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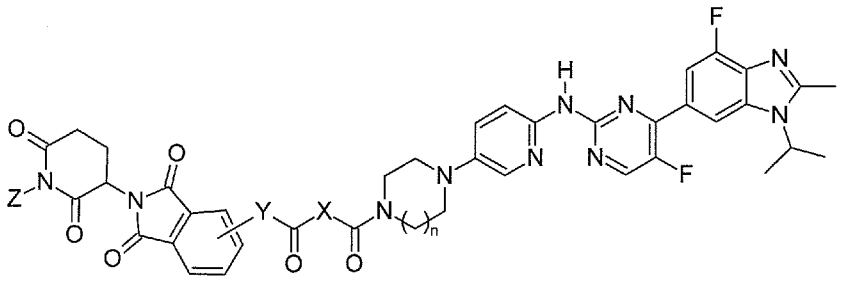
(54) Title: NOVEL CDK DEGRADERS



(I)

(57) Abstract: The present invention relates to novel CDK degraders of general formula (I). These novel compounds are useful in the treatment of diseases that are associated with CDK activity, such as cancer and inflammation.

Novel CDK Degraders

The present invention relates to novel CDK4/6/9 degraders. These novel compounds are useful in the treatment of diseases that are associated with CDK activity, such as cancer and inflammation.

A key hallmark of cancer is the dysregulated cell division, which leads to aberrant cell proliferation. Cell division is mainly controlled by a complex composed of cyclin and cyclin dependent kinases (CDKs). Todays approved drugs mostly inhibit CDK4 and 6, e.g., palbociclib, ribociclib, and abemaciclib. They have expanded treatment options for patients with advanced breast cancer, for patients with hormone receptor–positive, human epidermal growth factor receptor 2–negative advanced breast cancer. Thus, drugs inhibiting the CDKs are an important part of the clinician's armamentarium to fight cancer.

Besides direct kinase inhibitors, degraders of CDKs have been also described (Olson, C. M., Jiang, B., Erb, M. A.; et al. Pharmacological Perturbation of CDK9 Using Selective CDK9 Inhibition or Degradation. Nat. Chem. Biol. 2018, 14, 163–170.). While direct kinase inhibitors only inhibit the kinase enzyme activity, molecular glues or PROTAC degraders can also inhibit scaffolding and other functions of CDKs and thus exert better antiproliferative activity and suppress resistance occurrence.

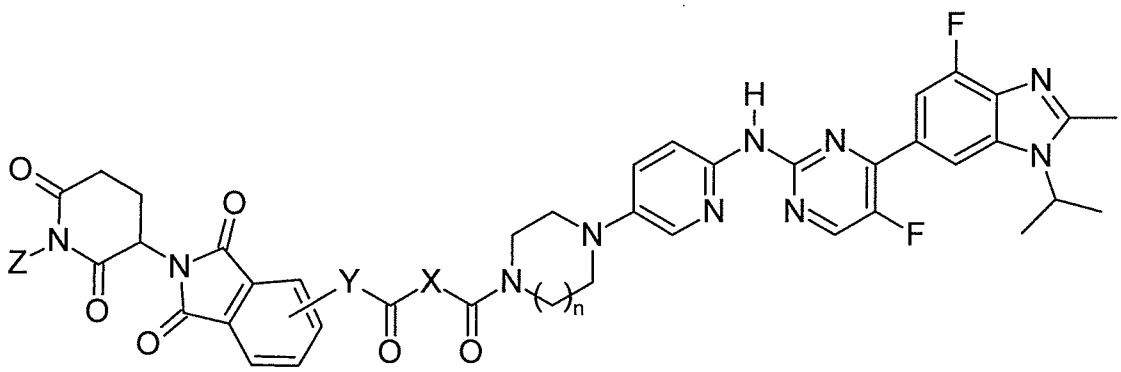
Concomitant inhibition of several CDKs can have advantageous antiproliferative effects. For example, inhibition of CDK9 suppresses transcription, leading to disruption of the pTEFb complex, and by extension, RNA PolII. CDK9 regulates cellular transcriptional elongation and mRNA maturation and has become an attractive therapeutic target for many cancers, especially those caused by dysregulation of transcription.

Due to the occurrence of resistance to current CDK-targeting drugs there is a great need for novel and potent drugs overcoming those issues.

It has therefore been the object of the present invention to provide novel CDK degraders which may be used for the treatment of diseases that are associated with CDK activity,

such as cancer and inflammation. Thus, the following invention preferably describes triple targeting PROTAC degraders against CDK4/6/9 with superior anticancer efficacies.

The present invention provides compounds of formula (I):



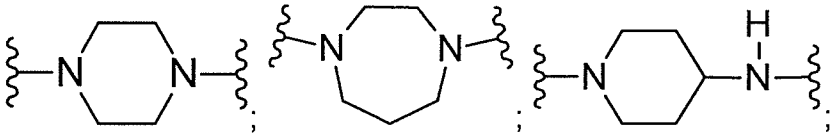
(I)

wherein

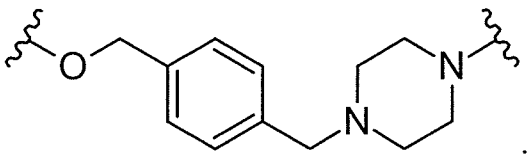
n is 1 or 2;

X is a C_{1-6} alkylene group, a C_{2-6} alkenylene group, a C_{1-6} heteroalkylene group, an optionally substituted C_{3-5} cycloalkylene group, or an optionally substituted aralkylene group;

Y is a NH group or is selected from the following groups:



; and



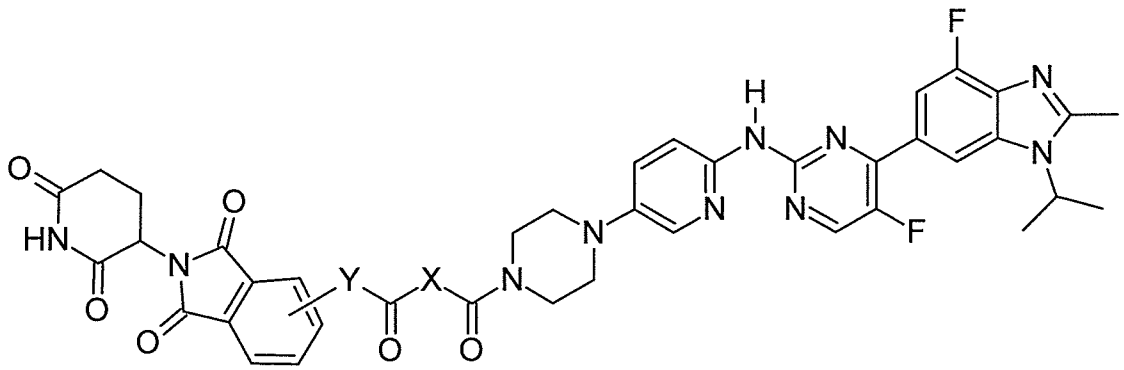
; and

Z is hydrogen or a methyl group;

or a salt thereof.

Preferably, Z is hydrogen.

The present invention further provides compounds of formula (Ia):

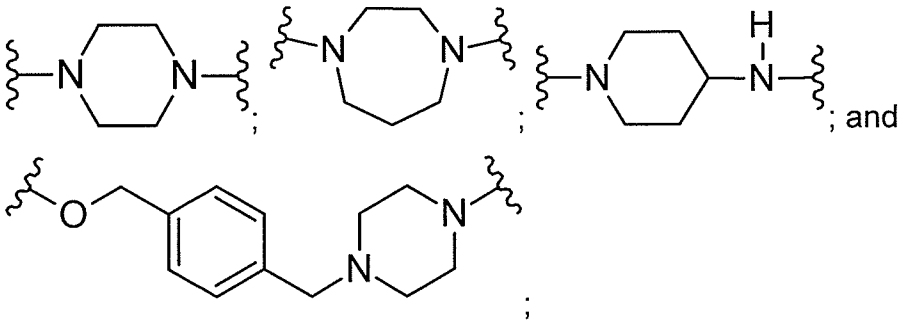


(Ia)

wherein

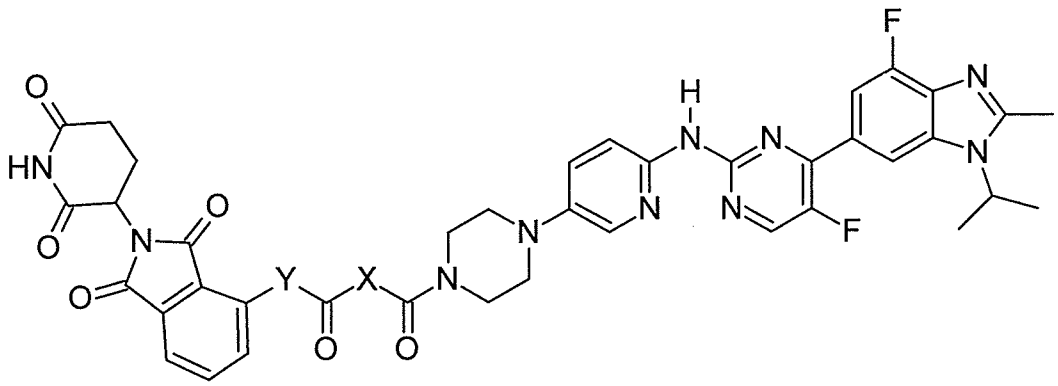
X is a C_{1-6} alkylene group, a C_{2-6} alkenylene group, a C_{1-6} heteroalkylene group, an optionally substituted C_{3-5} cycloalkylene group, or an optionally substituted aralkylene group; and

Y is a NH group or is selected from the following groups:



or a salt thereof.

The present invention moreover provides compounds of formula (II):

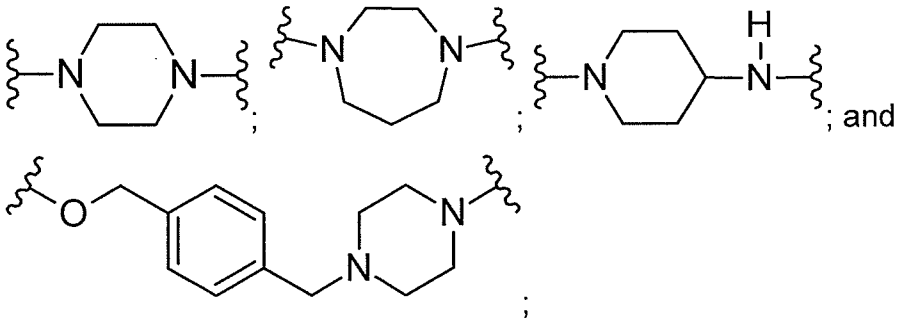


(II).

wherein

X is a C_{1-6} alkylene group, a C_{2-6} alkenylene group, a C_{1-6} heteroalkylene group, an optionally substituted C_{3-5} cycloalkylene group, or an optionally substituted aralkylene group; and

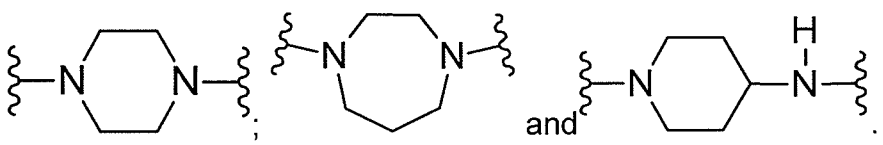
Y is a NH group or is selected from the following groups:



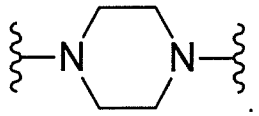
or a salt thereof.

Preferably, Y is a NH group.

Moreover preferably, Y is selected from the following groups:

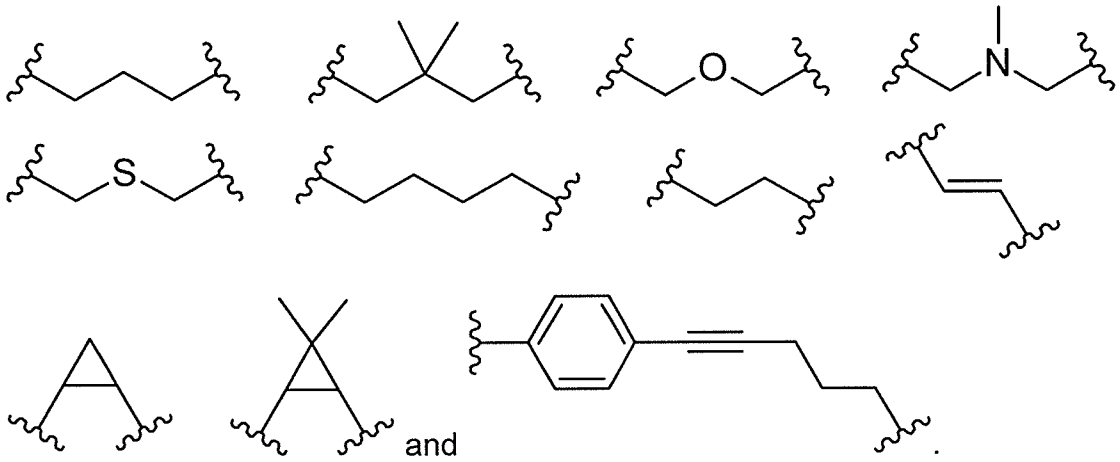


Further preferably, Y is the following group:



Moreover preferably, X is a C_{1-6} alkylene group or a C_{1-6} heteroalkylene group.

Further preferably, X is selected from the following groups:



Especially preferably, X is selected from the following groups:

The most preferred compounds of the present invention are the compounds disclosed in the examples, or a salt thereof.

It is further preferred to combine the preferred embodiments of the present invention in any desired manner (e.g., any embodiment for X may be combined with any embodiment of Y).

The expression alkyl refers to a saturated, straight-chain or branched hydrocarbon group that contains from 1 to 20 carbon atoms, preferably from 1 to 10 carbon atoms, especially from 1 to 6 (e.g. 1, 2, 3 or 4) carbon atoms, for example a methyl (Me, CH_3), CF_3, CD_3, ethyl (Et), *n*-propyl (nPr), *iso*-propyl (iPr), *n*-butyl (nBu), *iso*-butyl (iBu), *sec*-butyl (sBu), *tert*-butyl (tBu), *n*-pentyl, *iso*-pentyl, *n*-hexyl, 2,2-dimethylbutyl or *n*-octyl group.

The expression C_{1-6} alkyl refers to a saturated, straight-chain or branched hydrocarbon group that contains from 1 to 6 carbon atoms. The expression C_{1-4} alkyl refers to a saturated, straight-chain or branched hydrocarbon group that contains from 1 to 4 carbon atoms. Examples are a methyl, CF_3, CD_3, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *sec*-butyl or *tert*-butyl group.

The expressions alkenyl and alkynyl refer to at least partially unsaturated, straight-chain or branched hydrocarbon groups that contain from 2 to 20 carbon atoms, preferably from 2 to 10 carbon atoms, especially from 2 to 6 (e.g., 2, 3 or 4) carbon atoms, for example an ethenyl (vinyl), propenyl (allyl), iso-propenyl, butenyl, ethynyl (acetylenyl), propynyl (e.g., propargyl), butynyl, isoprenyl or hex-2-enyl group. Preferably, alkenyl groups have one or two (especially preferably one) double bond(s), and alkynyl groups have one or two (especially preferably one) triple bond(s). The expression C_{2-6} alkenyl refers to a straight-chain or branched hydrocarbon group that contains from 2 to 6 (e.g., 2, 3 or 4) carbon atoms and at least one double bond.

Furthermore, the terms alkyl, alkenyl and alkynyl refer to groups in which one or more hydrogen atoms have been replaced by a halogen atom (preferably F or Cl) such as, for example, a 2,2,2-trichloroethyl or a trifluoromethyl group.

The expression heteroalkyl refers to an alkyl, alkenyl or alkynyl group in which one or more (preferably 1 to 8; especially preferably 1, 2, 3 or 4) carbon atoms have been replaced by an oxygen, nitrogen, phosphorus, boron, selenium, silicon or sulfur atom (preferably by an oxygen, sulfur or nitrogen atom) or by a SO or a SO_2 group. The expression heteroalkyl furthermore refers to a carboxylic acid or to a group derived from a carboxylic acid, such as, for example, acyl, acylalkyl, alkoxycarbonyl, acyloxy, acyloxyalkyl, carboxyalkylamide or alkoxycarbonyloxy. Furthermore, the term heteroalkyl refers to groups in which one or more hydrogen atoms have been replaced by a halogen atom (preferably F or Cl).

Preferably, a heteroalkyl group contains from 1 to 12 carbon atoms and from 1 to 8 heteroatoms selected from oxygen, nitrogen and sulfur (especially oxygen and nitrogen). Especially preferably, a heteroalkyl group contains from 1 to 6 (e.g. 1, 2, 3 or 4) carbon

atoms and 1, 2, 3 or 4 (especially 1, 2 or 3) heteroatoms selected from oxygen, nitrogen and sulfur (especially oxygen and nitrogen). The term C_{1-6} heteroalkyl refers to a heteroalkyl group containing from 1 to 6 carbon atoms and 1, 2, 3 or 4 heteroatoms selected from O, S and/or N (especially O and/or N). The term C_{1-4} heteroalkyl refers to a heteroalkyl group containing from 1 to 4 carbon atoms and 1, 2 or 3 heteroatoms selected from O, S and/or N (especially O and/or N).

Examples of heteroalkyl groups are groups of formulae: R^a-O-Y^a-, R^a-S-Y^a-, R^a-SO-Y^a-, R^a-SO_2-Y^a-, R^a-N(R^b)-SO_2-Y^a-, R^a-SO_2-N(R^b)-Y^a-, R^a-N(R^b)-Y^a-, R^a-CO-Y^a-, R^a-O-CO-Y^a-, R^a-CO-O-Y^a-, R^a-CO-N(R^b)-Y^a-, R^a-N(R^b)-CO-Y^a-, R^a-O-CO-N(R^b)-Y^a-, R^a-N(R^b)-CO-O-Y^a-, -Y^a-CN, R^a-N(R^b)-CO-N(R^c)-Y^a-, R^a-O-CO-O-Y^a-, R^a-N(R^b)-C(=NR^d)-N(R^c)-Y^a-, R^a-CS-Y^a-, R^a-O-CS-Y^a-, R^a-CS-O-Y^a-, R^a-CS-N(R^b)-Y^a-, R^a-N(R^b)-CS-Y^a-, R^a-O-CS-N(R^b)-Y^a-, R^a-N(R^b)-CS-O-Y^a-, R^a-N(R^b)-CS-N(R^c)-Y^a-, R^a-O-CS-O-Y^a-, R^a-S-CO-Y^a-, R^a-CO-S-Y^a-, R^a-S-CO-N(R^b)-Y^a-, R^a-N(R^b)-CO-S-Y^a-, R^a-S-CO-O-Y^a-, R^a-O-CO-S-Y^a-, R^a-S-CO-S-Y^a-, R^a-S-CS-Y^a-, R^a-CS-S-Y^a-, R^a-S-CS-N(R^b)-Y^a-, R^a-N(R^b)-CS-S-Y^a-, R^a-S-CS-O-Y^a-, R^a-O-CS-S-Y^a-, wherein R^a being a hydrogen atom, a C_1-C_6 alkyl, a C_2-C_6 alkenyl or a C_2-C_6 alkynyl group; R^b being a hydrogen atom, a C_1-C_6 alkyl, a C_2-C_6 alkenyl or a C_2-C_6 alkynyl group; R^c being a hydrogen atom, a C_1-C_6 alkyl, a C_2-C_6 alkenyl or a C_2-C_6 alkynyl group; R^d being a hydrogen atom, a C_1-C_6 alkyl, a C_2-C_6 alkenyl or a C_2-C_6 alkynyl group and Y^a being a bond, a C_1-C_6 alkylene, a C_2-C_6 alkenylene or a C_2-C_6 alkynylene group, wherein each heteroalkyl group contains at least one carbon atom and one or more hydrogen atoms may be replaced by fluorine or chlorine atoms.

Specific examples of heteroalkyl groups are methoxy, trifluoromethoxy, ethoxy, *n*-propyloxy, *iso*-propyloxy, *n*-butoxy, *tert*-butyloxy, methoxymethyl, -CH_2CH_2OH, -CH_2OH, -SO_2Me, -NHAc, -OCD_3, -$C(CH_3)_2CN$, methoxyethyl, ethoxymethyl, 1-methoxyethyl, 1-ethoxyethyl, 2-methoxyethyl or 2-ethoxyethyl, methylamino, ethylamino, propylamino, isopropylamino, dimethylamino, diethylamino, isopropylethylamino, methylamino methyl, ethylamino methyl, diisopropylamino ethyl, methylthio, ethylthio, isopropylthio, enol ether, dimethylamino methyl, dimethylamino ethyl, acetyl, propionyl, butyryloxy, acetyloxy, methoxycarbonyl, ethoxycarbonyl, propionyloxy, acetylamino or propionylamino, carboxymethyl,

carboxyethyl or carboxypropyl, *N*-ethyl-*N*-methylcarbamoyl or *N*-methylcarbamoyl. Further examples of heteroalkyl groups are nitrile (-CN), isonitrile, cyanate, thiocyanate, isocyanate, isothiocyanate and alkylnitrile groups.

The expression cycloalkyl refers to a saturated or partially unsaturated (for example, a cycloalkenyl group) cyclic group that contains one or more rings (preferably 1 or 2), and contains from 3 to 14 ring carbon atoms, preferably from 3 to 10 (especially 3, 4, 5, 6 or 7) ring carbon atoms. The expression cycloalkyl refers furthermore to groups in which one or more hydrogen atoms have been replaced by fluorine, chlorine, bromine or iodine atoms or by OH, =O, SH, =S, NH_2, =NH, N_3 or NO_2 groups, thus, for example, cyclic ketones such as, for example, cyclohexanone, 2-cyclohexenone or cyclopentanone. Further specific examples of cycloalkyl groups are a cyclopropyl, cyclobutyl, cyclopentyl, spiro[4,5]decanyl, norbornyl, cyclohexyl, cyclopentenyl, cyclohexadienyl, decalinyl, bicyclo[4.3.0]nonyl, tetraline, cyclopentylcyclohexyl, fluorocyclohexyl or cyclohex-2-enyl group. Preferably, the expression cycloalkyl refers to a saturated cyclic group that contains one or more rings (preferably 1 or 2; especially preferably one), and contains from 3 to 14 ring carbon atoms, preferably from 3 to 10 (especially 3, 4, 5, 6 or 7) ring carbon atoms.

The expression heterocycloalkyl refers to a cycloalkyl group as defined above in which one or more (preferably 1, 2 or 3) ring carbon atoms have been replaced by an oxygen, nitrogen, silicon, selenium, phosphorus or sulfur atom (preferably by an oxygen, sulfur or nitrogen atom) or a SO group or a SO_2 group. A heterocycloalkyl group has preferably 1 or 2 ring(s) (especially preferably one) and 3 to 10 (especially 3, 4, 5, 6 or 7) ring atoms (preferably selected from C, O, N and S). The expression heterocycloalkyl refers furthermore to groups that are substituted by fluorine, chlorine, bromine or iodine atoms or by OH, =O, SH, =S, NH_2, =NH, N_3 or NO_2 groups. Examples are a piperidyl, prolinyl, imidazolidinyl, piperazinyl, morpholinyl (e.g. $-N(CH_2CH_2)_2O$), urotropinyl, pyrrolidinyl, tetrahydrothiophenyl, tetrahydropyranyl, tetrahydrofuryl or 2-pyrazolinyl group and also lactames, lactones, cyclic imides and cyclic anhydrides.

The expression alkylcycloalkyl refers to groups that contain both cycloalkyl and alkyl, alkenyl or alkynyl groups in accordance with the above definitions, for example

alkylcycloalkyl, cycloalkylalkyl, alkylcycloalkenyl, alkenylcycloalkyl and alkynylcycloalkyl groups. An alkylcycloalkyl group preferably contains a cycloalkyl group that contains one or two rings and from 3 to 10 (especially 3, 4, 5, 6 or 7) ring carbon atoms, and one or two alkyl, alkenyl or alkynyl groups (especially alkyl groups) having 1 or 2 to 6 carbon atoms.

The expression heteroalkylcycloalkyl refers to alkylcycloalkyl groups as defined above in which one or more (preferably 1, 2 or 3) carbon atoms have been replaced by an oxygen, nitrogen, silicon, selenium, phosphorus or sulfur atom (preferably by an oxygen, sulfur or nitrogen atom) or a SO group or a SO_2 group. A heteroalkylcycloalkyl group preferably contains 1 or 2 rings having from 3 to 10 (especially 3, 4, 5, 6 or 7) ring atoms, and one or two alkyl, alkenyl, alkynyl or heteroalkyl groups (especially alkyl or heteroalkyl groups) having from 1 or 2 to 6 carbon atoms. Examples of such groups are alkylheterocycloalkyl, alkylheterocycloalkenyl, alkenylheterocycloalkyl, alkynylheterocycloalkyl, heteroalkylcyclo-alkyl, heteroalkylheterocycloalkyl and heteroalkylheterocycloalkenyl, the cyclic groups being saturated or mono-, di- or tri-unsaturated.

The expression aryl refers to an aromatic group that contains one or more rings and from 6 to 14 ring carbon atoms, preferably from 6 to 10 (especially 6) ring carbon atoms. The expression aryl refers furthermore to groups that are substituted by fluorine, chlorine, bromine or iodine atoms or by OH, SH, NH_2, N_3 or NO_2 groups. Examples are the phenyl (Ph), naphthyl, biphenyl, 2-fluorophenyl, anilinyl, 3-nitrophenyl or 4-hydroxyphenyl group.

The expression heteroaryl refers to an aromatic group that contains one or more rings and from 5 to 14 ring atoms, preferably from 5 to 10 (especially 5 or 6 or 9 or 10) ring atoms, comprising one or more (preferably 1, 2, 3 or 4) oxygen, nitrogen, phosphorus or sulfur ring atoms (preferably O, S or N). The expression heteroaryl refers furthermore to groups that are substituted by fluorine, chlorine, bromine or iodine atoms or by OH, SH, N_3, NH_2 or NO_2 groups. Examples are pyridyl (e.g. 4-pyridyl), imidazolyl (e.g. 2-imidazolyl), phenylpyrrolyl (e.g. 3-phenylpyrrolyl), thiazolyl, isothiazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, oxadiazolyl, thiadiazolyl, indolyl, indazolyl, tetrazolyl, pyrazinyl, pyrimidinyl, pyridazinyl, 4-hydroxypyridyl (4-pyridonyl), 3,4-hydroxypyridyl (3,4-pyridonyl), oxazolyl, isoxazolyl, triazolyl, tetrazolyl, isoxazolyl, indazolyl, indolyl, benzimidazolyl, benzoxazolyl,

benzisoxazolyl, benzthiazolyl, pyridazinyl, quinolinyl, isoquinolinyl, pyrrolyl, purinyl, carbazolyl, acridinyl, pyrimidyl, 2,3′-bifuryl, pyrazolyl (e.g. 3-pyrazolyl) and isoquinolinyl groups.

The expression aralkyl refers to groups containing both aryl and also alkyl, alkenyl, alkynyl and/or cycloalkyl groups in accordance with the above definitions, such as, for example, arylalkyl, arylalkenyl, arylalkynyl, arylcycloalkyl, arylcycloalkenyl, alkylarylcycloalkyl and alkylarylcycloalkenyl groups. Specific examples of aralkyls are phenylcyclopentyl, cyclohexylphenyl as well as groups derived from toluene, xylene, mesitylene, styrene, benzyl chloride, *o*-fluorotoluene, 1*H*-indene, tetraline, dihydronaphthalene, indanone, cumene, fluorene and indane. An aralkyl group preferably contains one or two aromatic ring systems (especially 1 or 2 rings; especially preferably one ring), each containing from 6 to 10 carbon atoms and one or two alkyl, alkenyl and/or alkynyl groups containing from 1 or 2 to 6 carbon atoms and/or one or two cycloalkyl group containing 3, 4, 5, 6 or 7 ring carbon atoms.

The expression heteroaralkyl refers to groups containing both aryl and/or heteroaryl groups and also alkyl, alkenyl, alkynyl and/or heteroalkyl and/or cycloalkyl and/or heterocycloalkyl groups in accordance with the above definitions containing at least one heteroatom, which is preferably selected from N, O and S. A heteroaralkyl group preferably contains one or two aromatic ring systems (especially 1 or 2 rings; especially preferably one ring), each containing from 5 or 6 to 9 or 10 ring atoms (preferably selected from C, N, O and S) and one or two alkyl, alkenyl and/or alkynyl groups containing 1 or 2 to 6 carbon atoms and/or one or two heteroalkyl groups containing 1 to 6 carbon atoms and 1, 2 or 3 heteroatoms selected from O, S and N and/or one or two cycloalkyl groups each containing 3, 4, 5, 6 or 7 ring carbon atoms and/or one or two heterocycloalkyl groups, each containing 3, 4, 5, 6 or 7 ring atoms comprising 1, 2, 3 or 4 oxygen, sulfur or nitrogen atoms.

Examples are arylheteroalkyl, arylheterocycloalkyl, arylheterocycloalkenyl, arylalkylhetero-cycloalkyl, arylalkenylheterocycloalkyl, arylalkynylheterocycloalkyl, arylalkylheterocyclo-alkenyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heteroarylheteroalkyl, heteroarylcycloalkyl, heteroarylcycloalkenyl, heteroaryl-

heterocycloalkyl, heteroaryl-heterocycloalkenyl, heteroarylalkylcycloalkyl, heteroaryl-alkylheterocycloalkenyl, heteroaryl-heteroalkylcycloalkyl, heteroarylheteroalkyl-cycloalkenyl and heteroarylheteroalkyl-heterocycloalkyl groups, the cyclic groups being saturated or mono-, di- or tri-unsaturated. Specific examples are a tetrahydroisoquinolinyl, benzoyl, phthalidyl, 2- or 3-ethylindolyl, 4-methylpyridino, 2-, 3- or 4-methoxyphenyl, 4-ethoxyphenyl, 2-, 3- or 4-carboxyphenylalkyl group.

As already stated above, the expressions cycloalkyl, heterocycloalkyl, alkylcycloalkyl, heteroalkylcycloalkyl, aryl, heteroaryl, aralkyl and heteroaralkyl also refer to groups that are substituted by fluorine, chlorine, bromine or iodine atoms or by OH, =O, SH, =S, NH_2, =NH, N_3 or NO_2 groups, unless otherwise specified.

According to a preferred embodiment, all alkyl, alkenyl, alkynyl, heteroalkyl, cycloalkyl, heterocycloalkyl, alkylcycloalkyl, heteroalkylcycloalkyl, aryl, heteroaryl, aralkyl and heteroaralkyl groups described herein may optionally be substituted.

The term halogen refers to F, Cl, Br or I.

When an aryl, heteroaryl, cycloalkyl, alkylcycloalkyl, heteroalkylcycloalkyl, heterocycloalkyl, aralkyl or heteroaralkyl group contains more than one ring, these rings may be bonded to each other via a single or double bond, or these rings may be annulated, fused or bridged.

The suffix "-ene" like e.g. in "phenylene" refers to the corresponding divalent group.

The term "optionally substituted" refers to a group which is unsubstituted or substituted by one or more (especially by one, two or three; preferably by one or two) substituents.

If a group comprises more than one substituent, these substituents are independently selected, i.e. they may be the same or different.

If a group is substituted by a cyclic group, such as e.g., a cycloalkyl group or a heterocycloalkyl group, this cyclic group may be bonded to said group via a single or double bond or this cyclic group may be annulated or fused to said group.

Specific examples for substituents are fluorine, chlorine, bromine and iodine and OH, SH, NH_2, $-SO_3H$, $-SO_2NH_2$, C_{1-4} alkyl (e.g., methyl), C_{1-4} heteroalkyl, -COOH, -COOMe, -COMe (Ac), $-NHSO_2Me$, $-SO_2NMe_2$, $-CH_2NH_2$, -NHAc, $-SO_2Me$, $-CONH_2$, -CN, $-NHCONH_2$, $-NHC(NH)NH_2$, $-NOHCH_3$, $-N_3$ and $-NO_2$ groups.

Further examples of substituents are C_1-C_{10} alkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_1-C_{10} heteroalkyl, C_3-C_{18} cycloalkyl, C_1-C_{17} heterocycloalkyl, C_4-C_{20} alkylcycloalkyl, C_1-C_{19} heteroalkylcycloalkyl, C_6-C_{18} aryl, C_1-C_{17} heteroaryl, C_7-C_{20} aralkyl and C_1-C_{19} heteroaralkyl groups; especially C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 heteroalkyl, C_3-C_{10} cycloalkyl, C_1-C_9 heterocycloalkyl, C_4-C_{12} alkylcycloalkyl, C_1-C_{11} heteroalkylcycloalkyl, C_6-C_{10} aryl, C_1-C_9 heteroaryl, C_7-C_{12} aralkyl and C_1-C_{11} heteroaralkyl groups, further preferably C_1-C_6 alkyl and C_1-C_6 heteroalkyl groups.

The therapeutic use of compounds according to formula (I), (Ia) or (II), their pharmacologically acceptable salts, solvates and hydrates, as well as formulations and pharmaceutical compositions also lie within the scope of the present invention.

The present invention further provides pharmaceutical compositions comprising one or more compounds of formula (I), (Ia) or (II) or a salt thereof as defined herein or a pharmaceutically acceptable ester, prodrug, hydrate or solvate thereof, optionally in combination with a pharmaceutically acceptable carrier and/or adjuvant.

It is a further object of the present invention to provide a compound of formula (I), (Ia) or (II) as defined herein or a pharmaceutical composition as defined herein for the preparation of a medicament for the treatment of one or more diseases specified herein.

Preferably the compounds of the present invention may be used for the treatment and/or prevention of diseases that are associated with CDK activity, such as cancer and inflammation.

A therapeutically effective amount of a compound in accordance with this invention means an amount of compound that is effective to prevent, alleviate or ameliorate symptoms of a disease or prolong the survival of the subject being treated. Determination of a therapeutically effective amount is within the skill in the art.

The therapeutically effective amount or dosage of a compound according to this invention can vary within wide limits and may be determined in a manner known in the art. Such dosage may be adjusted to the individual requirements in each particular case including the specific compound being administered, the route of administration, the condition being treated, as well as the patient being treated.

The salt of a compound of formula (I), (Ia) or (II) is preferably a pharmacologically acceptable salt. Examples of pharmacologically acceptable salts of sufficiently basic compounds of formula (I), (Ia) or (II) are salts of physiologically acceptable mineral acids like hydrochloric, hydrobromic, sulfuric and phosphoric acid; or salts of organic acids like methanesulfonic, p-toluenesulfonic, lactic, acetic, trifluoroacetic, citric, succinic, fumaric, maleic and salicylic acid. Further, a sufficiently acidic compound of formula (I), (Ia) or (II) may form alkali or earth alkali metal salts, for example sodium, potassium, lithium, calcium or magnesium salts; ammonium salts; or organic base salts, for example methylamine, dimethylamine, trimethylamine, triethylamine, ethylenediamine, ethanolamine, choline hydroxide, meglumin, piperidine, morpholine, tris-(2-hydroxyethyl)amine, lysine or arginine salts; all of which are also further examples of salts of formula (I), (Ia) or (II).

Compounds of formula (I), (Ia) or (II) may be solvated, especially hydrated. The hydratization/hydration may occur during the process of production or as a consequence of the hygroscopic nature of the initially water free compounds of formula (I), (Ia) or (II). The solvates and/or hydrates may e.g. be present in solid or liquid form.

It should be appreciated that certain compounds of formula (I), (Ia) or (II) may have tautomeric forms from which only one might be specifically mentioned or depicted in the following description, different geometrical isomers (which are usually denoted as cis/trans isomers or more generally as (E) and (Z) isomers) or different optical isomers

as a result of one or more chiral carbon atoms (which are usually nomenclatured under the Cahn-Ingold-Prelog or R/S system). All these tautomeric forms, geometrical or optical isomers (as well as racemates and diastereomers) and polymorphous forms are included in the invention. Since the compounds of formula (I), (Ia) or (II) may contain asymmetric C-atoms, they may be present either as achiral compounds, mixtures of diastereomers, mixtures of enantiomers or as optically pure compounds. The present invention comprises both all pure enantiomers and all pure diastereomers, and also the mixtures thereof in any mixing ratio.

According to a further embodiment of the present invention, one or more hydrogen atoms of the compounds of the present invention may be replaced by deuterium. Deuterium modification improves the metabolic properties of a drug with little or no change in its intrinsic pharmacology. Deuterium substitution at specific molecular positions improves metabolic stability, reduces formation of toxic metabolites and/or increases the formation of desired active metabolites. Accordingly, the present invention also encompasses the partially and fully deuterated compounds of formula (I), (Ia) or (II). The term hydrogen also encompasses deuterium.

The present invention also relates to pro-drugs which are composed of a compound of formula (I), (Ia) or (II) and at least one pharmacologically acceptable protective group which will be cleaved off under physiological conditions, such as an alkoxy-, arylalkyloxy-, acyl-, acyloxymethyl group (e.g. pivaloyloxymethyl), an 2-alkyl-, 2-aryl- or 2-arylalkyl-oxycarbonyl-2-alkylidene ethyl group or an acyloxy group as defined herein, e.g. ethoxy, benzyloxy, acetyl or acetyloxy or, especially for a compound of formula (I), (Ia) or (II), carrying a hydroxy group (-OH): a sulfate, a phosphate ($-OPO_3$ or $-OCH_2OPO_3$) or an ester of an amino acid.

As used herein, the term pharmaceutically acceptable ester especially refers to esters which hydrolyze in vivo and include those that break down readily in the human body to leave the parent compound or a salt thereof. Suitable ester groups include, for example, those derived from pharmaceutically acceptable aliphatic carboxylic acids, particularly alkanoic, alkenoic, cycloalkanoic and alkanedioic acids, in which each alkyl or alkenyl moiety advantageously has not more than 6 carbon atoms. Examples of particular esters

include, but are not limited to, formates, acetates, propionates, butyrates, acrylates and ethylsuccinates.

As mentioned above, therapeutically useful agents that contain compounds of formula (I), (Ia) or (II), their solvates, salts or formulations are also comprised in the scope of the present invention. In general, compounds of formula (I), (Ia) or (II) will be administered by using the known and acceptable modes known in the art, either alone or in combination with any other therapeutic agent.

For oral administration such therapeutically useful agents can be administered by one of the following routes: oral, e.g. as tablets, dragees, coated tablets, pills, semisolids, soft or hard capsules, for example soft and hard gelatine capsules, aqueous or oily solutions, emulsions, suspensions or syrups, parenteral including intravenous, intramuscular and subcutaneous injection, e.g. as an injectable solution or suspension, rectal as suppositories, by inhalation or insufflation, e.g. as a powder formulation, as microcrystals or as a spray (e.g. liquid aerosol), transdermal, for example via an transdermal delivery system (TDS) such as a plaster containing the active ingredient or intranasal. For the production of such tablets, pills, semisolids, coated tablets, dragees and hard, e.g. gelatine, capsules the therapeutically useful product may be mixed with pharmaceutically inert, inorganic or organic excipients as are e.g. lactose, sucrose, glucose, gelatine, malt, silica gel, starch or derivatives thereof, talc, stearinic acid or their salts, dried skim milk, and the like. For the production of soft capsules, one may use excipients as are e.g. vegetable, petroleum, animal or synthetic oils, wax, fat, polyols. For the production of liquid solutions, emulsions or suspensions or syrups one may use as excipients e.g. water, alcohols, aqueous saline, aqueous dextrose, polyols, glycerin, lipids, phospholipids, cyclodextrins, vegetable, petroleum, animal or synthetic oils. Especially preferred are lipids and more preferred are phospholipids (preferred of natural origin; especially preferred with a particle size between 300 to 350 nm) preferred in phosphate buffered saline (pH = 7 to 8, preferred 7.4). For suppositories one may use excipients as are e.g. vegetable, petroleum, animal or synthetic oils, wax, fat and polyols. For aerosol formulations one may use compressed gases suitable for this purpose, as are e.g. oxygen, nitrogen and carbon dioxide. The pharmaceutically useful agents may also contain additives for conservation, stabilization, e.g. UV stabilizers, emulsifiers,

sweetener, aromatizers, salts to change the osmotic pressure, buffers, coating additives and antioxidants.

In general, in the case of oral or parenteral administration to adult humans weighing approximately 80 kg, a daily dosage of about 1 mg to about 10,000 mg, preferably from about 10 mg to about 1,000 mg, should be appropriate, although the upper limit may be exceeded when indicated. The daily dosage can be administered as a single dose or in divided doses, or for parenteral administration, it may be given as continuous infusion or subcutaneous injection.

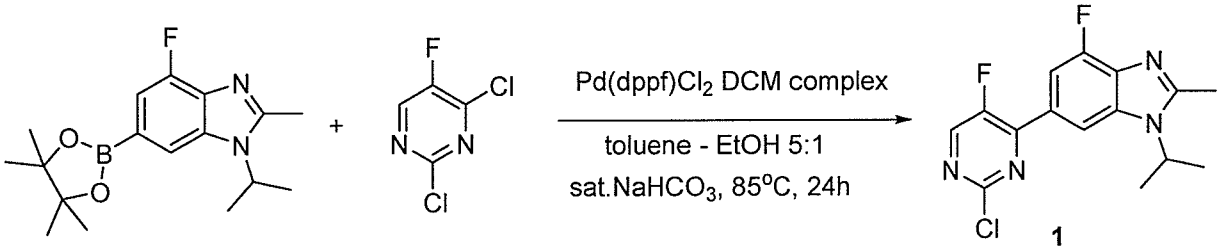
According to a moreover preferred embodiment, the present invention provides a method for treating one or more diseases specified herein which comprises administering to a subject in need of such treatment a therapeutically effective amount of a compound of formula (I), (Ia) or (II), or a pharmaceutically acceptable salt or solvate thereof.

According to a further preferred embodiment, the present invention provides a method for treating one or more diseases specified herein which comprises administering to a subject in need of such treatment a pharmaceutical composition comprising a compound of formula (I), (Ia) or (II), or a pharmaceutically acceptable salt or solvate thereof.

EXAMPLES

A. Synthesis of the compounds

Example 1: Synthesis of 6-(2-chloro-5-fluoropyrimidin-4-yl)-4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazole (1)



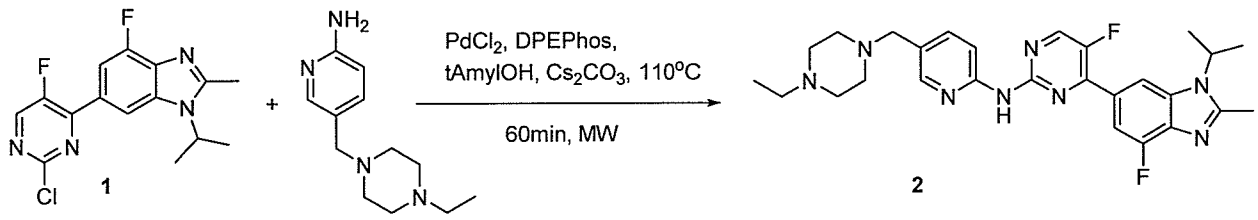
In a 3-neck round bottom flask 2,4-dichloro-5-fluoropyrimidine (1.1 equiv, 22 mmol, 3.6 g) was dissolved in a 5:1 mixture toluene: EtOH (30ml : 6ml) and then a saturated solution of $NaHCO_3$ (30 ml) and 4-fluoro-1-isopropyl-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-benzo[d]imidazole (1 equiv, 20 mmol, 6.3 g) were added. The white suspension was degassed by nitrogen for 30 min under vigorous stirring. Then $Pd(dppf)Cl_2 \cdot DCM$ complex (0.004 equiv., 0.08 mmol) was added, and the reaction mixture was heating overnight at 85°C under N_2 flow. The next day, the reaction mixture was allowed to reach rt and then H_2O was added, and the reaction mixture was extracted with EtOAc (x3). The combined organic phases were washed with Brine (x3), dried over $MgS0_4$ and the solvents were removed under vacuum to obtain a brown solid. The residue was purified by column chromatography (DCM – MeOH, 0 – 5% MeOH in DCM) to obtain the target compound.

20mmol scale: 6.3 g, 19.5 mmol, yield 98%, white solid.

^1H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, J = 3.4 Hz, 1H), 8.16 (d, J = 1.4 Hz, 1H), 7.80 (d, J = 11.4 Hz, 1H), 4.75 (hept, J = 7.0 Hz, 1H), 2.70 (s, 3H), 1.70 (d, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.6 (d, *J* = 3.5 Hz), 154.8 (d, *J* = 266.3 Hz), 154.2, 153.9 (dd, *J* = 8.5, 2.5 Hz), 153.2 (d, *J* = 252.9 Hz), 148.5, 148.3, 136.6 (d, *J* = 9.3 Hz), 134.7 (d, *J* = 17.4 Hz), 125.5 (dd, *J* = 7.4, 5.8 Hz), 108.8 (dd, *J* = 8.6, 3.4 Hz), 108.2 (dd, *J* = 20.5, 7.9 Hz), 48.7, 21.5, 15.2. HRMS (ESI): m/z calcd for $C_{15}H_{14}N_4ClF_2$ $[M+H]^+$: 323.08696; found 323.08698.

31.4 mmol scale: 8.4 g, 26.1 mmol, yield 83%, white solid. ^1H NMR (500 MHz, $CDCl_3$) δ 8.50 (d, J = 3.4 Hz, 1H), 8.15 (s, 1H), 7.77 (d, J = 11.5 Hz, 1H), 4.75 (hept, J = 7.0 Hz, 1H), 2.69 (s, 3H), 1.70 (d, J = 7.0 Hz, 6H).

Example 2: Synthesis of N-(5-((4-ethylpiperazin-1-yl) methyl) pyridin-2-yl)-5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-amine [abemaciclib] (2)

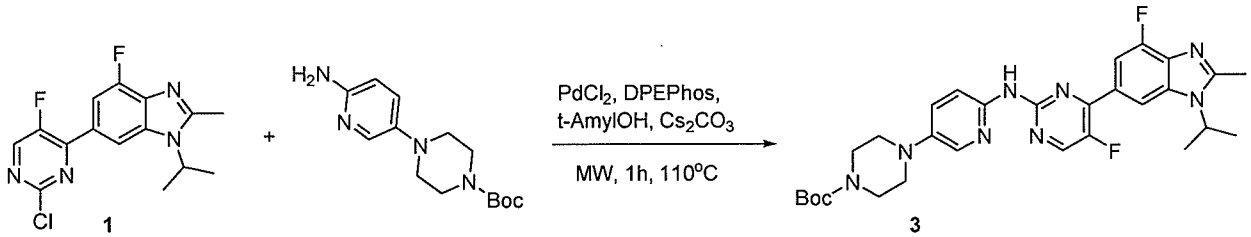


In a microwave vial 6-(2-chloro-5-fluoropyrimidin-4-yl)-4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazole **(1)** (1 equiv.), 5-((4-ethylpiperazin-1-yl)methyl)pyridin-2-amine (1.25

equiv.) and Cs_2CO_3 (2.5 equiv.) were suspended in *t*-AmylOH (0.2 M). The reaction mixture was degassed by nitrogen for 10 min under vigorous stirring and then DPEPhos (0.04 equiv.) and $PdCl_2$ (0.02 equiv.) were added. The microwave vial was sealed, and it was stirred for 10 min at room temperature under N_2. The reaction mixture was subjected to microwave irradiation for 1 h at 110°C. Then, the dark brown reaction mixture was filtered over silica under vacuum with DCM. The solvent was removed, and the obtained residue was purified by column chromatography DCM – MeOH – NH_3 (85: 10: 5) to obtain the pure product.

1 mmol scale: 300mg, 0.6 mmol, yield 60%, yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.00 (s, 1H), 8.48 (d, *J* = 3.8 Hz, 1H), 8.40 (d, *J* = 8.6 Hz, 1H), 8.32 (d, *J* = 2.0 Hz, 1H), 8.18 (d, *J* = 1.1 Hz, 1H), 7.78 (d, *J* = 11.5 Hz, 1H), 7.68 (dd, *J* = 8.6, 2.2 Hz, 1H), 4.71 (hept, *J* = 7.0 Hz, 1H), 3.49 (s, 2H), 2.68 (s, 3H), 2.58 – 2.47 (b, 8H),2.43 (q, *J* = 7.0 Hz, 2H), 1.70 (d, *J* = 7.0 Hz, 6H), 1.09 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.3 (d, *J* = 2.8 Hz), 153.5, 153.2 (d, *J* = 252.0 Hz), 152.1, 150.9 (d, *J* = 255.8 Hz), 151.30 (dd, *J* = 8.2, 2.2 Hz), 148.7, 147.2 (d, *J* = 27.2 Hz), 139.0, 136.4 (d, *J* = 9.3 Hz), 134.0 (d, *J* = 17.2 Hz), 127.4 (dd, *J* = 7.4, 5.8 Hz), 127.0, 111.4, 108.7 (dd, *J* = 8.5, 3.4 Hz), 108.1 (dd, *J* = 20.1, 7.3 Hz), 59.7, 52.7, 52.6, 52.2, 48.6 , 21.4, 15.0, 11.8. HRMS (ESI): m/z calcd for $C_{27}H_{33}N_8F_2$ $[M+H]^+$: 507.27908; found 507.27872

Example 3: Synthesis of *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate (3)



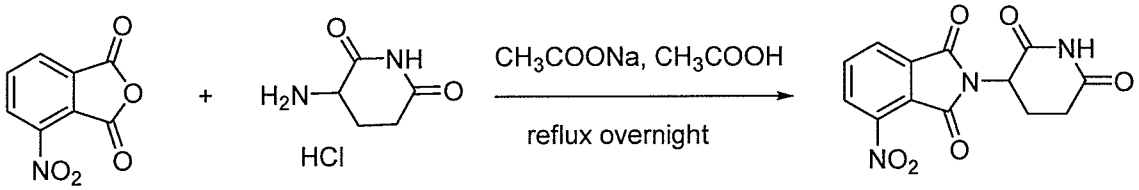
In a microwave vial 6-(2-chloro-5-fluoropyrimidin-4-yl)-4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazole **(1)** (1 equiv.), *tert*-butyl 4-(6-aminopyridin-3-yl)piperazine-1-carboxylate (1.25 equiv.) and Cs_2CO_3 (2.5 equiv.) were suspended in *t*-AmylOH (0.2 M). The reaction mixture was degassed by nitrogen for 10 min under vigorous stirring and then DPEPhos (0.04 equiv.) and $PdCl_2$ (0.02 equiv.) were added. The microwave vial was sealed, and it was stirred for 10min at room temperature under N_2. The reaction mixture was subjected to microwave irradiation for 1h at 110°C. Then, the dark brown reaction

mixture was filtered over silica under vacuum with DCM. Solvents were removed under reduced pressure and the crude was purified by column chromatography with (DCM - MeOH, 0 - 5% MeOH in DCM).

1 mmol scale: 312mg, 0.55 mmol, yield 55%, yellow solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 (d, *J* = 3.8 Hz, 1H), 8.31 (d, *J* = 9.0 Hz, 1H), 8.17 (s, 1H), 8.04 – 8.03 (m, 2H), 7.79 (d, *J* = 11.6 Hz, 1H), 7.35 (dd, *J* = 9.0, 3.0 Hz, 1H), 4.73 (hept, *J* = 7.0 Hz, 1H), 3.62 – 3.60 (m, 4H), 3.10 – 3.08 (m, 4H), 2.69 (s, 3H), 1.71 (d, *J* = 7.0 Hz, 6H), 1.49 (s, 9H).^{13}C NMR (126 MHz, $CDCl_3$) δ 155.4 (d, *J* = 2.8 Hz), 154.6, 153.5, 153.2 (d, *J* = 251.9 Hz), 151.3 (dd, *J* = 8.2, 1.8 Hz),150.7 (d, *J* = 255.8 Hz), 147.1 (d, *J* = 27.1 Hz), 146.9, 142.8, 137.4, 136.3 (d, *J* = 9.2 Hz), 133.9 (d, *J* = 17.1 Hz), 127.5 (dd, *J* = 7.4, 5.8 Hz), 127.1, 112.3, 108.7 (dd, *J* = 8.9, 3.1 Hz), 108.0 (dd, *J* = 20.0, 6.9 Hz), 80.0, 50.0, 48.6, 28.4, 21.4, 15.0. HRMS (ESI): m/z calcd for $C_{29}H_{35}O_2N_8F_2[M+H]^+$: 565.28456; found 565.28442.

Example 4: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-nitroisoindoline-1,3-dione (4)

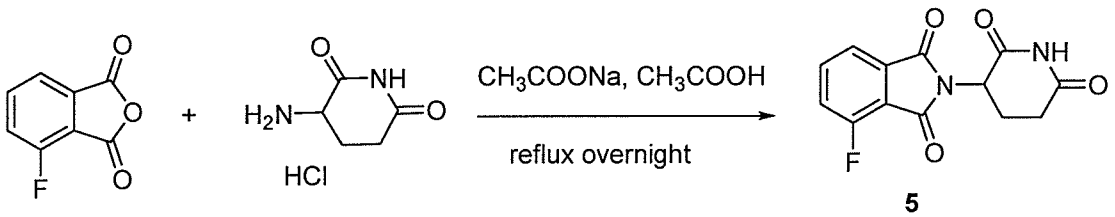


4

In a round-bottom flask, 3-nitrophthalic anhydride (1 equiv.), 3-aminopiperidine-2,6-dione hydrochloride (1 equiv.) and sodium acetate (1.2 equiv.) were mixed in AcOH (20 ml for 5 mmol scale). The resulting mixture was heated at 120°C overnight. After cooling to room temperature, most of the AcOH was removed under reduced pressure and the residue was dissolved in water, filtered, washed with water, and dried with vacuum to obtain the crude compound. The crude product was used directly in the next step.

10 mmol scale: 2.7 g, 8.9 mmol, yield 90%, purple solid.^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 11.20 (s, 1H), 8.36 (dd, J = 8.1, 0.7 Hz, 1H), 8.25 (dd, J = 7.5, 0.5 Hz, 1H), 8.14 – 8.11 (m, 1H), 5.21 (dd, J = 12.9, 5.4 Hz, 1H), 2.90 (ddd, J = 17.3, 14.0, 5.4 Hz, 1H), 2.64 – 2.61 (m, 1H), 2.56– 2.52 (m, 1H), 2.11 -2.06 (m, 1H).^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 172.8, 169.6, 165.3, 162.6, 144.5, 136.9, 133.1, 129.0, 127.4, 122.6, 49.5, 30.9, 21.8.

Example 5: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (5)

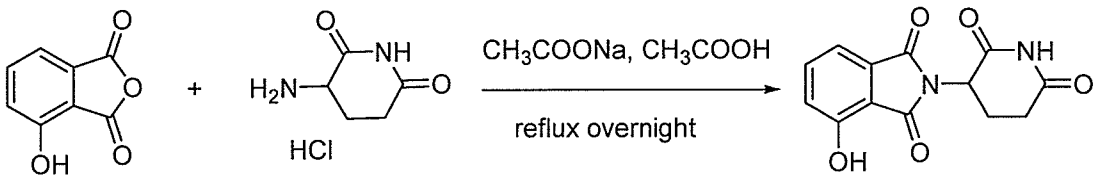


5

In a round-bottom flask, 3-fluorophthalic anhydride (1 equiv.), 3-aminopiperidine-2,6-dione hydrochloride (1 equiv.) and sodium acetate (1.2 equiv.) were mixed in AcOH (20 ml for 5mmol scale). The resulting mixture was heated at 120°C overnight. After cooling to room temperature, most of the AcOH was removed under reduced pressure and the residue was dissolved in water, filtered, washed with water, and dried with vacuum to obtain the crude compound. The crude product was purified by column chromatography (DCM – MeOH, 0 – 5% MeOH in DCM).

5 mmol scale: 1.2 g, 4.3 mmol, yield 85%, white solid. ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 11.16 (s, 1H), 7.96 – 7.92 (m, 1H), 7.79 (d, J = 7.3 Hz, 1H), 7.75 – 7.7.2 (m, 1H), 5.16 (dd, J = 13.0, 5.4 Hz, 1H), 2.91 – 2.85 (m, 1H), 2.63 – 2.58 (m, 1H), 2.53 – 2.51 (m, 1H), 2.07 – 2.04 (m, 1H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 172.8, 169.7, 166.1, 164.0, 156.8 (d, J = 262.3 Hz), 138.0 (d, J = 7.9 Hz), 133.5, 123.0 (d, J = 19.6 Hz), 120.1 (d, J = 3.2 Hz), 117.0 (d, J = 12.6 Hz), 49.1, 30.9, 21.9. HRMS (ESI): m/z calcd for $C_{13}H_{10}O_4N_2F$ $[M+H]^+$: 277.06191; found 277.06196.

Example 6: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (6)

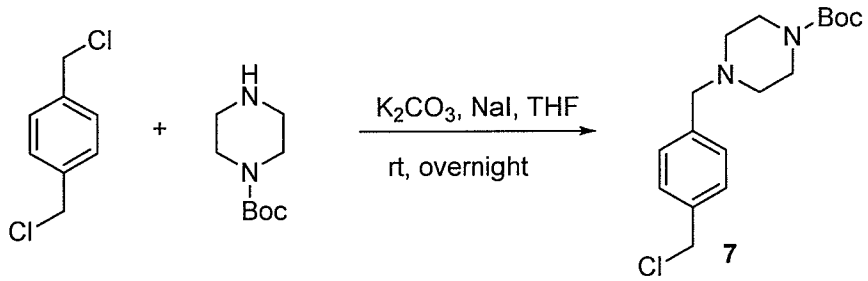


6

In a round-bottom flask, 3-hydroxyphthalic anhydride (1 equiv.), 3-aminopiperidine-2,6-dione hydrochloride (1 equiv.) and sodium acetate (1.2 equiv.) were mixed in AcOH (20 ml for 5 mmol scale). The resulting mixture was heated at 120°C overnight. After cooling to room temperature, most of the AcOH was removed under reduced pressure and the residue was dissolved in water, filtered, washed with water, and dried with vacuum to obtain the crude compound. The crude product was purified by column chromatography (DCM – MeOH, 0 – 5% MeOH in DCM).

20 mmol scale: 4.9 g, 18 mmol, yield 90%, white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 7.65 (dd, J = 8.3, 7.3 Hz, 1H), 7.32 (d, J = 7.1 Hz, 1H), 7.25 (d, J = 8.3 Hz, 1H), 5.07 (dd, J = 12.8, 5.4 Hz, 1H), 2.88 (ddd, J = 17.0, 13.9, 5.4 Hz, 1H), 2.60 – 2.50 (m, 2H), 2.02 (ddd, J = 10.4, 5.4, 3.1 Hz, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 172.9, 170.1, 167.1, 165.9, 155.5, 136.4, 133.2, 123.6, 114.4, 114.32, 48.7, 31.0, 22.1.

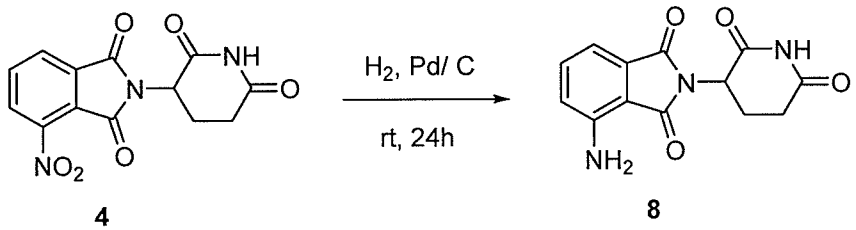
Example 7: Synthesis of *tert*-butyl 4-(4-(chloromethyl)benzyl)piperazine-1-carboxylate (7)



1,4-bis(chloromethyl)benzene (2 equiv.) was dissolved at 0°C in dry THF (10 ml). Potassium carbonate (2 equiv.), sodium iodide (1 equiv.) and *tert*-butyl piperazine-1-carboxylate (1 equiv.) were added as solids. The reaction mixture was stirred at 0°C for 30min and then rt overnight. Then, the reaction mixture was diluted with DCM and washed with H_2O (x3). The organic phase was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

2 mmol scale: 347 mg, 1.04 mmol, yield 52 %, white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.30 (m, 4H), 4.58 (s, 2H), 3.50 (s, 2H), 3.43 – 3.41 (m, 4H), 2.38 – 2.36 (m, 4H), 1.45 (s, 9H).^{13}C NMR (126 MHz, $CDCl_3$) δ 154.7, 138.3, 136.3, 129.4, 128.5, 79.5, 62.6, 52.8, 46.0, 28.4.HRMS (ESI): m/z calcd for $C_{17}H_{26}O_2N_2Cl$ $[M+H]^+$: 325.16773; found 325.16779.

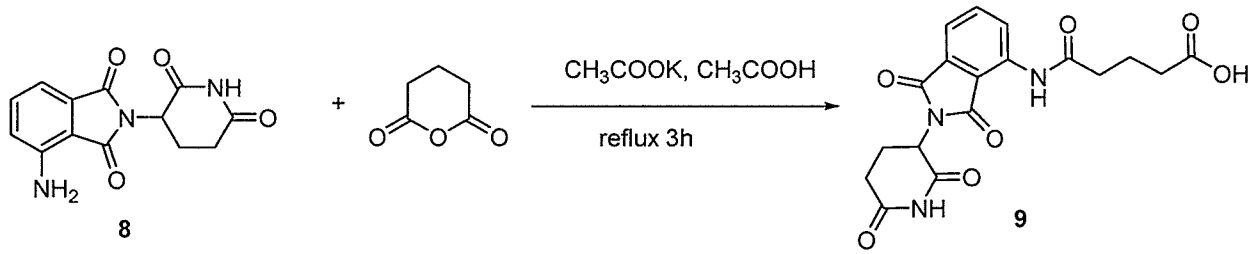
Example 8: Synthesis of 4-amino-2-(2,6-dioxopiperidin-3-yl) isoindoline-1,3-dione (8)



To a solution of 2-(2,6-dioxopiperidin-3-yl)-4-nitroisoindoline-1,3-dione **(4)** (8 mmol, 1.0 equiv.) in dry DMF (50 ml) was added Pd/C (1.6 mmol, 0.2 equiv.) under N_2. The reaction mixture was hydrogenated with 3.0 atm H_2 pressure at room temperature for 4 h. The progress of reaction was monitored by TLC. The reaction mixture was filtered over a pad of celite. The filtrate was diluted with EtOAc and the organic phase was washed with H_2O and Brine (x3) and was dried over $MgSO_4$. The solvent was removed under reduced pressure, to obtain a solid, which was used directly in the next step.

8 mmol scale: 2.0 g, 7.3 mmol, yield 92%, yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1H), 7.46 (dd, J = 8.4, 7.0 Hz, 1H), 7.02 – 6.99 (m, 2H), 6.52 (b, 2H), 5.04 (dd, J = 12.7, 5.4 Hz, 1H), 2.88 (ddd, J = 17.0, 13.9, 5.5 Hz, 1H), 2.54 – 2.50 (m, 1H), 2.04 – 2.00 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.9, 170.2, 168.6, 167.4, 146.8, 135.5, 132.0, 121.7, 111.0, 108.5, 48.5, 31.01, 22.2.

Example 9: Synthesis of 5-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl) amino)-5-oxopentanoic acid (9)



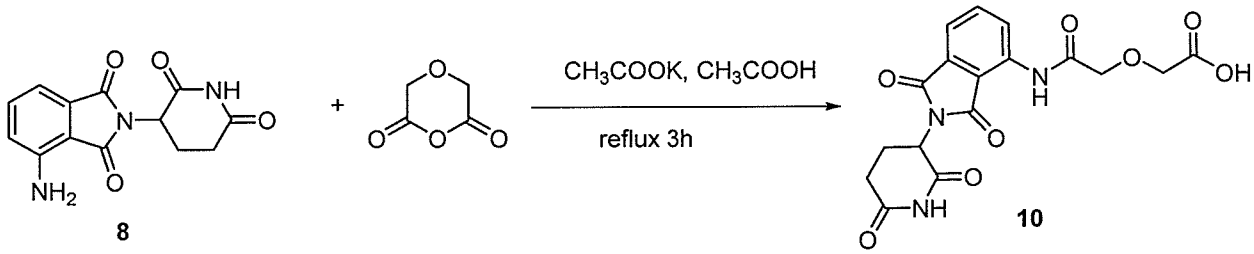
8 9

A mixture of 4-amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione **(8)** (7.3 mmol, 1.0 equiv), potassium acetate (29.3 mmol, 4.0 equiv.) and glutaric anhydride (29.23 mmol, 4.0 equiv.) in glacial AcOH (60 ml) was heated at reflux under nitrogen for 3h. After cooling to room temperature, acetic acid was removed under reduced pressure and the residue was extracted (EtOAc – H_2O). The organic phases were dried with $MgSO_4$, and solvents were removed under reduced pressure. The crude product was purified by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

7.3 mmol scale: 904 mg, 2.4 mmol, yield 32%, white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.14 (s, 1H), 9.73 (s, 1H), 8.43 (d, J = 8.3 Hz, 1H), 7.84 – 7.81 (m, 1H), 7.61 (d, J = 7.3 Hz, 1H), 5.14 (dd, J = 12.9, 5.4 Hz, 1H), 2.92 – 2.85 (m, 1H), 2.62 – 2.50 (m, 4H), 2.30 (t, J = 7.3 Hz, 2H), 2.08 – 2.05 (m, 1H), 1.86 – 1.80 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.0, 172.7, 171.5, 169.7, 167.5, 166.6, 136.3, 136.0, 131.4, 126.5, 118.3,

117.2, 48.8, 35.5, 32.7, 30.7, 21.9, 20.1. HRMS (ESI): m/z calcd for $C_{22}H_{25}O_7N_4$ $[M+H]^+$: 457.17178; found 457.17175.

Example 10: Synthesis of 2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl) amino)-2-oxoethoxy) acetic acid (10)

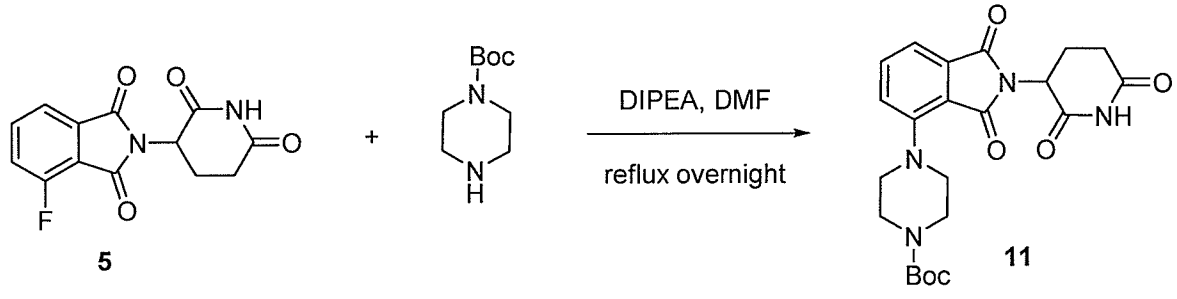


8 10

A mixture of 4-amino-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione **(8)** (7.3 mmol, 1.0 equiv), potassium acetate (29.3 mmol, 4.0 equiv.) and 1,4-dioxane-2,6-dione (29.23 mmol, 4.0 equiv.) in glacial AcOH (60 ml) was heated at reflux under nitrogen for 3h. After cooling to room temperature, acetic acid was removed under reduced pressure and the residue was extracted (EtOAc – H_2O). The organic phases were dried with $MgSO_4$, and solvents were removed under reduced pressure. The crude product was purified by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

7.3mmol scale: 850 mg, 2.2 mmol, yield 30%, white solid. ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 11.15 (s, 1H), 10.38 (s, 1H), 8.71 (d, J = 8.4 Hz, 1H), 7.87 (t, J = 7.9 Hz, 1H), 7.64 (d, J = 7.3 Hz, 1H), 5.16 (dd, J = 12.9, 5.4 Hz, 1H), 4.29 (s, 2H), 4.26 (s, 2H), 2.91 – 2.85 (m, 1H), 2.63 – 2.50 (m, 2H), 2.08 – 2.05 (m, 1H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 172.8, 171.1, 169.8, 168.9, 168.1, 166.7, 136.6, 135.9, 131.4, 124.5, 118.3, 116.2, 70.1, 67.9, 49.0, 31.0, 22.0. HRMS (ESI): m/z calcd for $C_{17}H_{16}O_8N_3$ $[M+H]^+$: 390.0932; found 390.0932.

Example 11: Synthesis of *tert*-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl) piperazine-1-carboxylate (11)

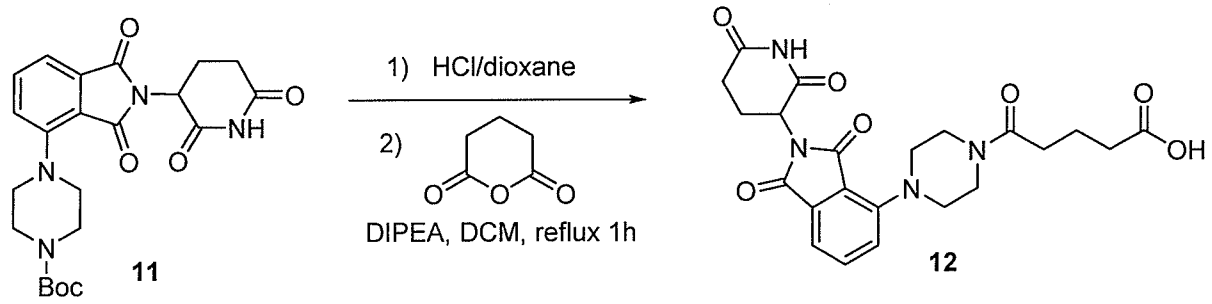


5 11

1-Boc-piperazine (11 mmol, 1.1 equiv.) was added to a stirred solution of 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione **(5)** (10 mmol, 1 equiv) and DIPEA (20 mmol, 2 equiv.) in DMF (1M). The reaction mixture was heated at reflux overnight. Then the mixture was cooled to room temperature, and it was diluted with DCM (100 ml) and was washed with H_2O (100ml x 3). The organic phase was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The residue was purified with column chromatography DCM – MeOH, 0 – 5% MeOH in DCM).

10 mmol scale: 3.1 g, 7 mmol, yield 70 %, yellow solid . ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (b, 1H), 7.61 (dd, *J* = 8.3, 7.3 Hz, 1H), 7.43 (d, *J* = 7.1 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 1H), 4.96 (dd, *J* = 12.4, 5.4 Hz, 1H), 3.66 – 3.64 (m, 4H), 3.30 – 3.26 (m, 4H), 2.95 – 2.93 (m, 1H), 2.86 – 2.72 (m, 2H), 2.15 – 2.09 (m, 1H), 1.48 (s, 9H).^{13}C NMR (126 MHz, $CDCl_3$) δ 171.1, 168.2, 167.3, 166.5, 154.7, 150.2, 135.7, 134.1, 123.4, 117.9, 116.2, 80.1, 50.8, 49.1, 31.4, 28.4, 28.3, 22.6. HRMS (ESI): m/z calcd for $C_{22}H_{27}O_6N_4$ $[M+H]^+$: 443.19251; found 443.19272.

Example 12: Synthesis of 5-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl) piperazin-1-yl)-5-oxopentanoicacid (12)

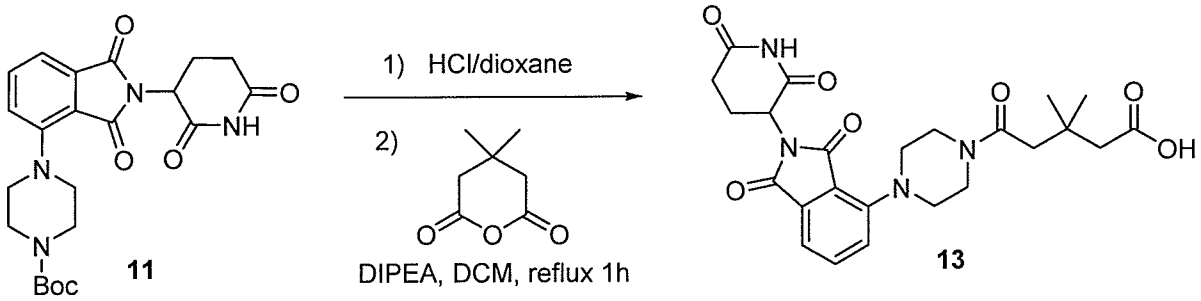


Tert-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate **(11)** was deprotected with 4 N HCl in dioxane (3 ml for 0.5 mmol scale). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt of 2-(2,6-dioxopiperidin-3-yl)-4-(piperazin-1-yl) isoindoline-1,3-dione (0.5 mmol, 1 equiv.) was suspended in 3 ml DCM. DIPEA (1 mmol, 2 equiv.) was added and after 10 min stirring at room temperature, glutaric anhydride (0.55 mmol, 1.1 equiv.) was added. The reaction mixture was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was

extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure.

0.5 mmol scale: 216 mg, 0.475 mmol, yield 95 %, yellow solid. ^1H NMR (500 MHz, DMSO-d_6) δ 12.04 (b, 1H), 11.09 (s, 1H), 7.72 (dd, J = 8.2, 7.4 Hz, 1H), 7.39 (d, J = 7.1 Hz, 1H), 7.35 (d, J = 8.4 Hz, 1H), 5.11 (dd, J = 12.7, 5.5 Hz, 1H), 3.64 – 3.59 (m, 4H), 3.34 – 3.30 (m, 4H), 2.88 (ddd, J = 16.8, 13.9, 5.3 Hz, 1H), 2.61 – 2.53 (m, 2H), 2.39 (t, J = 7.4 Hz, 2H), 2.29 – 2.23 (m, 2H), 2.05 – 2.02 (m, 1H), 1.76 – 1.70 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 174.3, 172.8, 170.4, 170.0, 167.0, 166.4, 149.5, 136.1, 133.6, 123.9, 116.9, 115.3, 50.9, 50.3, 48.8, 44.9, 40.9, 33.0, 31.5, 31.0, 22.1, 20.3. HRMS (ESI): m/z calcd for $C_{22}H_{25}O_7N_4$ $[M+H]^+$: 457.17178; found 457.17175.

Example 13: Synthesis of 5-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl) piperazin-1-yl)-3,3-dimethyl-5-oxopentanoicacid (13)

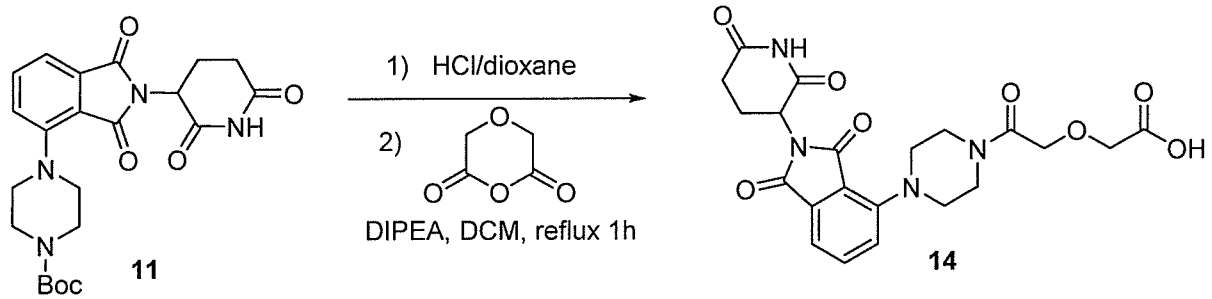


Tert-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate **(11)** was deprotected with 4 N HCl in dioxane (3 ml for 0.5 mmol scale). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt of 2-(2,6-dioxopiperidin-3-yl)-4-(piperazin-1-yl) isoindoline-1,3-dione (0.5 mmol, 1 equiv.) was suspended in 3 ml DCM. DIPEA (1 mmol, 2 equiv.) was added and after 10 min stirring at room temperature 3,3-dimethylglutaric anhydride (0.55 mmol, 1.1 equiv.) was added. The reaction mixture solution was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, and the solvent was removed under reduced pressure.

0.5 mmol scale: 232 mg, 0.48 mmol, yield 96%, yellow solid. ^1H NMR (500 MHz, DMSO-d_6) δ 12.03 (b, 1H), 11.10 (s, 1H), 7.72 (t, *J* = 7.8 Hz, 1H), 7.40 – 7.34 (m, 2H), 5.11 (dd, *J* = 12.7, 5.4 Hz, 1H), 3.71 – 3.69 (m, 4H), 3.47 – 3.35 (m, 4H), 2.92 – 2.81 (m, 1H), 2.61

– 2.56 (m, 1H), 2.36 – 2.34 (m, 4H), 2.04 – 2.02 (m, 1H), 1.26 – 1.23 (m, 1H), 1.07 (s, 3H), 1.05 (s, 3H).^{13}C NMR (126 MHz, DMSO-d_6) δ 173.2, 172.9, 170.0, 169.7, 167.0, 166.4, 149.4, 136.0, 133.6, 123.9, 117.0, 115.3, 50.9, 50.3, 48.8, 45.7, 44.9, 41.2, 32.6, 31.0, 27.6, 22.1. HRMS (ESI): m/z calcd for $C_{24}H_{29}O_7N_4$ $[M+H]^+$: 485.20308; found 485.20303.

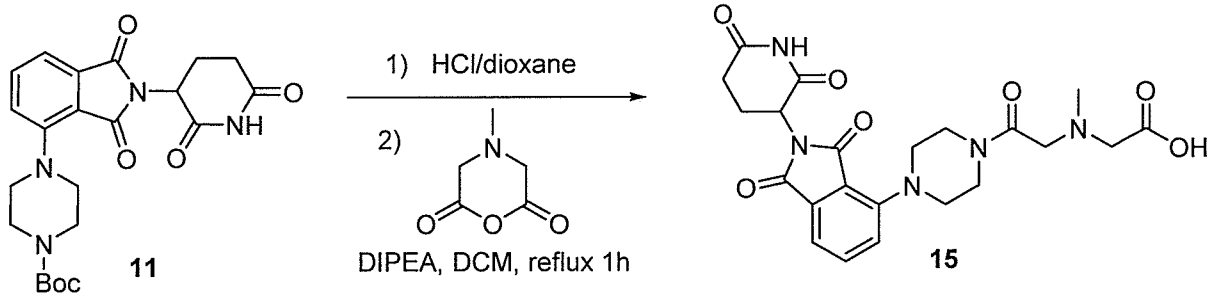
Example 14: Synthesis of 2-(2-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazin-1-yl)-2-oxoethoxy)acetic acid (14)



Tert-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate **(11)** was deprotected with 4 N HCl in dioxane (3 ml for 0.5 mmol scale). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt of 2-(2,6-dioxopiperidin-3-yl)-4-(piperazin-1-yl) isoindoline-1,3-dione (0.5 mmol, 1 equiv.) was suspended in 3 ml DCM. DIPEA (1 mmol, 2 equiv.) was added and after 10 min stirring at room temperature 1,4-dioxane-2,6-dione (0.55 mmol, 1.1 equiv.) was added. The reaction mixture solution was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, and the solvent was removed under reduced pressure.

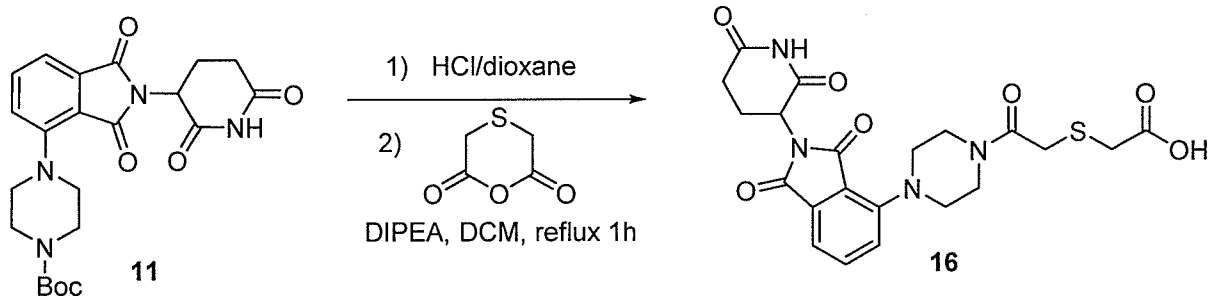
0.5 mmol scale: 178 mg, 0.39 mmol, yield 78%, yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 7.74 – 7.71 (m, 1H), 7.40 – 7.34 (m, 2H), 5.11 (dd, J = 12.8, 5.5 Hz, 1H), 4.31 (s, 2H), 4.11 (s, 2H), 3.64 – 3.62 (m, 4H), 3.33 – 3.28 (m, 4H), 2.61 – 2.55 (m, 2H), 2.04 – 2.02 (m, 1H), 1.26 – 1.23 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 172.3, 171.4, 170.0, 167.3, 167.0, 166.4, 149.4, 135.9, 133.6, 123.9, 117.0, 115.3, 69.2, 50.7, 50.2, 48.8, 44.3, 35.8, 31.0, 22.1. HRMS (ESI): m/z calcd for $C_{21}H_{23}O_8N_4$ $[M+H]^+$: 459.1510; found 459.1508

Example 15: Synthesis of N-(2-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazin-1-yl)-2-oxoethyl)-N-methylglycine (15)



Tert-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate **(11)** was deprotected with 4 N HCl in dioxane (3 ml for 0.5 mmol scale). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt of 2-(2,6-dioxopiperidin-3-yl)-4-(piperazin-1-yl) isoindoline-1,3-dione (0.5 mmol, 1 equiv.) was suspended in 3 ml DCM. DIPEA (1 mmol, 2 equiv.) was added and after 10 min stirring at room temperature 4-methylmorpholine-2,6-dione (0.55 mmol, 1.1 equiv.) was added. The reaction mixture solution was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was used directly in the next step without further characterization (0.2 mmol scale).

Example 16: Synthesis of 2-((2-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazin-1-yl)-2-oxoethyl)thio)acetic acid (16)

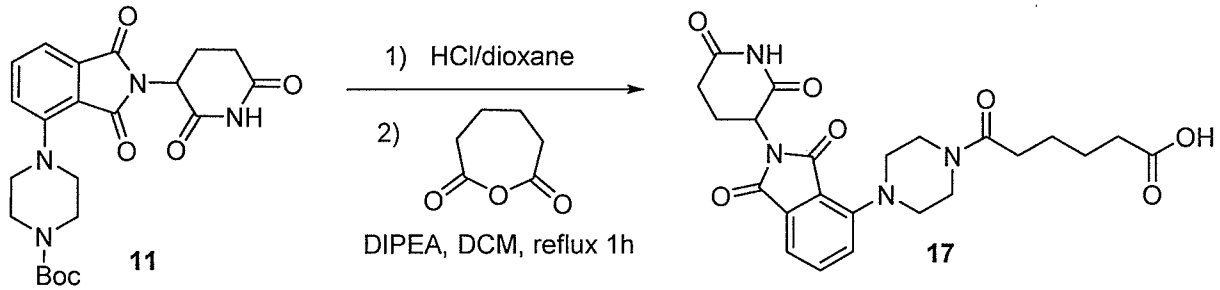


Tert-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate **(11)** was deprotected with 4 N HCl in dioxane (3 ml for 0.5 mmol scale). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt of 2-(2,6-

dioxopiperidin-3-yl)-4-(piperazin-1-yl) isoindoline-1,3-dione (0.5 mmol, 1 equiv.) was suspended in 3 ml DCM. DIPEA (1 mmol, 2 equiv.) was added and after 10 min stirring at room temperature thiodiglycolic anhydride (0.55 mmol, 1.1 equiv.) was added. The reaction mixture solution was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, and the solvent was removed under reduced pressure.

0.5 mmol scale: 178 mg, 0.38mmol, yield 75%, orange solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 7.72 (t, J = 7.5 Hz, 1H), 7.37 (dd, J = 20.9, 7.5 Hz, 2H), 5.11 (dd, J= 12.6, 5.2 Hz, 1H), 3.67– 3.59 (m, 6H), 3.46 – 3.34 (m, 6H), 2.88 – 2.84 (m, 1H), 2.61 – 2.55 (m, 1H), 2.04 – 2.02 (m, 1H), 1.27 – 1.25 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 172.8, 171.1, 170.0, 167.0, 166.4, 149.4, 135.9, 133.6, 123.9, 117.0, 115.3, 50.7, 50.2, 48.8, 45.7, 41.3, 33.3, 31.0, 22.1. HRMS (ESI): m/z calcd for $C_{21}H_{23}O_7N_4S$ $[M+H]^+$: 475.1282; found 475.12802.

Example 17: Synthesis of 6-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazin-1-yl)-6-oxohexanoic acid (17)

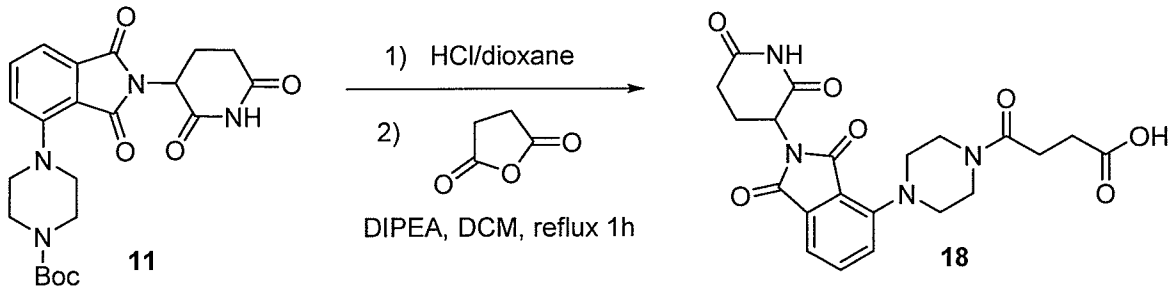


Tert-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate **(11)** was deprotected with 4 N HCl in dioxane (3 ml for 0.5 mmol scale). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt of 2-(2,6-dioxopiperidin-3-yl)-4-(piperazin-1-yl) isoindoline-1,3-dione (0.5 mmol, 1 equiv.) was suspended in 3 ml DCM. DIPEA (1 mmol, 2 equiv.) was added and after 10 min stirring at room temperature 2,7-oxepanedione (0.55 mmol, 1.1 equiv.) was added. The reaction mixture solution was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was

extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, and the solvent was removed under reduced pressure.

0.5 mmol scale: 212 mg, 0.45mmol, yield 90%, yellow solid. ^1H NMR (500 MHz, DMSO-d_6) δ 12.00 (b, 1H), 11.11 (s, 1H), 7.74 – 7.72 (m, 1H), 7.39 – 7.35 (m, 2H), 5.11 (dd, J = 12.6, 5.3 Hz, 1H), 3.64 – 3.57 (m, 4H), 3.45 – 3.38 (m, 4H), 2.90 – 2.88 (m, 1H), 2.61 – 2.58 (m, 1H), 2.37 – 2.34 (m, 2H), 2.25 – 2.22 (m, 2H), 2.05 – 2.03 (m, 1H), 1.54 – 1.52 (m, 4H), 1.24 – 1.22 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 174.5, 172.9, 170.7, 170.0, 167.1, 166.4, 149.5, 136.0, 133.6, 123.9, 116.9, 115.3, 51.0, 50.3, 48.9, 45.0, 41.0, 33.6, 32.1, 31.0, 24.3, 22.1. HRMS (ESI): m/z calcd for $C_{23}H_{27}O_7N_4[M+H]^+$: 471.18743; found 471.18738.

Example 18: Synthesis of 4-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazin-1-yl)-4-oxobutanoic acid (18)

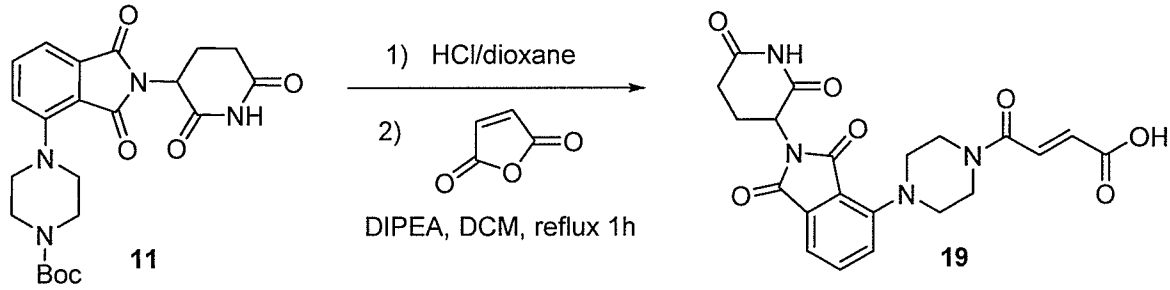


Tert-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate **(11)** was deprotected with 4 N HCl in dioxane (3 ml for 0.5 mmol scale). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt of 2-(2,6-dioxopiperidin-3-yl)-4-(piperazin-1-yl) isoindoline-1,3-dione (0.5 mmol, 1 equiv.) was suspended in 3 ml DCM. DIPEA (1 mmol, 2 equiv.) was added and after 10 min stirring at room temperature succinic anhydride (0.55 mmol, 1.1 equiv.) was added. The reaction mixture solution was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, and the solvent was removed under reduced pressure.

0.5 mmol scale: 190 mg, 0.43mmol, yield 86%, yellow solid. ^1H NMR (500 MHz, DMSO-d_6) δ 12.04 (s, 1H), 11.10 (s, 1H), 7.74 – 7.71 (m, 1H), 7.40 – 7.34 (m, 2H), 5.11 (dd, J = 12.7, 5.4 Hz, 1H), 3.66 – 3.58 (m, 4H), 3.34 – 3.31 (m, 4H), 3.25 – 3.23 (m, 2H), 2.61 –

2.58 (m, 3H), 2.48 – 2.44 (m, 2H), 2.04 – 2.02 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 174.0, 172.9, 170.0, 169.8, 167.1, 166.4, 149.5, 136.0, 133.6, 123.9, 117.0, 115.3, 50.8, 50.3, 48.9, 44.7, 41.1, 31.0, 29.0, 27.5, 22.1. HRMS (ESI): m/z calcd for $C_{21}H_{23}O_7N_4$ $[M+H]^+$: 443.15613; found 443.15585.

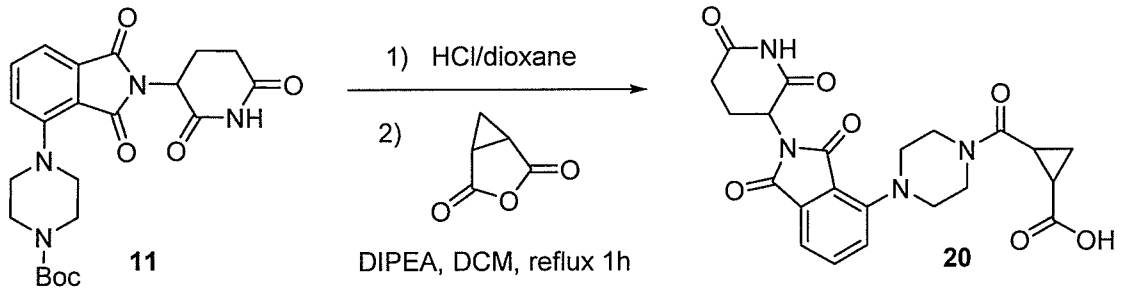
Example 19: Synthesis of (E)-4-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazin-1-yl)-4-oxobut-2-enoic acid (19)



Tert-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate **(11)** was deprotected with 4 N HCl in dioxane (3 ml for 0.5 mmol scale). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt of 2-(2,6-dioxopiperidin-3-yl)-4-(piperazin-1-yl) isoindoline-1,3-dione (0.5 mmol, 1 equiv.) was suspended in 3 ml DCM. DIPEA (1 mmol, 2 equiv.) was added and after 10 min stirring at room temperature maleic anhydride (0.55 mmol, 1.1 equiv.) was added. The reaction mixture solution was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, and the solvent was removed under reduced pressure.

0.5 mmol scale: 155 mg, 0.35mmol, yield 70%, yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (s, 1H), 7.73 – 7.70 (m, 1H), 7.43 – 7.34 (m, 2H), 6.69 (d, J = 11.6 Hz, 1H), 6.03 – 5.99 (m, 1H), 5.12 – 5.10 (m, 1H), 3.68 – 3.66 (m, 2H), 3.56 – 3.53 (m, 2H), 3.30 – 3.28 (m, 4H), 2.88 – 2.85 (m, 1H), 2.62 – 2.58 (m, 2H), 2.04 – 2.02 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 172.9, 170.0, 167.1, 166.4, 166.2, 165.7, 149.5, 136.2, 133.6, 133.5, 123.9, 117.1, 115.4, 50.4, 50.1, 48.9, 45.7, 40.7, 31.0, 22.1. HRMS (ESI): m/z calcd for $C_{21}H_{21}O_7N_4[M+H]^+$: 441.14048; found 441.14041.

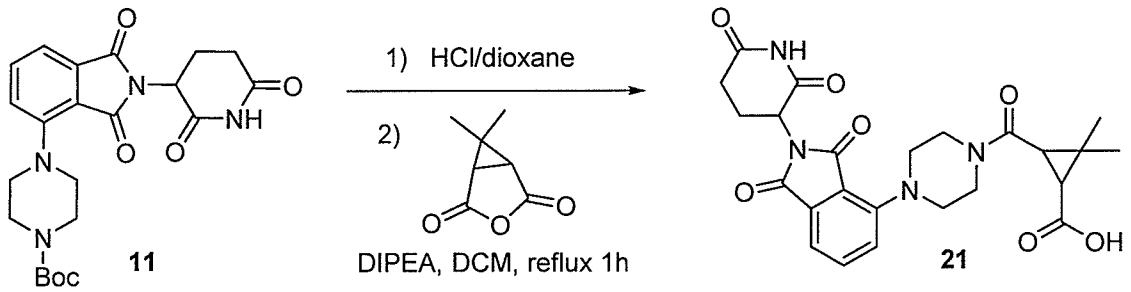
Example 20: Synthesis of 2-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carbonyl)cyclopropane-1-carboxylic acid (20)



Tert-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate **(11)** was deprotected with 4 N HCl in dioxane (3 ml for 0.5 mmol scale). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt of 2-(2,6-dioxopiperidin-3-yl)-4-(piperazin-1-yl) isoindoline-1,3-dione (0.5 mmol, 1 equiv.) was suspended in 3 ml DCM. DIPEA (1 mmol, 2 equiv.) was added and after 10 min stirring at room temperature 3-oxabicyclo[3.1.0]hexane-2,4-dione (0.55 mmol, 1.1 equiv.) was added. The reaction mixture solution was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, and the solvent was removed under reduced pressure.

0.5 mmol scale: 206 mg, 0.46 mmol, yield 91%, yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.23 (b, 1H), 11.10 (s, 1H), 7.73 (t, J = 7.7 Hz, 1H), 7.41 - 33 (m, 2H), 5.11 (dd, J = 12.6, 5.3 Hz, 1H), 3.78 – 3.76 (m, 2H), 3.68 – 3.66 (m, 1H), 3.55 – 3.52 (m, 1H), 3.35 – 3.32 (m, 4H), 3.20 – 3.17 (m, 1H), 3.11 – 3.06 (m, 1H), 2.61 – 2.57 (m, 1H), 2.29 – 2.24 (m, 1H), 2.04 – 1.96 (m, 2H), 1.36 – 1.33 (m, 1H), 1.18 - 1.15 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 172.6, 170.0, 167.0, 166.4, 166.2, 149.5, 136.1, 133.6, 123.9, 117.1, 115.3, 55.0, 50.9, 50.2, 48.8, 44.9, 41.3, 31.0, 22.7, 22.1. HRMS (ESI): m/z calcd for $C_{22}H_{23}O_7N_4[M+H]^+$: 455.15613; found 455.15613.

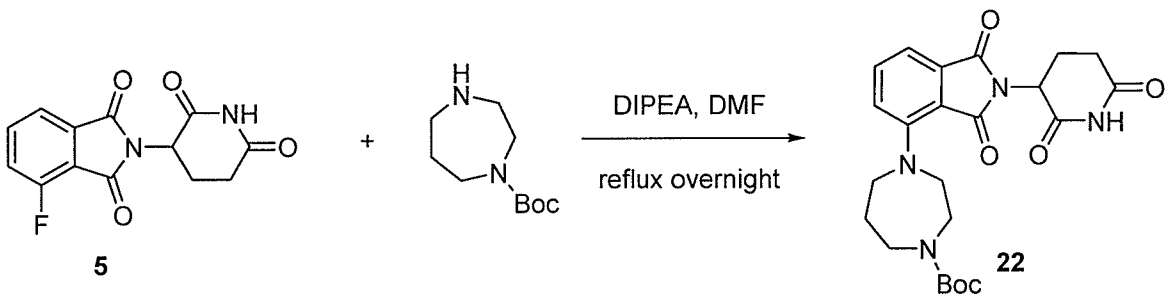
Example 21: Synthesis of 2-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carbonyl)cyclopropane-1-carboxylic acid (21)



Tert-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate **(11)** was deprotected with 4 N HCl in dioxane (3 ml for 0.5 mmol scale). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt of 2-(2,6-dioxopiperidin-3-yl)-4-(piperazin-1-yl) isoindoline-1,3-dione (0.5 mmol, 1 equiv.) was suspended in 3 ml DCM. DIPEA (1 mmol, 2 equiv.) was added and after 10 min stirring at room temperature caronic anhydride (0.55 mmol, 1.1 equiv.) was added. The reaction mixture solution was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, and the solvent was removed under reduced pressure.

0.5 mmol scale: 207 mg, 0.43mmol, yield 85%, orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.96 (s, 1H), 7.62 (t, *J* = 7.7 Hz, 1H), 7.42 (d, *J* = 7.1 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 1H), 4.96 (dd, *J* = 11.1, 4.9 Hz, 1H), 4.00 – 3.96 (m, 1H), 3.90 – 3.72 (m, 5H), 3.56 – 3.51 (m, 1H), 3.37 – 3.34 (m, 1H), 3.26 – 3.22 (m, 1H), 2.76 – 2.73 (m, 1H), 2.10 – 2.08 (m, 1H), 2.04 – 2.02 (m, 1H), 1.92 – 1.90 (m, 2H), 1.36 (s, 3H), 1.19 (s, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ 171.4, 170.7, 168.5, 167.0, 166.6, 149.3, 135.9, 133.9, 123.4, 118.1, 116.7, 51.8, 50.0, 49.1, 46.4, 42.2, 35.2, 32.2, 31.3, 27.4, 26.1, 22.5, 16.1. HRMS (ESI): m/z calcd for $C_{24}H_{27}O_7N_4$ $[M+H]^+$: 483.18743; found 483.18744.

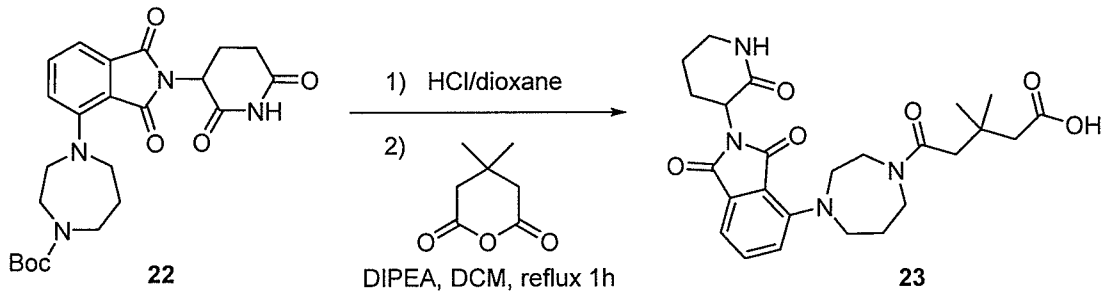
Example 22: Synthesis of *tert*-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-1,4-diazepane-1-carboxylate (22)



Tert-butyl 1,4-diazepane-1-carboxylate (1.1 equiv., 6.49 mmol) was added to a stirred solution of 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione **(5)** (1.0 equiv., 5.90 mmol) in DMF (36 ml) and DIPEA (2.0 equiv.,11.8 mmol). The reaction mixture was stirred at 90°C overnight. Then the mixture was cooled to room temperature, poured into H_2O, and extracted with EtOAc (3 x 50 ml). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and filtered. The crude compound was purified by column chromatography (PE – EtOAc, 0 – 50% EtOAc in PE).

5.90 mmol scale; 1.73 g, 3.80 mmol, yield 64 %, yellow solid. In the NMR two rotamers can be seen;^{1}H NMR (500 MHz, $CDCl_3$) δ 8.82 (s, 1H), 7.47 (t, J = 7.8 Hz, 1H), 7.25 (d, J = 7.0 Hz, 1H), 7.11 (d, J = 8.7 Hz, 1H), 4.10 (qd, J = 7.1, 0.7 Hz, 1H), 3.84 – 3.36 (m, 8H), 2.99 – 2.64 (m, 4H), 2.02 (d, J = 0.8 Hz, 2H), 1.46 – 1.32 (m, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.1, 168.3, 167.4, 166.6, 150.4, 135.0, 134.4, 122.2, 114.2, 113.9, 60.4, 49.1, 43.4, 31.4, 22.7, 21.0, 14.2.

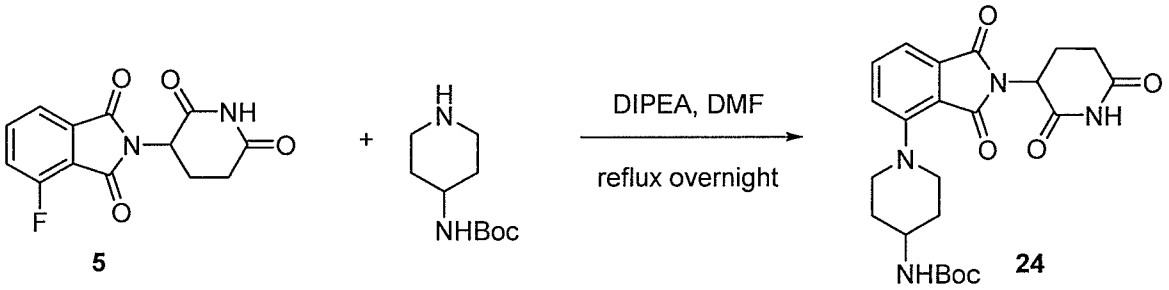
Example 23: Synthesis of 5-(4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-1,4-diazepan-1-yl)-3,3-dimethyl-5-oxopentanoic acid (23)



Tert-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-1,4-diazepane-1-carboxylate **(22)** (1 equiv., 3 mmol), was deprotected with 4N HCl in dioxane (15 ml). Stirring at rt overnight. Solvent was removed under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt of 4-(1,4-diazepan-1-yl)-2-(2,6-dioxopiperidin-3-yl) isoindoline-1,3-dione (1 equiv., 2.62 mmol) was suspended in 44 ml DCM. DIPEA (2 equiv., 5.24 mmol) was added and after 10 min stirring at room temperature, 3,3-dimethylglutaric anhydride (1.1 equiv., 2.89 mmol) was added. The reaction mixture was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure.

3.0 mmol scale; 822 mg, 1.65 mmol, yield 55 %, yellow solid. In the NMR two rotamers can be seen; ^{1}H NMR (500 MHz, DMSO-d$_6$) δ 12.00 (s, 1H), 11.09 (s, 1H), 7.67 – 7.52 (m, 1H), 7.32 (d, J = 8.6 Hz, 1H), 7.22 (t, J = 6.8 Hz, 1H), 5.10 (dt, J = 12.4, 5.2 Hz, 1H), 3.74 – 3.52 (m, 6H), 3.39 (dd, J = 14.0, 7.0 Hz, 2H), 2.66 – 2.52 (m, 4H), 2.41 – 2.21 (m, 4H), 1.10 (t, J = 7.0 Hz, 2H), 1.03 – 0.93 (m, 6H). ^{13}C NMR (126 MHz, DMSO-d$_6$) δ 173.7, 173.3, 170.9, 170.5, 167.4, 167.1, 148.8, 148.2, 134.5, 123.6, 113.5, 112.8, 112.5, 49.3, 45.1, 41.3, 32.9, 31.4, 28.0.

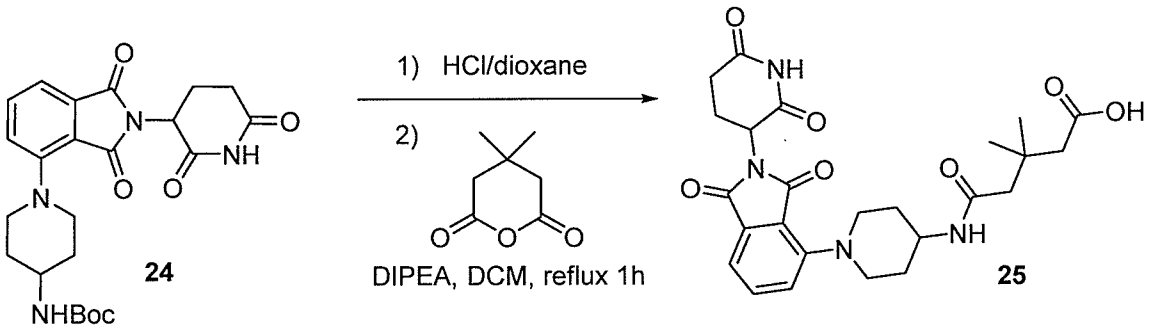
Example 24: Synthesis of *tert*-butyl (1-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl) piperidin-4-yl) carbamate (24)



Tert-butyl piperidin-4-yl carbamate (1.1 equiv., 6.49 mmol) was added to a stirred solution of 2-(2,6-dioxopiperidin- 3-yl)-4-fluoroisoindoline-1,3-dione **(5)** (1.0 equiv., 5.90 mmol) in DMF (36 ml) and DIPEA (2.0 equiv., 11.8 mmol). The reaction mixture was heated at reflux overnight. Then the mixture was cooled to room temperature, poured into H_2O, and extracted with EtOAc (3 x 50 ml). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and filtered. The crude compound was purified by column chromatography (PE – EtOAc, 0 – 50% EtOAc in PE).

5.9 mmol scale; 1.77 g, 3.83 mmol, yield 65 %, yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) 8.65 (s, 1H), 7.59 – 7.54 (m, 1H), 7.38 (d, J = 7.1 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H), 4.98 (dd, J = 12.1, 5.3 Hz, 1H), 4.65 (d, J = 8.1 Hz, 1H), 3.00 (tdd, J = 14.4, 5.7, 2.7 Hz, 2H), 2.93 – 2.67 (m, 4H), 2.15 – 2.03 (m, 4H), 1.68 (dddd, J = 14.8, 11.1, 7.5, 3.5 Hz, 2H), 1.46 (s, 9H).^{13}C NMR (126 MHz, $CDCl_3$) δ 171.3, 168.4, 167.8, 166.7, 150.5, 135.5, 134.1, 123.7, 117.5, 115.7, 49.1, 32.5, 31.4, 28.4, 22.7.

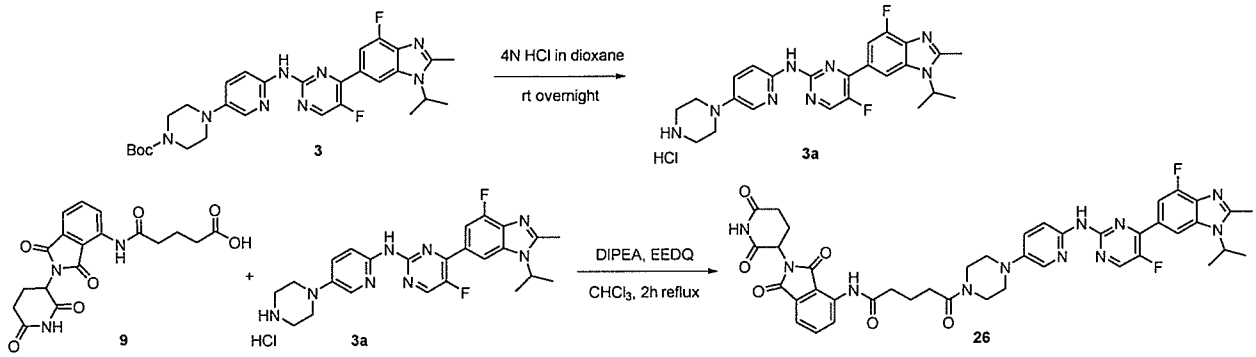
Example 25: Synthesis of 5-((1-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl) piperidin-4-yl) amino)-3,3-dimethyl-5-oxopentanoic acid (25)



Tert-butyl (1-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl) piperidin-4-yl) carbamate **(24)** (1 equiv., 3.4 mmol) was deprotected with 4N HCl in dioxane. Stirring at rt overnight. Solvent was removed under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt of 4-(4-aminopiperidin-1-yl)-2-(2,6-dioxopiperidin-3-yl) isoindoline-1,3-dione (1 equiv., 3.28 mmol) was suspended in 44 ml DCM. DIPEA (2 equiv., 6.56 mmol) was added and after 10 min stirring at room temperature, 3,3-dimethylglutaric anhydride (1.1 equiv., 3.60 mmol) was added. The reaction mixture solution was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure.

3.4 mmol scale; 983 mg, 1.96 mmol, yield 58 %, yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.04 (s, 1H), 11.10 (s, 1H), 7.99 (s, 1H), 7.70 (t, J = 7.5 Hz, 1H), 7.36 (s, 1H), 7.35 (s, 1H), 5.10 (dd, J = 12.7, 4.6 Hz, 1H), 3.80 (s, 1H), 3.64 (d, J = 11.3 Hz, 2H), 3.00 (t, J = 10.7 Hz, 2H), 2.68 – 2.54 (m, 4H), 2.21 (d, J = 66.4 Hz, 4H), 1.86 (d, J = 10.4 Hz, 2H), 1.61 (d, J = 10.7 Hz, 2H), 1.04 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 173.7, 173.3, 170.9, 170.5, 167.4, 167.0, 148.8, 148.2, 134.5, 123.6, 113.5, 112.8, 112.5, 49.3, 45.1, 41.4, 32.9, 31.4, 28.0.

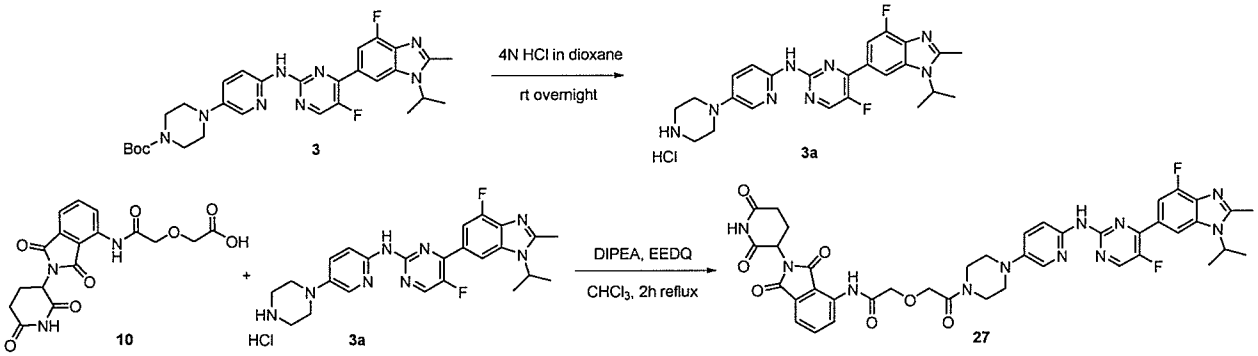
Example 26: Synthesis of N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-5-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazin-1-yl)-5-oxopentanamide (26)



In a small round-bottom flask containing *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1 equiv) 4N HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv) was added, followed by the addition of the carboxylic acid **(9)** (1 equiv) and EEDQ (2 equiv). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.2 mmol scale; 122 mg, 0.15 mmol, yield 75%, yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.64 (s, 1H), 9.42 (s, 1H), 8.80 (d, J = 8.5 Hz, 1H), 8.55 (s, 1H), 8.40 (d, J = 3.8 Hz, 1H), 8.33 (d, J = 9.1 Hz, 1H), 8.16 (s, 1H), 8.07 (d, J = 2.9 Hz, 1H), 7.78 (d, J = 11.6 Hz, 1H), 7.72 – 7.69 (m, 1H), 7.54 (d, J = 7.3 Hz, 1H), 7.35 (dd, J = 9.1, 2.9 Hz, 1H), 4.95 (dd, J = 12.3, 5.4 Hz, 1H), 4.72 (hept, J = 7.0 Hz, 1H), 3.80 – 3.78 (m, 2H), 3.65 – 3.63 (m, 2H), 3.14 – 3.09 (m, 4H), 2.94 – 2.91 (m, 1H), 2.78 (ddd, J = 10.7, 10.2, 3.9 Hz, 2H), 2.68 (s, 3H), 2.60 (td, J = 6.9, 1.8 Hz, 2H), 2.50 (t, J = 7.1 Hz, 2H), 2.18 – 2.09 (m, 3H), 1.70 (d, J = 7.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.8, 171.4, 170.5, 169.1, 168.2, 166.7, 155.31 (d, *J* = 2.8 Hz), 153.5, 153.3 (d, *J* = 251.7 Hz), 151.3 (dd, *J* = 8.5, 1.8 Hz), 150.8 (d, *J* = 255.2 Hz), 147.2, 147.0, 142.4, 137.7, 137.1, 136.4, 134.01 (d, *J* = 17.6 Hz), 131.2, 127.5 (dd, *J* = 7.4, 5.8 Hz), 127.4, 125.3, 118.5, 115.4, 112.5, 108.8 (dd, *J* = 9.4, 3.2 Hz), 108.0 (dd, *J* = 20.3, 6.4 Hz), 50.3, 50.1, 49.3, 48.6, 45.3, 41.42, 36.8, 31.9, 31.4, 22.7, 21.5, 20.5, 15.0. HRMS (ESI): m/z calcd for $C_{42}H_{42}O_6N_{11}F_2$ $[M+H]^+$: 834.32821; found 834.32819.

Example 27: Synthesis of N-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-2-(2-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazin-1-yl)-2-oxoethoxy) acetamide (27)

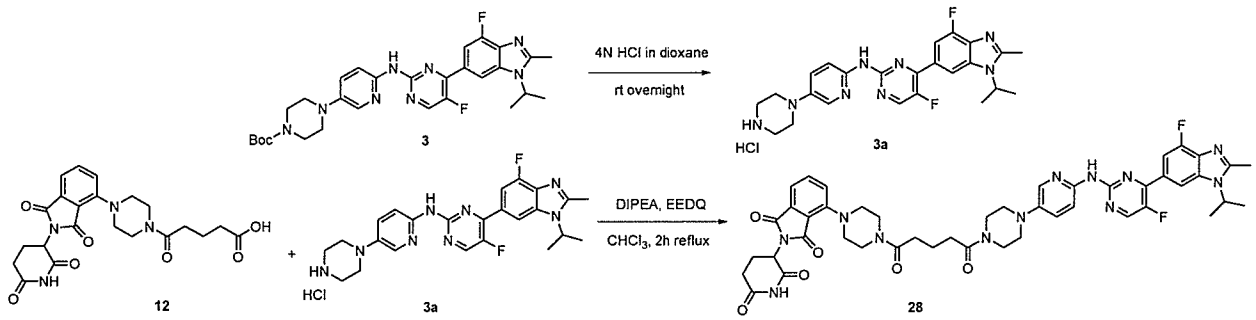


In a small round-bottom flask containing *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv) 4N HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic acid **(10)** (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt. The product precipated in the reaction mixture. Instead of column chromatography, solvent was removed under reduced pressure and the remaining solid was suspended in diethylether. Et_2O was decanted and then the residue was treated with methanol, which was also decanted. The remaining solid was dried under vacuum to obtain product (27).

0.1 mmol scale; 52 mg, 0.062 mmol, yield 62%, yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.16 (s, 1H), 10.47 (s, 1H), 9.84 (s, 1H), 8.73 (d, J = 8.4 Hz, 1H), 8.63 (d, J = 3.8 Hz, 1H), 8.25 (s, 1H), 8.06 (dd, J = 14.1, 5.9 Hz, 2H), 7.88 – 7.85 (m, 1H), 7.67 – 7.62 (m, 2H), 7.43 (dd, J = 9.1, 2.9 Hz, 1H), 5.14 (dd, J = 12.9, 5.4 Hz, 1H), 4.82 (hept, J = 7.0 Hz, 1H), 4.53 (s, 2H), 4.25 (s, 2H), 3.62 – 3.53 (m, 5H), 3.15 – 3.11 (m, 5H), 2.90 – 2.83 (m, 1H), 2.63 (s, 3H), 2.08 – 2.05 (m, 1H), 1.61 (d, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.8, 169.7, 170.0, 168.1, 166.9, 166.7, 155.7(d, *J* = 2.7 Hz), 154.6, 152.3 (d, *J* = 255.8 Hz), 150.5, 150.3 (d, *J* = 259.8 Hz), 149.2, 147.6 (d, *J* = 26.9 Hz), 146.2, 136.6, 136.0, 133.2 (d, *J* = 16.9 Hz), 131.4, 129.5, 128.9, 126.6 (dd, *J* = 7.4, 5.8 Hz), 125.8, 124.5, 121.5, 118.4, 116.1, 113.0, 108.9 (dd, *J* = 8.5, 3.4 Hz), 106.9 (d, *J* = 5.7

Hz), 70.2, 69.0, 53.3, 48.9, 48.1, 41.6, 30.9, 21.0, 18.0, 16.7, 14.6, 12.2. HRMS (ESI): m/z calcd for $C_{41}H_{40}O_7N_{11}F_2$ $[M+H]^+$: 836.30748; found 836.30756.

Example 28: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-(4-(5-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazin-1-yl)-5-oxopentanoyl) piperazin-1-yl) isoindoline-1,3-dione (28) [lot 1, lot 2]

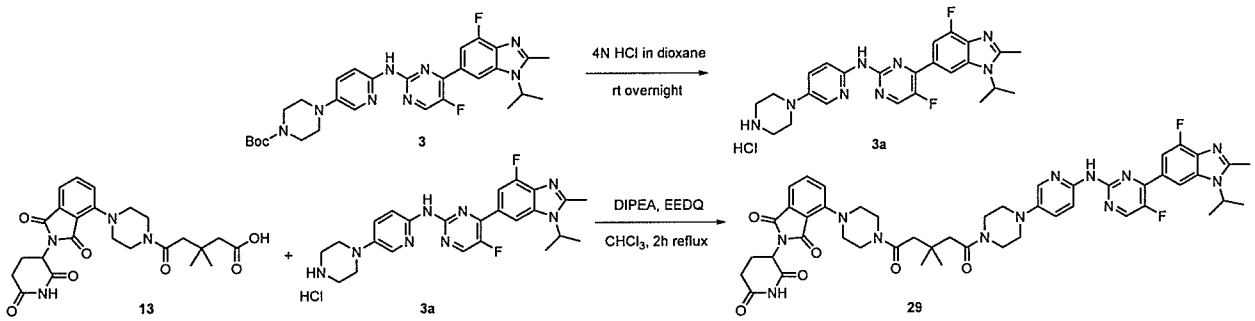


In a small round-bottom flask containing *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic acid **(12)** (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.2 mmol scale; 126mg, 0.14 mmol, yield 70%, yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.39 (s, 1H), 9.22 (s, 1H), 8.42 – 8.40 (m, 1H), 8.32 (d, *J* = 9.0 Hz, 1H), 8.14 – 8.11 (m, 2H), 7.73 (d, *J* = 11.5 Hz, 1H), 7.55 (t, *J* = 7.7 Hz, 1H), 7.38 (d, *J* = 7.1 Hz, 1H), 7.32 – 7.30 (m, 1H), 7.10 (d, *J* = 8.4 Hz, 1H), 4.95 (dd, *J* = 11.9, 5.2 Hz, 1H), 4.71 – 4.68 (m, 1H), 3.80 – 3.63 (m, 8H), 3.30 – 3.24 (m, 4H), 3.08 – 3.05 (m, 4H), 2.88 – 2.73 (m, 3H), 2.65 (s, 3H), 2.48 - 2.45 (m, 4H), 2.11 – 2.08 (m, 1H), 1.98 – 1.95 (m, 2H), 1.67 (t, *J* = 9.7 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 171.4, 171.3, 171.1, 168.3, 167.2, 166.6, 155.3(d, *J* = 2.7 Hz),153.6, 153.3 (d, *J* = 252.0 Hz), 151.30 (dd, *J* = 8.2, 2.2 Hz), 150.8 (d, *J* = 255 Hz), 149.9, 147.2, 147.0, 142.5,137.2, 136.4(d, *J* = 9.5 Hz), 135.8, 134.1, 127.5 (dd, *J* = 7.4, 5.8 Hz), 127.4, 123.3, 118.1, 116.4, 112.4, 108.76 (dd, *J* = 9.1, 3.3 Hz), 108.02 (dd, *J* = 20.1, 6.7 Hz),51.7, 50.5, 50.1, 49.2, 48.6, 45.6, 45.4, 41.4, 32.5, 32.4,

31.4, 22.7, 21.5, 20.7, 15.0. HRMS (ESI): m/z calcd for $C_{46}H_{49}O_6N_{12}F_2$ $[M+H]^+$: 903.38606; found 903.38599.

Example 29: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-(4-(5-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazin-1-yl)-3,3-dimethyl-5-oxopentanoyl) piperazin-1-yl) isoindoline-1,3-dione (29)

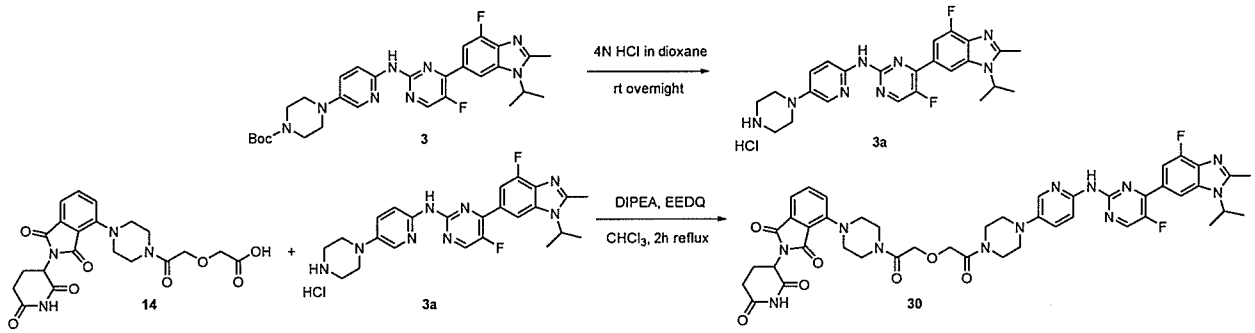


In a small round-bottom flask containing *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic **(13)** acid (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.1 mmol scale; 56 mg, 0.06 mmol, yield 60%, yellow solid.^{1}H NMR (500 MHz, $CDCl_3$) δ 9.84 (s, 1H), 8.86 (b, 1H), 8.42 (s, 1H), 8.34 (d, *J* = 7.0 Hz, 1H), 8.16 (s, 1H), 8.09 (s, 1H), 7.77 (d, *J* = 11.4 Hz, 1H), 7.58 (t, *J* = 7.5 Hz, 1H), 7.41 (d, *J* = 7.0 Hz, 1H), 7.34 (d, *J* = 7.1 Hz, 1H), 7.12 (d, *J* = 7.9 Hz, 1H), 4.96 (dd, *J* = 12.0, 5.0 Hz, 1H), 4.75 – 4.70 (m, 1H), 3.83 – 3.71 (m, 8H), 3.35 – 3.31 (m, 2H), 3.26 – 3.24 (m, 2H), 3.12 – 3.08 (m, 4H), 2.91 – 2.88 (m, 1H), 2.83 – 2.74 (m, 2H), 2.68 (s, 3H), 2.61 – 2.59 (m, 4H), 2.13 – 2.10 (m, 1H), 1.69 (d, *J* = 6.7 Hz, 6H), 1.19 (s, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 171.93, 170.6, 170.4, 168.6, 167.2, 166.6, 155.3 (d, *J* = 2.7 Hz), 153.5, 153.2 (d, *J* = 251.9 Hz), 151.2 (d, *J* = 7.8 Hz),150.7 (d, *J* = 255.2 Hz), 149.8, 147.1 (d, *J* = 26.9 Hz), 147.0, 142.4, 136.8, 136.3 (d, *J* = 6.7 Hz), 135.7, 134.1, 133.9 (d, *J* = 17.4 Hz), 127.5 (dd, *J* = 7.4, 5.8 Hz), 127.2, 123.3, 117.9, 116.2, 112.4, 108.74 (dd, *J* = 8.6, 2.0 Hz), 107.97 (dd, *J* = 20.0, 6.6

Hz), 51.7, 50.5, 50.3, 50.1, 49.2, 48.6, 41.7, 41.2, 33.6, 31.4, 28.8, 22.7, 21.4, 15.0. HRMS (ESI): m/z calcd for $C_{48}H_{53}O_6N_{12}F_2[M+H]^+$: 931.41736; found 931.41772.

Example 30: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-(4-(2-(2-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)-2-oxoethoxy)acetyl)piperazin-1-yl)isoindoline-1,3-dione (30)

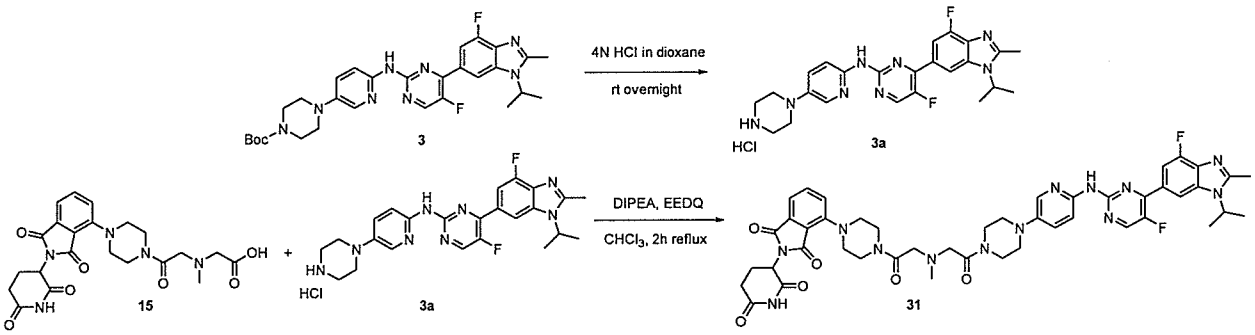


In a small round-bottom flask containing *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic **(14)** acid (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.1 mmol scale: 54 mg, 0.06 mmol, yield 60%, yellow solid.^{1}H NMR (500 MHz, $CDCl_3$) δ 9.70 (b, 1H), 8.70 (b, 1H), 8.41 (d, J = 3.4 Hz, 1H), 8.35 (d, J = 9.0 Hz, 1H), 8.17 (s, 1H), 8.06 (s, 1H), 7.78 (d, J = 11.6 Hz, 1H), 7.61 (t, J = 7.7 Hz, 1H), 7.44 (d, J = 7.2 Hz, 1H), 7.36 (d, J = 8.7 Hz, 1H), 7.15 (d, J = 8.2 Hz, 1H), 4.97 – 4.94 (m, 1H), 4.74 – 4.70 (m, 1H), 4.36 – 4.32 (m, 4H), 3.92 – 3.90 (m, 1H), 3.78 – 3.70 (m, 8H), 3.42 – 3.40 (m, 1H), 3.32 – 3.30 (m, 3H), 3.16 – 3.13 (m, 3H), 2.92 – 2.89 (m, 1H), 2.80 – 2.74 (m, 2H), 2.68 (s, 3H), 2.13 – 2.11 (m, 1H), 1.70 (d, J = 6.8 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 171.8, 168.5, 167.3, 167.2, 167.1, 166.7, 155.3, 153.6, 153.3 (d, *J* = 253.8 Hz),151.3 (d, *J* = 5.4 Hz), 150.8(d, *J* = 255.9 Hz), 149.8, 147.3, 147.1, 142.3, 136.8, 136.4 (d, *J* = 10.0 Hz), 135.8, 134.1, 133.9, 127.6, 123.4, 118.1, 116.5, 112.5, 108.8 (dd, *J* = 9.5, 3.8 Hz), 108.0 (dd, *J* = 18.8, 7.2 Hz), 70.1, 51.8, 50.4, 50.3, 50.0, 49.3, 48.6, 45.2, 44.8, 41.7, 31.4, 29.7,

22.8, 21.4, 15.0. HRMS (ESI): m/z calcd for $C_{45}H_{47}O_7N_{12}F_2$ $[M+H]^+$: 905.36532; found 905.36542.

Example 31: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-(4-(N-(2-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)-2-oxoethyl)-N-methylglycyl)piperazin-1-yl)isoindoline-1,3-dione (31)

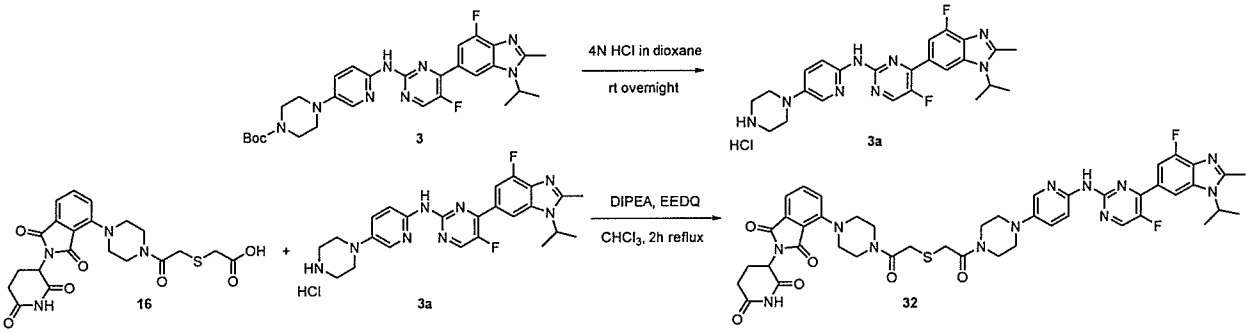


In a small round-bottom flask containing *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic **(15)** acid (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.2 mmol scale: 110 mg, 0.12 mmol, yield 70% (over 3 steps), yellow solid.^{1}H NMR (500 MHz, $CDCl_3$) δ 10.15 (b, 1H), 8.74 (b, 1H), 8.41 (d, *J* = 3.4 Hz, 1H), 8.34 (d, *J* = 9.0 Hz, 1H), 8.17 (s, 1H), 8.08 (s, 1H), 7.78 (d, *J* = 11.5 Hz, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 7.44 (d, *J* = 7.0 Hz, 1H), 7.36 (d, *J* = 8.8 Hz, 1H), 7.15 (d, *J* = 8.3 Hz, 1H), 4.98 – 4.94 (m, 1H), 4.72 (hept, *J* = 7.0 Hz, 1H), 3.95 – 3.89 (m, 2H), 3.82 – 3.73 (m, 6H), 3.45 – 3.34 (m, 4H), 3.32 – 3.28 (m, 4H), 3.15 – 3.08 (m, 4H), 2.90 – 2.88 (m, 1H), 2.79 – 2.74 (m, 2H), 2.68 (s, 3H), 2.43 (s, 3H), 2.13 – 2.11 (m, 1H), 1.70 (d, *J* = 6.9 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 172.2, 168.6, 168.4, 168.3, 167.2, 166.7, 155.3 (d, *J* = 2.7 Hz),153.6, 153.2 (d, *J* = 252.0 Hz), 151.3 (dd, *J* = 8.2, 2.2 Hz), 150.8 (d, *J* = 255.3 Hz), 149.9, 147.2 (d, *J* = 26.9 Hz), 147.0, 142.3, 136.6, 136.4 (d, *J* = 9.2 Hz), 135.8, 134.2, 134.0 (d, *J* = 10.8 Hz), 127.6 (dd, *J* = 7.4, 5.8 Hz), 127.4, 123.4, 118.2, 116.5, 112.6, 108.8 (dd, *J* = 9.5, 2.9

Hz), 108.0 (dd, *J* = 20.1, 6.5 Hz), 59.8, 52.2, 50.3, 50.1, 49.3, 48.6, 45.6, 45.1, 43.4, 41.6, 31.5, 22.9, 21.5, 15.1.HRMS (ESI): m/z calcd for $C_{46}H_{50}O_6N_{13}F_2$ $[M+H]^+$: 918.39696; found 918.3974.

Example 32: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-(4-(2-((2-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)-2-oxoethyl)thio)acetyl)piperazin-1-yl)isoindoline-1,3-dione (32)

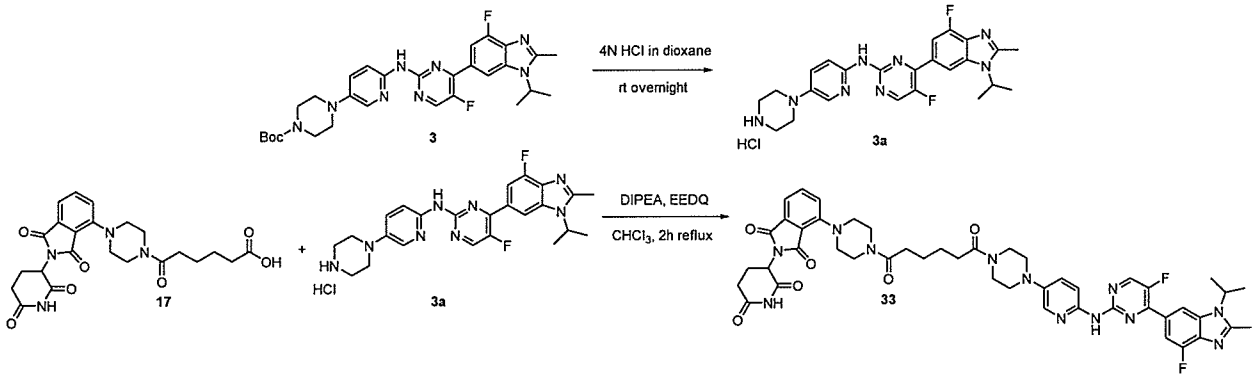


In a small round-bottom flask containing *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic **(16)** acid (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.1 mmol scale: 55 mg, 0.06 mmol, yield 60%, yellow solid.^{1}H NMR (500 MHz, $CDCl_3$) δ 10.43 (b, 1H), 9.22 (b, 1H), 8.41 (d, *J* = 3.3 Hz, 1H), 8.33 (d, *J* = 8.9 Hz, 1H), 8.14 (s, 1H), 8.11 (s, 1H), 7.74 (d, *J* = 11.5 Hz, 1H), 7.56 (t, *J* = 7.6 Hz, 1H), 7.39 (d, *J* = 7.0 Hz, 1H), 7.32 (d, *J* = 8.7 Hz, 1H), 7.10 (d, *J*= 8.3 Hz, 1H), 4.96 – 4.94 (m, 1H), 4.70 (hept, *J* = 7.0 Hz, 1H), 3.86 – 3.83 (m, 1H), 3.75– 3.71 (m, 5H), 3.66 – 3.64 (m, 2H), 3.52 (d, *J* = 7.1 Hz, 4H), 3.40 – 3.38 (m, 1H), 3.32 – 3.26 (m, 3H), 3.14 – 3.08 (m, 4H), 2.88 – 2.86 (m, 1H), 2.79 – 2.75 (m, 2H), 2.66 (s, 3H), 2.11 – 2.09 (m, 1H), 1.67 (d, *J* = 6.9 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 172.2, 168.8, 167.2, 167.1, 167.0, 166.6, 155.3 (d, *J* = 2.8 Hz), 153.5, 153.1 (d, *J* = 251.8 Hz), 151.1 (d, *J* =7.8 Hz), 150.6 (d, *J* = 255.5 Hz), 149.6, 147.1 (t, *J* = 13.5 Hz), 142.2, 136.8, 136.3 (d, *J* = 9.3 Hz), 135.7,134.0, 133.8 (d, *J* =17.1 Hz),

127.4, 123.3, 118.0, 116.3, 112.4, 108.7 (dd, *J* = 8.5, 3.4 Hz), 107.9 (dd, *J* = 20.0, 6.6 Hz), 51.5, 50.3, 50.1, 49.8, 49.2, 48.5, 46.3, 46.0, 41.7, 33.7, 31.4, 22.7, 21.4, 14.9. HRMS (ESI): m/z calcd for $C_{45}H_{47}O_6N_{12}F_2S[M+H]^+$: 921.34248; found 921.34247.

Example 33: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-(4-(6-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)-6-oxohexanoyl)piperazin-1-yl)isoindoline-1,3-dione (33)

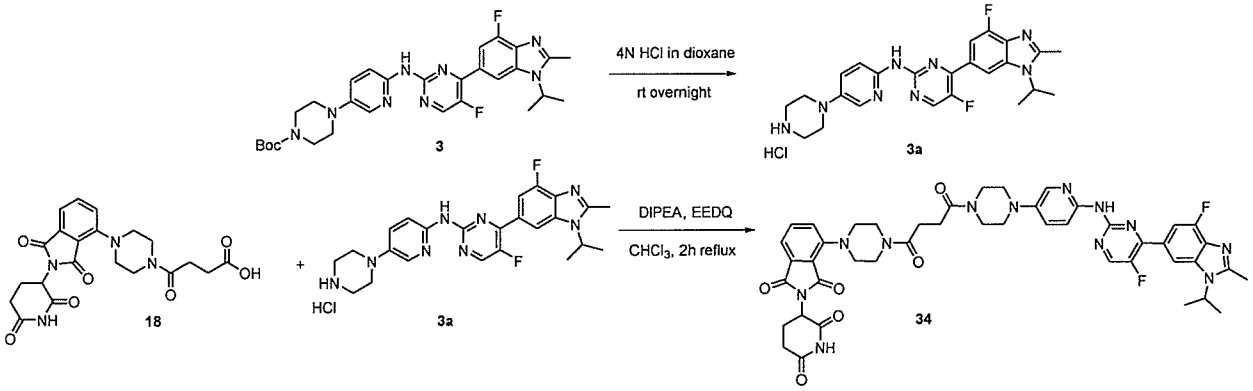


In a small round-bottom flask containing *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic **(17)** acid (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.1 mmol scale; 46 mg, 0.05 mmol, yield 50%, yellow solid.^{1}H NMR (500 MHz, $CDCl_3$) δ 9.94 (s, 1H), 8.86 (s, 1H), 8.42 (s, 1H), 8.35 (d, *J* = 7.8 Hz, 1H), 8.16 (s, 1H), 8.09 (d, *J* = 4.5 Hz, 1H), 7.77 (d, *J* = 11.5 Hz, 1H), 7.60 (t, *J* = 7.5 Hz, 1H), 7.43 (d, *J* = 7.0 Hz, 1H), 7.35 (d, *J* = 7.7 Hz, 1H), 7.14 (d, *J* = 7.7 Hz, 1H), 4.97 (dd, *J* = 12.0, 5.0 Hz, 1H), 4.72 (hept, *J* = 7.0 Hz, 1H), 3.90 – 3.88 (m, 1H), 3.83 – 3.63 (m, 7H), 3.40 – 3.38 (m, 1H), 3.28 – 3.25 (m, 3H), 3.12 – 3.08 (m, 4H), 2.92 – 2.88 (m, 1H), 2.83 – 2.78 (m, 2H), 2.68 (s, 3H), 2.42 – 2.40 (m, 4H), 2.14 – 2.11 (m, 1H), 1.70 – 1.69 (m, 4H), 1.68 (d, *J* = 6.8 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 171.3, 171.1, 168.6, 167.1, 166.6,155.3 (d, *J* = 2.8 Hz), 153.5, 153.3 (d, *J* = 252.0 Hz), 151.2 (dd, *J* = 8.2, 2.2 Hz), 150.8 (d, *J* = 255.2

Hz), 149.8, 147.0 (d, J = 27.2 Hz), 146.8, 142.4, 136.7, 136.3 (d, J = 9.3 Hz), 135.8, 134.1, 133.8 (d, J = 17.2 Hz), 127.5, 123.3, 118.0, 116.3, 112.6, 108.8 (dd, J = 8.5, 3.4 Hz), 108.0 (dd, J = 20.1, 7.3 Hz), 51.9, 50.4, 50.2, 49.9, 49.2, 48.6, 45.7, 45.4, 41.3, 33.0, 32.9, 31.4, 24.9, 22.7, 21.4, 15.0. HRMS (ESI): m/z calcd for $C_{47}H_{51}O_6N_{12}F_2[M+H]^+$: 917.40171; found 917.40179.

Example 34: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-(4-(4-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)-4-oxobutanoyl)piperazin-1-yl)isoindoline-1,3-dione (34)

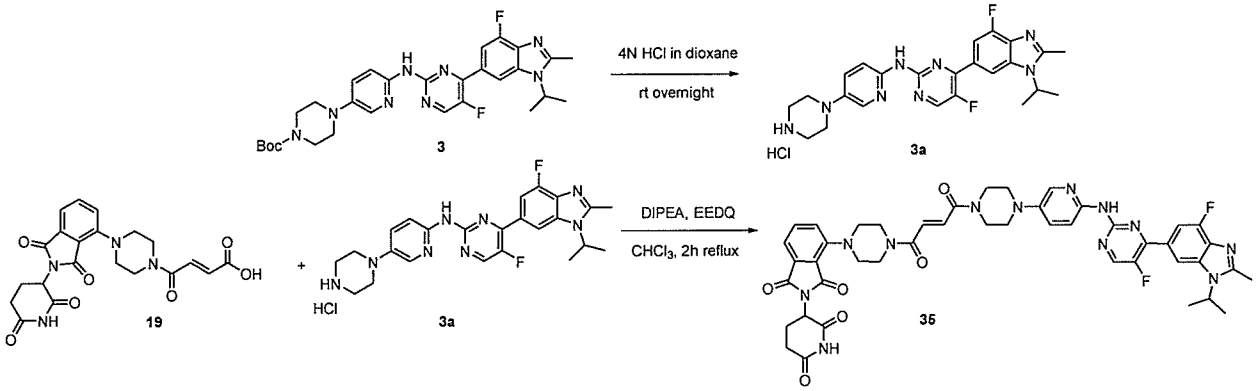


In a small round-bottom flask containing tert-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic **(18)** acid (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.1 mmol scale; 50 mg, 0.055 mmol, yield 55%, yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.20 (s, 1H), 9.13 (b, 1H), 8.41 (s, 1H), 8.33 (d, J = 8.6 Hz, 1H), 8.15 (s, 1H), 8.11 (s, 1H), 7.75 (d, J = 11.5 Hz, 1H), 7.57 (t, J = 7.6 Hz, 1H), 7.40 (d, J = 7.1 Hz, 1H), 7.32 (d, J = 8.3 Hz, 1H), 7.11 (d, J = 8.2 Hz, 1H), 4.97 (dd, J = 11.9, 5.2 Hz, 1H), 4.70 (hept, J = 7.0 Hz, 1H), 3.83 – 3.68 (m, 8H), 3.34 – 3.26 (m, 4H), 3.12 – 3.07 (m, 4H), 2.90 – 2.88 (m, 3H), 2.73 (b, 4H), 2.66 (s, 3H), 2.12 – 2.08 (m, 1H), 1.68 (d, J = 6.8 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 170.5, 107.4, 168.7, 167.2, 166.5,155.3 (d, J = 2.8 Hz), 153.5,

153.1 (d, *J* = 252.0 Hz), 151.1 (d, *J* = 7.8 Hz), 150.6 (d, *J* = 255.4 Hz), 147.2, 147.0, 142.4, 136.9, 136.3 (d, *J* = 9.4 Hz), 135.7, 134.0, 133.8 (d, *J* = 17.1 Hz), 127.5 (dd, *J* = 7.4, 5.8 Hz), 127.2, 123.3, 117.9, 116.2, 112.4, 108.6 (dd, *J* = 8.5, 3.4 Hz), 107.9 (dd, *J* = 19.9, 6.7 Hz),51.4, 50.5, 50.1, 49.8, 49.1, 48.5, 45.4, 45.1, 41.6, 41.5, 31.40, 27.9, 22.6, 21.4, 14.9. HRMS (ESI): m/z calcd for $C_{45}H_{47}O_6N_{12}F_2$ $[M+H]^+$: 889.37041; found 889.37048.

Example 35: Synthesis of (E)-2-(2,6-dioxopiperidin-3-yl)-4-(4-(4-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)-4-oxobut-2-enoyl)piperazin-1-yl)isoindoline-1,3-dione (35)

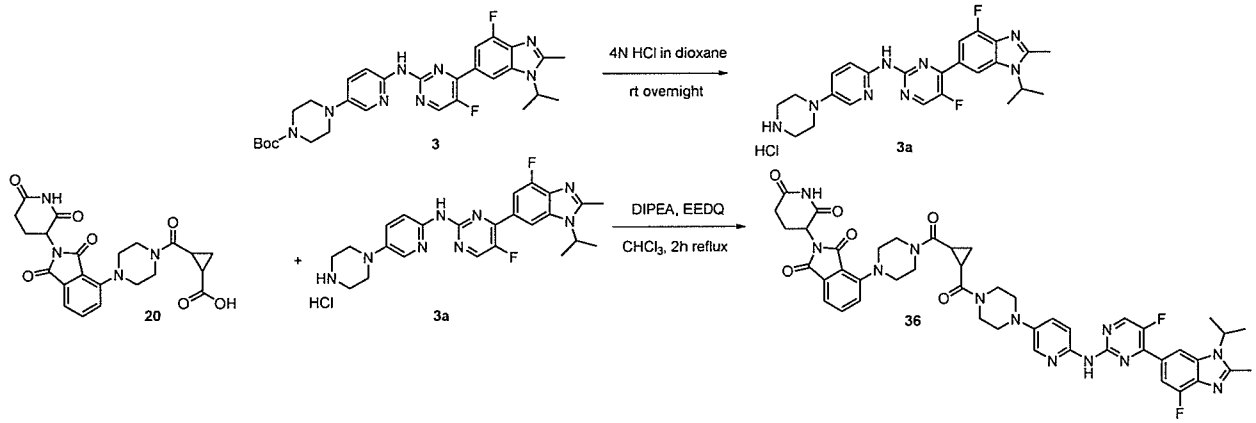


In a small round-bottom flask containing tert-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic **(19)** acid (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.1 mmol scale; 58 mg, 0.066 mmol, yield 66%, yellow solid.^{1}H NMR (500 MHz, $CDCl_3$) δ 9.75 (s, 1H), 8.92 (b, 1H), 8.43 (t, *J* = 13.1 Hz, 1H), 8.36 (d, *J* = 8.9 Hz, 1H), 8.16 (d, *J* = 22.0 Hz, 1H), 8.08 (d, *J* = 15.2 Hz, 1H), 7.77 (d, *J* = 11.3 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 7.41 (d, *J* = 6.2 Hz, 1H), 7.35 (d, *J* = 8.3 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 1H), 6.43 (b, 2H), 4.96 (dd, *J* = 11.9, 5.2 Hz, 1H), 4.72 (hept, *J* = 7.0 Hz, 1H), 3.90 – 3.66 (m, 8H), 3.37 – 3.32 (m, 4H), 3.14 – 3.11 (m, 4H), 2.91 – 2.88 (m, 1H), 2.82 – 2.77 (m, 2H), 2.68 (s,

3H), 2.13 – 2.10 (m, 1H), 1.67 (d, *J* = 6.8 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 171.9, 168.6, 167.2, 166.5, 165.4, 165.1, 155.3 (d, *J* = 2.8 Hz), 153.5, 153.2 (d,*J* = 252.0 Hz),151.30 (dd, *J* = 8.2, 2.2 Hz), 150.7 (d, *J* = 255.0 Hz), 149.8, 147.2, 147.0, 142.3, 137.0, 136.3 (d, *J* = 9.5 Hz), 135.7, 134.0, 133.9 (d, *J* = 17.1 Hz), 129.3, 128.8, 127.5, 123.4, 117.9, 116.2, 112.4, 108.7(dd, *J* = 8.5, 3.4 Hz), 107.9 (dd, *J* = 19.9, 6.7 Hz), 53.6, 51.45, 50.2, 49.8, 49.2, 48.6, 46.4, 46.1, 41.8, 41.3, 31.4, 22.6, 21.4, 18.6, 17.31, 15.0. HRMS (ESI): m/z calcd for $C_{45}H_{45}O_6N_{12}F_2[M+H]^+$: 887.35476; found 887.35504.

Example 36: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-(4-(2-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazine-1-carbonyl)cyclopropane-1-carbonyl)piperazin-1-yl)isoindoline-1,3-dione (36)

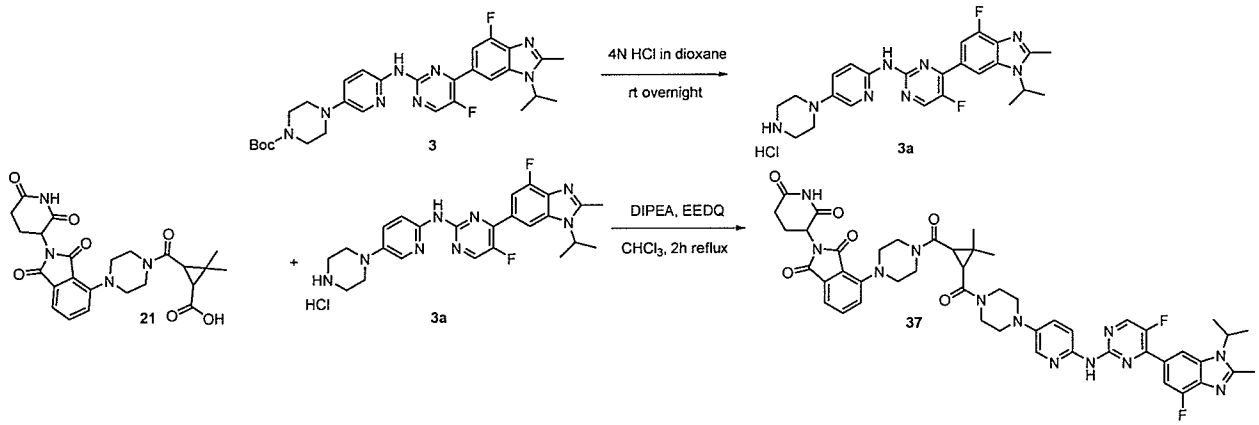


In a small round-bottom flask containing *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic **(20)** acid (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.1 mmol scale; 56 mg, 0.062 mmol, yield 62%, yellow solid.^{1}H NMR (500 MHz, $CDCl_3$) δ 9.36 (s, 1H), 8.64 (b, 1H), 8.41 (s, 1H), 8.33 (d, *J* = 8.2 Hz, 1H), 8.16 (s, 1H), 8.08 (s, 1H), 7.78 (d, *J* = 11.5 Hz, 1H), 7.60 (t, *J* = 7.5 Hz, 1H), 7.42 (d, *J* = 7.0 Hz, 1H), 7.35 (d,

J = 8.4 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 1H), 4.96 (dd, *J*= 11.9, 5.0 Hz, 1H), 4.72 (hept, *J* = 7.0 Hz, 1H), 3.90 – 3.79 (m, 8H), 3.44 – 3.10 (m, 8H), 2.91 – 2.74 (m, 3H), 2.68 (s, 3H), 2.12 – 2.10 (m, 3H), 1.82 – 1.75 (m, 2H), 1.70 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.6, 168.5, 168.0, 167.9, 167.2, 166.6, 155.3 (d, *J* = 2.8 Hz), 153.6, 153.3 (d, *J* = 252.0 Hz), 151.3 (dd, *J* = 8.2, 2.2 Hz), 150.8 (d, *J* = 255.2 Hz), 149.3, 147.0 (d, *J* = 27.2 Hz), 142.6, 137.0, 136.3 (d, *J* = 9.3 Hz), 135.8, 134.1, 133.9 (d, *J* = 17.2 Hz), 127.4, 123.4, 117.8, 116.2, 112.5, 108.8 (dd, *J* = 8.5, 3.4 Hz), 108.0 (dd, *J* = 20.1, 7.3 Hz), 51.9, 50.2, 49.9, 49.2, 48.6, 45.8, 45.5, 41.8, 31.4, 22.7, 21.5, 21.2 21.0, 15.0, 11.5. HRMS (ESI): m/z calcd for $C_{46}H_{47}O_6N_{12}F_2$ $[M+H]^+$: 901.37041; found 901.37048.

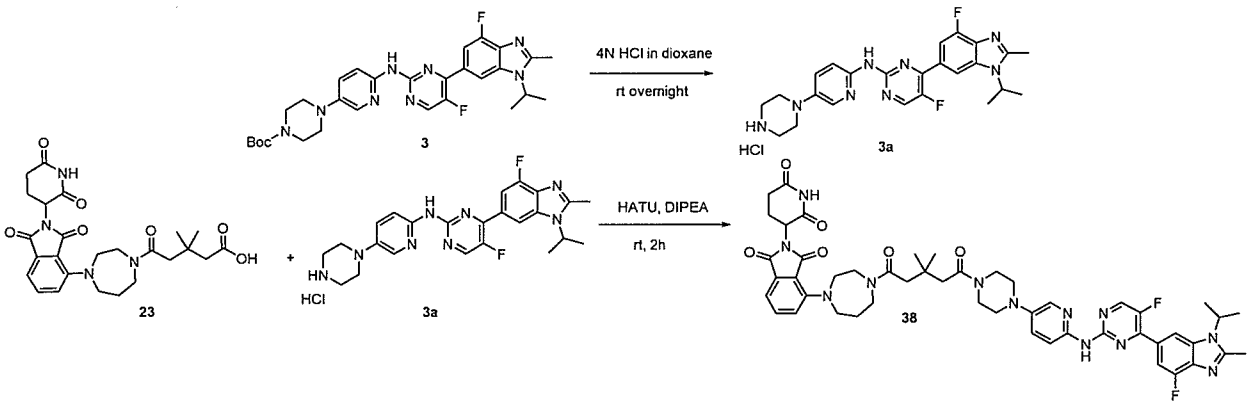
Example 37: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-(4-(3-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazine-1-carbonyl)-2,2-dimethylcyclopropane-1-carbonyl)piperazin-1-yl)isoindoline-1,3-dione (37)



In a small round-bottom flask containing *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic **(21)** acid (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.1 mmol scale; 60 mg, 0.065 mmol, yield 65%, yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.97 (b, 1H), 8.99 (b, 1H), 8.41 (s, 1H), 8.33 (d, *J* = 8.3 Hz, 1H), 8.16 (s, 1H), 8.11 (s, 1H), 7.76 (d, *J* = 11.5 Hz, 1H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.41 (d, *J* = 7.1 Hz, 1H), 7.33 (d, *J* = 8.7 Hz, 1H), 7.16 (t, *J* = 10.6 Hz, 1H), 4.97 (dd, *J* = 12.0, 5.2 Hz, 1H), 4.71(hept, *J* = 7.0 Hz, 1H), 3.83 – 3.69 (m, 8H), 3.52 – 3.47 (m, 2H), 3.34 – 3.31 (m, 1H), 3.26 – 3. 18 (m, 4H), 3.06 – 2.99 (m, 2H), 2.90 – 2.74 (m, 3H), 2.67 (s, 3H), 2.13– 2.10 (m, 1H), 1.82 – 1.80 (m, 2H), 1.68 (d, *J* = 6.8 Hz, 6H), 1.37 (s, 3H), 1.29 (s, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 168.6, 167.9, 167.8, 167.2, 166.6, 155.3 (d, *J* = 2.8 Hz), 153.5, 153.3 (d, *J* = 252.0 Hz), 151.3 (dd, *J* = 8.2, 2.2 Hz), 150.8 (d, *J* = 255.2 Hz), 150.0, 147.0 (d, *J* = 27.2 Hz), 146.8, 142.5, 136.9, 136.3 (d, *J* = 9.3 Hz), 135.7, 134.1, 133.8 (d, *J* = 17.2 Hz), 127.5 (dd, *J* = 7.4, 5.8 Hz), 127.2, 123.4, 117.7, 116.1, 112.5, 108.8 (dd, *J* = 8.5, 3.4 Hz), 108.0 (dd, *J* = 20.1, 7.3 Hz), 52.0, 50.2, 50.0, 49.1, 48.6, 45.7, 45.3, 41.2, 31.5, 31.4, 27.9, 24.6, 22.6, 21.4, 17.1, 15.0. HRMS (ESI): m/z calcd for $C_{48}H_{51}O_6N_{12}F_2$ $[M+H]^+$: 929.40171; found 929.40173.

Example 38: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-(4-(5-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazin-1-yl)-3,3-dimethyl-5-oxopentanoyl)-1,4-diazepan-1-yl) isoindoline-1,3-dione (38)

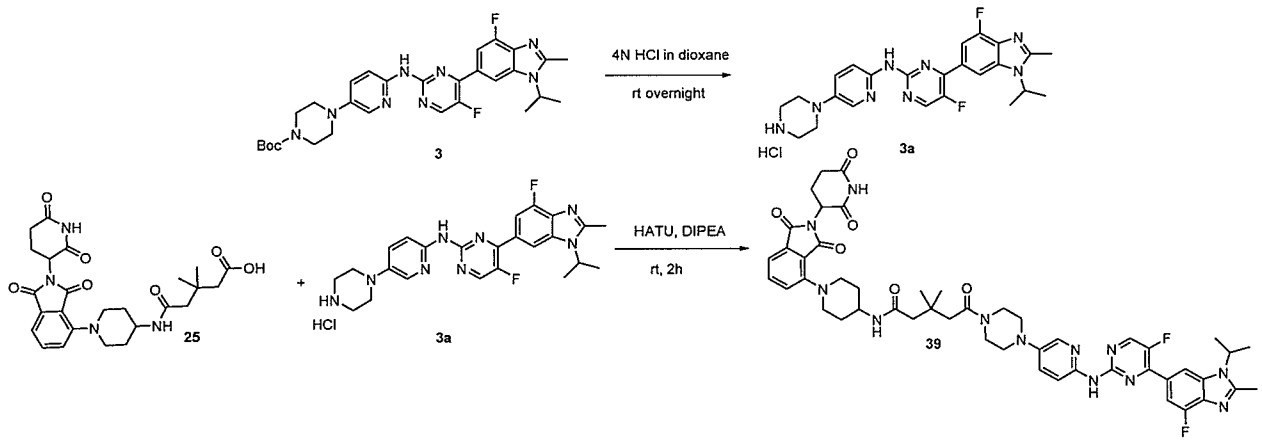


Tert-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) was deprotected with 4M HCl in dioxane. Stirring at rt overnight. The reaction mixture was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt (**3a**) (1.185 equiv., 0.71 mmol) was suspended in DMF (3 ml). Under stirring DIPEA (2

equiv., 1.42 mmol) was added, followed by the addition of the carboxylic acid **(23)** (1 equiv., 0.62 mmol) and HATU (1.2 equiv., 0.86 mmol). The reaction mixture was stirred at room temperature for 2h. The crude reaction was purified by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM) to get a yellow solid, which was recrystallized with 20% EtOAc in ethanol.

0.62 mmol scale; 220 mg, 0.23 mmol, yield 38 %, yellow solid. In the NMR two rotamers can be seen; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (d, J = 3.6 Hz, 1H), 9.86 (s, 1H), 8.65 (d, J = 3.7 Hz, 1H), 8.27 (s, 1H), 8.07 (ddd, J = 10.0, 8.1, 2.6 Hz, 2H), 7.68 (d, J = 12.1 Hz, 1H), 7.62 – 7.54 (m, 1H), 7.47 – 7.40 (m, 1H), 7.31 (d, J = 8.7 Hz, 1H), 7.20 (dd, J = 16.2, 6.9 Hz, 1H), 5.10 (ddd, J = 12.9, 10.3, 5.3 Hz, 1H), 4.84 (dt, J = 13.7, 6.7 Hz, 1H), 4.01 (dt, J = 36.3, 10.7 Hz, 2H), 3.78 – 3.38 (m, 12H), 3.07 (d, J = 18.9 Hz, 4H), 2.65 (s, 3H), 2.37 (dd, J = 79.1, 8.9 Hz, 4H), 2.03 – 1.99 (m, 2H), 1.96 – 1.80 (m, 2H), 1.63 (d, J = 6.8 Hz, 6H), 1.01 (d, J = 26.9 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.2, 171.9, 171.6 , 170.6, 168.8, 167.3, 166.9, 155.4, 154.3, 153.6, 152.3, 151.8, 151.3, 149.8, 149.1, 148.7, 147.3, 147.0, 142.5, 136.7, 136.4, 135.3, 135.1, 134.6, 134.0, 127.6, 127.4, 122.8, 114.2, 113.8, 113.6, 112.5, 108.8, 108.1, 60.4, 55.0, 53.3, 52.2, 51.8, 50.4, 50.1, 49.3, 48.8, 48.6 – 48.5 (m), 47.7, 45.8, 44.4, 41.8, 41.5, 41.2, 33.5, 31.5, 29.2 – 28.7 (m), 28.4, 26.4, 22.7, 21.5, 21.1, 15.0, 14.2.

Example 39: Synthesis of N-(1-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl) piperidin-4-yl)-5-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazin-1-yl)-3,3-dimethyl-5-oxopentanamide (39)

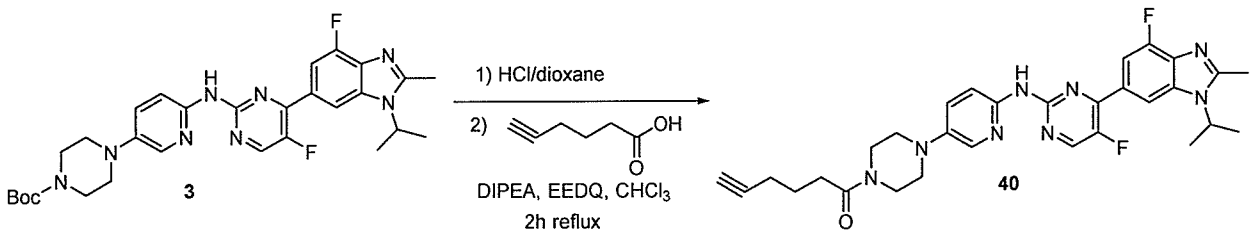


Tert-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) was

deprotected with 4M HCl in dioxane. Stirring at rt overnight. The reaction mixture was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1.185 equiv., 1 mmol) was suspended in DMF (5 ml). Under stirring DIPEA (2 equiv., 1.68 mmol) was added, followed by the addition of the carboxylic acid **(25)** (1equiv, 0.84 mmol) and HATU (1.2 equiv., 1.1 mmol). The reaction mixture was stirred at room temperature for 2h. The crude reaction was purified by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM) to get a yellow solid, which was recrystallized with 20% EtOAc in ethanol.

0.84 mmol scale; 301 mg, 0.31 mmol, yield 39%, yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.12 (s, 1H), 8.78 (s, 1H), 8.43 (d, J = 3.6 Hz, 1H), 8.37 (d, J = 9.1 Hz, 1H), 8.18 (s, 1H), 8.11 (d, J = 2.5 Hz, 1H), 7.91 (d, J = 7.7 Hz, 1H), 7.80 (d, J = 11.6 Hz, 1H), 7.58 (t, J = 7.8 Hz, 1H), 7.38 (d, J = 7.0 Hz, 2H), 7.18 (d, J = 8.5 Hz, 1H), 4.98 (dd, J = 12.1, 5.3 Hz, 1H), 4.74 (dt, J = 13.9, 6.9 Hz, 1H), 4.07 – 3.58 (m, 8H), 3.50 (s, 1H), 3.23 – 3.04 (m, 7H), 2.93 – 2.71 (m, 4H), 2.70 (s, 3H), 2.47 – 2.28 (m, 4H), 1.72 (d, J = 6.9 Hz, 6H), 1.13 (d, J = 5.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.1, 170.9, 168.8, 167.4, 166.9, 155.3, 154.3, 153.6, 152.3, 151.8, 151.3, 150.5, 149.8, 147.2, 142.2, 137.1, 137.0, 137.0, 136.1, 135.6, 134.1, 127.6, 123.6, 117.3, 115.5, 112.6, 108.8, 108.0, 50.7, 50.4, 49.9, 49.2, 48.6, 47.3, 46.5, 45.3, 41.4, 40.7, 34.4, 32.0, 31.4, 29.6, 29.3, 22.8, 21.5, 15.0.

Example 40: Synthesis of 1-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)hex-5-yn-1-one (40)

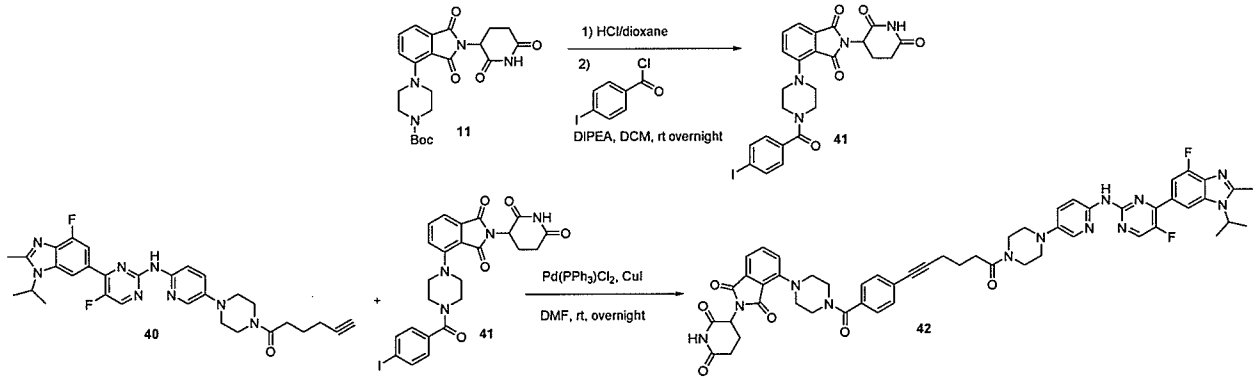


In a small round-bottom flask containing *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling.

The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic acid (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.5 mmol scale; 200 mg, 0.36 mmol, yield 72 %, yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (s, 1H), 8.42 (d, *J* = 3.7 Hz, 1H), 8.34 (d, *J* = 9.1 Hz, 1H), 8.16 (d, *J* = 0.9 Hz, 1H), 8.07 (d, *J* = 2.6 Hz, 1H), 7.78 (d, *J* = 11.6 Hz, 1H), 7.35 (dd, *J* = 9.1, 2.9 Hz, 1H), 4.73 (hept, *J* = 7.0 Hz, 1H), 3.80 – 3.78 (m, 2H), 3.68 – 3.66 (m, 2H), 3.15 – 3.09 (m, 4H), 2.69 (s, 3H), 2.52 (t, *J* = 7.4 Hz, 2H), 2.31 (td, *J* = 6.7, 2.6 Hz, 2H), 1.99 (t, *J* = 2.6 Hz, 1H), 1.91 (dd, *J* = 14.2, 7.0 Hz, 2H), 1.70 (d, *J* = 7.0 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 170.7, 155.3, 153.5, 153.3 (d, *J* = 252.3 Hz), 151.4, 150.5 (d, *J* = 255.3 Hz), 147.1 (d, *J* = 17.7 Hz), 147.0, 142.5, 137.3, 136.4 (d, *J* = 9.4 Hz), 134.0 (d, *J* = 17.0 Hz), 127.4 (dd, *J* = 7.4, 5.8 Hz), 127.3, 112.4, 108.7 (dd, *J* = 9.2, 2.7 Hz), 108.0 (dd, *J* = 20.2, 6.5 Hz), 83.71, 69.1, 50.4, 50.1, 48.6, 45.3, 41.4, 31.5, 23.8, 21.4, 18.0, 15.01.HRMS (ESI): m/z calcd for $C_{30}H_{33}ON_8F_2$ $[M+H]^+$: 559.27399; found 559.27393.

Example 42: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-(4-(4-(6-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)-6-oxohex-1-yn-1-yl)benzoyl)piperazin-1-yl)isoindoline-1,3-dione (42)

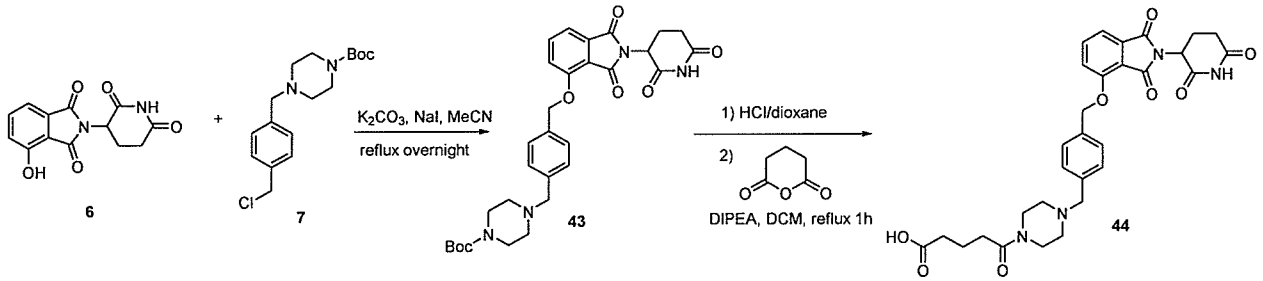


Tert-butyl 4-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate **(11)** was deprotected with 4 N HCl in dioxane (3 ml for 0.5 mmol scale). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt of 2-(2,6-dioxopiperidin-3-yl)-4-(piperazin-1-yl) isoindoline-1,3-dione was suspended in DCM (3 ml) at 0°C and DIPEA (1.1 mmol, 2.2 equiv.) was added, followed by the addition of 4-

iodobenzoyl chloride (0.6 mmol, 1.2 equiv.). The reaction mixture was stirred at 0°C for 30 min and then rt overnight. Then, it was diluted with DCM and washed with H_2O (x3). The organic phase was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product **(41)** was used directly in the next step, as follows: in a dry microwave vial under nitrogen, the alkyne intermediate **(40)** (0.1 mmol, 1equiv.) and the iodide intermediate **(41)** (0.1 mmol, 1equiv.) were added as solutions in 3ml of dry DMF, followed by the addition of triethylamine (3ml). Then, $Pd(PPh_3)Cl_2$ (10%) and CuI (15%) were added. The sealed microwave vial was stirred rt overnight under nitrogen. The reaction mixture was quenched with saturated NH_4Cl (10 ml) and extracted with DCM (3 x 10 ml). The combined organic phases were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude was purified by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

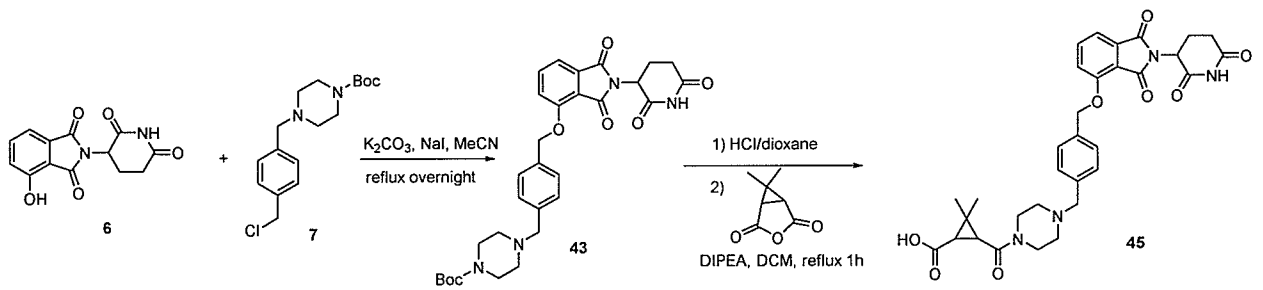
0.1 mmol scale; 70 mg, 0.07 mmol, yield 70%, yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.35 (b, 1H), 8.96 (b, 1H), 8.41 (d, *J* = 3.6 Hz, 1H), 8.35 (d, *J* = 9.0 Hz, 1H), 8.16 (s, 1H), 8.07 (s, 1H), 7.77 (d, *J* = 11.6 Hz, 1H), 7.61 (t, *J* = 7.8 Hz, 1H), 7.44 – 7.41 (m, 3H), 7.34 – 7.32 (m, 3H), 7.16 (d, *J* = 8.4 Hz, 1H),4.95 (dd, *J* = 12.2, 5.3 Hz, 1H), 4.72 (hept, *J* = 7.0 Hz, 1H), 4.10 – 3.78 (m, 3H), 3.72 – 3.66 (m, 5H), 3.38 – 3.36 (m, 3H), 3.17 – 3.09 (m, 5H), 2.92 – 2.88 (m, 1H), 2.81 – 2.74 (m, 2H), 2.68 (s, 3H), 2.58 – 2.52 (m, 4H), 2.11 – 2.09 (m, 1H), 2.00 – 1.95 (m, 2H), 1.69 (d, *J* = 6.9 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 172.3, 170.8, 169.9, 168.6, 167.1, 166.6, 155.3 (d, *J* = 2.8 Hz), 153.5, 153.3 (d, *J* = 252.0 Hz), 151.2 (dd, *J* = 8.2, 2.2 Hz), 150.8 (d, *J* = 255.2 Hz), 149.8, 147.3, 147.0 (d, *J* = 27.2 Hz), 142.4, 137.8, 137.0, 136.2 (d, *J* = 9.3 Hz), 135.8, 134.4, 134.1, 133.9 (d, *J* = 17.2 Hz), 131.7, 131.6, 128.8, 127.5 (dd, *J* = 7.4, 5.8 Hz), 127.2, 125.5, 123.4, 118.1, 116.5, 116.2, 112.5, 108.8 (dd, *J* = 8.5, 3.4 Hz), 108.0 (dd, *J* = 20.1, 7.3 Hz),91.2, 80.8, 50.5, 49.9, 49.2, 48.6, 45.3, 41.4, 31.5, 24.0, 22.7, 21.4, 18.9, 15.0. HRMS (ESI): m/z calcd for $C_{54}H_{53}O_6N_{12}F_2$ $[M+H]^+$: 1003.41736; found 1003.41742.

Example 44: Synthesis of 5-(4-(4-(((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)methyl)benzyl)piperazin-1-yl)-5-oxopentanoic acid (44)



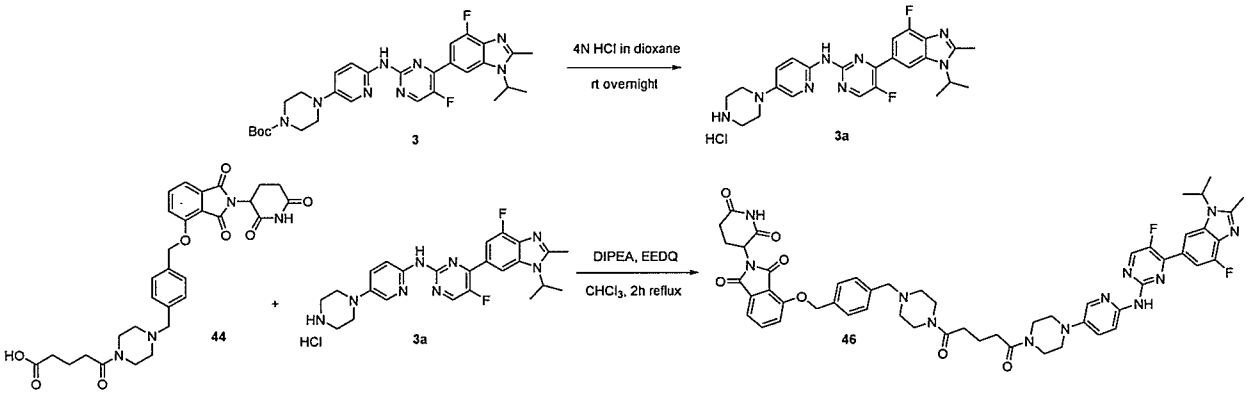
2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione **(6)** (1.02 mmol, 1 equiv.), *tert*-butyl 4-(4-(chloromethyl)benzyl)piperazine-1-carboxylate **(7)** (1.02 mmol, 1 equiv.), potassium carbonate (2.04 mmol, 2 equiv.), and sodium iodide (1.02 mmol, 1 equiv.) were suspended in acetonitrile (10 ml). The reaction mixture was heated at reflux overnight. Solvent was removed under reduced pressure. The residue was diluted with DCM and washed with H_2O (x3). The organic phase was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The obtained intermediate **(43)**, due to low solubility, was deprotected directly with 4N HCl in dioxane (5 ml). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt (0.2 mmol) was suspended in 3 ml DCM. DIPEA (2 equiv.) was added and after 10 min stirring at room temperature, glutaric anhydride (1.1 equiv.) was added. The reaction mixture was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The obtained intermediate **(44)** was used directly in the next step, due to low solubility.

Example 45: Synthesis of 3-(4-(4-(((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)methyl)benzyl)piperazine-1-carbonyl)-2,2-dimethylcyclopropane-1-carboxylic acid (45)



2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione **(6)** (1.02 mmol, 1 equiv.), *tert*-butyl 4-(4-(chloromethyl)benzyl)piperazine-1-carboxylate **(7)** (1.02 mmol, 1 equiv.), potassium carbonate (2.04 mmol, 2 equiv.), and sodium iodide (1.02 mmol, 1 equiv.) were suspended in acetonitrile (10 ml). The reaction mixture was heated at reflux overnight. Solvent was removed under reduced pressure. The residue was diluted with DCM and washed with H_2O (x3). The organic phase was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The obtained intermediate **(43)**, due to low solubility, was deprotected directly with 4N HCl in dioxane (5 ml). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt (0.2 mmol) was suspended in 3 ml DCM. DIPEA (2 equiv.) was added and after 10 min stirring at room temperature, caronic anhydride (1.1 equiv.) was added. The reaction mixture was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The obtained intermediate **(45)** was used directly in the next step, due to low solubility.

Example 46: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-((4-((4-(5-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)-5-oxopentanoyl)piperazin-1-yl)methyl)benzyl)oxy)isoindoline-1,3-dione (46)

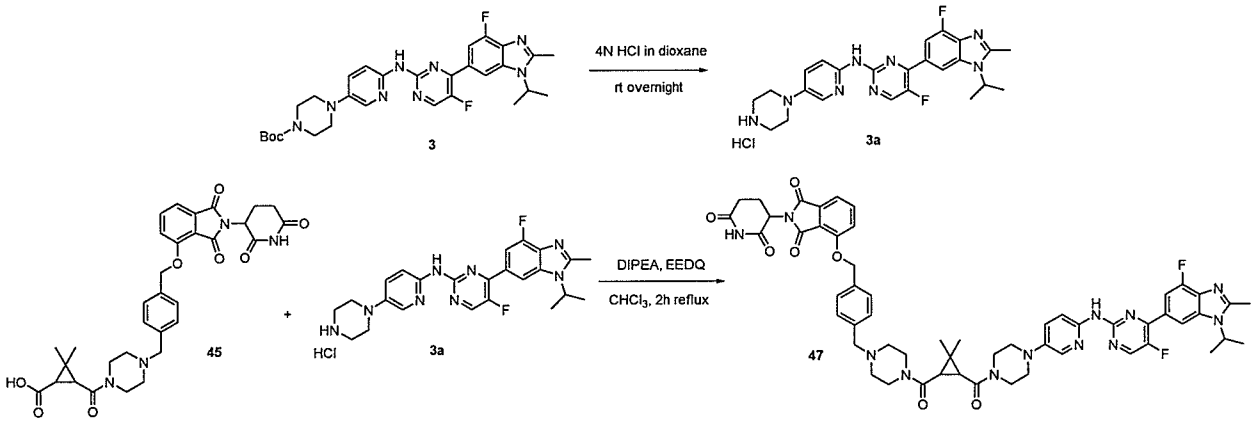


In a small round-bottom flask containing *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added

(x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic **(44)** acid (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.2 mmol scale: 122 mg, 0.12 mmol, yield 60% (over 2 steps), yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.92 (b, 1H), 8.72 (b, 1H), 8.41 (d, *J* = 3.5 Hz, 1H), 8.35 (d, *J* = 9.0 Hz, 1H), 8.18 – 8.16 (m, 1H), 8.06 (s, 1H), 7.78 (d, *J* = 11.5 Hz, 1H), 7.65 – 7.62 (m, 1H), 7.46 – 7.43 (m, 3H), 7.36 – 7.32 (m, 3H), 7.24 – 7.22 (m, 1H), 5.31 (s, 4H), 4.98 (dd, *J* = 12.3, 5.3 Hz, 1H), 4.73 (hept, *J* = 7.0 Hz, 1H), 3.78 – 3.76 (m, 2H), 3.66 – 3.62 (m, 4H), 3.53 – 3.49 (m, 4H), 3.11 – 3.08 (m, 4H), 2.94 – 2.77 (m, 3H), 2.68 (s, 3H), 2.64 – 2.39 (m, 6H), 2.16 – 2.13 (m, 1H), 1.97 – 1.95 (m, 2H), 1.70 (d, *J* = 6.9 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 171.1, 171.0, 168.5, 167.0, 165.6, 156.0, 155.3 (d, *J* = 2.8 Hz), 153.5, 153.3 (d, *J* = 252.0 Hz),151.2 (dd, *J* = 8.2, 2.2 Hz), 150.8 (d, *J* = 255.2 Hz), 147.0 (d, *J* = 27.2 Hz), 147.1, 136.8, 136.4, 136.3 (d, *J* = 9.3 Hz), 134.6, 133.8 (d, *J* = 17.2 Hz),129.6, 127.7, 127.1, 119.5, 117.6, 116.2, 112.50, 108.8 (dd, *J* = 8.5, 3.4 Hz), 108.0 (dd, *J* = 20.1, 7.3 Hz), 70.5, 62.4, 52.8, 50.5, 50.0, 49.2, 48.6, 45.3, 41.4, 32.4, 31.5, 29.7, 22.7, 21.4, 20.8, 15.0. HRMS (ESI): m/z calcd for $C_{54}H_{57}O_7N_{12}F_2$ $[M+H]^+$: 1023.44357; found 1023.44373.

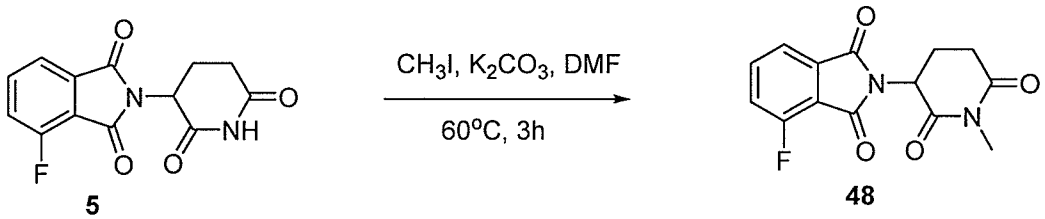
Example 47: Synthesis of 2-(2,6-dioxopiperidin-3-yl)-4-((4-((4-(3-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazine-1-carbonyl)-2,2-dimethylcyclopropane-1-carbonyl)piperazin-1-yl)methyl)benzyl)oxy)isoindoline-1,3-dione (47)



In a small round-bottom flask containing *tert*-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic **(45)** acid (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.2 mmol scale; 130 mg, 0.124 mmol, yield 62% (over 2 steps), yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.73 (b, 1H), 8.62 (b, 1H), 8.41 (d, *J* = 3.5 Hz, 1H), 8.34 (dd, *J* = 9.1, 4.2 Hz, 1H), 8.18 (s, 1H), 8.06 (s, 1H), 7.79 (d, *J* = 11.5 Hz, 1H), 7.67 – 7.62 (m, 1H), 7.47 – 7.42 (m, 3H), 7.37 – 7.33 (m, 3H), 7.25 – 7.24 (m, 1H), 5.34 – 5.30 (m, 2H), 4.98 (dd, *J* = 12.3, 5.3 Hz, 1H), 4.73 (hept, *J* = 7.0 Hz, 1H), 3.88 – 3.71 (m, 6H), 3.63 – 3.49 (m, 6H), 3.19 – 3.17 (m, 2H), 3.10 – 3.05 (m, 2H), 2.94 – 2.76 (m, 3H), 2.68 (s, 3H), 2.56 – 2.53 (m, 2H), 2.34 – 2.31 (m, 2H), 2.15 – 2.12 (m, 1H), 1.70 (d, *J* = 6.9 Hz, 6H), 1.35 (s, 3H), 1.26 (s, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 168.3, 167.9, 167.5, 167.0, 165.6, 156.0, 155.3 (d, *J* = 2.8 Hz), 153.5, 153.3 (d, *J* = 252.0 Hz), 151.2 (dd, *J* = 8.2, 2.2 Hz), 150.8 (d, *J* = 255.2 Hz), 147.0 (d, *J* = 27.2 Hz), 147.1, 136.8, 136.4, 136.3 (d, *J* = 9.3 Hz), 134.6, 133.8 (d, *J* = 17.2 Hz), 129.5, 127.4, 127.1, 119.5, 117.6, 116.2, 112.50, 108.8 (dd, *J* = 8.5, 3.4 Hz), 108.0 (dd, *J* = 20.1, 7.3 Hz),70.6, 70.5, 62.4, 52.8, 52.6, 50.6, 50.1, 49.8, 49.2, 48.6, 45.5, 41.4, 41.2, 32.0, 31.9, 31.5, 31.1, 31.0, 29.7, 28.0, 24.6, 22.7, 21.5, 17.1, 15.0. HRMS (ESI): m/z calcd for $C_{56}H_{59}O_7N_{12}F_2$ $[M+H]^+$:1049.45922; found 1049.45911.

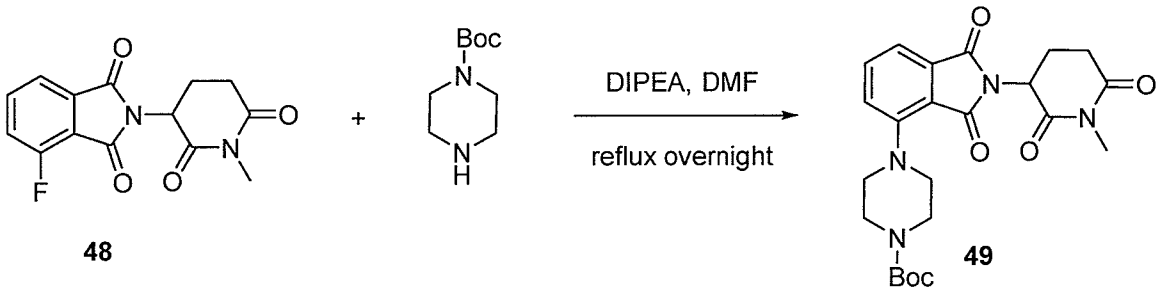
Example 48: Synthesis of 4-fluoro-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (48)



2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione **(5)** (2 mmol, 1.0 equiv.) and K_2CO_3 (2.4 mmol, 1.2 equiv.) were dissolved in DMF (8 ml). Then, iodomethane (2.4 mmol, 1.2 equiv.) was carefully added into the reaction mixture. The reaction mixture was stirred at 60 °C for 3 hours. The residue was diluted with EtOAc and was washed with H_2O (50 ml x 3). The organic phase was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified column chromatography PE : EtOAc (0-60% EtOAc in PE) to obtain compound **(48)**.

4 mmol scale: 985 mg, 3.4 mmol, yield 85%, white solid.^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 7.97 – 7.93 (m, 1H), 7.80 – 7.72 (m, 2H), 5.22 (dd, *J* = 13.1, 5.4 Hz, 1H), 3.02 (s, 3H), 2.99 – 2.92 (m, 1H), 2.80 – 2.75 (m, 1H), 2.58 – 2.51 (m, 1H), 2.11 – 2.05 (m, *J* = 12.9, 5.4, 2.5 Hz, 1H).^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 171.7, 169.4, 166.1, 164.0, 156.8 (d, *J* = 262.7 Hz),138.1, 133.4, 123.0 (d, *J*= 19.4 Hz), 120.1 (d, *J* = 12.3 Hz), 117.0 (d, *J* = 12.6 Hz), 49.7, 31.1, 26.67, 21.0. HRMS (ESI): m/z calcd for $C_{14}H_{12}O_4N_2F$ $[M+H]^+$: 291.07756; found 291.07776.

Example 49: Synthesis of *tert*-butyl 4-(2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate (49)



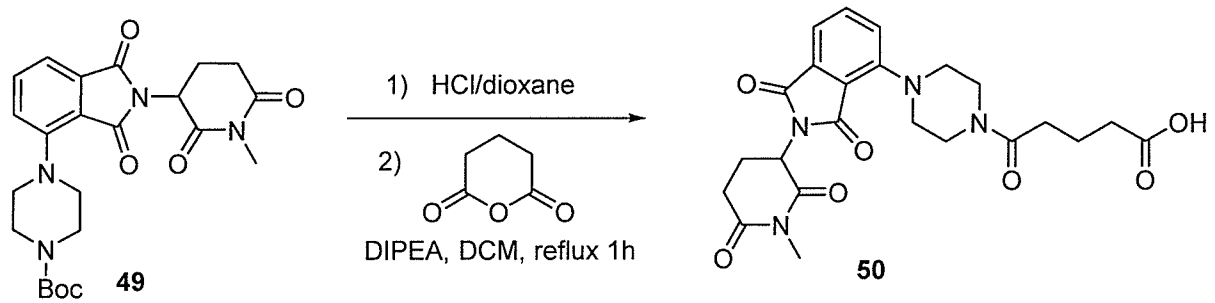
48 49

1-Boc-piperazine (2.2 mmol, 1.1 equiv.) was added to a stirred solution of 4-fluoro-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**48**) (2 mmol, 1 equiv.) and DIPEA (4 mmol, 2 equiv.) in DMF (1M). The reaction mixture was heated at reflux overnight. Then the mixture was cooled to room temperature, and it was diluted with diethylether and washed with H_2O (30 ml x 3). The organic phase was dried over $MgSO_4$, filtered and solvent was removed under reduced pressure. The residue was purified with column chromatography DCM – MeOH, 0 – 5% MeOH in DCM).

2 mmol scale; 730 mg, 1.6 mmol, yield 80%, light yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (dd, *J* = 8.3, 7.3 Hz, 1H), 7.42 (d, *J* = 7.1 Hz, 1H), 7.15 (d, *J* = 8.3 Hz, 1H), 4.95 (dd, *J* = 12.6, 5.3 Hz, 1H), 3.65 – 3.63 (m, 4H), 3.28 – 3.26 (m, 4H), 3.20 (s, 3H), 2.97

(dd, *J*= 13.2, 2.6 Hz, 1H), 2.78 – 2.70 (m, 2H), 2.10 – 2.05 (m, 1H), 1.47 (s, 9H).^{13}C NMR (126 MHz, $CDCl_3$) δ 171.1, 168.8, 167.4, 166.7, 154.7, 150.2, 135.6, 134.2, 123.3, 118.0, 116.1, 80.0, 49.9, 31.9, 28.4, 27.2, 21.9. HRMS (ESI): m/z calcd for $C_{23}H_{29}O_6N_4[M+H]^+$: 457.20816; found 457.208

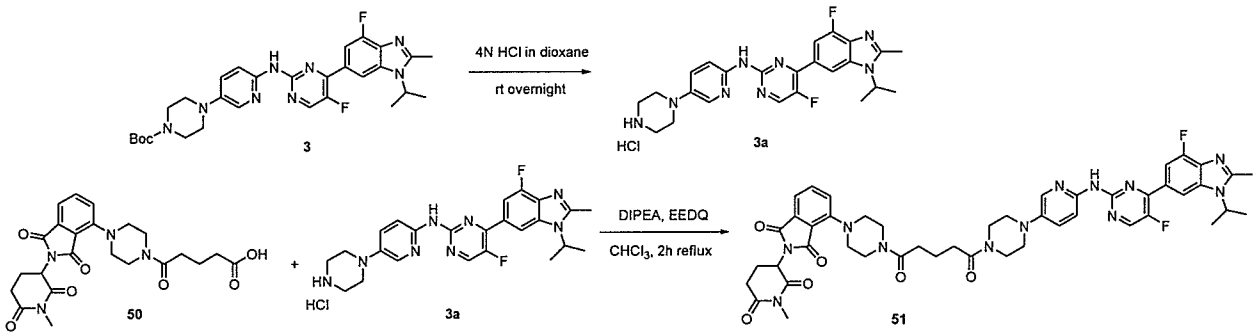
Example 50: Synthesis of 5-(4-(2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazin-1-yl)-5-oxopentanoic acid (50)



Tert-butyl 4-(2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)piperazine-1-carboxylate **(49)** was deprotected with 4 N HCl in dioxane (5 ml). Stirring rt overnight. The reaction mixture was dried under reduced pressure. Diethylether was added (x2) and was removed under reduced pressure. The HCl salt (0.98 mmol, 1 equiv.) was suspended in 5 ml DCM. DIPEA (1.96 mmol, 2 equiv.) was added and after 10 min stirring at room temperature, glutaric anhydride (1.08 mmol, 1.1 equiv.) was added. The reaction mixture was heated at 40 °C for 1h. The reaction was allowed to reach rt and it was extracted x2 (DCM – H_2O). The aqua phase was acidified with 2N HCl and it was extracted with DCM (x3). The combined organic phases were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure.

0.98 mmol scale: 300 mg, 0.64 mmol, yield 65%, yellow solid.^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (t, *J* = 7.8 Hz, 1H), 7.45 (d, *J* = 7.2 Hz, 1H), 7.16 (d, *J* = 8.4 Hz, 1H), 4.96 (dd, *J* = 12.3, 5.3 Hz, 1H), 3.88 – 3.80 (m, 2H), 3.72 – 3.70 (m, 2H), 3.39 – 3.31 (m, 2H), 3.30 – 3.27 (m, 2H), 3.21 (s, 3H), 3.00 – 2.97 (m, 1H), 2.81 – 2.74 (m, 2H), 2.50 – 2.46 (m, 4H), 2.11 – 2.06 (m, 1H), 1.99 (p, *J* = 7.0 Hz, 2H).^{13}C NMR (126 MHz, $CDCl_3$) δ 177.3, 171.1, 171.1, 168.8, 167.3, 166.7, 149.8, 135.8, 134.2, 123.3, 118.2, 116.5, 51.8, 50.3, 49.9, 45.6, 41.5, 33.1, 32.1, 31.9, 27.3, 22.0, 20.1. HRMS (ESI): m/z calcd for $C_{23}H_{27}O_7N_4[M+H]^+$: 471.18743; found 471.18735.

Example 51: Synthesis of 4-(4-(5-(4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl)pyrimidin-2-yl)amino)pyridin-3-yl)piperazin-1-yl)-5-oxopentanoyl)piperazin-1-yl)-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (51)



3 3a 50 3a 51

In a small round-bottom flask containing tert-butyl 4-(6-((5-fluoro-4-(4-fluoro-1-isopropyl-2-methyl-1H-benzo[d]imidazol-6-yl) pyrimidin-2-yl) amino) pyridin-3-yl) piperazine-1-carboxylate **(3)** (1equiv.) 4M HCl in dioxane was added. Stirring at rt overnight. The reaction mixture (orange suspension) was dried under vacuum. Diethylether was added (x2) and was removed under reduced pressure. The obtained HCl salt was used directly in the amide coupling. The HCl salt **(3a)** (1 equiv.) was suspended in $CHCl_3$ (0.1M). Under stirring DIPEA (2 equiv.) was added, followed by the addition of the carboxylic **(50)** acid (1 equiv.) and EEDQ (2 equiv.). The reaction mixture was heated at reflux for 2h. Then it was allowed to reach rt and was purified directly by column chromatography (DCM – MeOH, 0 – 10% MeOH in DCM).

0.2 mmol scale: 128 mg, 0.14 mmol, yield 70 %, yellow solid.^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 – 8.38 (m, 2H), 8.18 (s, 1H), 7.97 (s, 1H), 7.78 (d, *J* = 11.4 Hz, 1H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.45 (d, *J* = 7.2 Hz, 2H), 7.15 (d, *J* = 8.0 Hz, 1H), 4.96 (dd, *J* = 11.9, 4.9 Hz, 1H), 4.75 – 4.72 (m, 1H), 3.85 – 3.80 (m, 4H), 3.75 – 3.72 (m, 4H), 3.38 – 3.33 (m, 2H), 3.31 – 3.27 (m, 2H), 3.21 (s, 3H), 3.17 – 3.11 (m, 4H), 3.00 – 2.97 (m, 2H), 2.82 – 2.75 (m, 2H), 2.70 (s, 3H), 2.52 – 2.50 (m, 4H), 2.10 – 2.08 (m, 1H), 2.03 – 2.01 (m, 2H), 1.71 (d, *J* = 6.6 Hz, 6H).^{13}C NMR (126 MHz, $CDCl_3$) δ 171.2, 171.1, 168.8, 167.3, 166.7, 154.9 (d, *J* = 2.8 Hz), 153.6, 153.4 (d, *J* = 251.7 Hz), 151.6 (dd, *J* = 8.5, 1.8 Hz), 150.8 (d, *J* = 255.2 Hz), 149.8, 147.1, 146.5, 142.4, 136.3 (d, *J* = 9.3 Hz), 135.7, 134.1, 134.01 (d, *J* = 17.6 Hz), 128.4, 127.3 (dd, *J* = 7.4, 5.8 Hz), 123.3, 118.1, 116.4, 113.0, 108.8 (dd, *J* = 9.4, 3.2 Hz), 108.0 (dd, *J* = 20.3, 6.4 Hz),51.7, 50.5, 50.2, 49.8, 48.7, 45.6, 45.3, 41.4, 41.3, 32.5, 31.9, 27.3, 22.0, 21.4, 20.7, 15.0. HRMS (ESI): m/z calcd for $C_{47}H_{51}O_6N_{12}F_2$ $[M+H]^+$: 917.40171; found 917.40198.

B. Biological evaluation

Cell lines and cell culture

The neuroblastoma cell lines CHP-134, GI-M-EN, NGP, SJNB-1, SJNB-6 and TR14 and the breast cancer cell lines SK-BR-3 and MDA-MB-231 were acquired from the American Type Culture Collection or via historic collaborations. All cells, except MDA-MB-231, were cultured in DMEM high glucose (41965, Life Technologies), supplemented with 10% (v/v) FBS (F0804, Sigma-Aldrich), 2 mM L-glutamine (25030081, Life Technologies), 1x MEM-NEAA (11140050, Life Technologies) and 100 u/mL penicillin and 100 mg/mL streptomycin (15140122, Life Technologies) at 37°C in a humidified environment containing 5% CO2. MDA-MB-231 was cultured in Leibovitz L15 (11415, Life Technologies), supplemented with 10% (v/v) FBS (F0804, Sigma-Aldrich) and 100 u/mL penicillin and 100 mg/mL streptomycin (15140122, Life Technologies) at 37°C in a humidified environment containing 0% CO_2. The identity of all cell lines was verified with short tandem repeat (STR) profiling and the cultures were regularly checked for mycoplasma contamination.

KINOMEscan

The KINOMEscan was performed using the scanMAX kinase assay panel by Eurofins Discovery. Images were generated using TREE*spot*™ Software Tool and reprinted with permission from KINOME*scan*®, a division of DiscoveRx Corporation, © DISCOVERX CORPORATION 2010.

Immunoblotting

Cells were lysed in 1x Laemmli buffer (100 mM Tris-HCl, 4% (w/v) SDS, 20% (v/v) glycerol, pH 6.8) supplemented with cOmplete Protease Inhibitor Cocktail (11836153001, Roche Diagnostics). The cell lysates were passed through 23g and 27g Microlance needles (300800, 300635 (respectively), BD Biosciences) consecutively and heated at 50°C for 10 minutes. Protein concentration was determined using the DC protein assay (5000112, Bio-Rad) according to manufacturer's protocol. Protein samples were prepared by adding 5x loading buffer (250 mM Tris-HCl, 10% (w/v) SDS, 50% (v/v) glycerol, 500 mM DTT, 0.25% (w/v) bromophenol blue, pH 6.8), after which samples were

boiled at 95°C for 5 minutes. 10 ug protein was loaded on 10% Mini-PROTEAN TGX stain-free gels (4568036, Bio-Rad), which were run in 1x running buffer (5 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3). Proteins were transferred onto PVDF membranes (1704273, Trans-Blot Turbo RTA Transfer kit, Bio-Rad) using the Trans-Blot Turbo Transfer System (Bio-Rad) according to manufacturer's protocol (Mixed MW protocol). Membranes were blocked in 1x TBS-T (20 mM Tris-HCl, 150 mM NaCl, 0.1% (v/v) Tween20) supplemented with 2% (w/v) Amersham ECL Prime Blocking Reagent (RPN418, GE Healthcare Life Sciences) for 1 hour. Next, the membranes were incubated with primary antibody overnight at 4°C, followed by incubation with secondary antibody for 1 hour at RT. Membranes were incubated with Amersham ECL Prime Detection Reagent (RPN2232, GE Healthcare Life Sciences) and visualized by chemiluminescence on the ChemiDoc imager (Bio-Rad).

Antibodies

Antibody	Catalogue number	Dilution	Source	Molecular weight (kDa)	Company
CDK2 (55)	610146	1:5000	Mouse, mAb	33	BD Biosciences
CDK4 (DCS-31)	AHZ0202	1:1000	Mouse, mAb	30	Thermo Fisher Scientific
CDK6 (D4S8S)	13331	1:1000	Rabbit, mAb	36	Cell Signaling
CDK9 (C12F7)	2316	1:1000	Rabbit, mAb	42, 55	Cell Signaling
Alpha-tubulin (DM1A)	3873	1:10000	Mouse, mAb	52	Cell Signaling
Anti-Mouse-IgG (HRP conjugated)	NXA931	1:5000	Sheep	n.a.	GE Healthcare Life Sciences
Anti-Rabbit-IgG (HRP conjugated)	NA9340	1:5000	Donkey	n.a.	GE Healthcare Life Sciences

In vitro drug screening

40 µl of cells were seeded in 384-well microplates (3764, Corning Inc.) using the Multidrop combi reagent dispenser (Thermo Scientific). After overnight incubation to allow the cells to attach, cells were treated with various concentrations of PROTACs using either the

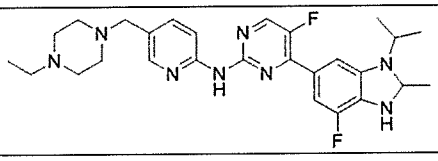
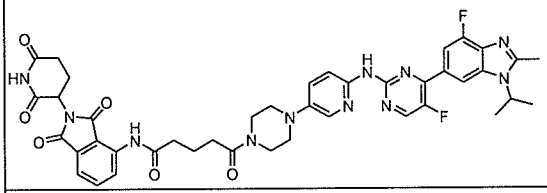
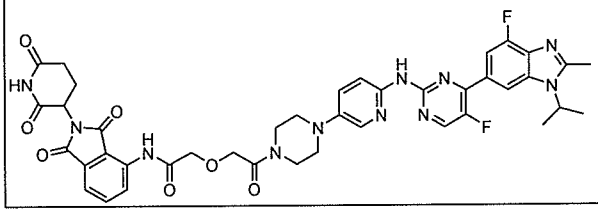
D300e digital dispenser (Tecan) or Echo 550 acoustic liquid handler (Labcyte Inc.) as part of our in-house high-throughput screening facility (Beckman-Coulter)[1]. Cell viability was determined after 72 hours using CellTiter-Glo 2.0 (G9242, Promega Corp.) according to manufacturer's protocol. The data was normalization to the DMSO-treated cells (defined as 100% viability) and the empty controls (0% viability) and curves were fit using the extension package drc in the statistic environment of R Studio (version 4.0.2)[2]. The half maximal concentration that inhibits the viability (IC50) and the area under the curve (AUC) were determined.

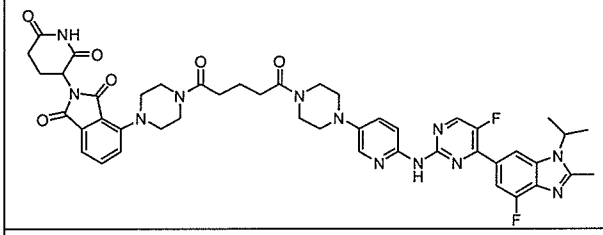
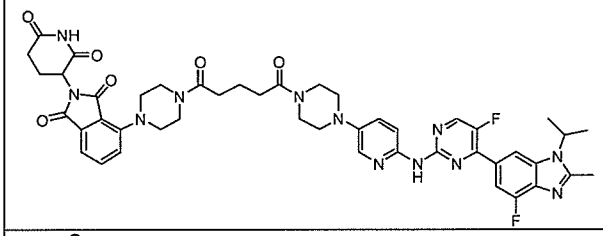
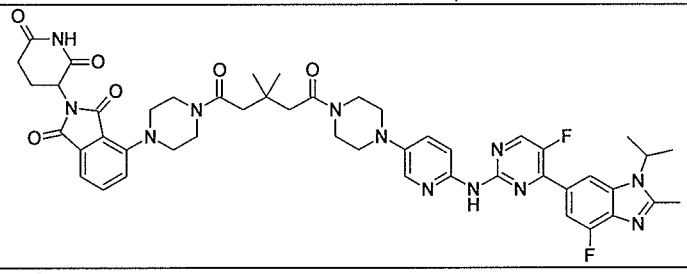
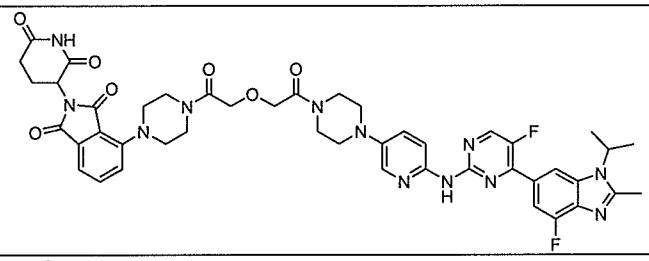
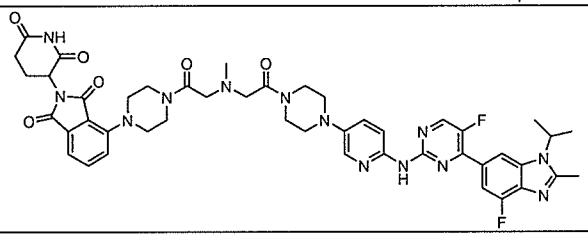
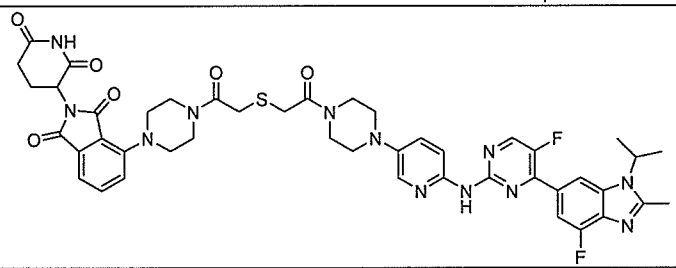
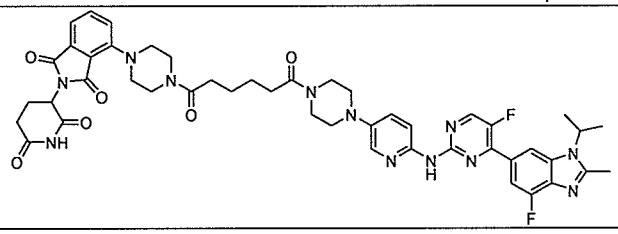
References

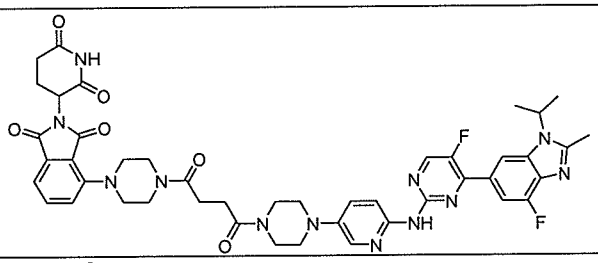
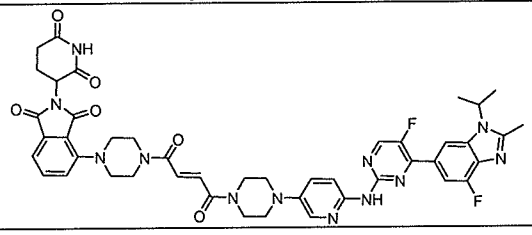
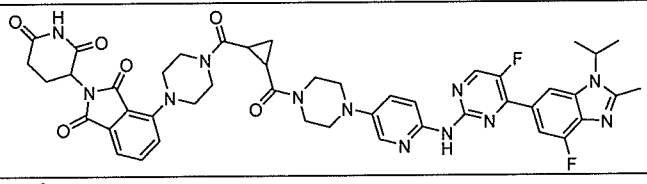
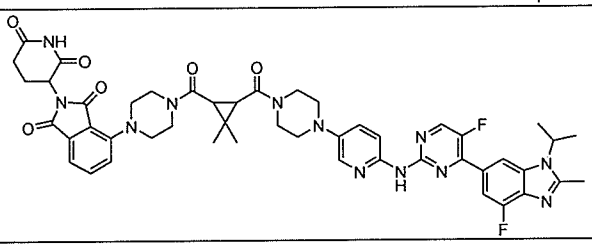
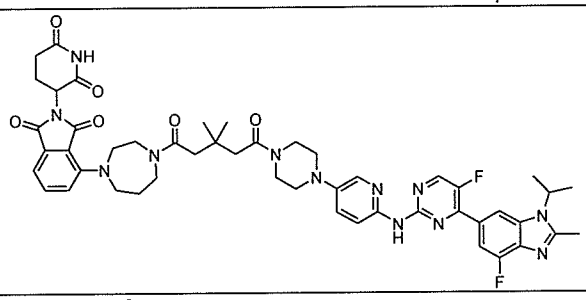
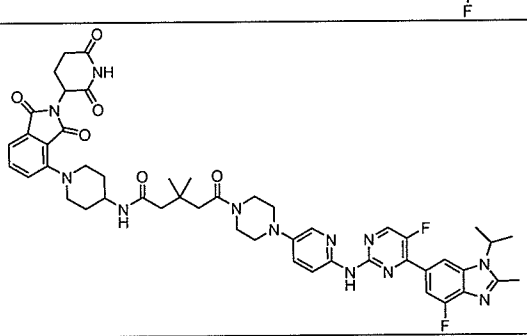
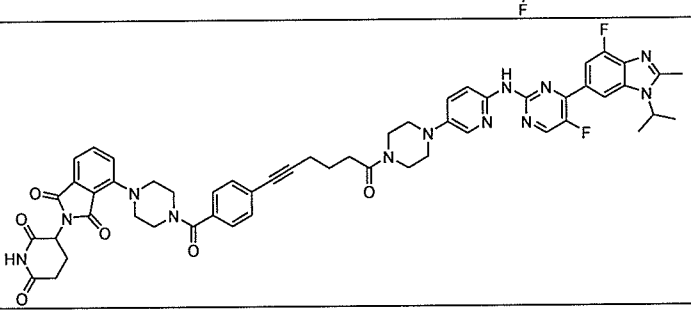
1. High-throughput screening (HTS) - Prinses Máxima Centrum - Research. https://research.prinsesmaximacentrum.nl/en/core-facilities/high-throughput-screening.

2. Ritz, C., Baty, F., Streibig, J. C. & Gerhard, D. Dose-Response Analysis Using R. PLoS One 10, e0146021 (2015).

Table 1: Overview of compounds described in the patent.

Structure	Example
	(2)
	(26)
	(27)

	(28) LOT1
	(28) LOT2
	(29)
	(30)
	(31)
	(32)
	(33)

	(34)
	(35)
	(36)
	(37)
	(38)
	(39)
	(42)

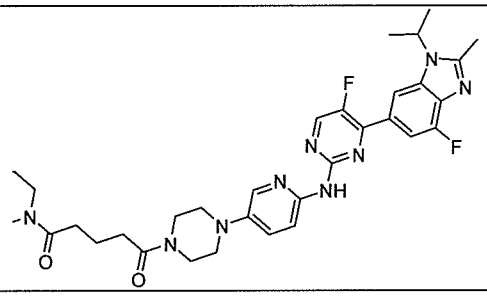
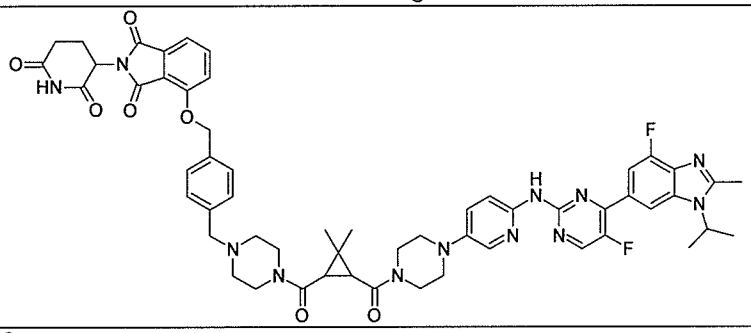
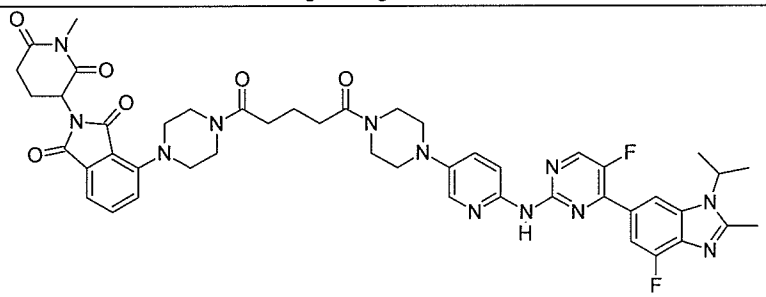
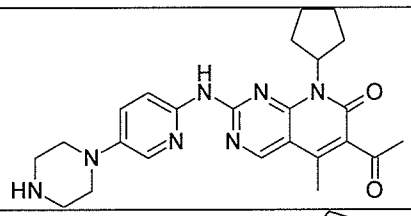
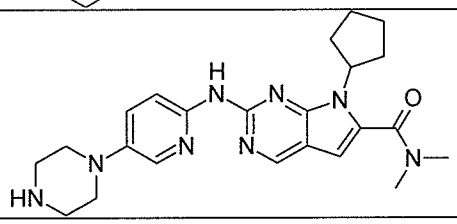
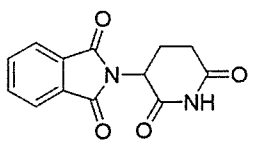
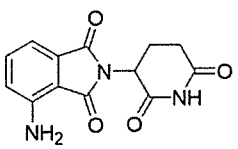
	(46)
	(47)
	(51)

Table 2. Compounds from the literature used in cell assays.

	Palbociclib
	Ribociclib
	Thalidomide
	Pomalidomide

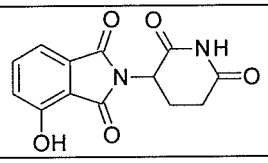
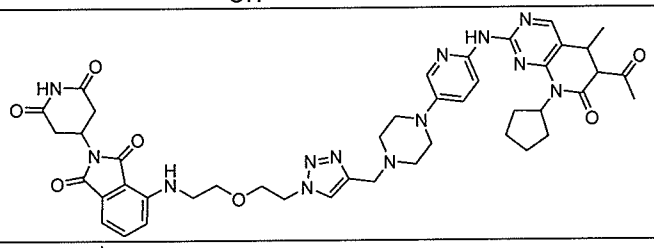
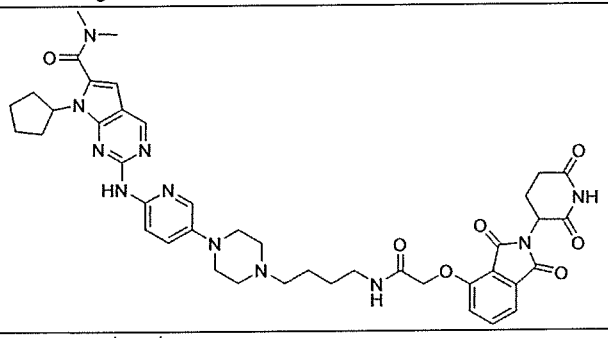
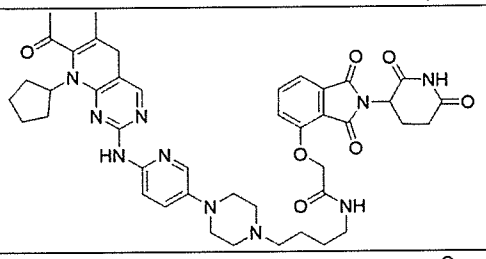
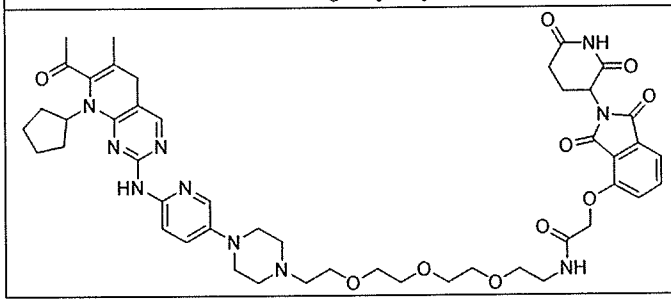
	Thalidomide-4-OH
	CP-10
	BSJ-04-132
	BSJ-03-204
	BSJ-03-123

Figure 1. Abemaciclib-based PROTACs exhibit differential efficacy depending on the neuroblastoma cell line. Heatmap showing the area-under-the-curve (AUC) of the cell viability curve of six neuroblastoma cell lines, after treatment with PROTACs and inhibitors for 72 h, using the Cell-Titer Glo 2.0 assay (n=1). In the heatmap three sets of abemaciclib-based PROTACs (numbered compounds) could be identified based on their efficacy (highly efficacious (bottom), moderately efficacious (middle), and non-efficacious (top). Each set shows an increased effect in cell lines with high CRBN E3 ligase expression (NGP, SJNB6, CHP134 (left)) compared to low CRBN E3 ligase expressing cell lines (SJNB1, GIMEN, TR14 (right)), except in case of the non-efficacious set.

CDK4/6 inhibitors (abemaciclib **(2)**, palbociclib, ribociclib), commercially available CDK4/6 PROTACs (compounds starting with BSJ and CP-10), CRBN inhibitors (pomalidomide, thalidomide(-4-OH)) and an inactive PROTAC **(51)** (inactive variant of **(28)**) were taken along for comparison. The CDK4/6 inhibitors are either highly or moderately efficacious, whereas the latter two are non-efficacious. **(51)** is moderately efficacious but is outperformed by its active counterpart **(28)**.

Figure 2. Abemaciclib-based PROTACs reduce cell viability more effectively than CDK4/6 inhibitors and commercially available CDK4/6 PROTACs in neuroblastoma cell lines. A-B. Cells were treated for 72 h, after which cell viability was determined using Cell-Titer Glo 2.0 (n=1). Curve fitting was performed using Graphpad Prism 9, after which the absolute IC50 was determined. A. Differential effect on cell viability upon (**29**) treatment in 6 neuroblastoma cell lines. NGP, SJNB6 and CHP134 are highly responsive, whereas SJNB1 has a moderate response and TR14 and GIMEN are relatively unresponsive. B. Top. **(29)** (gray circles) and other abemaciclib-based PROTACs (numbered compounds, gray circles) outperform abemaciclib **(2)** (black circles) and the other CDK4/6 inhibitors ribociclib and palbociclib (diamonds and squares, respectively) in NGP. Bottom. **(29)** also outperforms commercially available CDK4/6 PROTACs (compounds starting with BSJ and CP-10, gray squares and diamonds) in NGP, that contain ribociclib and palbociclib as mother compound (shape matched to PROTACs). The commercially available PROTACs exert smaller effects than the CDK4/6 inhibitors in the neuroblastoma line.

Figure 3. Degradation of CDK4/6/9 upon treatment with abemaciclib-based PROTACs in a concentration- and time-dependent manner. A-C. Target proteins were detected in cell lysate of SJNB6, a neuroblastoma cell line, via immunoblotting (n=1). A. (**29**) (top) and (**28, lot 1)** (bottom) induce CDK4 and CDK6 degradation after 24 h treatment at concentrations as low as 0.1 and 10 nM, respectively. B. CDK9 is degraded upon treatment with (**29**) and (**28, lot 1)** for 24 h at 10 and 1000 nM, respectively, whilst CDK2 is unaffected. C. Time series showing differential, but persistent CDK4/6/9 degradation patterns after **(29)** treatment at 100 nM.

Figure 4. (**29**) exhibits broad binding selectivity for kinases, including CDK4 and CDK9. KINOMEscan TREEspot maps depicting the binding profile of (**29**) at 100nM and 1000nM, probed against 468 wildtype and mutant kinases (scanMAX kinase panel, n=1).

Figure 5. Abemaciclib-based PROTACs reduce cell viability more effectively than CDK4/6 inhibitors and commercially available CDK4/6 PROTACs in HER2-positive (HER2+) and triple negative (TNBC) breast cancer cell lines. Breast cancer cells (**A.** SK-BR-3 (HR-,HER2+), and **B.** MDA-MB-231 (TNBC)) were treated for 72 h, after which cell viability was determined using Cell-Titer Glo 2.0 (n=4). Curve fitting was performed using Graphpad Prism 9, after which the absolute IC50 was determined. **A-B. (29)** (gray circles) and other abemaciclib-based PROTACs (**(34)** and **(28, lot 1)**, gray circles) outperform abemaciclib (black circles) and the other CDK4/6 inhibitors ribociclib and palbociclib (diamonds and squares, respectively), as well as commercially available CDK4/6 PROTACs (compounds starting with BSJ and CP-10, gray squares and diamonds), that contain ribociclib and palbociclib as mother compound (shape matched to PROTACs) in **A.** SK-BR3 and **B**. MDA-MB-231. The commercially available PROTACs exert smaller effects than the CDK4/6 inhibitors in the breast cancer cell lines.

The PROTAC degraders described herein are triple targeting as they potently induce degradation of CDK4, CDK6, and CDK9. CDKs are generally involved in regulation of the cell cycle (e.g. CDK4/6) or transcription regulation (e.g. CDK9). As such, deregulated CDK signaling is often implicated in cancer to allow for uncontrolled proliferation (Roskoski. Cyclin-Dependent Protein Serine/Threonine Kinase Inhibitors as Anticancer Drugs. Pharmacol. Res. 2019. 139. 471-488.; Chou et al. Transcription-Associated Cyclin-Dependent Kinases as Targets and Biomarkers for Cancer Therapy. Cancer Discov. 2020. 10. 351-370.). Therefore, hampering CDK signaling shows great promise for cancer therapy, as illustrated by the FDA approval of CDK4/6 inhibitors to treat hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer.

CDK4 and CDK6 are directly involved in cell cycle progression and as such, they are the regulators with the most direct effect on cell division (Roskoski. Cyclin-Dependent Protein Serine/Threonine Kinase Inhibitors as Anticancer Drugs. Pharmacol. Res. 2019. 139.

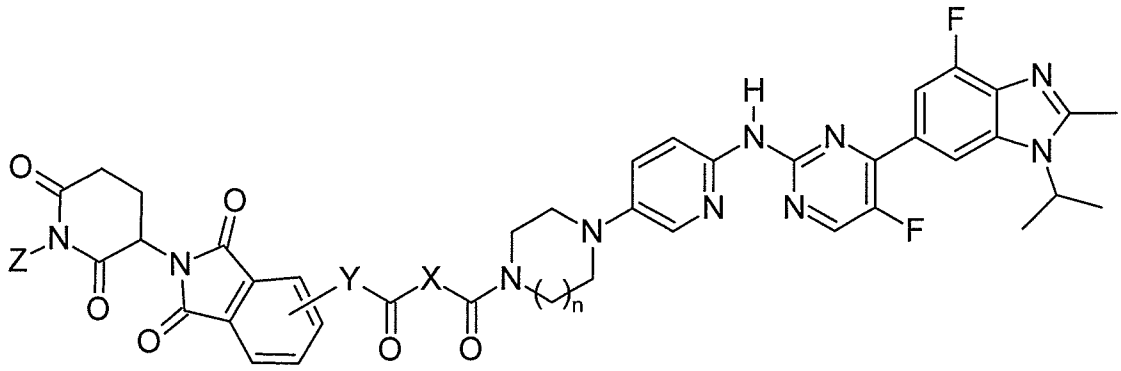
471-488.). CDK9 regulates transcription elongation and shows particular importance in cancers dependent on oncogenic transcriptional networks (e.g. MYC(N)-driven cancers) that drive cancer proliferation (Chou et al. Transcription-Associated Cyclin-Dependent Kinases as Targets and Biomarkers for Cancer Therapy. Cancer Discov. 2020. 10. 351-370.). CDK9 also regulates the expression of anti-apoptotic proteins, contributing to resistance to cell death (Whittaker et al. Molecular Profiling and Combinatorial Activity of CCT068127: a Potent CDK2 and CDK9 Inhibitor. Mol. Oncol. 2018. 12. 287-304.).

Resistance mechanisms associated with CDK inhibitors demand further development of treatment modalities, such as PROTAC degraders. Additionally, degradation of CDK9 was shown to have greater impact on hampering the MYC-dependent oncogenic transcriptional network and subsequently, cancer cell proliferation than CDK9 inhibition (Toure et al. Targeted Degradation of CDK9 Potently Disrupts the MYC Transcriptional Network. bioRxiv. 2024.).

Concluding, simultaneous targeting of CDKs regulating the cell cycle, oncogenic transcriptional networks and apoptotic signaling, embodied by CDK4, CDK6 and CDK9, using PROTAC degrader technology is a novel approach, distinct from existing inhibitors and degraders of CDK4/6 or CDK9, that hampers cell proliferation at multiple levels concurrently, inducing superior anti-proliferative effects.

CLAIMS

1. A compound of formula (I):



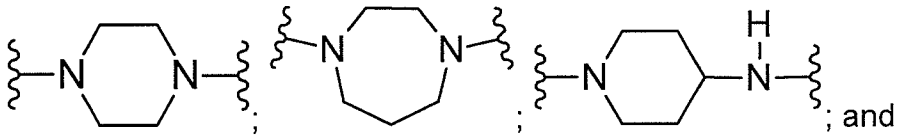
(I)

wherein

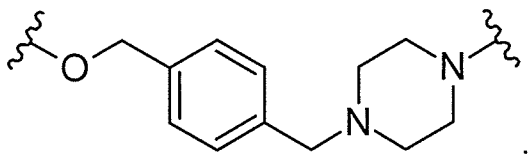
n is 1 or 2;

X is a C_{1-6} alkylene group, a C_{2-6} alkenylene group, a C_{1-6} heteroalkylene group, an optionally substituted C_{3-5} cycloalkylene group, or an optionally substituted aralkylene group;

Y is a NH group or is selected from the following groups:



; and

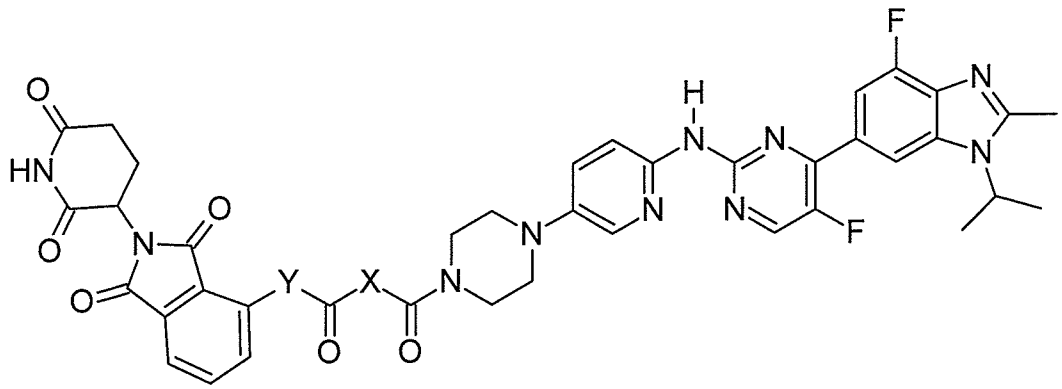


; and

Z is hydrogen or a methyl group;

or a salt thereof.

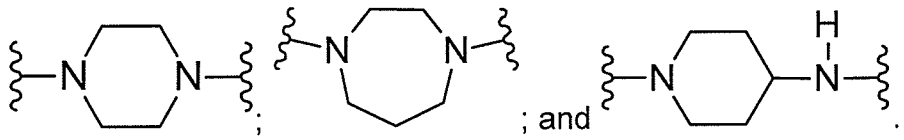
2. A compound according to claim 1 of formula (II) or a salt thereof:



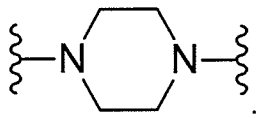
(II).

3. A compound according to claim 1 or 2, wherein Y is a NH group.

4. A compound according to claim 1 or 2, wherein Y is selected from the following groups:

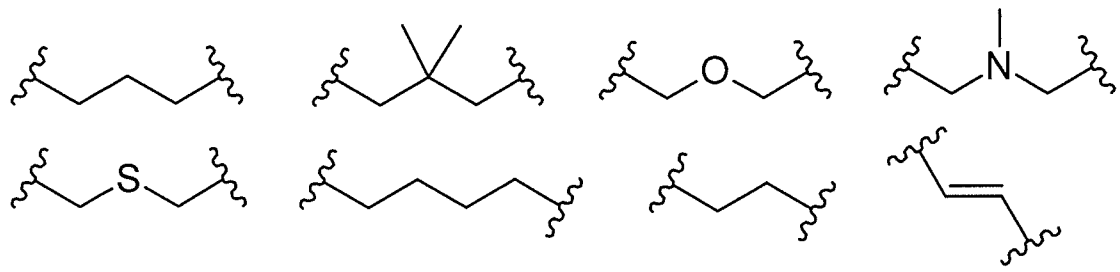


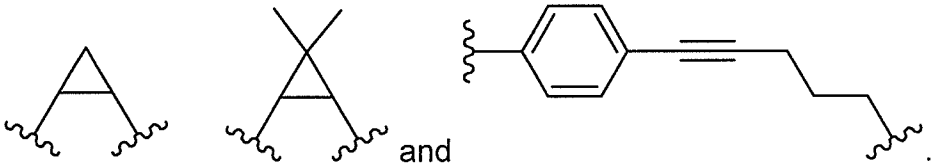
5. A compound according to claim 1 or 2, wherein Y is the following group:



6. A compound according to any one of the preceding claims, wherein X is a C_{1-6} alkylene group or a C_{1-6} heteroalkylene group.

7. A compound according to any one of the preceding claims 1 to 5, wherein X is selected from the following groups:





8. A compound according to any one of the preceding claims 1 to 5, wherein X is selected from the following groups:

9. Pharmaceutical composition comprising a compound according to anyone of the preceding claims and optionally one or more carrier substances and/or one or more adjuvants.

10. Compound according to any one of claims 1 to 8 or pharmaceutical composition according to claim 9 for use in the treatment of diseases that are associated with CDK activity.

11. Compound according to any one of claims 1 to 8 or pharmaceutical composition according to claim 9 for use in the treatment of cancer.

12. Compound according to any one of claims 1 to 8 or pharmaceutical composition according to claim 9 for use in the treatment of inflammation.

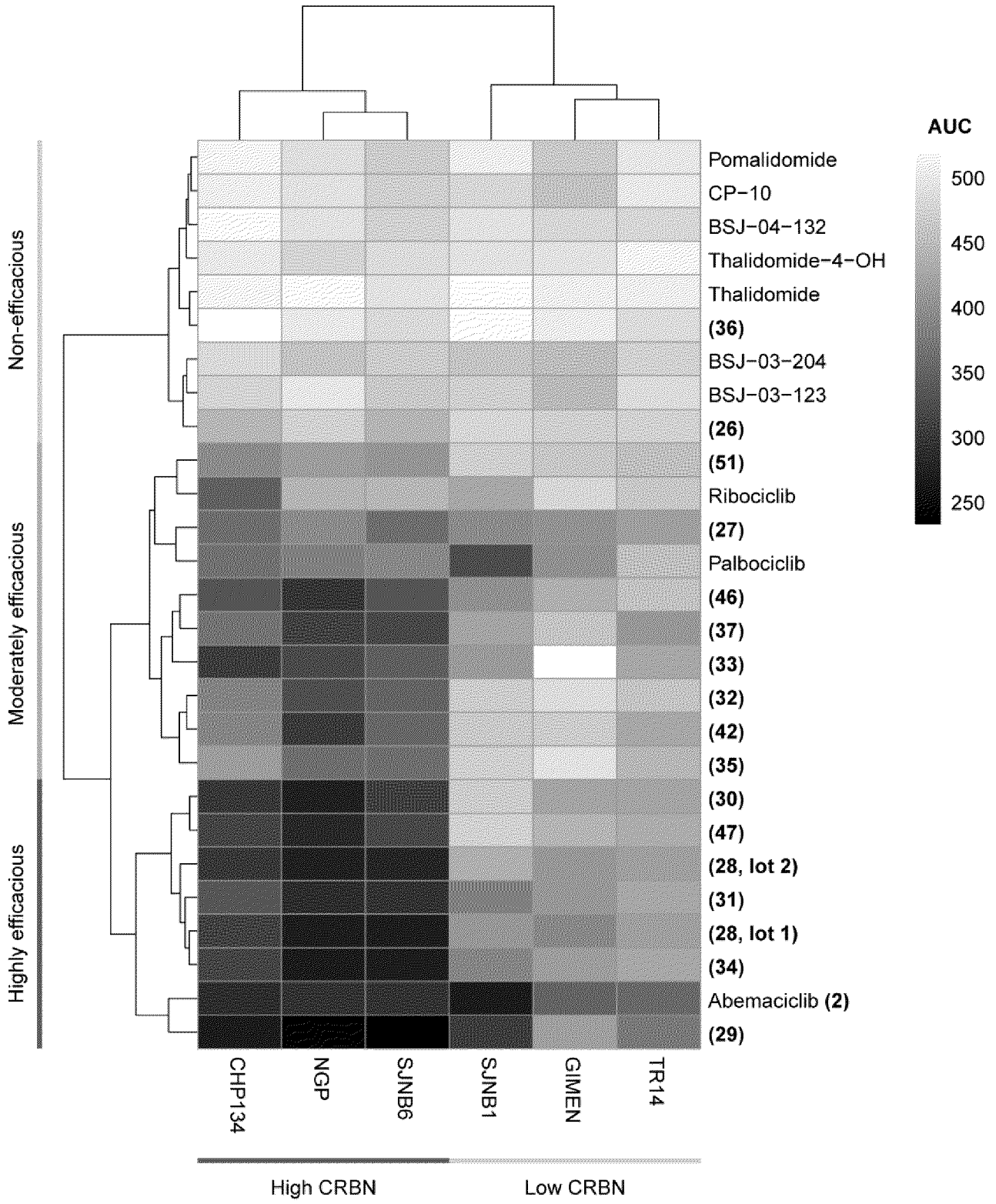
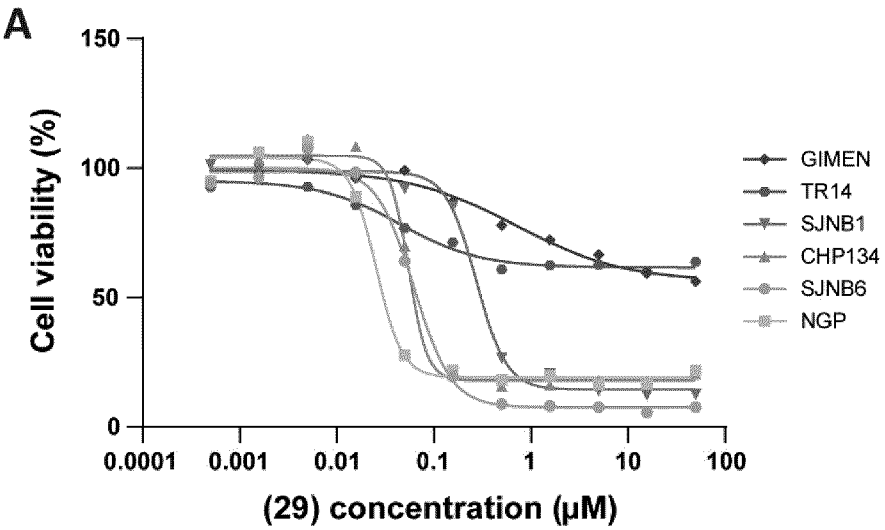
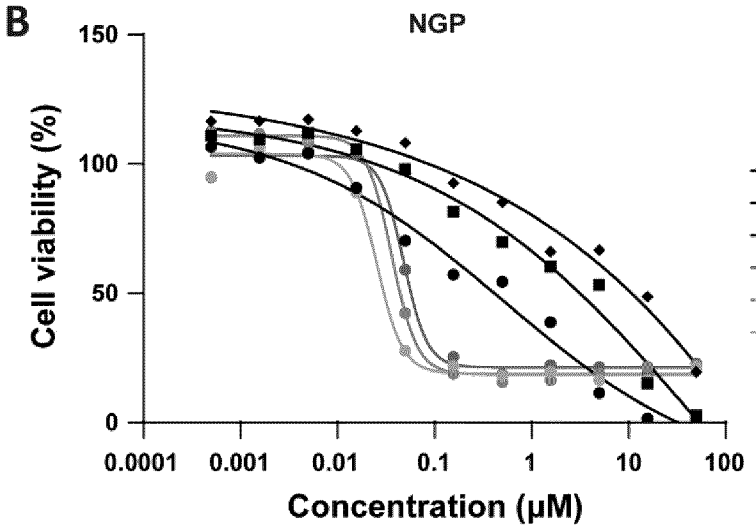

AUC
500
450
400
350
300
250
Pomalidomide
CP-10
BSJ-04-132
Thalidomide-4-OH
Thalidomide
(36)
BSJ-03-204
BSJ-03-123
(26)
(51)
Ribociclib
(27)
Palbociclib
(46)
(37)
(33)
(32)
(42)
(35)
(30)
(47)
(28, lot 2)
(31)
(28, lot 1)
(34)
Abemaciclib (2)
(29)
Non-efficacious
Moderately efficacious
Highly efficacious
CHP134
NGP
SJNB6
SJNB1
GIMEN
TR14
High CRBN
Low CRBN

Figure 1



A

Cell line	IC50 (72h, nM)
NGP	29.2
CHP134	60.0
SJNB6	67.2
SJNB1	309
GIMEN	n.a.
TR14	n.a.



B

Compound	IC50 (72h, nM)
(29)	29.2
(34)	41.0
(28, lot 1)	55.9
Abemaciclib **(2)**	223
Palbociclib	1582
Ribociclib	3555
BSJ-03-123	n.a.
BSJ-03-204	n.a.
CP-10	n.a.
BSJ-04-132	n.a.

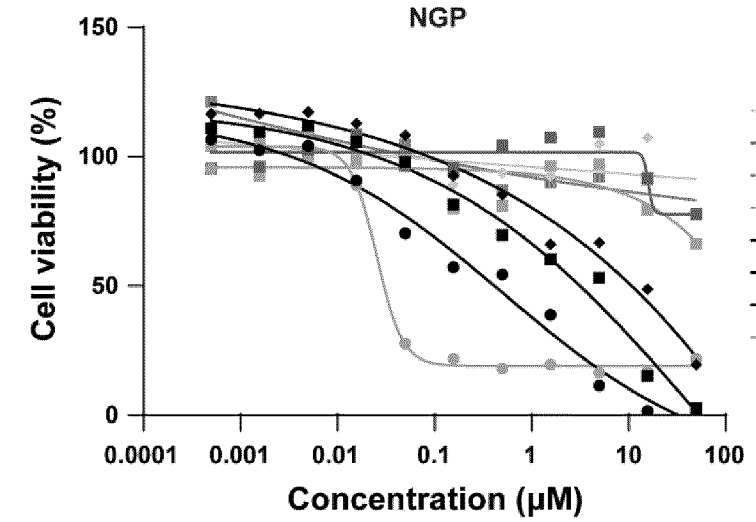


Figure 2

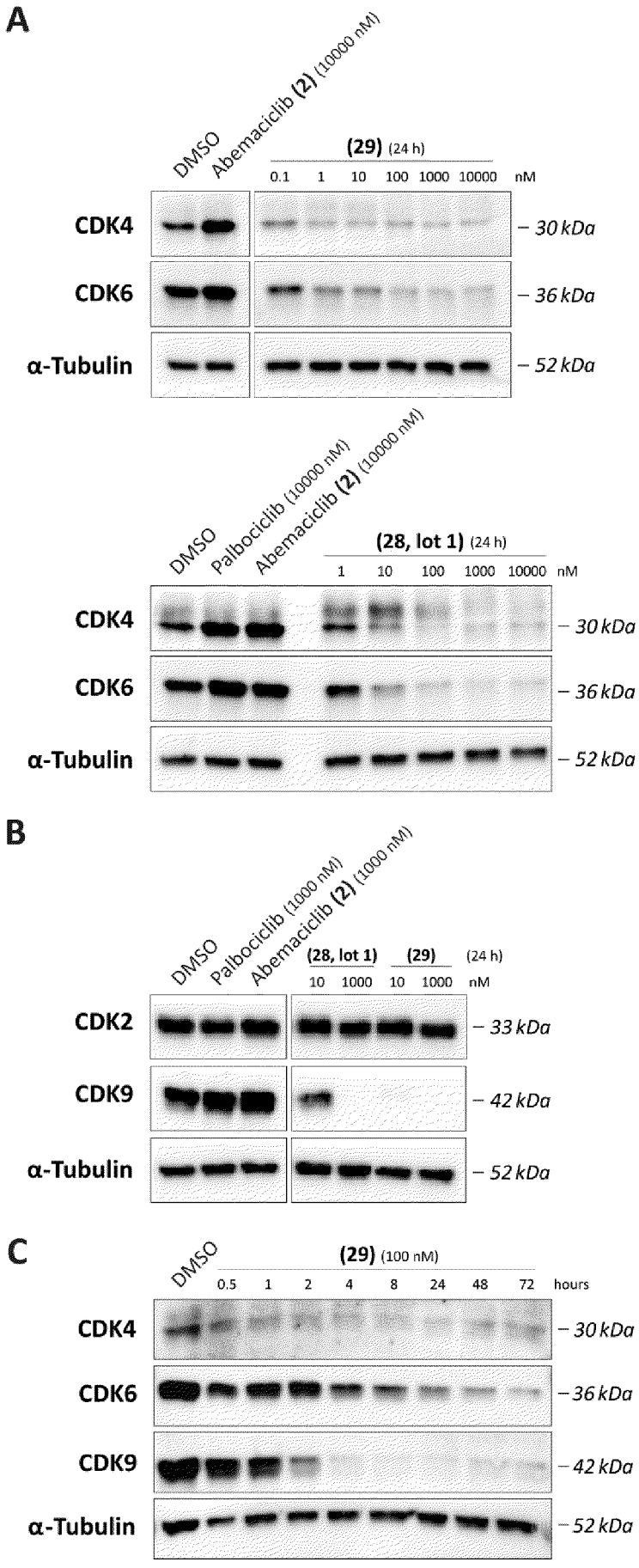

A
DMSO
Abemaciclib (2) (10000 nM)
(29) (24 h)
0.1 1 10 100 1000 10000 nM
CDK4
CDK6
α-Tubulin
30 kDa
36 kDa
52 kDa
DMSO
Palbociclib (10000 nM)
Abemaciclib (2) (10000 nM)
(28, lot 1) (24 h)
1 10 100 1000 10000 nM
CDK4
CDK6
α-Tubulin
30 kDa
36 kDa
52 kDa
B
DMSO
Palbociclib (1000 nM)
Abemaciclib (2) (1000 nM)
(28, lot 1)
(29)
(24 h)
10 1000 10 1000 nM
CDK2
CDK9
α-Tubulin
33 kDa
42 kDa
52 kDa
C
DMSO
(29) (100 nM)
0.5 1 2 4 8 24 48 72 hours
CDK4
CDK6
CDK9
α-Tubulin
30 kDa
36 kDa
42 kDa
52 kDa

Figure 3

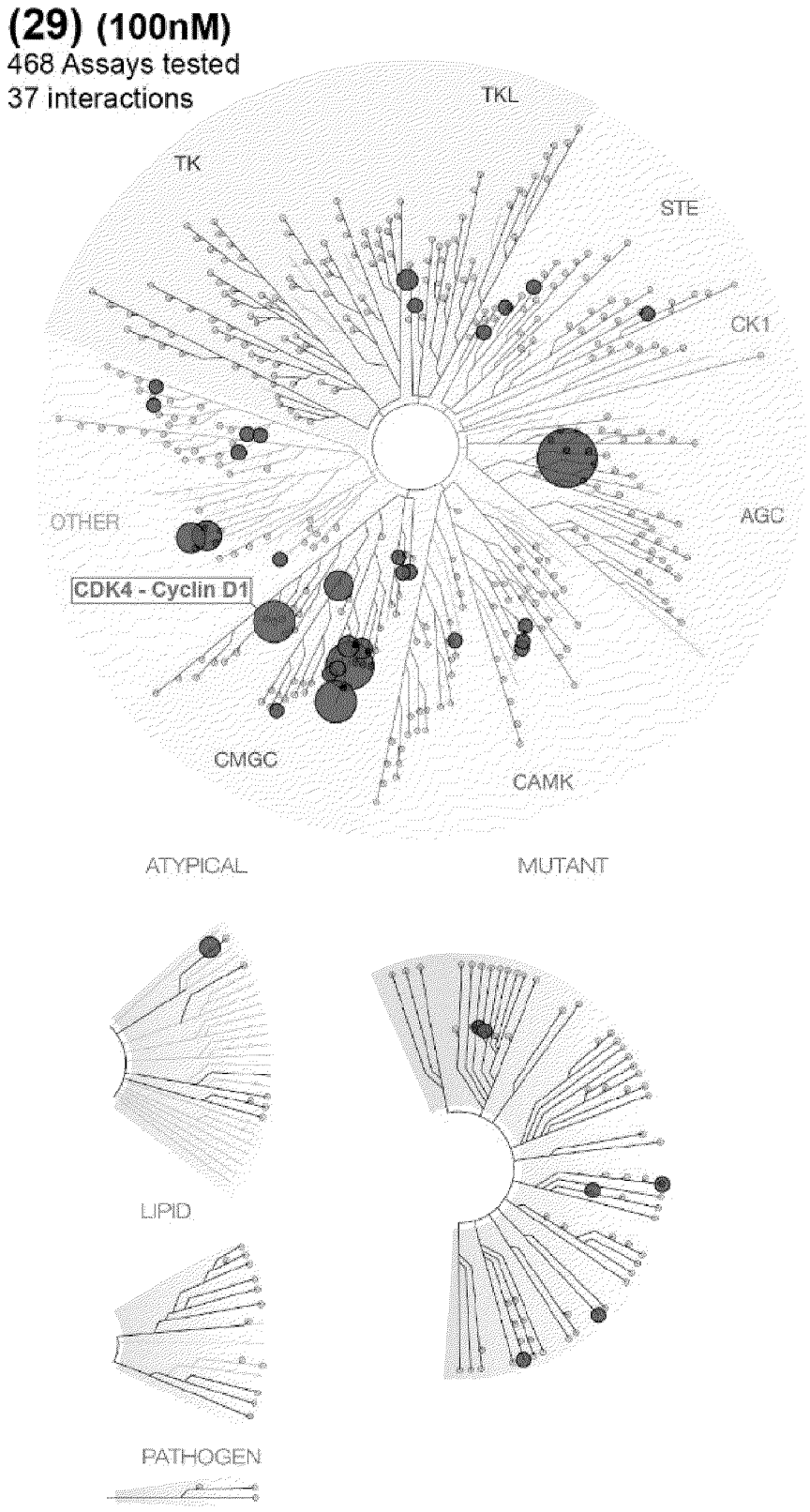

(29) (100nM)
468 Assays tested
37 interactions
TKL
TK
STE
CK1
AGC
OTHER
CDK4 - Cyclin D1
CMGC
CAMK
ATYPICAL
MUTANT
LIPID
PATHOGEN

Figure 4A

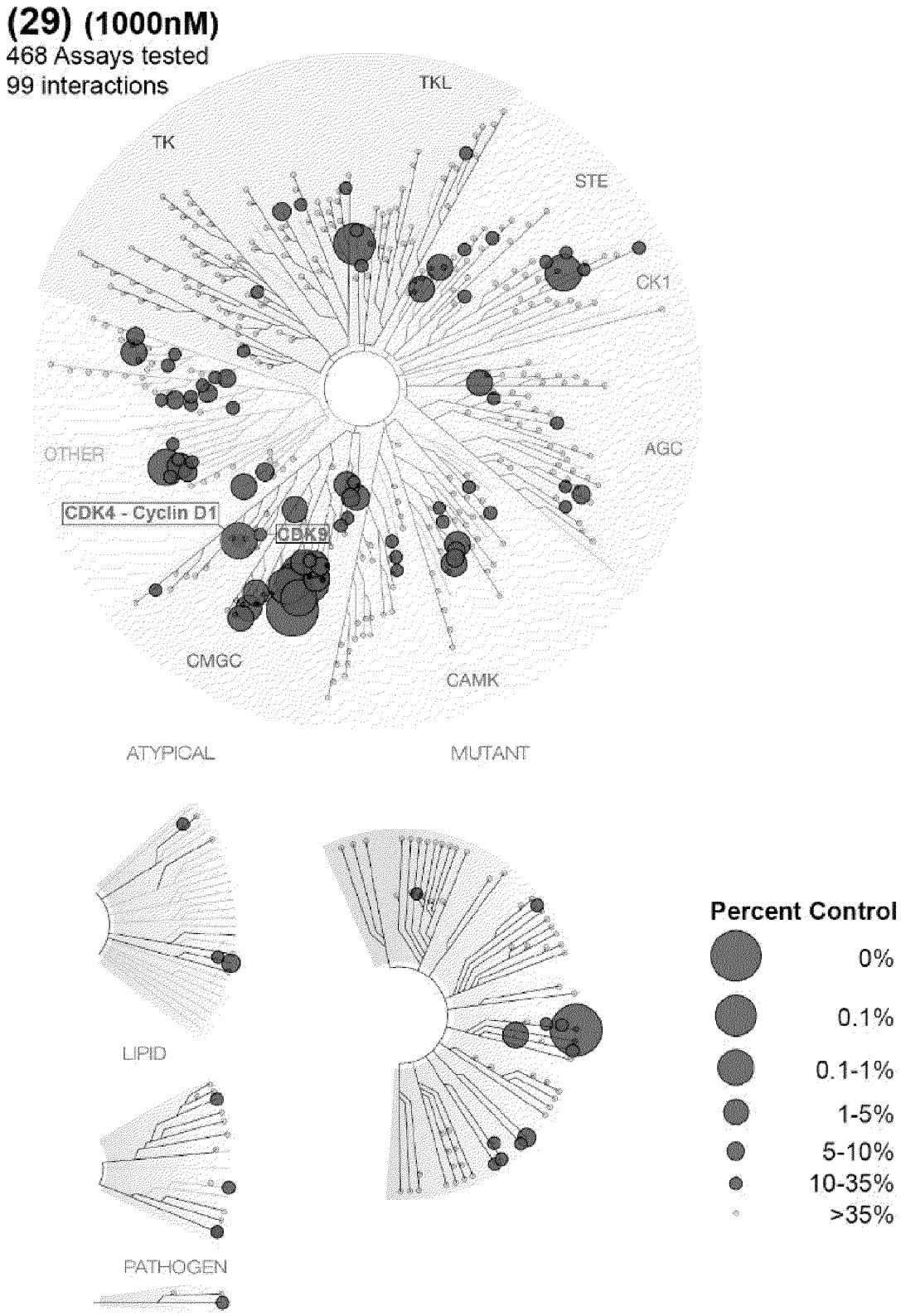
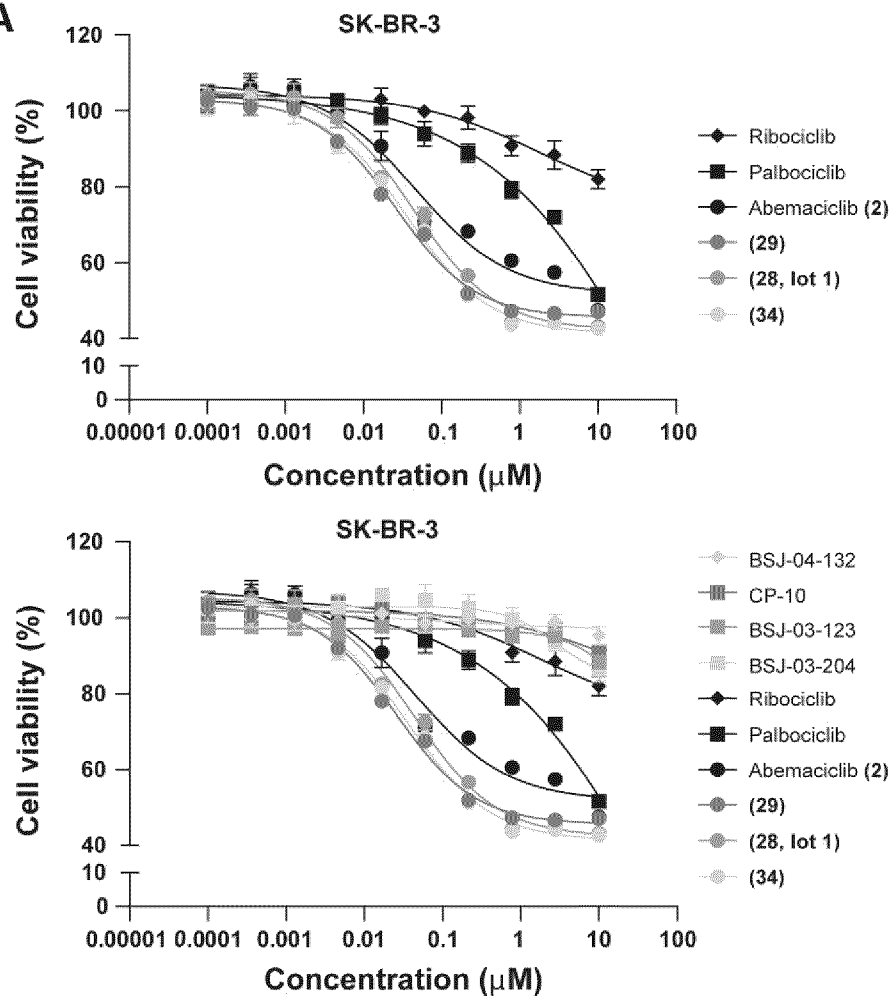

(29) (1000nM)
468 Assays tested
99 interactions
TKL
TK
STE
CK1
AGC
OTHER
CDK4 - Cyclin D1
CDK9
CMGC
CAMK
ATYPICAL
MUTANT
LIPID
PATHOGEN
Percent Control
0%
0.1%
0.1-1%
1-5%
5-10%
10-35%
>35%

Figure 4B

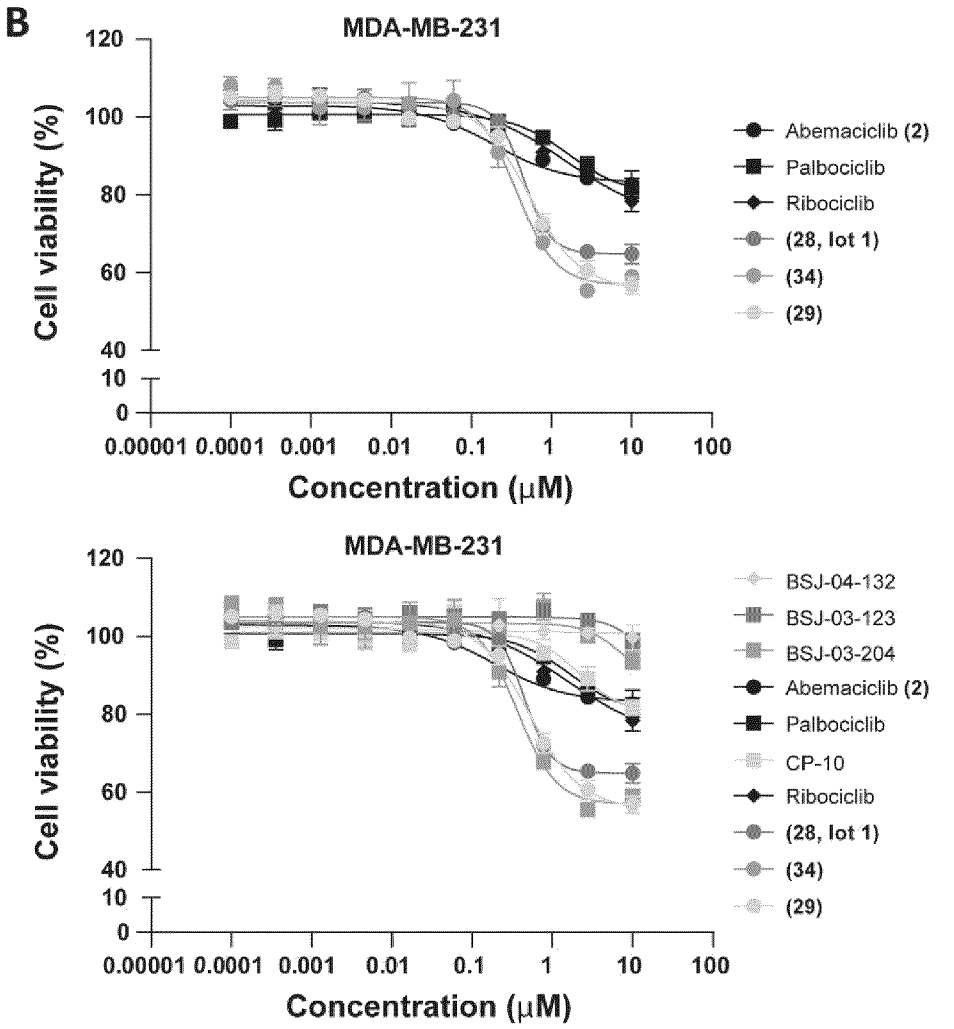
A



A

Compound	IC50 (72h, nM)
(34)	269.5
(28, lot 1)	328.7
(29)	335.2
Abemaciclib **(2)**	n.a.
Palbociclib	n.a.
Ribociclib	n.a.
BSJ-03-123	n.a.
BSJ-03-204	n.a.
CP-10	n.a.
BSJ-04-132	n.a.

Figure 5A



B

Compound	IC50 (72h, nM)
(29)	n.a.
(34)	n.a.
(29, lot 1)	n.a.
Ribociclib	n.a.
CP-10	n.a.
Palbociclib	n.a.
Abemaciclib **(2)**	n.a.
BSJ-03-204	n.a.
BSJ-03-123	n.a.
BSJ-04-132	n.a.

Figure 5B

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/066424

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D401/12 A61P35/00 A61K31/505 C07D401/14
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D A61P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/106870 A1 (ICAHN SCHOOL MED MOUNT SINAI [US]) 14 June 2018 (2018-06-14) claims 1-40; example 31	1-12
X	WO 2020/023480 A1 (DANA FARBER CANCER INST INC [US]) 30 January 2020 (2020-01-30) claims 1-40; examples I-8	1-12

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 June 2024	04/07/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sáez Díaz, R

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/066424

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018106870 A1	14-06-2018	AU 2017370694 A1	25-07-2019
		CA 3045037 A1	14-06-2018
		CN 110267659 A	20-09-2019
		EP 3551185 A1	16-10-2019
		JP 2020514252 A	21-05-2020
		US 2019336503 A1	07-11-2019
		US 2022054488 A1	24-02-2022
		WO 2018106870 A1	14-06-2018
WO 2020023480 A1	30-01-2020	AU 2019309909 A1	04-02-2021
		CA 3106523 A1	30-01-2020
		EP 3827000 A1	02-06-2021
		US 2021340140 A1	04-11-2021
		WO 2020023480 A1	30-01-2020