germany, 2004).

Preferred is the herein described use of lithium bis(trimethylsilyl)amide, tris(dibenzylideneacetone)di-palladium(0) and 2-(dicyclohexylphosphino)-2’,4’,6’-triisopropylbiphenyl in THF. The reactions are preferably run under an atmosphere of argon for 3-24 hours at 60°C in an oil bath.

In the third step, a compound of formula (4) is converted to a compound of formula (5), by means of cleaving the methyl ether present in compounds of formula (4).

Preferred is the herein described use of boron tribromide in DCM. The reactions are preferably run for 1-24 hours at 0°C to room temperature.

In the fourth step, a compound of formula (5) is coupled with a compound of formula (6), in which R1, R2 and L are as defined for the compound of general formula (1), to give a compound of formula (7). This reaction can be carried out by a Mitsunobu reaction (see for example: a) K.C.K. Swamy et al, Chem. Rev. 2009, 109, 2551).

Preferred is the herein described use of diisopropyl azodicarboxylate and triphenylphosphine in THF. The reactions are preferably run for 1-24 hours at 0°C to room temperature.
Compounds of the formula (6) can be prepared as outlined in Scheme 2, *infra.*

In the fifth step, a compound of formula (7) is converted to a macrocycle of formula (8). This cyclization reaction can be carried out by a Palladium-catalyzed C-N cross-coupling reaction (for a review on C-N cross coupling reactions see for example: a) L. Jiang, S.L. Buchwald in 'Metal-Catalyzed Cross-Coupling Reactions', 2nd ed.: A. de Meijere, F. Diederich, Eds.: Wiley-VCH: Weinheim, Germany, 2004).

Preferred is the herein described use of chloro(2-dicyclohexylphosphino-2',4',6'-tri-z, y-propyl-1 ,1'-biphenyl)[2-(2-aminoethyl)phenyl] palladium(II) methyl-feri-butylether adduct, 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl as catalyst and ligand, an alkali carbonate or an alkali phosphate, preferably potassium phosphate, as a base, in a mixture of a Ci-C3-alkylbenzene and a carboxamide based solvent, preferably a mixture of toluene and NMP, as a solvent. The reactions are preferably run under an atmosphere of argon for 2-24 hours at 100-130°C in a microwave oven or in an oil bath.


Preferred is the herein described use of periodic acid und iron(III)chloride.


*N*-unprotected sulfoximines of formula (10) (R₅ = H) may be further converted into *N*-functionalized derivatives of formula (11). There are multiple methods for the preparation of *N*-functionalized sulfoximines by functionalization of the nitrogen of the sulfoximine group:

- Reaction with isocyanates: see for example: a) V.J. Bauer et al, J. Org. Chem. 1966, 31, 3440; b) C. R.
Thioethers of formula (8) can also be oxidized to the corresponding sulfoines of formula (12). The oxidation can be prepared analogously to known processes (see for example: Sammond et al; Bioorg. Med. Chem. Lett. 2005, 15, 3519).

Compounds of the formula (6), in which R₁, R₂ and L are as defined for the compound of general formula (I) according to the present invention, can be prepared according to Scheme 2, starting e.g. from a 2,6-dichloroisonicotinic acid derivative of formula (13), in which R₂ is as defined for the compound of general formula (I), which is reduced to the corresponding pyridinemethanol of formula (14), by means of reduction. Preferred is the herein described use of sulfanediylidimethane - borane (1:1 complex) in tetrahydrofuran.

Derivatives of isonicotinic acid of formula (13), and esters thereof, are well known to the person skilled in the art, and are often commercially available.

In a second step, pyridinemethanol of formula (14) is reacted to give a compound of formula (15), in which LG represents a leaving group such as chloro, bromo, iodo, C₁-C₄-alkyl-S(=0)₂-, trifluoromethanesulfonyloxy-, benzenesulfonyloxy-, or para-toluenesulfonyloxy-. Such conversions are well known to the person skilled in the art; preferred is the herein described use of methanesulfonyl chloride in the presence of triethylamine as a base, in dichloromethane as a solvent, to give a compound of formula (15) in which LG represents methanesulfonyloxy-.
In a third step, a compound of formula (15) is reacted with a thiol of the formula \( R'\text{-SH} \), in which \( R' \) is as defined for the compound of general formula (I), to give a thioether derivative of formula (16). Thiols of the formula \( R'\text{-SH} \) are well known to the person skilled in the art and are commercially available in considerable variety.

In a fourth step, a thioether derivative of formula (16) is reacted with an anion formed \textit{in situ} from a diol of the formula \( \text{HO-L-OH} \), in which \( L \) is as defined for the compound of general formula (I), and an alkali metal, preferably sodium, in tetrahydrofuran as a solvent, to give intermediate compounds of formula (6) which can be further processed as outlined in Schemes 1b and 1c.

![Scheme 2](image-url)
The syntheses of the pyrimidine derivatives of formula (1a), constituting a further sub-set of the general formula (I) according to the present invention, are preferably carried out according to the general synthetic sequences as shown in Schemes 3a, 3b and 3c.

Scheme 3a
Scheme 3b
Schemes 3a, 3b and 3c, wherein \( R_1, R_2, R_3, R_4 \) and \( R_5 \) are as defined for the compound of general formula (I) according to the present invention, outline the preparation of pyrimidine compounds of the general formula (I) from 2,4-dichloro-5-fluoropyrimidine (CAS# 2927-71-1, 17). Said starting material (17) is reacted with a boronic acid derivative of formula (2) to give a compound of formula (18). The boronic acid derivative (2) may be a boronic acid (\( R = -H \)) or an ester of the boronic acid, e.g. its isopropyl ester (\( R = -CH\left(CH\left(CH_3\right)\right)_2 \)), preferably an ester derived from pinacol in which the boronic acid intermediate forms a 2-aryl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (\( R-\underline{R} = -C\left(CH_3\right)_2-C\left(CH_3\right)_2- \)). Boronic acids and their esters are commercially available and well-known to the person skilled in the art; see e.g. D.G. Hall, Boronic Acids, 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, ISBN 3-527-30991-8 and references cited therein.

The coupling reaction is catalyzed by Pd catalysts, e.g. by Pd(0) catalysts such as tetrakis(triphenylphosphine)palladium(0) [Pd(PPh$_3$)$_4$], tris(dibenzylideneacetone)di-palladium(0) [Pd$_2$(dba)$_3$], or by Pd(II) catalysts such as dichlorobis(triphenylphosphine)-palladium(II) [Pd(PPh$_3$)$_2$Cld], palladium(II) acetate and triphenylphosphine or by [1,1'-bis(diphenylphosphino)ferrocene]palladium dichloride [Pd(dppf)Cl$_2$]. The reaction is preferably carried out in a mixture of a solvent such as 1,2-dimethoxyethane, dioxane, DMF, DME, THF, or isopropanol with water and in the presence of a base such as aqueous potassium carbonate, aqueous sodium bicarbonate or potassium phosphate.

The reaction is performed at temperatures ranging from room temperature (\( \approx 20^\circ C \)) to the boiling point of the solvent. Further on, the reaction can be performed at temperatures above the boiling point using pressure tubes and a microwave oven, (review: D.G. Hall, Boronic Acids, 2005 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, ISBN 3-527-30991-8 and references cited therein).

The reaction is preferably completed after 1 to 36 hours of reaction time.
In the second step, a compound of formula (18) is converted to a compound of formula (19). Preferred is the herein described use of boron tribromide in DCM. The reactions are preferably run for 1-24 hours at 0°C to room temperature.

In the third step, a compound of formula (19) is coupled with a compound of formula (20) to give a compound of formula (21). This reaction can be carried out by a Mitsunobu reaction (see for example: a) K.C.K. Swamy et al, Chem. Rev. 2009, 109, 2551).

Compounds of the formula (20) can be prepared as outlined in Scheme 5, infra.


Rhodium-catalyzed imination of sulfoxides of formula (22) gives the corresponding N-trifluoroacetamide sulfoximines of formula (23) (see for example: Bolm et al, Org. Lett. 2004, 6, 1305).

Thioethers of formula (21) can also be oxidized to the corresponding sulfones of formula (24). The oxidation can be prepared analogously to known processes (see for example: Sammond et al; Bioorg. Med. Chem. Lett. 2005, 15, 3519).

Compounds of formula (25), wherein R\(^1\), R\(^2\), R\(^3\), R\(^4\), L and A are as defined for the compound of general formula (I) according to the present invention (rendering formulae (21), (22), (23) and (24) being subsets of formula (25)), can be reduced to give anilines of formula (26). The reduction can be prepared analogously to known processes (see for example: (a) Sammond et al; Bioorg. Med. Chem. Lett. 2005, 15, 3519; (b) R.C. Larock, Comprehensive Organic Transformations, VCH, New York, 1989, 411-415). Preferred is the herein described use of titanium(III)chloride in a mixture of aqueous hydrochloric acid and tetrahydrofuran.

Compounds of formula (26), wherein R\(^1\), R\(^2\), R\(^3\), R\(^4\), L and A are as defined for the compound of general formula (I) according to the present invention, can be converted to a macrocycle of formula (I). This cyclization reaction can be carried out by a Palladium-catalyzed C-N cross-coupling reaction (for a review on C-N cross coupling reactions see for example: a) L. Jiang, S.L. Buchwald in 'Metal-Catalyzed
Preferred is the herein described use of chloro(2-dicyclohexylphosphino-2',4',6'-tri-z,yo-propyl-1 \textsuperscript{1}biphenyl)[2-(2-aminoethyl)phenyl] palladium(II) methyl-fert-butylether adduct, 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl as catalyst and ligand, an alkali carbonate or an alkali phosphate, preferably potassium phosphate, as a base, in a mixture of a Ci-C3alkylbenzene and a carboxamide based solvent, preferably a mixture of toluene and NMP, as a solvent. The reactions are preferably run under an atmosphere of argon for 2-24 hours at 100-130°C in a microwave oven or in an oil bath.

Scheme 4

Scheme 4, wherein R\textsuperscript{1}, R\textsuperscript{2}, R\textsuperscript{3}, R\textsuperscript{4} and L are as defined for the compound of general formula (I) according to the present invention, outlines the preparation of N-substituted sulfoximine compounds of the general formula (I) from N-unsubstituted sulfoximine compounds.

N-unprotected sulfoximines of formula (lb) (R\textsuperscript{5} = H) may be reacted to to give N-functionalized derivatives of formula (lc). Formulae (lb) and (lc) both constitute subsets of the general formula (I).

There are multiple methods for the preparation of N-functionalized sulfoximines by functionalization of the nitrogen of the sulfoximine group:

- Reaction with isocyanates: see for example: a) V.J. Bauer et al, J. Org. Chem. 1966, 31, 3440; b) C. R.
Compounds of the formula (20), in which R¹, R² and L are as defined for the compound of general formula (I) according to the present invention, can be prepared according to Scheme 5, starting e.g. from a benzylic alcohol derivative of formula (27), in which R² is as defined for the compound of general formula (I), is reacted to give a compound of formula (28), in which LG represents a leaving group such as chloro, bromo, iodo, C₁-C₄-alkyl-S(=O)20-, trifluoromethanesulfonyloxy-, benzenesulfonyloxy-, or para-toluenesulfonyloxy-. Such conversions are well known to the person skilled in the art; preferred is the herein described use of thionyl chloride in N,N-dimethylformamide (DMF) as a solvent, to give a compound of formula (28) in which LG represents chloro.

Benzylic alcohol derivative of formula (27), or the corresponding carboxylic acids and their esters, are known to the person skilled in the art, and are commercially available in certain cases.

In a second step, a compound of formula (28) is reacted with a thiol of the formula R'-SH, in which R¹ is as defined for the compound of general formula (I), to give a thioether derivative of formula (29). Thioles of the formula R'SH are well known to the person skilled in the art and are commercially available in considerable variety.

In a third step, a thioether derivative of formula (29) is reacted with a carboxylic ester of formula (30), in which L' represents a Ci-Cs-alkylene group featuring one carbon atom less as compared to the corresponding group L in formula (31), R⁵ represents a C₁-C₄-alkyl group, and in which LG represents a leaving group such as chloro, bromo, iodo, C₁-C₄-alkyl-S(=O)20-, trifluoromethanesulfonyloxy-, benzenesulfonyloxy-, or para-toluenesulfonyloxy-, in the presence of a base, such as an alkali carbonate, preferably potassium carbonate, in N,N-dimethylformamide (DMF) as a solvent, to give a compound of formula (31).
In a fourth step, an ester of the formula (31) can be reduced using a reducing agent such as lithium aluminium hydride or di-zso-butylaluminiumhydride (DIBAL), in an ether, preferably tetrahydrofuran, as a solvent, to give compound of the formula (20) which can be further processed as shown in the Schemes 3a, 3b and 3c.

Alternatively, a thioether derivative of formula (29) can be directly converted into a compound of formula (20), if reacted with a compound of the formula HO-L-LG, in which L is as defined for the compound of general formula (I) according to the present invention, and in which LG represents a leaving group such as chloro, bromo, iodo, C

Compounds of formula (8) can also be synthesized according to Schemes 6 and 7 where phenol (5) is reacted with an alcohol (32) according to Scheme 1b to give ether (33). (33) can then be cyclised accordingly to give macrocyclic compound (34). PG is a protecting group which can be cleaved later on to yield the corresponding free hydroxyl compound. PG can e.g. be a cleavable group like methoxymethyl, ethoxymethyl, benzyl, p-methoxybenzyl, 2,4-dimethoxybenzyl, acetyl, allyl, tert-butylidimethylsilyl (TBS), tert-butyldipheylsilyl (TBDPS), triisopropylsilyl (TIPS) (see also e.g. P. J. Kocienski, Protecting Groups, 3rd Edition, Thieme). Alcohol (32) can be synthesized by protection the hydroxyl group of pyridine (14) followed by nucleophilic displacement of one of the chlorine atoms at the pyridine with a HO-L-OH diol under basic conditions (e.g. NaH, DMF).
After cleavage of protecting group PG the liberated free hydroxyl group in (35) can then be converted into a leaving group LG like halogen (e.g. Cl, Br) or sulphonlate (e.g. methylsulfonate, p-tosylsulphonate) facilitating the introduction of the sulphur moiety with a suitable thiol R'-SH via nucleophilic displacement (see Scheme 7). Alternatively, (35) can be converted into (8) via a Mitsunobu-type reaction using thioacetic acid as nucleophile followed by basic cleavage of the thioester and subsequent alkylation of the sulphhydryl group.
Scheme 7

General Structure sulfoximine-\(N\)-linked maleimides.
General Structure sulfonalkyl-linked maleimides.

*N*-Acyl-maleimides of structure (40) can be synthesized by coupling sulfoximine (10) with a maleimide-bearing carboxylic acid of structure (39) via common amide coupling methods, e.g. EDCI, HOBr and *N*,*N*-diisopropylamine in DMF or HATU and 4-methylmorpholine in DMF (see Scheme 8). Alternatively, carboxylic acid (39) can be converted in a previous step to an activated acid equivalent (e.g. acyl chloride, *N*-hydroxysuccinimidy ester) by methods known to those skilled in the art and then reacted with the sulfoximine.

Scheme 8

*N*-peptidic-maleimides of structure (45) can be synthesized as depicted in Scheme 9. Sulfoximine (10) is coupled with an *N*-protected amino acid of structure (41) (e.g., *R*<sup>L-1</sup> may be -CH<sub>3</sub>, -CH<sub>(CH<sub>3</sub>)</sub><sub>3</sub>, -<br>(CH<sub>2</sub>)<sub>3</sub>NHCONH<sub>2</sub>) via common amide coupling methods, e.g. EDCI, HOBr and *N*,*N*-diisopropylamine in DMF or HATU and 4-methylmorpholine in DMF. Upon deprotection of the amino acid nitrogen in (42) (e.g. TFA) the free amino group is then coupled with a maleimide bearing carboxylic acid of type (39). Alternatively, for the synthesis of polypeptidic linkers (*m* > 1) steps 1 and 2 in Scheme 9 are repeated. Alternatively to the last step in Scheme 9, carboxylic acid (39) can be converted in a previous step to an
activated acid equivalent (e.g. acyl chloride, \(N\)-hydroxysuccinimidyl ester) by methods known to those skilled in the art and then reacted with the amino acid amino group in structure (43) or (44).

**Scheme 9**

For the synthesis of \(N\)-sulfoximine ureas of structure (47) (see Scheme 10) sulfoximine (10) is reacted with a maleimide bearing compound of structure (46) wherein \(R^G\) is isocyanate, chloroformylamino or \(p\)-nitrophenylcarbamoyl.

**Scheme 10**

For the synthesis of maleimides of structure (51) (see Scheme 11) sulfoximine (10) is reacted with a compound of structure (48) wherein \(R^G\) is isocyanate, chloroformylamino or \(p\)-nitrophenylcarbamoyl and \(P^G_1\) or \(P^G_2\) are a nitrogen protecting group like Boc, Fmoc, benzyl, /\-methoxybenzyl etc. or together
form a protecting group like phthalimidoyl. In the next step the nitrogen protecting group PGVPG\(^2\) in (49) is removed under suitable conditions known to those skilled in the art and the liberated amine group in (50) is then acylated with a maleimide bearing carboxylic acid of type (39) as described before.

For the synthesis of maleimides of structure (55) (see Scheme 12) sulfoximine (10) is reacted with a compound of structure (52) wherein RG is isocyanate, chloroformylamino or p-nitrophenylcarbamoyl and RE is C1-C4 alkyl, allyl, or phenyl-Ci-C3-alkyl to give an urea-type sulfoximine of structure (53). After cleavage of the RE group by means of esterification or saponification known to those skilled in the art (e.g. potassium carbonate in methanol) the carboxylic acid moiety in (54) is then coupled with an maleimide bearing amine by common amidation procedures (e.g. EDCI, HOBt and N,N-diisopropylamine in DMF or HATU and 4-methylmorpholine in DMF) to give maleimide (55).
For the synthesis of maleimides of structure (60) (see Scheme 13) sulfoximine (10) is reacted with a chloroalkyl ester of chloroformic acid of structure (56) to give a sulfoximine-carbamate of type (57). The alkyl chloride in (57) is the substituted via nucleophilic displacement under basic conditions with an amine HNPG\(^2\) wherein PG\(^1\) or PG\(^2\) are a nitrogen protecting group like Boc, Fmoc, benzyl, \(p\)-methoxybenzyl etc. or together form a protecting group like phthalimidoyl. After deprotection of the amine group by suitable methods (see e.g. P. J. Kocienski, Protecting Groups, 3rd Edition, Thieme) the liberated amine is the coupled with maleimide bearing carboxylic acid of type (39) as described previously to give the maleimide (60).
Scheme 13
For the synthesis of sulfone derivatives (64), compound (36) is reacted with an N-protected amino alkyl hydrogen sulfide of type (61) or a corresponding alkylamine metal salt thereof under basic conditions to give benzylic sulfide (62) which is then oxidized in a further step to the corresponding sulfone (63) by methods known to those skilled in the art (e.g., w-chloroperbenzoic acid or sodium periodate) (see Scheme 14). Alternatively, sulfide (62) can also be oxidized in a two-step procedure via the corresponding sulfoxide. Removal of the nitrogen-protecting groups PG₁ and PG₂ by suitable methods yields free amine (64).

Scheme 14

Sulfone amine (64) can then be reacted with N-methoxycarbonylmaleimide to give maleimide (65) (see Scheme 15).
Coupling of sulfone amine (64) with maleimide carboxylic acid of type (39) as described earlier yields maleimide (66) (see Scheme 16).

Coupling of sulfone amine (64) with N-protected amino acids of type (41) by methods described earlier (see Scheme 9) followed by coupling with maleimide carboxylic acid of type (39) as described earlier yields peptidic maleimide (70) (see Scheme 17).
Coupling of sulfone amine (64) with a compound of structure (46) wherein $R^G$ is isocyanate, chloroformylamino or $p$-nitrophenylcarbamoyl gives urea-type maleimide of type (71) (see Scheme 18).

Scheme 18

Coupling of sulfone amine (64) with a maleimide-bearing PEG-linked activated carboxylic acid of type
(72) gives maleimide of structure (73) (see Scheme 19). Alternatively, the corresponding free carboxylic acid of (72) can be coupled to amine (64) by common amidation methods (e.g. EDCI, HOBt and N,N-diisopropylamine in DMF or HATU and 4-methylmorpholine in DMF) to give maleimide (73).

Scheme 19

Reaction of electrophilic compound (36) with thiol-bearing carboxylic acid (61A) under basic conditions gives sulfide (74) (see Scheme 20). Alternatively, a corresponding protected carboxylic acid can be employed instead of (61A). In a next step sulfide (74) is oxidized to the corresponding sulfone (75) by methods described previously.
a-\(N,\omega\)-\(N\)-bisprotected amino acid (76) like ornithine, lysine, or homolysine, is coupled to an PEGylated amine of type (77) by common amidation methods to give a compound of structure (78) (see Scheme 21). Cleavage of the co-protecting group gives amine of structure (79).
Coupling of carboxylic acid of structure (75) with amine of structure (79) by common amidation methods gives amide of structure (80) (see Scheme 22a). Removal of the α-protecting group gives amine (81) which can then be converted by coupling to a maleimide bearing activated carboxylic acid of type (39) as shown before to give maleimide of type (82).
$N$-Hydroxysuccinimidylesters of type (83) can be synthesized by coupling carboxylic acid of type (54) with $N$-hydroxysuccinimide under common esterification conditions like $N,N$-diisopropylcarbodiimide and $N$-methylmorpholine in DMF (see Scheme 22b).

Peptidic $N$-hydroxysuccinimidylesters of type (85) can be synthesized by reacting peptidic amine (44)
with a bifunctional \( N \)-hydroxysuccinimide ester of type (84) (see Scheme 23) under basic conditions, e.g. \( N,N \)-diisopropylamine in DMF.

![Scheme 23](image)

Compounds of type (46) in which \( R^{G} \) is \( p \)-nitrophenylcarbamoyl (46a) can be synthesized by reacting amine of type (86) with \( p \)-nitrophenylchloroformate in a suitable solvent, e.g. THF, diethylether, dioxane, dichloromethane, or chloroform, (see Scheme 24).

![Scheme 24](image)

Similarly amines of type (48) in which \( R^{G} \) is \( p \)-nitrophenylcarbamoyl (48a) can be synthesized by reacting amine (87) with \( p \)-nitrophenylchloroformate in a suitable solvent, e.g. THF, diethylether, dioxane, dichloromethane, or chloroform (see Scheme 25).

![Scheme 25](image)

The cysteine metabolites of type (89) and (90) can be synthesized by reacting maleimides of type (37)
or (38) with cysteine (88) in a suitable solvent, e.g. DMF, acetonitrile, THF, water, or mixtures thereof (see Scheme 26).

Scheme 26
HPLC methods:

Method 1 (preparative HPLC):
Instrument: Abimed/Gilson 305 / 306 binary pumps 0-100ml/min + 806 manometric module;
Knauer UV-detector K-2501; ISCO Foxy 200 fraction collector; SCPA PrepCon 5 software
Column: Chromatorex C18 10µm 125x30mm
Mobile phase A: water + 0.05% TFA, mobile phase B: acetonitrile + 0.05% TFA
Gradient: 10% B → 50% B; 50% B isocratic; 50% B → 80% B
Flow rate: 50 ml/ min

Column temperature: room temperature
UV-detection: 210 nm

Method 2 (preparative HPLC)
Instrument: Abimed/Gilson 305 / 306 binary pumps 0-100ml/min + 806 manometric module;
Knauer UV-detector K-2501; ISCO Foxy 200 fraction collector; SCPA PrepCon 5 software
Column: Kromasil-IOOA C18 5µ 125x20mm
Mobile phase A: water + 0.05% TFA, mobile phase B: acetonitrile + 0.05% TFA
Gradient: 15% B → 50% B; 50% B isocratic; 50% B → 80% B
Flow rate: 25 ml/ min

Column temperature: room temperature
UV-detection: 210 nm

Method 3 (preparative HPLC)
Instrument: Abimed/Gilson 305 / 306 binary pumps 0-100ml/min + 806 manometric module;
Knauer UV-detector K-2501; ISCO Foxy 200 fraction collector; SCPA PrepCon 5 software
Column: Kromasil-IOOA C18 5µ 125x20mm
Mobile phase A: water + 0.05% TFA, mobile phase B: acetonitrile + 0.05% TFA
Gradient: 15% B → 60% B; 60% B isocratic; 60% B → 80% B
Flow rate: 50 ml/ min

Column temperature: room temperature
UV-detection: 210 nm

Method 4 (preparative HPLC):
Instrument: Abimed/Gilson 305 / 306 binary pumps 0-100ml/min + 806 manometric module;
Knauer UV-detector K-2501; ISCO Foxy 200 fraction collector; SCPA PrepCon 5 software
Column: Chromatorex C18 10µm 125x30mm
Mobile phase A: water + 0.05% TFA, mobile phase B: acetonitrile + 0.05% TFA
Gradient: 15% B → 70% B; 70% B isocratic
Flow rate: 50 ml/min
Column temperature: room temperature
UV-detection: 210 nm

Method 5 (preparative HPLC)
Instrument: Abimed/Gilson 305 / 306 binary pumps 0-100ml/min + 806 manometric module; Knauer UV-detector K-2501; ISCO Foxy 200 fraction collector; SCPA PrepCon 5 software
Column: Kromasil-100A C18 5µ 125x20mm
Mobile phase A: water + 0.05% TFA, mobile phase B: acetonitrile + 0.05% TFA
Gradient: 15% B → 70% B; 70% B isocratic
Flow rate: 20 ml/min
Column temperature: room temperature
UV-detection: 210 nm

Method 6 (analytical HPLC)
Instrument: Waters Acquity UPLCMS SingleQuad
Column: Acquity UPLC BEH C18 1.7 50x2.1mm
Mobile phase A: water + 0.1%vol. HCOOH (99%), mobile phase B: acetonitrile
Gradient: 0-1.6 min 1-99% B; 1.6-2.0 min 99% B
Flow rate: 0.8 ml/min
Column temperature: 60°C
UV-detection: DAD scan 210-400 nm

Method 7 (analytical HPLC)
Instrument: Waters Acquity UPLCMS SingleQuad
Column: Phenomenex, Kinetex C18, 2.6 µm, 50x2.1mm
Mobile phase A: water + 0.1%vol. HCOOH (99%), mobile phase B: acetonitrile
Gradient: 0-1.9 min 1-99% B; 1.9-2.1 min 99% B
Flow rate: 1.3 ml/min
Column temperature: 60°C
UV-detection: DAD scan 210-400 nm

Method 8 (preparative HPLC)
Instrument: Labomatic HD-3000, pump head HDK-280, gradient module NDB-1000, fraction collector
Column: Chromatex C18 125mm x 30mm
Mobile phase A: water + 0.1%vol. TFA, mobile phase B: acetonitrile
Gradient: 30% B → 70% B → 100% B isocratic
Flow rate: 150 mL/min
Column temperature: r.t.
UV-detection: 254 nm

Method 9 (preparative HPLC)
5 Instrument: Labomatic HD-3000, pump head HDK-280, gradient module NDB-1000, fraction collector
Labomatic Labocol Vario 4000, Knauer UV detector Azura UVD 2.15, Prepcon 5 software
Column: Chromatex C18 125mm x 20mm
Mobile phase A: water + 0.1% vol. HCOOH, mobile phase B: acetonitrile
Gradient: 30% B -> 70% B -> 100% B isocratic
10 Flow rate: 30 mL/min
Column temperature: r.t.
UV-detection: 254 nm

Method 10 (preparative HPLC)
15 Instrument: Labomatic HD-3000, pump head HDK-280, gradient module NDB-1000, fraction collector
Labomatic Labocol Vario 4000, Knauer UV detector Azura UVD 2.15, Prepcon 5 software
Column: Chromatex C18 125mm x 20mm
Mobile phase A: water + 0.1% vol. HCOOH, mobile phase B: acetonitrile
Gradient: 40% B -> 80% B -> 100% B isocratic
20 Flow rate: 30 mL/min
Column temperature: r.t.
UV-detection: 254 nm

Method 11 (preparative HPLC)
25 Instrument: Labomatic HD-3000, pump head HDK-280, gradient module NDB-1000, fraction collector
Labomatic Labocol Vario 4000, Knauer UV detector Azura UVD 2.15, Prepcon 5 software
Column: Chromatex C18 125mm x 20mm
Mobile phase A: water + 0.1% vol. HCOOH, mobile phase B: acetonitrile
Gradient: 15% B -> 55% B -> 100% B isocratic
30 Flow rate: 30 mL/min
Column temperature: r.t.
UV-detection: 254 nm

Method 12 (LC/MS) (analytical HPLC)
35 Instrument MS: Thermo Scientific FT-MS; Instrument UHPLC+: Thermo Scientific UltiMate 3000;
Column: Waters, HSST3, 2.1 x 75 mm, C18 1.8 μm; Mobile phase A: 11 Water + 0.01% Formic acid;
Mobile phase B: 11 Acetonitrile + 0.01% Formic acid; Gradient: 0.0 min 10% B -> 2.5 min 95% B ->
Method 13 (analytical HPLC)
Instrument: Mobile phase s ACQUITY SQD UPLC System; Column: Mobile phase s Acquity UPLC HSS T3 1.8 µ 50 x 1 mm; Mobile phase A: 1 1 Water + 0.25 ml 99% Formic acid , Mobile phase B: 1 1 Acetonitrile + 0.25 ml 99%ige Formic acid; Gradient: 0.0 min 90% A → 1.2 min 5% A → 2.0 min 5% A Column temperature: 50°C; Flow rate: 0.90 ml/min; UV-Detection: 210 nm/ Optimum Integration Path 210-300 nm

Method 14 (analytical HPLC)
Instrument MS: Mobile phase s (Micromass) Quattro Micro; Instrument Mobile phase s UPLC Acquity; Column : Mobile phase s BEH C18 1.7 µ 50 x 2.1 mm; Mobile phase A: 1 1 Water + 0.01 mol Ammonium formate, Mobile phase B: 1 1 Acetonitrile; Gradient: 0.0 min 95% A → 0.1 min 95% A → 2.0 min 15% A → 2.5 min 15% A→ 2.51 min 10% A → 3.0 min 10% A; Column temperature: 40°C; Flow rate: 0.5 ml/min; UV-Detection: 210 nm

Method 15 (analytical HPLC)
Instrument: Agilent MS Quad 6150;HPLC: Agilent 1290; Column: Mobile phase s Acquity UPLC HSS T3 1.8 µ 50 x 2.1 mm; Mobile phase A: 1 1 Water + 0.25 ml 99% Formic acid , Mobile phase B: 1 1 Acetonitrile + 0.25 ml 99%ige Formic acid; Gradient: 0.0 min 90% A → 0.3 min 90% A → 1.7 min 5% A → 3.0 min 5% A Column temperature: 50°C; Flow rate: 1.20 ml/min; UV-Detection: 205 - 305 nm.

Method 16 (analytical HPLC)
Instrument: Mobile phase s ACQUITY SQD UPLC System; Column: Mobile phase s Acquity UPLC HSS T3 1.8 µ 50 x 1 mm; Mobile phase A: 1 1 Water + 0.25 ml 99%ige Formic acid , Mobile phase B: 1 1 Acetonitrile + 0.25 ml 99% Formic acid; Gradient: 0.0 min 95% A → 6.0 min 5% A → 7.5 min 5% A Column temperature: 50°C; Flow rate: 0.35 ml/min; UV-Detection: 210 - 400 nm.

Method 17 (LC/MS) (analytical HPLC)
Instrument MS: Waters SQD: Waters UPLC; Column: Zorbax SB-Aq (Agilent), 50 mm x 2.1 mm, 1.8 μµ; Mobile phase A: Water + 0.025%, Formic acid, Mobile phase B: Acetonitrile (ULC) + 0.025%, Formic acid; Gradient: 0.0 min 98%A - 0.9 min 25%A - 1.0 min 5%A - 1.4 min 5%A - 1.41 min 98%A - 1.5 min 98%A; Column temperature: 40°C; Flow rate: 0.600 ml/min; UV-Detection: DAD; 210 nm.
A-1 Preparative examples

Intermediate 1:

(2,6-Dichloropyridin-4-yl)methanol

To a stirred solution of 2,6-dichloroisonicotinic acid (10.0 g, 52.1 mmol) in THF (300 mL) at 0°C was added a solution of sulfanediyldimethane - borane (1:1) (16.0 g, 210.5 mmol) in THF. The mixture was allowed to react at room temperature overnight. Then MeOH (22 mL) was cautiously added to the stirred mixture while cooling with an ice bath. The reaction mixture was diluted with ethyl acetate (300 mL), washed with an aqueous sodium hydroxide solution (IN, 100 mL) and saturated aqueous sodium chloride solution. The organic layer was concentrated and the residue was purified by column chromatography on silica gel (hexane / ethyl acetate = 7:1 to 3:1) to give desired product (8.3 g; 46.6 mmol).

³¹ NMR (300MHz, CDC1₃, 300K) δ = 7.25 (2H); 4.72 (2H); 2.24 (1H).

Intermediate 2:

(2,6-dichloropyridin-4-yl)methyl methanesulfonate

(2,6-Dichloropyridin-4-yl)methanol (1.0 g; 5.62 mmol; see Intermediate 2) was dissolved in DCM (20 mL) and triethylamine (1.0 g; 9.88 mmol) was added. The resulting mixture was cooled to 0°C and methanesulfonyl chloride (0.9 g, 7.89 mmol) was added. The mixture was stirred at room temperature for 1 hour. By adding an aqueous hydrogen chloride solution (IN), the pH value of the mixture was adjusted to 3, before it was extracted three times with ethyl acetate. The combined organic layers were concentrated to give the crude product (1.4 g) that was used without further purification.
Intermediate 3:

**2,6-Dichloro-4-[(methylsulfanyl)methyl]pyridine**

(2,6-Dichloropyridin-4-yl)methyl methanesulfonate (1.40 g; 5.47 mmol, see Intermediate 2) was dissolved in THF (20 mL) and a mixture of sodium thiomethoxide and sodium hydroxide (wt 1/1, 0.70 g, 5 mmol, supplied by Shanghai DEMO Medical Tech Co., Ltd) was added. The resulting mixture was stirred overnight at room temperature. The reaction mixture was diluted with Water (10 mL) and extracted three times with ethyl acetate. The combined organic layers were concentrated and the residue was purified by column chromatography on silica gel (hexane / ethyl acetate = 6:1 to 3:1) to give the desired product (0.54 g; 2.60 mmol).

¾ NMR (300MHz, CDCl$_3$, 300K) $\delta$ = 7.18 (2H), 3.55 (2H), 1.98 (3H).

Intermediate 4:

**3-({6-Chloro-4-[(methylsulfanyl)methyl]pyridin-2-yl}oxy)propan-1-ol**

To a solution of 1,3-propanediol (660 mg; 8.68 mmol) in THF (10 mL) was added sodium (33 mg; 1.43 mmol) and the reaction mixture was refluxed for 3 hours. After cooling, 2,6-dichloro-4-[(methylsulfanyl)methyl]pyridine (300 mg, 1.44 mmol; see Intermediate 3) was added and the reaction mixture was refluxed for 16 hours. After cooling, the mixture was diluted with Water (10 mL) and extracted three times with ethyl acetate. The combined organic layers were concentrated and the residue was purified by flash column chromatography on silica gel (hexane / ethyl acetate = 5:1 to 2:1) to give the desired product (180 mg; 0.72 mmol).

¾ NMR (400MHz, CDCl$_3$, 300K) $\delta$ = 6.86 (1H), 6.56 (1H), 4.42 (2H), 3.71 (2H), 3.50 (2H), 3.27 (1H), 1.96 (5H).
Intermediate 5:
2-Chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyridine

A batch with 2-chloro-5-fluoro-4-iodopyridine (1000 mg; 3.88 mmol; APAC Pharmaceutical, LLC), (4-fluoro-2-methoxyphenyl)boronic acid (660 mg; 3.88 mmol; Aldrich Chemical Company Inc.) and tetrakis(triphenylphosphin)palladium(0) (449 mg; 0.38 mmol) in 1,2-dimethoxyethane (10.0 mL) and 2 M solution of potassium carbonate (5.8 mL) was degassed using argon. The batch was stirred under an atmosphere of argon for 4 hours at 100 °C. After cooling, the batch was diluted with ethyl acetate and THF and washed with a saturated aqueous solution of sodium chloride. The organic layer was filtered using a Whatman filter and concentrated. The residue was purified by column chromatography (hexane to hexane / ethyl acetate 50%) to give the desired product (947 mg; 3.70 mmol).

¾ NMR (400MHz, CDCl3, 300K) δ = 8.27 (m, 1H), 7.33 (m, 1H), 7.24 (m, 1H), 6.75 (m, 2H), 3.83 (s, 3H).

Intermediate 6:
5-Fluoro-4-(4-fluoro-2-methoxyphenyl)pyridin-2-amine

A solution of lithium bis(trimethylsilyl)amide in THF (1M; 20.5 mL; 20.53 mmol; Aldrich Chemical Company Inc.) was added to a mixture of 2-chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyridine (2.50 g; 9.78 mmol; see Intermediate 5), tris(dibenzylideneacetone)dipalladium (0) (0.18 g; 0.20 mmol; Aldrich Chemical Company Inc.) and 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl (0.19 g; 0.39 mmol; Aldrich Chemical Company Inc.) in THF (16.3 mL) under an atmosphere of argon at room temperature. The mixture was stirred at 60 °C for 6 hours. The mixture was cooled to -40 °C and Water (10 ml) was added. The mixture was slowly warmed to room temperature under stirring, solid sodium chloride was added and the mixture was extracted twice with ethyl acetate. The combined organic layers were filtered using a Whatman filter and concentrated. The residue was purified by column chromatography on silica gel (hexane to hexane / ethyl acetate 60%) to give the desired product (2.04 g; 8.64 mmol).

¾ NMR (400MHz, CDCl3, 300K) δ = 7.95 (1H), 7.20 (1H), 6.72 (2H), 6.46 (1H), 4.33 (2H), 3.61 (3H).
Intermediate 7:
2-(2-Amino-5-fluoropyridin-4-yl)-5-fluorophenol

A solution of boron tribromide in DCM (1M; 47.1 mL; 47.1 mmol; Aldrich Chemical Company Inc.) was added dropwise to a stirred solution of 5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyridin-2-amine (2.00 g, 8.47 mmol; see Intermediate 6) in DCM (205 mL) at 0°C. The mixture was slowly warmed to room temperature while stirring overnight. The mixture was cautiously diluted with an aqueous solution of sodium bicarbonate under stirring at 0°C and stirred at room temperature for 1 hour. A saturated solution of sodium chloride was added and the mixture was extracted with ethyl acetate. The combined organic layers were filtered using a Whatman filter and concentrated to give the crude product (1.92 g) that was used without further purification.

³¹NMR (400MHz, DMSO-de, 300K) δ = 10.21 (1H), 7.84 (1H), 7.19 (1H), 6.71 (2H), 6.39 (1H), 5.80 (2H).

Intermediate 8:
4-{2-[3-{(6-Chloro-4-[(methylsulfanyl)methyl]pyridin-2-yl}oxy]propoxy]-4-fluorophenyl}-5-fluoropyridin-2-amine

A solution of diisopropyl azodicarboxylate (1.70 mL; 8.64 mmol) in THF (6.8 mL) was added dropwise to a mixture of 3-{(6-chloro-4-[(methylsulfanyl)methyl]pyridin-2-yl}oxy)propan-1-ol (1.96 g; 7.89 mmol, see Intermediate 4), 2-(2-amino-5-fluoropyridin-4-yl)-5-fluorophenol (1.92 g; 8.64 mmol; see Intermediate 7) and triphenylphosphine (2.27 g; 8.64 mmol) in THF (34.0 mL) and the batch was stirred at room temperature for 5 hours. Additional triphenylphosphine (1.04 g; 3.94 mmol) and diisopropyl
azodicarboxylate (0.78 mL; 3.95 mmol) were added and the mixture was stirred at room temperature overnight. Additional diisopropyl azodicarboxylate (0.78 mL; 3.95 mmol) was added and the mixture was stirred at room temperature for 3 hours. Finally, additional triphenylphosphine (2.07 g; 7.89 mmol) and diisopropyl azodicarboxylate (1.55 mL; 7.89 mmol) were added and the mixture was stirred at room temperature for 3 hours before it was concentrated. The residue was by column chromatography on silica gel (hexane to hexane / ethyl acetate 75%) to give the desired product (2.37 g; 5.24 mmol).

¾ NMR (400MHz, CDCl3, 300K) δ = 7.98 (1H), 7.25 (1H), 6.92 (1H), 6.76 (2H), 6.59 (1H), 6.51 (1H), 4.41 (4H), 4.16 (2H), 3.56 (2H), 2.21 (2H), 2.04 (3H).

Intermediate 9:
15,19-Difluoro-8-[(methylsulfanyl)methyl]-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine

Methode A

A mixture of 4-{2-[3-((6-chloro-4-((methylsulfanyl)methyl)pyridin-2-yl)oxy)propoxy]-4-fluorophenyl}-5-fluoropyridin-2-amine (300 mg, 0.66 mmol; see Intermediate 8), chloro(2-dicyclohexylphosphino-2',4',6'-tri-z,yo-propyl-1,1'-biphenyl)[2-(2-aminoethyl)phenyl] palladium(II) methyl-fert-butylether adduct (55 mg; 0.07 mmol; ABCR GmbH & CO. KG) and 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl (32 mg; 0.07 mmol; Aldrich Chemical Company Inc.) and potassium phosphate (705 mg; 3.32 mmol) in toluene (50 ml) and NMP (6 ml) was stirred under an atmosphere of argon at 110°C in a closed vessel for 150 minutes. After cooling, the batch was diluted with DCM and ethyl acetate and washed with aqueous sodium chloride solution. The organic layer was filtered using a Whatman filter and concentrated. The residue was purified by column chromatography on silica gel (hexane to hexane / ethyl acetate 50%) to give the desired product (192 mg; 0.46 mmol).

¾ NMR (400MHz, CDCl3, 300K) δ = 8.81 (1H), 8.18 (1H), 7.63 (1H), 7.11 (1H), 6.79 (1H), 6.72 (1H), 6.23 (2H), 4.63 (2H), 4.07 (2H), 3.55 (2H), 2.29 (2H), 2.06 (3H).
Methode B

Under Argon, a mixture of 4-\{2-\{6-chloro-4-\{(methylsulfanyl)methyl\}pyridin-2-yl\}oxy\}propoxy\}-4-fluorophenyl\}-5-fluoropyridin-2-amine (2.8 g; 6.196 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-tri-\{1',1'-biphenyl\}[2-(2-aminoethyl)phenyl] palladium(II) 2nd generation precatalyst (389.9 mg; 0.496 mmol; Aldrich Chemical Company Inc.) and 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl (236 mg; 0.496 mmol; Aldrich Chemical Company Inc.) and potassium phosphate (6.58 g; 30.98 mmol) in toluene (350 ml) and NMP (50 mL) was stirred overnight at 100°C. After cooling, the reaction mixture was diluted with Water (250 ml). The resulting mixture was filtered through Celite. After phase separation, the aqueous phase was extracted twice with MTBE. The combined organic phases were dried over Na2S04, filtered, and concentrated in vacuo. The residue was dissolved in MeOH and after crystallization the white solid was filtered off and dried under high vacuum. 1.80 g (4.33 mmol) of the desired compound was obtained (70 % yield).

Intermediate 10:

(rac)-15,19-Difluoro-8-\{(methylsulfanyl)methyl\}-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine

Iron(III)chloride (2 mg; 0.01 mmol) was added to a mixture of 15,19-difluoro-8-\{(methylsulfanyl)methyl\}-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine (192 mg, 0.46 mmol; see Intermediate 9) in acetonitrile (11.3 mL) and the batch was stirred at room temperature for 10 minutes. The batch was cooled to 0°C and periodic acid (112 mg; 0.49 mmol) was added under stirring in one portion. After 10 minutes the ice bath was removed and the mixture was stirred at room temperature for 90 minutes before it was added to a stirred solution of sodium thiosulfate pentahydrate (642 mg; 2.59 mmol) in ice Water (14.0 mL). The batch was saturated with solid sodium chloride and extracted twice with THF and twice with ethyl acetate. The combined organic layers were filtered using a Whatman filter and concentrated. The residue was purified by column chromatography on silica gel (DCM to DCM / ethanol 50%) to give the desired product (173 mg) with a
purity of approximately 65% (HNMR analysis) which was used in the next step without further purification.

¾ NMR (400MHz, CDCl₃, 300K) δ = 8.76 (1H), 8.20 (1H), 7.62 (1H), 7.21 (1H), 6.79 (1H), 6.70 (1H), 6.18 (2H), 4.63 (2H), 4.07 (2H), 3.91 (1H), 3.81 (1H), 2.58 (3H), 2.28 (2H).

The reaction was repeated using 440 mgs of 15,19-difluoro-8-[(methylsulfanyl)methyl]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine. After work up, the residue was purified by column chromatography on silica gel (DCM to DCM / ethanol 50%) to give the desired product in two batches: 195 mg with a purity of 92%> and 88 mg with a purity of 97%>.

**Intermediate 11**

(rac)-15,19-Difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,HH-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine

Concentrated sulphuric acid (0.13 mL) was added dropwise to a stirred mixture of (rac)-15,19-difluoro-8-[(methylsulfanyl)methyl]-3,4-dihydro-2H, 11H-10,6-(azeno)- 12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine (102 mg; 0.24 mmol; see Intermediate 10) and sodium azide (31 mg; 0.47 mmol) in chloroform (0.40 mL) at 0°C. The ice bath was removed and the mixture was stirred for 24 hours at 45°C. After cooling additional sodium azide (61 mg; 0.95 mmol) was added and the mixture was stirred for additional 16 hours at 45°C. The mixture was again cooled to 0°C and additional chloroform (0.2 mL) and sodium azide (61 mg; 0.95 mmol) were added before additional concentrated sulphuric acid (0.10 mL) was added dropwise. The mixture was stirred at 45°C for additional 3 hours. While cooling with an ice bath, saturated aqueous sodium bicarbonate solution and saturated aqueous sodium chloride solution was added dropwise under stirring. The mixture was extracted twice with ethyl acetate and twice
with THF. The combined organic layers were filtered using a Whatman filter and concentrated. The residue was purified by preparative HPLC to give the desired product (26 mg; 0.06 mmol).

<table>
<thead>
<tr>
<th>System: Mobile phase</th>
<th>Autopurification system: Pump 254, Sample Manager 2767, CFO, DAD 2996, SQD 3100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column: XBridge C18 5μm 100x30 mm</td>
<td></td>
</tr>
<tr>
<td>Solvent: H₂O + 0.2% NH₃ (32%)</td>
<td>B = MeCN</td>
</tr>
<tr>
<td>Gradient: 0.5 min inlet (19% B, 25 to 50 mL/min); 0.5 – 5.5 min 38-50% B</td>
<td></td>
</tr>
<tr>
<td>Flow: 70 mL/min</td>
<td></td>
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<tr>
<td>Temperature: RT</td>
<td></td>
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<tr>
<td>Solution: 98 mg / 2.5 mL DMSO</td>
<td></td>
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<tr>
<td>Injection: 5 x 0.5 mL</td>
<td></td>
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<tr>
<td>Detection: DAD scan range 210–400 nm</td>
<td>MS ESI+, ESI-, scan range 160-1000 m/z</td>
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<tr>
<td>Retention time in min</td>
<td>Purity in %</td>
</tr>
<tr>
<td>4.3 – 4.7</td>
<td>99</td>
</tr>
</tbody>
</table>

¾ NMR (400MHz, DMSO-d6, 300K) δ = 9.70 (IH), 8.69 (IH), 8.31 (IH), 7.58 (IH), 7.07 (IH), 6.89 (IH), 6.59 (IH), 6.26 (IH), 4.51 (2H), 4.28 (2H), 4.12 (2H), 3.72 (IH), 2.88 (3H), 2.11 (2H).

**Intermediate 12**

tert-butyl [(25)-l-[[15,19-difluoro-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-(metheno)-1,5,ll,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido -¹⁸-sulfanylidene]amino]-l-oxopropan-2-yl carbamate

![Intermediate 12 structure](image-url)
To a cooled solution of (rac)-15,19-difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine (30.0 mg, 67.2 µmol; see Intermediate 11) in DMF (1.2 ml) was added N-(terti-butoxycarbonyl)-L-alanine (15.3 mg, 80.6 µmol), HATU (38.3 mg, 101 µmol) and 4-methylmorpholine (22 µl, 200 µmol) at 0°C. The reaction mixture was stirred for 2.5 h at 0°C and was then diluted with a mixture of Water/TFA/acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 4] and the desired compound (35 mg, 0.06 mmol) was obtained as a mixture of 2 diastereoisomers.

LC-MS [Method 12]: R_t = 2.26 min, 2.30 min; MS (ESIpos): m/z = 618 [M+H]^+

1H-NMR (500 MHz, DMSO-d6) δ [ppm]: -0.007 (2.56), 0.007 (1.72), 1.180 (3.02), 1.195 (3.02), 1.206 (2.88), 1.220 (2.67), 1.330 (0.99), 1.384 (15.84), 1.400 (16.00), 2.069 (1.17), 2.073 (9.08), 2.097 (1.64), 3.186 (4.82), 3.240 (9.84), 3.911 (1.21), 3.923 (1.40), 3.938 (1.03), 4.112 (2.66), 4.122 (3.20), 4.131 (2.46), 4.506 (1.53), 4.523 (1.57), 4.745 (0.71), 4.771 (1.60), 4.782 (2.68), 4.835 (0.87), 6.262 (2.43), 6.297 (1.72), 6.591 (1.67), 6.614 (2.61), 6.796 (0.64), 6.812 (0.59), 6.879 (1.08), 6.884 (1.26), 6.896 (2.11), 6.901 (2.56), 6.913 (1.37), 6.918 (1.69), 7.063 (1.83), 7.068 (1.85), 7.085 (1.85), 7.090 (1.76), 7.561 (0.91), 7.569 (1.03), 7.575 (1.13), 7.578 (1.30), 7.583 (1.26), 7.586 (1.07), 7.592 (0.96), 7.600 (0.83), 8.301 (1.45), 8.305 (1.47), 8.316 (2.05), 8.321 (1.90), 8.677 (2.61), 8.689 (2.52), 9.625 (1.39), 9.709 (1.37).

**Intermediate 13**

N-[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl][methyl](methyl)oxido-λ₅-sulfanylidene]-L-alaninamide trifluoroacetate

![Chemical Structure](image)
A solution of tert-butyl \((2S)-1-[[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclocotadecin-8-yl]methyl} (methyl)oxido^{ \text{6-sulfanylidene} }\) amino\)-1-oxopropan-2-yl]carbamate (33.0 mg, 53.4 µmol, see Intermediate 12) in DCM/TFA (solution 30% TFA in DCM) was stirred at room temperature for 30 min. The reaction mixture was concentrated \textit{in vacuo} and the desired TFA salt (34.0 mg) was used without further purification.

LC-MS [Method 12]: \( R_t = 1.35 \) min; MS (ESIpos): \text{m/z} = 518 [M+H-TFA] \(+

\text{1H-NMR (500 MHz, DMSO-\(d_6\)) \( \delta \) [ppm]: -0.007 (2.77), 0.006 (1.41), 1.234 (0.67), 1.338 (8.23), 1.352 (8.52), 1.362 (8.51), 1.376 (8.19), 2.086 (5.77), 2.095 (3.05), 3.375 (16.00), 3.835 (1.65), 3.849 (1.92), 3.860 (1.55), 4.117 (4.44), 4.127 (5.34), 4.136 (4.40), 4.530 (2.78), 4.845 (5.26), 4.873 (7.24), 6.287 (10.13), 6.609 (8.37), 6.886 (1.63), 6.892 (1.96), 6.903 (3.19), 6.908 (3.57), 6.920 (1.83), 6.925 (1.99), 7.072 (2.91), 7.077 (3.13), 7.094 (3.03), 7.099 (3.03), 7.566 (1.43), 7.574 (1.73), 7.580 (1.99), 7.584 (2.29), 7.588 (2.27), 7.592 (2.08), 7.597 (1.79), 7.605 (1.50), 8.055 (5.79), 8.334 (7.24), 8.339 (7.37), 8.684 (5.61), 8.696 (5.69), 9.766 (4.81), 9.776 (5.04).}

\textbf{Intermediate 14}

\text{N-(tert-butoxycarbonyl)-L-valyl-N-[[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclocotadecin-8-yl]methyl](methyl)oxido^{ \text{6-sulfanylidene} }]-L-alaninamide}

To a cooled solution of N-[[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclocotadecin-8-yl]methyl} (methyl)oxido^{ \text{6-sulfanylidene} }]-L-alaninamide
trifluoroacetate (33.0 mg, 52.2 µmol; see Intermediate 13) in DMF (1.5 ml) was added N-(tert-butoxycarbonyl)-L-valine (13.6 mg, 62.7 µmol), HATU (29.8 mg, 78.4 µmol) and 4-methylmorpholine (23 µl, 210 µmol) at 0°C. The reaction mixture was stirred for 2.5 h at 0°C and was then diluted with a mixture of Water/TFA/acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 4] and the desired compound (31 mg, 0.04 mmol) was obtained as a mixture of 2 diastereoisomers.

LC-MS [Method 13]: R_t = 1.31, 1.32 min; MS (ESIpos): m/z = 717 [M+H]^+

1H-NMR (500 MHz, DMSO-ifc) δ [ppm]: 0.723 (1.47), 0.736 (1.51), 0.830 (2.86), 0.843 (2.99), 0.903 (1.72), 0.916 (1.78), 1.263 (2.60), 1.271 (2.87), 1.278 (2.82), 1.285 (2.59), 1.420 (16.00), 2.093 (0.81), 3.122 (3.03), 3.143 (3.66), 4.121 (1.66), 4.148 (0.58), 4.162 (0.68), 4.175 (0.70), 4.189 (0.62), 4.527 (1.35), 4.752 (0.71), 4.780 (1.01), 4.812 (0.95), 4.925 (0.97), 4.953 (0.69), 6.287 (1.35), 6.305 (1.47), 6.594 (1.61), 6.606 (1.53), 6.904 (1.04), 7.070 (0.98), 7.088 (0.95), 7.093 (0.94), 7.601 (0.64), 8.310 (0.83), 8.324 (0.83), 8.642 (0.86), 8.654 (0.85), 8.751 (0.68), 8.763 (0.65), 9.778 (1.06), 9.894 (0.91).

Intermediate 15

L-valyl-N-[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido^6-sulfanylidene]-L-alaninamide trifluoroacetate

A solution of N-(tert-butoxycarbonyl)-L-valyl-N-[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido^6-sulfanylidene]-L-alaninamide (29.0 mg, 40.5 µmol, see Intermediate 14) in DCM/TFA (solution 30%
TFA in DCM) was stirred at room temperature for 30 min. The reaction mixture was concentrated *in vacuo* and the desired TFA salt (31.0 mg) was used without further purification.

**LC-MS [Method 12]:** R<sub>t</sub> = 1.41 min; MS (ESIpos): m/z = 617 [M+H-TFA]<sup>+</sup>

**1H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ ppm:**
-0.007 (1.06), 0.938 (6.19), 0.952 (16.00), 0.966 (14.35), 0.978 (6.18), 1.273 (6.16), 1.288 (11.78), 1.302 (6.21), 2.088 (3.41), 3.586 (1.68), 3.597 (2.36), 3.609 (1.53), 4.115 (4.10), 4.124 (4.65), 4.133 (3.89), 4.269 (1.69), 4.274 (1.72), 4.283 (2.35), 4.289 (2.28), 4.298 (1.63), 4.304 (1.50), 4.501 (2.26), 4.518 (2.72), 4.534 (2.12), 4.792 (4.23), 4.799 (5.24), 6.254 (3.97), 6.265 (4.03), 6.608 (6.25), 6.886 (1.31), 6.891 (1.46), 6.903 (2.49), 6.908 (2.66), 6.920 (1.37), 6.925 (1.41), 7.070 (2.30), 7.075 (2.35), 7.093 (2.35), 7.098 (2.23), 7.566 (1.20), 7.574 (1.36), 7.583 (1.74), 7.587 (1.69), 7.591 (1.49), 7.597 (1.29), 7.604 (1.09), 8.047 (4.62), 8.057 (4.63), 8.328 (3.68), 8.332 (4.89), 8.336 (3.44), 8.532 (3.18), 8.547 (3.10), 8.667 (2.37), 8.674 (2.86), 8.679 (2.74), 8.686 (2.45), 9.758 (4.00), 9.765 (3.84).

**Intermediate 16**

**tert-butyl [[(25)-5-(carbamoylamino)-l-[[[(15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-l,5,ll,13-benzodioxadiazacyclooctadecin-8-yl]methyl}(methyl)oxido -26.**

sulfanylidene]amino]-l-oxopentan-2-yl]carbamate

To a cooled solution of (rac)-15,19-difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,HH-10,6-(azeno)-12,16-(metheno)-1,5,ll,13-benzodioxadiazacyclooctadecine (35.0 mg, 78.4 μmol; see
Intermediate 11) in DMF (1.5 ml) was added N²-(tert-butoxycarbonyl)-N⁵-carbamoyl-L-ornithine (43.2 mg, 157 µmol), HATU (59.6 mg, 157 µmol) and 4-methylmorpholine (26 µl, 240 µmol) at 0° C. The reaction mixture was stirred for 2.5 h at 0° C and was then stirred overnight at room temperature. After dilution with a mixture of Water /TFA/acetonitrile (1ml), the resulting mixture was directly purified by preparative HPLC [Method 4, flow rate: 20 ml/min] and the desired compound (17 mg, 0.02 mmol) was obtained as a mixture of 2 diastereoisomers.

LC-MS [Method 13]: R₁ = 1.03 min, 1.05 min; MS (ESIpos): m/z = 704 [M+H]+

1H-NMR (500 MHz, DMSO-ifc) δ [ppm]: -0.007 (1.14), 1.319 (1.30), 1.382 (16.00), 1.397 (15.84), 2.073 (11.86), 2.104 (1.50), 2.900 (1.82), 2.914 (1.82), 3.187 (4.54), 3.246 (7.34), 3.701 (1.29), 3.846 (1.11), 4.113 (2.34), 4.122 (2.82), 4.132 (2.11), 4.515 (1.55), 4.736 (0.90), 4.762 (2.23), 4.772 (1.27), 4.832 (0.78), 6.267 (1.83), 6.305 (1.64), 6.601 (1.55), 6.618 (2.43), 6.744 (0.66), 6.760 (0.62), 6.841 (0.70), 6.857 (0.68), 6.878 (0.93), 6.883 (1.01), 6.895 (1.67), 6.900 (1.78), 6.912 (0.89), 6.917 (0.92), 7.062 (1.54), 7.067 (1.57), 7.085 (1.58), 7.090 (1.47), 7.563 (0.82), 7.571 (0.94), 7.576 (1.07), 7.580 (1.18), 7.584 (1.13), 7.588 (0.99), 7.594 (0.86), 7.602 (0.69), 8.302 (1.39), 8.306 (1.44), 8.314 (1.79), 8.319 (1.65), 8.676 (2.53), 8.688 (2.45), 9.643 (1.35), 9.719 (1.28).

Intermediate 17
N⁵-carbamoyl-N-{[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl}(methyl)oxido-λ₆-sulfanylidene]-L-ornithinamide trifluoroacetate
A solution of tert-butyl (2S)-5-(carbamoylamino)-L-[{{[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl}(methyl)oxido^6.-sulfanylidene]amino}-L-oxopentan-2-yl]carbamate (16.0 mg, 22.7 µmol; see Intermediate 16) in DCM/TFA (solution 30% TFA in DCM) was stirred at room temperature for 30 min. The reaction mixture was concentrated in vacuo and the desired TFA salt (16.5 mg) was used without further purification.

LC-MS [Method 12]: R_t = 1.25 min; MS (ESIneg): m/z = 602 [M-H-TFA]^-

1H-NMR (500 MHz, DMSO-d6) δ [ppm]: -0.007 (2.95), 0.006 (1.71), 0.922 (0.82), 0.937 (1.87), 0.951 (0.92), 1.234 (0.81), 1.318 (2.63), 1.444 (1.74), 1.456 (1.69), 1.728 (1.52), 1.739 (1.52), 1.750 (1.88), 1.763 (1.87), 2.105 (3.29), 2.958 (3.41), 3.358 (15.71), 3.390 (16.00), 3.797 (2.64), 4.117 (6.02), 4.126 (6.87), 4.135 (5.74), 4.511 (3.34), 4.528 (4.00), 4.544 (3.01), 4.798 (0.69), 4.825 (4.79), 4.854 (6.35), 6.008 (1.30), 6.294 (5.80), 6.300 (5.78), 6.626 (8.16), 6.885 (1.82), 6.890 (1.99), 6.902 (3.44), 6.907 (3.60), 6.919 (1.85), 6.924 (1.87), 7.071 (3.16), 7.076 (3.11), 7.094 (3.16), 7.099 (3.02), 7.566 (1.68), 7.574 (1.95), 7.584 (2.52), 7.588 (2.44), 7.597 (1.73), 7.605 (1.46), 8.064 (6.37), 8.331 (7.80), 8.336 (7.30), 8.681 (4.05), 8.684 (4.22), 8.693 (4.13), 9.800 (5.18), 9.809 (4.97).

**Intermediate 18**

N-(tert-butoxycarbonyl)-L-valyl-N⁵-carbamoyl-N-[{{[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl}(methyl)oxido^-6.-sulfanylidene]-L-ornithinamide
To a cooled solution of N⁵-carbamoyl-N-[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,13-benzodioxadiazacyclooctadecin-8-yl]methyl] (methyl)oxido-X-sulfanylidene]-L-ornithinamide trifluoroacetate (15.0 mg, 20.9 µmol; see Intermediate 17) in DMF (1 ml) was added N-(tert-butoxycarbonyl)-L-valine (5.45 mg, 25.1 µmol), HATU (11.9 mg, 31.4 µmol) and 4-methylmorpholine (9.2 µl, 84 µmol) at 0°C. The reaction mixture was stirred for 2.5 h at 0°C and was then diluted with a mixture of Water/TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 3] and the desired compound (11.5 mg, 0.01 mmol) was obtained as a mixture of 2 diastereoisomers.

LC-MS [Method 12]: R<sub>t</sub> = 2.16 min, 2.20 min; MS (ESIpos): m/z = 803 [M+H]<sup>+</sup>

³¹P-NMR (500 MHz, DMSO-de) δ [ppm]: -0.007 (0.73), 0.006 (0.58), 0.714 (1.84), 0.728 (1.92), 0.822 (4.04), 0.835 (4.20), 0.893 (1.98), 0.908 (2.07), 1.234 (0.52), 1.422 (16.00), 1.427 (15.86), 1.705 (0.50), 1.716 (0.55), 1.733 (0.46), 1.924 (0.45), 1.937 (0.43), 1.986 (0.44), 2.000 (0.42), 2.949 (1.07), 2.964 (2.06), 2.978 (1.14), 3.124 (4.18), 3.143 (4.61), 4.034 (0.47), 4.041 (0.49), 4.053 (0.43), 4.073 (0.50), 4.086 (0.69), 4.092 (0.80), 4.101 (1.24), 4.112 (1.94), 4.120 (2.60), 4.132 (2.08), 4.502 (1.23), 4.745 (1.05), 4.772 (1.34), 4.778 (1.25), 4.805 (1.27), 4.915 (0.99), 4.924 (0.99), 4.942 (0.81), 4.952 (0.69), 6.295 (1.74), 6.313 (1.85), 6.585 (1.68), 6.587 (1.60), 6.611 (1.85), 6.883 (0.57), 6.888 (0.68), 6.900 (1.12), 6.905 (1.32), 6.917 (0.66), 6.922 (0.71), 6.975 (0.54), 6.994 (0.65), 7.033 (0.53), 7.052 (0.54), 7.066 (1.17), 7.071 (1.27), 7.088 (1.18), 7.094 (1.23), 7.594 (0.67), 7.602 (0.81), 7.612 (0.82), 7.620 (0.71), 7.625 (0.43), 8.217 (0.53), 8.232 (0.49), 8.307 (1.31), 8.312 (1.33), 8.321 (1.38), 8.326 (1.20), 8.641 (1.09), 8.653 (1.12), 8.752 (0.86), 8.765 (0.87), 9.783 (1.42), 9.900 (1.16).

**Intermediate 19**

L-valyl-N⁵-carbamoyl-N-[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido-X-sulfanylidene]-L-ornithinamide trifluoroacetate
A solution of N-(teri-butoxycarbonyl)-L-valyl-N carbamoyl-N-[[15,19-difluoro-3,4-dihydro-2H,1 1H-10,6-(azeno)-12,1-6-(metheno)-1,5,11,13-benzodiazacyclooctadecin-8-yl][methyl] (methyl) oxido-λ₆-sulfanylidene]-L-ornithinamide (12.0 mg, 14.9 µmol; see Intermediate 18) in DCM/TFA (solution 30% TFA in DCM) was stirred at room temperature for 30 min. The reaction mixture was concentrated in vacuo and the desired TFA salt (12 mg) was used without further purification.

LC-MS [Method 14]: Rᵣ = 1.62 min; MS (ESIpos): m/z = 703 [M+H-TFA]⁺

1H-NMR (500 MHz, DMSO-d₆) δ [ppm]: 0.007 (0.94), 0.924 (6.58), 0.940 (12.56), 0.955 (16.00), 0.969 (6.35), 1.234 (1.91), 1.434 (2.39), 1.578 (1.20), 1.767 (1.23), 2.086 (4.28), 2.097 (3.91), 2.948 (3.35), 3.244 (15.27), 3.248 (15.27), 3.593 (9.79), 4.114 (3.89), 4.125 (4.69), 4.224 (1.09), 4.240 (2.00), 4.250 (2.04), 4.267 (0.99), 4.503 (2.49), 4.518 (3.14), 4.534 (2.27), 4.789 (6.46), 4.811 (0.62), 5.977 (1.62), 6.262 (4.38), 6.273 (4.41), 6.609 (7.43), 6.885 (1.41), 6.890 (1.63), 6.902 (2.72), 6.907 (2.84), 6.919 (1.52), 6.924 (1.50), 7.070 (2.57), 7.074 (2.56), 7.092 (2.63), 7.097 (2.50), 7.566 (1.29), 7.574 (1.54), 7.584 (1.99), 7.588 (1.97), 7.597 (1.47), 7.605 (1.17), 8.050 (5.76), 8.330 (5.36), 8.512 (3.27), 8.527 (3.17), 8.665 (2.54), 8.674 (3.50), 8.686 (2.58), 9.771 (4.22), 9.779 (4.06).

Intermediate 20
tert-buty 1 [(2R)-1-[[15,19-difluoro-3,4-dihydro-2H,1H-Q₆-(azeno)-l₂,1₆-(metheno)-1,5,1i,13-benzodiazacyclooctadecin-8-yl][methyl](methyl)oxido-λ₆-sulfanylidene]amino]-3-methyl-l-oxobutan-2-yl] carbamate
To a cooled solution of (rac)-15,19-difluoro-8-[{(S-methylsulfonimidoyl)methyl}-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine (10.0 mg, 22.4 µmol; see Intermediate 11) in DMF (0.5 ml) was added N-(feri-butoxycarbonyl)-D-valine (5.84 mg, 26.9 µmol), HATU (12.8 mg, 33.6 µmol) and 4-methylmorpholine (7.4 µl, 67 µmol) at 0 °C. The reaction mixture was stirred for 2 h at 0°C and was then stirred overnight at room temperature. After dilution with a mixture of Water /acetonitrile (1ml), the resulting mixture was directly purified by preparative HPLC [Method 3] and the desired compound (10.5 mg, 0.02 mmol) was obtained as a mixture of 2 diastereoisomers.

LC-MS [Method 12]: Rt = 2.42 min, 2.46 min; MS (ESIpos): m/z = 646 [M+H]⁺

1H-NMR (400 MHz, DMSO-ifc) δ [ppm]: -0.009 (2.50), 0.008 (2.03), 0.776 (6.12), 0.793 (6.32), 0.825 (2.64), 0.842 (3.31), 0.848 (4.43), 0.865 (3.81), 1.234 (1.14), 1.383 (16.00), 1.389 (15.75), 2.072 (4.20), 2.097 (1.88), 3.209 (4.01), 3.279 (6.17), 3.823 (0.91), 4.111 (1.91), 4.124 (2.41), 4.134 (1.97), 4.493 (1.35), 4.514 (1.60), 4.535 (1.27), 4.732 (1.24), 4.759 (1.35), 4.774 (1.21), 4.793 (1.36), 6.277 (2.01), 6.304 (1.65), 6.445 (0.58), 6.518 (0.64), 6.540 (0.60), 6.634 (3.27), 6.874 (0.78), 6.880 (0.90), 6.895 (1.49), 6.901 (1.68), 6.916 (0.85), 6.922 (0.92), 7.059 (1.38), 7.064 (1.45), 7.087 (1.45), 7.093 (1.38), 7.557 (0.71), 7.567 (0.86), 7.579 (1.07), 7.584 (1.03), 7.596 (0.83), 7.606 (0.71), 8.307 (1.42), 8.317 (2.23), 8.323 (1.74), 8.675 (1.83), 8.689 (1.85), 9.661 (1.15), 9.718 (1.16).
Intermediate 21

N-[[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-
benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido-λ6-sulfanylidene]-D-valinamide
trifluoroacetate

A solution of tert-butyl [(2R)-1-[[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-
benzodioxadiazacyclooctadecin-8-yl]methyl] (methyl)oxido-λ6-sulfanylidene] amino]-3-methyl-
1-oxobutan-2-yl]carbamate (9.0 mg, 13.9 µmol, see Intermediate 18) in DCM/TFA (solution 30% TFA in
DCM) was stirred at room temperature for 30 min. The reaction mixture was concentrated in vacuo and
the desired TFA salt (8.6 mg) was used without further purification.

LC-MS [Method 13]: R₁ = 0.80 min; MS (ESIpos): m/z = 546 [M+H-TFA]⁺

1H-NMR (500 MHz, DMSO-de) δ [ppm]: -0.007 (2.68), 0.007 (1.49), 0.901 (9.46), 0.905 (9.73), 0.915
(9.90), 0.919 (9.67), 0.929 (7.77), 0.943 (7.98), 0.952 (7.87), 0.966 (7.55), 1.234 (1.57), 2.095 (2.81),
2.158 (0.71), 2.171 (1.03), 2.180 (1.11), 2.197 (1.09), 2.202 (1.08), 2.211 (1.04), 2.217 (0.72), 2.225
(0.65), 3.386 (16.00), 3.396 (15.26), 3.640 (1.21), 3.651 (1.71), 3.663 (1.79), 3.671 (1.71), 3.683 (1.25),
4.118 (4.99), 4.128 (5.65), 4.136 (4.71), 4.505 (2.58), 4.522 (3.12), 4.538 (2.44), 4.810 (1.06), 4.839
(8.61), 4.855 (3.13), 4.882 (0.91), 6.289 (4.69), 6.306 (4.87), 6.307 (4.77), 6.626 (4.66), 6.635 (4.50),
6.887 (1.66), 6.892 (1.85), 6.904 (3.08), 6.909 (3.29), 6.920 (1.74), 6.925 (1.84), 7.072 (2.81), 7.077
(2.86), 7.095 (2.91), 7.099 (2.74), 7.113 (0.58), 7.567 (1.45), 7.575 (1.72), 7.581 (2.00), 7.584 (2.21),
7.588 (2.15), 7.592 (1.88), 7.598 (1.65), 7.606 (1.39), 7.995 (5.52), 8.334 (7.16), 8.339 (6.81), 8.682
(5.07), 8.694 (4.99), 9.766 (4.77), 9.775 (4.60).
Intermediate 22
4-nitrophenyl [2-(2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)ethyl]carbamate

To a solution of 4-Nitrophenyl chloroformate (228 mg, 97 % purity, 1.10 mmol) in THF (30 ml) was added 1-(2-aminoethyl)-lH-pyrrole-2,5-dione hydrochloride (1:1) (100 mg, 97 % purity, 549 µmol) at room temperature. The reaction mixture was stirred for 1 h, then filtered and concentrated in vacuo. The residue was diluted with ether and the suspension was filtered off and dried under high vacuum. 134 mg (0.43 mmol) of the desired compound was obtained as a white solid.

1H-NMR (400 MHz, DMSO-de) δ [ppm]: -0.008 (1.15), 0.008 (1.11), 3.196 (1.03), 3.211 (2.44), 3.225 (2.77), 3.240 (1.25), 3.559 (2.37), 3.571 (2.75), 3.574 (3.38), 3.587 (2.11), 7.056 (16.00), 7.347 (5.63), 7.353 (1.78), 7.365 (1.90), 7.370 (6.05), 8.111 (1.66), 8.262 (5.91), 8.268 (1.89), 8.279 (2.07), 8.285 (5.74).

Intermediate 23
tert-butyl 4-nitrophenyl ethane-1,2-diylbiscarbamate

To a solution of 4-Nitrophenyl chloroformate (838 mg, 97 % purity, 4.03 mmol) in THF (80 ml) was added tert-butyl (2-aminoethyl)carbamate (340 mg, 95 % purity, 2.02 mmol) at room temperature. The reaction mixture was stirred for 1.5 h, then filtered and concentrated in vacuo. The residue was diluted with ether and the suspension was filtered off and dried under high vacuum. 599 mg of the desired compound was obtained as a white solid.

1H-NMR (400 MHz, DMSO-de) δ [ppm]: 1.341 (1.08), 1.385 (16.00), 3.078 (1.03), 3.089 (1.32), 3.099 (1.15), 7.391 (2.08), 7.397 (0.71), 7.409 (0.75), 7.414 (2.27), 8.252 (2.16), 8.257 (0.81), 8.269 (0.78), 8.274 (2.14).
Under Argon, to a solution of (rflc)-15,19-difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine (20.0 mg, 44.8 μmol; see Intermediate 11) in DMF (5 ml) was added tert-butyl 4-nitrophenyl ethane-1,2-diylbiscarbamate (21.9 mg, 67.2 μmol, see Intermediate 23) and N,N-diisopropylethylamine (23 μl, 130 μmol) at room temperature. The reaction mixture was stirred overnight at room temperature, prior to the addition of a further portion of 4-nitrophenyl [2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]carbamate (20 mg, 61.5 μmol), due to incomplete conversion. The reaction mixture was further stirred for 2 days at room temperature and then heated to 80 °C. Over this reaction time, 2 additional portions of 4-nitrophenyl [2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]carbamate (40 mg in total, 123 μmol) and N,N-diisopropylethylamine (23 μl, 130 μmol) were added. Finally, the reaction mixture was diluted with a mixture of Water /TFA/acetonitrile (1 ml) and was then directly purified by preparative HPLC [Method 2] and the desired compound (7.7 mg, 0.01 mmol) was obtained.

LC-MS [Method 15]: R₁ = 1.39 min; MS (ESIneg): m/z = 631 [M-H]⁻

1H-NMR (500 MHz, DMSO-de) δ [ppm]: 1.368 (16.00), 2.088 (1.14), 2.971 (1.37), 3.007 (1.51), 3.129 (6.34), 4.112 (1.73), 4.120 (2.05), 4.496 (1.27), 4.513 (1.49), 4.529 (1.20), 4.718 (2.05), 4.727 (1.92), 6.273 (2.96), 6.619 (2.99), 6.738 (0.66), 6.821 (0.74), 6.878 (0.56), 6.883 (0.64), 6.895 (1.05), 6.900 (1.15), 6.912 (0.60), 6.917 (0.62), 7.067 (1.01), 7.071 (1.07), 7.089 (1.03), 7.094 (1.01), 7.576 (0.82), 8.318 (2.24), 8.322 (2.18), 8.667 (1.82), 8.679 (1.83), 9.744 (2.31).
Intermediate 25

\[(\text{rac}) \text{1-}(2\text{-aminoethyl})\text{-3-}[[[15,19\text{-difluoro-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl}]\text{methyl})(\text{methyl})\text{oxydi}-\lambda^6\text{-sulfanylidene}]\text{urea}\]

trifluoroacetate

A solution of \textit{tert}-butyl \[2-\text{-}[[[15,19\text{-difluoro-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl}]\text{methyl}]\text{(methyl)}\text{oxydi}^6\text{-sulfanylidene}]\text{carbamoyl}\text{amino}\text{ethyl}\text{carbamate} \text{(30.0 mg, 47 \text{\textmu}mol, see Intermediate 24)} \text{in DCM/TFA} \text{(solution 30\% TFA in DCM)} \text{was stirred at room temperature for 30 min. The reaction mixture was concentrated \textit{in vacuo} and the desired TFA salt (28 mg) was used without further purification.}

LC-MS [Method 15]: R\textsubscript{t} = 1.03 min; MS (ESIneg): m/z = 531 [M-H-TFA]\textsuperscript{-}.

\[\begin{align*}
1\text{H-NMR} \text{ (500 MHz, DMSO-de) } \delta \text{ [ppm]}: & \ -0.007 \ (1.62), \ 0.007 \ (0.94), \ 1.234 \ (2.16), \ 2.073 \ (1.87), \ 2.088 \ (2.43), \ 2.843 \ (2.52), \ 2.858 \ (2.47), \ 3.171 \ (16.00), \ 3.201 \ (1.93), \ 3.213 \ (4.17), \ 3.226 \ (4.37), \ 4.116 \ (4.35), \ 4.125 \ (4.92), \ 4.134 \ (4.15), \ 4.501 \ (2.51), \ 4.517 \ (3.00), \ 4.534 \ (2.38), \ 4.740 \ (6.92), \ 4.767 \ (0.79), \ 6.283 \ (7.84), \ 6.616 \ (6.45), \ 6.885 \ (1.38), \ 6.890 \ (1.51), \ 6.902 \ (2.57), \ 6.907 \ (2.75), \ 6.919 \ (1.36), \ 6.924 \ (1.51), \ 6.989 \ (1.34), \ 7.000 \ (2.55), \ 7.012 \ (1.19), \ 7.074 \ (2.36), \ 7.079 \ (2.41), \ 7.096 \ (2.51), \ 7.102 \ (2.24), \ 7.563 \ (1.24), \ 7.571 \ (1.46), \ 7.580 \ (1.83), \ 7.584 \ (1.83), \ 7.594 \ (1.40), \ 7.601 \ (1.15), \ 7.714 \ (2.85), \ 8.326 \ (5.90), \ 8.330 \ (5.54), \ 8.669 \ (3.51), \ 8.681 \ (3.58), \ 9.750 \ (5.12). \end{align*}\]
Intermediate 26

4-nitrophenyl [3-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)propyl]carbamate

To a solution of 4-Nitrophenyl chloroformate (335 mg, 97 % purity, 1.61 mmol) in THF (35 ml) was added 2-(3-aminopropyl)-1H-isoindole-1,3(2H)-dione hydrochloride (1:1) (200 mg, 97 % purity, 806 µmol) at room temperature. The reaction mixture was stirred for 2 h, then filtered and concentrated in vacuo. The residue was diluted with ether and the suspension was filtered off and dried under high vacuum. 170 mg (90 % purity) of the desired compound was obtained as a white solid.

1H-NMR (400 MHz, DMSO-de) δ [ppm]: -0.008 (2.52), 0.008 (2.26), 1.817 (1.48), 1.834 (5.17), 1.852 (7.54), 1.869 (5.62), 1.887 (1.95), 2.889 (1.18), 2.906 (2.44), 2.922 (1.18), 3.118 (2.95), 3.135 (6.93), 3.150 (6.80), 3.167 (2.78), 3.409 (6.37), 3.631 (6.41), 3.649 (11.90), 3.666 (6.65), 3.833 (1.17), 3.849 (2.24), 3.866 (1.09), 6.922 (6.15), 6.928 (2.00), 6.940 (1.96), 6.945 (6.49), 6.954 (0.83), 7.371 (1.57), 7.379 (14.93), 7.385 (5.16), 7.397 (4.99), 7.402 (16.00), 7.410 (1.67), 7.729 (1.00), 7.752 (1.09), 7.825 (5.99), 7.833 (7.54), 7.839 (7.48), 7.846 (13.83), 7.857 (4.50), 7.861 (5.00), 7.871 (13.80), 7.879 (7.43), 7.885 (7.21), 7.893 (5.46), 7.901 (0.94), 7.912 (1.61), 7.920 (0.85), 7.926 (0.82), 7.934 (0.65), 8.052 (2.33), 8.067 (4.56), 8.081 (2.29), 8.108 (5.52), 8.113 (1.83), 8.125 (1.74), 8.131 (5.37), 8.241 (1.78), 8.249 (15.78), 8.255 (4.87), 8.266 (4.85), 8.272 (15.22), 8.281 (1.41), 8.372 (0.98), 8.395 (0.90), 11.030 (3.02).

Intermediate 27

(rac)-[15,19-difluoro-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-(metheno)-1,5,ll,13-benzodioxadiazacyclooctadecin-8-yl]methyl)(methyl)oxido-1,5-sulfanylidene]-3-[3-(1,3-dioxo-1,3-dihydro-2H-isoindol-2-yl)propyl]urea
Under Argon, to a solution of (rflc)-15,19-difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine (20 mg, 44.8 μmol; see Intermediate 11) in DMF (5 ml) was added 4-nitrophenyl [3-(1,3-dioxo-1,3-dihydro-2H-isindol-2-yl)propyl]carbamate (27.6 mg, 90 % purity, 67.2 μmol, see Intermediate 26) and N,N-diisopropylethylamine (23 μl, 130 μmol) at room temperature. The reaction mixture was stirred overnight at room temperature, prior to the addition of a further portion of 4-nitrophenyl [3-(1,3-dioxo-1,3-dihydro-2H-isindol-2-yl)propyl]carbamate (9.191 mg, 90 % purity, 22.4 μmol, see Intermediate 26), due to incomplete conversion. After 5 h of stirring, the reaction mixture was diluted with a mixture of Water /TFA/acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 3] and the desired compound (9 mg, 0.01 mmol) was obtained.

LC-MS [Method 15]: R_t = 1.38 min; MS (ESIneg): m/z = 675 [M-H]

1H-NMR (400 MHz, DMSO-de) δ [ppm]: 1.236 (0.89), 1.691 (2.37), 1.709 (3.26), 1.728 (2.18), 2.072 (3.32), 3.026 (3.52), 3.039 (2.63), 3.124 (16.00), 3.200 (1.39), 3.555 (2.88), 3.574 (4.36), 3.592 (2.56), 4.093 (3.64), 4.468 (2.65), 4.730 (5.40), 6.272 (5.95), 6.621 (5.58), 6.846 (2.48), 6.877 (1.66), 6.899 (2.67), 6.914 (1.33), 7.054 (2.11), 7.078 (1.92), 7.554 (1.98), 7.832 (11.56), 7.838 (11.60), 8.240 (5.20), 8.246 (5.05), 8.636 (3.24), 8.651 (2.92), 9.689 (6.91).
Intermediate 28

(rac) 1-(3-aminopropyl)-3-[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,13-benzodioxadiazaacyclooctadecin-8-yl]methyl](methyl)oxido-λ^6^-sulfanylidene]urea trifluoroacetate

To a solution of (rac)-1-[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,13-benzodioxadiazaacyclooctadecin-8-yl]methyl](methyl)oxido-λ^6^-sulfanylidene]-3-[3-(1,3-dioxo-1,3-dihydro-2H-isindol-2-yl)propyl]urea (21.0 mg, 31.0 µmol, see Intermediate 27) in ethanol (3.1 ml) was added methylamine (58 µl, 470 µmol) (solution 33% wt. in anhydrous ethanol) at room temperature. The reaction mixture was heated to 50 °C and stirred overnight at this temperature, prior to the addition of a further portion of methylamine (63 µl, due to incomplete conversion. The reaction mixture was further stirred for 3 days at 50 °C. Over this reaction time, 2 additional portions of methylamine (60 µl in total) were added. The reaction mixture was concentrated in vacuo and the crude was directly purified by preparative HPLC [Method 5]. The desired compound (9 mg, 0.01 mmol) was obtained as its TFA salt.

LC-MS [Method 12]: R_t = 1.32 min; MS (ESIpos): m/z = 547 [M+H-TFA]^+

1H-NMR (500 MHz, DMSO-de) δ [ppm]: -0.120 (0.67), -0.007 (6.28), 0.007 (4.03), 1.147 (0.98), 1.236 (1.17), 1.640 (0.88), 1.654 (2.23), 1.668 (2.94), 1.682 (2.09), 1.696 (0.64), 2.068 (0.96), 2.087 (2.14), 2.101 (2.11), 2.120 (0.88), 2.358 (0.91), 2.362 (1.06), 2.365 (0.81), 2.371 (0.83), 2.383 (0.71), 2.635 (0.75), 2.768 (1.48), 2.782 (2.48), 2.795 (2.27), 2.808 (1.14), 3.037 (1.48), 3.050 (3.63), 3.063 (3.50), 3.076 (1.30), 3.144 (16.00), 3.214 (0.59), 3.898 (1.25), 4.114 (3.68), 4.124 (4.10), 4.133 (3.49), 4.499 (2.07), 4.516 (2.43), 4.533 (1.94), 4.708 (0.78), 4.735 (4.39), 4.743 (4.20), 4.769 (0.68), 6.277 (7.18), 6.616 (6.37), 6.885 (1.22), 6.890 (1.39), 6.902 (2.26), 6.907 (2.49), 6.919 (1.23), 6.924 (1.33), 7.015 (1.19), 7.027 (2.32), 7.039 (1.11), 7.074 (2.10), 7.079 (2.17), 7.097 (2.13), 7.102 (2.06), 7.563 (1.26), 7.571 (1.46), 7.576 (1.67), 7.580 (1.91), 7.584 (1.87), 7.588 (1.74), 7.594 (1.62), 7.602 (1.55), 7.640 (2.23), 8.325 (5.34), 8.330 (5.13), 8.670 (3.71), 8.682 (3.68), 9.742 (4.85).
Under an atmosphere of argon, to a solution of (rac)-15,19-Difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,1IH-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine (10.0 mg, 22.4 µmol; see Intermediate 11) in DMF (0.5 ml) was added dropwise Ethyl 3-isocyanatopropionate (3.0 µl, 98 % purity, 22 µmol) and triethylamine (3.1 µl, 22 µmol) at room temperature. The reaction mixture was stirred overnight at room temperature and was then diluted with a mixture of Water /TFA/acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 1] and the desired compound (13 mg, 0.02 mmol) was obtained.

LC-MS [Method 15]: Rₜ = 1.35 min; MS (ESIneg): m/z = 588 [M-H]⁻

1H-NMR (500 MHz, DMSO-d₆) δ [ppm]: 1.106 (0.61), 1.162 (5.50), 1.177 (11.02), 1.191 (5.73), 1.232 (0.80), 2.073 (4.43), 2.098 (2.86), 2.419 (2.37), 2.434 (4.72), 2.448 (2.63), 2.560 (1.19), 3.120 (16.00), 3.214 (4.02), 4.033 (2.06), 4.047 (5.35), 4.061 (5.33), 4.075 (2.02), 4.111 (3.95), 4.121 (4.95), 4.130 (4.29), 4.492 (2.47), 4.508 (3.17), 4.525 (2.61), 4.730 (9.47), 6.273 (6.54), 6.615 (6.65), 6.864 (1.89), 6.878 (2.10), 6.883 (1.89), 6.895 (2.67), 6.900 (2.98), 6.912 (1.53), 6.917 (1.63), 7.066 (2.32), 7.071 (2.60), 7.088 (2.51), 7.093 (2.58), 7.559 (1.12), 7.566 (1.37), 7.576 (2.00), 7.580 (1.97), 7.589 (1.49), 7.598 (1.22), 8.319 (5.59), 8.323 (5.89), 8.668 (4.51), 8.680 (4.66), 9.734 (5.02).
Intermediate 30

\((\text{rac})\) N-\{[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,1,13-
benzodioxadiazacyclooctadecin-8-yl]methyl] (methyl)oxido-\(\delta^6\)-sulfanylidene]carbamoyl\}-beta-
alanine

To a solution of ethyl N-\{[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-
1,5,1,13-benzodioxadiazacyclooctadecin-8-yl]methyl] (methyl)oxido-\(\delta^6\)-sulfanylidene]carbamoyl\} -beta-
alaninate (13.0 mg, 22.0 \(\mu\)mol; see Intermediate 29) in a mixture of THF/Water (2/1, 1ml) was added
Lithium hydroxide monohydrate (1.85 mg, 44.1 \(\mu\)mol) at room temperature. The reaction mixture was
stirred for 3 hours at room temperature and was then diluted with a mixture of Water /TFA/acetonitrile (1 ml).
The resulting mixture was directly purified by preparative HPLC [Method 2] and the desired compound (10 mg, 0.02 mmol) was obtained.

LC-MS [Method 5]: \(R_t = 1.23\) min; MS (ESIneg): \(m/z = 560\) [M-H]

1H-NMR (500 MHz, DMSO-d\(_6\)) \(\delta\) [ppm]: 2.088 (2.94), 2.101 (2.87), 2.361 (3.40), 2.376 (5.41), 2.390
(2.87), 3.123 (16.00), 3.192 (3.93), 4.112 (4.62), 4.121 (5.27), 4.131 (4.46), 4.495 (3.45), 4.512 (3.94),
4.528 (3.18), 4.729 (9.00), 4.757 (0.97), 6.279 (8.37), 6.619 (7.71), 6.797 (1.72), 6.879 (1.55), 6.884
(1.78), 6.896 (2.98), 6.901 (3.23), 6.913 (1.64), 6.918 (1.71), 7.066 (2.83), 7.071 (2.88), 7.089 (2.88),
7.094 (2.81), 7.561 (1.38), 7.569 (1.64), 7.574 (1.89), 7.578 (2.13), 7.582 (2.04), 7.592 (1.57), 7.600
(1.32), 8.319 (6.70), 8.324 (6.58), 8.669 (5.43), 8.681 (5.44), 9.742 (7.27).

Intermediate 31

(rac)-2-chloroethyl \{[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,1,13-
benzodioxadiazacyclooctadecin-8-yl]methyl] (methyl)oxido-\(\delta^6\)-sulfanylidene]carbamate
To a solution of (rac)-15,19-difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine (25 mg, 0.056 mmol; see Example 5) in pyridine (0.84 ml) was added 2-Chloroethyl chloroformate (7.5 µl, 0.078 mmol; CAS-RN 627-11-2) at room temperature. The reaction mixture was stirred overnight at room temperature, prior to the addition of more ethyl chloroformate (5 µl, 0.056 mmol, 1 eq) due to incomplete conversion. After 5 hours of stirring, the reaction mixture was quenched with a mixture of Water/acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 1] and the desired compound (23 mg, 0.04 mmol) was obtained.

$^1$H NMR (500 MHz, DMSO-$d_6$) δ ppm 9.84 (s, 1 H), 8.68 (d, 1 H), 8.34 (d, 1 H), 7.56 - 7.62 (m, 1 H), 7.09 (dd, 1 H), 6.91 (td, 1 H), 6.61 (s, 1 H), 6.28 (s, 1 H), 4.77 - 4.85 (m, 2 H), 4.49 - 4.56 (m, 2 H), 4.19 - 4.28 (m, 2 H), 4.10 - 4.15 (m, 2 H), 3.81 (t, 2 H), 3.30 (s, 3 H), 2.05 - 2.15 (m, 2 H).

**Intermediate 32**

(rac)-2-(1,3-dioxo-1,3-dihydro-2H-isindoI-2-yl)ethyl [[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido -$_{6ℓ}$-sulfanylidene] carbamate
Under an atmosphere of argon, to a solution of (rac)-2-chloroethyl [[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-l,5,ll,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido-\(\lambda^6\)-sulfanylidene]carbamate (24 mg, 0.043 mmol; see Example 21) in DMF (0.5 ml) was added potassium phtalimide (8.84 mg, 0.048 mmol). The reaction mixture was heated to 80 °C and stirred overnight at this temperature. After cooling, the resulting mixture was directly purified by preparative HPLC [Method 1] and the desired compound (20 mg, 0.03 mmol) was obtained.

\[3\] NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) ppm 9.78 (s, 1 H), 8.67 (d, 1 H), 8.33 (d, 1 H), 7.76 - 7.95 (m, 4 H), 7.51 - 7.67 (m, 1 H), 7.08 (dd, 1 H), 6.90 (td, 1 H), 6.58 (s, 1 H), 6.25 (s, 1 H), 4.72 (br. s, 2 H), 4.48 - 4.55 (m, 2 H), 4.17 - 4.25 (m, 2 H), 4.10 - 4.15 (m, 2 H), 3.85 (t, 2 H), 3.19 (s, 3 H), 2.04 - 2.14 (m, 2 H).

**Intermediate 33**

(rac)-2-aminoethyl [[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-l,5,ll,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido-\(\lambda^6\)-sulfanylidene]carbamate

To a solution of (rac)-2-(1,3-dioxo-1,3-dihydro-2H-isooindol-2-yl)ethyl [[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-l,5,ll,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido-X \(\lambda^6\)-sulfanylidene]carbamate (18 mg, 0.027 mmol) in ethanol (0.8 ml) was added methylamine (50 \(\mu\)l, 0.407 mmol, 15 eq) (solution 33% wt. in anhydrous ethanol) at room temperature. The reaction mixture was heated to 50 °C and stirred for 90 min at this temperature. The reaction mixture was concentrated in vacuo and the crude was directly purified by preparative HPLC [Method 1]. The desired compound was obtained as its TFA salt, which was then dissolved in ethyl acetate and washed with an aqueous solution of sodium bicarbonate. After phase separation, the aqueous phase was extracted twice with ethyl acetate and the combined organic phases were dried over sodium sulfate, filtered, and concentrated in vacuo. The free amine (8 mg, 0.01 mmol) was obtained.
\[
\text{NMR (400 MHz, DMSO-d6)} \delta \text{ ppm } 9.83 \text{ (s, 1 H), 8.68 \text{ (d, 1 H), 8.33 \text{ (d, 1 H), 7.52 - 7.67 \text{ (m, 1 H), 7.08 \text{ (dd, 1 H), 6.90 \text{ (td, 1 H), 6.61 \text{ (s, 1 H), 6.27 \text{ (s, 1 H), 4.71 - 4.88 \text{ (m, 2 H), 4.44 - 4.63 \text{ (m, 2 H), 4.09 - 4.17 \text{ (m, 2 H), 3.88 - 3.99 \text{ (m, 2 H), 3.27 \text{ (s, 3 H), 2.75 \text{ (t, 2 H), 2.05 - 2.15 \text{ (m, 2 H).}}}}}}}}}
\]

5 **Intermediate 34:**

2,6-dichloro-4-[(ethoxymethoxy)methyl]pyridine

\[
\text{O} \quad \text{O} \quad \text{CH}_3 \\
\text{Cl} \quad \text{N} \quad \text{Cl}
\]

To a solution of (2,6-dichloropyridin-4-yl)methanol (1g, 5.617 mmol; see Intermediate 1, CAS-RN 101990-69-6) and N,N-diisopropylethylamine (3.91 ml, 22.47 mmol) in DCE (25 ml) was added dropwise chloromethyl ethyl ether (1.042 ml, 11.235 mmol) at room temperature. The reaction mixture was heated to 70 °C and stirred for 1 h at this temperature. The reaction mixture was then quenched with an aqueous solution of ammonium chloride and diluted with DCM. After phase separation, the aqueous phase was extracted twice with DCM. The combined organic phases were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The desired compound (1.30 g, 5.34 mmol) was obtained without any further purification.

\[
\text{NMR (500 MHz, DMSO-d6)} \delta \text{ ppm } 7.51 \text{ (t, 2 H), 4.74 \text{ (s, 2 H), 4.64 \text{ (s, 2 H), 3.56 \text{ (q, 2 H), 1.12 \text{ (t, 3 H).}}}}}
\]

10 **Intermediate 35:**

3-((6-chloro-4-[(ethoxymethoxy)methyl]pyridin-2-yl)oxy)propan-1-ol

\[
\text{O} \quad \text{O} \quad \text{CH}_3 \\
\text{Cl} \quad \text{N} \quad \text{Cl}
\]

To a suspension of sodium hydride (330 mg, 8.25 mmol, 60 % in oil) in THF (140 ml) was added dropwise a solution of 1,3-propanediol (2.39 ml, 33.037 mmol) in THF (10 ml) at room temperature. The reaction mixture was stirred for 30 min, after which a solution of 2,6-dichloro-4-[(ethoxymethoxy)methyl]pyridine (1.30 g, 5.34 mmol; see Intermediate 34) in THF (10 ml) was added.
dropwise at room temperature. The reaction mixture was stirred at reflux for 3 h, prior to the addition of a further portion of sodium hydride (330 mg, 8.25 mmol, 60 % in oil) at room temperature, due to incomplete conversion. After 48 h of stirring under reflux, the reaction mixture was cooled, quenched with water (120 ml) and extracted three times with ethyl acetate. After phase separation, the combined organic phases were dried over sodium sulfate, filtered, and concentrated in vacuo. The crude was purified by preparative HPLC [Method 1] and the desired compound (1.154 g, 4.06 mmol) was obtained.

\[
\text{Intermediate 36:} \\
4-\{2-\{3-\{6-chloro-4-\{(ethoxymethoxy)methyl\}pyridin-2-yl\}oxy\}propoxy\}-4-fluorophenyl\}-5-fluoropyridin-2-amine
\]

Under an atmosphere of argon, a solution of diisopropyl azodicarboxylate (1.12 ml, 5.67 mmol) in THF (3.9 mL) was added dropwise to a mixture of 3-\{(6-chloro-4-\{(ethoxymethoxy)methyl\}pyridin-2-yl\}oxy\}propan-1-ol (1.25 g, 4.53 mmol; see Intermediate 35), 2-(2-amino-5-fluoropyridin-4-yl)-5-fluorophenol (1.103 g, 4.96 mmol; see Intermediate 7) and triphenylphosphine (1.486 g; 5.67 mmol) in THF (19.5 mL). The reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo and the crude product was diluted with a mixture of water / acetonitrile. The resulting mixture was directly purified by preparative HPLC [Method 1] and the desired compound (1.88 g, 3.58 mmol) was obtained.

\[
\text{Intermediate 37:} \\
8-\{(ethoxymethoxy)methyl\}-15,19-difluoro-3,4-dihydro-2H,IIH-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine
\]
Under an atmosphere of argon, a mixture of 4-{2-[3-(6-chloro-4-[(ethoxymethoxy)methyl]pyridin-2-yl] oxy)propoxy]-4-fluorophenyl} -5-fluoropyridin-2-amine (1.70 g, 3.542 mmol; see Intermediate 36), chloro(2-dicyclohexylphosphino-2',4',6'-triisopropyl- 1,1'-biphenyl)[2-(2'-amino- 1,1'-biphenyl)]palladium (II) (223 mg, 0.283 mmol, Aldrich Chemical Company Inc. CAS-RN 1310584-14-5), 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl (135 mg, 0.283 mmol, Aldrich Chemical Company Inc. CAS-RN 564483-18-7) and potassium phosphate (3.76 g; 17.71 mmol) in toluene (210 ml) and NMP (28 mL) was stirred overnight at 100 °C. After cooling, the reaction mixture was diluted with Water (170 ml) and filtered through celite. The resulting mixture was extracted twice with MTBE. The combined organic phases were dried over magnesium sulfate, filtered, and concentrated in vacuo. The residue was diluted with hot methanol and after cooling; the suspension was filtered off and dried under high vacuum. 1.16 g (2.57 mmol) of the desired compound was obtained.

¾ NMR (500 MHz, DMSO-<i>d</i><sub>6</sub>) δ ppm 9.64 (s, 1 H), 8.72 (d, 1 H), 8.31 (d, 1 H), 7.55 - 7.62 (m, 1 H), 7.08 (dd, 1 H), 6.90 (td, 1 H), 6.57 (s, 1 H), 6.14 (s, 1 H), 4.71 (s, 2 H), 4.50 (t, 2 H), 4.46 (s, 2 H), 4.12 (t, 2 H), 3.58 (q, 2 H), 2.05 - 2.14 (m, 2 H), 1.15 (t, 3 H).

Intermediate 38:
[15,19-difluoro-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-(metheno)-1,5,ll,13-benzodioxadiazacyclooctadecine-8-yl]methanol

To a solution of 8-[(ethoxymethoxy)methyl]-1 5,19-difluoro-3,4-dihydro-2H, 11H-1 0,6-(azeno)-12, 16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine (577 mg, 1.30 mmol, see Intermediate 37) in
MeOH (60 ml) was added concentrated 37% HCl (1.087 ml, 13.01 mmol) at room temperature. The reaction mixture was heated to 60 °C and stirred at this temperature for 15 min. After cooling, the reaction mixture was concentrated in vacuo and the residue was diluted in DCM. The resulting solution was dried over sodium sulfate and concentrated in vacuo. The isolated solid was dried under high vacuum to give 536 mg (1.39 mmol) of the desired compound, which was used without additional purification.

\[ \text{Intermediate 39:} \]

\[ [15,19\text{-difluoro-3,4-dihydro-2H,1\text{-IH}-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl} \text{methyl methanesulfonate}} \]

To a solution of [15,19-difluoro-3,4-dihydro-2H,1\text{-IH}-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methanol (125 mg, 0.32 mmol, see Intermediate 38) and triethylamine (109 \( \mu \)l, 0.78 mmol) in THF (6.5 ml) was added dropwise mesyl chloride (30 \( \mu \)l, 0.39 mmol) at 0 °C. The reaction mixture was stirred for 1 h at room temperature. The reaction mixture was quenched with an aqueous solution of sodium bicarbonate, diluted with Water and extracted three times with ethyl acetate. After phase separation, the combined organic phases were dried over sodium sulfated, filtered, and concentrated in vacuo. 133 mg (0.29 mmol) of the desired compound was obtained, which was used without additional purification.

\[ \text{\( ^{1}H\) NMR (500 MHz, DMSO-\( d_6 \)) \( \delta \) ppm 9.76 (s, 1 H), 8.71 (d, 1 H), 8.33 (d, 1 H), 7.55 - 7.62 (m, 1 H), 7.09 (dd, 1 H), 6.86 - 6.95 (m, 1 H), 6.60 (s, 1 H), 6.21 (s, 1 H), 5.19 (s, 2 H), 4.52 (t, 2 H), 4.13 (t, 2 H), 3.29 (s, 3 H), 2.05 - 2.13 (m, 2 H).} \]
**Intermediate 40:**
8-(chloromethyl)-15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine

To a solution of [15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methanol (200 mg, 0.519 mmol; see Intermediate 38) in DCM (20 ml) was added a drop of DMF and thionyl chloride (379 µl, 5.19 mmol). The reaction mixture was stirred for 48 h at room temperature and then concentrated in vacuo. The residue was diluted with hot hexane and the suspension filtered off and dried under high vacuum. 140 mg (0.3 mmol) of the desired compound was obtained.

\[ \text{\textsuperscript{1}H NMR (500 MHz, DMSO-\textit{d}_6)} \delta \text{ ppm: } 9.77 \text{ (s, 1 H)}, 8.70 \text{ (d, 1 H)}, 8.33 \text{ (d, 1 H)}, 7.52 - 7.66 \text{ (m, 1 H)}, 7.09 \text{ (dd, 1 H)}, 6.85 - 6.97 \text{ (m, 1 H)}, 6.62 \text{ (s, 1 H)}, 6.24 \text{ (s, 1 H)}, 4.63 \text{ (s, 2 H)}, 4.51 \text{ (t, 2 H)}, 4.13 \text{ (t, 2 H)}, 2.04 - 2.14 \text{ (m, 2 H)}. \]

**Intermediate 41**

Methode A

To a solution of 2-(Boc-amino)ethanethiol (8 µmol, 0.047 mmol, CAS-RN 67385-09-5) in ethanol (1 ml) was added cesium carbonate (30.9 mg, 0.095 mmol). After 30 min of stirring at room temperature, a solution of [15,19-difluoro-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-(metheno)-1,5,ll,13-benzodioxadiazacyclooctadecin-8-yl]methyl methanesulfonate (20 mg, 0.043 mmol; see Intermediate 39) in ethanol (1 ml) was added dropwise. The reaction mixture was stirred for 48 h at room temperature. The reaction mixture was concentrated in vacuo and the crude product was diluted with a mixture of Water / acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 1] and the desired compound (10 mg, 0.02 mmol) was obtained.

\[ \text{NMR (400 MHz, DMSO-}^d) \] δ ppm: 9.60 (s, 1 H), 8.71 (d, 1 H), 8.31 (d, 1 H), 7.54 - 7.63 (m, 1 H), 7.08 (dd, 1 H), 6.85 - 6.96 (m, 2 H), 6.54 (s, 1 H), 6.16 (s, 1 H), 4.50 (t, 2 H), 4.12 (br. s., 2 H), 3.61 (s, 2 H), 3.11 (q, 2 H), 2.04 - 2.14 (m, 2 H), 1.38 (s, 9 H).

Methode B

To a solution of 8-(chloromethyl)-15,19-difluoro-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-(metheno)-1,5,ll,13-benzodioxadiazacyclooctadecine (110 mg, 0.27 mmol, see Intermediate 40) and 2-(Boc-amino)ethanethiol (92 µmol, 0.545 mmol, CAS-RN 67385-09-5) in ethanol (10 ml) was added cesium carbonate (195.2 mg, 0.599 mmol). The reaction mixture was stirred overnight at room temperature. After 24 h of stirring, the reaction mixture was concentrated in vacuo and washed with an aqueous solution of sodium bicarbonate. After phase separation, the aqueous phase was extracted twice with DCM and the combined organic phases were dried over sodium sulfate, filtered, and concentrated in vacuo. The desired compound (510 mg) was obtained and used without additional purification.

Intermediate 42
To a solution of tert-butyl [2-([15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,1,13-benzodioxadiazacyclooctadecin-8-yl]methyl)sulfonyl)ethyl]carbamate (300 mg, 0.551 mmol; see Intermediate 41) in DCM (13 ml) was added 3-chloroperoxybenzoic acid (272 mg, 1.21 mmol, 77% max) at 0 °C. After 30 min of stirring at 0 °C, the reaction mixture was stirred overnight at room temperature. The reaction mixture was quenched with an aqueous solution of sodium bicarbonate, diluted with Water and extracted twice with DCM. After phase separation, the combined organic phases were dried over sodium sulfate, filtered, and concentrated in vacuo. The crude was purified by preparative HPLC [Method 1] and the desired compound (132 mg, 0.23 mmol) was obtained.

\[ \text{NMR (500 MHz, DMSO-}d_6) \delta \text{ ppm 9.78 (s, 1 H), 8.69 (d, 1 H), 8.33 (d, 1 H), 7.55 - 7.62 (m, 1 H), 7.09 (dd, 1 H), 7.03 (t, 1 H), 6.91 (td, 1 H), 6.60 (s, 1 H), 6.24 (s, 1 H), 4.52 (t, 2 H), 4.45 (s, 2 H), 4.09 - 4.16 (m, 2 H), 3.38 (q, 2 H), 3.24 (t, 2 H), 2.06 - 2.14 (m, 2 H), 1.39 (s, 9 H).} \]

Intermediate 43

2-([15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,1,13-benzodioxadiazacyclooctadecin-8-yl]methyl)sulfonyl)ethanamine

To a solution of tert-butyl ([15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,1,13-benzodioxadiazacyclooctadecin-8-yl]methyl)sulfonyl)acetate (14 mg, 0.026 mmol; see Intermediate 42) in dioxane (0.5 ml) was added a solution of HCl 4N in dioxane (64 µl, 0.256 mmol). The reaction mixture was stirred for 48 h at room temperature and then concentrated in vacuo. The crude product was diluted with a mixture of Water/acetonitrile (1 ml) and the resulting mixture was directly purified by preparative HPLC [Method 2]. The desired compound (4 mg, 0.01 mmol) was obtained.

\[ \text{NMR (500 MHz, DMSO-}d_6) \delta \text{ ppm 13.54 (br. s., 1 H), 9.78 (s, 1 H), 8.68 (d, 1 H), 8.32 (d, 1 H), 7.52 - 7.63 (m, 1 H), 7.08 (dd, 1 H), 6.90 (td, 1 H), 6.60 (s, 1 H), 6.20 (s, 1 H), 4.54 (s, 2 H), 4.52 (t, 2 H), 4.27 (s, 2 H), 4.13 (t, 2 H), 2.05 - 2.14 (m, 2 H).} \]
**Intermediate 44**

methyl N-(tert-butoxycarbonyl)-L-valyl-L-alaninate

To a cooled solution of N-(tert-butoxycarbonyl)-L-valine (500 mg, 2.30 mmol) in DMF (1.2 ml) was added methyl L-alaninate hydrochloride (1:1) (385 mg, 2.76 mmol), HATU (1.31 g, 3.45 mmol) and 4-methylmorpholine (760 µl, 6.9 mmol) at 0°C. The reaction mixture was stirred for 1.5 h at 0°C. The reaction mixture was quenched with a saturated aqueous solution of ammonium chloride, diluted with Water and extracted twice with MTBE. After phase separation, the combined organic phases were dried over sodium sulfate, filtered, and concentrated in vacuo. The crude was purified by preparative HPLC [Method 1] and the desired compound (440 mg, 1.46 mmol) was obtained.

LC-MS [Method 16]: R_t = 2.10 min; MS (ESIpos): m/z = 303 [M+H]^+

**Intermediate 45**

N-(tert-butoxycarbonyl)-L-valyl-L-alanine

To a solution of methyl N-(tert-butoxycarbonyl)-L-valyl-L-alaninate (428 mg, 1.42 mmol, see Intermediate 44) in a mixture of THF/Water (2/1, 10 ml) was added Lithium hydroxide monohydrate (119 mg, 2.83 mmol) at 0°C. The reaction mixture was stirred for 3 hours at 0°C and was then diluted with Water. The reaction mixture was acidified with TFA (0.327 ml, 4.25 mmol) and extracted twice with ethyl acetate. After phase separation, the combined organic phases were dried over sodium sulfate, filtered, and concentrated in vacuo. The crude was purified by preparative HPLC and the desired compound (299 mg, 1.04 mmol) was obtained.

LC-MS [Method 15]: R_t = 0.91 min; MS (ESIneg): m/z = 287 [M-H]^−
Intermediate 46

To a cooled solution of 2-[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl]sulfonyl]ethanamine (30.0 mg, 63.0 µmol; see Intermediate 43) in DMF (1.2 ml) was added (N-(fert-butoxycarbonyl)-L-valyl-L-alanine (27.2 mg, 94.4 µmol; see Intermediate 45), HATU (35.9 mg, 94.4 µmol) and 4-methylmorpholine (28 µl, 250 µmol) at 0°C. The reaction mixture was stirred for 1.5 h at 0°C and was then diluted with a mixture of Water/TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 4, flow: 20 ml/min] and the desired compound (36.1 mg, 0.05 mmol) was obtained.

LC-MS [Method 12]: R_t = 2.25 min; MS (ESIpos): m/z = 747 [M+H]^+

1H-NMR (500 MHz, DMSO-de) δ [ppm]: -0.007 (5.37), 0.006 (2.84), 0.785 (2.84), 0.799 (2.90), 0.851 (3.99), 0.865 (3.98), 1.147 (0.99), 1.217 (4.01), 1.231 (4.19), 1.374 (16.00), 2.089 (0.78), 2.358 (0.64), 2.361 (0.88), 2.365 (0.64), 2.631 (0.63), 2.635 (0.87), 2.639 (0.63), 3.173 (0.81), 3.186 (1.56), 3.199 (0.94), 4.118 (1.21), 4.128 (1.47), 4.136 (1.17), 4.246 (0.67), 4.450 (2.14), 4.456 (2.02), 4.508 (0.70), 4.526 (0.70), 6.234 (2.74), 6.599 (1.91), 6.898 (0.79), 6.903 (0.97), 7.015 (0.57), 7.034 (0.57), 7.068 (0.79), 7.073 (0.85), 7.090 (0.85), 7.096 (0.84), 7.585 (0.61), 8.090 (0.61), 8.105 (0.58), 8.278 (1.02), 8.325 (1.20), 8.725 (1.02), 8.737 (1.02), 9.899 (1.21).
To a solution of N-(tert-butoxycarbonyl)-L-valyl-N-[[15,19-difluoro-3,4-dihydro-2H,1IH-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl] sulfonyl]ethyl]-L-alaninamide (34.2 mg, 45.8 µmol; see Intermediate 46) in DCM (1 ml) was added dropwise a solution of TFA in DCM (235 µl, 0.92 mmol, 30% TFA in DCM). The reaction mixture was stirred for 30 min at room temperature, and then concentrated in vacuo. The desired TFA salt (35.6 mg, 0.05 mmol) was used without further purification.

LC-MS [Method 15]: R<sub>t</sub> = 1.06 min; MS (ESIpos): m/z = 647 [M+H-TFA]<sup>+</sup>

1H-NMR (500 MHz, DMSO-de) δ [ppm]: -0.120 (0.68), -0.007 (7.47), 0.006 (3.91), 0.117 (0.60), 0.900 (11.03), 0.914 (16.00), 0.930 (10.58), 1.147 (0.97), 1.238 (11.90), 1.252 (10.84), 2.020 (1.02), 2.034 (1.49), 2.046 (1.51), 2.060 (1.10), 2.088 (2.06), 2.101 (2.01), 2.358 (0.74), 2.362 (0.97), 2.366 (0.71), 2.632 (0.63), 2.635 (0.86), 3.234 (2.15), 3.248 (4.43), 3.263 (2.80), 3.475 (2.64), 3.489 (3.69), 3.502 (5.17), 3.515 (5.96), 3.525 (6.68), 3.536 (7.50), 3.552 (7.78), 3.576 (9.34), 3.587 (10.01), 3.598 (9.12), 4.117 (3.08), 4.126 (3.49), 4.136 (2.97), 4.310 (1.93), 4.324 (2.88), 4.339 (1.81), 4.447 (9.27), 4.499 (1.96), 4.516 (2.28), 4.533 (1.87), 6.230 (6.93), 6.231 (7.15), 6.587 (6.18), 6.589 (6.18), 6.885 (1.23), 6.890 (1.38), 6.902 (2.26), 6.907 (2.51), 6.919 (1.27), 6.924 (1.41), 7.071 (2.11), 7.076 (2.18), 7.094 (2.18), 7.099 (2.11), 7.564 (1.08), 7.572 (1.23), 7.578 (1.35), 7.582 (1.59), 7.586 (1.51), 7.590 (1.38), 7.595 (1.24), 7.603 (1.05), 8.021 (3.90), 8.031 (3.91), 8.307 (1.41), 8.319 (3.06), 8.326 (6.00), 8.331 (6.48), 8.569 (2.80), 8.583 (2.77), 8.684 (4.18), 8.696 (4.21), 9.779 (6.48).
Intermediate 48

3-([15,19-difluoro-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-(metheno)-l,5,1143-benzodioxadiaza-
cyclooctadecin-8-yl][methyl]sulfanyl)propanoic acid

1.81 g (4.48 mmol; see Intermediate 40) 8-(chloromethyl)-15,19-difluoro-3,4-dihydro-2H,1 1H-10,6-(azeno)-12,16-(metheno)-l,5,ll,13-benzodioxazacyclooctadecine were dissolved in ethanol. 590 µl (6.7 mmol) 3-mercaptopropionic acid and 4.38 g (13.4 mmol) cesium carbonate were added and the mixture was stirred for 16 h at room temperature. Most of the solvent was removed under reduced pressure and the residue was treated with a 5% solution of potassium hydrogen sulfate in Water until the pH reached approximately at 2. The formed precipitate was filtered off and dried under high vacuum.

Product: 1.87 g (90 % purity, 80 % yield).

LC-MS [Method 15]: R_t = 1.43 min; MS (ESIpos): m/z = 474 [M+H]^+.

¾-NMR (400 MHz, DMSO-de) δ [ppm]: 1.140 (0.75), 2.315 (0.47), 2.319 (0.61), 2.324 (0.52), 2.358 (1.13), 2.594 (1.18), 2.609 (1.55), 2.627 (0.61), 2.657 (0.56), 2.661 (0.66), 2.666 (0.52), 2.701 (1.18), 3.278 (4.42), 3.613 (2.87), 4.110 (0.85), 4.484 (0.56), 6.148 (1.51), 6.525 (1.41), 6.883 (0.56), 6.890 (0.61), 7.049 (0.52), 7.055 (0.52), 7.077 (0.52), 8.292 (1.18), 8.298 (1.18), 8.689 (0.99), 8.704 (1.04), 9.620 (1.46).
**Intermediate 49**

3-([(1549-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,1143-benzodioxadiaza-cyclooctadecin-8-yl)methyl]sulfonyl)propanoic acid

51.00 g (purity 90%, 1.90 mmol; see Intermediate 48) 3-([(15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazcyclooctadecin-8-yl)methyl]sulfanyl)propanoic acid was dissolved in 40 ml DCM and 8 ml methanol. 984 mg (purity 70%, 3.99 mmol) m-chloroperbenzoic acid was added and the mixture was stirred for 75 min at room temperature. DCM was added and mixture was washed with saturated sodium hydrogen sulfide. The organic layer was separated, treated with DMF and CAN. The precipitate was filtered off and dried under high vacuum.

Product: 570 mg (74 % purity, 44 % yield).

LC-MS [Method 13]: $R_t = 0.98$ min; MS (ESIpos): $m/z = 506$ [M+H]+.

**Intermediate 50**


'H-NMR (400 MHz, DMSO-de) $\delta$ [ppm]: 2.101 (13.12), 4.117 (15.68), 4.434 (16.00), 6.244 (9.44), 7.089 (8.64), 8.318 (12.48), 8.687 (9.44), 9.777 (8.96).
87.6 mg (224 µmol) N^2-(tert-butoxycarbonyl)-N^1-(9H-fluoren-9-ylmethoxy)carbonyl]-L-lysine and 86 mg (224 µmol) 2,5,8,11,14,17,20,23-octaoxapentacosan-25-amine were dissolved in 2.3 ml DMF. 85.3 mg (224 µmol) HATU and 160 µl (930 µmol) N,N-diisopropylethylamine were added. The mixture was stirred at room temperature for 135 min. Ethyl acetate was added and the mixture was washed with saturated aq. sodium hydrogen carbonate solution and two times with 5% aq. potassium hydrogen sulfate solution. The organic layer was separated, dried over sodium sulfate and evaporated under reduced pressure. The resulting product was used in the next step without further purification.

Product: 105 mg (96 % purity, 65 % yield).

LC-MS [Method 13]: R_t = 1.08 min; MS (ESIpos): m/z = 834 [M+H]^+.

**Intermediate 51**

*tert-butyl [(28S)-32-amino-27-oxo-2,5,8,11,14,17,20,23-octaoxa-26-azadotriacontan-28-yl]carbamate*

105 mg (126 µmol; see Intermediate 50) 9H-fluoren-9-ylmethyl [(28S)-28-[(tert-butoxycarbonyl)amino]-27-oxo-2,5,8,11,14,17,20,23-octaoxa-26-azadotriacontan-32-yl]carbamate were dissolved in 1.00 ml DMF and treated with 100 µl (101 µmol) piperidine. The reaction mixture was stirred at room temperature for lh. The solvent was removed under reduced pressure. The resulting product was used in the next step without further purification.

Product: 109 mg (purity 70%, yield: quant.).

LC-MS [Method 15]: R_t = 0.79 min; MS (ESIpos): m/z = 612 [M+H]^+.
**Intermediate 52** tert-butyl \( \{(285)-38-[15,19\text{-difluoro}-3,4\text{-dihydro}-2H,1\text{H}-10,6-(azeno)-12,16-(metheno)-1,5,11,13\text{-benzodioxadiazacyclooctadecin-8-yl]}-37,37\text{-dioxido}-27,34\text{-dioxo}-2,5,8,11,14,17,20,23\text{-octaoxa-37}\lambda\text{-thia}-26,33\text{-diazaoctatriacontan-28-yl}\}\) carbamate

\[
\begin{align*}
\text{H} & \quad \text{O} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{O} & \quad \text{O} \\
\text{S} & \quad \text{O} \\
\text{N} & \quad \text{N} \\
\text{F} & \quad \text{F}
\end{align*}
\]

28.3 mg (56.1 μg; see Intermediate 49) \( 3-\{(15,19\text{-difluoro}-3,4\text{-dihydro}-2H,1\text{H}-10,6-(azeno)-12,16-(metheno)-1,5,11,13\text{-benzodioxadiazacyclooctadecin-8-yl}]methyl\} sulfonyl\)propanoic acid were dissolved in 900 μl DMF. 25.6 mg (67.3 μg) HATU were added to the mixture. After stirring for 10 min, 220 μg \( N,N\)-diisopropylethylamine and 49.0 mg (purity 70%, 56.1 μg; see Intermediate 51) tert-butyl \( [(28S)-32\text{-amino-27-oxo-2,5,8,11,14,17,20,23\text{-octaoxa-37}\lambda\text{-thia}-26,33\text{-diazaoctatriacontan-28-yl}]\) carbamate were added and the mixture was placed in a refrigerator at -4°C for 16 h. The product was isolated by reversed-phase HPLC (ACN/Water with 0.1% TFA, gradient 35-95%).

Product: 19.0 mg (76% purity, 24% yield).

LC-MS [Method 13]: \( R_t = 1.07 \text{ min; } M \text{S (ESIpos): } m/z = 1099 \ [M+H]^+ \).
19.0 mg, (purity 76%, 13.1 µmol) tert-butyl {(28S)-38-[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]-37,37-dioxido-27,34-dioxo-2,5,8,11,14,17,20,23-octaoxa-37lamba 6-thia-26,33-diazaoctatriacontan-28-yl}carbamate (19.0 mg, 76 % purity, 13.1 µmol; see Intermediate 52) were dissolved in 380 µL DCM and 66 µL (260 µmol) of 4N hydrochloric acid in 1,4-dioxane. After stirring for 1 h the solvent was removed under reduced pressure. Product: 10.0 mg (82 % purity, 60 % yield).

LC-MS [Method 13]: Rₜ = 0.85 min; MS (ESIpos): m/z = 999 [M+H]+.

**Intermediate 54**

Methyl 6-{{[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl}(methyl)oxido-6-sulfanylidene|carbamoyl|amino)hexanoate

To a solution of 15,19-difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2 H₁₁H₁₀,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine (178 mg, 399 µmol; see Intermediate 11) in DMF (7 mL) was added at r.t. triethylamine (56 µL, 400 µmol) and methyl 6-isocyanatohexanoate (68.3
mg, 399 µmol). After stirring for 14 h at r.t. trifluoroacetic acid (3 drops) was added and the mixture was purified by preparative HPLC (Method 10) to give the title compound (170 mg, 0.28 mmol).

LC-MS (Method 6): R<sub>t</sub> = 1.31 min; MS (ESI+): m/z = 618.4.

Intermediate 55
6-[[[15,19-Difluoro-3,4-dihydro-2H,11 H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctaderin-8-yl]methyl](methyl)oxido-lambda<sup>6</sup>-sulfanylidene][carbamoyl]amino)hexanoic acid trifluoroacetate

To a solution of methyl 6-[[[15,19-difluoro-3,4-dihydro-2H,11 H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl] (methyl)oxido<sup>6</sup> sulfanylidene][carbamoyl]amino)hexanoate (170 mg, 275 µmol; see Intermediate 54) in THF (9 mL) and Water (4.5 mL) was added at r.t. lithium hydroxide monohydrate (23.1 mg, 550 µmol). After stirring for 14 h trifluoroacetic acid (42 µl, 550 µmol) was added and the solvent was removed under reduced pressure. The residue was taken up in acetonitrile (3 mL) and purified by preparative HPLC (Method 8) to give the title compound (130 mg, 0.18 mmol).

1H-NMR (400 MHz, acetonitrile-<i>d</i><sub>3</sub>) δ [ppm]: 0.545 (0.51), 0.630 (2.04), 0.644 (2.85), 0.660 (2.39), 0.681 (1.12), 0.741 (1.12), 0.758 (2.45), 0.777 (2.75), 0.796 (1.89), 0.850 (1.02), 0.869 (2.50), 0.887 (3.06), 0.906 (2.09), 0.925 (0.66), 1.479 (2.45), 1.495 (2.60), 1.570 (3.16), 1.589 (5.61), 1.608 (2.70), 1.718 (0.92), 1.723 (2.14), 1.728 (2.85), 1.732 (2.09), 1.737 (0.92), 1.924 (6.22), 1.948 (0.82), 2.060 (0.97), 2.065 (2.14), 2.070 (3.01), 2.074 (2.19), 2.079 (0.97), 2.340 (2.70), 2.506 (16.00), 2.602 (0.66), 3.505 (3.46), 3.517 (4.03), 3.528 (3.41), 3.884 (2.34), 3.906 (2.80), 3.926 (2.24), 4.096 (0.61), 4.133 (6.22), 4.169 (0.56), 5.666 (9.89), 5.669 (9.63), 6.020 (6.73), 6.244 (1.68), 6.276 (1.83), 6.282 (2.04), 6.297 (3.06), 6.303 (3.46), 6.317 (1.73), 6.324 (1.99), 6.464 (2.60), 6.471 (2.70), 6.493 (2.75), 6.499 (2.60), 6.954 (1.48), 6.965 (1.73), 6.972 (1.83), 6.976 (2.14), 6.982 (2.04), 6.986 (1.83), 6.994 (1.68), 7.003 (1.48), 7.720 (7.44), 7.726 (7.13), 8.069 (5.55), 8.084 (5.61), 9.160 (5.50).
Final Intermediate F1

(rac)-N-[[15,19-difluoro-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-(metheno)-1,5,ll,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido-\(\lambda^6\)-sulfanylidene]-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamide

Under Argon, to a solution of (rflc)-15,19-difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-(metheno)-1,5,ll,13-benzodioxadiazacyclooctadecine 10.0 mg, 22.4 \(\mu\)mol; see Intermediate 11) in DMF (1 ml) was added 3-maleimidopropionic acid 7.6 mg, 44.8 \(\mu\)mol, HOBT 10.3 mg, 53.8 \(\mu\)mol, EDCI 10.3 mg, 53.8 \(\mu\)mol and finally \(\text{N,N-diisopropylethylamine}\) (23 \(\mu\)l, 130 \(\mu\)mol) at room temperature. The reaction mixture was stirred overnight at room temperature and was then diluted with a mixture of Water/TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 2] and the desired compound (9 mg, 14.76 \(\mu\)mol) was obtained.

LC-MS [Method 15]: \(R_t = 1.35\) min; MS (ESIpos): \(m/z = 598\) [M+H]+

IH-NMR (500 MHz, DMSO-d6) \(\delta\) [ppm]: 1.246 (0.82), 1.260 (0.94), 2.083 (1.23), 2.086 (1.24), 2.100 (1.21), 2.462 (1.96), 2.475 (3.42), 2.478 (3.25), 2.731 (1.04), 2.891 (1.27), 3.610 (2.56), 3.624 (4.06), 3.639 (2.31), 4.112 (1.92), 4.121 (2.17), 4.130 (1.82), 4.499 (1.49), 4.516 (1.70), 4.533 (1.43), 4.741 (5.96), 6.242 (4.12), 6.244 (3.90), 6.594 (3.82), 6.596 (3.65), 6.879 (0.72), 6.884 (0.82), 6.896 (1.34), 6.901 (1.47), 6.913 (0.75), 6.918 (0.80), 6.992 (16.00), 7.065 (1.25), 7.070 (1.28), 7.088 (1.35), 7.093 (1.23), 7.579 (0.94), 7.583 (0.90), 8.322 (3.16), 8.326 (2.99), 8.672 (2.57), 8.684 (2.52), 9.809 (3.84).

Final Intermediate F2

(rac)-N-[[15,19-difluoro-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-(metheno)-1,5,ll,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido-\(\lambda^6\)-sulfanylidene]-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide
To a cooled solution of (rac)-15,19-difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine (12.0 mg, 26.9 μmol; see Intermediate 1) in DMF (0.5 ml) was added (2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)acetic acid (6.25 mg, 40.3 μmol), HATU (15.3 mg, 40.3 μmol) and 4-methylmorpholine (12 μl, 110 μmol) at 0°C. The reaction mixture was stirred for 2 h at 0°C and was then diluted with a mixture of Water/TFA/acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 3] and the desired compound (13 mg, 0.02 mmol) was obtained.

LC-MS [Method 12]: Rₜ = 2.03 min; MS (ESIpos): m/z = 584 [M+H]⁺

1H-NMR (500 MHz, DMSO-d6) δ [ppm]: 2.073 (6.77), 2.103 (1.44), 2.116 (1.43), 4.062 (0.82), 4.097 (6.66), 4.100 (6.63), 4.119 (2.43), 4.129 (2.72), 4.135 (2.42), 4.511 (1.39), 4.528 (1.64), 4.545 (1.32), 4.767 (6.75), 6.243 (4.59), 6.245 (4.63), 6.581 (4.26), 6.583 (4.31), 6.881 (0.78), 6.887 (0.93), 6.898 (1.49), 6.903 (1.64), 6.915 (0.85), 6.920 (0.88), 7.070 (1.44), 7.075 (1.47), 7.092 (1.56), 7.097 (1.75), 7.108 (16.00), 7.573 (0.83), 7.579 (0.95), 7.582 (1.05), 7.587 (1.02), 7.590 (0.91), 7.596 (0.79), 8.327 (3.43), 8.332 (3.29), 8.682 (2.75), 8.694 (2.70), 9.706 (4.20).

Final Intermediate F3

N-[6-(2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)hexanoyl]-L-valyl -N-[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido-λ⁵-sulfanylidene]-L-alaninamide
To a cooled solution of L-valyl-N-[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl] (methyl)oxido^6-sulfanylidene]-L-alaninamide trifluoroacetate (10.5 mg, 14.4 µmol; see Intermediate 15) in DMF (0.8 ml) was added 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoic acid (4.55 mg, 21.6 µmol), HATU (10.9 mg, 28.7 µmol) and 4-methylmorpholine (5.5 µl, 50 µmol) at 0°C. The reaction mixture was stirred for 2 h at 0°C and was then diluted with a mixture of Water/TFA/acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 3] and the desired compound (11 mg, 0.01 mmol) was obtained as a mixture of 2 diastereoisomers.

LC-MS [Method 12]: Rt = 2.11, 2.13 min; MS (ESI+): m/z = 810 [M+H]^+

1H-NMR (500 MHz, DMSO-d6) δ [ppm]: -0.007 (2.12), 0.006 (1.24), 0.794 (5.78), 0.807 (5.96), 0.827 (6.39), 0.841 (6.89), 0.849 (6.11), 0.862 (6.12), 0.873 (6.25), 0.886 (5.97), 1.173 (2.39), 1.187 (3.63), 1.203 (2.96), 1.219 (1.48), 1.232 (7.09), 1.246 (12.38), 1.261 (6.69), 1.445 (1.39), 1.460 (3.48), 1.475 (4.95), 1.491 (4.60), 1.505 (2.93), 1.517 (1.44), 1.951 (1.20), 1.965 (1.50), 1.980 (1.48), 1.993 (1.15), 2.091 (2.64), 2.120 (1.88), 2.131 (1.76), 2.145 (1.64), 2.158 (1.47), 2.174 (1.52), 2.180 (1.61), 2.202 (0.78), 3.181 (12.70), 3.199 (11.90), 3.349 (3.44), 3.363 (6.58), 3.378 (3.29), 4.114 (3.69), 4.123 (4.62), 4.132 (3.63), 4.157 (1.85), 4.164 (1.85), 4.171 (2.55), 4.178 (2.42), 4.185 (1.90), 4.193 (1.71), 4.219 (1.63), 4.232 (1.81), 4.237 (1.94), 4.249 (2.37), 4.262 (2.01), 4.266 (2.09), 4.280 (1.84), 4.514 (3.13).
4.748 (1.85), 4.776 (3.76), 4.796 (2.48), 4.823 (1.10), 4.834 (2.17), 4.861 (1.30), 6.268 (3.86), 6.290 (4.10), 6.615 (6.97), 6.884 (1.41), 6.901 (2.44), 6.906 (2.54), 6.918 (1.36), 6.922 (1.38), 6.983 (16.00), 6.988 (14.82), 7.066 (2.43), 7.071 (2.51), 7.089 (2.53), 7.094 (2.39), 7.583 (1.77), 7.669 (1.70), 7.687 (1.65), 7.728 (1.54), 8.053 (1.61), 8.068 (1.56), 8.107 (1.74), 8.121 (1.68), 8.297 (3.08), 8.302 (3.07), 8.313 (2.96), 8.318 (2.84), 8.668 (2.25), 8.680 (2.28), 8.702 (2.45), 8.714 (2.45), 9.756 (3.43), 9.780 (3.66).

**Final Intermediate F4**

N-[(2,5-dioxo-2,5-dihydro-lH-pyrrol-1-yl)acetyl]-L-valyl -N-[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido-λ^5^-sulfanylidene]-L-alaninamide

To a cooled solution of L-valyl-N-[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl] (methyl)oxido-λ^5^-sulfanylidene]-L-alaninamide trifluoroacetate (8.00 mg, 10.9 µmol; see Intermediate 15) in DMF (1 ml) was added (2,5-dioxo-2,5-dihydro-lH-pyrrol-1-yl)acetic acid (2.55 mg, 16.4 µmol), HATU (6.24 mg, 16.4 µmol) and 4-methylmorpholine (4.8 µL, 44 µmol) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C and was then diluted with a mixture of Water /TFA/acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 3, flow 20 ml/min] and the desired compound (4.2 mg, 0.01 mmol) was obtained as a mixture of 2 diastereoisomers.

LC-MS [Method 13]: R_t = 1.14 min; MS (ESIpos): m/z = 754 [M+H]^+
$^1$H-NMR (500 MHz, DMSO-d$_6$) $\delta$ [ppm]: -0.120 (0.57), -0.007 (5.14), 0.007 (4.18), 0.815 (4.16), 0.828 (4.43), 0.840 (4.50), 0.854 (4.73), 0.864 (4.33), 0.878 (5.42), 0.881 (5.68), 0.895 (4.26), 1.147 (0.68), 1.233 (5.25), 1.249 (8.74), 1.263 (4.69), 1.975 (0.88), 1.989 (1.15), 2.003 (1.13), 2.016 (0.85), 2.073 (2.12), 2.099 (1.65), 2.362 (0.62), 3.190 (9.06), 3.215 (8.88), 4.097 (0.71), 4.114 (2.67), 4.131 (5.09), 4.141 (3.63), 4.147 (3.71), 4.152 (3.40), 4.174 (1.39), 4.182 (1.06), 4.188 (1.57), 4.196 (1.45), 4.203 (0.99), 4.211 (0.89), 4.230 (0.83), 4.243 (0.95), 4.248 (1.67), 4.261 (1.67), 4.267 (1.00), 4.279 (0.78), 4.502 (1.48), 4.518 (1.97), 4.535 (1.46), 4.747 (0.88), 4.777 (4.31), 4.814 (1.51), 4.841 (0.75), 6.265 (2.97), 6.615 (2.72), 6.627 (2.81), 6.881 (0.97), 6.886 (1.10), 6.980 (1.83), 6.903 (2.07), 6.915 (1.00), 6.920 (1.10), 7.066 (1.77), 7.071 (1.93), 7.085 (16.00), 7.094 (1.97), 7.565 (0.75), 7.572 (0.88), 7.580 (1.20), 7.596 (0.83), 7.604 (0.69), 8.155 (1.32), 8.171 (2.19), 8.185 (1.40), 8.193 (1.45), 8.204 (1.50), 8.211 (1.51), 8.218 (1.35), 8.341 (4.22), 8.346 (4.08), 8.672 (1.68), 8.684 (1.70), 8.697 (1.76), 8.709 (1.71), 9.755 (2.71), 9.765 (2.73).

**Final Intermediate F5**

N-[6-(2,5-dioxo-2,5-dihydro-lH-pyrrol-1-yl)hexanoyl]-L-valyl -$N^5$-carbamoyl-N-[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azono)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido-$\lambda^5$-sulfanylidene]-L-ornithinamide
To a cooled solution of L-valyl-N\(^5\)-carbamoyl-N-[[15,19-difluoro-3,4-dihydro-2H,IIH-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl] (methyl)oxido\(^6\) -sulfanylidene]-L-ornithinamide trifluoroacetate (5.00 mg, 6.12 \(\mu\)mol; see Intermediate 19) in DMF (0.8 ml) was added 6-(2,5-dioxo-2,5-dihydro-L-pyrrol-l-yl)hexanoic acid (1.94 mg, 9.18 \(\mu\)mol), HATU (4.66 mg, 12.2 \(\mu\)mol) and 4-methylmorpholine (2.4 \(\mu\)l, 21 \(\mu\)mol) at 0\(^\circ\) C. The reaction mixture was stirred for 2 h at 0\(^\circ\) C and was then diluted with a mixture of Water/TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 3, flow: 20 ml/min] and the desired compound (4.3 mg, 0.005 mmol) was obtained as a mixture of 2 diastereoisomers.

\[\text{N-}[(2,5\text{-dioxo-2,5\text{-dihydro-L-pyrrol-l-yl})acetyl]-L\text{-valyl-N}^5\text{-carbamoyl-N-}[[15,19\text{-difluoro-3,4-dihydro-2H,IIH-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl}(methyl)oxido-\lambda^2\text{-sulfanylidene]-L-ornithinamide}}\]
To a cooled solution of L-valyl-N⁵-carbamoyl-N-[[(15,19-difluoro-3,4-dihydro-2H,12H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl)methyl] (methyl)oxido⁻⁶-
5
sulfanylidene]-L-ornithinamide trifluoroacetate (5.00 mg, 6.12 µmol; see Intermediate 19) in DMF (0.5 ml) was added (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid (1.42 mg, 9.18 µmol), HATU (3.49 mg, 9.18 µmol) and 4-methylmorpholine (2.7 µl, 24 µmol) at 0°C. The reaction mixture was stirred for 2 h at 0°C and was then diluted with a mixture of Water/acetonitrile (1:1). The resulting mixture was directly purified by preparative HPLC [Method 3, flow 20 ml/min] and the desired compound (3 mg, 0.004 mmol) was obtained as a mixture of 2 diastereoisomers.

LC-MS [Method 12]: $R_t = 1.79$ min, 1.80 min; MS (ESIpos): $m/z = 840$ [M+H]⁺

1H-NMR (500 MHz, DMSO-d₆) δ [ppm]: -0.007 (4.52), 0.007 (2.80), 0.804 (3.58), 0.818 (3.75), 0.834 (4.00), 0.847 (7.20), 0.860 (4.05), 0.867 (3.97), 0.881 (3.63), 1.147 (0.83), 1.237 (0.95), 1.389 (1.16), 1.993 (0.76), 2.007 (0.98), 2.020 (0.95), 2.034 (0.72), 2.106 (1.37), 2.357 (4.49), 2.362 (1.38), 2.366 (1.81), 2.369 (13.31), 2.381 (12.77), 2.393 (3.92), 2.635 (0.70), 2.708 (0.57), 2.941 (1.90), 3.188 (7.80), 3.211 (7.54), 4.126 (2.89), 4.134 (2.99), 4.145 (4.05), 4.150 (3.61), 4.161 (4.99), 4.276 (0.70), 4.289 (0.79), 4.295 (1.38), 4.307 (1.37), 4.313 (0.81), 4.326 (0.70), 4.503 (1.24), 4.521 (1.68), 4.537 (1.20), 4.739 (0.81), 4.767 (3.36), 4.813 (1.20), 4.841 (0.66), 5.919 (0.69), 6.276 (2.41), 6.302 (2.50), 6.622 (2.89), 6.881 (0.83), 6.886 (1.00), 6.897 (1.56), 6.902 (1.78), 6.914 (0.91), 6.919 (0.99), 7.066 (1.54), 7.071 (1.68), 7.084 (16.00), 7.089 (2.14), 7.094 (1.73), 7.491 (0.77), 7.566 (1.05), 7.574 (1.16), 7.581 (1.41), 7.598 (1.02), 7.605 (0.87), 8.122 (0.94), 8.136 (1.87), 8.155 (1.61), 8.176 (1.46), 8.194 (1.03), 8.339 (3.79), 8.345 (3.62), 8.672 (1.45), 8.684 (1.47), 8.699 (1.56), 8.711 (1.56), 9.769 (2.17), 9.783 (2.27).
Final Intermediate F7

N-[[15,19-(3,4-dihydro-2H,11H-10,6-(3-ζεηο)-12,16-(ηθενο)-1,5,11,13-
benzodioxadiazacyclooctadecin-8-yl)methyl](methyl)oxido-λ^6-sulfanylidene]amino]-3-methyl-l-
oxobutan-2-yl]-6-(2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)hexanamide

To a cooled solution of N-[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-
1,5,11,13-benzodioxadiazacyclooctadecin-8-yl)methyl] (methyl)oxido-λ^6-sulfanylidene]-D-valinamide
trifluoroacetate (7.00 mg, 10.6 μmol; see Intermediate 21) in DMF (0.8 ml) was added 6-(2,5-dioxo-2,5-
dihydro-lH-pyrrol-l-yl)hexanoic acid (3.36 mg, 15.9 μmol), HATU (8.07 mg, 21.2 μmol) and 4-
methylmorpholine (4.1 μl, 37 μmol) at 0°C. The reaction mixture was stirred for 2 h at 0°C and was then diluted with a mixture of Water /TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 3] and the desired compound (3.2 mg, 0.004 mmol) was obtained as a mixture of 2 diastereoisomers.

LC-MS [Method 12]: R_t = 2.13 min, 2.15 min; MS (ESIpos): m/z = 739 [M+H]^+

1H-NMR (500 MHz, DMSO-de) δ [ppm]: 0.804 (11.45), 0.819 (16.00), 0.834 (7.76), 0.838 (8.05), 0.852 (6.60), 1.146 (1.93), 1.161 (3.21), 1.176 (3.50), 1.191 (2.24), 1.234 (0.62), 1.455 (3.56), 1.470 (3.26), 1.486 (2.77), 1.496 (2.48), 2.048 (0.96), 2.073 (6.31), 2.084 (4.37), 2.096 (4.11), 2.131 (2.06), 2.144 (1.74), 2.158 (2.22), 2.173 (2.25), 2.191 (1.89), 2.206 (1.21), 2.220 (0.78), 3.200 (12.59), 3.284 (11.62), 3.319 (2.49), 3.326 (3.16), 3.333 (4.75), 3.340 (5.22), 3.347 (2.91), 3.354 (2.56), 4.124 (9.20), 4.134 (8.28), 4.149 (4.50), 4.496 (2.63), 4.513 (3.38), 4.529 (2.55), 4.720 (0.65), 4.747 (3.43), 4.757 (3.13), 4.778 (2.70), 4.816 (2.41), 4.843 (1.18), 6.267 (4.08), 6.299 (4.53), 6.613 (4.54), 6.620 (4.14), 6.878...
Final Intermediate F8

(rac)-[[(15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,ll,13-benzodioxadiazacyclooctadecin-8-yl)methyl](methyl)oxido-\(\lambda^5\)-sulfanylidene]-3-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]urea

Under Argon, to a solution of (rac)-15,19-difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,ll,13-benzodioxadiazacyclooctadecine (7.50 mg, 16.8 \(\mu\)mol; see Intermediate 11) in DMF (1 ml) was added 4-nitrophenyl [2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]carbamate (7.69 mg, 25.2 \(\mu\)mol, see Intermediate 22) and \(N,N\)-diisopropylethylamine (8.8 \(\mu\)l, 50 \(\mu\)mol) at room temperature. The reaction mixture was stirred overnight at room temperature, prior to the addition of a further portion of 4-nitrophenyl [2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]carbamate (3 mg, 9.8 \(\mu\)mol), due to incomplete conversion. After 4 h of stirring, the reaction mixture was diluted with a mixture of Water /TFA/acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 2] and the desired compound (8.5 mg, 13.87 \(\mu\)mol) was obtained.

LC-MS [Method 12]: \(R_t = 1.90\) min; MS (ESIpos): \(m/z = 613\) [M+H]+

\(^1\)H-NMR (500 MHz, DMSO-de) \(\delta\) [ppm]: -0.007 (1.49), 0.007 (0.85), 2.069 (1.24), 2.073 (4.51), 2.088 (2.11), 2.102 (1.98), 3.096 (15.84), 3.111 (1.74), 3.123 (3.55), 3.135 (3.64), 3.147 (1.60), 3.174 (1.12), 3.454 (2.78), 3.467 (4.79), 3.479 (2.18), 4.112 (3.21), 4.121 (3.72), 4.130 (2.96), 4.496 (2.31), 4.512 (2.72), 4.529 (2.20), 4.697 (8.08), 6.286 (5.99), 6.612 (6.59), 6.614 (6.61), 6.879 (1.26), 6.884 (1.42), 6.882 (1.53), 6.894 (2.61), 6.899 (2.86), 6.916 (1.53), 6.967 (12.23), 6.978 (10.93), 7.062 (2.56), 7.066 (2.64), 7.084 (2.60), 7.089 (2.57), 7.560 (1.16), 7.568 (1.46), 7.578 (2.02), 7.591 (1.40), 7.599 (1.13), 7.656 (1.69), 7.673 (1.62), 7.706 (1.86), 7.723 (1.83), 8.306 (3.18), 8.311 (3.68), 8.314 (3.54), 8.319 (2.94), 8.673 (2.66), 8.679 (2.87), 8.685 (3.09), 8.691 (2.46), 9.712 (3.85), 9.730 (3.48).
Final Intermediate F9

(rac) N-[2-[[15,19-difluoro-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-(metheno)-1,5,ll,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido -sulfanylidene]carbamoyl]amino)ethyl]-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide

To a cooled solution of (rac) 1-(2-aminoethyl)-3-[15,19-difluoro-3,4-dihydro-2H, 1H-10,6-(azeno)-12,16-(metheno)-1,5,ll,13-benzodioxadiazacyclooctadecin-8-yl]methyl] (methyl)oxido -sulfanylidene]urea trifluoroacetate (13.0 mg, 20.1 µmol; see Intermediate 25) in DMF (1 ml) was added (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid (4.68 mg, 30.2 µmol), HATU (11.5 mg, 30.2 µmol) and 4-methylmorpholine (8.8 µl, 80 µmol) at 0°C. The reaction mixture was stirred for 2 h at 0°C and was then diluted with a mixture of Water/TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 3, flow 20 ml/min] and the desired compound (10.5 mg, 0.02 mmol) was obtained.

LC-MS [Method 13]: R_t = 0.94 min; MS (ESIpos): m/z = 670 [M+H]^+

1H-NMR (500 MHz, DMSO-de) δ [ppm]: -0.007 (2.35), 0.007 (1.51), 2.073 (1.81), 2.086 (2.12), 3.042 (2.37), 3.085 (2.19), 3.096 (3.26), 3.108 (2.70), 3.130 (12.51), 4.016 (12.52), 4.111 (3.18), 4.120 (3.63), 4.129 (3.03), 4.494 (2.38), 4.512 (2.77), 4.528 (2.34), 4.732 (6.88), 6.281 (6.80), 6.623 (5.57), 6.868...
Final Intermediate F10

\[ \text{(rac) N-[2-\{[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl\}(methyl)oxido}  \]^6. \]

\text{sulfanylidene]carbamoyl[amino][ethyl]-6-(2,5-dioxo-2,5-dihydro-L-pyrrol-1-yl)hexanoamide}

To a cooled solution of (rac) 1-(2-aminoethyl)-3-[ [[15,19-difluoro-3,4-dihydro-2H,11H-1 O,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl] (methyl)oxido\(^6\) 6.

\text{sulfanylidene]urea trifluoroacetate (13.0 mg, 20.1 \( \mu \)mol; see Intermediate 25) in DMF (1 ml) was added}

\text{6-(2,5-dioxo-2,5-dihydro-L-pyrrol-1-yl)hexanoic acid (6.37 mg, 30.2 \( \mu \)mol), HATU (15.3 mg, 40.2}

\text{\( \mu \)mol) and 4-methylmorpholine (7.7 \( \mu l \), 70 \( \mu \)mol) at 0° C. The reaction mixture was stirred for 2 h at 0°}

\text{C and was then diluted with a mixture of Water /TFA/acetonitrile (1ml). The resulting mixture was}

\text{directly purified by preparative HPLC [Method 3, flow 20 ml/min] and the desired compound (10 mg,}

\text{0.01 mmol) was obtained.}

LC-MS [Method 12]: \( R_t = 1.81 \) min; MS (ESIpos): \text{m/z = 726} \([\text{M+H}]^+\)

\text{1H-NMR (500 MHz, DMSO-de)} \( \delta \) [ppm]: -0.007 (2.24), 0.007 (1.23), 1.150 (1.46), 1.167 (1.90), 1.182

\text{(1.36), 1.442 (2.03), 1.458 (3.05), 1.473 (2.72), 1.492 (1.39), 2.016 (1.97), 2.031 (2.97), 2.045 (1.56),}

\text{2.073 (4.90), 2.084 (1.50), 2.097 (1.47), 3.026 (1.63), 3.057 (1.22), 3.069 (1.68), 3.082 (1.55), 3.096}

\text{(1.31), 3.115 (9.19), 3.345 (23.2), 3.359 (3.87), 3.373 (1.97), 3.765 (2.97), 4.111 (2.40), 4.120 (2.69),}

\text{4.129 (2.22), 4.493 (1.48), 4.511 (1.73), 4.526 (1.37), 4.734 (4.08), 6.278 (5.19), 6.280 (5.09), 6.624}

\text{(3.05), 6.830 (0.85), 6.877 (0.89), 6.882 (0.97), 6.893 (1.61), 6.898 (1.69), 6.910 (0.87), 6.915 (0.91),}
Final Intermediate Fll

\[ \text{(rac)} \quad \text{N-}[3-\{[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl}(methyl)oxido-6sulfanylidene} \text{carbamoyl} \text{-amino)} \text{propyl}-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide \]

To a cooled solution of \((\text{rac})\) \(1-(3\text{-aminopropyl})-3-[[15,19\text{-difluoro-3,4-dihydro-2H, 1H-1 0,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl}]methyl}\) (methyl)oxido-6. sulfanylidene[urea trifluoroacetate (9.00 mg, 13.6 \(\mu\text{mole}\); see Intermediate 28) in DMF (0.5 ml) was added (2,5-dioxo-2,5-dihydro-1H-pyrrol-1 -yl)acetic acid (3.17 mg, 20.4 \(\mu\text{mole}\)), HATU (7.8 mg, 20.4 \(\mu\text{mole}\)) and 4-methylmorpholine (6 \(\mu\text{l}, 54 \(\mu\text{mole}\)) at 0° C. The reaction mixture was stirred for 2 h at 0° C and was then diluted with a mixture of Water /TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 3, flow 20 ml/min] and the desired compound (8.5 mg, 0.01mmol) was obtained.

LC-MS [Method 12]: \(R_t = 1.73\) min; MS (ESIpos): \(m/z = 684 \ [\text{M+H}]^+\)
Final Intermediate F12

\[\text{N-[3-}{{\text{[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl}(methyl)oxido}}{}^6.\text{sulfanylidene}}\text{carbamoyl}]\text{amino}\text{propyl}\text{-6-(2,5-dioxo-2,5-dihydro-1H-pyrrl-yl)hexanamide} \]

To a cooled solution of (rac) 1-(3-aminopropyl)-3-[ [[15,19-difluoro-3,4-dihydro-2H, 11H-1 0,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl] (methyl)oxido\textsuperscript{a} 6.

sulfanylidene]urea trifluoroacetate (9.00 mg, 13.6 \(\text{m} \mu\text{mol}) see Intermediate 28) in DMF (1 ml) was added 6-(2,5-dioxo-2,5-dihydro-1H-pyrrl-yl)hexanoic acid (4.32 mg, 20.4 \(\text{m} \mu\text{mol}), \text{HATU} (10.4 mg, 27.2 \(\text{m} \mu\text{mol}) and 4-methylmorpholine (5.2 \(\mu\text{l}, 48 \(\mu\text{mol}) at 0\text{°C}. The reaction mixture was stirred for 2 h at 0\text{°C} and was then diluted with a mixture of Water /TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 3, flow 20 ml/min] and the desired compound (10 mg, 0.01 mmol, 96% purity) was obtained.

LC-MS [Method 12]: \(R_t = 1.83 \text{ min;} \text{ MS (ESIpos): } \text{m/z = 740 [M+H]}^+\)

\(\text{\textsuperscript{3}H-NMR (500 MHz, DMSO-de) } \delta \text{ [ppm]}: 1.171 (1.23), 1.463 (2.66), 1.475 (2.93), 2.009 (1.13), 2.024 (1.74), 2.083 (1.08), 2.967 (1.39), 3.016 (1.35), 3.116 (4.62), 3.350 (1.49), 3.364 (2.63), 3.379 (1.32), 4.109 (1.66), 4.119 (1.87), 4.489 (1.11), 4.506 (1.31), 4.522 (1.05), 4.733 (3.26), 5.043 (0.50), 6.274 (3.56), 6.622 (2.93), 6.789 (0.42), 6.875 (0.69), 6.880 (0.79), 6.892 (1.25), 6.897 (1.38), 6.909 (0.71), 6.914 (0.76), 6.987 (16.00), 7.062 (1.16), 7.067 (1.18), 7.085 (1.20), 7.090 (1.15), 7.555 (0.59), 7.564 (0.68), 7.572 (0.86), 7.586 (0.66), 7.594 (0.58), 7.712 (0.75), 8.311 (3.01), 8.316 (2.87), 8.665 (2.37), 8.677 (2.35), 9.755 (2.41).\)
Final Intermediate F13

(rac) N^3-[[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido^6-sulfanylidene]carbamoyl-N-[2-(2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)ethyl]-beta-alaninamide

To a solution of (rac) N-[[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido^6-sulfanylidene]carbamoyl-N-[2-(2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)ethyl]-beta-alanine (3.0 mg, 5.34 µmol; see Intermediate 30) in DMF (0.5 ml) was added l-(2-aminoethyl)-lH-pyrrrole-2,5-dione trifluoroacetate (2.72 mg, 10.7 µmol), HATU (4.06 mg, 10.7 µmol) and 4-methylmorpholine (2.3 µl, 21 µmol) at room temperature. The reaction mixture was stirred for 2 h at room temperature and was then diluted with a mixture of Water/TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 2] and the desired compound (3.5 mg, 5.12 µmol) was obtained.

LC-MS [Method 15]: R_t = 1.22 min; MS (ESIneg): m/z = 682 [M-H]^-

1H-NMR (400 MHz, DMSO-de) δ [ppm]: -0.008 (2.75), 0.008 (2.59), 1.148 (1.26), 1.237 (2.38), 2.072 (3.78), 2.144 (2.38), 2.162 (4.69), 2.181 (2.50), 2.328 (1.47), 2.669 (1.35), 3.114 (14.06), 3.165 (2.61), 3.180 (4.31), 3.196 (4.64), 3.434 (4.34), 3.449 (5.81), 3.463 (3.15), 4.121 (3.17), 4.510 (2.29), 4.720 (6.88), 6.277 (6.34), 6.626 (4.69), 6.698 (1.82), 6.880 (1.26), 6.894 (1.87), 6.901 (2.29), 6.922 (1.24), 6.996 (16.00), 7.069 (2.03), 7.096 (2.01), 7.582 (1.42), 7.962 (1.84), 8.312 (4.62), 8.318 (4.50), 8.668 (4.10), 8.683 (3.94), 9.741 (4.80).
Final Intermediate F14

2-\{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethyl [\{[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl\}(methyl)oxido-\lambda^6-sulfanylidene]carbamate

To a cooled solution of (rac)-2-aminoethyl \{[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl\} (methyl) oxido-\lambda^6-sulfanylidene]carbamate (5.90 mg, 11.1 µmol; see Intermediate 33) in DMF (1 ml) was added (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid (2.57 mg, 16.6 µmol), HATU (6.3 mg, 16.6 µmol) and 4-methylmorpholine (4.9 µl, 44 µmol) at 0° C. The reaction mixture was stirred for 2 h at 0° C and was then diluted with a mixture of Water /TFA/acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 3, flow 20 ml/min] and the desired compound (5.3 mg, 0.01 mmol) was obtained.

LC-MS [Method 13]: R_t = 0.98 min; MS (ESIpos): m/z = 671 [M+H]^+

\(^1\)H-NMR (500 MHz, DMSO-de) δ [ppm]: -0.008 (1.14), 0.005 (0.90), 2.071 (1.74), 2.104 (1.05), 2.369 (0.45), 3.290 (2.30), 3.301 (2.25), 3.312 (0.86), 3.956 (3.04), 3.966 (3.89), 3.976 (3.71), 3.987 (2.48), 4.024 (9.43), 4.123 (2.08), 4.503 (1.05), 4.520 (1.22), 4.775 (4.45), 6.275 (3.63), 6.608 (3.30), 6.883 (0.77), 6.895 (1.21), 6.900 (1.32), 6.912 (0.67), 6.917 (0.72), 7.065 (1.18), 7.070 (1.24), 7.083 (16.00), 7.577 (0.83), 7.591 (0.66), 7.599 (0.55), 8.320 (2.86), 8.325 (2.79), 8.344 (1.36), 8.668 (2.22), 8.680 (2.18), 9.817 (3.34).
Final Intermediate F15

1-{[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl}sulfonyl)ethyl]-1H-pyrrole-2,5-dione

To a cooled solution of 2-{[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl}sulfonyl)ethanamine (8.50 mg, 17.8 µmol; see Intermediate 43) and N-Methoxycarbonylmaleimide (2.77 mg, 17.8 µmol) in a mixture of dioxane/Water (1.2 ml, 10/1) was added a saturated aqueous solution of sodium bicarbonate (79 µl) at 0°C. The reaction mixture was stirred for 2.5 h at 0°C, and then stirred for 1 h at room temperature. The reaction mixture was concentrated in vacuo and directly purified by preparative HPLC [Method 3, flow 20 ml/min]. 6.5 mg (0.01 mmol) of the desired compound was obtained.

LC-MS [Method 15]: Rₜ = 1.38 min; MS (ESIpos): m/z = 557 [M+H]⁺


Final Intermediate F16

N-[2-{[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl}sulfonyl)ethyl]-6-(2,5-dioxo-2,5-dihydro-1H-pyrrolo-1-yl)hexanamide
To a cooled solution of 2-({[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl}sulfonyl)ethanamine (10.0 mg, 2.1 µmol; see Intermediate 43) in DMF (1 ml) was added 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoic acid (6.65 mg, 31.5 µmol), HATU (16 mg, 42.2 µmol) and 4-methylmorpholine (8.1 µl, 73 µmol) at 0° C. The reaction mixture was stirred for 2 h at 0° C and was then diluted with a mixture of Water/TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 3, flow 20 ml/min] and the desired compound (7.6 mg, 0.01 mmol) was obtained.

**LC-MS [Method 12]:** R<sub>t</sub> = 1.98 min; MS (ESIpos): m/z = 670 [M+H]<sup>+</sup>

1H-NMR (500 MHz, DMSO-de) δ [ppm]: -0.007 (1.22), 1.160 (1.03), 1.175 (1.86), 1.190 (1.26), 1.444 (1.72), 1.459 (2.76), 1.477 (2.57), 1.493 (1.41), 2.030 (2.07), 2.045 (3.54), 2.060 (1.95), 2.088 (1.18), 3.223 (1.57), 3.236 (2.98), 3.251 (1.97), 3.344 (2.48), 3.358 (4.40), 3.372 (2.41), 3.441 (1.66), 3.453 (2.71), 3.468 (3.09), 3.480 (2.49), 4.112 (1.75), 4.122 (1.99), 4.131 (1.71), 4.437 (6.18), 4.496 (1.15), 4.513 (1.35), 4.530 (1.06), 6.229 (3.98), 6.230 (4.04), 6.593 (3.65), 6.878 (0.71), 6.884 (0.80), 6.895 (1.25), 6.900 (1.41), 6.912 (0.72), 6.917 (0.79), 6.985 (16.00), 7.064 (1.17), 7.069 (1.25), 7.078 (1.25), 7.092 (1.18), 7.576 (0.88), 7.581 (0.85), 7.584 (0.78), 8.034 (0.80), 8.045 (1.55), 8.316 (3.05), 8.321 (2.94), 8.671 (2.46), 8.683 (2.43), 9.760 (3.76).

**Final Intermediate F17**

N-[2-({[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl}sulfonyl)ethyl]-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamide
To a cooled solution of 2-((15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl)methyl)sulfonyl)ethanamine (10.0 mg, 21 µmol; see Intermediate 43) in DMF (1 ml) was added 4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanoic acid (5.77 mg, 31.5 µmol), HATU (16 mg, 42 µmol) and 4-methylmorpholine (8.1 µl, 73 µmol) at 0°C. The reaction mixture was stirred for 5 h at 0°C and was then diluted with a mixture of Water/TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 2] and the desired compound (10.5 mg, 0.02 mmol) was obtained.

LC-MS [Method 13]: R_t = 1.03 min; MS (ESIpos): m/z = 642 [M+H]^+

\(^1\)H-NMR (500 MHz, DMSO-de) δ [ppm]: 1.686 (0.49), 1.700 (1.60), 1.714 (2.27), 1.729 (1.61), 1.744 (0.52), 2.045 (2.15), 2.060 (3.31), 2.075 (2.39), 3.217 (1.44), 3.230 (2.74), 3.245 (1.83), 3.377 (2.29), 3.391 (4.57), 3.404 (2.14), 3.430 (1.01), 3.442 (1.87), 3.457 (1.83), 3.470 (0.75), 4.111 (1.65), 4.121 (1.87), 4.130 (1.56), 4.437 (5.84), 4.495 (1.12), 4.512 (1.29), 4.528 (1.06), 6.224 (3.84), 6.589 (3.49), 6.877 (0.66), 6.882 (0.78), 6.894 (1.23), 6.899 (1.36), 6.911 (0.70), 6.916 (0.75), 6.988 (16.00), 7.063 (1.15), 7.068 (1.18), 7.086 (1.18), 7.090 (1.14), 7.559 (0.58), 7.566 (0.66), 7.576 (0.84), 7.580 (0.82), 7.590 (0.64), 7.598 (0.55), 8.059 (0.73), 8.071 (1.46), 8.082 (0.71), 8.317 (2.92), 8.321 (2.79), 8.670 (2.32), 8.682 (2.29), 9.757 (3.39).

Final Intermediate F18

N-[2-((15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl)methyl)sulfonyl]ethyl]-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamide
To a cooled solution of 2-(((15,19-difluoro-3,4-dihydro-2H,1H,10,6-(azeno)-12,16-(metheno)-
1,5,1,13-benzodioadiazaacyclooctadecin-8-yl)methyl)sulfonyl)ethanamine (12.0 mg, 25.2 µmol; see
Intermediate 43) in DMF (0.5 ml) was added (2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid (5.86 mg,
37.8 µmol), HATU (14.4 mg, 37.8 µmol) and 4-methylmorpholine (11 µl, 100 µmol) at 0 °C. The
reaction mixture was stirred for 1 h at 0 °C and was then diluted with a mixture of Water
/TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 2] and
the desired compound (6.6 mg, 0.01 mmol) was obtained.

**LC-MS [Method 15]:** \( R_t = 1.30 \) min; MS (ESIpos): \( m/z = 614 \ [\text{M+H}]^+ \)

**1H-NMR (500 MHz, DMSO-de) \( \delta \ [\text{ppm]}: \) -0.007 (1.60), 0.007 (1.02), 2.105 (1.10), 3.254 (1.50), 3.267
(2.84), 3.282 (1.83), 3.481 (1.13), 3.493 (2.04), 3.508 (2.02), 3.520 (0.92), 3.737 (3.11), 4.032 (9.19),
4.114 (1.72), 4.124 (1.92), 4.133 (1.64), 4.440 (5.90), 4.500 (1.07), 4.517 (1.25), 4.535 (1.05), 6.228
(3.80), 6.230 (3.77), 6.590 (3.51), 6.592 (3.37), 6.880 (0.69), 6.885 (0.77), 6.897 (1.25), 6.902 (1.40),
6.913 (0.72), 6.918 (0.79), 7.066 (1.22), 7.072 (1.20), 7.089 (1.48), 7.094 (1.64), 7.100 (16.00), 7.575
(0.76), 7.579 (0.85), 8.321 (3.00), 8.326 (2.83), 8.450 (1.50), 8.674 (2.38), 8.686 (2.33), 9.759 (3.55).

**Final Intermediate F19**

N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl -N-[2-(((15,19-difluoro-3,4-dihydro-
2H,1H,10,6-(azeno)-12,16-(metheno)-1,5,1,13-benzodioadiazaacyclooctadecin-8-
yl)methyl)sulfonyl)ethyl]-L-alaninamide
To a cooled solution of L-valyl-N-[2-{{[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-
(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl[methyl] sulfonyl}ethyl}-L-alaninamide
trifluoroacetate (12 mg, 15.8 µmol; see Intermediate 47) in DMF (1 ml) was added 6-(2,5-dioxo-2,5-
dihydro-1H-pyrrol-1-yl)hexanoic acid (5.00 mg, 23.7 µmol), HATU (12 mg, 31.5 µmol) and 4-
methylmorpholine (6.1 µl, 55 µmol) at 0°C. The reaction mixture was stirred for 2 h at 0°C and was then diluted with a mixture of Water/TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 2, flow 20 ml/min] and the desired compound (7.9 mg, 96% purity, 0.01 mmol) was obtained.

LC-MS [Method 2]: R<sub>t</sub> = 2.01 min; MS (ESIpos): m/z = 840 [M+H]<sup>+</sup>

1H-NMR (500 MHz, DMSO-de) δ [ppm]: -0.007 (3.43), 0.006 (2.00), 0.788 (6.39), 0.801 (6.46), 0.827 (6.09), 0.840 (6.11), 1.148 (1.76), 1.164 (2.05), 1.179 (1.84), 1.194 (7.11), 1.208 (6.50), 1.445 (2.16), 1.461 (3.24), 1.476 (2.69), 1.492 (1.18), 1.932 (1.10), 1.946 (1.07), 2.071 (1.23), 2.085 (2.19), 2.100 (2.37), 2.114 (1.46), 2.126 (1.06), 2.141 (1.40), 2.156 (0.92), 3.209 (1.57), 3.222 (3.18), 3.236 (1.76), 3.337 (2.33), 3.351 (4.36), 3.365 (2.23), 3.480 (1.06), 3.493 (2.25), 3.506 (2.11), 3.519 (0.83), 3.906 (1.06), 4.116 (2.34), 4.131 (3.10), 4.145 (1.77), 4.149 (1.76), 4.162 (1.32), 4.185 (1.26), 4.199 (1.80), 4.213 (1.17), 4.439 (5.94), 4.497 (1.26), 4.514 (1.42), 4.530 (1.13), 6.225 (4.20), 6.227 (4.23), 6.603 (3.68), 6.879 (0.78), 6.884 (0.84), 6.896 (1.44), 6.901 (1.48), 6.913 (0.78), 6.918 (0.79), 6.984 (16.00), 6.994 (0.61), 7.004 (0.92), 7.066 (1.30), 7.071 (1.30), 7.088 (1.32), 7.093 (1.23), 7.562 (0.69), 7.570 (0.80), 7.576 (0.89), 7.579 (0.98), 7.584 (0.95), 7.587 (0.83), 7.593 (0.75), 7.796 (1.70), 7.813 (1.61), 8.019 (1.70), 8.033 (1.65), 8.118 (0.86), 8.130 (1.73), 8.141 (0.83), 8.318 (3.12), 8.323 (2.97), 8.691 (2.44), 8.703 (2.47), 9.796 (3.65).
**Final Intermediate F20**

N-[[2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl]acetyl]-L-valyl -N-2-[[[15,19-difluoro-3,4-dihydro-2H,10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl]sulfonyl]ethyl-L-alaninamide

To a cooled solution of L-valyl-N-2-[[[15,19-difluoro-3,4-dihydro-2H,10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl]sulfonyl]ethyl-L-alaninamide trifluoroacetate (16.0 mg, 21.0 μmol; see Intermediate 47) in DMF (1 ml) was added (2,5-dioxo-2,5-dihydro-lH-pyrrol-l-yl)acetic acid (4.89 mg, 31.5 μmol), HATU (12 mg, 31.5 μmol) and 4-methylmorpholine (9.2 μl, 84 μmol) at 0° C. The reaction mixture was stirred for 1 h at 0° C and was then diluted with a mixture of Water/TFA/acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 2, flow 20 ml/min] and the desired compound (3 mg, 3.8 μmol) was obtained.

LC-MS [Method 13]: R_t = 1.01 min; MS (ESIpos): m/z = 784 [M+H]^+

1H-NMR (500 MHz, DMSO-de) δ [ppm]: -0.007 (3.59), 0.007 (2.34), 0.794 (6.72), 0.808 (6.81), 0.832 (6.62), 0.846 (6.39), 1.198 (6.83), 1.212 (6.68), 1.236 (1.01), 1.926 (0.79), 1.940 (1.20), 1.953 (1.15), 1.966 (0.67), 2.105 (1.42), 3.212 (1.71), 3.226 (3.62), 3.240 (1.98), 3.475 (1.05), 3.488 (1.92), 3.496 (2.04), 3.507 (1.87), 3.521 (1.07), 3.659 (2.28), 4.073 (0.79), 4.107 (4.98), 4.116 (6.35), 4.125 (2.68), 4.135 (2.08), 4.149 (0.90), 4.172 (1.35), 4.185 (1.74), 4.190 (1.67), 4.198 (1.56), 4.203 (1.61), 4.212 (1.88), 4.226 (1.16), 4.441 (6.44), 4.499 (1.35), 4.515 (1.63), 4.532 (1.25), 6.231 (4.59), 6.601 (3.96), 6.880 (0.83), 6.885 (0.90), 6.896 (1.47), 6.901 (1.63), 6.913 (0.83), 6.918 (0.86), 7.066 (1.98), 7.072 (16.00), 7.089 (1.49), 7.093 (1.40), 7.563 (0.70), 7.571 (0.81), 7.580 (1.08), 7.585 (1.03), 7.594 (0.76), 7.602 (0.63), 8.103 (0.97), 8.114 (1.84), 8.126 (0.91), 8.168 (1.86), 8.182 (1.77), 8.235 (1.85), 8.252 (1.79), 8.328 (3.25), 8.333 (3.10), 8.687 (2.55), 8.699 (2.54), 9.787 (3.82).
Final Intermediate F21

1-[[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl]methyl]sulfonyl]ethyl]-3-[[2-(2,5-dioxo-2,5-dihydro-1H-pyror-1-yl)ethyl]urea

Under Argon, to a solution of 2-(((15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl)methyl)sulfonyl)ethanamine (5 mg, 10.5 µmol; see Intermediate 43) in DMF (1.6 ml) was added 4-nitrophenyl [2-(2,5-dioxo-2,5-dihydro-1H-pyror-1-yl)ethyl]carbamate (4.80 mg, 15.7 µmol, see Intermediate 22) and N,N-diisopropylethylamine (5.5 µl, 31 µmol) at room temperature. The reaction mixture was stirred overnight at room temperature, and then diluted with a mixture of Water /TFA/acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 2] and the desired compound (2.5 mg, 3.9 µmol) was obtained.

LC-MS [Method 15]: R_t = 1.27 min; MS (ESIpos): m/z = 643 [M+H]^+

1H-NMR (400 MHz, DMSO-de) δ [ppm]: 0.009 (2.29), 1.148 (0.78), 1.236 (1.10), 2.106 (1.48), 2.670 (0.69), 3.122 (1.00), 3.136 (2.64), 3.150 (2.80), 3.165 (2.71), 3.182 (3.78), 3.198 (2.28), 3.383 (1.22), 3.400 (2.73), 3.410 (4.22), 3.424 (5.27), 3.439 (2.43), 3.889 (3.30), 4.111 (2.63), 4.122 (3.02), 4.133 (2.56), 4.408 (7.24), 4.495 (1.56), 4.517 (1.84), 4.537 (1.50), 6.084 (1.29), 6.238 (5.74), 6.595 (4.62), 6.873 (0.78), 6.880 (0.89), 6.894 (1.55), 6.901 (1.73), 6.916 (0.88), 6.922 (0.93), 6.973 (16.00), 7.061 (1.44), 7.067 (1.46), 7.089 (1.52), 7.095 (1.41), 7.555 (0.74), 7.565 (0.86), 7.577 (1.12), 7.582 (1.09), 7.594 (0.83), 7.604 (0.72), 8.316 (3.54), 8.322 (3.51), 8.672 (2.81), 8.688 (2.82), 9.743 (4.24).
Final Intermediate F22

N-[2-([15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl][methyl]sulfonyl)ethyl]-19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-amide

To a cooled solution of 2-([15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl][methyl]sulfonyl)ethanamine (10.0 mg, 21.0 µmol, see Intermediate 43) in DMF (1 ml) was added 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-{15-[(2,5-dioxopyrrolidin-1-yl)oxy]-15-oxo-3,6,9,12-tetraoxapentadec-1-yl}propanamide (10.8 mg, 21 µmol, Aldrich, CAS-RN 6525-99-2) and 4-methylmorpholine (9.2 µl, 84 µmol) at 0°C. The reaction mixture was stirred overnight at 0°C and was then diluted with a mixture of Water/TFA/acetonitrile (1 ml). The resulting mixture was directly purified by preparative HPLC [Method 2, flow 20 ml/min] and the desired compound (12.5 mg, 0.01 mmol) was obtained.

LC-MS [Method 12]: Rf = 1.76 min; MS (ESIpos): m/z = 875 [M+H]+

1H-NMR (500 MHz, DMSO-de) δ [ppm]: 2.072 (0.88), 2.102 (0.72), 2.307 (2.46), 2.321 (4.82), 2.335 (2.16), 3.118 (0.64), 3.130 (1.85), 3.142 (1.96), 3.153 (0.72), 3.236 (0.95), 3.249 (1.86), 3.263 (1.17), 3.336 (1.64), 3.348 (3.02), 3.360 (1.27), 3.458 (11.28), 3.471 (16.00), 3.491 (1.92), 3.504 (0.54), 3.570 (1.75), 3.578 (1.70), 3.585 (2.74), 3.590 (3.21), 3.599 (1.94), 3.603 (1.58), 4.113 (1.09), 4.122 (1.25), 4.432 (3.68), 4.498 (0.72), 4.514 (0.85), 4.531 (0.69), 6.230 (2.50), 6.592 (2.28), 6.877 (0.43), 6.882 (0.49), 6.894 (0.80), 6.899 (0.88), 6.910 (0.46), 6.915 (0.48), 6.990 (9.80), 7.063 (0.75), 7.068 (0.77), 7.086 (0.77), 7.090 (0.74), 7.558 (0.37), 7.566 (0.44), 7.576 (0.56), 7.589 (0.43), 7.597 (0.36), 7.990 (0.72), 8.124 (0.48), 8.135 (0.97), 8.147 (0.48), 8.318 (1.90), 8.322 (1.81), 8.673 (1.49), 8.685 (1.46), 9.762 (2.23).
1 Final Intermediate F23 N\textsuperscript{6}·[3-((15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,l3-benzodioxadiazacyclooctadecin-8-yl)methyl)sulfonyl]propanoyl]-N\textsuperscript{2}-[6-(2,5-dioxo-2,5-dihydro-[1H-pyrrol-1-yl])hexanoyl]-N-2,5,8,14,17,20,23-octaoxapentacosan-25-yl-L-lysinamide

10.0 mg (9.66 µmol; see Intermediate 53) N\textsuperscript{6}·[3-((15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,l3-benzodioxadiazacyclooctadecin-8-yl)methyl] sulfonyl]propanoyl]-N-2,5,8,14,17,20,23-octaoxapentacosan-25-yl-L-lysinamide hydrochloride were dissolved in 1.1 ml DMF. Then, 5.36 mg (17.4 µmol) l-[6-[(2,5-dioxopyrrolidin-1-yl)oxy]-6-oxohexyl]-lH-pyrrole-2,5-dione and 8.4 µl N,N-diisopropylamine were added and the reaction mixture was stirred for 2 h at room temperature. The product was isolated by reversed-phase HPLC (ACN/Water with 0.1% TFA, gradient 35-80%).

Product: 8.07 mg (85 % purity, 60 % yield).

LC-MS [Method 12]: R\textsubscript{t} = 1.89 min; MS (ESIpos): m/z = 1192 [M+H]\textsuperscript{+}.

\textsuperscript{3}H-NMR (500 MHz, DMSO-de) δ [ppm]: 1.170 (5.12), 1.235 (4.14), 1.369 (4.14), 1.464 (9.15), 1.571 (1.89), 2.094 (7.34), 3.016 (4.14), 3.190 (3.96), 3.232 (16.00), 3.329 (5.42), 3.363 (8.58), 3.375 (8.98), 3.419 (8.40), 3.718 (5.05), 4.123 (5.56), 4.182 (2.57), 4.431 (6.80), 4.516 (3.84), 5.753 (6.20), 6.165 (0.77), 6.239 (3.91), 6.506 (0.67), 6.595 (3.85), 6.899 (2.42), 6.990 (9.27), 7.067 (2.34), 7.089 (2.41), 7.575 (2.16), 7.827 (2.63), 7.868 (2.84), 8.022 (2.37), 8.319 (3.99), 8.687 (2.98), 9.777 (3.49).
To a solution of Intermediate 30 (N-N-{[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,1,13-benzodioxazacyclooctadecin-8-yl]methyl}(methyl)oxido-\(\lambda^6\)-sulfanylidene|carbamoyl| -beta-alanine) (18.0 mg, 32.1 \(\mu\)mol) in DMF (0.4 mL) was added at r.t. 1-hydroxypyrrolidine-2,5-dione (5.53 mg, 48.1 \(\mu\)mol) and N,N-diisopropylcarbodiimide (7.4 \(\mu\)l, 48 \(\mu\)mol). After stirring for 14 h more 1-hydroxypyrrolidine-2,5-dione (5.53 mg, 48.1 \(\mu\)mol) and N,N-diisopropylcarbodiimide (7.4 \(\mu\)l, 48 \(\mu\)mol) was added and the mixture was stirred for further 24 h. After filtration of the mixture the filtrate was directly purified by preparative HPLC (Method 11) to give the title compound (18.0 mg, 0.03 mmol).

LC-MS (Method 6): \(R_t = 1.13\) min; MS (ESI+): \(m/z = 659.4\).
To a solution of 6-(([[15,19-difluoro-3,4-dihydro-2H,l1H-10,6-(azeno)-12,16-(metheno)-l,5,l1,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido-lambda 6-sulfanylidene)-carbamoyl) amino)hexanoic acid (24.0 mg, 0.04 mmol; see Intermediate 55) in DMF (1.2 mL) was added atr.t. N,N-diisopropylcarbodiimide (7 µl, 48 µmol) and 1-hydroxypyrrolidine-2,5-dione (5.49 mg, 0.048 mmol). After stirring for 14 h more N,N-diisopropylcarbodiimide (7 µl, 48 µmol) and 1-hydroxypyrrolidine-2,5-dione (5.49 mg, 0.048 mmol) was added and stirring continued for further 7 h. Then N-methylmorpholine (5.5 µl, 50 µmol) was added and stirring continued for further 14 h. After that the reaction mixture was purified by preparative HPLC (Method 9) to give the title compound (9.00 mg, 0.01 mmol).

LC-MS (Method 7): R_t = 1.20 min; MS (ESI+): m/z = 701.

1H-NMR (400 MHz, DMSO-d6) δ [ppm]: 1.144 (0.72), 1.231 (1.16), 1.336 (1.52), 1.352 (1.52), 1.377 (1.16), 1.394 (1.61), 1.410 (1.43), 1.607 (1.43), 1.626 (1.70), 1.643 (1.34), 2.085 (1.34), 2.318 (1.70), 2.322 (3.66), 2.327 (5.36), 2.332 (3.84), 2.337 (1.61), 2.518 (16.00), 2.523 (10.99), 2.641 (1.97), 2.660 (5.27), 2.665 (4.65), 2.669 (5.90), 2.673 (4.56), 2.678 (3.31), 2.808 (7.96), 2.931 (0.72), 2.948 (1.34), 2.962 (1.88), 2.976 (1.43), 3.107 (12.16), 3.199 (0.89), 4.114 (2.23), 4.485 (1.25), 4.504 (1.52), 4.527 (1.25), 4.738 (4.11), 6.265 (5.36), 6.268 (5.27), 6.621 (3.93), 6.840 (0.89), 6.855 (1.88), 6.869 (0.98), 6.875 (1.07), 6.881 (1.07), 6.896 (1.61), 6.903 (1.79), 6.917 (0.89), 6.924 (0.98), 7.061 (1.43), 7.067 (1.34), 7.089 (1.43), 7.095 (1.43), 7.553 (0.80), 7.563 (0.80), 7.570 (0.98), 7.575 (1.07), 7.580 (1.07), 7.592 (0.80), 7.603 (0.72), 8.319 (3.84), 8.326 (3.66), 8.668 (2.77), 8.683 (2.68), 9.758 (3.84).

Final Intermediate F26

N-[[5-[(2,5-Dioxopyrrolidin-1-yl)oxy]-5-oxopentanoyl]-L-valyl-A-[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-l,5,ll,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido-X6-sulfanylidene]-L-alaninamide
To a solution of L-valyl-N-[[[15,19-difluoro-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxazacyclotadecin-8-yl]methyl] (methyl)oxido^-6-sulfanylidene]-L-alaninamide trifluoroacetate (15.6 mg, 21.3 μmol; see Intermediate 15) in DMF (0.25 mL) was added atr.t. 1,1’-[(1,5-dioxopentane-1,5-diyl)bis(oxy)]dipyrrolidine-2,5-dione (12.5 mg, 38.4 μmol) and N,N-diisopropylethylamine (11 μL, 64 μmol). After stirring for 1h at that temperature the mixture was filtered and purified by preparative HPLC (Method 8) to give the title compound (7.50 mg, 0.01 mmol).

LC-MS (Method 6): R_t = 1.22 min; MS (ESI+): m/z = 828.6.

1H-NMR (400 MHz, DMSO-d_6) δ [ppm]: 0.806 (3.92), 0.823 (4.17), 0.840 (4.42), 0.857 (5.00), 0.863 (4.42), 0.881 (4.42), 0.888 (4.67), 0.905 (4.33), 1.145 (0.67), 1.232 (4.92), 1.246 (6.00), 1.251 (5.83), 1.265 (5.00), 1.827 (1.42), 1.839 (2.00), 1.846 (2.17), 1.859 (1.50), 1.865 (1.50), 1.961 (0.75), 1.978 (0.92), 1.991 (0.92), 2.007 (0.83), 2.081 (1.42), 2.284 (0.67), 2.303 (1.67), 2.314 (2.00), 2.318 (3.25), 2.323 (4.50), 2.327 (6.33), 2.332 (4.75), 2.337 (2.42), 2.347 (0.67), 2.518 (16.00), 2.523 (10.42), 2.660 (1.58), 2.665 (3.92), 2.669 (6.67), 2.674 (5.42), 2.679 (2.33), 2.689 (3.17), 2.693 (3.33), 2.707 (1.75), 2.712 (1.67), 2.804 (12.00), 3.177 (9.42), 3.195 (8.83), 3.789 (2.83), 4.121 (2.75), 4.156 (1.25), 4.163 (1.25), 4.174 (1.75), 4.181 (1.67), 4.192 (1.17), 4.199 (1.08), 4.238 (0.92), 4.254 (1.00), 4.261 (1.00), 4.268 (1.08), 4.277 (1.00), 4.284 (1.08), 4.290 (1.00), 4.307 (0.92), 4.513 (1.67), 4.747 (1.25), 4.781 (2.92), 4.798 (1.58), 4.833 (1.58), 4.867 (0.75), 6.266 (2.83), 6.268 (2.92), 6.288 (3.17), 6.290 (3.00), 6.611 (5.25), 6.880 (1.00), 6.886 (1.25), 6.901 (1.83), 6.907 (2.08), 6.922 (1.08), 6.929 (1.17), 7.067 (1.75), 7.074 (2.00), 7.096 (1.75), 7.102 (1.75), 7.564 (0.75), 7.571 (0.75), 7.582 (1.17), 7.592 (1.08), 7.603 (0.75), 7.610 (0.67), 7.789 (1.25), 7.812 (1.17), 7.852 (1.17), 7.875 (1.17), 8.123 (1.17), 8.141 (1.17), 8.176 (1.25), 8.194 (1.17), 8.316 (2.58), 8.323 (2.58), 8.328 (2.58), 8.334 (2.33), 8.666 (1.83), 8.682 (1.83), 8.703 (2.00), 8.718 (2.00), 9.778 (2.58), 9.802 (2.75).
Metabolite M1

2-((15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-
benzodioxadiazacyclooctadecin-8-yl)methyl)sulfonyl)ethanamine

Synthesis; see Intermediate 43

Metabolite M2

S-[1-(3-[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-
benzodioxadiazacyclooctadecin-8-yl)methyl](methyl)oxido-5'-sulfanylidene]amino)-3-oxopropyl)-
2,5-dioxopyrroolidin-3-yl]-L-cysteine trifluoroacetate

To a solution of (rac)-N-[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-
benzodioxadiazacyclooctadecin-8-yl)methyl](methyl)oxido-5'-sulfanylidene]-3-(2,5-dioxo-2,5-dihydro-
1H-pyrrol-1-yl)propanamide (4.20 mg, 7.03 µmol; see Fl) in DMF (4 ml) was added L-cysteine (17 mg, 141 µmol) at room temperature. The reaction mixture was stirred for 2 days at room temperature and was
directly purified by preparative HPLC [Method 2] and the desired compound (2.1 mg, 2.5 µmol) was obtained.

LC-MS [Method 12]: R_t = 1.51 min; MS (ESIpos): m/z = 719 [M+H-TFA]^+

¾-NMR (500 MHz, DMSO-de) δ [ppm]: -0.121 (1.13), -0.007 (1.61), 0.006 (7.89), 0.116 (1.02), 1.147 (1.39), 1.235 (1.84), 2.085 (3.79), 2.357 (2.52), 2.369 (5.29), 2.381 (4.81), 2.394 (1.58), 2.559 (3.45), 2.635 (1.16), 2.731 (4.13), 2.890 (5.03), 3.041 (1.61), 3.056 (1.75), 3.070 (1.89), 3.085 (1.78), 3.142 (1.61), 3.160 (3.02), 3.179 (2.97), 3.198 (3.02), 3.214 (2.23), 3.230 (3.42), 3.243 (6.30), 3.369 (0.85), 3.393 (2.01), 3.403 (2.06), 3.422 (1.81), 3.432 (1.67), 3.593 (5.85), 3.609 (9.02), 3.624 (5.43), 4.064 (3.08), 4.073 (4.92), 4.082 (5.71), 4.091 (4.81), 4.100 (3.62), 4.113 (6.36), 4.122 (7.10), 4.270 (2.54), 4.500 (3.76), 4.517 (4.35), 4.534 (3.53), 4.757 (16.00), 6.256 (13.46), 6.601 (11.82), 6.883 (2.43), 6.888 (2.69), 6.900 (4.38), 6.905 (4.81), 6.917 (2.43), 6.922 (2.66), 7.067 (3.96), 7.072 (4.16), 7.090 (4.13), 7.095 (4.41), 7.198 (0.88), 7.563 (2.20), 7.570 (2.52), 7.580 (3.11), 7.584 (3.00), 7.594 (2.43), 7.601 (2.12), 8.324 (9.87), 8.329 (9.39), 8.389 (4.30), 8.674 (8.25), 8.686 (8.14), 9.810 (11.56).

Metabolite M3

S-1-[2-(((15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl)methyl](methyl)oxido-X6-sulfanylidene)carbamoyl]amino)ethyl]-2,5-dioxopyrrolidin-3-yl]-L-cysteine trifluoroacetate

To a solution of (rac)-[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecin-8-yl)methyl](methyl)oxido-λ6-sulfanylidene]-3-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl]urea (3.50 mg, 5.71 µmol; see F8) in DMF (3 ml) was added L-cysteine (13.8 mg, 114 µmol) at room temperature. The reaction mixture was stirred for 2 days at room temperature and was directly purified by preparative HPLC [Method 2] and the desired compound (1.4 mg, 1.6 µmol) was obtained.
LC-MS [Method 12]: \( R_t = 1.47 \) min; MS (ESIpos): \( m/z = 734 \) [M+H-TFA]⁺

\[ \delta \text{ ppm: } 0.852 \ (2.59), \ 1.146 \ (1.30), \ 1.235 \ (16.00), \ 1.550 \ (1.36), \ 2.086 \ (2.68), \ 2.369 \ (1.59), \ 3.114 \ (9.83), \ 3.156 \ (4.20), \ 3.268 \ (1.93), \ 3.461 \ (7.00), \ 4.073 \ (1.72), \ 4.120 \ (4.19), \ 4.263 \ (1.32), \ 4.513 \ (2.79), \ 4.720 \ (5.58), \ 5.753 \ (2.93), \ 6.286 \ (4.32), \ 6.613 \ (4.05), \ 6.902 \ (2.22), \ 6.957 \ (1.61), \ 7.069 \ (2.03), \ 7.094 \ (2.44), \ 7.577 \ (1.84), \ 8.321 \ (4.41), \ 8.384 \ (2.58), \ 8.673 \ (2.92), \ 8.685 \ (2.95), \ 9.722 \ (4.08). \]

**Metabolite M4**

S-[(6-[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,1l,13-benzodioxadiazacyclooctadecin-8-yl][methyl]sulfonyl]ethyl]amino]-6-oxohexyl)-2,5-dioxopyrrolidin-3-yl]-L-cysteine trifluoroacetate

To a solution of N-2-([15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,1l,13-benzodioxadiazacyclooctadecin-8-yl][methyl] sulfonyl)ethyl]-6-(2,5-dioxo-5-dihydro-1H-pyrrol-1-yl)hexanamide (4 mg, 5.97 \( \mu \)mol; see F16) in DMF (1.5 ml) was added L-cysteine (14.5 mg, 119 \( \mu \)mol) at room temperature. The reaction mixture was stirred for 48 h at room temperature and was then diluted with a mixture of Water/TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 2] and the desired compound (4.1 mg, 4.5 \( \mu \)mol) was obtained.

LC-MS [Method 12]: \( R_t = 1.56 \) min; MS (ESIneg): \( m/z = 789 \) [M-H-TFA]⁻

\[ \delta \text{ ppm: } 1.147 \ (1.39), \ 1.203 \ (4.12), \ 1.217 \ (3.25), \ 1.235 \ (2.71), \ 1.455 \ (3.36), \ 1.469 \ (6.13), \ 1.484 \ (5.31), \ 1.498 \ (3.16), \ 2.038 \ (4.23), \ 2.053 \ (7.18), \ 2.068 \ (4.97), \ 2.088 \ (3.36), \ 2.361 \ (1.24), \ 2.635 \ (1.00), \ 3.035 \ (1.28), \ 3.050 \ (1.41), \ 3.064 \ (1.56), \ 3.079 \ (1.48), \ 3.152 \ (1.58), \ 3.170 \ (2.13), \ 3.189 \ (2.06), \ 3.207 \ (2.12), \ 3.234 \ (7.11), \ 3.246 \ (10.91), \ 3.260 \ (4.94), \ 3.339 \ (4.62), \ 3.353 \ (7.39), \ 3.367 \ (4.51), \ 3.412 \ (2.84), \ 3.463 \ (6.98), \ 3.476 \ (6.77), \ 3.655 \ (16.00), \ 4.076 \ (3.14), \ 4.123 \ (5.79), \ 4.273 \]
(2.10), 4.439 (13.61), 4.515 (3.75), 6.229 (9.28), 6.592 (8.63), 6.886 (1.69), 6.903 (3.17), 6.920 (1.69),
7.070 (2.91), 7.089 (3.16), 7.578 (2.34), 8.054 (3.81), 8.320 (6.57), 8.325 (6.46), 8.389 (6.57), 8.673 (5.01),
8.685 (5.09), 9.762 (7.98).

5 Metabolite M5
S-[[4-[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,13-benzodioxadiazacyclooctadecin-8-yl]methyl)sulfonyl]ethyl]amino]-4-oxobutyl]-2,5-dioxopyrrolidin-3-yl]-L-cysteine trifluoroacetate

To a solution of N-[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,13-benzodioxadiazacyclooctadecin-8-yl]methyl] sulfonyl]ethyl]-4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl]butanamide (3.5 mg, 5.45 µmol; see F17) in DMF (2 ml) was added L-cysteine (13.2 mg, 109 µmol) at room temperature. The reaction mixture was stirred for 48 h at room temperature and was then diluted with a mixture of Water/TFA/acetonitrile (1ml). The resulting mixture was directly purified by preparative HPLC [Method 2] and the desired compound (4.8 mg, 5.4 µmol) was obtained.

LC-MS [Method 12]: Rt = 1.52 min; MS (ESIpos): m/z = 763 [M+H-TFA]⁺

Metabolite M6

S-(l-[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,1,13-
benzodioxadiazacyclocotadecin-8-yl]-2,2-dioxido-6,22-dioxo-9,12,15,18-tetraoxa-2X-
thia-5,21-diazatetracosan-24-yl)-2,5-dioxopyrrolidin-3-yl)-L-cysteine trifluoroacetate

To a solution of N-[2-([15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,1,13-
benzodioxadiazacyclocotadecin-8-yl]methyl] sulfonyl)ethyl]- 19-(2,5-dioxo-2,5-dihydro- 1H-pyrrolo- 1-yl)-
17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-amide (4 mg, 4.57 µmol; see F22) in DMF (1.7 ml) was
added L-cysteine (11.1 mg, 91.4 µmol) at room temperature. The reaction mixture was stirred for 48 h at
room temperature and was then diluted with a mixture of Water/TFA/acetonitrile (1ml). The resulting
mixture was directly purified by preparative HPLC [Method 2] and the desired compound (3 mg, 2.7
µmol) was obtained.

LC-MS [Method 12]: R<sub>t</sub> = 1.51 min; MS (ESIpos): m/z = 996 [M+H-TFA]<sup>+</sup>

¾-NMR (500 MHz, DMSO-de) δ [ppm]: -0.008 (1.38), 1.177 (0.42), 2.090 (1.11), 2.311 (2.45), 2.323
(4.91), 2.336 (2.62), 2.523 (0.58), 2.560 (2.00), 3.040 (0.41), 3.055 (0.46), 3.069 (0.50), 3.084 (0.53),
3.131 (0.56), 3.155 (2.12), 3.167 (2.96), 3.178 (0.89), 3.186 (1.01), 3.205 (0.60), 3.228 (0.94), 3.242
(1.81), 3.250 (2.90), 3.264 (1.66), 3.364 (2.01), 3.376 (3.67), 3.388 (1.66), 3.403 (0.63), 3.423 (0.57),
3.432 (0.64), 3.459 (16.00), 3.474 (15.86), 3.486 (10.62), 3.559 (1.24), 3.579 (3.13), 3.592 (4.72), 3.605 (1.97), 4.055 (0.69), 4.064 (1.17), 4.073 (1.37), 4.082 (1.13), 4.091 (0.76), 4.123 (2.01), 4.273 (0.77), 4.433 (5.26), 4.516 (1.41), 6.154 (0.46), 6.230 (3.46), 6.499 (0.40), 6.592 (3.16), 6.884 (0.72), 6.896 (1.20), 6.901 (1.28), 6.913 (0.69), 6.918 (0.70), 7.065 (1.10), 7.088 (1.14), 7.093 (1.08), 7.560 (0.58), 7.568 (0.68), 7.577 (0.85), 7.591 (0.66), 7.599 (0.56), 8.024 (0.77), 8.038 (0.81), 8.131 (0.68), 8.143 (1.35), 8.154 (0.65), 8.319 (2.76), 8.324 (2.61), 8.397 (1.32), 8.674 (2.12), 8.686 (2.14), 9.719 (0.39), 9.762 (3.23).

Metabolite 7
S-(3R)-L-[285]-28-(4-{3-[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacycloctadecin-8-yl]methyl]sulfonyl]propanoyl}amino]butyl)-27,30-dioxo-2,5,8,11,14,17,20,23-octaoxa-26,29-diazapentriacontan-35-yl]-2,5-dioxopyrrolidin-3-yl]-L-cysteine

T a solution of N°-[3-[[15,19-difluoro-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacycloctadecin-8-yl]methyl]sulfonyl]propanoyl]-N°-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-N°-2,5,8,11,14,17,20,23-octaoxapentacosan-25-yl-L-lysinamide (3.00 mg, 2.52 µmol; see F23) in 500 µl DMSO was added L-cysteine (910 µg, 7.5 µmol). The mixture was stirred at
room temperature for 16h. The product was isolated by reversed-phase HPLC (ACN/Water with 0.1% TFA, gradient 35-80%).

Product: 1.90 mg (100 % purity, 57 % yield).

5

LC-MS [Method 17]: R_t = 0.94 min; MS (ESIpos): m/z = 1313 [M+H]^+.

Procedure 1: General process for coupling to cysteine side chains

The following antibodies were used for the coupling reactions:

- cetuximab (anti EGFR AK), A
- nimotuzumab (anti-EGFR AK), B
- anti-TWEAKR TPP-2090, C
- cetuximab TPP-4030 (anti-EGFR AK), D
- cetuximab TPP-5653 (anti-EGFR AK), E

Between 2 and 5 equivalents of tris(2-carboxyethyl)phosphine hydrochloride (TCEP), dissolved in PBS buffer, were added to a solution of the appropriate antibody in PBS buffer in the concentration range between 1 mg/ml and 20 mg/ml, preferably in the range of about 10 mg/ml to 15 mg/ml, and the mixture was stirred at RT for 1h. For this purpose, the solution of the respective antibody used can be employed at the concentrations stated in the working examples, or it may optionally also be diluted with PBS buffer to about half of the stated starting concentrations in order to get into the preferred concentration range. Subsequently, depending on the intended loading from 2 to 12 equivalents, preferably about 5-10 equivalents of the maleinimide precursor compound or halide precursor compound to be coupled can be added as a solution in DMSO. Here, the amount of DMSO should not exceed 10% of the total volume.

The reaction was stirred in the case of maleinimide precursors for 60-240 min at RT and then applied to PBS-equilibrated PD 10 columns (Sephadex® G-25, GE Healthcare) and eluted with PBS buffer. Generally, unless indicated otherwise, 5 mg of the antibody in question in PBS buffer were used for the reduction and the subsequent coupling. Purification on the PD10 column thus in each case afforded solutions of the respective ADCs in 3.5 ml PBS buffer. The sample was then concentrated by ultracentrifugation and optionally rediluted with PBS buffer. If required, for better removal of low-molecular weight components, concentration by ultrafiltration was repeated after redilution with PBS buffer. For biological tests, if required, the concentrations of the final ADC samples were optionally...
adjusted to the range of 0.5-15 mg/ml by redilution. The respective protein concentrations, stated in the working examples, of the ADC solutions were determined. Furthermore, antibody loading (drug/mAb ratio) was determined using the methods described under Procedure 2.

Unless indicated otherwise, the immunoconjugates shown in the examples were prepared by this process. Depending on the linker, the ADCs shown in the examples may also be present to a lesser or higher degree in the form of the hydrolysed open-chain succinamides attached to the antibodies. Both isomeric forms could be present.

In particular the ADCs containing the linker substructure

\[
\begin{align*}
\text{#}_1 & \quad \text{H} \quad \text{N} \quad \text{O} \\
\text{#}_2 & \quad \text{O} \\
\text{N} & \quad \text{O} \\
\text{O} & \quad \text{N}
\end{align*}
\]

10 to thiol groups of the antibodies may optionally also be prepared in a targeted manner by rebuffering after the coupling and stirring at pH 8 for about 20 h according to Scheme 26 via the ADCs attached via open-chain succinamides.

#1 represents the sulphur bridge to the antibody, and #2 the point of attachment to the modified payload.

Such ADCs where the linker is attached to the antibodies through hydrolysed open-chain succinamides may optionally also be prepared in a targeted manner by an exemplary procedure as follows:

Under argon, a solution of 0.344 mg TCEP in 100 µl of PBS buffer was added to 60 mg of the antibody in question in 5 ml of PBS buffer (c~12 mg/ml). The reaction was stirred at RT for 30 min, and 0.003 mmol of a maleimide precursor compound dissolved in 600 µl of DMSO was then added. After a further 1.5 h - 2 h of stirring at RT, the reaction was diluted with 1075 µl of PBS buffer which had been adjusted to pH 8 beforehand.

This solution was then applied to PD 10 columns (Sephadex® G-25, GE Healthcare) which had been equilibrated with PBS buffer pH 8 and was eluted with PBS buffer pH 8. The eluate was diluted with PBS buffer pH 8 to a total volume of 14 ml. This solution was stirred at RT under argon overnight. If required, the solution was then rebuffered to pH 7.2. The ADC solution was concentrated by ultracentrifugation, rediluted with PBS buffer (pH 7.2) and then optionally concentrated again to a concentration of about 10 mg/ml.

Other potentially hydrolysis-sensitive thianylsuccinimide bridges to the antibody in the working examples contain the following linker substructures, where #1 represents the thioether linkage to the antibody and #2 the point of attachment to the modified payload:
These linker substructures represent the linking unit to the antibody and have (in addition to the linker composition) a significant effect on the structure and the profile of the metabolites formed in the tumour cells.

5

**Procedure 2: Determination of the antibody, the toxophore loading and the proportion of open cysteine adducts**

For protein identification in addition to molecular weight determination after deglycosylation and/or denaturing, a tryptic digestion was carried out which, after denaturing, reduction and derivatization, confirms the identity of the protein via the tryptic peptides found.

The toxophore loading of the PBS buffer solutions obtained of the conjugates described in the working example was determined as follows:

Determination of toxophore loading of lysine-linked ADCs was carried out by mass spectrometric determination of the molecular weights of the individual conjugate species. Here, the antibody conjugates were first deglycosylated with PNGaseF, and the sample was acidified and, after HPLC separation/desalting, analysed by mass spectrometry using ESI-MicroTofQ (Broker Daltonik). All spectra over the signal in the TIC (Total Ion Chromatogram) were added and the molecular weight of the
different conjugate species was calculated based on MaxEnt deconvolution. The DAR (drug/antibody ratio) was then calculated after signal integration of the different species.

The toxophore loading of cysteine-linked conjugates was determined by reversed-phase chromatography of the reduced and denatured ADCs. Guanidinium hydrochloride (GuHCl) (28.6 mg) and a solution of DL-dithiothreitol (DTT) (500 mM, 3 µl) were added to the ADC solution (1 mg/ml, 50 µl). The mixture was incubated at 55°C for one hour and analysed by HPLC.

HPLC analysis was carried out on an Agilent 1260 HPLC system with detection at 220 nm. A Polymer Laboratories PLRP-S polymeric reversed-phase column (catalogue number PL.1912-3802) (2.1 x 150 mm, 8 µm particle size, 1000 A) was used at a flow rate of 1 ml/min with the following gradient: 0 min, 25%B; 3 min, 25%B; 28 min, 50%B. Mobile phase A consisted of 0.05% trifluoroacetic acid (TFA) in water, mobile phase B of 0.05% trifluoroacetic acid in acetonitrile.

The detected peaks were assigned by retention time comparison with the light chain (L0) and the heavy chain (H0) of the non-conjugated antibody. Peaks detected exclusively in the conjugated sample were assigned to the light chain with one toxophore (LI) and the heavy chains with one, two and three toxophores (HI, H2, H3).

Average loading of the antibody with toxophores was calculated from the peak areas determined by integration as double the sum of the toxophore number weighed integration results of all peaks divided by the sum of the singly weighed integration results of all peaks. In individual cases, it may be possible that, owing to co-elution of some peaks, it is not possible to determine toxophore loading accurately.

In the cases where light and heavy chains could not be separated sufficiently by HPLC, determination of toxophore loading of cysteine-linked conjugates was carried out by mass spectrometric determination of the molecular weights of the individual conjugate species at light and heavy chain.

Guanidinium hydrochloride (GuHCl) (28.6 mg) and a solution of DL-dithiothreitol (DTT) (500 mM, 3 µl) were added to the ADC solution (1 mg/ml, 50 µl). The mixture was incubated for one hour at 55°C and analysed by mass spectrometry after online desalting using ESI-MicroTofQ (Broker Daltonik).

For the DAR determination, all spectra were added over the signal in the TIC (Total Ion Chromatogram), and the molecular weight of the different conjugate species at light and heavy chain was calculated based on MaxEnt deconvolution. The average loading of the antibody with toxophores was calculated by integration of certain molecular weight areas as double the sum of the toxophore number weighed integration results of all peaks divided by the sum of the singly weighed integration results of all peaks.
To determine the proportion of the open cysteine adduct, the molecular weight area ratio of closed to open cysteine adduct (molecular weight delta 18 Dalton) of all singly conjugated light and heavy chains was determined. The mean of all variants yielded the proportion of the open cysteine adduct.

**Working Examples ADCs**

**Example 1A**

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate Fl, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS. Some of the ADC may also be present in the form of the hydrolysed open-chain succinamides attached to the antibody.

Protein concentration: 1.86 mg/ml

Drug/mAb ratio: 3.4

**Example 1B**

Here, 5.0 mg of nimotuzumab in PBS (c=16.6 mg/ml) were used for coupling with Intermediate Fl, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS. Some of the ADC may also be present in the form of the hydrolysed open-chain succinamides attached to the antibody.

Protein concentration: 1.86 mg/ml

Drug/mAb ratio: 3.7
Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F2, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.93 mg/ml
Drug/mAb ratio: 2.8

Example 2C
Here, 5 mg of anti-TWEAKR TPP-2090 in PBS (c=12.87 mg/ml) were used for coupling with Intermediate F2, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation, rediluted with PBS and concentrated again.

Protein concentration: 1.46 mg/ml
Drug/mAb ratio: 2.9
Example 3A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F3, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 2.01 mg/ml
Drug/mAb ratio: 3.2

Example 3B

Here, 5.0 mg of nimotuzumab in PBS (c=16.6 mg/ml) were used for coupling with Intermediate F3, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.97 mg/ml
Drug/mAb ratio: 3.5
Example 4A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F4, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

5 Protein concentration: 1.46 mg/ml
Drug/mAb ratio: 2.3

Example 4B

Here, 5.0 mg of nimotuzumab in PBS (c=16.6 mg/ml) were used for coupling with Intermediate F4, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.46 mg/ml
Drug/mAb ratio: 4.6
Example 5A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F5, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 2.00 mg/ml
Drug/mAb ratio: 2.8

Example 5B

Here, 5.0 mg of nimotuzumab in PBS (c=20.6 mg/ml) were used for coupling with Intermediate F5, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 2.07 mg/ml
Drug/mAb ratio: 3.6

Example 5D

Here, 5.0 mg of cetuximab TPP-4030 in PBS (c=13.2 mg/ml) were used for coupling with Intermediate F5, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.93 mg/ml
Drug/mAb ratio: 3.4
Example 6A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F6, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.85 mg/ml
Drug/mAb ratio: 2.4

Example 6B

Here, 5.0 mg of nimotuzumab in PBS (c=20.6 mg/ml) were used for coupling with Intermediate F6, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.58 mg/ml
Drug/mAb ratio: 3.3

Example 6E

Here, 5.0 mg of cetuximab TPP-5653 in PBS (c=10.5 mg/ml) were used for coupling with Intermediate F6, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.42 mg/ml
Drug/mAb ratio: 2.9
Example 7A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F7, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 2.03 mg/ml

Drug/mAb ratio: 3.2

Example 7B

Here, 5.0 mg of nimotuzumab in PBS (c=16.6 mg/ml) were used for coupling with Intermediate F7, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.98 mg/ml

Drug/mAb ratio: 3.5
Example 8A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F8, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS. Some of the ADC may also be present in the form of the hydrolysed open-chain succinamides attached to the antibody.

Protein concentration: 1.37 mg/ml
Drug/mAb ratio: 5.8

Example 8D

Here, 5.0 mg of cetuximab TPP-4030 in PBS (c=13.2 mg/ml) were used for coupling with Intermediate F8, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS. Some of the ADC may also be present in the form of the hydrolysed open-chain succinamides attached to the antibody.

Protein concentration: 1.99 mg/ml
Drug/mAb ratio: 2.7
Example 9A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F9, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.93 mg/ml
Drug/mAb ratio: 3.1

Example 9B

Here, 5.0 mg of nimotuzumab in PBS (c=16.6 mg/ml) were used for coupling with Intermediate F9, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.65 mg/ml
Drug/mAb ratio: 3.5

Example 9E

Here, 5.0 mg of cetuximab TPP-5653 in PBS (c=10.5 mg/ml) were used for coupling with Intermediate F9, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.64 mg/ml
Drug/mAb ratio: 2.8
Example 10A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F10, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

5 Protein concentration: 1.97 mg/ml

Drug/mAb ratio: 2.9

Example 10B

Here, 5.0 mg of nimotuzumab in PBS (c=16.6 mg/ml) were used for coupling with Intermediate F10, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.91 mg/ml

Drug/mAb ratio: 2.9

Example 11A
Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate Fll, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.90 mg/ml
Drug/mAb ratio: 2.7

**Example 11B**

Here, 5.0 mg of nimotuzumab in PBS (c=16.6 mg/ml) were used for coupling with Intermediate Fll, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.62 mg/ml
Drug/mAb ratio: 2.8

**Example IIP**

Here, 5.0 mg of cetuximab TPP-4030 in PBS (c=13.2 mg/ml) were used for coupling with Intermediate Fll, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.76 mg/ml
Drug/mAb ratio: 2.8

**Example HE**

Here, 5.0 mg of cetuximab TPP-5653 in PBS (c=10.5 mg/ml) were used for coupling with Intermediate Fll, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.71 mg/ml
Drug/mAb ratio: 2.7
Example 12A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F12, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.95 mg/ml
Drug/mAb ratio: 2.7

Example 12B

Here, 5.0 mg of nimotuzumab in PBS (c=16.6 mg/ml) were used for coupling with Intermediate F12, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.93 mg/ml
Drug/mAb ratio: 3.1

Example 12E

Here, 5.0 mg of cetuximab TPP-5653 in PBS (c=10.5 mg/ml) were used for coupling with Intermediate F12, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.84 mg/ml
Drug/mAb ratio: 2.4
Example 13A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F13, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS. Some of the ADC may also be present in the form of the hydrolysed open-chain succinamides attached to the antibody.

Protein concentration: 1.49 mg/ml

Drug/mAb ratio: 5.2

Example 13D

Here, 5.0 mg of cetuximab TPP-4030 in PBS (c=13.2 mg/ml) were used for coupling with Intermediate F13, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS. Some of the ADC may also be present in the form of the hydrolysed open-chain succinamides attached to the antibody.

Protein concentration: 2.01 mg/ml

Drug/mAb ratio: 2.4
Example 14A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F14, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.85 mg/ml
Drug/mAb ratio: 2.6

Example 14B

Here, 5.0 mg of nimotuzumab in PBS (c=20.6 mg/ml) were used for coupling with Intermediate F14, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.74 mg/ml
Drug/mAb ratio: 2.3

Example 15A
Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F15, and the
reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.
Some of the ADC may also be present in the form of the hydrolysed open-chain succinamides attached to
the antibody.

Protein concentration: 1.83 mg/ml
Drug/mAb ratio: 4.3

Example 15C
Here, 5 mg of anti-TWEEKR TPP-2090 in PBS (c=12.87 mg/ml) were used for coupling with
Intermediate F15, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation,
rediluted with PBS and concentrated again. Some of the ADC may also be present in the form of the
hydrolysed open-chain succinamides attached to the antibody.

Protein concentration: 1.29 mg/ml
Drug/mAb ratio: 3.3

Example 15D
Here, 5.0 mg of cetuximab TPP-4030 in PBS (c=13.2 mg/ml) were used for coupling with Intermediate
F15, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted
with PBS. Some of the ADC may also be present in the form of the hydrolysed open-chain succinamides
attached to the antibody.

Protein concentration: 1.90 mg/ml
Drug/mAb ratio: 3.4
Example 16A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F16, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

5 Protein concentration: 1.77 mg/ml

Drug/mAb ratio: 3.1

Example 16B

Here, 5.0 mg of nimotuzumab in PBS (c=16.6 mg/ml) were used for coupling with Intermediate F16, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

10 Protein concentration: 1.93 mg/ml

Drug/mAb ratio: 3.1

Example 17A
Here, 5 mg of cetuximab in PBS (c=21.88 mg/ml) were used for coupling with Intermediate F17, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS. Some of the ADC may also be present in the form of the hydrolysed open-chain succinamides attached to the antibody.

Protein concentration: 1.86 mg/ml
Drug/mAb ratio: 3.6

**Example 18A**

![Chemical structure of a monoclonal antibody conjugate with cetuximab](image URL)

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F18, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.62 mg/ml
Drug/mAb ratio: 2.8

**Example 18B**

Here, 5.0 mg of nimotuzumab in PBS (c=16.6 mg/ml) were used for coupling with Intermediate F18, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.43 mg/ml
Drug/mAb ratio: 2.9
Example 19A

Here, 5 mg of cetuximab in PBS (c=21.88 mg/ml) were used for coupling with Intermediate F19, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

5 Protein concentration: 1.82 mg/ml

Drug/mAb ratio: 2.2

Example 20A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F20, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.47 mg/ml

Drug/mAb ratio: 2.8
**Example 20B**

Here, 5.0 mg of nimotuzumab in PBS (c=16.6 mg/ml) were used for coupling with Intermediate F20, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.27 mg/ml

Drug/mAb ratio: 3.4

**Example 20D**

Here, 5.0 mg of cetuximab TPP-4030 in PBS (c=13.2 mg/ml) were used for coupling with Intermediate F20, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.51 mg/ml

Drug/mAb ratio: 2.7

**Example 20E**

Here, 5.0 mg of cetuximab TPP-5653 in PBS (c=10.5 mg/ml) were used for coupling with Intermediate F20, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.41 mg/ml

Drug/mAb ratio: 2.7

**Example 21A**
Here, 5 mg of cetuximab in PBS (c=21.88 mg/ml) were used for coupling with Intermediate F21, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS. Some of the ADC may also be present in the form of the hydrolysed open-chain succinamides attached to the antibody.

Protein concentration: 1.90 mg/ml

Drug/mAb ratio: 3.0

Example 22A

Here, 5 mg of cetuximab in PBS (c=21.88 mg/ml) were used for coupling with Intermediate F22, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS. Some of the ADC may also be present in the form of the hydrolysed open-chain succinamides attached to the antibody.

Protein concentration: 1.72 mg/ml

Drug/mAb ratio: 2.3
Example 23A

Here, 5 mg of cetuximab in PBS (c=10.92 mg/ml) were used for coupling with Intermediate F23, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

5 Protein concentration: 1.71 mg/ml
Drug/mAb ratio: 2.9

Example 23C

Here, 5 mg of anti-TWEAKR TPP-2090 in PBS (c=18.6 mg/ml) were used for coupling with Intermediate F23, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation, rediluted with PBS and concentrated again. Some of the ADC may also be present in the form of the hydrolysed open-chain succinamides attached to the antibody.

Protein concentration: 1.54 mg/ml
Drug/mAb ratio: 3.7

Example 23D

Here, 5.0 mg of cetuximab TPP-4030 in PBS (c=13.2 mg/ml) were used for coupling with Intermediate F23, and the reaction was, after Sephadex purification, concentrated by ultracentrifugation and rediluted with PBS.

Protein concentration: 1.66 mg/ml
Drug/mAb ratio: 2.0
A-2 Examples of preparation of the CDK9 inhibitors of the ADCs according to the present invention and test of their biological efficacy

Materials and Methods:

The percentage data in the following tests and examples are percentages by weight unless otherwise indicated; parts are parts by weight. Solvent ratios, dilution ratios and concentration data of liquid/liquid solutions are in each case based on volume.

Examples were tested in selected biological assays one or more times. When tested more than once, data are reported as either average values or as median values, wherein

- the average value, also referred to as the arithmetic mean value, represents the sum of the values obtained divided by the number of times tested, and
- the median value represents the middle number of the group of values when ranked in ascending or descending order. If the number of values in the data set is odd, the median is the middle value. If the number of values in the data set is even, the median is the arithmetic mean of the two middle values.

Examples were synthesized one or more times. When synthesized more than once, data from biological assays represent average values or median values calculated utilizing data sets obtained from testing of one or more synthetic batch.

The in vitro pharmacological properties of the compounds can be determined according to the following assays and methods.

Noteworthily, in the CDK9 assays described below the resolution power is limited by the enzyme concentrations, the lower limit for IC50s is about 1-2 nM in the CDK9 high ATP assay and 2-4 nM in the CDK low ATP assays. For compounds exhibiting IC50s in this range the true affinity to CDK9 and thus the selectivity for CDK9 over CDK2 might be even higher, i.e. for these compounds the selectivity factors calculated in columns 4 and 7 of Table 2, infra, are minimal values, they could be also higher.

1a. CDK9/CycT1 kinase assay:

CDK9/CycT1 inhibitory activity of compounds of the present invention was quantified employing the CDK9/CycT1 TR-FRET assay as described in the following paragraphs:

Recombinant full-length His-tagged human CDK9 and CycT1, expressed in insect cells and purified by Ni-NTA affinity chromatography, were purchased from Invitrogen (Cat. No PV4131). As substrate for
the kinase reaction biotinylated peptide biotin-Ttds-YISPLKSPYKISEG (C-terminus in amid form) was used which can be purchased e.g. from the company JERINI Peptide Technologies (Berlin, Germany).

For the assay 50 nL of a 1000-fold concentrated solution of the test compound in DMSO was pipetted into a black low volume 384-well microtiter plate (Greiner Bio-One, Frickenhausen, Germany), 2 µL of a solution of CDK9/CycTl in aqueous assay buffer [50 mM Tris/HCl pH 8.0, 10 mM MgCl₂, 10 mM dithiothreitol, 0.1 mM sodium ortho-vanadate, 0.01% (v/v) Nonidet-P40 (Sigma)] were added and the mixture was incubated for 15 min at 22°C to allow pre-binding of the test compounds to the enzyme before the start of the kinase reaction. Then the kinase reaction was started by the addition of 3 µL of a solution of adenosine-tri-phosphate (ATP, 16.7 µM => final cone, in the 5 µL assay volume is 10 µM) and substrate (1.67 µM => final cone, in the 5 µL assay volume is 1 µM) in assay buffer and the resulting mixture was incubated for a reaction time of 25 min at 22°C. The concentration of CDK9/CycTl was adjusted depending on the activity of the enzyme lot and was chosen appropriate to have the assay in the linear range, typical concentrations were in the range of 1 µg/mL. The reaction was stopped by the addition of 5 µL of a solution of TR-FRET detection reagents (0.2 µM streptavidine-XL665 [Cisbio Bioassays, Codelet, France] and 1 nM anti-RB(pSer807/pSer811)-antibody from BD Pharmingen [#558389]) and 1.2 nM LANCE EU-W1024 labeled anti-mouse IgG antibody [Perkin-Elmer, product no. AD0077]) in an aqueous EDTA-solution (100 mM EDTA, 0.2 % (w/v) bovine serum albumin in 100 mM HEPES pH 7.5).

The resulting mixture was incubated 1 h at 22°C to allow the formation of complex between the phosphorylated biotinylated peptide and the detection reagents. Subsequently the amount of phosphorylated substrate was evaluated by measurement of the resonance energy transfer from the Eu-chelate to the streptavidine-XL. Therefore, the fluorescence emissions at 620 nm and 665 nm after excitation at 350 nm was measured in a HTRF reader, e.g. a Rubystar (BMG Labtechnologies, Offenburg, Germany) or a Viewlux (Perkin-Elmer). The ratio of the emissions at 665 nm and at 622 nm was taken as the measure for the amount of phosphorylated substrate. The data were normalised (enzyme reaction without inhibitor = 0 % inhibition, all other assay components but no enzyme = 100 % inhibition). Usually the test compounds were tested on the same microtiterplate in 11 different concentrations in the range of 20 µM to 0.1 nM (20 µM, 5.9 µM, 1.7 µM, 0.51 µM, 0.15 µM, 44 nM, 13 nM, 3.8 nM, 1.1 nM, 0.33 nM and 0.1 nM, the dilution series prepared separately before the assay on the level of the 1000-fold concentrated solutions in DMSO by serial 1:3.4 dilutions) in duplicate values for each concentration and IC50 values were calculated by a 4 parameter fit using an inhouse software.

**lb. CDK9/CycTl high ATP kinase assay**

see method C-3 described below

**2a. CDK2/CycE kinase assay:**
CDK2/CycE inhibitory activity of compounds of the present invention was quantified employing the CDK2/CycE TR-FRET assay as described in the following paragraphs:

Recombinant fusion proteins of GST and human CDK2 and of GST and human CycE, expressed in insect cells (Sf9) and purified by Glutathion-Sepharose affinity chromatography, were purchased from ProQinase GmbH (Freiburg, Germany). As substrate for the kinase reaction biotinylated peptide biotin-Tds-YISPLKSPYKISEG (C-terminus in amid form) was used which can be purchased e.g. from the company JERINI Peptide Technologies (Berlin, Germany).

For the assay 50 nL of a 1,000 fold concentrated solution of the test compound in DMSO was pipetted into a black low volume 384well microtiter plate (Greiner Bio-One, Frickenhuisen, Germany). 2 µL of a solution of CDK2/CycE in aqueous assay buffer [50 mM Tris/HCl pH 8.0, 10 mM MgCl₂, 1.0 mM dithiothreitol, 0.1 mM sodium ortho-vanadate, 0.01% (v/v) Nonidet-P40 (Sigma)] were added and the mixture was incubated for 15 min at 22°C to allow pre-binding of the test compounds to the enzyme before the start of the kinase reaction. Then the kinase reaction was started by the addition of 3 µL of a solution of adenosine-tri-phosphate (ATP, 16.7 µM => final cone, in the 5 µL assay volume is 10 µM) and substrates (1.25 µM => final cone, in the 5 µL assay volume is 0.75 µM) in assay buffer and the resulting mixture was incubated for a reaction time of 25 min at 22°C. The concentration of CDK2/CycE was adjusted depending of the activity of the enzyme lot and was chosen appropriate to have the assay in the linear range, typical concentrations were in the range of 130 ng/mL. The reaction was stopped by the addition of 5 µL of a solution of TR-FRET detection reagents (0.2 µM streptavidine-XL665 [Cisbio Bioassays, Codolet, France] and 1 nM anti-RB(pSer807/pSer81)1)-antibody from BD Pharmingen [#558389] and 1.2 nM LANCE EU-W1024 labeled anti-mouse IgG antibody [Perkin-Elmer, product no. AD0077]) in an aqueous EDTA-solution (100 mM EDTA, 0.2 % (w/v) bovine serum albumin in 100 mM HEPES pH 7.5).

The resulting mixture was incubated 1 h at 22°C to allow the formation of complex between the phosphorylated biotinylated peptide and the detection reagents. Subsequently the amount of phosphorylated substrate was evaluated by measurement of the resonance energy transfer from the Eu-chelate to the streptavidine-XL. Therefore, the fluorescence emissions at 620 nm and 665 nm after excitation at 350 nm was measured in a TR-FRET reader, e.g. a Rubystar (BMG Labtechnologies, Offenburg, Germany) or a Viewlux (Perkin-Elmer). The ratio of the emissions at 665 nm and at 622 nm was taken as the measure for the amount of phosphorylated substrate. The data were normalised (enzyme reaction without inhibitor = 0 % inhibition, all other assay components but no enzyme = 100 % inhibition). Usually the test compounds were tested on the same microtiterplate in 11 different concentrations in the range of 20 µM to 0.1 nM (20 µM, 5.9 µM, 1.7 µM, 0.51 µM, 0.15 µM, 44 nM, 13 nM, 3.8 nM, 1.1 nM, 0.33 nM and 0.1 nM, the dilution series prepared separately before the assay on the level of the 1000 fold concentrated solutions in DMSO by serial 1:3.4 dilutions) in duplicate values for each concentration and IC50 values were calculated by a 4 parameter fit using an inhouse software.
2b. CDK2/CycE high ATP kinase assay:

CDK2/CycE -inhibitory activity of compounds of the present invention at 2 mM adenosine-tri-phosphate (ATP) was quantified employing the CDK2/CycE TR-FRET (TR-FRET = Time Resolved Fluorescence Resonance Energy Transfer) assay as described in the following paragraphs.

Recombinant fusion proteins of GST and human CDK2 and of GST and human CycE, expressed in insect cells (Sf9) and purified by Glutathion-Sepharose affinity chromatography, were purchase from ProQinase GmbH (Freiburg, Germany). As substrate for the kinase reaction, biotinylated peptide biotin-Tdts-Y1SPLKSPYKISEG (C-terminus in amido form) was used which can be purchased e.g. from the company JERINI peptide technologies (Berlin, Germany).

For the assay 50 nl of a lOOfold concentrated solution of the test compound in DMSO was pipetted into a black low volume 384well microtiter plate (Greiner Bio-One, Frickenhausen, Germany), 2 µl of a solution of CDK2/CycE in aqueous assay buffer [50 mM Tris/HCl pH 8.0, 10 mM MgCl2, 1.0 mM dithiothreitol, 0.1 mM sodium ortho- vanadate, 0.01% (v/v) Nonidet-P40 (Sigma)] were added and the mixture was incubated for 15 min at 22°C to allow pre-binding of the test compounds to the enzyme before the start of the kinase reaction. Then the kinase reaction was started by the addition of 3 µl of a solution ATP (3.33 mM => final cone, in the 5 µl assay volume is 2 mM) and substrate (1.25 µM => final cone, in the 5 µl assay volume is 0.75 µM) in assay buffer and the resulting mixture was incubated for a reaction time of 25 min at 22°C. The concentration of CDK2/CycE was adjusted depending of the activity of the enzyme lot and was chosen appropriate to have the assay in the linear range, typical concentrations were in the range of 15 ng/ml. The reaction was stopped by the addition of 5 µl of a solution of TR-FRET detection reagents (0.2 µM streptavidine-XL665 [Cisbio Bioassays, Codolet, France] and 1 nM anti-RB(pSer807/pSer811)-antibody from BD Pharmingen [# 558389] and 1.2 nM LANCE EU-W1024 labeled anti-mouse IgG antibody [Perkin-Elmer, product no. AD0077, as an alternative a Terbium-cryptate-labeled anti-mouse IgG antibody from Cisbio Bioassays can be used]) in an aqueous EDTA-solution (100 mM EDTA, 0.2 % (w/v) bovine serum albumin in 100 mM HEPES pH 7.5).

The resulting mixture was incubated 1 h at 22°C to allow the formation of complex between the phosphorylated biotinylated peptide and the detection reagents. Subsequently the amount of phosphorylated substrate was evaluated by measurement of the resonance energy transfer from the Eu-chelate to the streptavidine-XL. Therefore, the fluorescence emissions at 620 nm and 665 nm after excitation at 350 nm were measured in a TR-FRET reader, e.g. a Rubystar (BMG Labtechnologies, Offenburg, Germany) or a Viewlux (Perkin-Elmer). The ratio of the emissions at 665 nm and at 622 nm was taken as the measure for the amount of phosphorylated substrate. The data were normalised (enzyme reaction without inhibitor = 0 % inhibition, all other assay components but no enzyme = 100 % inhibition). Usually the test compounds were tested on the same microtiter plate in 11 different concentrations in the range of 20 µM to 0.1 nM (20 µM, 5.9 µM, 1.7 µM, 0.51 µM, 0.15 µM, 44 nM, 13 nM, 3.8 nM, 1.1 nM, 0.33 nM and 0.1 nM, the dilution series prepared separately before the assay on the
level of the 100-fold concentrated solutions in DMSO by serial 1:3.4 dilutions) in duplicate values for each concentration and IC50 values were calculated by a 4 parameter fit using an inhouse software.

3. Proliferation Assay:

Cultivated tumour cells (HeLa, human cervical tumour cells, ATCC CCL-2; NCI-H460, human non-small cell lung carcinoma cells, ATCC HTB-177; A2780, human ovarian carcinoma cells, ECACC # 93112519; DU 145, hormone-independent human prostate carcinoma cells, ATCC HTB-81; HeLa-MaTu-ADR, multidrug-resistant human cervical carcinoma cells, EPO-GmbH Berlin; Caco-2, human colorectal carcinoma cells, ATCC HTB-37; B16F10, mouse melanoma cells, ATCC CRL-6475) were plated at a density of 5,000 cells/well (DU145, HeLa-MaTu-ADR), 3,000 cells/well (NCI-H460, HeLa), 2,500 cells/well (A2780), 1,500 cells/well (Caco-2), or 1,000 cells/well (B16F10) in a 96-well multititer plate in 200 µL of their respective growth medium supplemented 10% fetal calf serum. After 24 hours, the cells of one plate (zero-point plate) were stained with crystal violet (see below), while the medium of the other plates was replaced by fresh culture medium (200 µi), to which the test substances were added in various concentrations (0 µM, as well as in the range of 0.001 - 10 µM). The cells were incubated for 4 days in the presence of test substances. Cell proliferation was determined by staining the cells with crystal violet: the cells were fixed by adding 20 µi/measuring point of an 11% glutaric aldehyde solution for 15 minutes at room temperature. After three washing cycles of the fixed cells with water, the plates were dried at room temperature. The cells were stained by adding 100 µi/measuring point of a 0.1% crystal violet solution (pH 3.0). After three washing cycles of the stained cells with water, the plates were dried at room temperature. The dye was dissolved by adding 100 µi/measuring point of a 10% acetic acid solution. The extinction was determined by photometry at a wavelength of 595 nm. The change of cell number, in percent, was calculated by normalization of the measured values to the extinction values of the zero-point plate (=0%) and the extinction of the untreated (0 µM) cells (=100%). The IC50 values (inhibitory concentration at 50% of maximal effect) were determined by means of a 4 parameter fit.

Non-adherent MOLM-13 human acute myeloid leukemia cells (DSMZ ACC 554) were seeded at a density of 5,000 cells/well in a 96-well multititer plate in 100 µL of growth medium supplemented 10% fetal calf serum. After 24 hours, cell viability of one plate (zero-point plate) was determined with the Cell Titre-Glo Luminescent Cell Viability Assay (Promega), while 50 µL of test compound containing medium was added to the wells of the other plates (final concentrations in the range of 0.001 - 10 µM and DMSO controls). Cell viability was assessed after 72-hour exposure with the Cell Titre-Glo Luminescent Cell Viability Assay (Promega). IC50 values (inhibitory concentration at 50% of maximal effect) were determined by means of a 4 parameter fit on measurement data which were normalized to vehicle (DMSO) treated cells (=100%) and measurement readings taken immediately before compound exposure (=0%).
4. Equilibrium Shake Flask Solubility Assay:

4a) High Throughput determination of aqueous drug solubility (100 mmolar in DMSO)

The high throughput screening method to determine aqueous drug solubility is based on:

Thomas Onofrey and Greg Kazan, Performance and correlation of a 96-well high throughput screening method to determine aqueous drug solubility,

http://www.millipore.com/publications.nsf/a73664f9f98 Iaf8c852569b9005b4eee/e565516fb76e743585256da30052db77/$FILE/AN1731EN00.pdf

The assay was run in a 96-well plate format. Each well was filled with an individual compound. All pipetting steps were performed using a robot platform.

100 µl of a 10 mmolar solution of drug in DMSO were concentrated by vacuum centrifugation and resolved in 10 µl DMSO. 990 µl phosphate buffer pH 6.5 were added. The content of DMSO amounts to 1%. The multititer plate was put on a shaker and mixed for 24 hrs at room temperature. 150 µl of the suspension were transferred to a filtration plate. After filtration using a vacuum manifold the filtrate was diluted 1:400 and 1:8000. A second microtiter plate with 20 µl of a 10 mM solution of drug in DMSO served for calibration. Two concentrations (0.005 µM and 0.0025 µM) were prepared by dilution in DMSO / water 1:1 and used for calibration. Filtrate and calibration plates were quantified by HPLC-MS/MS.

Chemicals:

Preparation of 0.1 M phosphate buffer pH 6.5:
61.86 g NaCl and 39.54 mg KH2PO4 were solved in water and filled up to 1 l. The mixture was diluted 1:10 with water and the pH adjusted to 6.5 by NaOH.

Materials:

Millipore MultiScreen HTS-HV Plate 0.45 µm

Chromatographic conditions were as follows:

HPLC column: Ascentis Express C18 2.7 µm 4.6 x 30 mm
Injection volume: 1 µl
Flow: 1.5 ml/min
Mobile phase: acidic gradient

A: Water / 0.05% HCOOH
B: Acetonitrile / 0.05% HCOOH
0 min → 95%A 5%B
0.75 min → 5%A 95%B
2.75 min → 5%A 95%B
2.76 min → 95%A 5%B
3 min → 95%A 5%B
The areas of sample- and calibration injections were determined by using mass spectrometry software (AB SCIEX: Discovery Quant 2.1.3. and Analyst 1.6.1). The calculation of the solubility value (in mg/l) was executed by an inhouse developed Excel macro.

5 4b) Thermodynamic solubility in water from powder

The thermodynamic solubility of compounds in water was determined by an equilibrium shake flask method (see for example: E.H. Kerns, L. Di: Drug-like Properties: Concepts, Structure Design and Methods, 276-286, Burlington, MA, Academic Press, 2008). A saturated solution of the drug was prepared and the solution was mixed for 24 h to ensure that equilibrium was reached. The solution was centrifuged to remove the insoluble fraction and the concentration of the compound in solution was determined using a standard calibration curve. To prepare the sample, 2 mg solid compound was weighed in a 4 mL glass vial. 1 mL phosphate buffer pH 6.5 was added. The suspension was stirred for 24 hrs at room temperature. The solution was centrifuged afterwards. To prepare the sample for the standard calibration, 2 mg solid sample was dissolved in 30 mL acetonitrile. After sonification the solution was diluted with water to 50 mL. Sample and standards were quantified by HPLC with UV-detection. For each sample two injection volumes (5 and 50 µl) in triplicates were made. Three injection volumes (5 µl, 10 µl and 20 µl) were made for the standard.

Chromatographic conditions:

HPLC column: Xterra MS C18 2.5 µm 4.6 x 30 mm

Injection volume: Sample: 3x5µl and 3x50µl
Standard: 5µl, IOµl, 20µl

Flow: 1.5mL/min

Mobile phase: acidic gradient:

A: Water/ 0.01% TFA

B: Acetonitrile / 0.01% TFA

0 min → 95%A 5%B

0-3 min → 35%A 65%B, linear gradient

3-5 min → 35%A 65%B, isocratic

5-6 min → 95%A 5%B, isocratic

UV detector: wavelength near the absorption maximum (between 200 and 400nm)

The areas of sample- and standard injections as well as the calculation of the solubility value (in mg/l) were determined by using HPLC software (Waters Empower 2 FR).
4c) Thermodynamic solubility in Citrate buffer pH 4


A saturated solution of the drug was prepared and the solution was mixed for 24 h to ensure that equilibrium has been reached. The solution was centrifuged to remove the insoluble fraction and the concentration of the compound in solution was determined using a standard calibration curve.

To prepare the sample, 1.5 mg solid compound was weighed in a 4 ml glass vial. 1 ml Citrate buffer pH 4 was added. The suspension was put on a stirrer and mixed for 24 hrs at room temperature. The solution was centrifuged afterwards. To prepare the sample for the standard calibration, 0.6 mg solid sample was dissolved in 19 ml acetonitrile/water 1:1. After sonification the solution was filled up with acetonitrile/water 1:1 to 20 ml.

Sample and standards were quantified by HPLC with UV-detection. For each sample two injection volumes (5 and 50 µl) in triplicates were made. Three injection volumes (5 µl, 10 µl and 20 µl) were made for the standard.

Chemicals:

Citrate buffer pH 4 (MERCK Art. 109435; 1 L buffer consisting of 11,768 g citric acid, 4,480 g sodium hydroxide, 1,604 g hydrogen chloride)

Chromatographic conditions were as follows:

HPLC column: Xterra MS C18 2.5 µm 4.6 x 30 mm
Injection volume: Sample: 3x5µl and 3x50µl
Standard: 5µl, 10µl, 20µl
Flow: 1.5ml/min
Mobile phase: acidic gradient:
A: Water/ 0.01% TFA
B: Acetonitrile / 0.01% TFA
Omin: 95%A  5%B
0-3 min: 35%A 65%B, linear gradient
3-5 min: 35%A 65%B, isocratic
5-6 min: 95%A  5%B, isocratic
UV detector: wavelength near the absorption maximum (between 200 and 400nm)

The areas of sample- and standard injections as well as the calculation of the solubility value (in mg/l) were determined by using HPLC software (Waters Empower 2 FR).

The areas of sample- and standard injections as well as the calculation of the solubility value (in mg/l) were determined by using HPLC software (Waters Empower 2 FR).
5. Caco-2 Permeation Assay:
Caco-2 cells (purchased from DSMZ Braunschweig, Germany) were seeded at a density of 4.5 x 10^4 cells per well on 24 well insert plates, 0.4 μm pore size, and grown for 15 days in DMEM medium supplemented with 10% fetal bovine serum, 1% GlutaMAX (100X, GIBCO), 100 U/mL penicillin, 100μg/mL streptomycin (GIBCO) and 1% non essential amino acids (100 x). Cells were maintained at 37°C in a humified 5% CO₂ atmosphere. Medium was changed every 2-3 day. Before running the permeation assay, the culture medium was replaced by a FCS-free hepes-carbonate transport buffer (pH 7.2). For assessment of monolayer integrity the transepithelial electrical resistance (TEER) was measured. Test compounds were predissolved in DMSO and added either to the apical or basolateral compartment in final concentration of 2 μM in transport buffer. Before and after 2h incubation at 37°C samples were taken from both compartments. Analysis of compound content was done after precipitation with methanol by LC/MS/MS analysis. Permeability (Papp) was calculated in the apical to basolateral (A → B) and basolateral to apical (B → A) directions. The apparent permeability was calculated using following equation:

\[ P_{\text{app}} = \frac{(V_r/P_o)(l/S)(P_2/t)}{ } \]

Where \( V_r \) is the volume of medium in the receiver chamber, \( P_o \) is the measured peak area or height of the test drug in the donor chamber at \( t=0 \), \( S \) the surface area of the monolayer, \( P_2 \) is the measured peak area of the test drug in the acceptor chamber after 2h of incubation, and \( t \) is the incubation time. The efflux ratio basolateral (B) to apical (A) was calculated by dividing the Papp B-A by the Papp A-B. In addition the compound recovery was calculated.

6. Investigation of in vitro metabolic stability in rat hepatocytes
Hepatocytes from Han Wistar rats were isolated via a 2-step perfusion method. After perfusion, the liver was carefully removed from the rat: the liver capsule was opened and the hepatocytes were gently shaken out into a Petri dish with ice-cold Williams medium E (purchased from Sigma Aldrich Life Science, St Louis, MO). The resulting cell suspension was filtered through sterile gauze in 50 ml falcon tubes and centrifuged at 50 x g for 3 min at room temperature. The cell pellet was resuspended in 30 ml WME and centrifuged through a Percoll® gradient for 2 times at 100 x g. The hepatocytes were washed again with Williams' medium E (WME) and resuspended in medium containing 5% Fetal calf serum (FCS, purchased from Invitrogen, Auckland, NZ). Cell viability was determined by trypan blue exclusion.

For the metabolic stability assay liver cells were distributed in WME containing 5% FCS to glass vials at a density of 1.0 x 10^6 vital cells/ml. The test compound was added to a final concentration of 1 μM. During incubation, the hepatocyte suspensions were continuously shaken and aliquots were taken at 2, 8, 16, 30, 45 and 90 min, to which equal volumes of cold acetonitrile were immediately added. Samples were frozen at -20°C over night, after subsequently centrifuged for 15 minutes at 3000 rpm and the supernatant was analyzed with an Agilent 1200 HPLC-system with LCMS/MS detection.
The half-life of a test compound was determined from the concentration-time plot. From the half-life the intrinsic clearances were calculated. Together with the additional parameters liver blood flow, amount of liver cells in vivo and in vitro, the maximal oral bioavailability (Fmax) was calculated using the following scaling parameters: Liver blood flow (rat) - 4.2 L/h/kg; specific liver weight - 32 g/kg rat body weight; liver cells in vivo- 1.1 x 10^8 cells/g liver, liver cells in vitro - 0.5 x 10^9/ml.

7. In vivo pharmacokinetics in rats

For in vivo pharmacokinetic experiments test compounds were administered to male Wistar rats intravenously at doses of 0.3 to 1 mg/kg formulated as solutions using either rat plasma or solubilizers such as PEG400 in well-tolerated amounts.

For pharmacokinetics after intravenous administration test compounds were given as i.v. bolus and blood samples were taken at 2 min, 8 min, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h after dosing. Depending on the expected half-life additional samples were taken at later time points (e.g. 48 h, 72 h).

Blood was collected into Lithium-Heparin tubes (Monovetten® , Sarstedt) and centrifuged for 15 min at 3000 rpm. An aliquot of 100 µL from the supernatant (plasma) was taken and precipitated by addition of 400 µL ice cold acetonitrile and frozen at -20 °C over night. Samples were subsequently thawed and centrifuged at 3000 rpm, 4 °C for 20 minutes. Aliquots of the supernatants were taken for analytical testing using an Agilent 1200 HPLC-system with LCMS/MS detection. PK parameters were calculated by non-compartmental analysis using a PK calculation software.

PK parameters derived from concentration-time profiles after i.v.: CLplasma: Total plasma clearance of test compound (in L/kg/h); CLblood: Total blood clearance of test compound; CLplasma*Cp/Cb (in L/kg/h) with Cp/Cb being the ratio of concentrations in plasma and blood, AUCnorm: Area under the concentration-time curve from t=0h to infinity (extrapolated) divided by the administered dose (in kg*h/L); t1/2: terminal half-life (in h).

8. Surface Plasmon Resonance PTEFb

Definitions

The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of the reversible associations of biological molecules in real time within a biosensor matrix, for example using the Biacore® system (GE Healthcare Biosciences, Uppsala, Sweden). Biacore® uses the optical properties of surface plasmon resonance (SPR) to detect alterations in the refractive index of a buffer, which changes as molecules in solution interact with the target immobilized on the surface. In brief, proteins are covalently bound to the dextran matrix at a known concentration and a ligand for the protein is injected through the dextran matrix. Near infrared light, directed onto the opposite side of the sensor chip surface is reflected and also induces an evanescent wave in the gold film, which in turn, causes an intensity dip in the reflected light at a particular angle known as the resonance angle. If the
refractive index of the sensor chip surface is altered (e.g. by compound binding to the bound protein) a
shift occurs in the resonance angle. This angle shift can be measured. These changes are displayed with
respect to time along the y-axis of a sensorgram, which depicts the association and dissociation of any
biological reaction.

The term "KD", as used herein, is intended to refer to the equilibrium dissociation constant of a particular
compound / target protein complex.

The term "koff", as used herein, is intended to refer to the off-rate, i.e. the dissociation rate constant of a
particular compound / target protein complex.

The term "target residence time", as used herein, is intended to refer to the inverse of the rate of
dissociation rate constant (1/koff) of a particular compound / target protein complex.

For further descriptions see:
Day Y et al, Protein Science, 2002;11, 1017-1025
Myskza DG, Anal Biochem., 2004; 329, 316-323

Biological activity
The biological activity (e.g. as inhibitors of PETFb) of the compounds according to the invention can be measured using the SPR assay described.

The level of activity exhibited by a given compound in the SPR assay can be defined in terms of the KD
value, and preferred compounds of the present invention are compounds having a KD value of less than 1
micromolar, more preferably less than 0.1 micromolar. Furthermore, the time in residence at its target of a given compound can be defined in terms of the target
residence time (TRT), and preferred compounds of the present invention are compounds having a TRT
value of more than 10 minutes, more preferably more than 1 hour.
The ability of the compounds according to the invention to bind human PTEFb may be determined using surface plasmon resonance (SPR). $k_D$ values and $k_{off}$ values may be measured using a Biacore® T200 instrument (GE Healthcare, Uppsala, Sweden).

For SPR measurements, recombinant human PTEFb (CDK9/Cyclin T1 recombinant human active protein kinase purchased from ProQinase, Freiburg, Germany) is immobilized using standard amine coupling (Johnsson B et al, Anal Biochem. 1991 Nov 1;198(2):268-77). Briefly, carboxymethylated dextran biosensor chips (CM7, GE Healthcare) are activated with N-ethyl-N’-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Human PTEFb is diluted in 1x HBS-EP+ (GE Healthcare) into 30 μg / ml and injected on the activated chip surface. Subsequently, a 1:1 solution of 1 M ethanolamine-HCl (GE Healthcare) and 1x HBS-EP is injected to block unreacted groups, resulting in approximately 4000 response units (RU) of immobilized protein. A reference surface is generated by treatment with NHS-EDC and ethanolamine-HCl. Compounds are dissolved in 100% dimethylsulfoxide (DMSO, Sigma-Aldrich, Germany) to a concentration of 10 mM and subsequently diluted in running buffer (1x HBS-EP+ pH 7.4 [generated from HBS-EP+ Buffer 10x (GE Healthcare): 0.1 M HEPES, 1.5 M NaCl, 30 mM EDTA and 0.5% v/v Surfactant P20], 1% v/v DMSO). For kinetic measurements, four-fold serial dilutions of compound (0.39 nM to 100 nM) are injected over immobilized protein. Binding kinetics is measured at 25 °C with a flow rate of 50 μl/min in running buffer. Compound concentrations are injected for 60 s followed by a dissociation time of 1800 s. The resulting sensorgrams are double-referenced against the reference surface as well as against blank injections.

The double-referenced sensorgrams are fit to a simple reversible Langmuir 1:1 reaction mechanism as implemented in the Biacore® T200 evaluation software 2.0 (GE Healthcare). In cases where full compound dissociation has not occurred at the end of the dissociation phase, the $R_{max}$ parameter (response at saturation) is fit as local variable. In all other cases, $R_{max}$ is fit as global variable.

**Abbreviations used in the description of the chemistry and in the Examples that follow are:**

- br. (broad, ¾ NMR signal)
- CDCl$_3$ (deuterated chloroform)
- cHex (cyclohexane)
- DCE (dichloroethane)
- d (doublet, ¾ NMR signal)
- DCM (dichloromethane)
- DIPEA (di-wo-propylethylamine)
- DMAP (4-$N,N$-dimethylaminopyridine)
- DME (1,2-dimethoxyethane)
- DMF ($N,N$-dimethylformamide)
- DMSO (dimethyl sulfoxide)
- ES (electrospray)
- EtOAc (ethyl acetate)
- EtOH (ethanol)
- h (hour(s))
- ¾ NMR (proton nuclear magnetic resonance spectroscopy)
- HPLC (High Performance Liquid Chromatography)
- iPrOH (iso-propanol)
- m (multiplet, ¾ NMR signal)
- mCPBA (me/a-chloroperoxybenzoic acid)
- MeCN (acetonitrile)
- MeOH (methanol)
- min (minute(s))
- MS (mass spectrometry)
- MTBE (methyl tert-butyl ether)
- NMP ($N$-Methylpyrrolidin-2-one)
- NMR (nuclear magnetic resonance)
- Pd(dppf)Cl$_2$ ([1,1'-bis(diphenylphosphino)ferrocene]dichloro palladium(II) complex with dichloromethane)
- p (quartet, ¾
NMR signal); quin (quintet, ¾ NMR signal); rac (racemic); RT (room temperature); s (singlet, ¾ NMR signal); sat. aq. (saturated aqueous); SiO2 (silica gel); t (triplet, ¾ NMR signal); TFA (trifluoroacetic acid); TFAA (trifluoroacetic anhydride), THF (tetrahydrofuran); UV (ultraviolet).

5 **Chemical naming:**
The IUPAC names of the examples were generated using the program 'ADC/Name batch version 12.01' from ADC LABS.

**Salt stoichiometry:**
In the present text, in particular in the Experimental Section, for the synthesis of intermediates and of examples of the present invention, when a compound is mentioned as a salt form with the corresponding base or acid, the exact stoichiometric composition of said salt form, as obtained by the respective preparation and/or purification process, is, in most cases, unknown.

15 Unless specified otherwise, suffixes to chemical names or structural formulae such as "hydrochloride", "trifluoroacetate", "sodium salt", or "x HC1", "x CF3COOH", "x Na++", for example, are to be understood as not a stoichiometric specification, but solely as a salt form.

This applies analogously to cases in which synthesis intermediates or example compounds or salts thereof have been obtained, by the preparation and/or purification processes described, as solvates, such as hydrates with (if defined) unknown stoichiometric composition.

**HPLC methods:**
Method 1 (preparative HPLC):

25 Instrument: Abimed/Gilson 305 / 306 binary pumps 0-100ml/min + 806 manometric module; Knauer UV-detector K-2501; ISCO Foxy 200 fraction collector; SCPA PrepCon 5 software

Column: Chromatorex C18 10μm 125x30mm
Mobile phase A: water + 0.05% TFA, mobile phase B: acetonitrile + 0.05% TFA
Gradient: 10% B → 50% B; 50% B isocratic; 50% B → 80% B
Flow rate: 50 ml/ min
Column temperature: room temperature
UV-detection: 210 nm

35 Method 2 (preparative HPLC)

Instrument: Abimed/Gilson 305 / 306 binary pumps 0-100ml/min + 806 manometric module; Knauer UV-detector K-2501; ISCO Foxy 200 fraction collector; SCPA PrepCon 5 software
Column: Kromasil-IOOA C18 5µ 125x20mm
Mobile phase A: water + 0.05% TFA, mobile phase B: acetonitrile + 0.05% TFA
Gradient: 15% B → 50% B; 50% B isocratic; 50% B → 80% B
Flow rate: 25 ml/ min
Column temperature: room temperature
UV-detection: 210 nm

Example 1:
(rac)- 16,20-Difluoro-9-[(S-methylsulfonimidoyl)methyl]-2,3,4,5-tetrahydro-12H-13,17-(azeno)-ll,7-(metheno)-1,6,12,14-benzodioxadiazacyclononadecine
Preparation of Intermediate 1.1:
3-(Chloromethyl)-5-nitrophenol

Thionyl chloride (84.0 g; 712 mmol) was added dropwise to a stirred solution of 3-(hydroxymethyl)-5-nitrophenol (60.0 g; 355 mmol; CAS-No. 180628-74-4 purchased from Struchem) in DMF (1200 mL) at 0°C. The mixture was stirred at 10°C for 3 hours. The mixture was concentrated, diluted with water and extracted three times with ethyl acetate. The combined organic layers were washed twice with water and concentrated to afford the crude product (60.0 g, 320 mmol) that was used without further purification.

Preparation of Intermediate 1.2:
3-[(Methylsulfanyl)methyl]-5-nitrophenol

To a solution of crude 3-(chloromethyl)-5-nitrophenol (60.0 g; 320 mmol) in acetone (600 mL) at room temperature was added an aqueous solution of sodium thiomethoxide (21%, 180 mL). The mixture was stirred at room temperature for 3 hours before additional aqueous solution of sodium thiomethoxide (21%, 180 mL) was added and the mixture was stirred at room temperature overnight. Finally, additional aqueous solution of sodium thiomethoxide (21%; 90 mL) was added and the mixture was stirred at room temperature for 6 hours. The batch was diluted with ethyl acetate and an aqueous solution of sodium chloride and extracted three times with ethyl acetate. The combined organic layers were concentrated and the residue was purified by column chromatography on silica gel (pentane / ethyl acetate 4:1) to afford the desired product (60.0 g, 302 mmol).

¾ NMR (300MHz, CDCl3, 300K) δ = 7.71 (1H), 7.57(1H), 7.15 (1H), 3.66 (2H), 1.99 (3H).
**Preparation of Intermediate 1.3:**

Ethyl 4-{3-[(methylsulfanyl)methyl]-5-nitrophenoxy}butanoate

Ethyl 4-bromobutanoate (15.8 g; 81 mmol) was added dropwise to a stirred mixture of 3-[(methylsulfanyl)methyl]-5-nitrophenol (15.0 g; 75 mmol) and potassium carbonate (12.5 g; 90 mmol) in DMF (150 mL) at 0°C. The mixture was stirred at room temperature overnight. The mixture diluted with water and extracted three times with ethyl acetate. The combined organic layers were washed twice with water and concentrated to afford the crude product (17.6 g) that was used without further purification.

¾ NMR (300MHz, DMSO-d6, 300K) δ = 7.74 (1H), 7.53 (1H), 7.30 (1H), 4.03 (3H), 3.75 (2H), 3.50 (1H), 2.42 (3H), 1.99 (1H), 1.92 (3H), 1.14 (3H).

**Preparation of Intermediate 1.4:**

4-{3-[(Methylsulfanyl)methyl]-5-nitrophenoxy}butan-1-ol

A solution of DIBAL in hexane (1N; 176 mL) was added dropwise to a stirred solution of crude ethyl 4-{3-[(methylsulfanyl)methyl]-5-nitrophenoxy}butanoate (17.6 g) in dry THF (400 mL) at -25°C. The mixture was stirred at 0°C for 150 minutes. Water (200 mL) was added dropwise, the mixture was acidified with an aqueous solution of hydrogen chloride (1N) to pH 4-5 and extracted three times with ethyl acetate. The combined organic layers were concentrated and the residue was purified by column chromatography on silica gel (pentane / ethyl acetate = 4:1 to 2:1) to afford the desired product (14.0 g, 51.7 mmol)

¾ NMR (300MHz, DMSO-d6, 300K) δ = 7.71 (1H), 7.50 (1H), 7.28 (1H), 4.43 (1H), 4.03 (2H), 3.73 (2H), 3.43 (2H), 1.92 (3H), 1.74 (2H), 1.54 (2H).
Preparation of Intermediate 1.5:

2-Chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidine

A batch with 2,4-dichloro-5-fluoropyrimidine (200 mg; 1.20 mmol; Aldrich Chemical Company Inc.), (4-fluoro-2-methoxyphenyl)boronic acid (224 mg; 1.31 mmol; Aldrich Chemical Company Inc.) and tetrakis(triphenylphosphin)palladium(0) (138 mg; 0.12 mmol) in 1,2-dimethoxyethane (3.6 ml) and 2M solution of potassium carbonate (1.8 ml) was degassed using argon. The batch was stirred under an atmosphere of argon for 16 hours at 90°C. After cooling the batch was diluted with ethyl acetate and washed with saturated aqueous sodium chloride solution. The organic layer was filtered using a Whatman filter and concentrated. The residue was purified by column chromatography (hexane / ethyl acetate 1:1) to give the desired product (106 mg; 0.41 mmol).

¾ NMR (400MHz, CDCl3, 300K) δ = 8.47 (1H), 7.51 (1H), 6.82 (1H), 6.73 (1H), 3.85 (3H).

Preparation of Intermediate 1.6:

2-(2-Chloro-5-fluoropyrimidin-4-yl)-5-fluorophenol

A solution of boron tribromide in DCM (1M; 43.3 mL; 47.1 mmol; Aldrich Chemical Company Inc.) was added dropwise to a stirred solution of 2-chloro-5-fluoro-4-(4-fluoro-2-methoxyphenyl)pyrimidine (2.00 g; 7.79 mmol) in DCM (189 mL) at 0°C. The mixture was slowly warmed to room temperature while stirring overnight. The mixture was cautiously diluted with an aqueous solution of sodium bicarbonate under stirring at 0°C and stirred at room temperature for 1 hour. Solid sodium chloride was added and the mixture filtered using a Whatman filter. The organic layer was concentrated to give the crude product (1.85 g) that was used without further purification.

¾ NMR (400MHz, DMSO-d6, 300K) δ = 10.80 (1H), 8.90 (1H), 7.50 (1H), 6.83 (1H), 6.78 (1H).
Preparation of Intermediate 1.7:
2-Chloro-5-fluoro-4-[4-fluoro-2-(4-{3-[(methylsulfanyl)methyl]-5-nitrophenox}butoxy)phenyl]pyrimidine

A solution of diisopropyl azodicarboxylate (0.41 mL; 2.06 mmol) in THF (1.6 mL) was added dropwise to a mixture of 4-{3-[(methylsulfanyl)methyl]-5-nitrophenoxy}butan-1-ol (511 mg; 1.88 mmol), 2-(2-chloro-5-fluoropyrimidin-4-yl)-5-fluorophenol (500 mg; 2.06 mmol) and triphenylphosphine (541 mg; 2.06 mmol) in THF (8.1 mL) and the batch was stirred at room temperature overnight. The mixture was concentrated and the residue was purified by column chromatography on silica gel (hexane to hexane / ethyl acetate 50%) to give the desired product (579 mg; 1.11 mmol).

$\delta$ NMR (400MHz, DMSO-d$_6$, 300K) δ = 8.87 (1H), 7.77 (1H), 7.54 (2H), 7.31 (1H), 7.16 (1H), 6.97 (1H), 4.14 (2H), 4.08 (2H), 3.78 (2H), 1.95 (3H), 1.79 (4H).

Preparation of Intermediate 1.8:
(rac)-2-Chloro-5-fluoro-4-[4-fluoro-2-(4-{3-[(methylsulfanyl)methyl]-5-nitrophenox}butoxy)phenyl]pyrimidine

Iron(III)chloride (5 mg; 0.03 mmol) was added to a mixture of 2-chloro-5-fluoro-4-[4-fluoro-2-(4-{3-[(methylsulfanyl)methyl]-5-nitrophenox}butoxy)phenyl]pyrimidine (545 mg; 1.10 mmol) in acetonitrile (27 mL) and the batch was stirred at room temperature for 10 minutes. The batch was cooled to 0°C and periodic acid (268 mg; 1.18 mmol) was added under stirring in one portion. After 10 minutes the ice bath was removed and the mixture was stirred at room temperature for 3 hours before it was added to a stirred solution of sodium thiosulfate pentahydrate (1527 mg; 6.15 mmol) in ice water (32 mL). The batch was saturated with solid sodium chloride and extracted twice with THF and twice with ethyl acetate. The
combined organic layers were filtered using a Whatman filter and concentrated to give the crude product (636 mg) which was used in the next step without further purification.

$\frac{3}{4}$ NMR (400MHz, CDCl3, 300K) $\delta = 8.50$ (1H), 7.77 (1H), 7.72 (1H), 7.54 (1H), 7.20 (1H), 6.84 (1H), 6.76 (1H), 4.08 (5H), 3.92 (1H), 2.57 (3H), 1.96 (4H).

Preparation of Intermediate 1.9:

$\text{(rac)}$-$N\text{-}[3\text{-}[4\text{-}[2\text{-}(2\text{-chloro-5-fluoropyrimidin-4-yl})\text{-}5\text{-fluorophenoxy}]butoxy]\text{-}5\text{-nitrobenzyl}]\text{(methyl)oxido-}\lambda^6\text{-sulfanylidene}\text{-}2\text{-}2\text{-trifluoroacetamide}$

To a suspension of crude (rac)-2-chloro-5-fluoro-4-[4-fluoro-2-[4-[3-[(methylsulfinyl)methyl]-5-nitrophenoxyl]butoxy]phenyl]pyrimidine (330 mg; 0.65 mmol), trifluoroacetamide (146 mg; 1.29 mmol), magnesium oxide (104 mg; 2.58 mmol) and rhodium(II)-acetate dimer (7 mg; 0.02 mmol) in DCM (4.8 mL) was added iodobenzene diacetate (311 mg; 0.97 mmol) at room temperature. The batch was stirred for 18 hours at room temperature, filtered and concentrated to give the crude product (340 mg) which was used in the next step without further purification.

Preparation of Intermediate 1.10:

$\text{(rac)}$-$N\text{-}[3\text{-}amino-5\text{-}[4\text{-}[2\text{-}(2\text{-chloro-5-fluoropyrimidin-4-yl})\text{-}5\text{-fluorophenoxy}]butoxy]benzyl]\text{(methyl)oxido-}\lambda^6\text{-sulfanylidene}\text{-}2\text{-}2\text{-trifluoroacetamide}$

Titanium(III)chloride solution (approx. 15% in approx. 10% hydrochloric acid, 7.1 mL; Merck Schuchardt OHG) was added to a stirred solution of (rac)-$N\text{-}[3\text{-}[4\text{-}[2\text{-}(2\text{-chloro-5-fluoropyrimidin-4-yl})\text{-}5\text{-fluorophenoxy}]butoxy]\text{-}5\text{-nitrobenzyl}]\text{(methyl)oxido-}\lambda^6\text{-sulfanylidene}\text{-}2\text{-}2\text{-trifluoroacetamide}$ (640
mg; 1.03 mmol) in THF (15 mL) at room temperature. The batch was stirred for 3 hours. By addition of solid sodium carbonate the pH of the mixture was adjusted to approximately 6. A saturated aqueous sodium chloride solution was added and the mixture was extracted three times with ethyl acetate/THF 1:1. The combined organic layers were filtered using a Whatman filter and concentrated. The residue was purified by column chromatography on silica gel (hexane to hexane/ethyl acetate 80%) to give the desired product (176 mg; 0.30 mmol).

\[ \text{NMR} \ (400\text{MHz}, \ \text{CDCl}_3, \ 300\text{K}) \ \delta = 8.47 \ (1\text{H}), \ 7.54 \ (1\text{H}), \ 6.84 \ (1\text{H}), \ 6.74 \ (1\text{H}), \ 6.30 \ (2\text{H}), \ 6.24 \ (1\text{H}), \ 4.67 \ (1\text{H}), \ 4.58 \ (1\text{H}), \ 4.11 \ (2\text{H}), \ 3.93 \ (2\text{H}), \ 3.83 \ (2\text{H}), \ 3.17 \ (3\text{H}), \ 1.90 \ (4\text{H}). \]

### Preparation of end product:

A mixture of (rac)-N-[(3-amino-5-[(4-[(2-chloro-5-fluoropyrimidin-4-yl)-5-fluorophenoxyl]butoxy]benzyl)(methyl)oxido-\(\lambda^2\)-sulfanylidene]-2,2,2-trifluoroacetamide (143 mg; 0.24 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-tri-z,yo-propyl-l,1'-biphenyl)[2-(2-aminoethyl)phenyl]palladium(II) methyl-tert-butylether adduct (20 mg; 0.02 mmol; ABCR GmbH & CO. KG) and 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl (11 mg; 0.02 mmol; Aldrich Chemical Company Inc.) and potassium phosphate (256 mg; 1.21 mmol) in toluene (18.0 ml) and NMP (2.2 mL) was stirred under an atmosphere of argon at 110°C in a closed vessel for 3 hours. After cooling, the batch was diluted with THF and ethyl acetate and washed with aqueous sodium chloride solution. The organic layer was filtered using a Whatman filter and concentrated. The residue was purified by preparative HPLC to give the desired product (25 mg; 0.05 mmol).

| System: | Waters Autopurificationsystem: Pump 254, Sample Manager 2767, CFO, DAD 2996, SQD 3100 |
| Column: | XBrigde C18 5µm 100x30 mm |
| Solvent: | A = H₂O + 0.2% NH₃ (32%) |
|         | B = MeCN |
| Gradient: | 0.5 min inlet (18% B, 25 to 50 mL/min); 0.5 – 5.5 min 37-39% B |
| Flow: | 70 mL/min |
| Temperature: | RT |
| Solution: | 216 mg / 2.2 mL DMSO |
| Injection: | 6 x 0.37 mL |
| Detection: | DAD scan range 210–400 nm |
| MS ES⁺, ES⁻, scan range 160-1000 m/z |
| Retention time in min | Purity in % | Amount in mg |
| 4.5 – 4.8 | 99 | 25 |

\[ \text{NMR} \ (400\text{MHz}, \ \text{DMSO-d6}, \ 300\text{K}) \ \delta = 9.73 \ (1\text{H}), \ 8.63 \ (1\text{H}), \ 7.97 \ (1\text{H}), \ 7.38 \ (1\text{H}), \ 7.13 \ (1\text{H}), \ 6.86 \ (1\text{H}), \ 6.68 \ (1\text{H}), \ 6.50 \ (1\text{H}), \ 4.26 \ (2\text{H}), \ 4.22 \ (2\text{H}), \ 4.13 \ (2\text{H}), \ 3.51 \ (1\text{H}), \ 2.80 \ (3\text{H}), \ 2.11 \ (4\text{H}). \]
Example 2:

15,19-Difluoro-8-[(methylsulfanyl)methyl]-3,4-dihydro-2H,11H-12,16-(azeno)-10,6-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine

Preparation of Intermediate 2.1:

3-{3-[(Methylsulfanyl)methyl]-5-nitrophenoxy}propan-1-ol

Intermediate 2.1 was prepared from 3-[(methylsulfanyl)methyl]-5-nitrophenol (see Intermediate 1.2) under similar conditions as described in the preparation protocol for Intermediate 1.3, using 3-bromopropan-1-ol instead of ethyl 4-bromobutanoate.

$\delta$ NMR (300MHz, CDC1$_3$, 300K) = 7.72 (IH), 7.54 (IH), 7.13 (IH), 4.08 (2H), 3.85 (2H), 3.78 (2H), 2.03 (2H), 1.98 (3H).
Preparation of Intermediate 2.2:
2-Chloro-5-fluoro-4-[4-fluoro-2-(3-[3-[(methylsulfanyl)methyl]-5-nitrophenoxy]propoxy)phenyl]pyrimidine

A solution of diisopropyl azodicarboxylate (0.41 mL; 2.06 mmol) in THF (1.6 mL) was added dropwise to a mixture of 3-[3-[(methylsulfanyl)methyl]-5-nitrophenoxy]propan-1-ol (484 mg; 1.88 mmol), 2-(2-chloro-5-fluoropyrimidin-4-yl)-5-fluorophenol (500 mg; 2.06 mmol; see Intermediate 1.6) and triphenylphosphine (541 mg; 2.06 mmol) in THF (8.1 mL) and the batch was stirred at room temperature for 150 minutes. The mixture was concentrated and the residue was purified by column chromatography on silica gel (hexane to hexane/ethyl acetate 30%) to give the desired product (570 mg; 1.18 mmol).

¾ NMR (400MHz, CDCl₃, 300K) δ = 8.50 (1H), 7.81 (1H), 7.63 (1H), 7.54 (1H), 7.21 (1H), 6.85 (1H), 6.80 (1H), 4.26 (2H), 4.17 (2H), 3.72 (2H), 2.29 (2H), 2.04 (3H).

Preparation of Intermediate 2.3:
3-[2-(2-Chloro-5-fluoropyrimidin-4-yl)-5-fluorophenoxy]propoxy)-5-[(methylsulfanyl)methyl]aniline

Titanium(III)chloride solution (approx. 15% in approx. 10% hydrochloric acid, 8.2 mL; Merck Schuchardt OHG) was added to a stirred solution of 2-chloro-5-fluoro-4-[4-fluoro-2-(3-[(methylsulfanyl)methyl]-5-nitrophenoxy]propoxy)phenyl]pyrimidine (570 mg; 1.18 mmol).
[(methylsulfanyl)methyl]-5-nitrophenoxy)propoxy)phenyl]pyrimidine (570 mg; 1.18 mmol) in THF (17 mL) at room temperature. The batch was stirred for 3 hours. Additional titanium(III)chloride solution (2.0 mL) was added and the batch was stirred for one additional hour. By addition of solid sodium carbonate the pH of the mixture was adjusted to approximately 6. A saturated aqueous sodium chloride solution was added and the mixture was extracted three times with ethyl acetate/THF 1:1. The combined organic layers were filtered using a Whatman filter and concentrated give the crude product (552 mg) that was used without further purification.

¾ NMR (400MHz, CDCl₃, 300K) δ = 8.46 (1H), 7.54 (1H), 6.81 (2H), 6.28 (2H), 6.10 (1H), 4.23 (2H), 4.02 (2H), 3.56 (2H), 2.20 (2H), 2.03 (3H).

Preparation of end product:
A mixture of 3-[3-{2-(2-chloro-5-fluoropyrimidin-4-yl)-5-fluorophenoxy}propoxy]-5-[(methylsulfanyl)methyl] aniline (549 mg; 1.22 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-tri-O-propyl-L,l'-biphenyl)[2-(2-aminoethyl)phenyl] palladium(II) methyl-fert-butylether adduct (100 mg; 0.12 mmol; ABCR GmbH & CO. KG) and 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl (58 mg; 0.12 mmol; Aldrich Chemical Company Inc.) and potassium phosphate (1289 mg; 6.07 mmol) in toluene (91 ml) and NMP (11 mL) was stirred under an atmosphere of argon at 110°C in a closed vessel for 3 hours. After cooling, the batch was diluted with ethyl acetate and washed with aqueous sodium chloride solution. The organic layer was filtered using a Whatman filter and concentrated. The residue was purified by column chromatography on silica gel (hexane / ethyl acetate 10% to 65%) to give the desired product (304 mg; 0.73 mmol).

¾ NMR (400MHz, CDCl₃, 300K) δ = 8.72 (1H), 8.40 (1H), 7.62 (1H), 7.25 (1H), 6.81 (2H), 6.51 (1H), 6.44 (1H), 4.37 (2H), 4.14 (2H), 3.62 (2H), 2.34 (2H), 2.04 (3H).

Example 3:
15,19-Difluoro-8-[(methylsulfanyl)methyl]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine
Preparation of end product:

The synthesis of 15,19-Difluoro-8-[(methylsulfanyl)methyl]-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,II,13-benzodioxadiazacyclooctadecine corresponds to the synthesis of Intermediate 9 described in section A-1.

Example 4:

(rac)-15,19-Difluoro-8-[(methylsulfinyl)methyl]-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,II,13-benzodioxadiazacyclooctadecine

The synthesis of (rac)-15,19-Difluoro-8-[(methylsulfinyl)methyl]-3,4-dihydro-2H,1H-10,6-(azeno)-12,16-(metheno)-1,5,II,13-benzodioxadiazacyclooctadecine corresponds to the synthesis of Intermediate 10 described in section A-1.
**Example 5:**

(rac)-15,19-Difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine

The synthesis of (rac)-15,19-Difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine corresponds to the synthesis of Intermediate 11 described in section A-1.
Example 6 and 7:
Enantiomers of
15,19-Difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,llH-10,6-(azeno)-12,16-
(metheno)-l,5,II,13-benzodioxazacyclooctadecine

(rac)-15,19-Difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H, 11H-10,6-(azeno)-
12,16-(metheno)-l,5,II,13-benzodioxazacyclooctadecine (20 mg; see Example 5) was separated into the
single enantiomers by preparative chiral HPLC.

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<td>purity in %</td>
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<tr>
<td>Example 6</td>
<td>Enantiomer 1</td>
</tr>
<tr>
<td>Example 7</td>
<td>Enantiomer 2</td>
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</table>
Example 6: 15,19-Difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-l,5,11,13-benzodioxadiazacyclooctadecine; Enantiomer 1

\( ^1H\text{-NMR} \) (300 MHz, DMSO-d6, 300 K): \( \delta \text{ [ppm]} = 9.70 \text{ (IH)}, 8.69 \text{ (IH)}, 8.31 \text{ (IH)}, 7.58 \text{ (IH)}, 7.07 \text{ (IH)}, 6.89 \text{ (IH)}, 6.59 \text{ (IH)}, 6.26 \text{ (IH)}, 4.51 \text{ (2H)}, 4.28 \text{ (2H)}, 4.12 \text{ (2H)}, 3.72 \text{ (IH)}, 2.88 \text{ (3H)}, 2.11 \text{ (2H)}.\)

Example 7: 15,19-Difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-l,5,11,13-benzodioxadiazacyclooctadecine; Enantiomer 2

\( ^1H\text{-NMR} \) (300 MHz, DMSO-d6, 300 K): \( \delta \text{ [ppm]} = 9.70 \text{ (IH)}, 8.69 \text{ (IH)}, 8.31 \text{ (IH)}, 7.58 \text{ (IH)}, 7.07 \text{ (IH)}, 6.89 \text{ (IH)}, 6.59 \text{ (IH)}, 6.26 \text{ (IH)}, 4.51 \text{ (2H)}, 4.28 \text{ (2H)}, 4.12 \text{ (2H)}, 3.72 \text{ (IH)}, 2.88 \text{ (3H)}, 2.11 \text{ (2H)}.\)

Example 8:

(rac)-14,18-difluoro-7-[(S-methylsulfonimidoyl)methyl]-2,3-dihydro-10H-9,5-(azeno)-ll,15-(metheno)-l,4,10,12-benzodioxadiazacycloheptadecine

Intermediate 8a:

14,18-Difluoro-7-{(methylsulfanyl)methyl]-2,3-dihydro-10H-9,5-(azeno)-ll,15-(metheno)-l,4,10,12-benzodioxadiazacycloheptadecine
**Preparation of Intermediate 8a.1:**

2-({6-Chloro-4-[(methylsulfanyl)methyl]pyridin-2-yl}oxy)ethanol

Intermediate 8a.1 was prepared from 2,6-dichloro-4-[(methylsulfanyl)methyl]pyridine (see Intermediate 3.3) under similar conditions as described in the preparation protocol for Intermediate 3.4 using ethylene glycol.

\[ \text{NMR (300MHz, CDCl3, 300K)} \delta = 6.94 (1H), 6.66 (1H), 4.46 (2H), 3.96 (2H), 3.56 (2H), 2.54 (1H), 2.03 (3H). \]

**Preparation of Intermediate 8a.2:**

4-{{2-2-((6-Chloro-4-[(methylsulfanyl)methyl]pyridin-2-yl)oxy)ethoxy}-4-fluorophenyl}-5-fluoropyridin-2-amine

A solution of diisopropyl azodicarboxylate (0.87 mL; 4.50 mmol) in THF (3.5 mL) was added dropwise to a mixture of 2-{(6-chloro-4-[(methylsulfanyl)methyl]pyridin-2-yl}oxy)ethanol (0.96 g; 4.11 mmol), 2-(2-amino-5-fluoropyridin-4-yl)-5-fluorophenol (1.00 g; 4.50 mmol; see Intermediate 3.7) and triphenylphosphine (1.18 g; 4.50 mmol) in THF (17.7 mL) and the batch was stirred at room temperature for 6 hours. Additional triphenylphosphine (0.54 g; 2.06 mmol) and diisopropyl azodicarboxylate (0.41 mL; 1.03 mmol) were added and the mixture was stirred at room temperature for 6 hours before it was concentrated. The residue was purified by column chromatography on silica gel (hexane / ethyl acetate 15% to 65%) to give the desired product (1.00 g; 2.28 mmol).

\[ \text{NMR (400MHz, CDCl3, 300K)} \delta = 7.89 (1H), 7.26 (1H), 6.94 (1H), 6.80 (2H), 6.61 (1H), 6.57 (1H), 4.61 (2H), 4.34 (2H), 3.57 (2H), 2.04 (3H). \]
Preparation of end product intermediate 8a:
A mixture of 4-{2-{[(6-chloro-4-[(methylsulfanyl)methyl]pyridin-2-yl]oxy}ethoxy}-4-fluorophenyl}-5-fluoropyridin-2-amine (500 mg; 1.14 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-tri-2-ethylhexylbiphenyl) palladium(II) methyl-feri-butylether adduct (94 mg; 0.11 mmol, ABCR GmbH & Co. KG) and 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl (54 mg; 0.11 mmol, Aldrich Chemical Company Inc.) and potassium phosphate (1212 mg; 5.71 mmol) in toluene (85 ml) and NMP (10 ml) was stirred under an atmosphere of argon at 110°C in a closed vessel for 4.5 hours. After cooling, the batch was diluted with DCM and ethyl acetate and washed with aqueous sodium chloride solution. The organic layer was filtered using a Whatman filter and concentrated. The residue was purified by column chromatography on silica gel (hexane to hexane / ethyl acetate 50%) to give the desired product (445 mg; 1.11 mmol) that still contained some impurities. For biological testing, 50 mgs of this material were additionally purified by preparative HPLC to give the pure product.

| System: | Waters Autopurificationsystem: Pump 2545, Sample Manager 2767, CFO, DAD 2996, ELSD 2424, SQD |
| Column: | XBridge C18 5μm 100x30 mm |
| Solvent: | A = H2O + 0.2% NH3 (32%) |
|  | B = MeCN |
| Gradient: | 0.00 – 0.50 min 5% B, 25 mL/min |
|  | 0.51 – 5.50 min 10-100% B, 70 mL/min |
|  | 5.51 – 6.50 min 100% B, 70 mL/min |
| Temperature: | RT |
| Solution: | Max. 250 mg / max. 2.5 mL DMSO or DMF |
| Injection: | 1 x 2.5 mL |
| Detection: | DAD scan range 210–400 nm |
|  | MS ESI+, ESI-, scan range 160-1000 m/z |

¾ NMR (400MHz, CDCl3, 300K) δ = 8.36 (1H), 8.10 (1H), 7.74 (1H), 7.68 (1H), 6.82 (1H), 6.74 (1H), 6.41 (1H), 6.36 (1H), 4.73 (2H), 4.32 (2H), 3.57 (2H), 2.07 (3H).
Intermediate 8b:
(rac)-14,18-difluoro-7-[(methylsulfanyl)methyl]-2,3-dihydro-10H-9,5-(azeno)-ll,15-(metheno)-
1,4,10,12-benzodioxadiazacycloheptadecine

Iron(III)chloride (3 mg; 0.02 mmol) was added to a mixture of 14,18-difluoro-7-
[(methylsulfanyl)methyl]-2,3-dihydro- 10H-9,5-(azeno)- 11,15-(metheno)- 1,4,10,12-
benzodioxadiazacycloheptadecine (250 mg; 0.62 mmol; see Intermediate 8a) in acetonitrile (15.2 mL)
and the batch was stirred at room temperature for 10 minutes. The batch was cooled to 0°C and periodic
acid (151 mg; 0.66 mmol) was added under stirring in one portion. After 90 minutes the ice bath was
removed and the mixture was stirred at room temperature for 5 hours before it was added to a stirred
solution of sodium thiosulfate pentahydrate (865 mg; 3.49 mmol) in ice water (18.4 mL). The batch was
saturated with solid sodium chloride and extracted twice with THF and twice with ethyl acetate. The
combined organic layers were filtered using a Whatman filter and concentrated. The residue was purified
by column chromatography on silica gel (DCM to DCM / ethanol 50%) to give the desired product (141
mg; 0.34 mmol).

³¹NMR (400MHz, CDCl₃, 300K) δ = 8.29 (1H), 8.13 (1H), 7.66 (2H), 6.81 (1H), 6.73 (1H), 6.35 (1H),
6.31 (1H), 4.72 (2H), 4.41 (2H), 3.88 (2H), 2.59 (3H).

Preparation of end product

Concentrated sulphuric acid (0.13 mL) was added dropwise to a stirred mixture of (rac)-14,18-difluoro-7-
[(methylsulfanyl)methyl]-2,3-dihydro- 10H-9,5-(azeno)- 11,15-(metheno)- 1,4,10,12-
benzodioxadiazacycloheptadecine (100 mg; 0.24 mmol; see Intermediate 8b) and sodium azide (31 mg;
0.47 mmol) in chloroform (0.41 mL) at 0°C. The ice bath was removed and the mixture was stirred for
5.5 hours at 45°C. After cooling additional sodium azide (31 mg; 0.48 mmol) was added and the mixture
was stirred for additional 16 hours at 45°C. While cooling with an ice bath, saturated aqueous sodium bicarbonate solution and saturated aqueous sodium chloride solution was added dropwise under stirring.
The mixture was extracted twice with ethyl acetate and twice with THF. The combined organic layers
were filtered using a Whatman filter and concentrated. The residue was purified by preparative HPLC to
give the desired product (24 mg; 0.06 mmol).
**System:** Waters Autopurificationsystem: Pump 2545, Sample Manager 2767, CFO, DAD 2996, ELSD 2424, SQD

**Column:** XBrigde C18 5µm 100x30 mm

**Solvent:**

- **A** = H₂O + 0.2% NH₃ (32%)
- **B** = MeCN

**Gradient:**

- 0.00 - 0.50 min 5% B, 25 mL/min
- 0.51 - 5.50 min 10-100% B, 70 mL/min
- 5.51 - 6.50 min 100% B, 70 mL/min

**Temperature:** RT

**Solution:** Max. 250 mg / max. 2.5 mL DMSO or DMF

**Injection:** 1 x 2.5 mL

**Detection:** DAD scan range 210-400 nm

| MS ESI+, ESI-, scan range 160-1000 m/z |

\[\frac{1}{2}\text{-NMR} \ (300 \text{ MHz, DMSO-}d_6, 300 \text{ K}): \delta \text{ [ppm]} = 9.74 \text{ (IH)}, 8.24 \text{ (IH)}, 8.13 \text{ (IH)}, 7.63 \text{ (IH)}, 7.12 \text{ (IH)}, 6.94 \text{ (IH)}, 6.51 \text{ (IH)}, 6.36 \text{ (IH)}, 4.59 \text{ (2H)}, 4.36 \text{ (2H)}, 4.31 \text{ (2H)}, 3.75 \text{ (IH)}, 2.87 \text{ (3H)}.\]

**Example 9:**

(rac)-16,20-difluoro-9-[(S-methylsulfonimidoyl)methyl]-2,3,4,5-tetrahydro-12H-II,7-(azeno)-13,17-(metheno)-1,6,12,14-benzodioxadiazacyclononadecine

![Example 9](image)

**Intermediate 9a:**

16,20-Difluoro-9-[(methylsulfanyl)methyl]-2,3,4,5-tetrahydro-12H-II,7-(azeno)-13,17-(metheno)-1,6,12,14-benzodioxadiazacyclononadecine
Preparation of Intermediate 9a.1:

4-({6-Chloro-4-[(methylsulfanyl)methyl]pyridin-2-yl}oxy)butan-1-ol

Intermediate 9a.1 was prepared from 2,6-dichloro-4-[(methylsulfanyl)methyl]pyridine (see Intermediate 3.3) under similar conditions as described in the preparation protocol for Intermediate 3.4 using butane-1,4-diol.

1/4 NMR (300MHz, CDCl3, 300K) δ = 6.90 (1H), 6.59 (1H), 4.34 (2H), 3.74 (2H), 3.55 (2H), 2.03 (3H), 1.88 (2H), 1.74 (2H), 1.61 (1H).

Preparation of Intermediate 9a.2:

4-{2-[4-({6-chloro-4-[(methylsulfanyl)methyl]pyridin-2-yl}oxy)butoxy]-4-fluorophenyl}-5-fluoropyridin-2-amine

A solution of diisopropyl azodicarboxylate (0.89 mL; 4.50 mmol) in THF (3.5 mL) was added dropwise to a mixture of 4-{6-chloro-4-[(methylsulfanyl)methyl]pyridin-2-yl}oxy)butan-1-ol (1.08 g; 4.11 mmol), 2-(2-amino-5-fluoropyridin-4-yl)-5-fluorophenol (1.00 g; 4.50 mmol; see Intermediate 3.7) and
triphenylphosphine (1.18 g; 4.50 mmol) in THF (17.7 mL) and the batch was stirred at room temperature for 6 hours. Additional triphenylphosphine (0.54 g; 2.06 mmol) and diisopropyl azodicarboxylate (0.41 mL; 1.03 mmol) were added and the mixture was stirred at room temperature for 6 hours before it was concentrated. The residue was purified by column chromatography on silica gel (hexane to hexane / ethyl acetate 60%) to give the desired product (1.87 g; 4.01 mmol).

\[ \text{NMR (400MHz, CDCl}_3, 300K) \delta = 7.94 (1H), 7.23 (1H), 6.90 (1H), 6.74 (2H), 6.57 (1H), 6.52 (1H), 4.53 (2H), 4.31 (2H), 4.05 (2H), 3.56 (2H), 2.03 (3H), 1.88 (4H). \]

Preparation of end product intermediate 9a:

A mixture of 4-{2-[4-{[6-chloro-4-[{methylsulfanyl}methyl]pyridin-2-yl}oxy]butoxy}-4-fluorophenyl}-5-fluoropyridin-2-amine (1000 mg; 2.15 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-tri-zy,yo-propyl-l,b-biphenyl)[2-(2-aminoethyl)phenyl] palladium(II) methyl-fert-butylether adduct (177 mg; 0.22 mmol; ABCR GmbH & CO. KG) and 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl (102 mg; 0.22 mmol; Aldrich Chemical Company Inc.) and potassium phosphate (2278 mg; 10.73 mmol) in toluene (160 ml) and NMP (20 mL) was stirred under an atmosphere of argon at 110°C in a closed vessel for 4.5 hours. After cooling, the batch was diluted with DCM and ethyl acetate and washed with aqueous sodium chloride solution. The organic layer was filtered using a Whatman filter and concentrated. The residue was purified by column chromatography on silica gel (hexane to hexane / ethyl acetate 50%) to give the desired product (732 mg; 1.70 mmol).

\[ \text{NMR (400MHz, CDCl}_3, 300K) \delta = 8.36 (1H), 8.18 (1H), 7.51 (1H), 7.33 (1H), 6.79 (1H), 6.76 (1H), 6.25 (1H), 6.23 (1H), 4.42 (2H), 4.10 (2H), 4.07 (2H), 3.54 (2H), 2.19 (2H), 2.05 (3H), 1.95 (2H). \]

Intermediate 9b:

(rac)-16,20-Difluoro-9-[(methylsulfinyl)methyl]-2,3,4,5-tetrahydro-12H-ll,7-(azeno)-13,17-(metheno)-l,6,12,14-benzodioxadiazacyclonadecine

Iron(III)chloride (3 mg; 0.02 mmol) was added to a mixture of 16,20-difluoro-9-[(methylsulfinyl)methyl]-2,3,4,5-tetrahydro-12H-11,7-(azeno)-13,17-(metheno)-1,6,12,14-benzodioxadiazacyclonadecine (250 mg; 0.58 mmol; see Intermediate 9a) in acetonitrile (14.2 mL) and
the batch was stirred at room temperature for 10 minutes. The batch was cooled to 0°C and periodic acid (142 mg; 0.62 mmol) was added under stirring in one portion. After 4 hours at 0°C, the mixture was added to a stirred solution of sodium thiosulfate pentahydrate (809 mg; 3.26 mmol) in ice water (17.2 mL). The batch was saturated with solid sodium chloride and extracted twice with THF and twice with ethyl acetate. The combined organic layers were filtered using a Whatman filter and concentrated. The residue was purified by column chromatography on silica gel (DCM to DCM / ethanol 35%) to give the desired product (204 mg; 0.46 mmol).

¾ NMR (400MHz, CDC13, 300K) δ = 8.35 (1H), 8.19 (1H), 7.62 (1H), 7.33 (1H), 6.78 (1H), 6.75 (1H), 6.22 (1H), 6.19 (1H), 4.43 (2H), 4.10 (2H), 3.90 (1H), 3.80 (1H), 2.56 (3H), 2.18 (2H), 1.95 (2H).

**Preparation of end product**

Concentrated sulphuric acid (0.12 mL) was added dropwise to a stirred mixture of (rac)-16,20-difluoro-9-[(methylsulfmyl)methyl]-2,3,4,5-tetrahydro-12H-11,7-(azeno)-13,17-(metheno)-1,6,12,14-benzodioxadiazacyclonadecine (100 mg; 0.22 mmol; see Intermediate 9b) and sodium azide (29 mg; 0.45 mmol) in chloroform (0.38 mL) at 0°C. The ice bath was removed and the mixture was stirred for 6 hours at 45°C. After cooling additional sodium azide (29 mg; 0.45 mmol) was added and the mixture was stirred for additional 16 hours at 45°C. While cooling with an ice bath, saturated aqueous sodium bicarbonate solution and saturated aqueous sodium chloride solution was added dropwise under stirring. The mixture was extracted twice with ethyl acetate and twice with THF. The combined organic layers were filtered using a Whatman filter and concentrated. The residue was purified by preparative HPLC to give the desired product (10 mg; 0.02 mmol).

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<td><strong>Solvent:</strong></td>
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<td>B = MeCN</td>
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<td><strong>Gradient:</strong></td>
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<td>5.51 - 6.50 min 100% B, 70 mL/min</td>
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¾-NMR (300 MHz, DMSO-$d_6$, 300 K): $\delta$ [ppm] = 9.73 (IH), 8.33 (IH), 8.18 (IH), 7.34 (IH), 7.12 (IH), 6.87 (IH), 6.54 (IH), 6.25 (IH), 4.33 (2H), 4.27 (2H), 4.13 (2H), 3.74 (IH), 2.87 (3H), 2.01 (2H), 1.84 (2H).

Example 10:
(rac)-15,19-difluoro-8-[(S-methylsulfonimidoyl)methyl]-3,4-dihydro-2H,llH-12,16-(azeno)-10,6-(metheno)-1,5,il,13-benzodioxadiazacyclooctadecine

![Chemical Structure]

Preparation of Intermediate 10.1:
(rac)-2-Chloro-5-fluoro-4-[4-fluoro-2-(3-{3-[{(methylsulfinyl)methyl]-5-nitrophenoxy}propoxy)phenyl]pyrimidine

Iron(III)chloride (6 mg; 0.04 mmol) was added to a mixture of 2-chloro-5-fluoro-4-[4-fluoro-2-(3-{{(methylsulfinyl)methyl]-5-nitrophenoxy}propoxy}phenyl]pyrimidine (650 mg; 1.35 mmol; see Intermediate 2.2) in acetonitrile (33 mL) and the batch was stirred at room temperature for 10 minutes. The batch was cooled to 0°C and periodic acid (329 mg; 1.44 mmol) was added under stirring in one portion. The mixture was stirred 0°C for 40 minutes. The ice bath was removed and the mixture was stirred for additional 50 minutes at RT before it was added to a stirred solution of sodium thiosulfate pentahydrate (1874 mg; 7.55 mmol) in ice water (40 mL). The batch was saturated with solid sodium chloride and extracted twice with THF and twice with ethyl acetate. The combined organic layers were
filtered using a Whatman filter and concentrated to give the crude product (673 mg) which was used in the next step without further purification.

$\gamma$-NMR (400MHz, CDCl$_3$, 300K) $\delta = 8.54$ (1H), 7.78 (1H), 7.75 (1H), 7.54 (1H), 7.22 (1H), 6.85 (1H), 6.79 (1H), 4.26 (2H), 4.18 (2H), 4.06 (1H), 3.94 (1H), 2.56 (3H), 2.29 (2H).

**Preparation of Intermediate 10.2:**

(rac)-N-[(3-[3-[2-(2-chloro-5-fluoropyrimidin-4-yl)-5-fluorophenoxy]propoxy]-5-nitrobenzyl)(methyl)oxido-$\lambda^6$-sulfanylidene]-2,2,2-trifluoroacetamide

To a suspension of crude (rac)-2-chloro-5-fluoro-4-[4-fluoro-2-(3-[methylsulfinyl)methyl]-5-nitrophenoxy]propoxy)phenyl]pyrimidine (670 mg; 1.35 mmol), trifluoroacetamide (304 mg; 2.69 mmol), magnesium oxide (217 mg; 5.38 mmol) and rhodium(II)-acetate dimer (15 mg; 0.03 mmol) in DCM (10.0 mL) was added iodobenzene diacetate (650 mg; 2.02 mmol) at room temperature. The batch was stirred for 18 hours at room temperature, filtered and concentrated to give the crude product (997 mg) which was used in the next step without further purification.

**Preparation of Intermediate 10.3:**

(rac)-N-[(3-amino-5-[3-[2-(2-chloro-5-fluoropyrimidin-4-yl)-5-fluorophenoxy]propoxy]benzyl)(methyl)oxido-$\lambda^6$-sulfanylidene]-2,2,2-trifluoroacetamide

Titanium(III)chloride solution (approx. 15% in approx. 10% hydrochloric acid, 11.3 mL; Merck Schuchardt OHG) was added to a stirred solution of crude (rac)-N-[(3-[3-[2-(2-chloro-5-fluoropyrimidin-4-yl)-5-fluorophenoxy]propoxy]-5-nitrobenzyl)(methyl)oxido-$\lambda^6$-sulfanylidene]-2,2,2-trifluoroacetamide (997 mg) in THF (24 mL) at room temperature. The batch was stirred for 3 hours. By addition of solid
sodium carbonate the pH of the mixture was adjusted to approximately 6. A saturated aqueous sodium chloride solution was added and the mixture was extracted three times with ethyl acetate/THF 1:1. The combined organic layers were filtered using a Whatman filter and concentrated. The residue was purified by column chromatography on silica gel (hexane to hexane/ethyl acetate 50%) to give the desired product (368 mg; 0.64 mmol).

\[ \frac{3}{4} \text{NMR } (400\text{MHz, CDCl}_3, 300\text{K}) \delta = 8.48 (1\text{H}), 7.53 (1\text{H}), 6.84 (1\text{H}), 6.78 (1\text{H}), 6.30 (2\text{H}), 6.24 (1\text{H}), 4.64 (1\text{H}), 4.58 (1\text{H}), 4.22 (2\text{H}), 4.02 (2\text{H}), 3.84 (2\text{H}), 3.15 (3\text{H}), 2.21 (2\text{H}). \]

10 Preparation of end product:

A mixture of (rac)-N-[(3-amino-5-{3-[2-(2-chloro-5-fluoropyrimidin-4-yl)-5-fluorophenoxy]propoxy}benzyl)(methyl)oxido-\( \lambda^6 \)-sulfanylidene]-2,2,2-trifluoroacetamide (366 mg; 0.63 mmol), chloro(2-dicyclohexylphosphino-2',4',6'-tri-z,yo-propyl-l,l'-biphenyl)[2-(2-aminoethyl)phenyl]palladium(II) methyl-tert-butylether adduct (52 mg; 0.06 mmol; ABCR GmbH & CO. KG) and 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl (30 mg; 0.06 mmol; Aldrich Chemical Company Inc.) and potassium phosphate (671 mg; 3.16 mmol) in toluene (47.3 ml) and NMP (5.8 mL) was stirred under an atmosphere of argon at 110°C in a closed vessel for 3 hours. After cooling, the batch was diluted with THF and ethyl acetate and washed with aqueous sodium chloride solution. The organic layer was filtered using a Whatman filter. Water (10 mL), MeOH (10 mL) and potassium carbonate (500 mg) were added and the mixture was stirred for 45 minutes. The organic layer was filtered using a Whatman filter and concentrated. The residue was purified by preparative HPLC to give the desired product (65 mg; 0.25 mmol) and (rac)-N-[[15,19-difluoro-3,4-dihydro-2H,1-IH-12,16-(azeno)-10,6-(metheno)-1,5,1 1,13-benzodioxadiazacyclooctadecin-8-yl]methyl](methyl)oxido-\( \lambda^6 \)-sulfanylidene]-2,2,2-trifluoroacetamide (50 mg; 0.09 mmol; Example 16).

| System | Waters Autopurification system SQD |
| Column | Waters XBridge C18 5µ 100x30mm |
| Solvent | A: H₂O + 0.1% Vol. formic acid (99%) B: MeOH |
| Gradient | 0.00–0.50 min 50% B (25–70 mL/min), 0.51–5.50 min 50–80% B (70 mL/min), |
| Temperature | Rt |
| Solution | 664 mg / 5 mL DMSO |
| Injection | 5 x 1 mL |
| Detection | DAD scan: 210–400 nm; MS ESI-Pos., scan range 160–1000 m/z |

| Retention time in min | Purity in % | Amount in mg |
| Example 15 | 3.6 – 4.2 | > 99% | 65 |
| Example 16 | 5.2 – 5.7 | 98% | 50 |

\[ \frac{3}{4} \text{NMR } (400\text{MHz, DMSO-d6, } 300\text{K}) \delta = 9.81 (1\text{H}), 8.72 (1\text{H}), 8.67 (1\text{H}), 7.61 (1\text{H}), 7.20 (1\text{H}), 6.92 (1\text{H}), 6.75 (1\text{H}), 6.49 (1\text{H}), 4.21 (6\text{H}), 3.52 (1\text{H}), 2.82 (3\text{H}), 2.15 (2\text{H}). \]
The following Table 1 provides an overview on the compounds described in the example section:

**Table 1**

<table>
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<tr>
<th>Example No.</th>
<th>Structure</th>
<th>Name of compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Structure" /></td>
<td>(rac)-16,20-Difluoro-9-[(S-methylsulfonimidoyl)methyl]-2,3,4,5-tetrahydro-12H-13,17-(azeno)-11,7-(metheno)-1,6,12,14-benzodioxadiazacyclononadecine</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2" alt="Structure" /></td>
<td>15,19-Difluoro-8-[(methylsulfanyl)methyl]-3,4-dihydro-2H,11H-12,16-(azeno)-10,6-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine</td>
</tr>
<tr>
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<td><img src="image3" alt="Structure" /></td>
<td>15,19-Difluoro-8-[(methylsulfanyl)methyl]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine</td>
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<tr>
<td>Example No.</td>
<td>Structure</td>
<td>Name of compound</td>
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<td>-----------</td>
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</tr>
<tr>
<td>4</td>
<td><img src="image" alt="Structure 4" /></td>
<td>(rac)-15,19-Difluoro-8-[(methylsulfinyl)methyl]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="Structure 5" /></td>
<td>(rac)-15,19-Difluoro-8-[(S-methylsulfonyl)imido(methyl)]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine</td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="Structure 6" /></td>
<td>15,19-Difluoro-8-[(S-methylsulfonyl)imido(methyl)]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine; enantiomer 1</td>
</tr>
<tr>
<td>7</td>
<td><img src="image" alt="Structure 7" /></td>
<td>15,19-Difluoro-8-[(S-methylsulfonyl)imido(methyl)]-3,4-dihydro-2H,11H-10,6-(azeno)-12,16-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine; enantiomer 2</td>
</tr>
<tr>
<td>Example No.</td>
<td>Structure</td>
<td>Name of compound</td>
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<tr>
<td>------------</td>
<td>-----------</td>
<td>------------------</td>
</tr>
<tr>
<td>8</td>
<td><img src="image" alt="Structure 8" /></td>
<td>(rac)-14,18-difluoro-7-[(S-methylsulphonimidoyl)methyl]-2,3-dihydro-10H-9,5-(azeno)-11,15-(metheno)-1,4,10,12-benzodioxadiazacycloheptadecine</td>
</tr>
<tr>
<td>9</td>
<td><img src="image" alt="Structure 9" /></td>
<td>(rac)-16,20-difluoro-9-[(S-methylsulphonimidoyl)methyl]-2,3,4,5-tetrahydro-12H-11,7-(azeno)-13,17-(metheno)-1,6,12,14-benzodioxadiazacyclononadecine</td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="Structure 10" /></td>
<td>(rac)-15,19-difluoro-8-[(S-methylsulphonimidoyl)methyl]-3,4-dihydro-2H,11H-12,16-(azeno)-10,6-(metheno)-1,5,11,13-benzodioxadiazacyclooctadecine</td>
</tr>
</tbody>
</table>
Results:

Table 2: Inhibition for CDK9 and CDK2 of CDK9 inhibitors according to the present invention

The IC50 (inhibitory concentration at 50% of maximal effect) values are indicated in nM. "n.t." means that the compounds have not been tested in the respective assay.

©: Example Number
©: CDK9: CDK9/CycT1 kinase assay as described under Method 1a. of Materials and Methods
©: CDK2: CDK2/CycE kinase assay as described under Method 2a. of Materials and Methods
©: Selectivity CDK9 over CDK2: IC50 (CDK2) / IC50 (CDK9) according to Methods 1a. and 2a. of Materials and Methods
©: high ATP CDK9: CDK9/CycT1 kinase assay as described under Method 1b. of Materials and Methods
©: high ATP CDK2: CDK2/CycE kinase assay as described under Method 2b. of Materials and Methods
©: Selectivity high ATP CDK9 over high ATP CDK2: IC50 (high ATP CDK2) / IC50 (high ATP CDK9) according to Methods 1b. and 2b. of Materials and Methods

Noteworthily, in the CDK9 assays, as described supra in the Methods 1a. and 1b. of Materials and Methods, resolution power is limited by the enzyme concentrations, the lower limit for IC50s is about 1-2 nM in the CDK9 high ATP assay and 2-4 nM in the CDK low ATP assays. For compounds exhibiting IC50s in this range the true affinity to CDK9 and thus the selectivity for CDK9 over CDK2 might be even higher, i.e. for these compounds the selectivity factors calculated in columns 4 and 7 of Table 2, infra, are minimal values, they could be also higher.

Table 2

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<tr>
<td>7</td>
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Tables 3a and 3b: Inhibition of proliferation of HeLa, HeLa-MaTu-ADR, NCI-H460, DU145, Caco-2, B16F10, A2780 and MOLM-13 cells by compounds according to the present invention, determined as described under Method 3. of Materials and Methods. All IC50 (inhibitory concentration at 50% of maximal effect) values are indicated in nM, "n.t." means that the compounds have not been tested in the respective assay.
Φ: Example Number
©: Inhibition of HeLa cell proliferation
◎: Inhibition of HeLa-MaTu-ADR cell proliferation
◎: Inhibition of NCI-H460 cell proliferation
◎: Inhibition of DU145 cell proliferation
◎: Inhibition of Caco-2 cell proliferation
◎: Inhibition of B16F10 cell proliferation
◎: Inhibition of A2780 cell proliferation
◎: Inhibition of MOLM-13 cell proliferation

Table 3a: Indications represented by cell lines

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<td>HeLa-MaTu-ADR</td>
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<td>Multidrug-resistant human cervical carcinoma</td>
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<td>NCI-H460</td>
<td>ATCC</td>
<td>Human non-small cell lung carcinoma</td>
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<td>DU 145</td>
<td>ATCC</td>
<td>Hormone-independent human prostate carcinoma</td>
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<tr>
<td>Caco-2</td>
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<td>Human colorectal carcinoma</td>
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<td>B16F10</td>
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<td>ECACC</td>
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<td>MOLM-13</td>
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<tr>
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Table 3b: Inhibition of proliferation
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</table>
Table 4: Caco-2 permeation of compounds according to the present invention, determined as described under Method 5 of Materials and Methods.

©: Example Number

©: Concentration of test compound indicated in µM.

©: \( P_{pA-B} \) (Mad) indicated in [nm/s]

©: \( P_{pB-A} \) (Mari) indicated in [nm/s]

©: Efflux ratio (\( P_{app B-A} / P_{app A-B} \))

<table>
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<th>Structure</th>
<th>©</th>
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Table 5: Stability in rat hepatocytes and τ/2 in rats after iv dosing as determined by Method 6. and Method 7. as described in Materials and Methods.

①: Example Number
②: The maximal calculated oral bioavailability (Fmax) based on stability data in rat Hepatocytes.
③: τ/2: terminal half-life (in h) from in vivo study after i.v. bolus dosing to rats.

Table 5

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Table 6: Equilibrium dissociation constants \( K_D \) \( [1/s] \), dissociation rate constants \( k_{off} \) \( [1/s] \), and target resident times \([\text{min}]\) as determined by Method 8, as described in Materials and Methods.

Ø: Example Number
©: Equilibrium dissociation constant \( K_D \) \( [1/s] \)
®: Dissociation rate constant \( k_{off} \) \( [1/s] \)
©©: Target resident time \([\text{min}]\)

Table 6

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<td>&lt;5.0E-5</td>
<td>&gt;333</td>
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</table>
Dissociation rate constants below $5 \times 10^{-5}$ s$^{-1}$ are not resolvable with the present assay and are reported as $<5 \times 10^{-5}$ s$^{-1}$.

It is expected that the prolonged residence time of macrocyclic CDK9 inhibitors according to the invention will result in a sustained inhibitory effect on CDK9 signaling, ultimately contributing to sustained target engagement and anti-tumor efficacy.
**B:** **Preparation of antibody/active compound conjugates (ADC)**

**B-1** General process for generating anti-TWEAKR antibodies

The anti-TWEAKR antibodies were generated, for example, by screening of a phage display library for recombinant human TWEAKR. Particularly the antibody TPP-2090 is an important example. Generation and sequence of this antibody are disclosed in WO2014/198817A1. The antibody TPP2090 was used for the working examples described here. In addition, antibodies which bind to TWEAKR are known to the person skilled in the art; see, for example, WO2009/020933(A2) or WO2009140177 (A2).

Cetuximab variants were generated, for example, by single amino acid exchange as well as combinations within VL and VH region Positions which are critical for interaction to EGFR were selected. Humanization was performed by grafting of CDRs into the human frameworks derived from IGKV3-15-01-IGKJ2-01 and IGHV4-59-01-D-IGHJ1-01 germline sequences. The antibodies TPP-4030 and TPP-5653 were used for the working examples described here. These antibodies show reduced cytotoxic effects in in vitro assay.

The commercial antibodies Cetuximab (trade name: Erbitux), Trastuzumab (trade name: Herceptin) and Nimotuzumab (trade name: CIMAher) were also used for the working examples described here.

**B-2** General process for expressing anti-TWEAKR antibodies in mammalian cells

The antibodies, for example TPP-2090, were produced in transient mammalian cell cultures as described by Tom et al., Chapter 12 in Methods Express: Expression Systems edited by Micheal R. Dyson and Yves Durocher, Scion Publishing Ltd, 2007.

**B-3** General process for purifying antibodies from cell supernatants

The antibodies, for example TPP-2090 were obtained from the cell culture supernatants. The cell supernatants were clarified by centrifugation of cells. The cell supernatant was then purified by affinity chromatography on a MabSelect Sure (GE Healthcare) chromatography column. To this end, the column was equilibrated in DPBS pH 7.4 (Sigma/Aldrich), the cell supernatant was applied and the column was washed with about 10 column volumes of DPBS pH 7.4 + 500 mM sodium chloride. The antibodies were eluted in 50 mM sodium acetate pH 3.5 + 500 mM sodium chloride and then purified further by gel filtration chromatography on a Superdex 200 column (GE Healthcare) in DPBS pH 7.4.
The commercially available antibodies Cetuximab, Trastuzumab and Nimotuzumab were purified from the commercial product by standard chromatographic methods (protein A, preparative SEC).

C-1 Assessment of biological efficacy

The biological activity of the compounds according to the invention was analyzed in the assays described below.

C-la Determination of the cytotoxic effects of the ADCs directed against corresponding targets

The analysis of the cytotoxic effects of the anti-TWEAKR-ADCs, EGFR-ADCs and Erbitux-variant-ADCs was carried out with various cell lines:

NCI-H292: human mucoepidermoid lung carcinoma cells, ATCC-CRL-1848, standard medium: RPMI 1640 (Biochrom; #FG1215, stab. glutamine) + 10% FCS (Biochrom; #S0415), TWEAKR-positive, EGFR-positive.

The cells are cultivated by standard methods, as indicated in the American Tissue Type Collection (ATCC) for the respective cell lines.

MTT-Assay

Cultivation of the cells was performed after standard protocols using media described in C-1. The test was carried out by detaching the cells with a solution of Accutase in PBS (Biochrom AG #L2143), pelleting, resuspending in culture medium, counting and seeding the cells into a 96-well culture plate with white bottom (Costar #3610; NCI H292: 2500 cells/well in a total volume of 100 µL). The cells were then incubated in an incubator at 37°C and 5% carbon dioxide. After 48 h, the antibody drug conjugates were added in 10 µL of culture medium in concentrations of from 10⁻⁸M to 10⁻¹⁰M to the cells (triplicates) and incubated in an incubator at 37°C and 5% carbon dioxide. After 96h, the proliferation was measured using the MTT assay (ATCC, Manassas, Virginia, USA, catalogue No. 30-1010K). At the end of the selected incubation time (96h), the MTT reagent was added and incubated with the cells for 4h, followed by lysis of the cells overnight by addition of the detergent. The dye formed was detected at 570nm (Infinite M1000 pro, Fa. Tecan). Based on the measured data the IC₅₀ value was determined from the DRC (dose response curve). The proliferation of cells, which were not treated with test substance but were otherwise treated identically, was defined as the 100% value.
The IC50-values derived from selected EGFR-ADC examples utilizing EGFR antibody Cetuximab (Erbitux) are summarized in table 1.

Table 1

<table>
<thead>
<tr>
<th>example</th>
<th>NCI-H292 IC50 [M] MTT Assay</th>
<th>example</th>
<th>NCI-H292 IC50 [M] MTT Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>3.17E-08</td>
<td>13A</td>
<td>4.90E-10</td>
</tr>
<tr>
<td>2A</td>
<td>3.01E-09</td>
<td>14A</td>
<td>1.13E-10</td>
</tr>
<tr>
<td>4A</td>
<td>2.64E-10</td>
<td>15A</td>
<td>7.51E-10</td>
</tr>
<tr>
<td>5A</td>
<td>1.27E-10</td>
<td>16A</td>
<td>4.60E-09</td>
</tr>
<tr>
<td>6A</td>
<td>4.60E-10</td>
<td>17A</td>
<td>1.00E-09</td>
</tr>
<tr>
<td>7A</td>
<td>3.65E-10</td>
<td>18A</td>
<td>3.37E-10</td>
</tr>
<tr>
<td>8A</td>
<td>7.02E-10</td>
<td>19A</td>
<td>2.79E-09</td>
</tr>
<tr>
<td>9A</td>
<td>2.88E-10</td>
<td>20A</td>
<td>2.77E-10</td>
</tr>
<tr>
<td>10A</td>
<td>2.75E-10</td>
<td>21A</td>
<td>1.89E-09</td>
</tr>
<tr>
<td>11A</td>
<td>2.75E-10</td>
<td>22A</td>
<td>3.89E-10</td>
</tr>
<tr>
<td>12A</td>
<td>2.89E-10</td>
<td>23A</td>
<td>8.79E-11</td>
</tr>
</tbody>
</table>

The IC50-values derived from selected EGFR-ADC examples utilizing EGFR antibody Nimotuzumab (CIMAher) are summarized in table 2.

Table 2

<table>
<thead>
<tr>
<th>example</th>
<th>NCI-H292 IC50 [M] MTT Assay</th>
<th>example</th>
<th>NCI-H292 IC50 [M] MTT Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>5.60E-08</td>
<td>10B</td>
<td>3.73E-08</td>
</tr>
<tr>
<td>3B</td>
<td>2.12E-08</td>
<td>11B</td>
<td>1.40E-08</td>
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<tr>
<td>4B</td>
<td>3.20E-08</td>
<td>12B</td>
<td>8.27E-08</td>
</tr>
<tr>
<td>5B</td>
<td>1.51E-07</td>
<td>14B</td>
<td>4.49E-08</td>
</tr>
<tr>
<td>6B</td>
<td>5.90E-08</td>
<td>16B</td>
<td>5.10E-08</td>
</tr>
<tr>
<td>7B</td>
<td>6.69E-09</td>
<td>18B</td>
<td>5.24E-09</td>
</tr>
<tr>
<td>9B</td>
<td>1.41E-08</td>
<td>20B</td>
<td>5.90E-09</td>
</tr>
</tbody>
</table>

The IC50-values derived from selected EGFR-ADC examples utilizing EGFR antibodies TPP-4030 (D) and TPP-5653 (E) are summarized in table 3.
Table 3

<table>
<thead>
<tr>
<th>example</th>
<th>NCI-H292 IC₅₀ [M] MTT Assay</th>
<th>example</th>
<th>NCI-H292 IC₅₀ [M] MTT Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>5D</td>
<td>4.36E-08</td>
<td>6E</td>
<td>6.38E-08</td>
</tr>
<tr>
<td>8D</td>
<td>1.71E-08</td>
<td>9E</td>
<td>5.52E-08</td>
</tr>
<tr>
<td>11D</td>
<td>1.58E-08</td>
<td>11E</td>
<td>1.39E-08</td>
</tr>
<tr>
<td>13D</td>
<td>1.31E-08</td>
<td>12E</td>
<td>7.09E-08</td>
</tr>
<tr>
<td>15D</td>
<td>1.15E-08</td>
<td>20E</td>
<td>3.91E-09</td>
</tr>
<tr>
<td>20D</td>
<td>5.59E-09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23D</td>
<td>3.35E-09</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The IC₅₀-values derived from selected TWEAKR-ADC examples utilizing TWEAKR antibody (C) are summarized in table 4.

Table 4

<table>
<thead>
<tr>
<th>example</th>
<th>NCI-H292 IC₅₀ [M] MTT Assay</th>
<th>example</th>
<th>NCI-H292 IC₅₀ [M] MTT Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>15C</td>
<td>4.91E-09</td>
<td>23C</td>
<td>5.00E-07</td>
</tr>
</tbody>
</table>

The IC₅₀ values refer to the examples with the indicated drug/antibody ratios (DAR) described in the experimental part. These values can vary when different a DAR is present. The IC₅₀ values are means derived from different independent experiments or single values. The effects of the antibody-drug conjugates were selective and tested in comparison to the isotype control coupled to the same linker and payload.

In a preferred embodiment of the present invention, the conjugate of the present invention shows a significantly higher efficacy in inhibiting the growth of a tumour e.g. by reducing its size compared with the treatment with the antibody itself when measured in those tumor cells, especially in lung carcinoma cells, preferably NCI-H292 cells by MTT assay.

C-2 Internalization assay

Internalization is a key process which enables specific and efficient provision of the cytotoxic payload in antigen-expressing cancer cells via antibody drug conjugates (ADC). This process is monitored via fluorescent labelling of specific TWEAKR antibodies and an isotype control antibody. First, the fluorescent dye is conjugated to lysines of the antibody. Conjugation is carried out using a two-fold molar excess of CypHer 5E mono NHS ester (Batch 357392, GE Healthcare) at pH 8.3. After the coupling, the
reaction mixture was purified to remove excess dye and to adjust the pH using gel chromatography (Zeba Spin Desalting Columns, 40K, Fa. Thermo Scientific, No. 87768; elution buffer: DULBECCO'S PBS, Fa. Sima-Aldrich, No. D8537). The protein solution was then concentrated (VIVASPIN 500, from Sartorius stedim biotec). Determination of the dye load of the antibody was by spectrophotometric analysis (NanoDrop) and subsequent calculation (D: P = A \times \text{protene}(A_{280} - 0.16\text{dye})/\text{dye}). The dye load of the antibodies examined here and the isotype control were of a comparable order. In cell binding assays, it was confirmed that the conjugation did not lead to a change in the affinity of the antibody.

The labelled antibodies were used for the internalization assay. Prior to the start of the treatment, the cells (2 x 10⁴/well) were seeded in 100 µL medium in a 96-well MTP (fat, black, clear bottom No. 4308776, from Applied Biosystems). After 18 h of incubation at 37°C/5% CO₂, the medium was replaced and labelled anti-TWEAKR antibodies were added in different concentrations (10, 5, 2.5, 1, 0.1 µg/mL). The same treatment protocol was applied to the labeled isotype control (negative control). The chosen incubation times were 0 h, 0.25 h, 0.5 h, 1 h, 1.5 h, 2 h, 3 h, 6 h and 24 h. The fluorescence measurement was carried out using the InCell Analyser 1000 (from GE Healthcare). This was followed by kinetic evaluation via measurement of the parameters granule counts/cell and total granule intensity/cell.

Following binding to the respective target, antibodies were examined for their internalization ability. For this purpose, cells with different target expression levels were chosen. A target-mediated specific internalization with the antibodies was observed, whereas the isotype control showed no internalization (Figure 1).

1.1.1  C-3 CDK9/CycT1 high ATP kinase assay

CDK9/CycT1-inhibitory activity of compounds of the present invention at a high ATP concentration after preincubation of enzyme and test compounds was quantified employing the CDK9/CycT1 TR-FRET assay as described in the following paragraphs. Recombinant full-length His-tagged human CDK9 and CycT1, expressed in insect cells and purified by Ni-NTA affinity chromatography, were purchase from Life Technologies (Cat. No PV4131). As substrate for the kinase reaction biotinylated peptide biotin-Ttds-YISPLKSPYKISEG (C-terminus in amid form) was used which can be purchased e.g. from the company JERINI peptide technologies (Berlin, Germany). For the assay 50 nL of a 1000-fold concentrated solution of the test compound in DMSO was pipetted into a black low volume 384-well microtiter plate (Greiner Bio-One, Frickenhausen, Germany), 2 µL of a solution of CDK9/CycT1 in aqueous assay buffer [50 mM Tris/HCl pH 8.0, 1 mM MgCl₂, 10 mM dithiothreitol, 0.1 mM sodium ortho-vanadate, 0.01% (v/v) Nonidet-P40 (Sigma)] were added and the mixture was incubated for 15 min at 22°C to allow pre-binding of the test compounds to the enzyme before the start of the kinase reaction. Then the kinase reaction was started by the addition of 3 µL of a solution of adenosine-tri-phosphate (ATP, 3.3 mM => final cone, in the 5 µL assay volume is 2 mM) and
substrate (1.67 µM => final cone in the 5 µL assay volume is 1 µM) in assay buffer and the resulting mixture was incubated for a reaction time of 25 min at 22°C. The concentration of CDK9/CycT1 was adjusted depending of the activity of the enzyme lot and was chosen appropriate to have the assay in the linear range. typical concentrations were in the range of 0.5 µg/mL. The reaction was stopped by the addition of 5 µL of a solution of TR-FRET detection reagents (0.2 µM streptavidine-XL665 [Cisbio Bioassays, Codolet, France] and 1 nM anti-RB (pSer807/pSer811)-antibody from BD Pharmingen [# 558389] and 1.2 nM LANCE EU-W1024 labeled anti-mouse IgG antibody [Perkin-Elmer, product no. AD0077]) in an aqueous EDTA-solution (100 mM EDTA, 0.2 % (w/v) bovine serum albumin in 100 mM HEPES pH 7.5).

The resulting mixture was incubated 1 h at 22°C to allow the formation of complex between the phosphorylated biotinylated peptide and the detection reagents. Subsequently the amount of phosphorylated substrate was evaluated by measurement of the resonance energy transfer from the Eu-chelate to the streptavidine-XL. Therefore, the fluorescence emissions at 620 nm and 665 nm after excitation at 350 nm was measured in a TR-FRET reader, e.g. a Pherastar (BMG Labtechnologies, Offenburg, Germany) or a Viewlux (Perkin-Elmer). The ratio of the emissions at 665 nm and at 622 nm was taken as the measure for the amount of phosphorylated substrate. The data were normalised (enzyme reaction without inhibitor = 0 % inhibition, all other assay components but no enzyme = 100 % inhibition). Usually the test compounds were tested on the same microtiterplate in 11 different concentrations in the range of 20 µM to 0.1 nM (20 µM, 5.9 µM, 1.7 µM, 0.51 µM, 0.15 µM, 44 nM, 13 nM, 3.8 nM, 1.1 nM, 0.33 nM and 0.1 nM, the dilution series prepared separately before the assay on the level of the 100fold concentrated solutions in DMSO by serial 1:3.4 dilutions) in duplicate values for each concentration and IC₅₀ values were calculated by a 4 parameter fit using Genedata Screener™ software.

In Table 5 the inhibition for CDK9 of compounds according to the present invention is summarized.
Noteworthily, in the CDK9 assays described below the resolution power is limited by the enzyme concentrations, the lower limit for IC₅₀s is about 1-2 nM in the CDK9 high ATP assay. For compounds exhibiting IC₅₀s in this range the true affinity to CDK9 might be even higher. The IC₅₀ values are means derived from different independent experiments or single values.

### Biochemical characteristics of the antibodies

**Determination of binding affinities by Biacore analysis:**

Binding affinities of anti-TWEAKR antibodies were examined using surface plasmon resonance analysis on a Biacore T100 instrument (GE Healthcare Biacore, Inc.). The antibodies were immobilized on a CM5 sensor chip using an indirect capture reagent, anti-human IgG (Fc). Reagents of the "Human Antibody Capture Kit" (BR-1008-39, GE Healthcare Biacore, Inc.) were used as described by the manufacturer. Anti-TWEAKR antibodies were injected at a concentration of 10 µg/mL at 10 µL/min for 10 sec. All experimental steps were carried out at 25°C. After fixation of anti-TWEAKR antibodies the extracellular domain of TWEAKR (Analyt, 30R-AT080, Fitzgerald) was injected in a concentration range of 3.9 to 500 nM. Sensorgrams were generated after in-line reference cell correction, followed by subtraction of the buffer sample. The dissociation constant (KD) was calculated based on the ratio of association (kₒᵣ) and dissociation (kₒᵢ) constants, obtained by fitting sensorgrams using a 1:1 first order binding model.

The anti-EGFR antibodies were analyzed as described above. IgGs were captured via an amine coupled anti-Fc capture Ab. After fixation of anti-EGFR antibodies rh EGFR (Sino Biological Inc.) was used as analyt at concentrations ranging from 1.56 - 200 nM.

### Table 5

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>CDK9/CycT1 high ATP kinase assay [IC₅₀ nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>3.36</td>
</tr>
<tr>
<td>M2</td>
<td>2.58</td>
</tr>
<tr>
<td>M3</td>
<td>2.86</td>
</tr>
<tr>
<td>M4</td>
<td>1.18</td>
</tr>
<tr>
<td>M5</td>
<td>0.69</td>
</tr>
<tr>
<td>M6</td>
<td>1.35</td>
</tr>
<tr>
<td>M7</td>
<td>11.7</td>
</tr>
</tbody>
</table>
Table 6a: Recombinant antigens used for affinity measurements

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>Description</th>
<th>Origin</th>
<th>Cat.-No (Fitzgerald Inc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPP-2305</td>
<td>hTNFRSF12Aminosäuren a28-80</td>
<td>Human</td>
<td>30R-AT080</td>
</tr>
<tr>
<td>rhEGFR</td>
<td></td>
<td>Human</td>
<td>Sino Biological Inc.: 10001-H08</td>
</tr>
</tbody>
</table>

Table 6b: Monovalent $K_D$ values of described antibodies measured using Biacore

<table>
<thead>
<tr>
<th>antibody</th>
<th>$k_a$ (1/µs)</th>
<th>$k_d$ (1/s)</th>
<th>$K_D$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPP-2090</td>
<td>9.1E+06</td>
<td>1.1E-01</td>
<td>12.4</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>8.6E+05</td>
<td>1.47E-03</td>
<td>2</td>
</tr>
<tr>
<td>Nimotuzumab</td>
<td>3.3E+04</td>
<td>1.06E-03</td>
<td>32</td>
</tr>
<tr>
<td>TPP-4030</td>
<td>3.6E+05</td>
<td>1.3E-01</td>
<td>360</td>
</tr>
<tr>
<td>TPP-5653</td>
<td></td>
<td></td>
<td>168</td>
</tr>
</tbody>
</table>

Determination of cell binding of described antibodies measured by FACS analysis

The binding affinity of the antibodies was measured by FACS analysis using different cancer cell lines. Therefore cells ($5 \times 10^5$ cells/well) were incubated in FACS-buffer (PBS without Ca/Mg, 3% FCS, Biochrom) with $1 \mu$g/mL primary antibody solution (start concentration) on ice for 30-45min (protected from light). A dose response curve was generated using 1:5 dilution steps. After incubation 200µL ice-cold FACS-buffer was added and the cell suspension was centrifuged at 4°C, 400g, for 4min. A washing step with 300µL ice-cold FACS-buffer followed by second centrifugation was performed. Then the cell pellet was resuspended in 100µL FACS-buffer and incubated with the secondary antibody (1:10 dilution; monoclonal Anti-Kappa light chains-FITC antibody, Fa. Sigma, No. SAB4700605) for 30min on ice. Afterwards the cells were washed with FACS-buffer again before the cell concentration was adjusted to $5 \times 10^5$ cells/mL. The FACS analysis started directly using a Guava flow cytometer (Fa. Millipore). Propidium Iodid (final concentration $5 \mu$g/mL) was used as viability control. The EC50 values of the antibodies were determined using the DRC (Dose Response Curve).

In the following Table 6 the EC50- values for the described antibodies are summarized. EGFR- and TWEAKR-antibody cell binding was tested using human lung carcinoma cells NCI H292.
Table 7: Binding affinity of described antibodies to NCI-H292-cells

<table>
<thead>
<tr>
<th>Antibody</th>
<th>EC50 H292 [M]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPP-2090</td>
<td>1.0E-09</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>6.1E-10</td>
</tr>
<tr>
<td>Nimotuzumab</td>
<td>1.3E-08</td>
</tr>
<tr>
<td>TPP-4030</td>
<td>5.9E-09</td>
</tr>
<tr>
<td>TPP-5653</td>
<td>3.3E-10</td>
</tr>
</tbody>
</table>

---

**Diagram 1:**
- **Square:** TPP2090 1 µg/ml
- **Isotope control 1 µg/ml**

**Diagram 2:**
- **Square:** Cetuximab 1 µg/ml
- **Circle:** Nimotuzumab 1 µg/ml
- **Triangle:** TPP-4030 1 µg/ml
- **Isotope control 1 µg/ml**
Patent claims

1. Conjugate of a binder or a derivative thereof with one or more molecules of an active component, wherein the active component is a CDK9 kinase inhibitor, which is conjugated to the binder via a linker Z.

2. Conjugate according to claim 1, wherein the binder or a derivative thereof is a binding peptide or a derivative of a binding peptide or -protein.

3. Conjugate according to claim 2, wherein each molecule of the active component is binding to different amino acids of the binding peptide or -protein or their derivatives respectively, via a linker.

4. Conjugate according to any one or all of the above claims, wherein the conjugate averages 1.2 to 50 molecules of the active components per binder.

5. Conjugate according to any one of claims 2 to 4, wherein the binding peptide or protein represents an antibody or wherein the derivative or the derivative of the binding peptide or -protein comprises the group

\[
-nH-(\text{C}_2\text{H}_4)^4_{_{\text{NH}_2}} \text{ or } -s \text{c}_{\text{H}_2} \text{N}_{\text{H}_2}, \text{respectively.}
\]

6. Conjugate according to any one of the above claims, wherein the binder binds to a cancer target-molecule.

7. Conjugate according to claim 6, wherein the binder is binding to an extracellular target molecule.

8. Conjugate according to claim 7, wherein the binder, after binding to the extracellular target molecule, is internalized in the expressing cell of the target molecule and is processed intracellularly, preferably through the lysosomal pathway.

9. Conjugate according to any one of claims 2 to 8, wherein the binding peptide or -protein is a human, humanized or chimeric monoclonal antibody, or an antigen-binding fragment thereof.

10. Conjugate according to claim 9, wherein the binding peptide or -protein is, an anti-EGFR-antibody, anti-TWEAKR-antibody, or an antigen binding fragment thereof.
11. Conjugate according to any one or all of the above claims, wherein the conjugate of a binder with one or more molecules of an active component has the following formula

\[ AB - \frac{Z}{MC} \]

wherein \( AB \) stands for a binder, \( Z \) stands for a linker, \( n \) stands for a number between 1 and 50, preferably 1.2 to 20 and especially preferred 2 to 8, and \( MC \) stands for an active component of Formula (I):

\[
\text{I}
\]

wherein

- \( A \) represents a bivalent group selected from the group consisting of \(-S-, -S(=0)-, -S(=0)2-\) and \(-S(=0)(=NR \, \varphi)\);
- \( L \) represents a \( C2-C6 \)-alkylene group,

wherein said group is optionally substituted with

- (i) one substituent selected from hydroxy, \( C2-C3 \)-alkenyl, \( C2-C3 \)-alkynyl, \( C3-C4 \)-cycloalkyl, hydroxy-\( C1-C5 \)-alkyl, \(-\text{(CH}_2\text{)}_NR \, \varphi, \text{and/or} \)
- (ii) one or two or three substituents, identically or differently, selected from halogen and \( C1-C5 \)-alkyl,

with the proviso that a \( C2 \)-alkylene group is not substituted with a hydroxy group,

- or wherein
  - one carbon atom of said \( C2-C6 \)-alkylene group forms a three- or four-membered ring together with a bivalent group to which it is attached, wherein said bivalent group is selected from \(-\text{CH2CH2}-, -\text{CH2CH2CH2}-, -\text{CH2CH2CH2}-; \)
X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R\(^1\) represents \(-Z\#1\) or a group selected from Ci-C6-alkyl-, C3-C6-alkenyl, C3-C6-alkyl-, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, phenyl-Ci-C3-alkyl- and heteroaryl-Ci-C3-alkyl-,

wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, Ci-C6-alkyl-, halo-Ci-C3-alkyl-, Ci-C6-alkoxy-, Ci-C3-fluoroalkoxy-, \(-\text{NH}_2\), alkylamino-, dialkylamino-, acetylamino-, \(N\)-methyl-\(N\)-acetylamino-, cyclic amines, \(-\text{OP}(=\text{O})(\text{OH})_2\), \(-\text{C}(=\text{O})\text{OH}\), \(-\text{C}(=\text{O})\text{NH}_2\);

R\(^2\) represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-;

R\(^3\), R\(^4\) represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-;

R\(^5\) represents \(-Z\#1\) or a group selected from a hydrogen atom, cyano, \(-\text{C}(=\text{O})\text{R}\) \(^8\), \(-\text{C}(=\text{O})\text{OR}\) \(^8\), \(-\text{S}(=\text{O})_2\text{R}^8\), \(-\text{C}(=\text{O})\text{NR}^7\), Ci-C6-alkyl-, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, wherein said Ci-C6-alkyl-, C3-C7-cycloalkyl-, heterocyclyl-, phenyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, \(-\text{NH}_2\), alkylamino-, dialkylamino-, acetylamino-, \(N\)-methyl-\(N\)-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-;

R\(^6\), R\(^7\) represent, independently from each other, a group selected from a hydrogen atom, Ci-C6-alkyl-, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, benzyl and heteroaryl, wherein said Ci-C6-alkyl-, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, benzyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, Ci-C3-alkoxy-, \(-\text{NH}_2\), alkylamino-, dialkylamino-, acetylamino-, \(N\)-methyl-\(N\)-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-, or

R\(^6\) and R\(^7\), together with the nitrogen atom they are attached to, form a cyclic amine;

R\(^8\) represents a group selected from Ci-C6-alkyl-, halo-Ci-C3-alkyl-, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, benzyl and heteroaryl, wherein said group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, C1-C3-alkoxy-, \(-\text{NH}_2\), alkylamino-, dialkylamino-, acetylamino-, \(N\)-methyl-\(N\)-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-,
wherein one of the substituents R¹ or R₅ is Z-#1,
wherein Z stands for the linker and #1 for the bond to the binder,
or a salt, solvate or salt of a solvate thereof.

12. Conjugate according to claim 11, wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2- and -S(=0)(=NR ³₅)-;

L represents a C₂-C₆-alkylene group,

wherein said group is optionally substituted with one substituent selected from hydroxy, C₃-C₅-cycloalkyl, hydroxy-C₃-alkyl, -(CH₂)NR³R⁷, and optionally with one or two or three additional substituents, identically or differently, selected from halogen and Ci-C₃-alkyl,

with the proviso that a C₂-alkylene group is not substituted with a hydroxy group.

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R¹ represents -Z-#1 or a group selected from Ci-C₆-alkyl-, C₃-C₅-cycloalkyl-, phenyl and phenyl-Ci-C₃-alkyl-,

wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, Ci-C₃-alkyl-, fluoro-Ci-C₂-alkyl-, Ci-C₃-alkoxy-, Ci-C₂-fluoroalkoxy-, -N³⁄₄ alkylamino-, dialkylamino-, cyclic amines, -OP(=0)(OH)¾ -C(=0)OH, -C(=0)NH ²;

R² represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C₂-alkyl-, Ci-C₂-alkoxy-, fluoro-Ci-C₂-alkyl-, Ci-C₂-fluoroalkoxy-;

R³, R⁴ represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano Ci-C₂-alkyl-, Ci-C₂-alkoxy-, fluoro-Ci-C₂-alkyl-, Ci-C₂-fluoroalkoxy-;

R⁵ represents -Z-#1 or a group selected from, a hydrogen atom, cyano, -C(=0)R ⁸, -C(=0)OR ⁸, -S(=0)₂R ⁸, -C(=0)NR ³R⁷, Ci-Ce-alkyl-, C₃-C₅-cycloalkyl-, phenyl,

wherein said Ci-C₆-alkyl, C₃-C₅-cycloalkyl- or phenyl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, Ci-C₃-alkyl-, Ci-C₃-alkoxy-, -N³⁄₄ alkylamino-, dialkylamino-, cyclic amines, fluoro-Ci-C₂-alkyl-, Ci-C₂-fluoroalkoxy-;

R⁶, R⁷ represent, independently from each other, a group selected from a hydrogen atom, Ci-Ce-alkyl-, C₃-C₅-cycloalkyl-, phenyl and benzyl,
wherein said Ci-C6-alkyl-, Cs-Cs-cycloalkyl-, phenyl or benzyl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines, fluoro-Ci-C2-alkyl-, Ci-C2-fluoroalkoxy-, or
5
R6 and R7, together with the nitrogen atom they are attached to, form a cyclic amine;

R8 represents a group selected from Ci-C6-alkyl-, fluoro-Ci-C3-alkyl-, Cs-Cs-cycloalkyl-, phenyl and benzyl, wherein said group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, cyclic amines, fluoro-Ci-C2-alkyl-, Ci-C2-fluoroalkoxy-,
10
wherein one of the substituents R1 or R3 is Z-#1,

wherein Z stands for the linker and #1 for the bond to the binder.

13. Conjugate according to any one of claims 11 or 12, wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2- and -S(=0)(=NR5);

20
L represents a C2-C4-alkylene group,

wherein said group is optionally substituted with
(i) one substituent selected from C3-C4-cycloalkyl and hydroxymethyl-, and/or
(ii) one or two additional substituents, identically or differently, selected from C1-C2-alkyl,

25
X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R1 represents -Z-#1 or a group selected from C1-C4-alkyl-, C3-C5-cycloalkyl- and phenyl,

30
wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, Ci-C2-alkyl-, C1-C2-alkoxy-, -NH2, -C(=0)OH;

R2 represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, cyano, methyl, methoxy-, trifluoromethyl-, trifluoromethoxy-;

R3 represents a group selected from a hydrogen atom, a fluoro atom, a chloro atom, cyano, methyl, methoxy-, trifluoromethyl-, trifluoromethoxy-;
R^4 represents a hydrogen atom or a fluoro atom;
R^5 represents -Z-#1 or a group selected from a hydrogen atom, cyano, -C(=0)NR^7, -
C(=0)OR^8, -C(=0)OR^8, -C(=0)R^8, C_1-C_4-alkyl-.
wherein said C_1-C_4-alkyl- group is optionally substituted with one substituent selected from
the group consisting of halogen, hydroxy, cyano, C_3-C_5-alkoxy-, -NH_2, alkylamino-, dialkylamino-, cyclic amines;
R^6, R^7 represent, independently from each other, a group selected from a hydrogen atom, C_1-C_4-
alkyl- and C_3-C_5-cycloalkyl-,
wherein said C_1-C_4-alkyl- or C_3-C_5-cycloalkyl- group is optionally substituted with one or
two substituents, identically or differently, selected from the group consisting of hydroxy,
C_1-C_2-alkyl-, C_1-C_2-alkoxy-, -NH_2, alkylamino-, dialkylamino-, cyclic amines, or
R^6 and R^7, together with the nitrogen atom they are attached to, form a cyclic amine;
R^8 represents a group selected from C_1-C_6-alkyl-, fluoro-C_1-C_3-alkyl-, C_1-C_2-cycloalkyl- and
phenyl,
wherein said group is optionally substituted with one substituent selected from the group
consisting of halogen, hydroxy, C_1-C_2-alkyl-, C_1-C_2-alkoxy-, -NH_2,
wherein one of the substituents R^1 or R^5 is Z-#1,
wherein Z stands for the linker and # 1 for the bond to the binder.

14. Conjugate according to any one of claims 11 to 13, wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2- and
-S(=0)(=NR^5)-;
L represents a C_2-C_4-alkylene group;
X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and
Y represents N;
R^1 represents -Z-#1 or a group selected from C_1-C_4-alkyl-,
wherein said group is optionally substituted with one or two or three substituents, identically
or differently, selected from the group consisting of hydroxy, C_1-C_2-alkoxy-, -NH_2, -C(=0)OH;
R^2 represents a hydrogen atom;
R^3 represents a group selected from a hydrogen atom, a fluoro atom;
R^4 represents a hydrogen atom;
R^5 represents -Z-#l or a group selected from a hydrogen atom, cyano, -C(=0)NR^7, -C(=0)R^8, -C(=0)OR^8, -S(=0)_2R^8, C_1-C_4-alkyl-.

R^5, R^7 represent, independently from each other, a group selected from a hydrogen atom, C_1-C_4-alkyl- and C_3-C_5-cycloalkyl-, or R^6 and R^7, together with the nitrogen atom they are attached to, form a cyclic amine;

R^8 represents a group selected from C_1-C_6-alkyl-, fluoro-C_1-C_3-alkyl-, C_3-C_5-cycloalkyl- and phenyl,

wherein said group is optionally substituted with one substituent selected from the group consisting of halogen, hydroxy, C_1-C_2-alkyl-, C_1-C_2-alkoxy-, -NH_2.

wherein one of the substituents R^1 or R^5 is Z-#l,

wherein Z stands for the linker and # 1 for the bond to the binder.

15. Conjugate according to any one of claims 11 to 14, wherein

L represents a C_3-C_4-alkylene group.

16. Conjugate according to any one of claims 11 to 15, wherein

A represents a bivalent group selected from the group consisting of -S(=0)2- and -S(=0)(=NR^5)-;

L represents a C_3-C_4-alkylene group;

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R^1 represents -Z-#l, a methyl- group;

R^2 represents a hydrogen atom;

R^3 represents a group selected from a hydrogen atom, a fluoro atom;

R^4 represents a hydrogen atom;

R^5 represents -Z-#l or represents a group selected from a hydrogen atom, -C(=0)NR^7, -C(=0)R^8, -C(=0)OR^8, -S(=0)_2R^8, C_1-C_4-alkyl-.
R⁶, R⁷ represent, independently from each other, a group selected from a hydrogen atom, C₁-C₂-alkyl;

R⁸ represents a C₁-C₂-alkyl- group,

wherein one of the substituents R¹ or R⁵ is Z-#₁,

wherein Z stands for the linker and #₁ for the bond to the binder.

17. Conjugate according to any one of claims 11 to 16, wherein
R¹ represents -Z-#₁ or a methyl group;

wherein one of the substituents R¹ or R⁵ is Z-#₁,

wherein Z stands for the linker and #₁ for the bond to the binder,

or an enantiomer, diastereomer, salt, solvate or salt of a solvate thereof.

18. Conjugate according to any one of claims 11 to 17, wherein
R³ represents a fluoro atom, and
R⁴ represents a hydrogen atom.

19. Conjugate according to any one of claims 11 to 18, wherein
R² represents a hydrogen atom,

or a salt, solvate or salt of solvate thereof.

20. Conjugate according to any one of claims 11 to 19, in which the active component is a compound of general formula (la)

\[(\text{la})\]

wherein
A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2- and -S(=0)(=NR 5); 
X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N; 
R1 represents -Z-#l or a group selected from Ci-C6-alkyl-, C3-C6-alkenyl, C3-C6-alkynyl, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, phenyl-Ci-C3-alkyl- and heteroaryl-Ci-C3-alkyl-, wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, Ci-C6-alkyl-, halo-Ci-C3-alkyl-, Ci-Ce-alkoxy-, Ci-C3-fluoroalkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, -OP(=0)(OH)½-C(=0)OH, -C(=0)NH2; 
R5 represents -Z-#l or a group selected from a hydrogen atom, cyano, -C(=O)R8, -C(=O)OR8, -S(=O)2R8, -C(=0)NR 7, Ci-C6-alkyl-, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, wherein said Ci-Ce-alkyl, C3-C7-cycloalkyl-, heterocyclyl-, phenyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy--; wherein one of the substituents R1 or R5 is Z-#l, wherein Z stands for the linker and #1 for the bond to the binder; or the salts, solvates or salts of solvates thereof.

21. Conjugate according to claim 20, wherein

A represents a bivalent group selected from the group consisting of -S(=0)2- and -S(=0)(=NR 5); 
R1 represents -Z-#l or a methyl- group; 
R5 represents -Z-#l or a group selected from a hydrogen atom, -C(=0)NR 7, -C(=0)R 8, -C(=0)OR 8, -S(=O)2R8, methyl-;
R6 represents an ethyl- group; 
R7 represents a hydrogen atom; 
R8 represents a Ci-C2-alkyl- group; 
wherein one of the substituents R1 or R5 is Z-#l, wherein Z stands for the linker and #1 for the bond to the binder.

22. Conjugate according to any one or all of the above claims wherein the cyclin dependent kinase inhibitor is a compound of Formula (la) according to claim 20, wherein
X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R¹ represents -Z-# 1,
R⁶ represents an ethyl-group;
R⁷ represents a hydrogen atom;

R⁸ represents a Ci-C2-alkyl-group,

wherein Z stands for the linker and # 1 for the bond to the binder.

23. Conjugate according to any one or all of the above claims wherein the cyclin dependent kinase inhibitor is a compound of Formula (Ia) according to claim 20, wherein

A represents a bivalent group -S(=O)(=NR⁵)-;

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R¹ represents a methyl-group;
R⁵ represents -Z-# 1;

wherein Z stands for the linker and # 1 for the bond to the binder

or a salt, solvate or salt of solvate thereof.

24. Conjugate according to any one or all of the above claims wherein the cyclin dependent kinase inhibitor represents one of the following formulae:
25. Conjugate according to any one or all of the above claims wherein the cyclin dependent kinase inhibitor represents one of the following formulae:

![Chemical Structures](image)

26. Conjugate according to any one or all of the above claims, wherein the linker -Z- represents one of the following general structures (i) to (iv):

(v) §-(CH\(_2\))\(^m\)-(CO)\(^m\)-SG-L\(_1\)-L\(_2\)-§§
(vi) §-(CH\(_2\))\(^v\)-(CO)\(^m\)-L\(_1\)-SG-L\(_1\)-L\(_2\)-§§
(vii) §-(CH\(_2\))\(^v\)-(CO)\(^m\)-L\(_1\)-L\(_2\)-§§
(viii) §-(CH\(_2\))\(^v\)-(CO)\(^m\)-L\(_1\)-SG-L\(_2\)-§§

wherein

m is 0 or 1;
v is 0, 1 or 2;
represents the attachment point to the active component;
§§ represents the attachment point to the binder;
SG and SGI represent an *in vivo* cleavable group, LI represent independently of each other in vivo non-cleavable organic groups, and L2 represents an attachment group.

27. Conjugate according to claim 24, wherein the *in vivo* cleavable group SG represents a 2-8 oligopeptide group, preferably a dipeptide group or a disulfide, a hydrazine, an acetal or an aminal and SGI represents a 2-8 oligopeptide group, preferably a dipeptide group.

28. Conjugate according to any one or all of the above claims, wherein the linker Z is bound to the cysteine side chain or the cysteine rest respectively, and represents the following formula:

$$
\text{§}-(\text{CH}_2)_v-(\text{CO})_m-L_1-L_2-\text{§§}
$$

wherein
m is of 0 or 1;
v is 0, 1 or 2
§ represents the attachment point to the active component;
§§ represents the attachment point to the binder; and
L2 represents

![Diagram of molecular structures]

wherein
#1 represents the attachment point to the sulfur atom of the binder,
#2 represents the attachment point to the group L1,

L1 represents -(NR\(^{10}\))\(^n\)-(Gl)\(^o\)-G2-,
where R\(^{10}\) represents H, NH\(_2\) or Ci-C3-alkyl;

Gl represents -NHCO- or \[\begin{array}{c}
\text{N} \\
\text{N} \text{CO}
\end{array}\]
n is 0 or 1;
o is 0 or 1; and
G2 represents a straight-chain or branched hydrocarbon chain having 1 to 100 carbon atoms from arylene groups and/or straight-chain and/or branched and/or cyclic alkylene groups and which may be interrupted once or more than once by one or more of the groups -0-, -S-, -SO-, SO2, -NH-, -CO-, -NMe-, -NHNH-, -SO2NHNH-, -NHCO-, -CONH-, -CONHNNH- and a 5- to 10-membered aromatic or non-aromatic heterocycle having up to 4 heteroatoms selected from the group consisting of N, O and S, -SO- or -SO2- (preferably -N=N-), where the side chains, if present, may be substituted by -NHCONH2, -COOH, -OH, -NH2, NH-CN-NH2, sulphonamide, sulphone, sulphoxide or sulphonic acid, or represents one of the groups below:

where Rx represents H, C1-C3-alkyl or phenyl.

29. Conjugate according to claim 28, wherein
   LI represents -(Gl)n-G2-
   wherein
   G1 represents -NH- or -O-;
n is of 0 or 1;
G2 represents a straight or branched C1-C100 chain of aryl groups and/or straight an/or branched and/or cyclic alkylene groups, which may be substituted with one or more of the groups -0-, -S-, -SO-, -NH-, -CO-, -NHCO-, -CONH-, -NHCONH-, wherein the side chains, if present, may be substituted with -NHCONH2.

30. Conjugate according to any one of claims 26 to 28, wherein L2 represents one or more of the following three formulae:

wherein

#1 represents the attachment point to the sulfur atom of the binder,
#2 represents the attachment point to the group LI,
wherein in a preferred embodiment over 80% of the attachment points to the sulfur atom of the binder, in respect to the total number of attachments of the linker to the binder, are represented by one of the two structures:

\[ \text{Structure 1} \]

31. Conjugate according to any one of claims 26 to 30, wherein the carbon chain of G2 is intermitted by one of the following groups:

\[ \text{Structure 2} \]

\[ \text{Structure 3} \]

wherein X represents H or a Ci-Cio alkyl group that may be substituted with -NHCONH2, -COOH, -OH, -NH2, NH-CNNH2, sulfonamide, sulfone, sulfoxide or sulfonic acid.

32. Conjugate according to any one or all of the above claims, wherein the linker Z is \( R^5 \) and is bound to the cysteine side chain or the cysteine rest respectively, and represents the following formula:

\[ \text{Structure 4} \]

where

- #3 represents the bond to the active compound molecule,
- #4 represents the bond to the binder peptide or protein,
- \( R^{11} \) represents H or N\(^6\) ;
B represents \(-[(\text{CH}_2)_x \cdot (X^4)_y]w-\text{(CH}_2)_z,\)

\[ \begin{align*}
  w & = 0 \text{ to } 20; \\
  x & = 0 \text{ to } 5; \\
  y & = 0 \text{ or } 1; \\
  z & = 1 \text{ to } 5; \text{ and}
\end{align*} \]

\[ \text{X}^4 \text{ represents } -\text{O}, -\text{CONH}, -\text{NHCO} \text{ or } \]

33. Conjugate according to any one or all of the above claims, wherein the linker Z is R^1 and is bound to the cysteine side chain or the cysteine rest respectively, and represents the following formula:

\[ \begin{align*}
  \#3 & \text{ represents the bond to the active compound molecule,} \\
  \#4 & \text{ represents the bond to the binder peptide or protein,} \\
  R^{11} & \text{ represents } \text{H or} \text{NH}_2; \\
  B & \text{ represents } -[(\text{CH}_2)_x \cdot (X^4)_y]w-\text{(CH}_2)_z, \\
  w & = 0 \text{ to } 20; \\
  x & = 0 \text{ to } 5; \\
  y & = 0 \text{ or } 1; \\
  z & = 1 \text{ to } 5; \text{ and}
\end{align*} \]

\[ \text{X}^4 \text{ represents } -\text{O}, -\text{CONH}, -\text{NHCO} \text{ or } \]

34. Conjugate according to any one of claims 25 to 33, wherein the conjugate represents one of the following formulae:
wherein AB represents a binder that is attached via the sulfur atom of the binder; n is of 1 to 20; A represents a bivalent group selected from the group consisting of -S-, -S(=0)- and -S(=0)2-; and L1 represents a straight or branched C1-C100 chain of aryl groups and/or straight an/or branched and/or cyclic alkyne groups, which may be substituted with one or more of the groups -OH, -SO-, -NH-, -CO-, -NMe-, -NHNH-, -SO2NHNH-, -NHCO-, -CONH-, -NHCONH- and a 5 to 10 membered aromatic or non-aromatic heterocycle containing up to 4 hetero atoms chosen from N, O and S, -SO- or SO2, preferably \(-N-\)\(^\text{5-10} \)\(-\text{CO-}\), wherein the side chains, if present, may be substituted with -NHCONH2, -COOH, -OH, -NH\(_2\), NH-CNNH2, sulfonamide, sulfone, sulfoxide or sulfonic acid.

35. Conjugate according to any one of claims 25 to 33, wherein the conjugate represents one of the following formulae:

wherein AB represents a binder that is attached via the sulfur atom of the binder; n is of 1 to 20; A represents a bivalent group selected from the group consisting of -S-, -S(=0)- and -S(=0)2-; L1
represents a straight or branched C1-C100 chain of aryl groups and/or straight an/or branched and/or cyclic alkylene groups, which may be substituted with one or more of the groups -0-, -S-, -SO-, -NH-, -CO-, -NMe-, -NHNH-, -SO2NHNH-, -NHCO-, -CONH-, -NHCONH- and a 5 to 10 membered aromatic or non-aromatic heterocycle containing up to 4 hetero atoms chosen from N, O and S, -SO- or SO2, preferably -N=O-N=C-O-, wherein the side chains, if present, may be substituted with -NHCONH2, -COOH, -OH, -NH2, NH-CNNH2, sulfonamide, sulfone, sulfoxide or sulfonic acid; and SGI represents a 2-8 oligopeptide group, preferably a dipeptide group.

36. Conjugate according to any one or all of the above claims wherein the anti-TWEAKR-binder is an agonistic antibody.

37. Conjugate according to any one or all of the above claims containing,

a heavy chain variable domain comprising:

a. a CDR1 of the heavy chain having an amino acid sequence comprising a formula PYPMX (SEQ ID NO:171), wherein X is I or M;

b. a CDR2 of the heavy chain having an amino acid sequence comprising a formula YISPSGGXTHYADSVKG (SEQ ID NO: 172), wherein X is S or K;

c. a CDR3 of the heavy chain having an amino acid sequence comprising a formula GGDTYFDYFDY (SEQ ID NO: 173);

and a light chain variable domain comprising:

d. a CDR1 of the light chain having an amino acid sequence comprising a formula RASQSISXYLN (SEQ ID NO: 174), wherein X is G or S;

e. a CDR2 of the light chain having an amino acid sequence comprising a XASSLQS (SEQ ID NO: 175), wherein X is Q, A or N; and

f. a CDR3 of the light chain having an amino acid sequence comprising a QQSYXXPXIT (SEQ ID NO: 176), wherein X at position 5 is T or S, X at position 6 is T or S and X at position 8 is G or F.
38. Compounds of general formula (Γ)

wherein

A represents a bivalent group selected from the group consisting of \(-\text{S}-\), \(-\text{S}(=0)-\), \(-\text{S}(=0)2-\) and \(-\text{S}(=0)(=\text{NR}^5)-\);

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R\(^1\) represents \(-\text{Z}\) or a group selected from \text{Ci-C}6-alkyl-, \text{C}3-\text{C}6-alkenyl, \text{C}3-\text{C}6-alkynyl, \text{C}3-\text{C}7-cycloalkyl-, phenyl, heteroaryl, phenyl-\text{Ci-C}3-alkyl- and heteroaryl-\text{Ci-C}3-alkyl-,

wherein said group is optionally substituted with one or two or three substituents, identically or differently, selected from the group consisting of hydroxy, cyano, halogen, \text{Ci-C}6-alkyl-, \text{halo-Ci-C}3-alkyl-, \text{Ci-C}e-alkoxy-, \text{Ci-C}3-fluoroalkoxy-, \text{N}3/4 alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, \text{OP}(=0)(\text{OH})_n, \text{C}(=0)\text{OH}, \text{C}(=0)\text{NH}_2;

R\(^3\), R\(^4\) represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, \text{Ci-C}3-alkyl-, \text{Ci-C}3-alkoxy-, \text{halo-Ci-C}3-alkyl-, \text{Ci-C}3-fluoroalkoxy-;

R\(^5\) represents \(-\text{Z}\) or a group selected from a hydrogen atom, cyano, \text{C}(=0)\text{R}^8, \text{C}(=0)\text{OR}^8, \text{S}(=0)\text{R}^8, \text{C}(=0)\text{NR}^5\text{R}^7, \text{Ci-C}6-alkyl-, \text{C}3-\text{C}7-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, wherein said \text{Ci-C}e-alkyl, \text{C}3-\text{C}7-cycloalkyl-, heterocyclyl-, phenyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, cyano, \text{Ci-C}3-alkyl-, \text{Ci-C}3-alkoxy-, \text{N}3/4 alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-\text{Ci-C}3-alkyl-, \text{Ci-C}3-fluoroalkoxy-;

R\(^6\), R\(^7\) represent, independently from each other, a group selected from a hydrogen atom, \text{Ci-C}6-alkyl-, \text{C}3-\text{C}7-cycloalkyl-, heterocyclyl-, phenyl, benzyl and heteroaryl,
wherein said Ci-C6-alkyl-, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, benzyl or heteroaryl group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-, or R6 and R7, together with the nitrogen atom they are attached to, form a cyclic amine;

R8 represents a group selected from Ci-C6-alkyl-, halo-Ci-C3-alkyl-, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, benzyl and heteroaryl,

wherein said group is optionally substituted with one, two or three substituents, identically or differently, selected from the group consisting of halogen, hydroxy, Ci-C3-alkyl-, Ci-C3-alkoxy-, -NH2, alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, halo-Ci-C3-alkyl-, Ci-C3-fluoroalkoxy-,

wherein one of the substituents R1 or R5 is Z,

wherein Z stands for the linker;
or the enantiomers, diastereomers, salts, solvates or salts of solvates thereof; and

the linker -Z- represents one of the following general structures (v) to (viii):

\[ \text{§-(CH}_2\text{)}_v\text{-(CO)}_m\text{SGI}-\text{LI- L3} \]

\[ \text{§-(CH}_2\text{)}_v\text{-(CO)}_m\text{L1-SG-L1-L3} \]

\[ \text{§-(CH}_2\text{)}_v\text{-(CO)}_m\text{L1-L3} \]

\[ \text{§-(CH}_2\text{)}_v\text{-(CO)}_m\text{L1-SG-L3} \]

wherein

v is 0, 1 or 2

m is 0 or 1,

§ represents the attachment point to the active component;

SG and SGI represent a in vivo cleavable group, LI represent independently of each other in vivo non-cleavable organic groups, and

L3 represents
wherein

\[ \text{#1} \] represents the attachment site to the group L1.

39. Compounds according to claim 38, wherein the compound represents one of the following formulae:
wherein L1 represents a straight or branched C1-C100 chain of aryl groups and/or straight and/or branched and/or cyclic alkyne groups, which may be substituted with one or more of the groups -O-, -S-, -SO-, -NH-, -CO-, -NMe-, -NHNH-, -SO₂NHNH-, -NHCO-, -CONH-, -NHCONH- and a 5 to 10 membered aromatic or non-aromatic heterocycle containing up to 4 hetero atoms chosen from N, O and S, -SO- or SO₂, preferably \( \text{N}^{\text{CO}} \), wherein the side chains, if present, may be substituted with -NHCONH₂, -COOH, -OH, -NH₂, NH-CNNH₂, sulfonamide, sulfone, sulfoxide or sulfonic acid; and

SGI represents a 2-8 oligopeptide group, preferably a dipeptide group.

40. Method for forming the conjugate according to claims 11 to 39, wherein a compound of formula (I), preferably the trifluoroacetic acid salt of the compound, reacts with the cysteine rest of a binding peptide or -protein, wherein the compound is preferably used in a 2 to 12-fold molar excess in respect to the binding peptide or -protein.

41. Method according to claim 40, wherein a compound according to one of the following formulae, or a salt thereof (preferably the trifluoroacetic acid salt) of the compound, reacts with the cysteine rest of a binding peptide or -protein, wherein the compound is preferably used in a 2 to 12-fold molar excess in respect to the binding peptide or -protein.
wherein

A represents a bivalent group selected from the group consisting of -S-, -S(=0)-, -S(=0)2-;

X, Y represent CH or N with the proviso that one of X and Y represents CH and one of X and Y represents N;

R1 represents a group selected from Ci-C6-alkyl-, C3-C6-alkenyl, C3-C6-alkynyl, C3-C7-cycloalkyl-, heterocyclyl-, phenyl, heteroaryl, phenyl-Ci-C3-alkyl- and heteroaryl-Ci-C3-alkyl-, wherein said group is optionally substituted with one or two or three substituents, identically or differently,

selected from the group consisting of hydroxy, cyano, halogen, Ci-C6-alkyl-, halo-Ci-C3-alkyl-, Ci-C6-alkoxy-, Ci-C3-fluoroalkoxy-, -N3/4 alkylamino-, dialkylamino-, acetylamino-, N-methyl-N-acetylamino-, cyclic amines, -OP(=0)(OH)3/4 -C(=0)OH, -C(=0)NH2;

R3, R4 represent, independently from each other, a group selected from a hydrogen atom, a fluoro atom, a chloro atom, a bromo atom, cyano, Ci-C3-alkyl-, Ci-C3-alkoxy-, halo-Ci-C3-alkyl-

SGI represents a 2-8 oligopeptide group, preferably a dipeptide group;

L1 represents a straight or branched Ci-C10 chain of aryl groups and/or straight an/or branched and/or cyclic alkylene groups, which may be substituted with one or more of the groups -0-, -S-, -SO-, -NH-, -CO-, -NMe-, -NHNH-, -SO2NH2H2H2H2NH2, -NCO-, -CONH-, -NHCONH- and a 5 to 10 membered aromatic or non-aromatic heterocycle containing up to 4 hetero atoms chosen from N, O and S, -SO2 or

SO2, preferably - N\square/\square\square N\square/\square CO-\square, wherein the side chains, if present, may be substituted with -NHCONH2, -COOH, -OH, -NH22 NH-CNHH2, sulfonamide, sulfone, sulfoxide or sulfonic acid; and

L4 has the same meaning as L1.

42. Method according to any one of claims 40 or 41 wherein the binder and/or the inhibitor bound to the linker is optionally partially reduced prior to coupling.
43. Pharmaceutical composition comprising a conjugate according to any one or all of the above claims in combination with an inert, non-toxic, pharmaceutically acceptable excipient.

44. Conjugate according to any one or all of claims the above claims for use in the treatment and/or prophylaxis of diseases.

45. Conjugate according to any one or all of the above claims for use in the treatment and/or prophylaxis of hyperproliferative and/or angiogenic diseases.

46. Conjugate according to any one or all of the above claims for use in the treatment of cancer.

47. Conjugate according to any one or all of the above claims for use in the treatment of colon cancer, liver cancer and lung cancer.

48. Conjugate according to any one or all of the above claims for use in the treatment of lung cancer.

49. Compounds according to any one of claims 38 or 39 that have the structures below
50. A metabolite obtainable by the cleavage of any of the conjugates as defined in claims 1 to 37.

51. The metabolite according to claim 50, wherein the metabolite does not comprise a cysteine and/or a lysine residue of the binder protein or peptide.

52. A method of treating cancer comprising the administration of a conjugate according to any of claims 1 to 37.
53. The method treatment according to claim 52, wherein the cancer is one of the group of lung cancer, colon cancer and liver cancer, preferably lung cancer.

54. The method of treatment according to claims 52 or 53, wherein the conjugate according to any one of claims 1 to 37 targets the NCI-H292 tumor cell line.

55. The use of a conjugate according to any one of claims 1 to 37 for the manufacture of a medicament for the treatment of cancer.

56. The use according to claim 55 wherein the cancer is one of the group of lung cancer, colon cancer and liver cancer, preferably lung cancer.

57. The use according to claim 55 wherein the conjugate according to any one of claims 1 to 37 targets the NCI-H292 tumor cell line.