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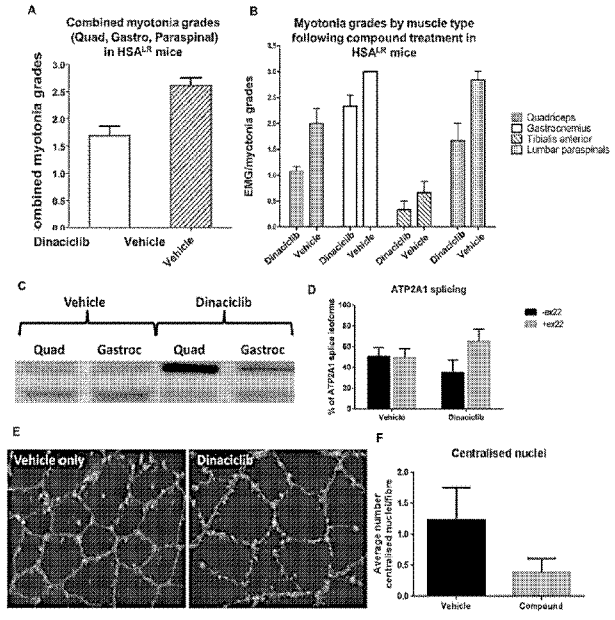
[Continued on next page]

(54) Title: INHIBITORS AND THEIR USES

Figure 7



(57) Abstract: The invention relates to inhibitors of CDK12 (cyclin-dependent kinase 12), and there use in the treatment or prevention of a disorder in a subject caused by the generation of repeat expansion transcripts.

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INHIBITORS AND THEIR USES

The present invention relates to inhibitors of cyclin-dependent kinase 12 (CDK12) and in particular, inhibitors of CDK12 for use in the treatment of disorders caused by the generation of RNA repeat expansion transcripts. The RNA repeat expansion transcript may be from a CTG DNA repeat expansion such as seen in Myotonic Dystrophy.

Myotonic Dystrophy, also referred to herein as DM, is the most common form of adult muscular dystrophy. DM type 1 (DM1) affects 1 in 8,000 people and is caused by a CTG repeat sequence in the 3' untranslated region of the *DMPK* (dystrophia myotonica protein kinase) gene, which is greatly expanded in patients who may have anything from 50 repeats to several thousand on affected chromosomes compared to between 5 and 37 repeats on wild-type chromosomes. The expanded DNA repeat is transcribed, and despite being correctly spliced the RNA repeat expansion transcripts remain sequestered in the nucleus forming distinct foci. These foci interact with cellular proteins, such as MBNL1 (muscleblind-like splicing regulator 1), a key splicing regulator, which in turn leads to downstream splicing abnormalities. In addition to the sequestration of proteins the mutant RNA causes activation of CELF1 (CUGBP, Elav-like family member 1), which is also implicated in splicing. Additional molecular pathways are thought to be affected by the toxic RNA, including inhibition of translation.

DM is an inherited and progressive autosomal dominant multisystem disorder and symptoms can be highly variable. Typical features include myotonia, muscle weakness, cardiac arrhythmias, cognitive dysfunction, diabetes and cataracts. There is currently no treatment for DM and clinical management relies on a fragmented approach utilising already marketed drugs to treat specific symptoms of the disorder, such as mexiletine to treat myotonia and modafinal to address daytime sleepiness. However, due to the complex and variable nature of the disorder, treatment of individual symptoms is not an efficient way to manage the condition.

Key components of the DM molecular pathway have been identified but how these factors interact is still unclear. Recently there has been considerable effort towards therapeutic treatments for this condition including targeting different points of the molecular pathway with varying levels of success. The most obvious approach is to directly target the repeat expansion transcript to neutralize the harmful repeats or

promote transcript degradation and subsequent clearance from the cell. To date this has been attempted using either ribozymes or antisense oligonucleotides (Langlois, M.A. *et al.* (2003). *Molecular therapy: the journal of the American Society of Gene Therapy*, 7: 670-680; Wheeler, T.M. *et al. Nature*, 488: 111-115; and Mulders, S.A. *et al.* (2009). *Proc Natl Acad Sci USA*, 106: 13915-13920). Other methods to target the repeat sequence directly have involved the introduction of a blocking molecule, such as morpholino oligonucleotides or small molecules that physically prevent binding of MBNL protein by sitting in the groove of the RNA and preventing protein association and binding (Wheeler, T.M. *et al.* (2009). *Science,* 325: 336-339). A series of compounds have been shown to successfully disrupt the CUG repeat:MBNL protein interaction including pentamidine, a bisamidinium inhibitor, a series of peptide ligands and two natural products, lomofugin and dilomofungin. Treatment of DM1-model-CUG-repeat cells with these compounds led to a loss of nuclear foci and a reversal of DM associated splicing events, consistent with release of MBNL protein from this complex. These compounds show that disruption of the RNA:protein interaction may be an option for therapeutic development. However these compounds do not represent suitable starting points for drug development due to high levels of toxicity, poor oral availability and, in the case of peptide ligands, instability in serum. To date there is no suitable treatment for DM.

An object of the present invention is to provide an alternative, preferably an improved, treatment and compound useful for treating disorder caused by the generation of repeat expansion transcripts, such as CTG repeat expansion transcripts as seen in DM-1, with an aim to address at least one of the aforementioned disadvantages.

According to a first aspect of the invention, there is provided an inhibitor for use in the treatment or prevention of a disorder in a subject caused by the generation of repeat expansion transcripts, wherein the inhibitor is an inhibitor of CDK12 (cyclin-dependent kinase 12).

The repeat expansion transcript may result in the transcript being retained in the nucleus. In one embodiment, the term “retained in the nucleus” may refer to no detectable repeat expansion transcript leaving the nucleus. In another embodiment, the

term may refer to a delay in the transcript leaving the nucleus (i.e. a transient retention in the nucleus).

The disorder may comprise any disorder associated with RNA repeat expansion transcripts, such as transcripts from CTG DNA repeat expansions, where the mutant transcripts do not get exported from the nucleus. The disorder may be any disorder selected from the group comprising Myotonic Dystrophy type 1 (CTG_n), Myotonic Dystrophy type 2 ($CCTG_n$), Fragile X associated tremor/ataxia syndrome (CGG_n), and amylotrophic lateral sclerosis (ALS) and frontotemporal dementia (C9ORF72) ($GGGGCC_n$) each of which shows or may show nuclear retention of transcripts from repeat DNA expansions. The disorder may be any disorder selected from the group comprising Spinocerebellar Ataxia Type 8 (CTG_n), Spinocerebellar Ataxia Type 10 ($ATTCT_n$) and Spinocerebellar Ataxia Type 31 ($TGGAA_n$) which may show nuclear retention. The disorder may be any disorder selected from the group comprising Huntington's Disease like 2 (CTG_n), and Huntington's Disease, Spinocerebellar Ataxia Types 1, 2, 3, 6, 7, 17, Dentatorubral-pallidoluysian atrophy and Spinal and Bulbar Muscular Atrophy (which are associated with (CAG_n) repeat expansions, but which may show nuclear retention of transcripts with repeat expansions at the longest end of the disease range. The disorder may comprise any one or more disorders provided in Table 1.

Table 1.

DISEASE	ASSOCIATED GENE/ORF	GENBANK ACCESSION NUMBER
Myotonic Dystrophy type 1 (CTG_n),	DMPK	NM_001081563 VERSION NM_001081563.2 GI:571026697
Myotonic Dystrophy type 2 ($CCTG_n$),	ZNF9	AY329622 AF389886 AF389887 AH010982 VERSION AY329622.1 GI:40738012
Fragile X associated tremor/ataxia syndrome (CGG_n),	FMR1	L19493 VERSION L19493.1 GI:388753
Amylotrophic lateral sclerosis (ALS) and frontotemporal dementia (C9ORF72) ($GGGGCC_n$)	C9ORF72	JN681271 VERSION JN681271.1 GI:356892155
Spinocerebellar Ataxia Type 8 (CTG_n)	SCA8	AF126749 VERSION AF126749.1 GI:4589125
Spinocerebellar Ataxia Type 10 ($ATTCT_n$)	SCA10	NM_013236 VERSION NM_013236.3 GI:266453258

Huntington's Disease like 2 (CTG_n)	Junctophilin3	NM_001271604 VERSION NM_001271604.2 GI:413082123
Huntington's Disease	Huntingtin	NM_002111 VERSION NM_002111.7 GI:588282786
Spinocerebellar Ataxia Type 1	Ataxin1	NM_000332 VERSION NM_000332.3 GI:189491746
Spinocerebellar Ataxia Type 2	Ataxin2	NM_002973 VERSION NM_002973.3 GI:171543894
Spinocerebellar Ataxia Type 3	Ataxin3	NM_004993 VERSION NM_004993.5 GI:189163490
Spinocerebellar Ataxia Type 6	$CACNA1_A$	NM_000068 VERSION NM_000068.3 GI:148536843
Spinocerebellar Ataxia Type 7	Ataxin 7	NM_000333 VERSION NM_000333.3 GI:189491740
Spinocerebellar Ataxia Type 17	Tata box binding protein	CR456776 VERSION CR456776.1 GI:48145668
Dentatorubral-pallidoluysian atrophy	Atrophin	D31840 VERSION D31840.1 GI:862329
Spinal and Bulbar Muscular Atrophy	Androgen receptor	M34233 VERSION M34233.1 GI:179033
Spinocerebellar Ataxia Type 31	BEAN1	NM_001178020

In one embodiment, the repeat expansion transcript may comprise RNA from a CTG repeat (i.e. the RNA may comprise a CUG repeat sequence). In another embodiment, the repeat expansion transcript may comprise RNA from a CCTG repeat (i.e. the RNA may comprise a CCUG repeat sequence). In another embodiment, the repeat expansion transcript may comprise RNA from a CGG repeat. In another embodiment, the repeat expansion transcript may comprise RNA from a GGGGCC repeat. In another embodiment, the repeat expansion transcript may comprise RNA from a ATTCT repeat (i.e. the RNA may comprise a AUUCU repeat sequence). In another embodiment, the repeat expansion transcript may comprise RNA from a CAG repeat. In another embodiment, the repeat expansion transcript may comprise RNA from TGGAA repeat (i.e. the RNA may comprise a UGGAA repeat sequence).

CDK12 is a transcription elongation associated C-terminal repeat domain kinase, which has shown to associate with elongating transcripts, rather than being involved

in the initiation of transcription. The invention herein has found that inhibition of CDK12 results in the removal of nuclear foci from DM cells. Further advantageously, as CDK12 is not required at the start of transcription and its inhibition does not result in global transcriptional arrest, it is suitable as a target for long term DM treatment, and other disorders caused by the generation of repeat expansion transcripts, such as transcripts from CTG repeat expansions.

The term "inhibit" or "inhibition" used in the context of CDK12 herein is understood to mean a reduction or complete elimination of CDK12 activity. The reduction in activity of CDK12 may be 100%. Alternatively, the reduction in activity of CDK12 may be at least 90%. The reduction in activity of CDK12 may be at least 80%. The reduction in activity of CDK12 may be at least 70%. The reduction in activity of CDK12 may be at least 60%. In some embodiments, the CDK12 inhibition may be measured by an assay measuring any inhibition of CDK12 consumption of ATP during the phosphorylation of a substrate peptide in the presence of the molecule to be screened.

The CDK12 inhibition may involve blocking the CDK12 active site directly or indirectly; changing the conformation of CDK12; blocking CDK12 interactions; preventing cyclin k binding; preventing phosphorylation of Ser2 on the C-terminal domain of RNA polymerase II; reducing the presence of CDK12; or sequestering the CDK12, for example through aggregation.

The inhibition of CDK12 activity may be by reduction in the presence of CDK12 (i.e. the activity of CDK12 itself may not be inhibited, but the amount of active CDK12 available in the cells and tissue may be reduced). Therefore, in some embodiments, the inhibition of CDK12 may be provided by reducing the expression of CDK12. Alternatively, CDK12 may be mutated to an inactive form. Alternatively, CDK12 may be targeted for degradation or sequestration to reduce the amount of active CDK12 available in the cells or tissue. The reduction in amount of CDK12 may be 100%. Alternatively, the reduction in amount of CDK12 may be at least 90%. The reduction in amount of CDK12 may be at least 80%. The reduction in amount of CDK12 may be at least 70%. The reduction in amount of CDK12 may be at least 60%. The inhibition of CDK12 activity by reduction in the presence of CDK12 may be measured by RT-PCR of CDK12 transcripts in a sample, or by western blot. The skilled person would

understand that there are several methods that may be used to determine the presence and level of any particular protein, or transcripts thereof, in a sample.

The term "inhibitor" used herein is understood to include an agent, such as a molecule, that it capable of causing the inhibition of CDK12 activity. Additionally, or alternatively, the term "inhibitor" used herein is understood to include an agent, such as a molecule, that it capable of causing the inhibition of CDK12 availability.

The inhibitor may be specific for CDK12. For example, the inhibitor may not inhibit, or not substantially inhibit other cyclin-dependent kinases. Alternatively, the activity of one or more other cyclin-dependent kinases may be inhibited by the inhibitor, but similar to or less than the activity of CDK12. Alternatively, the activity of one or more other cyclin-dependent kinases may be inhibited by the inhibitor, but significantly less than the activity of CDK12. In one embodiment, the inhibitor is not an inhibitor of CDK9 activity or availability. The other CDKs that may not be inhibited, or not significantly inhibited, may be selected from the group consisting of CDK1, CDK2, CDK3, CDK4, CDK5, CDK6, CDK7, CDK8, CDK9, CDK10, CDK11, and CDK13; or combinations thereof. The other CDKs that may not be inhibited, or not significantly inhibited, may be selected from the group comprising CDK1, CDK2, CDK5, and CDK9; or combinations thereof. Preferably CDK9 is not inhibited, or is not significantly inhibited by an inhibitor of the invention.

The inhibitor may comprise an inhibitor of CDK12 expression. The inhibitor may comprise an oligonucleotide, such as siRNA, capable of inhibiting CDK12 expression. The oligonucleotide may comprise a sequence capable of binding to nucleic acid of the CDK12 gene, or regulatory elements thereof, or a mRNA transcript thereof. The oligonucleotide may comprise a sequence substantially complementary to the CDK12 gene, or regulatory elements thereof. The oligonucleotide may comprise a sequence substantially complementary to CDK12 mRNA transcript. The sequence may be substantially complementary over a region of at least 5 nucleotides. The sequence may be substantially complementary over a region of at least 8 nucleotides. The sequence may be substantially complementary over a region of at least 10, 13, 15, or 18 nucleotides. The oligonucleotide may comprise a sequence capable of binding to SEQ ID NO: 1, and reducing translation thereof, e.g. siRNA gene silencing.

The inhibitor may comprise a molecule capable of binding to CDK12. The inhibitor molecule may be capable of blocking binding of CDK12 to its target molecule. The inhibitor may comprise a molecule capable of preventing CDK12 binding to cyclin K. The inhibitor may comprise a molecule capable of preventing CDK12 phosphorylating Ser2 on the c-terminal domain of RNA polymerase II. The binding of the inhibitor to CDK12 may be at, or adjacent to, the CDK12 active site, such that the active site is blocked. The binding of the inhibitor to CDK12 may be at amino acid position, 727-1020 (underlined in the sequence below). The binding of the inhibitor to CDK12 may be at a C terminal domain extension that extends around the N and C terminal lobes and contacts bound ATP (C terminal domain extension comprises residues 1011-1039 of CDK12). Such a domain is unique to CDK12 and is not present in CDK9. The binding of the inhibitor to CDK12 may be at any one or more of the ATP contact residues selected from Thr737, Lys756, Glu814, Met816 and Asp819 (in bold-type on the sequence below).

CDK12 binds to cyclin K and phosphorylates Ser2 on the C-terminal domain of RNA polymerase II. Advantageously, the inhibitor treatment will result in a shift in the relative proportions of wild type and mutant DMPK transcripts (CDK12 inhibition leads to a preferential loss of the expanded repeat transcript).

The inhibitor may comprise a therapeutically active agent. The inhibitor may comprise a small molecule. The term “small molecule” means any compound that has a molecular weight of less than 1kDa. The inhibitor may be an organic compound with a molecular weight of less than 1KDa. Molecular weight is understood to be the sum of the atomic weights of all the atoms in a molecule.

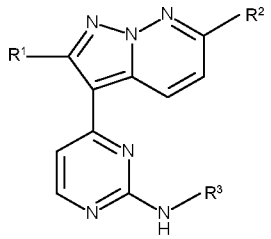
The inhibitor may comprise nucleic acid such as an oligonucleotide. The oligonucleotide may comprise DNA, RNA, or synthetic nucleic analogues such as PMO, LNA or PNA. The oligonucleotide may comprise siRNA or microRNA. The oligonucleotide may comprise the sequence of CGAAAUAAUGAUGUUGGCACCAGUU, or a variant thereof, for siRNA silencing of CDK12.

The inhibitor may comprise a peptide or protein capable of binding to CDK12. The inhibitor may comprise an antibody, or an antibody fragment, or antibody mimetic.

The inhibitor may be cell membrane permeable.

The inhibitor may comprise a pyrazolo[1,5b]pyridazine core structure, and be capable of inhibiting CDK12 activity.

In one embodiment, the inhibitor comprises a compound of Formula (I) or a pharmaceutically acceptable salt or solvate thereof:



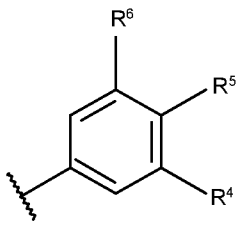
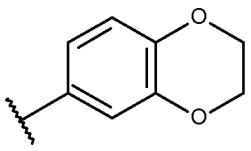
Formula I

wherein:

R^1 is H, -OH, -O-, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynl, C_{1-6}haloalkyl, halogen, -CN, -OC_{1-6}alkyl;

R^2 is H, -OH, -O-, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynl, C_{1-6}haloalkyl, halogen, -CN, -OC_{1-6}alkyl or a five or 6 membered cycloaryl, cycloalkyl or heterocycl having one, two or three heteroatoms selected from O, S and N, for example benzene, morpholinyl, piperidine, piperazine;

R^3 is C_{3-6}cycloalkyl, for example cyclopropyl,

 or  ;

wherein:

R^4 is H, -OH, -O-, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynl, C_{1-6}haloalkyl, halogen, -CN, -OC_{1-6}alkyl;

R^5 is H; -OH; -O-; C_{1-6}alkyl; C_{2-6}alkenyl; C_{2-6}alkynl; C_{1-6}haloalkyl; halogen; -CN; -OC_{1-6}alkyl;; C_{1-6}alkyl-N-(X)(Y); a five or six membered cycloaryl, cycloalkyl or heterocycl having one, two or three heteroatoms

selected from O, S and N and said cycloaryl, cycloalkyl or heterocycle being optionally substituted with a C_{1-3}alkyl, for example N-methylpiperazinyl; or -OC_{1-6}alkyl-N(X)(Y);

wherein X is H or C_{1-6}alkyl, and Y is H or C_{1-6}alkyl;

and wherein the alkyl groups are optionally substituted by one or more –OH groups; and

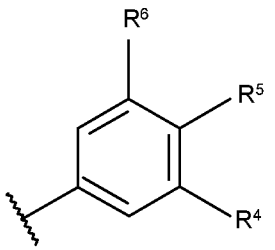
R^6 is H, -OH, -O-, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynl, C_{1-6}haloalkyl, halogen, -CN, -OC_{1-6}alkyl.

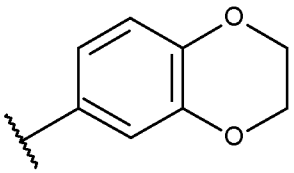
Preferably:

R^1 is H;

R^2 is H;

R^3 is C_{3-6}cycloalkyl, for example cyclopropyl,



or 

;

wherein:

R^4 is H, -CN, -OC_{1-6}alkyl, C_{1-6}haloalkyl;

R^5 is H, ; C_{1-6}alkyl-N-(X)(Y); a five or six membered cycloaryl, cycloalkyl or heterocycl having one, two or three heteroatoms selected from O, S and N and said cycloaryl, cycloalkyl or heterocycle being optionally substituted with a C_{1-3}alkyl, for example N–methylpiperazinyl, -OC_{1-6}alkyl-N(X)(Y);

wherein X is H or C_{1-6}alkyl, and Y is H or C_{1-6}alkyl;

and wherein the alkyl groups are optionally substituted by one or more –OH groups; and

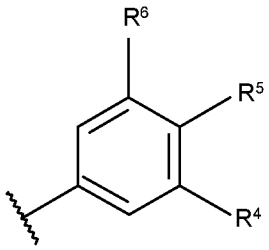
R^6 is H or -OC_{1-6}alkyl.

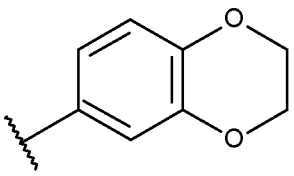
Preferably:

R^1 is H;

R^2 is H;

R^3 is cyclopropyl,



or 

;

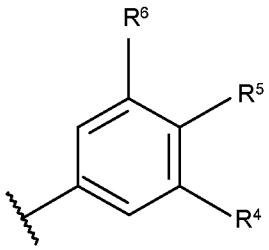
wherein:

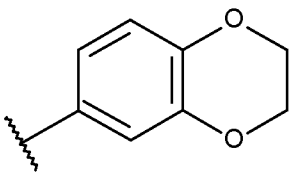
R^4 is H, -CN, $-OCH_3$, CF_3;

R^5 is H, $-CH_2N(CH_3)_2$, N–methylpiperazinyl, $-OCH_2CH(OH)CH_2N(CH_3)_2$; and

R^6 is H or $–OCH_3$.

In one embodiment R^1 is H, R^2 is H, R^3 is cyclopropyl,



or 

;

wherein:

R4 is H; R5 is $-CH_2NEt_2$, or $-OCH_2CH(OH)CH_2N(CH_3)_2$; and R6 is H;

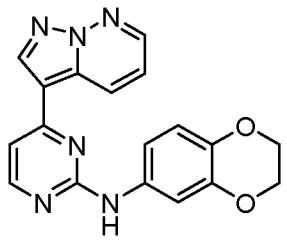
or

R4 is -CN, $-OCH_3$; R5 is H; and R6 is H; or

R4 is $-CF_3$; R5 is N–methylpiperazinyl; and R6 is H; or

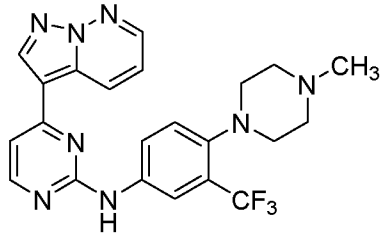
R4 is $-OCH_3$; R5 is H; and R6 is $–OCH_3$.

In one embodiment, the inhibitor of Formula (I) is of the following formula:



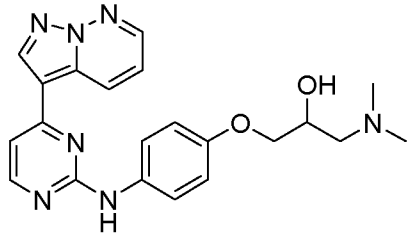
(II)

In another embodiment, the inhibitor of Formula (I) is of the following formula:



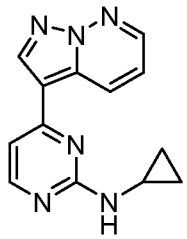
(III)

In another embodiment, the inhibitor of Formula (I) is of the following formula::



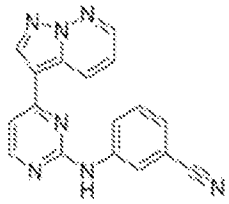
(IV)

In another embodiment, the inhibitor of Formula (I) is of the following formula:



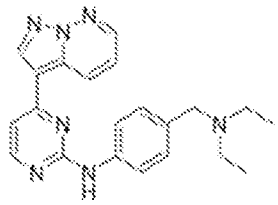
(V)

In one embodiment the inhibitor of Formula (I) is of the following formula:



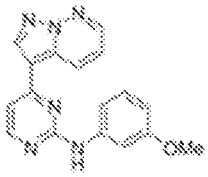
(VI)

In one embodiment the inhibitor of Formula (I) is of the following formula:



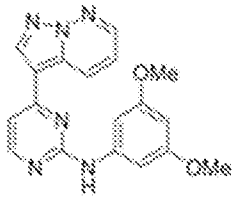
(VII)

In one embodiment the inhibitor of Formula (I) is of the following formula:



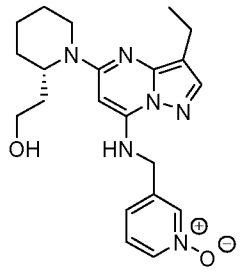
(VIII)

In one embodiment the inhibitor of Formula (I) is of the following formula:



(IX)

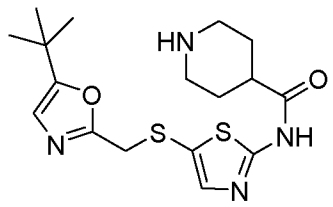
The inhibitor may comprise dinaciclib, or a derivative thereof. For example, in one embodiment the inhibitor comprises a compound of Formula (X) or a pharmaceutically acceptable salt or solvate thereof:



SCH 727965
(dinaciclib)

(X)

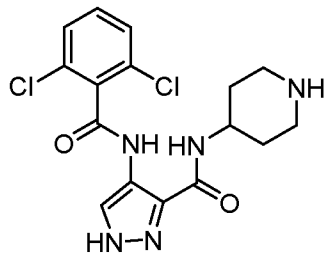
In one embodiment, the inhibitor comprises a compound of Formula (XI) or a pharmaceutically acceptable salt or solvate thereof:



SNS-032

(XI)

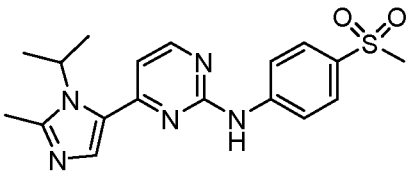
In one embodiment, the inhibitor comprises a compound of Formula (XII) or a pharmaceutically acceptable salt or solvate thereof:



AT7519

(XII)

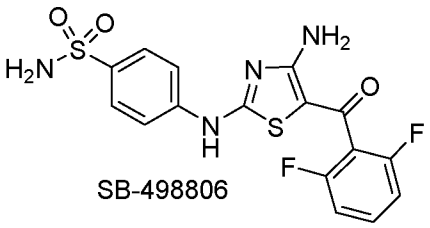
In one embodiment, the inhibitor comprises a compound of Formula (XIII) or a pharmaceutically acceptable salt or solvate thereof:



AZD 5438

(XIII)

In one embodiment, the inhibitor comprises a compound of Formula (XIV) or a pharmaceutically acceptable salt or solvate thereof:



SB-498806

(XIV)

As used herein, the term "C1-C3 alkyl" refers to a straight-chain or branched-chain alkyl group containing from 1 to 3 carbon atoms. Examples of alkyl groups include, but are not limited to methyl, ethyl, propyl or isopropyl.

As used herein, the term "6 member heterocyclyl" refers to a saturated monocyclic heterocyclic group having 6 ring members and containing at least one heteroatom as a ring member, wherein each of said at least one heteroatoms may be independently selected from the group consisting of nitrogen, oxygen, and sulfur. One group of heterocyclyls has 1 heteroatom as ring member. Another group of heterocyclyls has 2 heteroatoms as ring members. Examples of heterocyclyl groups include, but are not limited to piperazinyl, morpholinyl, piperidinyl, oxanyl, thianyl or dioxanyl.

As used herein, the term "haloalkyl" refers to an alkyl group having the meaning as defined above wherein one or more hydrogens are replaced with a halogen. A halo C1-3 alkyl group refers to a haloalkyl group wherein the alkyl moiety has from 1 to 3 C atoms. Specifically embraced are monohaloalkyl, dihaloalkyl or polyhaloalkyl groups. A monohaloalkyl group, for one example, may have an iodo, bromo, chloro or fluoro atom within the group. Dihalo or polyhaloalkyl groups may have two or more of the same halo atoms or a combination of different halo groups. Examples of haloalkyl groups include, but are not limited to, fluoromethyl, difluoromethyl, trifluoromethyl, chloromethyl, dichloromethyl, trichloromethyl, pentafluoroethyl, heptafluoropropyl, difluorochloromethyl, dichlorofluoromethyl, difluoroethyl, difluoropropyl, dichloroethyl or dichloropropyl.

Additionally, the compounds of Formulas I-XVI can contain asymmetric carbon atoms (chiral atoms) and can therefore exist in racemic and optically active forms. Thus, optical isomers or enantiomers, racemates, tautomers, and diastereomers are also encompassed in the compounds of Formulas I-XVI.

In one embodiment, the inhibitor is administered, or arranged to be administered, in combination with at least one other therapeutic agent. The one other therapeutic agent may comprise an oligonucleotide, such as siRNA or miRNA or equivalents thereof. The oligonucleotide may be capable of targeting expanded repeat transcript for degradation, for example through the RNA interference pathway. The oligonucleotide may comprise the sequence of (CAG)7. In another embodiment, the oligonucleotide may comprise the sequence of AGC AGC AGC AGC AG. In another embodiment, the oligonucleotide may comprise the sequence of CAG CAG CAG CAG CAG C. In another embodiment, the oligonucleotide may comprise the sequence of CGGAGCGGTTGTGAACTGGC.

The oligonucleotide may target the repeat sequence such as CUGn, CCUGn, CGGn, GGGGCCn, CAGn, or AUUCUn. Alternatively the siRNA oligonucleotide may bind anywhere in a target RNA which includes an expanded repeat transcript such as CUGn, CCUGn, CGGn, GGGGCCn, CAGn, AUUCUn. The skilled person will understand that U (uridine) residues will substitute T (thymine) residues in RNA relative to DNA. The siRNA oligonucleotide may bind anywhere in a target RNA which includes an expanded repeat transcript such as transcripts from any one of the transcripts from GenBank accession numbers: NM_001081563, AY329622, AF389886, AF389887, AH010982, L19493, JN681271, AF126749, NM_013236, NM_001271604, NM_002111, NM_000332, NM_002973, NM_004993, NM_000068, NM_000333, CR456776, D31840, or M34233.

The oligonucleotide may be at least 10, 11, 12, 13, 14, or 15 nucleotides long. In one embodiment, the oligonucleotide is at least 15 nucleotides long.

Advantageously, continuous exposure to inhibitor may be unnecessary to produce a beneficial effect in disorders caused by the generation of repeat expansion transcripts, such as DM-1. A pulsatile treatment may be sufficient. The inhibitor would remove

nuclear foci from afflicted cells after a short exposure, and this short exposure leads to a prolonged effect, wherein the inhibitor treatment results in a shift in the relative proportions of wild type and mutant DMPK transcripts (CDK12 inhibition leads to a preferential loss of the expanded repeat transcript). This may result from CDK12 removal from the repeat expansion transcript with subsequent dissociation of the nuclear foci and degradation of the mutant transcript. The nuclear foci protect repeat expansion transcripts from degradation and once released, they may be vulnerable to cellular or targeted degradation. Thus, a "two-hit" therapy regime may be provided in which a short treatment with a CDK12 inhibitor is used to disperse foci and expose repeat expansion transcripts to degradation via endogenous processes, or by, for example, antisense oligonucleotides.

The at least one other therapeutic agent may comprise a small molecule, drug, pro-drug, peptide, protein, antibody, nucleotide or vaccine. The at least one other therapeutic agent may comprise a sodium channel blocker, such as mexiletine, phenytoin or procainamide. The at least one other therapeutic agent may comprise a CNS stimulant drug, such as modafinil, for example for treating excessive daytime sleepiness. The at least one other therapeutic agent may comprise dehydroepiandrosterone (DHEA), creatine supplementation or mecasermin rinfabate (IPLEX, combination of recombinant insulin-like growth factor 1 and its binding protein, BP-3), which may be provided, for example, for improving muscle weakness. The at least one other therapeutic agent may comprise any one or more of pentamidine, a bisamidinium inhibitor, lomofugin or dilomofungin.

In an embodiment a CDK inhibitor may be used in combination with an oligonucleotide that targets the DMPK gene. The DMPK gene may be in the expanded repeat in the 3' untranslated region or it may be targeted anywhere in the gene.

The use in combination with at least one other therapeutic agent may comprise a combined dose formulation or a separate dose formulation. The use may be concurrent or sequential.

The inhibitor may be administered, or arranged to be administered, intermittently. For example, the inhibitor may be administered, or arranged to be administered, once in a three-day treatment cycle. Alternatively, the inhibitor may be administered, or

arranged to be administered, once in a 2-day treatment cycle. Alternatively, the inhibitor may be administered, or arranged to be administered, once in a 4-day treatment cycle. Alternatively, the inhibitor may be administered, or arranged to be administered, once in a treatment cycle of at least 3 days. Alternatively, the inhibitor may be administered, or arranged to be administered, once in a treatment cycle of at least 5 days. The inhibitor may be administered, or arranged to be administered, on day one of a three-day treatment cycle and wherein the dose is released within a 2-hour period immediately following administration.

The inhibitor may be provided and/or administered in a therapeutically effective dose. The dose may be sufficient to cause an alleviation or prevention of one or more symptoms of the disorder caused by the generation of repeat expansion transcripts, such as DM-1. The dose may be sufficient to cause an alleviation or prevention of all symptoms of the disorder caused by the generation of repeat expansion transcripts, such as DM-1. The dose may be sufficient to reduce the activity or presence of CDK12 by at least 50%. The dose may be sufficient to reduce the nuclear retention of repeat expansion transcripts by at least 50%. The dose may be sufficient to improve myotonia and muscle strength as judged by a six-minute walk test and hand grip test.

In one embodiment, the dose may comprise between about 2 and about 100 mg/kg. In one embodiment, the dose may comprise between about 5 and about 80 mg/kg. In one embodiment, the dose may comprise between about 2 and about 60 mg/kg. In one embodiment, the dose may comprise between about 5 and about 60 mg/kg. In one embodiment, the dose may comprise between about 5 and about 30 mg/kg. In one embodiment, the dose may comprise between about 5 and about 20 mg/kg. In one embodiment, the dose may comprise between about 5 and about 10 mg/kg. The dose may comprise no more than 60 mg/kg in a period of 24 hours. The dose may comprise no more than 30 mg/kg in a period of 24 hours. The dose may comprise no more than 10 mg/kg in a period of 24 hours. The dose may comprise no more than 5 mg/kg in a period of 24 hours. The dose may comprise no more than 100 mg/kg in a period of 3 days. The dose may comprise no more than 60 mg/kg in a period of 3 days. The dose may comprise no more than 30 mg/kg in a period of 3 days. The dose may comprise no more than 10 mg/kg in a period of 3 days. The dose may comprise no more than 5 mg/kg in a period of 3 days. The dose may comprise no more than 100 mg/kg in a period of 1 week. The dose may comprise no more than 60 mg/kg in a period of 1

week. The dose may comprise no more than 30 mg/kg in a period of 1 week. The dose may comprise no more than 20 mg/kg in a period of 1 week. The dose may comprise no more than 10 mg/kg in a period of 1 week. The dose may comprise no more than 100 mg/kg in a period of 2 or 4 weeks. The dose may comprise no more than 60 mg/kg in a period of 2 or 4 weeks. The dose may comprise no more than 30 mg/kg in a period of 2 or 4 weeks. The dose may comprise no more than 10 mg/kg in a period of 2 or 4 weeks. No more than 4 doses may be administered, or arranged to be administered, in a period of 24 hours. No more than 3 doses may be administered, or arranged to be administered, in a period of 24 hours. No more than 2 doses may be administered, or arranged to be administered, in a period of 24 hours. No more than 1 dose may be administered, or arranged to be administered, in a period of 24 hours. No more than 4 doses may be administered, or arranged to be administered, in a period of 3 days. No more than 3 doses may be administered, or arranged to be administered, in a period of 3 days. No more than 2 doses may be administered, or arranged to be administered, in a period of 3 days. No more than 1 dose may be administered, or arranged to be administered, in a period of 3 days. No more than 4 doses may be administered, or arranged to be administered, in a period of 1 week. No more than 3 doses may be administered, or arranged to be administered, in a period of 1 week. No more than 2 doses may be administered, or arranged to be administered, in a period of 1 week. No more than 1 dose may be administered, or arranged to be administered, in a period of 1 week. No more than 20 doses may be administered, or arranged to be administered, in a period of 2 or 4 weeks. No more than 15 doses may be administered, or arranged to be administered, in a period of 2 or 4 weeks. No more than 10 doses may be administered, or arranged to be administered, in a period of 2 or 4 weeks. No more than 8 doses may be administered, or arranged to be administered, in a period of 2 or 4 weeks. No more than 5 doses may be administered, or arranged to be administered, in a period of 2 or 4 weeks. No more than 4 doses may be administered, or arranged to be administered, in a period of 2 or 4 weeks. No more than 3 doses may be administered, or arranged to be administered, in a period of 2 or 4 weeks. No more than 2 doses may be administered, or arranged to be administered, in a period of 2 or 4 weeks. No more than 1 dose may be administered, or arranged to be administered, in a period of 2 or 4 weeks.

Advantageously, continuous exposure to the inhibitor may not be necessary to produce a beneficial effect in the treated subject and that a pulsatile treatment may be

sufficient. This method of administration may have the advantage of reducing side effects as it would allow a lower duration/frequency of inhibitor application.

The inhibitor may be administered orally or parenterally, for example, by intravenous, intramuscular or subcutaneous injection. In one embodiment, the inhibitor is suitable for oral administration.

The subject may be a mammal. In one embodiment the subject is human.

According to another aspect of the invention, there is provided the use of a CDK12 inhibitor in the preparation of a medicament for the treatment or prevention of a disorder caused by the generation of repeat expansion transcripts in a subject, for example RNA transcripts from a CTG repeat expansion.

According to another aspect of the invention, there is provided a method of treatment or prevention of a disorder caused by the generation of repeat expansion transcripts in a subject, comprising administering an inhibitor of CDK12 to the subject. The RNA repeat expansion transcripts may be from CTG repeat expansions.

The method may further comprise a subsequent administration of at least one other therapeutic agent. The at least one other therapeutic agent may comprise an oligonucleotide. The at least one other therapeutic agent may be suitable for enhancing degradation of nucleic acid repeat transcripts of DMPK.

According to another aspect of the invention, there is provided a method of screening for a therapeutic agent for a disorder caused by the generation of repeat expansion transcripts, such as RNA repeat expansion transcripts as in DM, comprising:

-providing a molecule for screening;

-detecting if the molecule inhibits the activity of CDK12,

wherein detection of inhibition of CDK12 selects the molecule as a potential candidate therapeutic agent.

Detecting if the molecule inhibits the activity of CDK12 may comprise the use of an assay measuring any inhibition of CDK12 consumption of ATP during the phosphorylation of a substrate peptide in the presence of the molecule to be screened.

The inhibition of CDK12 may be selective inhibition. The method of screening may further comprise the step of detecting if the molecule does not significantly inhibit other cyclin dependent kinases, such as CDK9.

According to another aspect of the invention, there is provided a composition comprising a CDK12 inhibitor and a pharmaceutically acceptable carrier.

The composition may further comprise at least one other therapeutic agent. The least one other therapeutic agent may be capable of enhancing repeat expansion transcript degradation through cellular or targeted degradation. The at least one other therapeutic agent may comprise any one or more therapeutic agent described herein.

According to another aspect of the invention, there is provided a CDK12 inhibitor as described herein.

The skilled person will understand that optional features of one embodiment or aspect of the invention may be applicable, where appropriate, to other embodiments or aspects of the invention.

There now follows by way of example only a detailed description of the present invention with reference to the following drawings.

Figure 1: Screening the PKIS compound collection identified 6 inhibitors that reduce nuclear foci. (A-F) Six inhibitors cause reduced nuclear foci across an 11 point dilution. Graphs show percentage of nuclear foci relative to DMSO treated cells. All six compounds share a pyrazolo[1,5b]pyridazine core structure.

Figure 2: Analysis of the PKIS inhibition profiles to identify common kinase targets. (A) Loading plot of 2-compound partial least squares model. Kinase activities correlating with the nuclear foci assay are labelled, with cyclin-dependent kinases highlighted with *. **(B-G)** Plots show the percentage inhibition of the kinase target at 0.1μM concentration of compounds, grouped according to activity in the nuclear foci assay, including the six active

compounds. Compounds with less than 25% inhibitory active activity on the kinase are not included on the graphs.

Figure 3: Focussed screening of CDK family inhibitors. 12 point dilution graphs plotting percentage of nuclear foci relative to DMSO treated cells for **(A)** SNS-032 **(B)** AT7519 **(C)** PD0332991 **(D)** R-roscovitine **(E)** dinaciclib **(F)** CDK9 inhibitor II.

Figure 4: Chemoproteomics target deconvolution. IC50 values were generated by affinity capturing of kinases from K562 or A204 cell extract using beads derivatized with SNS-032, in the presence of different concentrations of free competing compound or vehicle (DMSO). pIC50 values are plotted against the pIC50 in the foci inhibition assay for each compound. A good correlation of kinase binding affinity with the inhibitory activity on foci is observed for CDK5, CDK7, CDK9, and CDK12. r: Pearson correlation coefficient; p: p-value (calculated probability).

Figure 5: CDK9 and CDK12 protein expression in DM. Western blot of vastus lateralis muscle biopsy samples in non-DM and DM1 patients for **(A)** CDK9 and **(B)** CDK12. Both blots are normalised to α-tubulin. **(C)** Histogram to quantify levels of CDK9 protein normalised to α-tubulin. **(D)** Histogram to quantify levels of CDK12 protein normalised to α-tubulin. **(E)** Immunohistochemistry of CDK12 in non-DM and DM1 fibroblast cells. **(F)** Quantification of the number of CDK12 nuclear granules. **(G)** Immunohistochemistry and in situ hybridisation shows co-localisation of repeat expansion foci with CDK12 nuclear granules (CDK12 in green/bright spots, repeat expansion RNA (Arrow)) **(H)** CDK12 protein knockdown by shRNA results in reduced CDK12 protein granules (green) and a subsequent reduction in CUG repeat expansion RNA foci (red/Arrow).

Figure 6: Inhibitor treatment as a therapeutic for DM. (A) Ethidium bromide stained gel showing RT-PCR products from nuclear (N) and cytoplasmic (C) RNA fractions following amplification and *Bpm*I digest of a fragment of DMPK. GAPDH is used as a loading control. **(B)** Histograms showing the relative proportions of nuclear mutant *DMPK* transcripts

compared to wild type *DMPK* transcripts. The relative proportions of the mutant and wild type *DMPK* transcripts in DM1 fibroblast cells were assessed following treatment with dinaciclib (1μM) for 24 hours. **(C-F)** Histograms show percentage of cells in the population with 0, <2, <5 and 5+ foci per nucleus **(C)** Untreated DM1 cells. **(D)** DM1 cells treated with SNS-032 for 2 hours. **(E)** DM1 cells treated with SNS-032 for 2 hours with 48 hours recovery in growth media **(F)** DM1 cells treated with SNS-032 for 2 hours with 72 hours recovery in growth media.

Figure 7: Inhibitor treatment in a DM1 mouse model (A) Combined myotonia grade scores in quadriceps, gastrocnemius and paraspinal muscles from HSALR mice following vehicle and dinaciclib compound treatment (n=6 per treatment group) **(B)** Myotonia grades by muscle type in dinaciclib and vehicle treated HSALR mice. **(C)** Ethidium bromide stained gel to assess ATP2A1 splice isoforms in quadriceps and gastrocnemius muscle samples from vehicle and dinaciclib treated mice **(D)** Histogram showing the relative proportion of exon 22 exclusion and inclusion in vehicle and dinaciclib treated HSALR mice **(E-F)** Laminin stain demonstrates a reduction in centralised nuclei in muscle fibres of dinaciclib treated mice compared to vehicle control animals.

Figure 8: Screening the PKIS collection identifies the CMGC kinase family. Plots to analyse the PKIS inhibition profiles of kinases identified from the partial least squares model as possible cellular targets comparing active versus inactive compounds. Compounds with less than 25% inhibition are not included on the graphs.

Figure 9: Kinobead profiling of 11 compounds. Kinobeads profiling of a set of 11 compounds which represent a range of activities in the nuclear foci assay. Target profiles were generated by adding each compound to K562 cell extract at a concentration of 2 μM followed by incubation with kinobeads and quantification of bead-bound proteins. **A:** Values indicate target binding compared to a DMSO control where a value of 1 represents 100% binding and therefore no target inhibition and a value of 0 indicates 0% binding and 100%

inhibition of the target kinase. **B:** Shows the structure of each compound tested.

Figure 10: Comparison of protein binding profiles for immobilised inhibitors SNS-032 and AT7519.

Figure 11: Examples of dose-response competition binding curves for different compound/target combinations. An affinity matrix was generated by immobilisation of SNS-032 to sepharose beads and affinity capturing was performed from K562- or A204 cell extract in the presence of vehicle (DMSO) or different concentrations of inhibitor as indicated. IC50 concentrations and inflection points of the dose-response competition curves are indicated by dotted lines.

Figure 12: CDK12 protein knockdown by siRNA and shRNA. (A) CDK12 immunohistochemistry following knockdown with scrambled and CDK12 shRNA **(B)** Quantification of CDK12 protein granules within the nucleus in CDK12 shRNA cells shows a 56% reduction in CDK12 granule number compared to scrambled shRNA **(C)** In situ hybridization analysis following knockdown with scrambled and CDK12 shRNA **(D)** Quantification of CUG repeat RNA foci within the nucleus in CDK12 shRNA cells shows a 69% reduction compared to scrambled shRNA **(E)** Histogram show percentage of cells in the population with 0, 1-2, 3-4 and 5+ foci per nucleus, in scrambled and CDK12 shRNA treated cells. **(F)** CDK12 immunohistochemistry following knockdown with scrambled and CDK12 siRNA **(G)** Quantification of CDK12 granules within the nucleus in CDK12 siRNA cells shows a 47% reduction in CDK12 granule number compared to scrambled siRNA **(H)** siRNA validation by western blot analysis for CDK12 and α-tubulin **(I)** Histogram of intensity scan of western blot analysis showed a 34% reduction in CDK12 protein compared to scrambled controls. Data normalized to α-tubulin. **(J)** In situ hybridization analysis following knockdown with scrambled and CDK12 siRNA **(K)** Quantification of CUG repeat RNA foci within the nucleus in CDK12 siRNA cells shows a 46% reduction compared to scrambled siRNA **(L)** Histograms show percentage of cells in the population with 0, 1-2, 3-4 and 5+ foci per nucleus, in scrambled and CDK12 siRNA treated cells.

Figure 13: IC50 values of previously reported CDK inhibitors.

Figure 14: pIC50 values generated by affinity capturing with the SNS-032 affinity matrix in K562 cell extract for the different CDK inhibitors added to the cell extracts.

INTRODUCTION

Myotonic dystrophy is caused by a CTG repeat expansion within the 3' untranslated region of the *DMPK* gene, leading to the formation of distinct nuclear foci. The involvement of kinases has been linked to the pathophysiology of the condition but to date a definitive kinase target for drug development has not been identified. It has been observed herein that CDK12 is elevated in DM cell lines and in DM patient muscle biopsies. Repeat expansion transcripts accumulate at the periphery of nuclear speckles and CDK12 co-localises with these nuclear speckles. It has been found that inhibition of CDK12 leads to the dispersal of DM-associated nuclear foci and degradation of repeat expansion transcripts.

RESULTS AND DISCUSSION

Screening the PKIS collection

Using a previously reported assay the PKIS collection was screened for compounds that reduce nuclear foci and the compounds were then analysed for their known selectivity profiles to identify the common kinase targets (Ketley, A. *et al.* (2014). *Hum Mol Genet*, 23: 1551-1562). DM1 fibroblasts were treated with compounds in an 11 point dilution series from 2011M-19nM for 24 hours. Following treatment, fluorescent *in situ* hybridisation was performed with a cy3 labelled CAG10 probe, to visualize nuclear foci and cells were analysed on a Molecular Devices plate reader with customised MetaExpress software (Ketley, A. *et al.* (2014). *Hum Mol Genet*, 23: 1551-1562). Compounds that reduced nuclear foci in a concentration dependent manner, compared to DMSO treated cells were identified and prioritized for further study. Six compounds that share a pyrazolo[1,5b]pyridazine core were found to reduce the number of nuclear foci following 24 hour treatment of DM cells (Fig 1).

Target deconvolution

The known selectivity profile of the six active compounds was examined to identify common kinase targets. The pIC50 values generated from the foci assays were compared to the compound inhibition profiles against 224 kinase targets (Drewry, D.H. *et al.* (2014). *Current topics in medicinal chemistry*, 14: 340-342). A partial least squares (PLS) model was used to cluster the data which suggested that the common target was likely to be a member of the CMGC (cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAP kinases), glycogen synthase kinases (GSKs) and CDK-like kinases) family (Fig. 2A). Indeed, these compounds were originally designed to target CDK family members (Stevens, K.L. *et al.* (2008). *Bioorganic & medicinal chemistry letters*, 18: 5758-5762) and the inhibitors active in the nuclear foci assay all showed significant activity across many members of the CDK family. The kinase inhibition profiles for the six hit compounds were compared to their activity in the nuclear foci assay, which suggested that CDK1, CDK2, CDK3, CDK5 and CDK6 are unlikely to be responsible for foci formation (Fig. 2B-G). Of the targets covered by the PKIS collection, CDK4 appeared more likely to play a role (Fig. 2E) but other CDK family members required consideration as they are absent from the PKIS annotations.

Focussed screening of known CDK family inhibitor molecules

Next, additional small molecule CDK inhibitors with well-annotated selectivity profiles were tested (Figure 13) in the nuclear foci assay. This subset of inhibitors displayed differential activities in the nuclear foci assay and a diverse range of potencies across the CDK family members (Fig 3). To confirm involvement of the CDK family the kinobeads methodology was employed, which is based on sepharose beads derivatized with a combination of promiscuous kinase inhibitors (Bantscheff, M. *et al.* (2007). *Nat Biotechnol*, 25: 1035-1044; Werner, T. *et al.* (2012). *Analytical chemistry*, 84: 7188-7194; and Kruse, U. *et al.* (2011). *Leukemia*, 25: 89-100) to profile 10 compounds, which represent a range of activities in the nuclear foci assay. Target profiles were generated by adding each compound to K562 erythroleukemia cell extract at a concentration of 2 μM, followed by incubation with two variations of kinobeads and quantification of bead-bound proteins. The first type of bead was developed for the profiling of tyrosine and serine/threonine kinases of the eukaryotic protein kinase family (Bantscheff, M. *et al.* (2007). *Nat Biotechnol*, 25: 1035-1044), whereas the second type of bead captures additional kinases from the PI3K/lipid

kinase family (Bergamini, G. *et al.* (2012). *Nature chemical biology*, 8: 576-582). The profiling results clearly indicated that the most active compounds in this subset exhibited shared activities only across the CDK family (Fig. 9A), consistent with the PKIS data.

To refine the possible target kinases, the IC50 values were compared for the nuclear foci active inhibitors and the nuclear foci inactive inhibitors. Small molecules SNS-032 (also known as BMS 387032) and AT-7519 demonstrate high activity in the nuclear foci assay and display the highest potency against CDK2 and CDK9 (Fig. 3a and 3b and Figure 13). However CDK2 cannot be responsible for nuclear foci reduction due to significant overlap in the IC50 values of active and inactive compounds against this target. Likewise CDK1, CDK4, CDK5, CDK6 and CDK7 can all be discounted for similar reasons (Figure 13).

The analysis of this subset of known CDK inhibitor compounds confirms the results of the PKIS screen but raises the possibility that the target responsible for nuclear foci reduction is a less well described CDK family member. To investigate this possibility four additional CDK inhibitors, with a range of potencies, were tested to determine the potential involvement of CDK9 as a possible target (Fig. 3E-F and Figure 13). Interestingly, dinaciclib and SNS-032 have the same IC50 value against CDK9, however, dinaciclib displays a significantly increased activity in the nuclear foci assay. Furthermore, CDK9 Inhibitor II (Fig. 3F), which is a specific but less potent CDK9 inhibitor, with an IC50 value of 350nM, reduced nuclear foci at the highest concentration whereas two other compounds; PD0332991 and R-roscovitine, with comparable CDK9 IC50 values (400nM and 500nM, respectively) are inactive in the nuclear foci assay at the concentrations tested (Fig 3C and 3D). These data suggest an additional CDK family member as the target for nuclear foci reduction.

Chemoproteomics target resolution

To determine the specific CDKs responsible for the reduction of nuclear foci it was sought to expand the target coverage within the CDK family by the immobilization of two of the active compounds containing a suitable secondary amine; SNS-032 and AT7519 (Fig. 10). Beads derivatized with either compound showed good coverage of the CDK family, including family members which are not present in commercial kinase panels. For an in depth chemoproteomics study profiling inhibitor selectivity

across the CDK family, dose-response competition-binding profiles were generated for all active and one of the inactive compounds (PD0332991) in K562 cells or in A204 rhabdomyosarcoma cells. To confirm that the K562, A204 and DM1 cell lines express the same kinases a whole proteome analysis was conducted. All CDK/PCTK proteins identified by profiling were also identified in the cells used for the phenotypic foci assay as follows:

CDK family member proteins identified by whole proteome analysis of DM fibroblasts (protein accession numbers for CDK family proteins).

CDK1: P06493
CDK2: CAA43807.1
CDK4: CAG47043
CDK5: CAG33322
CDK6: Q00534
CDK7: P50613
CDK9: AAF72183
CDK10: AAH25301
CDK12: Q9NYV4.2
CDK13: Q14004.2
PCTK1: Q00536
PCTK2: Q00537.2

The resulting dataset comprises IC50 curves for 12 CDK family kinases (Fig. 11, Figure 14). The best correlation across the compound set of kinase IC50 values with the inhibitory activity on foci was observed for CDK12, followed by CDK9 (Figure 4). The IC50 values for CDK9 and CDK12 are closely in line with their observed activity in the foci assay, whereas the IC50 values for CDK2 and CDK7 appear too low to implicate them as targets. Consistent with the previous analysis, presented in Figure 3E, dinaciclib was the most active inhibitor against the CDK family, in particular for CDK12, suggesting it may be the most likely kinase target.

CDK12 in DM pathogenesis

Thus far the experiments point to CDK12 as the most likely kinase to have an association with repeat expansion foci. To examine the potential involvement in DM

pathogenesis, the endogenous levels of CDK12 were assessed, in addition to CDK9, in vastus lateralis muscle biopsy samples from four DM1 and four healthy volunteers using Western blots. No significant difference in CDK9 protein level compared to that in controls could be detected (Fig. 5A and 5C). However, there was a clear increase in levels of CDK12 in DM biopsies versus those from healthy volunteers, with 48% more CDK12 protein detected in DM samples ($p=0.0025$) (Fig. 5B and 5D).

To understand the relationship between nuclear foci and CDK12 in DM pathophysiology, the cellular location of the protein was established by immunohistochemistry in DM and non-DM fibroblasts. Consistent with previously published data in non-DM cells CDK12 was localised in the nucleus in granular structures in both DM and non-DM cells (Ko, T.K. *et al.* (2001). *Journal of cell science*, 114: 2591-2603) (Fig. 5E). Quantification of these structures in 400 cells showed that the number of granules was elevated in DM cells, with an average number of 18.74 (±3.88), compared to 12.07 (±2.27) in non-DM cells ($p<0.0001$) (Fig. 5f). The size of CDK12 granules was not significantly different in non-DM and DM cells. This increase in number is consistent with the increased overall levels of CDK12 protein detected by Western blot. Next immunohistochemistry of CDK12 was conducted followed by in situ hybridisation to detect the repeat expansion RNA foci. Co-localisation of the repeat expansion foci with CDK12 protein was found. Quantification using customised MetaXpress software revealed that 100% of repeat expansion foci co-localised with CDK12 protein (Fig. 5g). To understand the impact of increased CDK12 protein in DM and the relationship between CDK12 protein granules and repeat expansion foci shRNA and siRNA were used to reduce the number of CDK12 granules in DM cells. The effect on repeat expansion foci was then assessed. Following lentiviral infection expressing three shRNAs against CDK12 a 56% reduction in the number of CDK12 granules and a 69% reduction in repeat expansion foci compared to control cells was observed (Fig. 5H and Fig. 12A-E). This was verified by siRNA knockdown of CDK12 and quantification by Western blot analysis, which confirmed a 34% reduction in protein levels. This resulted in a 46% reduction in CDK12 protein granules and a 47% reduction in repeat expansion RNA foci indicating that specific inhibition of CDK12 and dissolution of CDK12 protein from nuclear granules leads to a dispersal of repeat expansion foci (Fig. 12F-L).

Inhibitor treatment as a therapeutic for DM

As an association between CDK12 and nuclear foci has been established, and nuclear foci comprise repeat expansion transcripts, it was sought to establish the effect of inhibitor treatment on the level of repeat expansion transcripts. For this an RT-PCR assay was employed that utilises a *Bpml* polymorphism to distinguish between wild-type and mutant DMPK transcripts (Fig. 6A) (Hamshere, M.G. *et al.* (1997). *Pro Natl Acad Sci USA,* 94: 7394-7399). Following treatment with dinaciclib, analysis of nuclear and cytoplasmic cell extracts showed that the repeat expansion transcripts were still retained within the nuclear fraction. However, quantification using Genescan analysis showed a 59% decrease in the relative proportion of repeat expansion transcripts compared to wild type transcripts in the nucleus (Fig 6B). These data indicate that exposure to dinaciclib, leads to preferential loss of the repeat containing transcript, which in turn suggests that targeting CDK12 provides a viable option for DM treatment development.

Loss of the repeat transcript may result from incomplete transcription of the expanded transcript following inhibitor treatment or it may be due to CDK12 removal from the repeat expansion transcript with subsequent dissociation of the nuclear foci and degradation of the mutant transcript. If the latter is correct it would suggest that nuclear foci protect repeat expansion transcripts from degradation and once released, they may be vulnerable to cellular or targeted degradation. Thus, a possible two-hit therapy regime is proposed in which short treatment with a CDK12 inhibitor is used to disperse foci and expose repeat expansion transcripts to degradation via endogenous processes or by antisense oligonucleotides (Mulders, S.A. *et al.* (2009). *Proc Natl Acad Sci USA*, 106: 13915-13920).

As CDK12 is a transcription-regulating kinase associated with nuclear foci in DM cells, it was sought to establish the effect of inhibiting this target on the kinetics of nuclear foci formation and dispersal. To do this DM1 fibroblast cells were exposed to the two most potent foci reducing compounds, dinaciclib and SNS-032, for different lengths of time from 2 hours to 48 hours. Both compounds produced a significant reduction in foci but this was most rapid in the case of SNS-032, which was effective following just 2 hours of treatment. Continuous exposure to transcription-regulating inhibitors, would not be a viable therapy option for DM, thus the effect of short term treatment on nuclear foci was examined. DM1 fibroblasts were exposed to SNS-032 for 2 hours, after which time the cells were washed thoroughly and allowed to recover

in complete growth media. Quantification of nuclear foci showed that 68% of untreated DM1 cells have more than 5 foci and only 5% have no detectable foci (Fig. 6C). When cells are treated with SNS-032 for 2 hours, with no recovery time, this distribution shifts to 28% of cells with more than 5 foci and 10% cells with no foci (Fig. 6D). However, following 48 and 72 hours of recovery following exposure to SNS-032, the proportion of cells without nuclear foci increases further to 14% and 36%, respectively (Fig. 6E and 6F). Taken together this data suggests that a short (2hr) treatment with inhibitor, followed by a prolonged (72hr) recovery leads to a significant reduction in numbers of nuclear foci and a preferential reduction in mutant transcripts, and therefore that pulsatile treatment could be an efficacious approach to DM therapy.

To establish the *in vivo* effect of this inhibitor HSA^{LR} mice were treated by intraperitoneal injection for a 28 day treatment period comprising 12 injections in total. Following inhibitor treatment the mice were analysed by EMG analysis to assess the functional effect on myotonia and demonstrated a significant improvement in the myotonia grade score across the four muscle types tested; quadriceps, gastrocnemius, tibialis anterior and the lumbar paraspinals ($p=0.0021$, $n=6$) (Fig. 7A-B). Molecular analysis demonstrated an improvement in the inclusion of exon 22 for the splice isoforms of ATP2A1 (Fig. 7C-D) and a reduction in the presence of centralised nuclei within muscle fibres following inhibitor treatment (Fig 7E-F).

MATERIALS AND METHODS

Cell Culture

Fibroblast cells were grown in Dulbecco's Modified Eagles Medium (DMEM) with penicillin and streptomycin, and 10% fetal calf serum (Sigma).

In situ hybridization protocol

Cells were exposed to compounds for 24hrs after which in situ hybridization was performed to identify foci using a Cy3 labelled $(CAG)_{10}$ probe. Plates were analysed on a Molecular Devices Micro High Content Imaging system, with nine fields imaged per well to give approximately 100 cells per well, per compound treatment. The nuclear area was identified by Hoechst stain and the number, size and intensity of foci

was determined by scoring adjacent pixels that were 80 grayscales or more above background.

Preparation of cell extracts

K562 and A204 cells were obtained from ATCC and cultured in RPMI medium containing 10% FCS. Cells were expanded to 1,5 x 106 cells/ml. A204 cells were cultured in McCoy's 5A medium containing 15% FCS. Cells were expanded to 100% confluency. Cells were harvested and subjected to 3 washes with ice-cold PBS. Aliquots were snap frozen in liquid nitrogen and stored at -80 °C. Cell extracts were prepared as described (Bantscheff, M. *et al.* (2011). *Nat Biotechnol*, 29: 255-265).

Chemoproteomics

Affinity profiling was performed as described previously (Bantscheff, M. *et al.* (2007). *Nat Biotechnol*, 25: 1035-1044 and Bantscheff, M. *et al.* (2011). *Nat Biotechnol*, 29: 255-265). Sepharose beads were derivatized with SNS-032 at a concentration of 1 mM to generate a bead matrix, or KinobeadsTM were used as a matrix for profiling. Beads (35 µl in case of KinobeadsTM or 5 µl in case of SNS-032) were washed and equilibrated in lysis buffer at 4 °C for 1 h with 1 ml (5 mg) K562 cell extract, which was pre-incubated with compound or buffer. Beads were transferred to disposable columns (MoBiTec), washed extensively with lysis buffer and eluted with SDS sample buffer. Proteins were alkylated, separated on 4-12 % NuPAGE (Invitrogen), stained with colloidal Coomassie, and quantified by isobaric mass tagging and LC-MS/MS.

Peptide and protein identification and quantification

Sample preparation and labeling with TMT isobaric mass tags was performed essentially as described (Bantscheff, M. *et al.* (2011). *Nat Biotechnol*, 29: 255-265). For mass spectrometric analyses samples were dried in vacuo and resuspended in 0.1% formic acid in water and aliquots of the sample were injected into a nano-LC system coupled to a mass spectrometer: Eksigent 1D+ coupled to LTQ-OrbitrapXL mass spectrometer, Waters nanoAcquity coupled to Orbitrap Elite mass spectrometer, or Ultimate 3000 RSLC nano coupled to Q Exactive mass spectrometer (Thermo Fisher Scientific). Peptides were separated on custom 50 cm x 75 µM (internal diameter) reversed-phase columns (Reprosil) at 40 °C. Gradient elution was performed from 3% acetonitrile to 40% acetonitrile in 0.1% formic acid over 120-270min. LTQ-Orbitrap

XL was operated with Xcalibur 2.0, Orbitrap Elite and Q Exactive instruments were operated with Xcalibur 2.2 software. Intact peptides were detected in the LTQ-OrbitrapXL/Orbitrap Elite at 30.000 resolution (measured at m/z = 400), in the Q Exactive at 70.000 resolution (m/z = 200). Internal calibration was performed with LTQ-OrbitrapXL using the ion signal from (Si(CH3)20)6H+ at m/z 445.120025. Data-dependent tandem mass spectra were generated for up to ten peptide precursors (LTQ-OrbitrapXL/Orbitrap Elite six precursor, Q Exactive ten) using a combined CID/HCD (LTQ-Orbitrap XL) approach or using HCD only (Orbitrap Elite/Q Exactive) at a resolution of 15.000/17.500. For CID up to 5,000 ions (LTQ-Orbitrap XL) were accumulated in the ion trap (maximum ion accumulation time = 150 msec), for HCD up to 50.000 ions (LTQ-OrbitrapXL, maximum ion accumulation time = 350 msec), up to 30.000 ions (Orbitrap Elite, maximum ion accumulation time = 150 msec) and 1e6 ions (Q Exactive, maximum ion accumulation time = 60 msec) were accumulated in the HCD cell. Mascot 2.3 and 2.4 (Matrix Science) was used for protein identification using 10 p.p.m. mass tolerance for peptide precursors and 0.6 Da (CID) or 20 mDa (HCD) tolerance for fragment ions. Carbamidomethylation of cysteine residues and TMT modification of lysine residues were set as fixed modifications and methionine oxidation, N-terminal acetylation of proteins and TMT modification of peptide N-termini were set as variable modifications. The search database consisted of a customized version of the International Protein Index database combined with a decoy version of this database created using a script supplied by Matrix Science. Criteria for protein quantification were: a minimum of 2 sequence assignments matching to unique peptides was required (FDR for quantified proteins <<0.1 %), Mascot ion score > 10, signal to background ratio of the precursor ion > 4, signal to interference > 0.5 (Savitski, M.M. *et al.* (2010). *Journal of the American Society for Mass Spectrometry*, 21: 1668-1679). Reporter ion intensities were multiplied with the ion accumulation time yielding an area value proportional to the number of reporter ions present in the mass analyser. Peptide fold changes were corrected for isotope purity as described and adjusted for interference caused by co-eluting nearly isobaric peaks as estimated by the signal-to-interference measure (Savitski. M.M. *et al.* (2013). *Journal of proteome research*, 12: 3586-3598). Protein quantification was achieved using a sum-based bootstrap algorithm (Savitski, M.M. (2011). *Analytical chemistry*, 83: 8959-8967).

Assay for Repeat Expansion Transcripts

Reverse transcription was performed using 1 μg total RNA from compound-treated and untreated cells. PCR was carried out using 1/20 of the synthesized cDNA with primers N11, 5'-CACTGTCGGACATTCGGGAAGGTGC and 133, 5'GCTTGCACGTGTGGCTCAAGCAGCTG. For Genescan analysis primer N11 was labelled with FAM. Amplification was performed with a Tm of 58°C. The PCR product was subsequently heated to 95°C for 2 minutes followed by cooling to 4°C. For *Bpml* restriction digestion analysis of DMPK PCR products, 8 μl of PCR mixture was digested overnight with restriction enzyme *Bpml* (NEB) in a total reaction volume of 20 μl at 37°C. The final products were analysed by electrophoresis at 90V with 3% agarose gels and the density of bands quantified using ImageJ software or by fragment analysis on an AB1377 sequencer followed by Genescan quantification.

Western Blots and detection

Western blotting was performed using a commercial NuPage system (Invitrogen, UK) according to the manufacturer's instructions. The primary antibodies used in this study were human CDK9 (Abcam, 1:1000 dilution), human CDK12 (Abcam, 1:400 dilution), human α-tubulin and human Lamin B (both obtained from Santa Cruz and used at dilutions of 1:500). Anti-mouse IgG-horseradish peroxidise (HRP) was used as the secondary antibody. ImageJ software was used for the quantification of bands on western blots.

Colocalisation studies

Cells were grown on coverslips for 24 hours before being fixed and permeabilised with 50:50 ice cold acetone:methanol. Cells were blocked in 5% BSA with 5% sheep serum. Anti-CDK12 antibody (Abcam) was used at 1:1000 dilution at 4°C overnight followed by staining with Alexafluor-488 anti-mouse secondary antibody (1:500). Cells were incubated in 4% PFA for 5 minutes, followed by 15 minutes in pre-hybridisation solution (40% formamide, 10% 20xSSC, 50% DEPC water) and incubated with a cy3 labelled CAGio probe overnight at 37°C. Coverslips were mounted on slides using Vectorshield Mounting Media with DAPI. Images were acquired using a Zeiss 710 confocal microscope and analysed using LSM image browser.

siRNA synthesis

The siRNA oligonucleotides were synthesized on an ABI 394 DNA/RNA synthesizer. Columns (SynBase™ CPG 1000Å, RNA: 0.2 μmol), standard 2'-OTBDMS RNA-phosphoramidites and reagents for the synthesizer were purchased from Link Technologies Ltd., $MeNH_2$ solution (33 wt.% in ethanol) was obtained from Fluka, NEt3•3HF, N-methylpyrrolidinone (NMP) were purchased from Aldrich, illustra Nap™-10 columns were obtained from GE Healthcare Europe GmbH. Dichloromethane and acetonitrile were freshly distilled from CaH_2 before use on the synthesizer.

The siRNA oligonucleotides were synthesized using a standard 0.2 μM scale protocol, but with a 10 min coupling time for each nucleotide addition step. The polymer-bound oligoribonucleotide was transferred from the synthesis column to a 1.5 mL microfuge tube and suspended in $MeNH_2$ solution (1 mL). The mixture was heated to 65 °C for 10 min, cooled to room temperature (water/icebath) and centrifuged for 1 min (10 000 g). The supernatant was separated from the CPG beads, the beads were washed with RNase free water (2 x 0.25 mL), all supernatants were combined and dried (2 h under nitrogen stream, then freeze dried). The oligoribonucleotide was resuspended in anhydrous NEt_3•3HF/NEt_3/NMP solution (250 μl of a solution of 1.5 mL NMP, 750 μl NEt3 and 1.0 mL NEt_3•3HF), heated to 65 °C for 1.5 h, cooled to room temperature and quenched with 3M NaOAc solution (25 μL). n-BuOH (1 mL) was added to the mixture, which was then thoroughly mixed, cooled to -70 °C for 1 – 2 h (dry ice) to encourage further precipitation and centrifuged for 30 min (4 °C, 13 000 g). The supernatant was removed, the pellet washed with 70% EtOH (2 x 500 μL) and then dried in vacuo (30 min). The dry precipitate was dissolved in RNase free water (1 mL) and desalted using a Nap™-10 column following the standard protocol. The resulting solution was freeze dried overnight leaving the oligoribonucleotide as a white foam/powder.

CDK12 siRNA knockdown

Scrambled: 5' ACGUGACACGUUCGGAGAAUU and CDK12: 5' CGAAAUAAUGAUGUUGGCACCAGUU siRNA sequences. Cells were electroporated on day 1 and day 4 with 800nM of scrambled or CDK12 siRNA using the Amaxa Nucleofector system. Cells were collected on day 7 for immunohistochemistry, in situ hybridisation and western blot analysis.

CDK12 shRNA knockdown

Cells were plated at 40% confluency the day before infection in 96 well format. Lentiviral titre (SantaCruz sc-44343-V) was added at an MOI of 10 in 5µg/ml polybrene diluted in DMEM media. Cells were spin inoculated by centrifugation at 2500rpm for 30 minutes. Following 24 hours incubation the virus was removed and replaced with fresh DMEM media. The infection was repeated on day 4 and cells were collected on day 7 for immunohistochemistry and in situ hybridisation analysis.

CDK12 sequence

```
         10         20         30         40         50
MPNSERHGGK KDGSGGASGT LQPSSGGGSS NSRERHRLVS KHKRHKSKHS
         60         70         80         90        100
KDMGLVTPEA ASLGTVIKPL VEYDDISSDS DTFSDDMAFK LDRRENDERR
        110        120        130        140        150
GSDRSDRLHK HRHHQHRRSR DLLKAKQTEK EKSQEVSSKS GSMKDRISGS
        160        170        180        190        200
SKRSNEETDD YGKAQVAKSS SKESRSSKLH KEKTRKEPEL KSGHKDRSKS
        210        220        230        240        250
HRKRETPKSY KTVDSPKRRS RSPHRKWSDS SKQDDSPSGA SYGQDYDLSP
        260        270        280        290        300
SRSHTSSNYD SYKKSPGSTS RRQSVSPPYK EPSAYQSSTR SPSPYSRRQR
        310        320        330        340        350
SVSPYSRRRS SSYERSGSYS GRSPSPYGRR RSSSPFLSKR SLSRSPLPSR
        360        370        380        390        400
KSMKSRSRSP AYSRHSSSHS KKKRSSSRSR HSSISPVRLP LNSSLGAELS
        410        420        430        440        450
RKKKERAAAA AAAKMDGKES KGSPVFLPRK ENSSVEAKDS GLESKKLPRS
        460        470        480        490        500
VKLEKSAPDT ELVNVTHLNT EVKNSSDTGK VKLDENSEKH LVKDLKAQGT
        510        520        530        540        550
PDSKPIALKE EIVTPKETET SEKETPPPLP TIASPPPPLP TTTPPPQTPP
        560        570        580        590        600
LPPLPPIPAL PQQPPLPPSQ PAFSQVPASS TSTLPPSTHS KTSAVSSQAN
        610        620        630        640        650
SQPFVQVSVK TQVSVTAAIP HLKTSTLPPL PLPPLLPGDD DMDSPKETLP
        660        670        680        690        700
SKPVKKEKEQ RTRHLLTDLP LPPELPGGDL SPPDSPEPKA ITPPQQPYKK
        710        720        730        740        750
RPKICCPRYG ERRQTESDWG KRCVDKFDII GIIGEGTYGQ VYKAKDKDTG
        760        770        780        790        800
ELVALKKVRL DNEKEGFPIT AIREIKILRQ LIHRSVVNMK EIVTDKQDAL
        810        820        830        840        850
DFKKDKGAFY LVFEYMDHDL MGLLESGLVH FSEDHIKSFM KQLMEGLEYC
        860        870        880        890        900
HKKNFLHRDI KCSNILLNNS GQIKLADFGL ARLYNSEESR PYTNKVITLW
        910        920        930        940        950
YRPPELLLGE ERYTPAIDVW SCGCILGELF TKKPIFQANL ELAQLELISR
        960        970        980        990       1000
LCGSPCPAVW PDVIKLPYFN TMKPKKQYRR RLREEFSFIP SAALDLLDHM
       1010       1020       1030       1040       1050
LTLDPSKRCT AEQTLQSDFL KDVELSKMAP PDLPHWQDCH ELWSKKRRRQ
```

```
      1060       1070       1080       1090       1100
RQSGVVVEEP PPSKTSRKET TSGTSTEPVK NSSPAPPQPA PGKVESGAGD
      1110       1120       1130       1140       1150
AIGLADITQQ LNQSELAVLL NLLQSQTDLS IPQMAQLLNI HSNPEMQQQL
      1160       1170       1180       1190       1200
EALNQSISAL TEATSQQQDS ETMAPEESLK EAPSAPVILP SAEQTTLEAS
      1210       1220       1230       1240       1250
STPADMQNIL AVLLSQLMKT QEPAGSLEEN NSDKNSGPQG PRRTPTMPQE
      1260       1270       1280       1290       1300
EAAACPPHIL PPEKRPPEPP GPPPPPPPPP LVEGDLSSAP QELNPAVTAA
      1310       1320       1330       1340       1350
LLQLLSQPEA EPPGHLPHEH QALRPMEYST RPRPNRTYGN TDGPETGFSA
      1360       1370       1380       1390       1400
IDTDERNSGP ALTESLVQTL VKNRTFSGSL SHLGESSSYQ GTGSVQFPGD
      1410       1420       1430       1440       1450
QDLRFARVPL ALHPVVGQPF LKAEGSSNSV VHAETKLQNY GELGPGTTGA
      1460       1470       1480       1490
SSSGAGLHWG GPTQSSAYGK LYRGPTRVPP RGGRGRGVPY
```

The kinase domain of CDK12 is from amino acid position, 727-1020 (underlined). CDK12 has an additional C terminal domain extension that extends around the N and C terminal lobes and contacts bound ATP (underlined and italics). This is unique to CDK12 and is not present in CDK9.

The contact residues with ATP are Thr737, Lys756, Glu814, Met816 and Asp819 (highlighted by bold type)

CDK12 transcript sequence (SEQ ID NO: 1)

1 gtgtgactgg gtctgtgtga gggagagagt gtgtgtggtg tggaggtgaa acggaggcaa

61 gaaaggggge tacctcagga gcgagggaca aaggggggcgt gaggcaccta ggccgcggca

121 ccccggcgac aggaagccgt cctgaaccgg gctaccgggt aggggaaggg cccgcgtagt

181 cctcgcaggg ccccagagct ggagtcggct ccacagcccc gggccgtcgg cttctcactt

241 cctggacctc cccggcgccc gggcctgagg actggctcgg cggagggaga agaggaaaca

301 gacttgagca gctccccgtt gtctcgcaac tccactgccg aggaactctc atttcttccc

361 tcgctccttc acccccacc tcatgtagaa gggtgctgag gcgtcgggag ggaggaggag

421 cctgggctac cgtccctgcc ctccccaccc ccttcccggg gcgctttggt gggcgtggag

481 ttggggttgg gggggtgggt gggggttgct ttttggagtg ctggggaact ttttccctt

541 cttcaggtca ggggaaaggg aatgcccaat tcagagagac atgggggcaa gaaggacggg

601 agtggaggag cttctggaac tttgcagccg tcatcgggag gcggcagctc taacagcaga

661 gagcgtcacc gcttggtatc gaagcacaag cggcataagt ccaaacactc caaagacatg

721 gggttggtga cccccgaagc agcatccctg ggcacagtta tcaaaccttt ggtggagtat

781 gatgatatca gctctgattc cgacaccttc tccgatgaca tggccttcaa actagaccga

841 agggagaacg acgaacgtcg tggatcagat cggagcgacc gcctgcacaa acatcgtcac

901 caccagcaca ggcgttcccg ggacttacta aaagctaaac agaccgaaaa agaaaaaagc

961 caagaagtct ccagcaagtc gggatcgatg aaggaccgga tatcgggaag ttcaaagcgt

1021 tcgaatgagg agactgatga ctatgggaag gcgcaggtag ccaaaagcag cagcaaggaa

1081 tccaggtcat ccaagctcca caaggagaag accaggaaag aacgggagct gaagtctggg

1141 cacaaagacc ggagtaaaag tcatcgaaaa agggaaacac ccaaaagtta caaaacagtg

1201 gacagcccaa aacggagatc caggagcccc cacaggaagt ggtctgacag ctccaaacaa

1261 gatgatagcc cctcgggagc ttcttatggc caagattatg accttagtcc ctcacgatct

1321 catacctcga gcaattatga ctcctacaag aaaagtcctg gaagtacctc gagaaggcag

1381 tcggtcagtc ccccttacaa ggagccttcg gcctaccagt ccagcacccg gtcaccgagc

1441 ccctacagta ggcgacagag atctgtcagt ccctatagca ggagacggtc gtccagctac

1501 gaaagaagtg gctcttacag cgggcgatcg cccagtccct atggtcgaag gcggtccagc

1561 agccctttcc tgagcaagcg gtctctgagt cggagtccac tccccagtag gaaatccatg

1621 aagtccagaa gtagaagtcc tgcatattca agacattcat cttctcatag taaaaagaag

1681 agatccagtt cacgcagtcg tcattccagt atctcacctg tcaggcttcc acttaattcc

1741 agtctgggag ctgaactcag taggaaaaag aaggaaagag cagctgctgc tgctgcagca

1801 aagatggatg gaaaggagtc caagggttca cctgtatttt tgcctagaaa agagaacagt

1861 tcagtagagg ctaaggattc aggtttggag tctaaaaagt tacccagaag tgtaaaattg

1921 gaaaaatctg ccccagatac tgaactggtg aatgtaacac atctaaacac agaggtaaaa

1981 aattcttcag atacagggaa agtaaagttg gatgagaact ccgagaagca tcttgttaaa

2041 gatttgaaag cacagggaac aagagactct aaacccatag cactgaaaga ggagattgtt

2101 actccaaagg agacagaaac atcagaaaag gagacccctc cacctcttcc cacaattgct

2161 tctcccccac cccctctacc aactactacc cctccacctc agacaccccc tttgccacct

2221 ttgcctccaa taccagctct tccacagcaa ccacctctgc ctccttctca gccagcattt

2281 agtcaggttc ctgcttccag tacttcaact ttgccccctt ctactcactc aaagacatct

2341 gctgtgtcct ctcaggcaaa ttctcagccc cctgtacagg tttctgtgaa gactcaagta

2401 tctgtaacag ctgctattcc acacctgaaa acttcaacgt tgcctccttt gcccctccca

2461 cccttattac ctggagatga tgacatggat agtccaaaag aaactcttcc ttcaaaacct

2521 gtgaagaaag agaaggaaca gaggacacgt cacttactca cagaccttcc tctcctcca

2581 gagctccctg gtggagatct gtctccccca gactctccag aaccaaaggc aatcacacca

2641 cctcagcaac catataaaaa gagaccaaaa atttgttgtc ctcgttatgg agaaagaaga

2701 caaacagaaa gcgactgggg gaaacgctgt gtggacaagt ttgacattat tgggattatt

2761 ggagaaggaa cctatggcca agtatataaa gccaaggaca aagacacagg agaactagtg

2821 gctctgaaga aggtgagact agacaatgag aaagagggct tcccaatcac agccattcgt

2881 gaaatcaaaa tccttcgtca gttaatccac cgaagtgttg ttaacatgaa ggaaattgtc

2941 acagataaac aagatgcact ggatttcaag aaggacaaag gtgcctttta ccttgtattt

3001 gagtatatgg accatgactt aatgggactg ctagaatctg gtttggtgca cttttctgag

3061 gaccatatca agtcgttcat gaaacagcta atggaaggat tggaatactg tcacaaaaag

3121 aatttcctgc atcgggatat taagtgttct aacattttgc tgaataacag tgggcaaatc

3181 aaactagcag attttggact tgctcggctc tataactctg aagagagtcg cccttacaca

3241 aacaaagtca ttactttgtg gtaccgacct ccagaactac tgctaggaga ggaacgttac

3301 acaccagcca tagatgtttg gagctgtgga tgtattcttg gggaactatt cacaaagaag

3361 cctatttttc aagccaatct ggaactggct cagctagaac tgatcagccg actttgtggt

3421 agcccttgtc cagctgtgtg gcctgatgtt atcaaactgc cctacttcaa caccatgaaa

3481 ccgaagaagc aatatcgaag gcgtctacga gaagaattct ctttcattcc ttctgcagca

3541 cttgatttat tggaccacat gctgacacta gatcctagta agcggtgcac agctgaacag

3601 accctacaga gcgacttcct taaagatgtc gaactcagca aaatggctcc tccagacctc

3661 ccccactggc aggattgcca tgagttgtgg agtaagaaac ggcgacgtca gcgacaaagt

3721 ggtgttgtag tcgaagagcc acctccatcc aaaacttctc gaaaagaaac tacctcaggg

3781 acaagtactg agcctgtgaa gaacagcagc ccagcaccac ctcagcctgc tcctggcaag

3841 gtggagtctg gggctgggga tgcaataggc cttgctgaca tcacacaaca gctgaatcaa

3901 agtgaattgg cagtgttatt aaacctgctg cagagccaaa ccgacctgag catccctcaa

3961 atggcacagc tgcttaacat ccactccaac ccagagatgc agcagcagct ggaagccctg

4021 aaccaatcca tcagtgccct gacggaagct acttcccagc agcaggactc agagaccatg

4081 gccccagagg agtctttgaa ggaagcaccc tctgccccag tgatcctgcc ttcagcagaa

4141 cagacgaccc ttgaagcttc aagcacacca gctgacatgc agaatatatt ggcagttctc

4201 ttgagtcagc tgatgaaaac ccaagagcca gcaggcagtc tggaggaaaa caacagtgac

4261 aagaacagtg ggccacaggg gccccgaaga actcccacaa tgccacagga ggaggcagca

4321 gcatgtcctc ctcacattct tccaccagag aagaggcccc ctgagccccc cggacctcca

4381 ccgccgccac ctccaccccc tctggttgaa ggcgatcttt ccagcgcccc ccaggagttg

4441 aacccagccg tgacagccgc cttgctgcaa cttttatccc agcctgaagc agagcctcct

4501 ggccacctgc cacatgagca ccaggccttg agaccaatgg agtactccac ccgaccccgt

4561 ccaaacagga cttatggaaa cactgatggg cctgaaacag ggttcagtgc cattgacact

4621 gatgaacgaa actctggtcc agccttgaca gaatccttgg tccagaccct ggtgaagaac

4681 aggaccttct caggctctct gagccacctt ggggagtcca gcagttacca gggcacaggg

4741 tcagtgcagt ttccagggga ccaggacctc cgttttgcca gggtcccctt agcgttacac

4801 ccggtggtcg ggcaaccatt cctgaaggct gagggaagca gcaattctgt ggtacatgca

4861 gagaccaaat tgcaaaacta tggggagctg gggccaggaa ccactggggc cagcagctca

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4981 gggcctacaa gagtcccacc aagaggggga agagggagag gagttcctta ctaacccaga

5041 gacttcagtg tcctgaaaga ttcctttcct atccatcctt ccatccagtt ctctgaatct

5101 ttaatgaaat catttgccag agcgaggtaa tcatctgcat ttggctactg caaagctgtc

5161 cgttgtattc cttgctcact tgctactagc aggcgactta cgaaataatg atgttggcac

5221 cagttccccc tggatgggct atagccagaa catttacttc aactctacct tagtagatac

5281 aagtagagaa tatggagagg atcattacat tgaaaagtaa atgttttatt agttcattgc

5341 ctgcacttac tgatcggaag agagaaagaa cagtttcagt attgagatgg ctcaggagag

5401 gctctttgat ttttaaagtt ttggggtggg ggattgtgtg tggtttcttt cttttgaatt

5461 ttaatttagg tgttttgggt ttttttcctt taaagagaat agtgttcaca aaatttgagc

5521 tgctctttgg cttttgctat aagggaaaca gagtggcctg gctgatttga ataaatgttt

5581 ctttcctctc caccatctca cattttgctt ttaagtgaac acttttttccc cattgagcat

5641 cttgaacata ctttttttcc aaataaatta ctcatcctta aagtttactc cactttgaca

5701 aaagatacgc ccttctccct gcacataaag caggttgtag aacgtggcat tcttgggcaa

5761 gtaggtagac tttacccagt ctctttcctt ttttgctgat gtgtgctctc tctctctctt

5821 tctctctctc tctctctctc tctctctctc tctctctctc tgtctcgctt gctcgctctc

5881 gctgtttctc tctctttgag gcatttgttt ggaaaaaatc gttgagatgc ccaagaacct

5941 gggataattc tttacttttt ttgaaataaa ggaaaggaaa ttcagactct tacattgttc

6001 tctgtaactc ttcaattcta aaatgttttg ttttttaaac catgttctga tggggaagtt

6061 gatttgtaag tgtggacagc ttggacattg ctgctgagct gtggttagag atgatgcctc

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6181 aaaaaaaaac aaaaaccttt caaacagagc attgtgatat tgtcaaagag aaaaacaaat

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6301 atacctgacc ttttttaaaa aaataatttt aaaacagcat actgtgagga agaacagtat

6361 tgacataccc acatcccagc atgtgtaccc tgccagttct tttagggatt tttcctccaa

6421 agagatttgg atttggtttt ggtaaaaggg gttaaattgt gcttccaggc aagaactttg

6481 ccttatcata aacaggaaat gaaaaaggga agggctgtca ggatgggata atttgggagg

6541 cttctcattc tggcttctat ttctatgtga gtaccagcat atagagtgtt ttaaaaacag

6601 atacatgtca tataatttat ctgcacagac ttagaccttc aggaaacata ggttaagccc

6661 ccttttacaa agaaaaagta aacatacttc agcatcttgg agggtagttt tcaaaactca

6721 agtttcatgt ttcaatgcca agttcttatt ttaaaaaata aaatctactt ataagagaaa

6781 ggtgcattac ttaaaaaaaa aaaactttaa agaaatgaaa gaagaaccct cttcagatac

6841 ttacttgaag actgttttcc cctgttaatg agatatagct agatatcggt gtgtgtattt

6901 ctttattatt ctctggtttt tgatctggcc ttgcctccag ggccaaacac tgatttagaa

6961 agagagcctt ctagctattt tggcattgat ggctttttat accagtgtgt ccagttagat

7021 ttactaggct tactgacatg ctattggtaa atcgcattaa agttcatctg aaccttctgt

7081 ctgttgactt cttagtcctc agacatgggc ctttgtgttt tagaatattt gaatttgagt

7141 tattgggccc cactccctgt tttttattaa agaacgtgag cctgggatac tttcagaagt

7201 atctgttcaa tgaaaaaaag ttggtttccc atcaaatatg aataaaattc tctatatatt

7261 tcattgtatt ttggttatca gcagtcatca ataatgtttt tccctcccct ctcccacctc

7321 ttatttttaa ttatgccaaa tatcctaaat aatatactta agcctccatt ccctcatccc

7381 tactagggaa gggggtgagt gtatgtgtga gtgtatgtgt atgtatgatc ccatctcacc

7441 cccaccccca ttttgggagt cttttaaaat gaaaacaaag tttggtagtt ttgactattt

7501 ctaaaagcag aggagaaaaa aaaacttatt taaatatcct ggaatctgta tggaggaaga

7561 aaaggtattt gttaattttt cagttacgtt atctataaac atgatggaag taaaggtttg

7621 gcagaatttc accttgacta tttgaaaatt acagacccaa ttaattccat tcaaaagtgg

7681 ttttcgtttt gttttaatta ttgtacaatg agagatattg tctattaaat acattatttt

7741 gaacagatga gaaatctgat tctgttcatg agtgggaggc aaaactggtt tgaccgtgat

7801 catttttgtg gttttgaaaa caaatatact tgacccagtt tccttagttt tttcttcaac

7861 tgtccatagg aacgataagt atttgaaagc aacatcaaat ctatacgttt aaagcagggc

7921 agttagcaca aatttgcaag tagaacttct attagcttat gccatagaca tcacccaacc

7981 acttgtatgt gtgtgtgtat atataatatg catatatagt taccgtgcta aaatggttac

8041 cagcaggttt tgagagagaa tgctgcatca gaaaagtgtc agttgccacc tcattctccc

8101 tgatttaggt tcctgacact gattcctttc tctctcgttt ttgaccccca ttgggtgtat

8161 cttgtctatg tacagatatt ttgtaatata ttaaattttt ttctttcagt ttataaaaat

8221 ggaaagtgga gattggaaaa ttaaatattt cctgttacta taccactttt gctccattgc

8281 att

CLAIMS

1. An inhibitor for use in the treatment or prevention of a disorder in a subject caused by the generation of repeat expansion transcripts, wherein the inhibitor is an inhibitor of CDK12 (cyclin-dependent kinase 12).

2. The inhibitor for use according to claim 1, wherein the repeat expansion transcript results in the transcript being retained in the nucleus.

3. The inhibitor for use according to claim 1 or claim 2, wherein the disorder comprises any disorder selected from the group comprising Myotonic Dystrophy type 1, Myotonic Dystrophy type 2, Fragile X associated tremor/ataxia syndrome, amylotrophic lateral sclerosis (ALS) and frontotemporal dementia (C9ORF72), Huntington's Disease like 2, Huntington's Disease, Spinocerebellar Ataxia Types 1, 2, 3, 6, 7, 8, 10, 31, 17, Dentatorubral-pallidoluysian atrophy and Spinal and Bulbar Muscular Atrophy.

4. The inhibitor for use according to any preceding claim, wherein the repeat expansion transcript comprises RNA from a CTG DNA repeat; a CCTG DNA repeat; a CGG DNA repeat; a GGGGCC DNA repeat; a ATTCT DNA repeat; a TGGAA DNA repeat or a CAG DNA repeat.

5. The inhibitor for use according to any preceding claim, wherein the inhibitor is specific for CDK12.

6. The inhibitor for use according to any preceding claim, wherein the inhibitor is not an inhibitor of CDK9 activity or availability.

7. The inhibitor for use according to any of claims 1 to 4, wherein the inhibitor comprises an inhibitor of CDK12 expression.

8. The inhibitor for use according to claim 7, wherein the inhibitor comprises an oligonucleotide capable of inhibiting CDK12 expression.

9. The inhibitor for use according to claim 8, wherein the oligonucleotide comprises a sequence substantially complementary to CDK12 mRNA transcript.

10. The inhibitor for use according to any of claims 1 to 6, wherein the inhibitor comprises a molecule capable of binding to CDK12 and/or capable of blocking binding of CDK12 to its target molecule.

11. The inhibitor for use according to any of claims 1 to 6 or 10, wherein the inhibitor comprises a molecule capable of preventing CDK12 binding to cyclin K.

12. The inhibitor for use according to any of claims 1 to 6, 10 or 11, wherein the inhibitor comprises a molecule capable of preventing CDK12 phosphorylating Ser2 on the c-terminal domain of RNA polymerase II.

13. The inhibitor for use according to any of claims 10 to 12, wherein the binding of the inhibitor to CDK12 is at, or adjacent to, the CDK12 active site, such that the active site is blocked.

14. The inhibitor for use according to any of claims 10 to 13, wherein the binding of the inhibitor to CDK12 is at amino acid position, 727-1020.

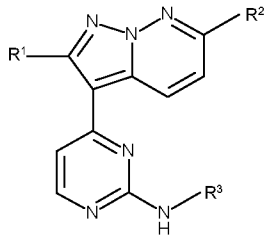
15. The inhibitor for use according to any of claims 10 to 14, wherein the binding of the inhibitor to CDK12 is at a C terminal domain extension that extends around the N and C terminal lobes and contacts bound ATP.

16. The inhibitor for use according to any of claims 10 to 15, wherein the binding of the inhibitor to CDK12 is at any one or more of the ATP contact residues selected from Thr737, Lys756, Glu814, Met816 and Asp819.

17. The inhibitor for use according to any of claims 1 to 6, or 10 to 16, wherein the inhibitor comprises a small molecule, oligonucleotide, peptide or protein capable of binding to CDK12.

18. The inhibitor for use according to any of claims 1 to 6, or 10 to 17, wherein the inhibitor comprises a pyrazolo[1,5b]pyridazine core structure, and is capable of inhibiting CDK12 activity.

19. The inhibitor for use according to any of claims 1 to 6, or 10 to 18, wherein the inhibitor comprises a compound of Formula (I) or a pharmaceutically acceptable salt or solvate thereof:



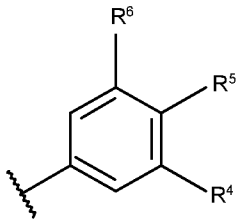
Formula I

wherein:

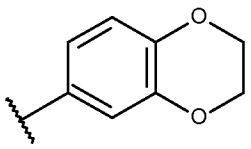
R^1 is H, -OH, -O-, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynl, C_{1-6}haloalkyl, halogen, -CN, -OC_{1-6}alkyl;

R^2 is H, -OH, -O-, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynl, C_{1-6}haloalkyl, halogen, -CN, -OC_{1-6}alkyl or a five or 6 membered cycloaryl, cycloalkyl or heterocycl having one, two or three heteroatoms selected from O, S and N, for example benzene, morpholinyl, piperidine, piperazine;

R^3 is C_{3-6}cycloalkyl, for example cyclopropyl,



or



;

wherein:

R^4 is H, -OH, -O-, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynl, C_{1-6}haloalkyl, halogen, -CN, -OC_{1-6}alkyl;

R^5 is H; -OH; -O-; C_{1-6}alkyl; C_{2-6}alkenyl; C_{2-6}alkynl; C_{1-6}haloalkyl; halogen; -CN; -OC_{1-6}alkyl; -; C_{1-6}alkyl-N-(X)(Y); a five or six membered cycloaryl, cycloalkyl or heterocycl having one, two or three heteroatoms selected from O, S and N and said cycloaryl, cycloalkyl or heterocycle being optionally substituted with a C_{1-3}alkyl, for example N-methylpiperazinyl; or -OC_{1-6}alkyl-N(X)(Y);

wherein X is H or C_{1-6}alkyl, and Y is H or C_{1-6}alkyl;

and wherein the alkyl groups are optionally substituted by one or more –OH groups; and

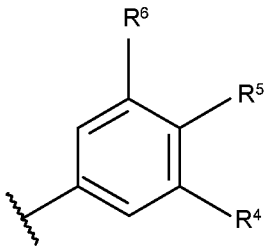
R^6 is H, -OH, -O-, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynl, C_{1-6}haloalkyl, halogen, -CN, -OC_{1-6}alkyl.

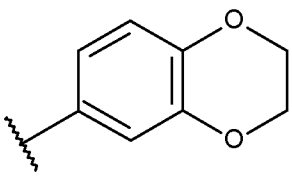
20. The inhibitor for use according to claim 19, wherein:

R^1 is H;

R^2 is H;

R^3 is C_{3-6}cycloalkyl, for example cyclopropyl,



or


;

wherein:

R^4 is H, -CN, -OC_{1-6}alkyl, C_{1-6}haloalkyl;

R^5 is H,; C_{1-6}alkyl-N-(X)(Y); a five or six membered cycloaryl, cycloalkyl or heterocycl having one, two or three heteroatoms selected from O, S and N and said cycloaryl, cycloalkyl or heterocycle being optionally substituted with a C_{1-3}alkyl, for example N–methylpiperazinyl,-OC_{1-6}alkyl-N(X)(Y);;

wherein X is H or C_{1-6}alkyl, and Y is H or C_{1-6}alkyl;

and wherein the alkyl groups are optionally substituted by one or more –OH groups; and

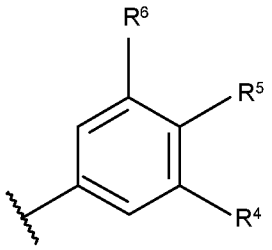
R^6 is H or -OC_{1-6}alkyl.

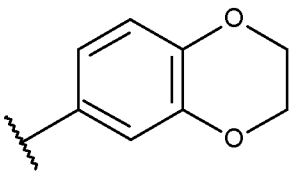
21. The inhibitor for use according to claim 19, wherein:

R^1 is H;

R^2 is H;

R^3 is cyclopropyl,



or 

;

wherein:

R^4 is H, -CN, -OCH_3, CF_3;

R^5 is H, -$CH_2N(CH_3)_2$, N–methylpiperazinyl, -$OCH_2CH(OH)CH_2N(CH_3)_2$; and

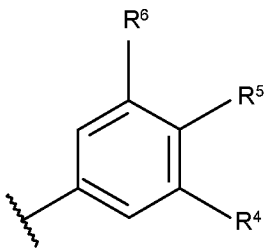
R^6 is H or –OCH_3.

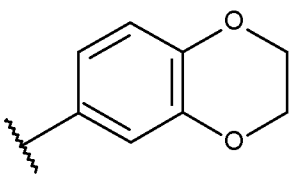
22. The inhibitor for use according to any claim 19, wherein:

R^1 is H,

R^2 is H,

R^3 is cyclopropyl,



or 

;

wherein:

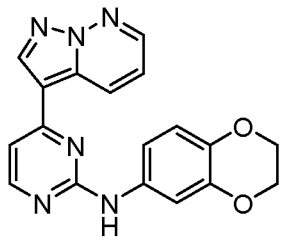
R4 is H; R5 is -CH_2NEt_2, or -$OCH_2CH(OH)CH_2N(CH_3)_2$; and R6 is H; or

R4 is -CN, -OCH_3; R5 is H; and R6 is H; or

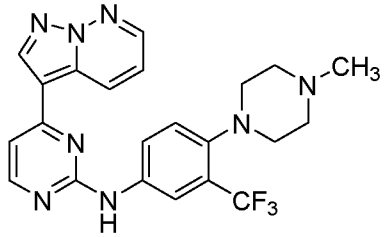
R4 is -CF_3; R5 is N–methylpiperazinyl; and R6 is H; or

R4 is -OCH_3; R5 is H; and R6 is –OCH_3.

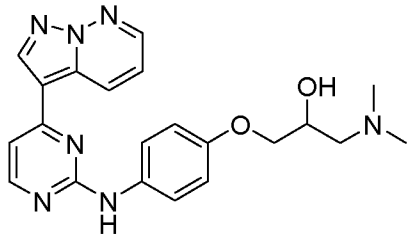
23. The inhibitor for use according to claim 19, wherein the inhibitor of Formula (I) is of the following formula:



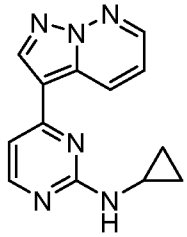
(II); or



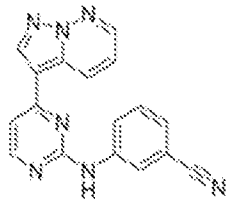
(III); or



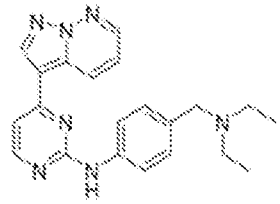
(IV); or



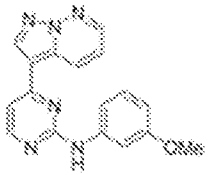
(V): or



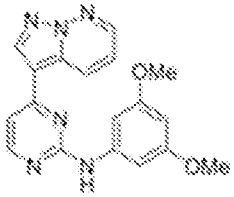
(VI); or



(VII); or

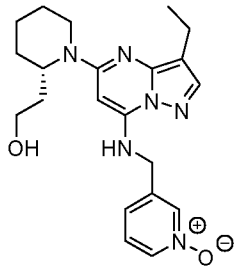


(VIII); or



(IX).

24. The inhibitor for use according to claim 1 to 6, or 10 to 16, wherein the inhibitor comprises a compound of Formula (X) or a pharmaceutically acceptable salt or solvate thereof:



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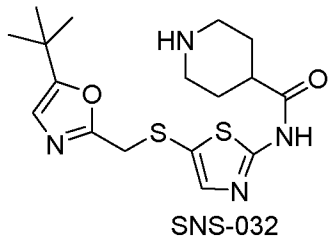
(X)

25. The inhibitor for use according to claim 1 to 6, or 10 to 16, wherein the inhibitor comprises a compound of Formula (XI) or a pharmaceutically acceptable salt or solvate thereof:



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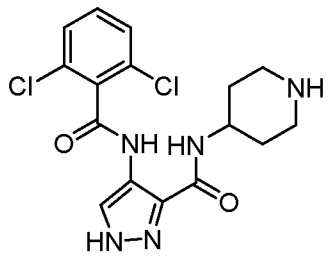
(XI).

26. The inhibitor for use according to claim 1 to 6, or 10 to 16, wherein the inhibitor comprises a compound of Formula (XII) or a pharmaceutically acceptable salt or solvate thereof:



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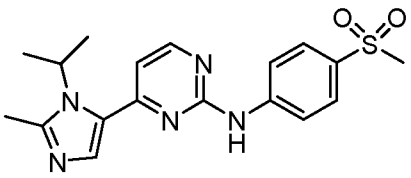
(XII).

27. The inhibitor for use according to claim 1 to 6, or 10 to 16, wherein the inhibitor comprises a compound of Formula (XIII) or a pharmaceutically acceptable salt or solvate thereof:



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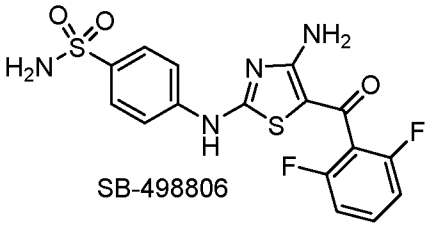
(XIII).

28. The inhibitor for use according to claim 1 to 6, or 10 to 16, wherein the inhibitor comprises a compound of Formula (XIV) or a pharmaceutically acceptable salt or solvate thereof:



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(XIV).

29. The inhibitor for use according to any preceding claim, wherein the inhibitor is administered, or arranged to be administered, in combination with at least one other therapeutic agent.

30. The inhibitor for use according to claim 29, wherein the one other therapeutic agent comprises an oligonucleotide, such as siRNA or miRNA or equivalents thereof; or

wherein the at least one other therapeutic agent may comprise a small molecule, drug, pro-drug, peptide, protein, antibody, nucleotide or vaccine.

31. The inhibitor for use according to claim 29, wherein the at least one other therapeutic agent comprises a sodium channel blocker; a CNS stimulant drug; dehydroepiandrosterone (DHEA); creatine supplementation; mecasermin rinfabate (IPLEX, combination of recombinant insulin-like growth factor 1 and its binding protein, BP-3); pentamidine; a bisamidinium inhibitor; lomofugin; or dilomofungin; or combinations thereof.

32. The inhibitor for use according to any preceding claim, wherein the use is in combination with an oligonucleotide that targets the DMPK gene.

33. The inhibitor for use according to any preceding claim, wherein the inhibitor is administered, or arranged to be administered, intermittently.

34. Use of a CDK12 inhibitor in the preparation of a medicament for the treatment or prevention of a disorder caused by the generation of repeat expansion transcripts in a subject.

35. A method of treatment or prevention of a disorder caused by the generation of repeat expansion transcripts in a subject, comprising administering an inhibitor of CDK12 to the subject.

36. The method according to claim 35, wherein the method further comprises a subsequent administration of at least one other therapeutic agent.

37. A method of screening for a therapeutic agent for a disorder caused by the generation of repeat expansion transcripts comprising:

-providing a molecule for screening;

-detecting if the molecule inhibits the activity of CDK12,

wherein detection of inhibition of CDK12 selects the molecule as a potential candidate therapeutic agent.

38. A composition comprising a CDK12 inhibitor and a pharmaceutically acceptable carrier.

39. The composition according to claim 38 further comprising at least one other therapeutic agent.

40. The inhibitor, method, use, or composition substantially as described herein, optionally with reference to the accompanying figures.

Figure 1

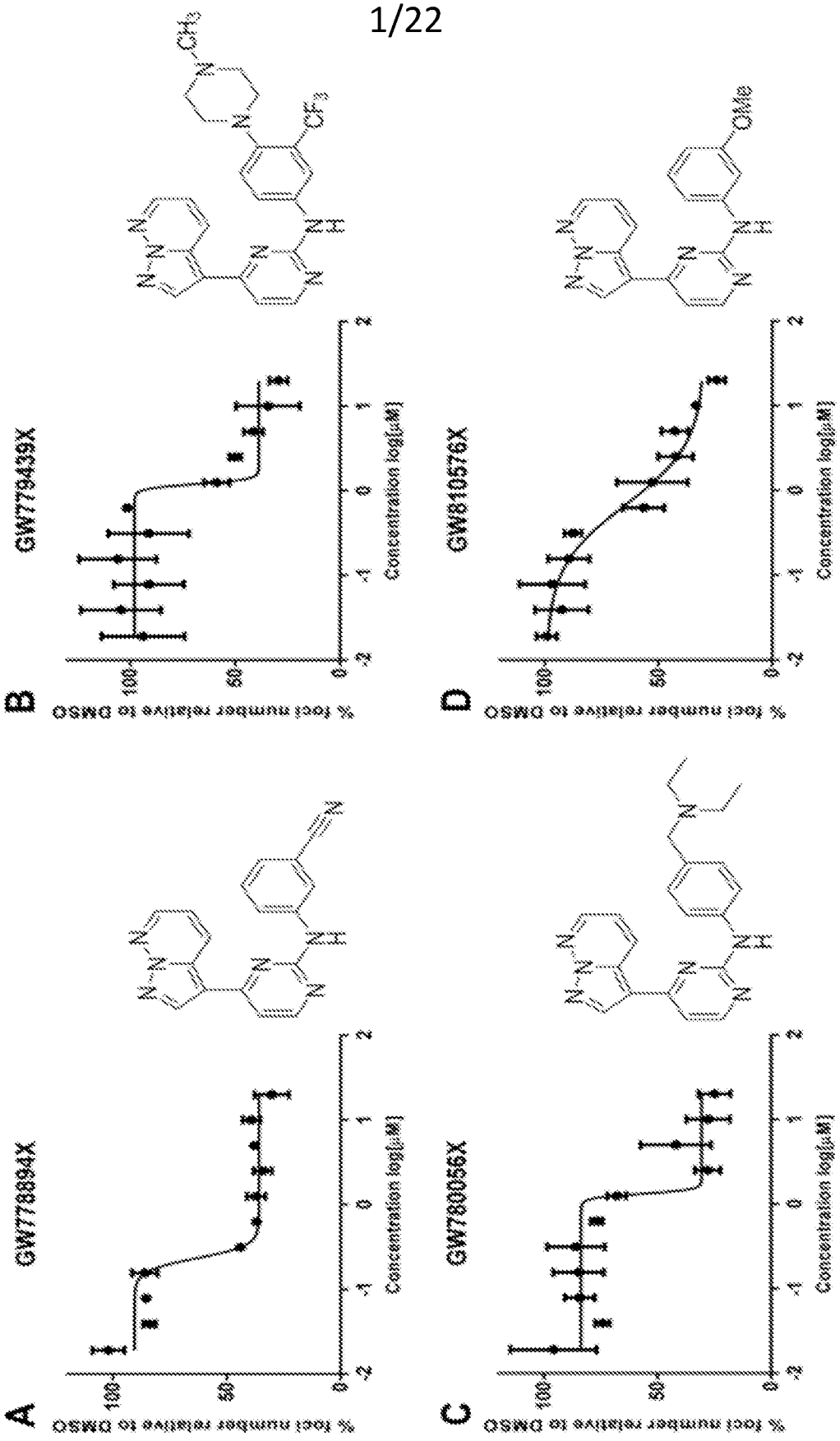
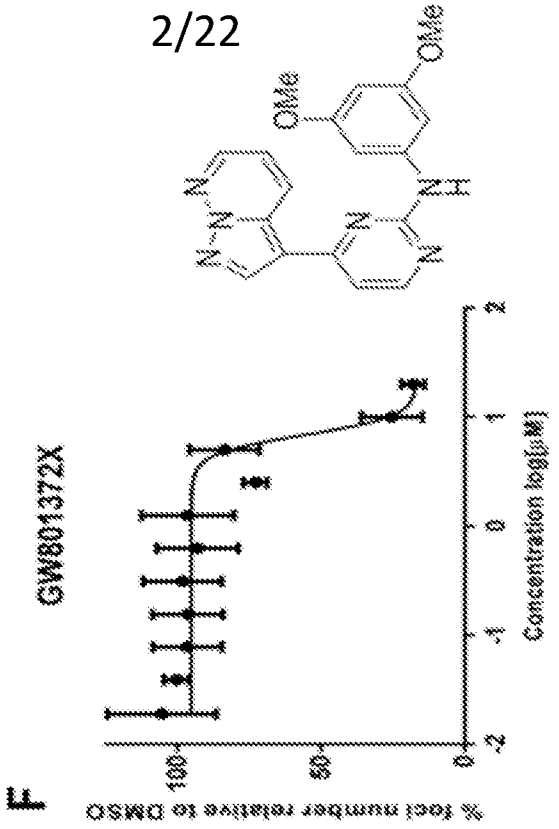

A
GW778894X
B
GW779439X
C
GW780056X
D
GW810576X
% foci number relative to DMSO
Concentration log[μM]
100
50
0
-2
-1
0
1
2
OMe
CF3

Figure 1 continued



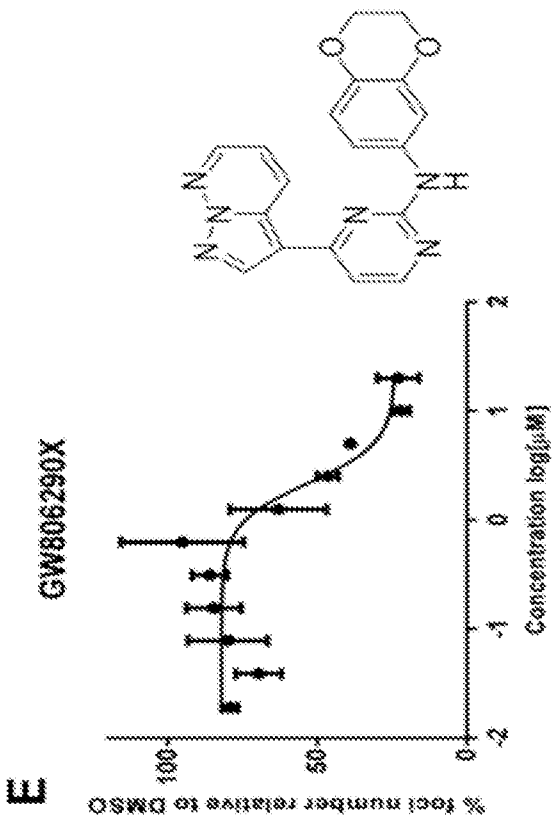


Figure 2A

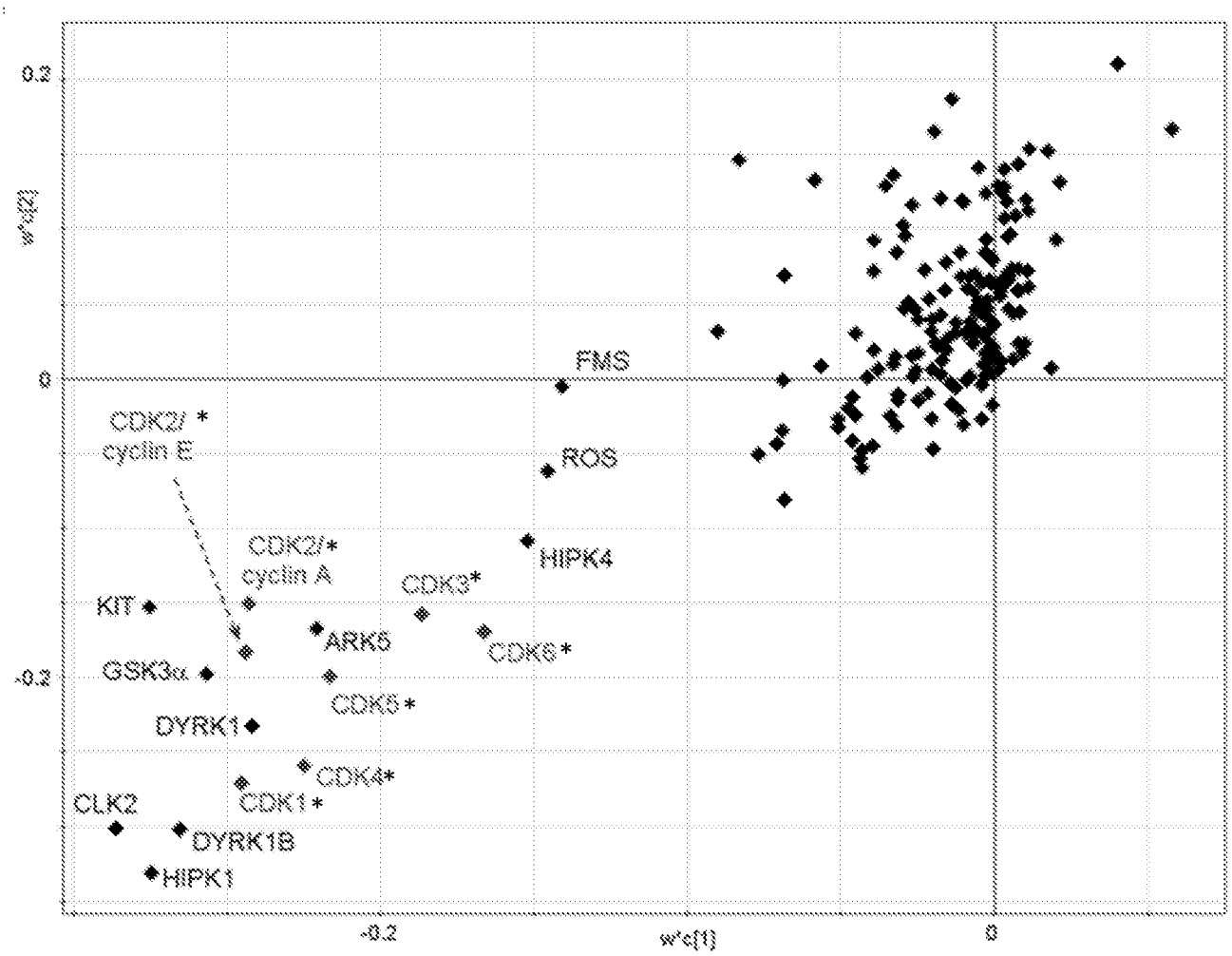

0.2
0
-0.2
w*c[2]
FMS
ROS
HIPK4
CDK3*
CDK8*
CDK2/ * cyclin E
CDK2/* cyclin A
KIT
ARK6
GSK3α
CDK5*
DYRK1
CDK4*
CDK1*
CLK2
DYRK1B
HIPK1
-0.2
w*c[1]
0

Figure 2 continued

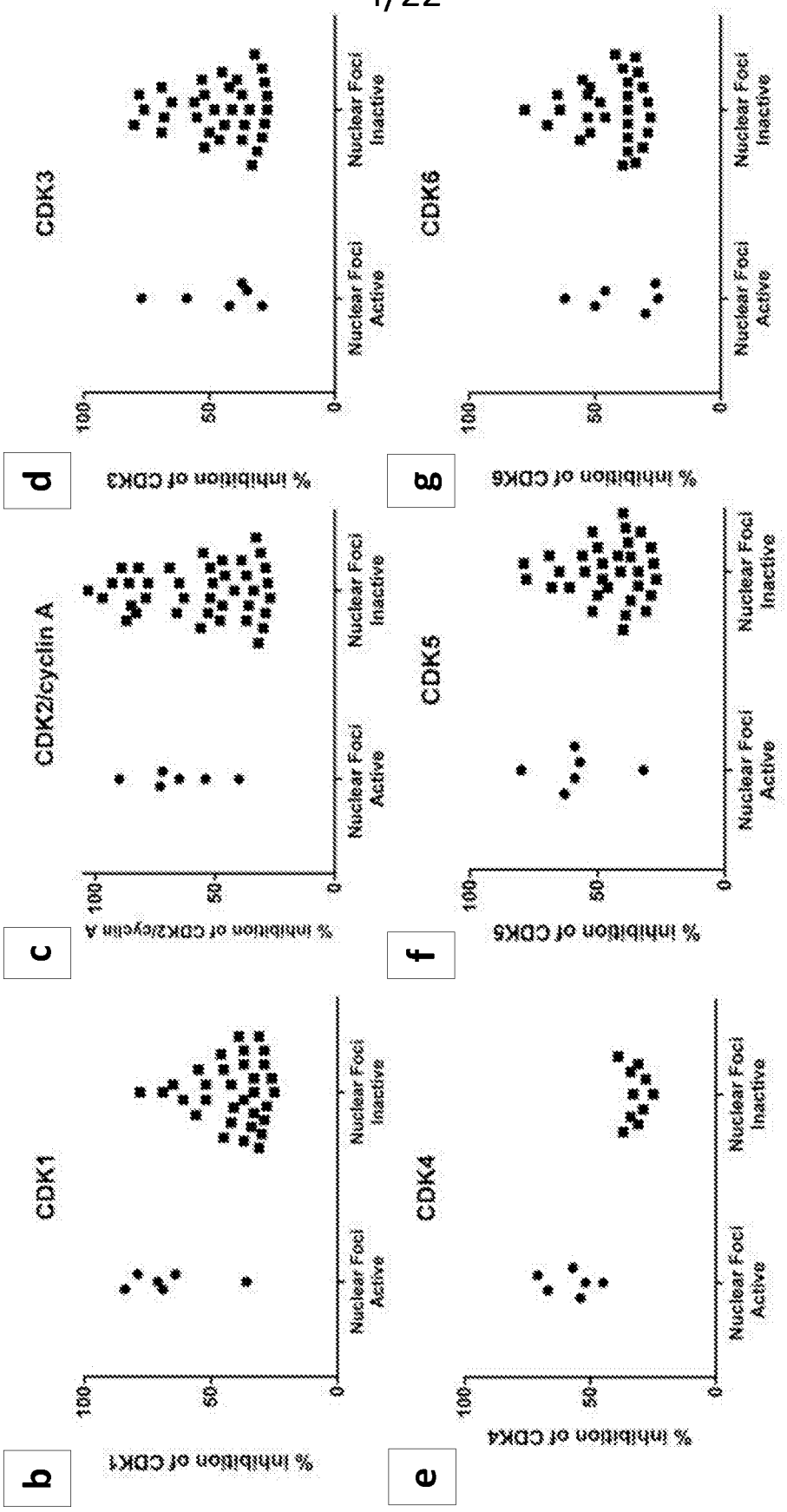


Figure 3

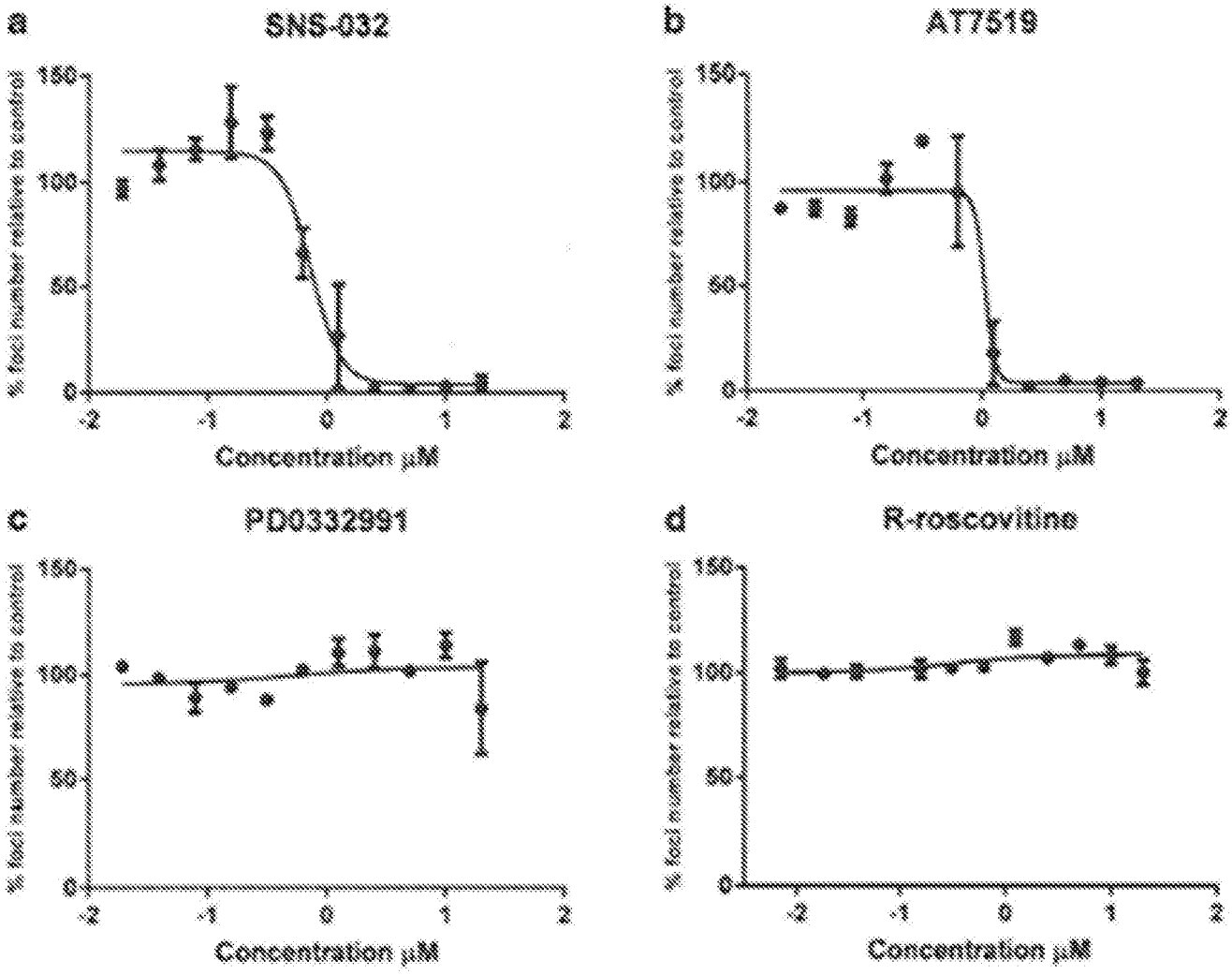

a
SNS-032
b
AT7519
c
PD0332991
d
R-roscovitine
% foci number relative to control
150
100
50
0
-2
-1
0
1
2
Concentration μM

Figure 3 continued

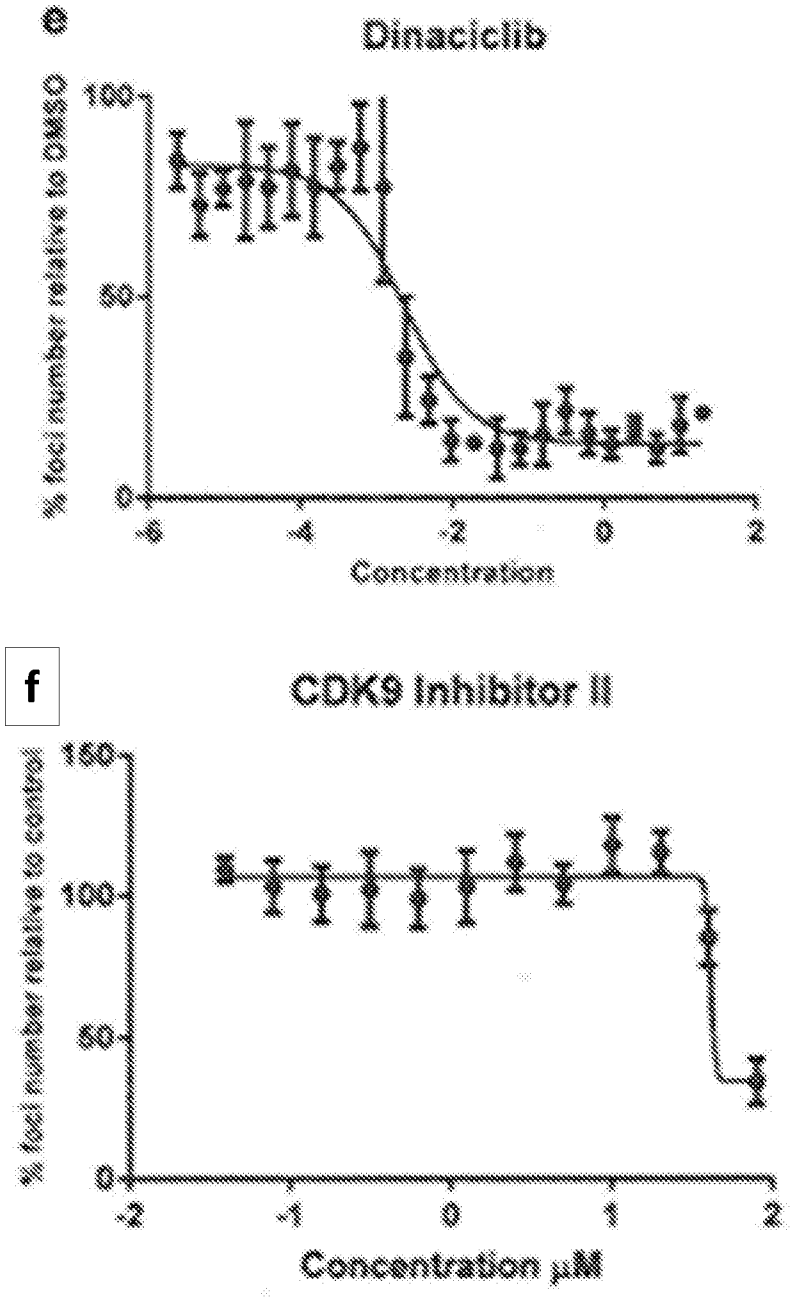


Figure 4

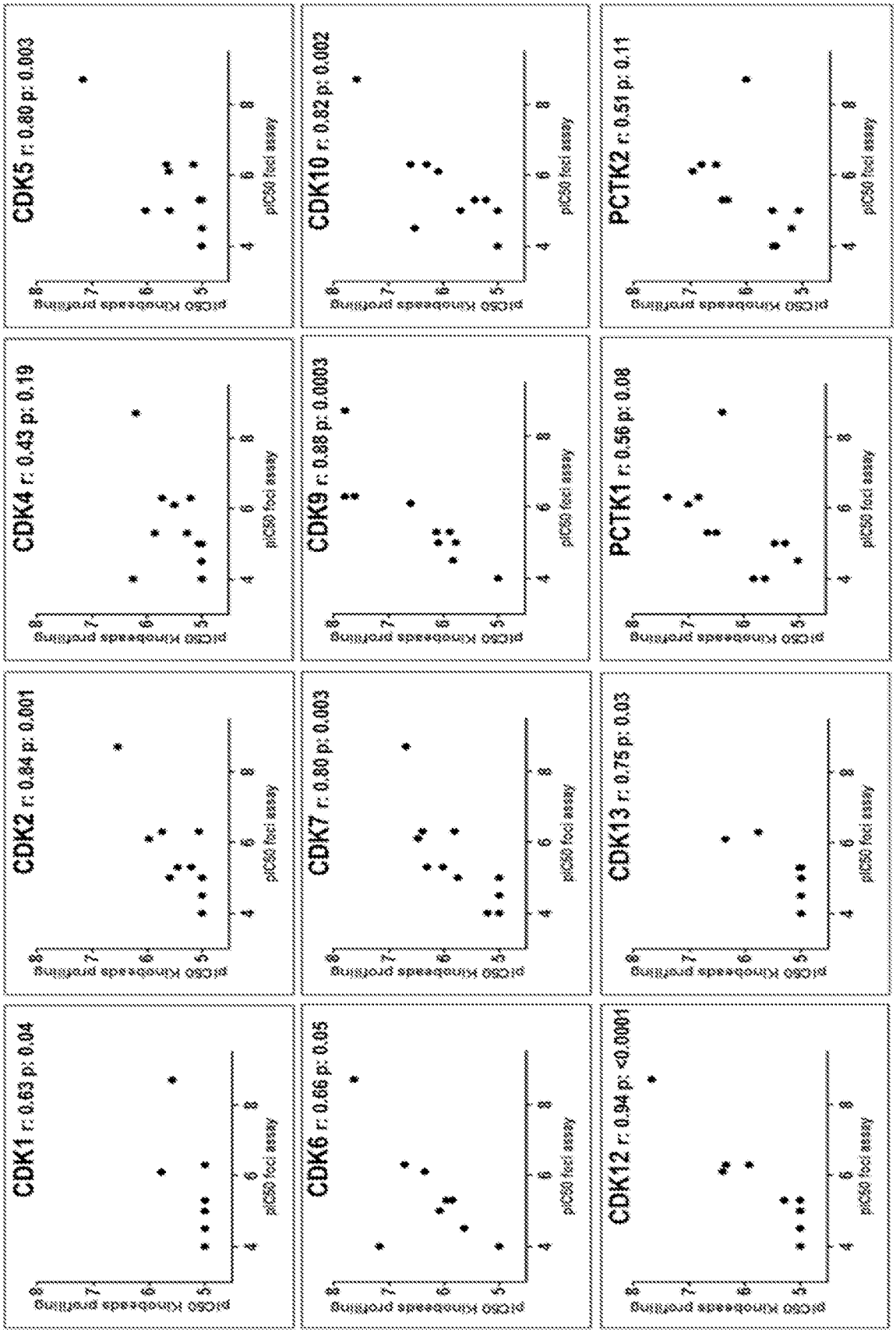

CDK1 r: 0.63 p: 0.04
CDK2 r: 0.84 p: 0.001
CDK4 r: 0.43 p: 0.19
CDK5 r: 0.80 p: 0.003
CDK6 r: 0.66 p: 0.05
CDK7 r: 0.80 p: 0.003
CDK9 r: 0.88 p: 0.0003
CDK10 r: 0.82 p: 0.002
CDK12 r: 0.94 p: <0.0001
CDK13 r: 0.75 p: 0.03
PCTK1 r: 0.56 p: 0.08
PCTK2 r: 0.51 p: 0.11
pIC50 kinobeads profiling
pIC50 foci assay

Figure 5

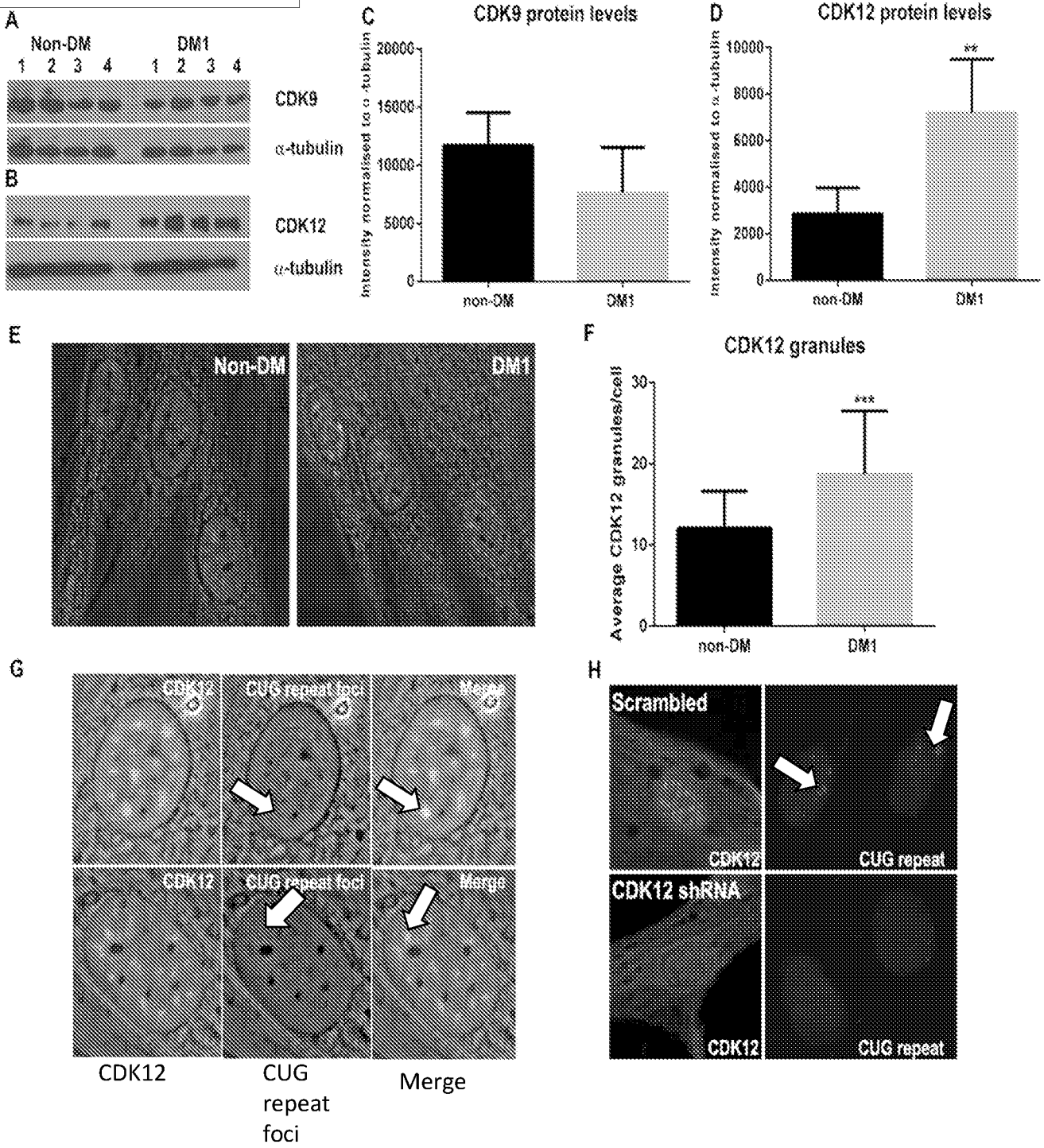

A
Non-DM
DM1
1 2 3 4 1 2 3 4
CDK9
α-tubulin
B
CDK12
α-tubulin
C
CDK9 protein levels
Intensity normalised to α-tubulin
20000
15000
10000
5000
0
non-DM
DM1
D
CDK12 protein levels
Intensity normalised to α-tubulin
10000
8000
6000
4000
2000
0
**
non-DM
DM1
E
Non-DM
DM1
F
CDK12 granules
Average CDK12 granules/cell
30
20
10
0
***
non-DM
DM1
G
CDK12
CUG repeat foci
Merge
CDK12
CUG repeat foci
Merge
CDK12
CUG repeat foci
Merge
H
Scrambled
CDK12
CUG repeat
CDK12 shRNA
CDK12
CUG repeat

Figure 6

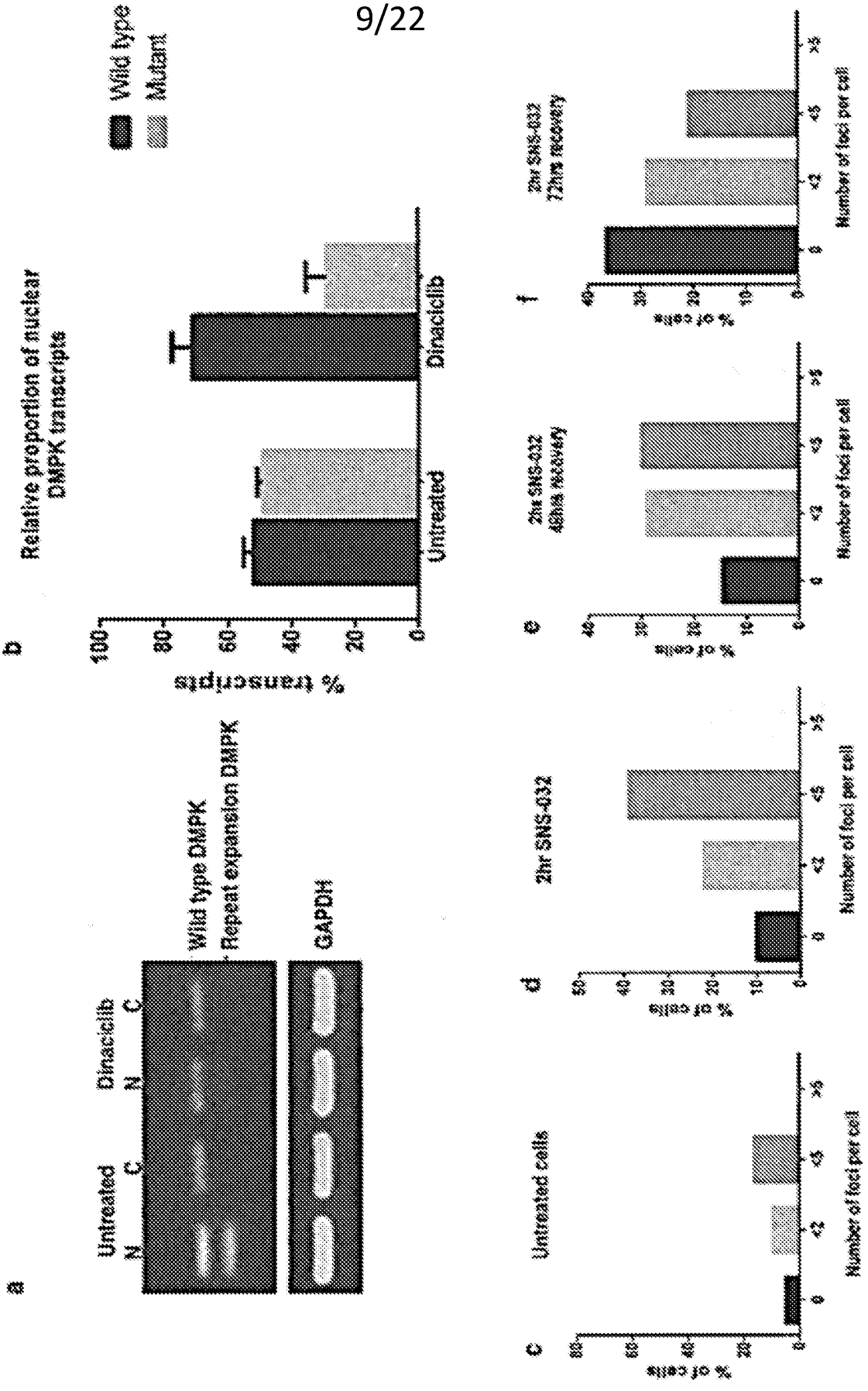

a
Untreated
Dinaciclib
N
C
N
C
Wild type DMPK
Repeat expansion DMPK
GAPDH
b
Relative proportion of nuclear DMPK transcripts
Wild type
Mutant
% transcripts
100
80
60
40
20
0
Untreated
Dinaciclib
c
Untreated cells
d
2hr SNS-032
e
2hr SNS-032
48hrs recovery
f
2hr SNS-032
72hrs recovery
% of cells
Number of foci per cell

Figure 7

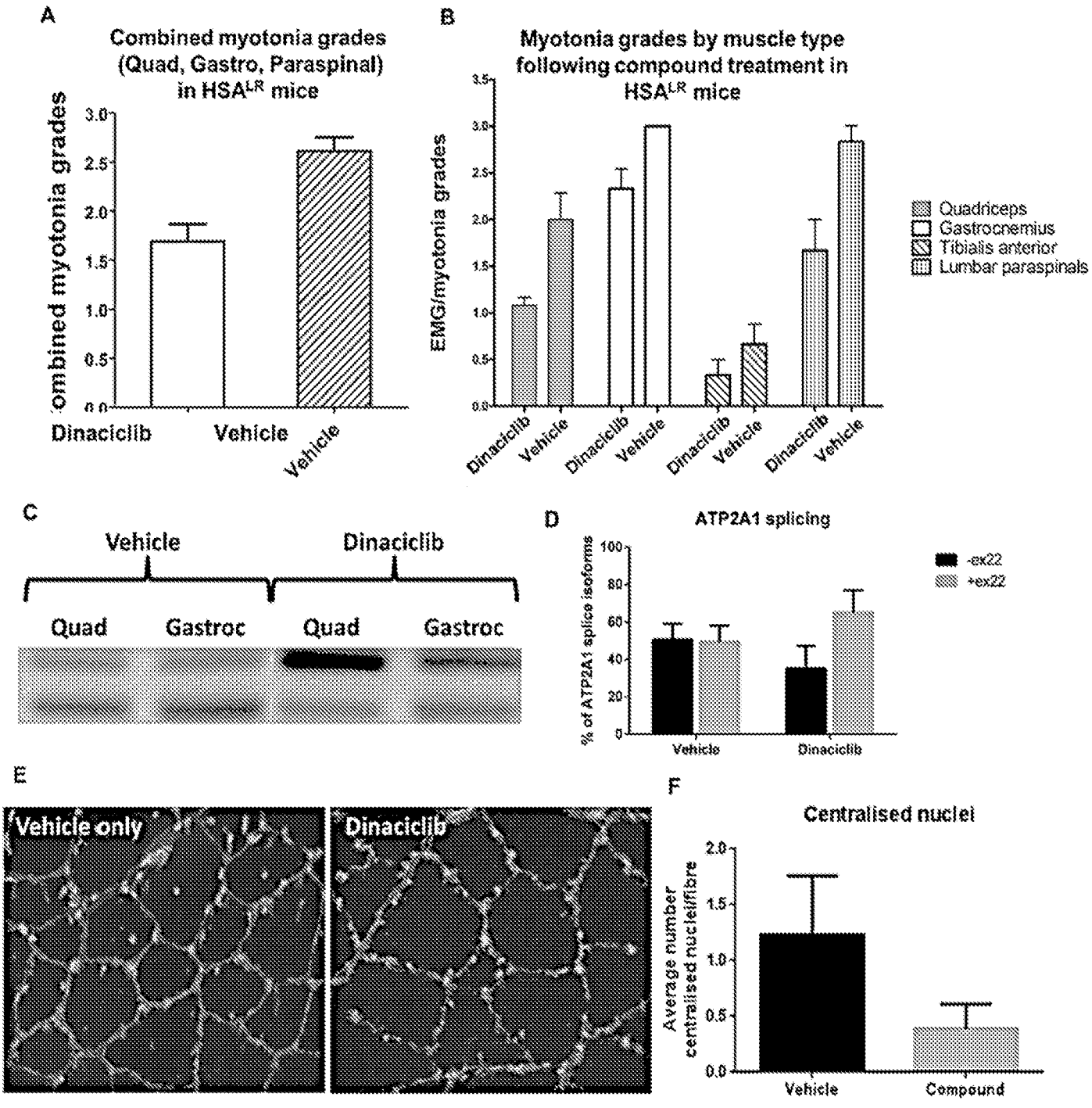

A
Combined myotonia grades (Quad, Gastro, Paraspinal) in HSA^LR mice
Combined myotonia grades
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Dinaciclib
Vehicle
Vehicle
B
Myotonia grades by muscle type following compound treatment in HSA^LR mice
EMG/myotonia grades
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Dinaciclib
Vehicle
Dinaciclib
Vehicle
Dinaciclib
Vehicle
Dinaciclib
Vehicle
Quadriceps
Gastrocnemius
Tibialis anterior
Lumbar paraspinals
C
Vehicle
Dinaciclib
Quad
Gastroc
Quad
Gastroc
D
ATP2A1 splicing
% of ATP2A1 splice isoforms
100
80
60
40
20
0
Vehicle
Dinaciclib
-ex22
+ex22
E
Vehicle only
Dinaciclib
F
Centralised nuclei
Average number centralised nuclei/fibre
2.0
1.5
1.0
0.5
0.0
Vehicle
Compound

Figure 8

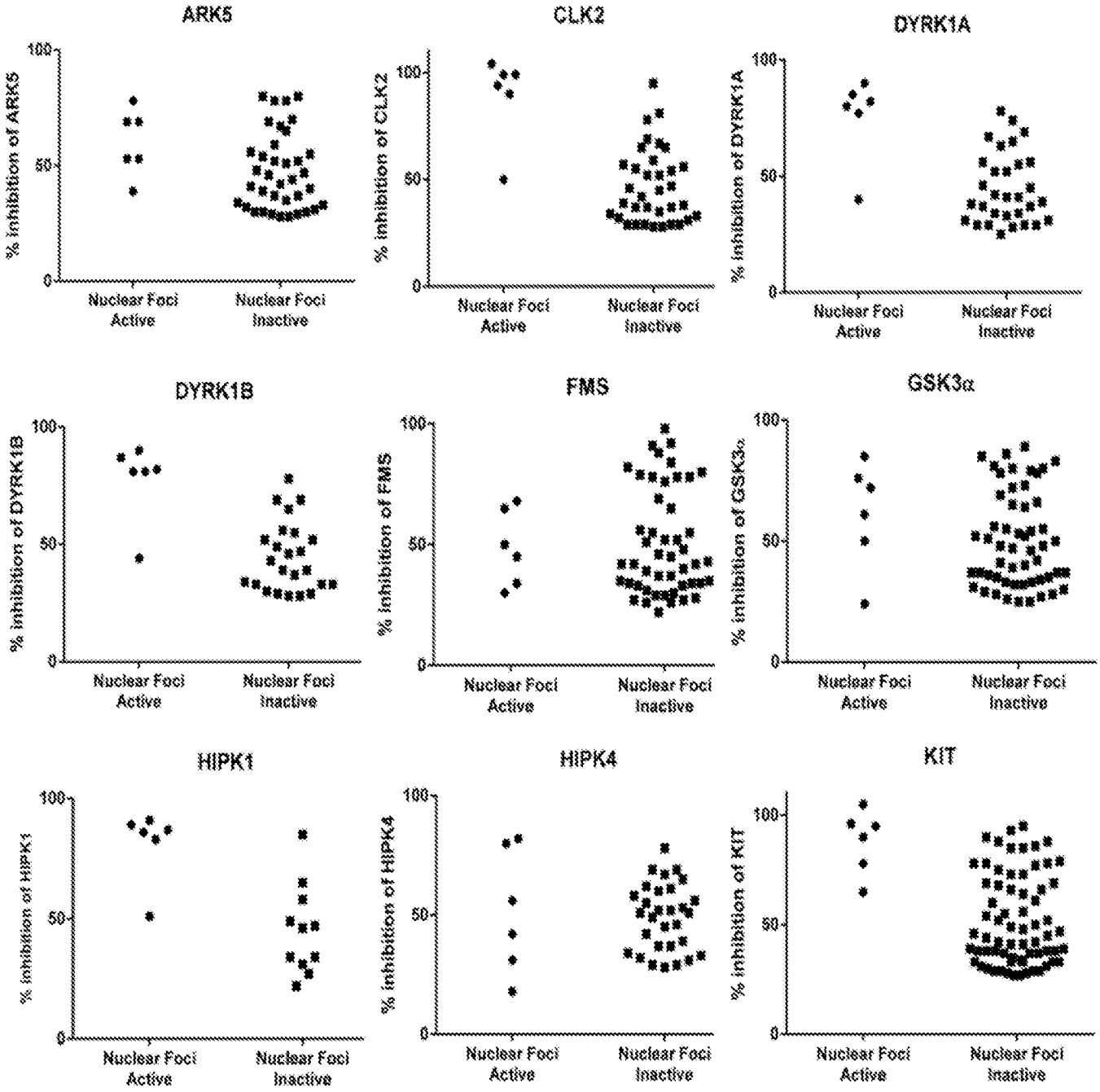

ARK5
% inhibition of ARK5
CLK2
% inhibition of CLK2
DYRK1A
% inhibition of DYRK1A
DYRK1B
% inhibition of DYRK1B
FMS
% inhibition of FMS
GSK3α
% inhibition of GSK3α
HIPK1
% inhibition of HIPK1
HIPK4
% inhibition of HIPK4
KIT
% inhibition of KIT
100
50
0
Nuclear Foci Active
Nuclear Foci Inactive

Figure 9A

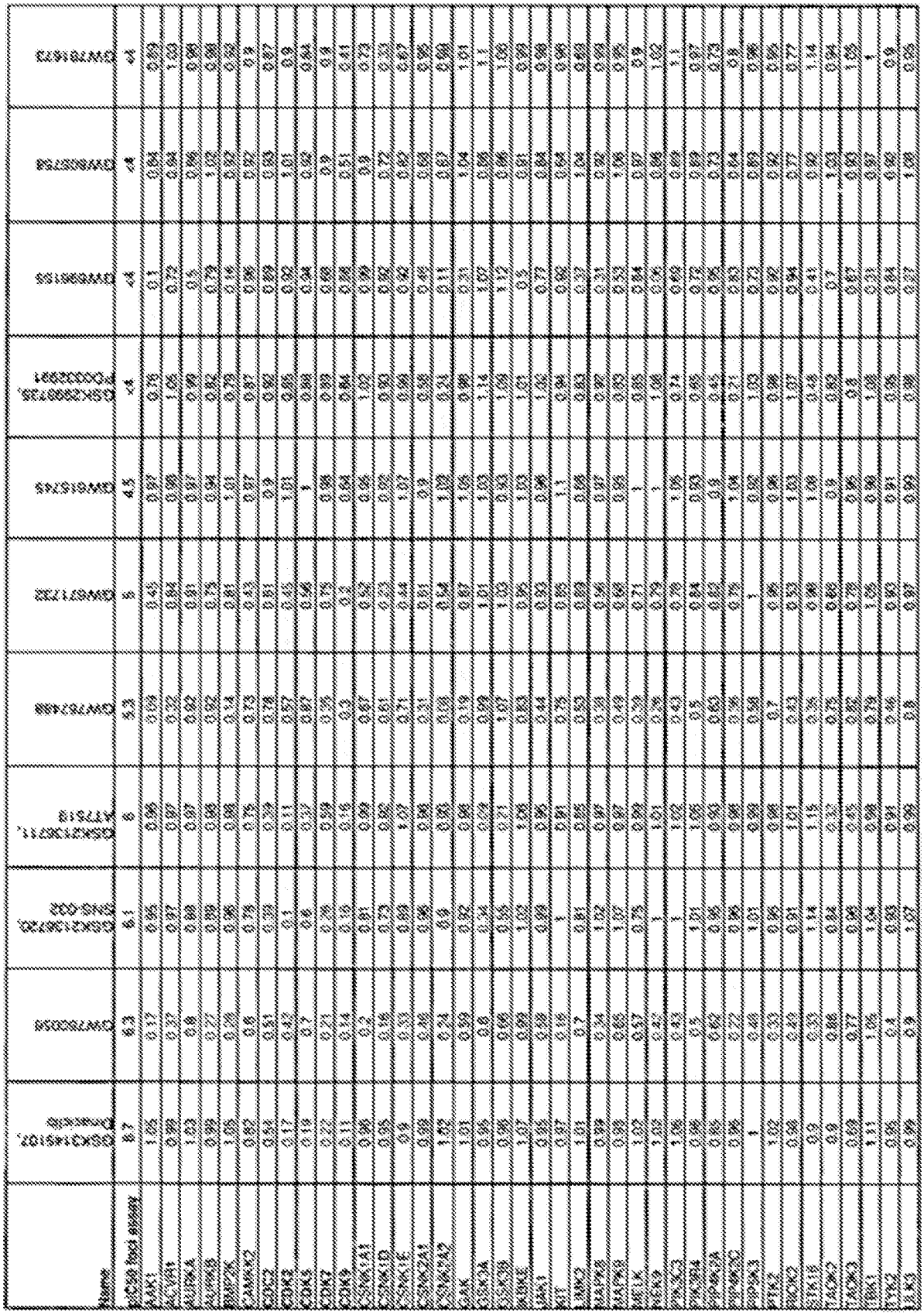
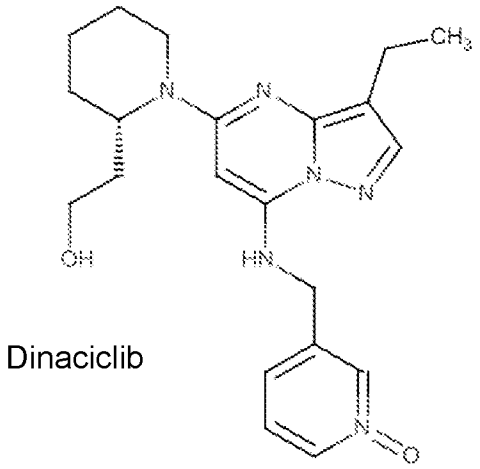
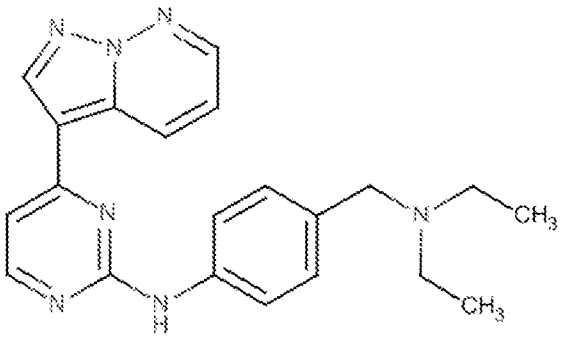


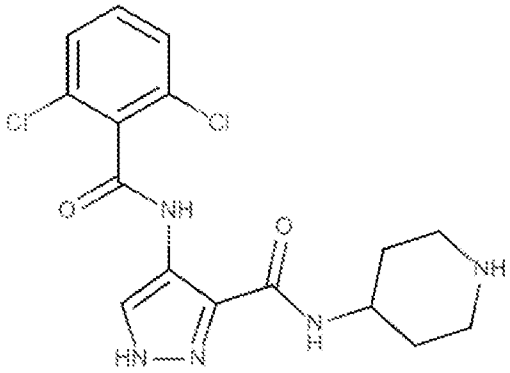
Figure 9B continued



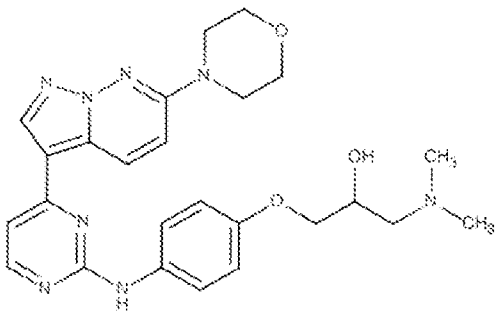
Dinaciclib



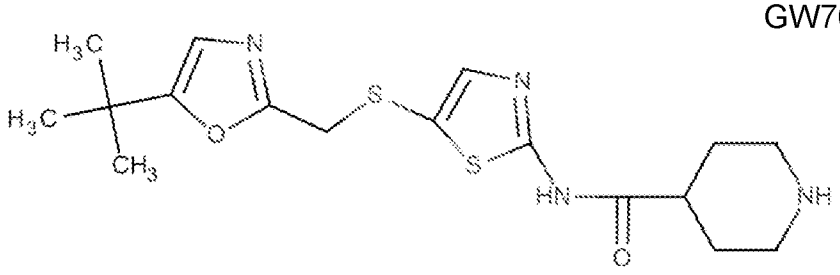
GW780056



AT7519

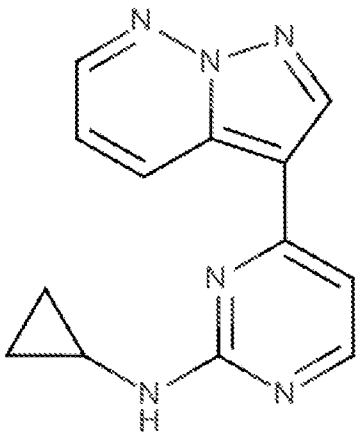


GW767488

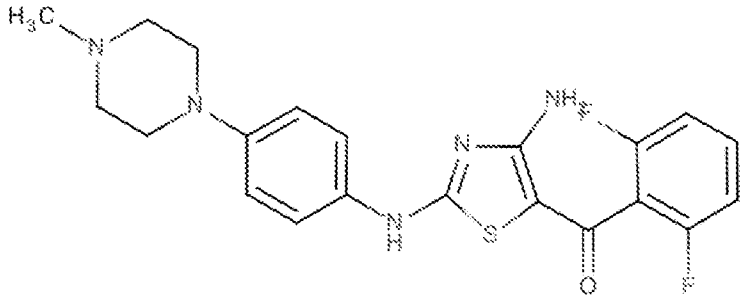


SNS-032

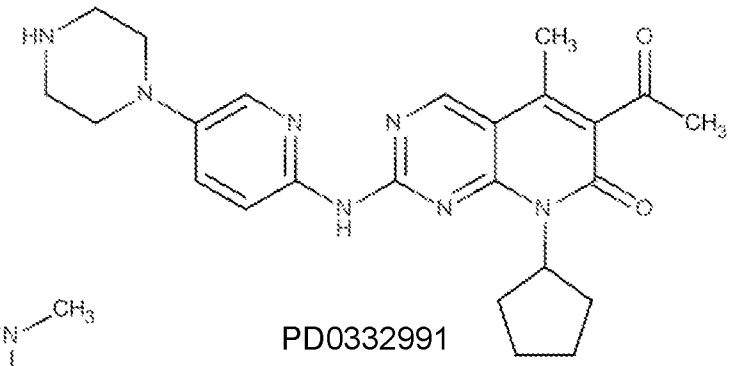
Figure 9B continued



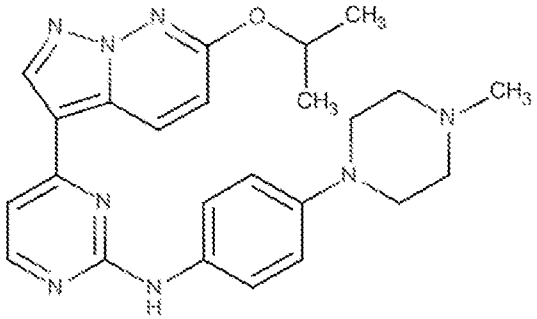
GW671732



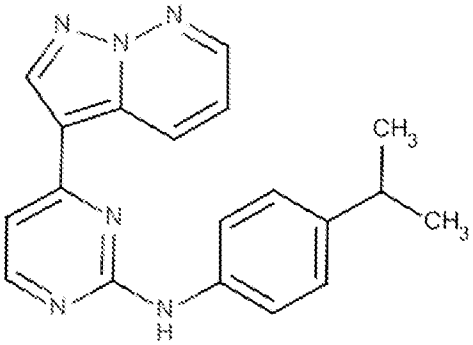
GW615745



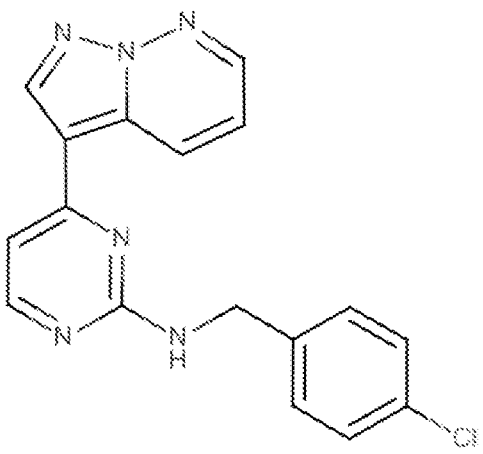
PD0332991



GW696155



GW805758



GW781673

Figure 10

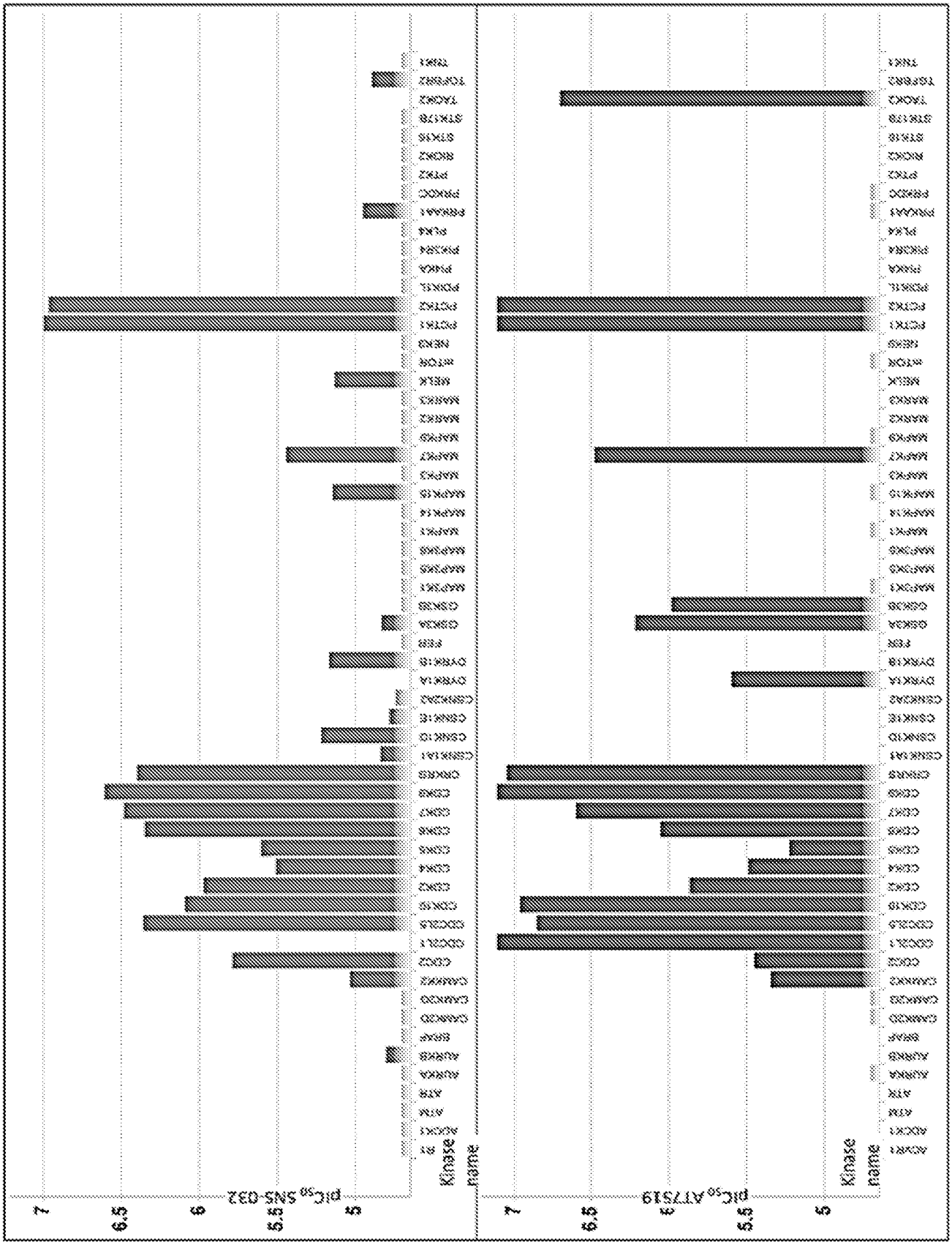


Figure 11

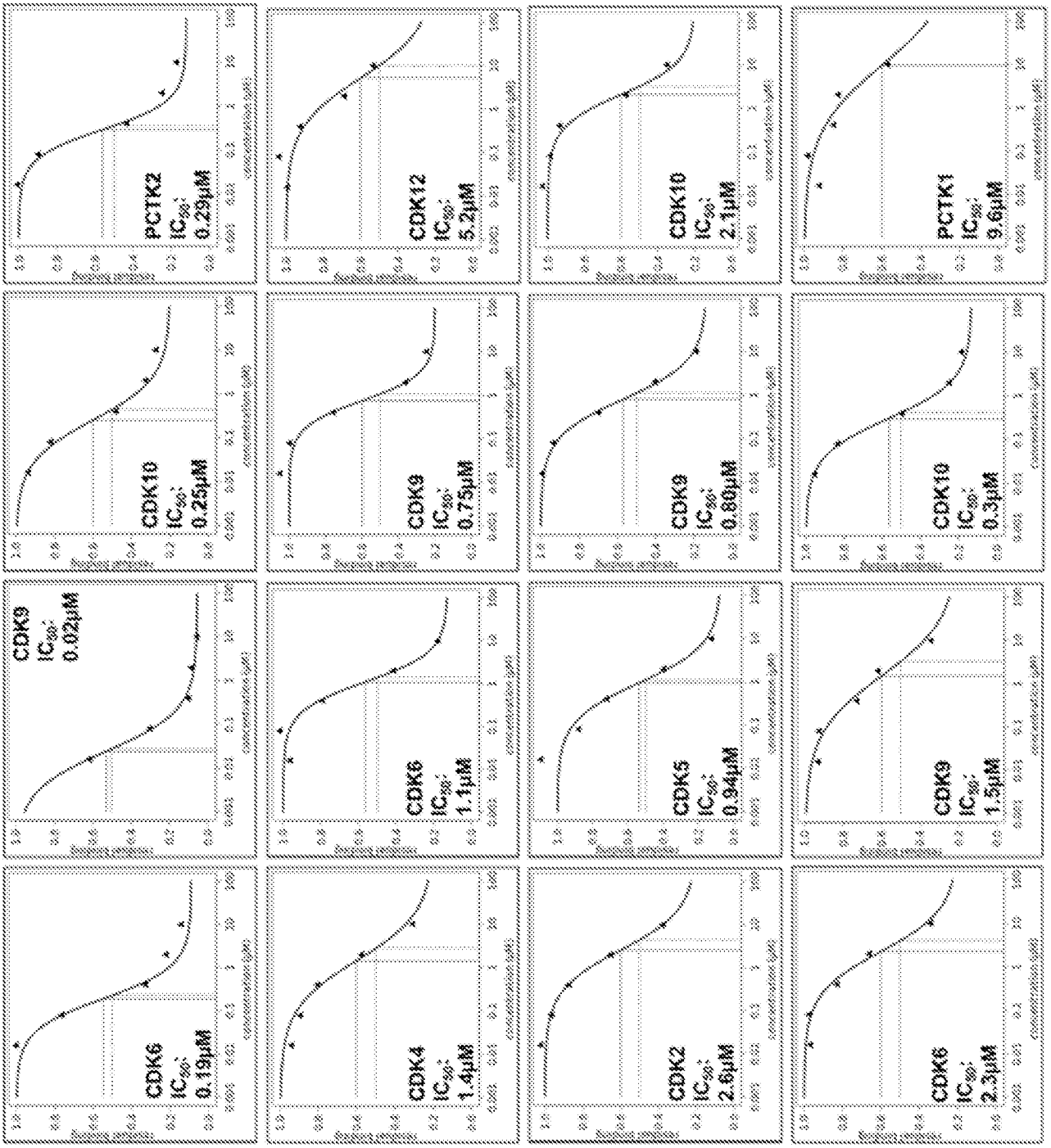

GW780056
K562 extract
X015636
CDK6
IC50:
0.19μM
CDK9
IC50:
0.02μM
CDK10
IC50:
0.25μM
PCTK2
IC50:
0.29μM
GW767488
K562 extract
X015861
CDK4
IC50:
1.4μM
CDK6
IC50:
1.1μM
CDK9
IC50:
0.75μM
CDK12
IC50:
5.2μM
GW671732
A204 extract
X015816
CDK2
IC50:
2.6μM
CDK5
IC50:
0.94μM
CDK9
IC50:
0.80μM
CDK10
IC50:
2.1μM
GW615745
K562 extract
X015637
CDK6
IC50:
2.3μM
CDK9
IC50:
1.5μM
CDK10
IC50:
0.3μM
PCTK1
IC50:
9.6μM

Figure 12

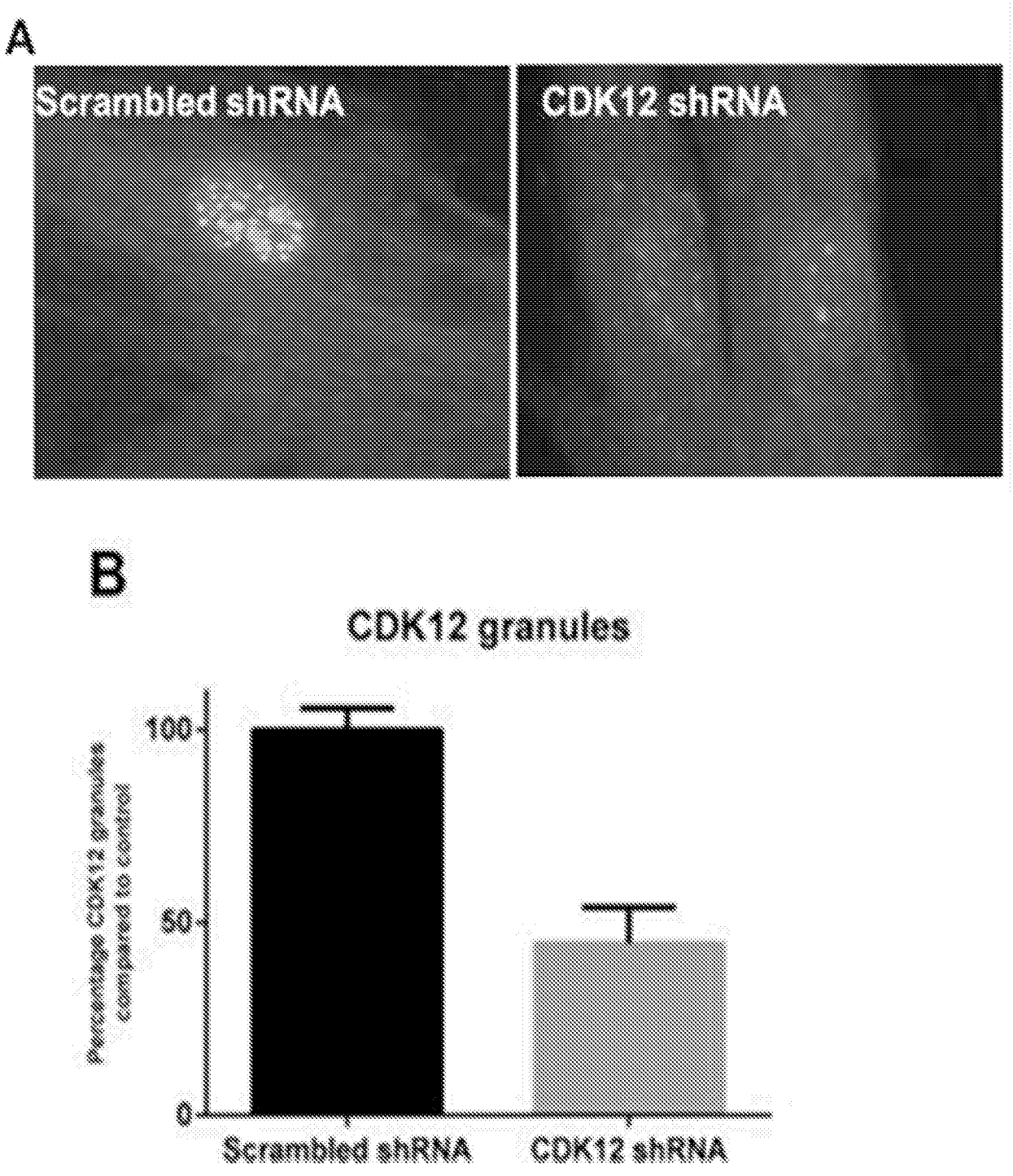

A
Scrambled shRNA
CDK12 shRNA
B
CDK12 granules
Percentage CDK12 granules compared to control
100
50
0
Scrambled shRNA
CDK12 shRNA

Figure 12 continued

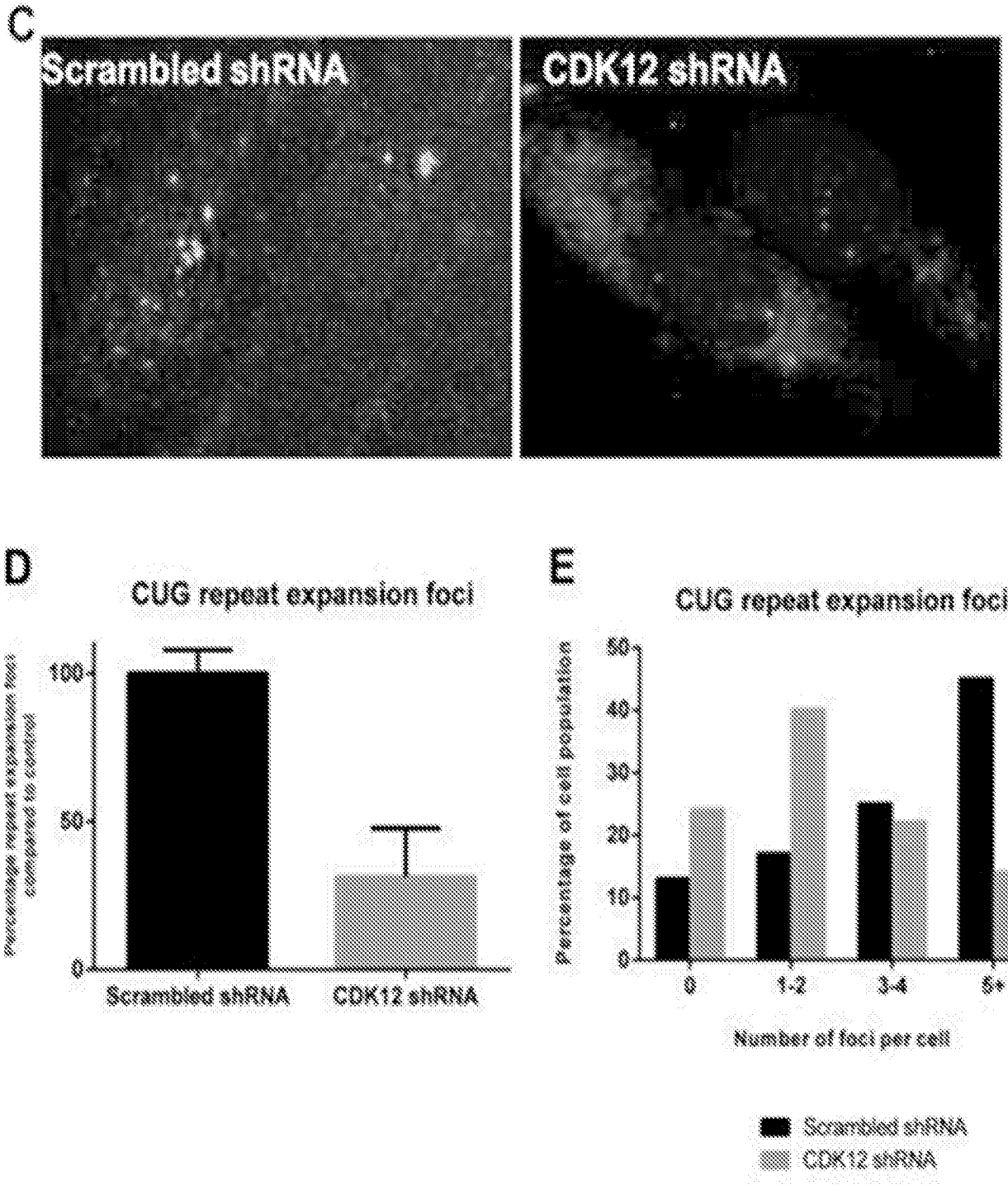


Figure 12 continued

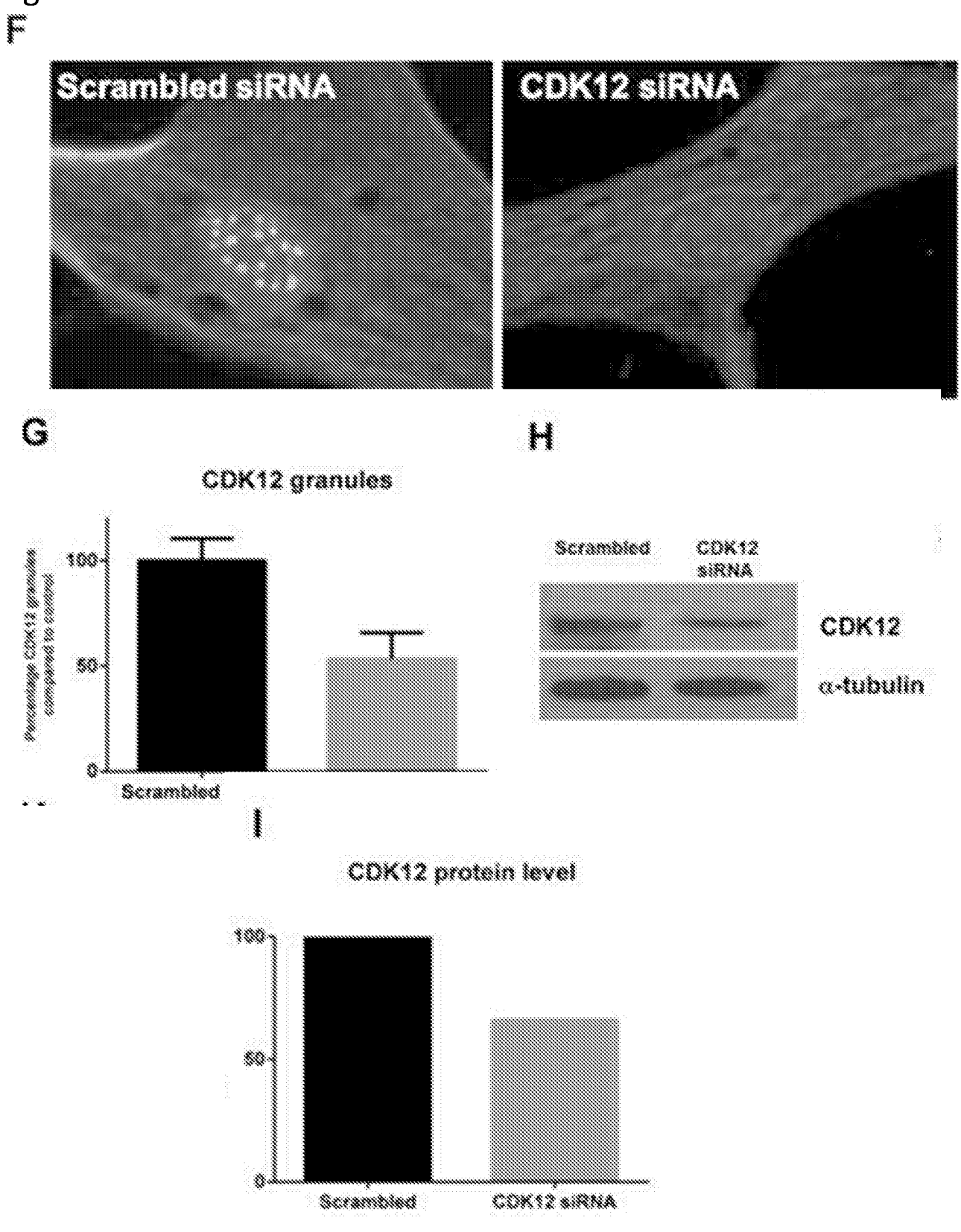
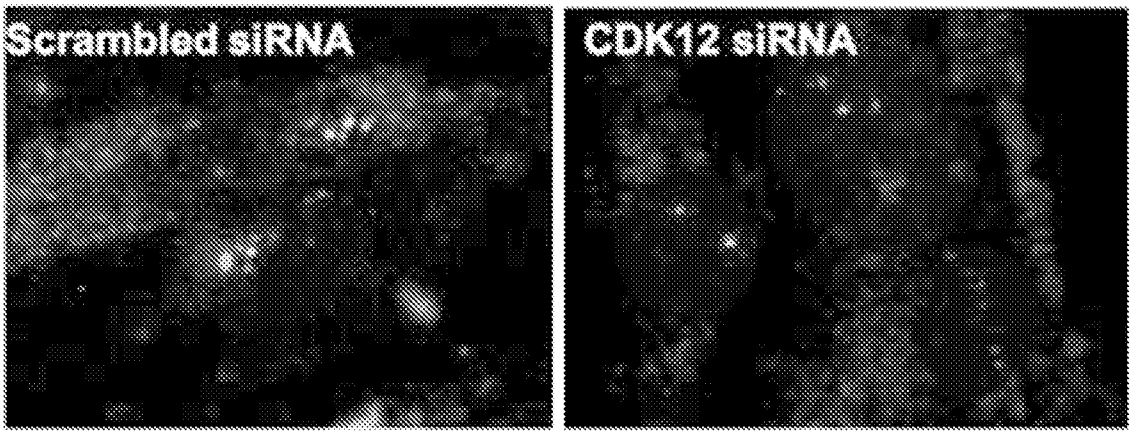
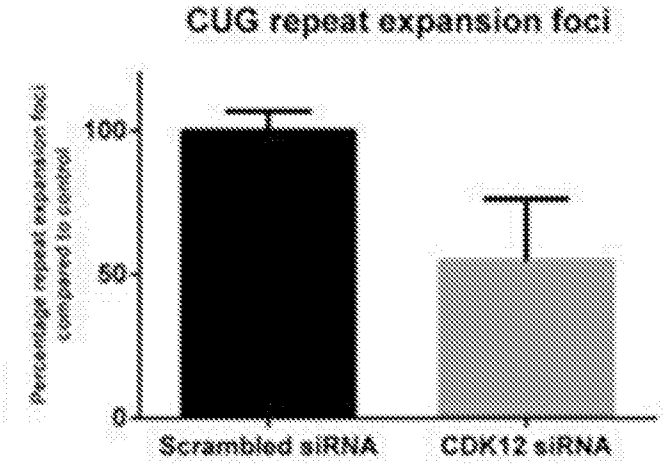


Figure 12 continued

J



K



L

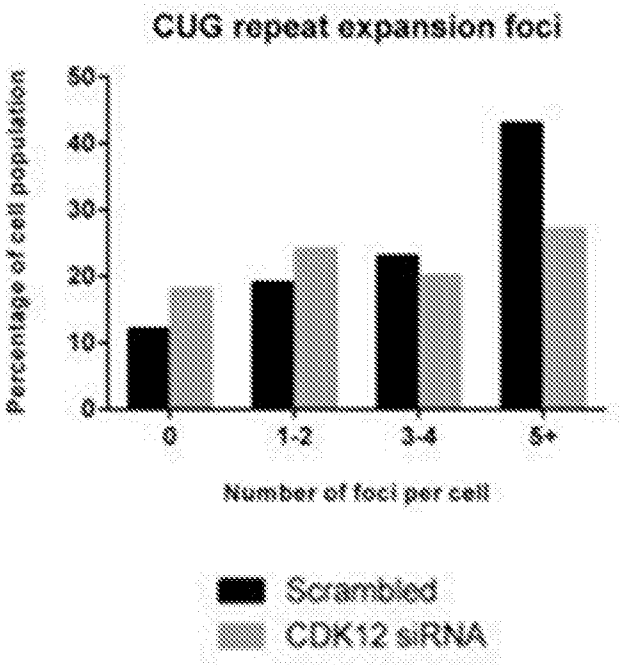


Figure 13

	CDK1/CyclinB	CDK2/CyclinA	CDK3/CyclinE	CDK4/Cyclin D	CDK5/p53	CDK6/cyclinD3	CDK7/cyclinH	CDK8	CDK9/CyclinT
Nuclear foci Active									
Dinaciclib	3	1		10	1		70		4
AT7519	170	40	400	63	15	130	2500		8
SNS-032	500	40		800	300	800	60		4
GW7674888X		500		40					
GW615745X		170		3					
SB-498806	300	6		15					
GW671732X	2	125		70					
Nuclear foci inactive									
PD0332991				8		15			400
Olomoucine	6200	6200			3200				2500
R-roscovitine		630			150		630	4000	500
GW7638870X		780		20					

Figure 14

experiment identifier	cell line	compound	alias	pIC50 fact assay	pIC50 values from SNS032-Kinobeads profiling in K562 and A204 extract											
					CDK1	CDK2	CDK4	CDK5	CDK6	CDK7	CDK9	CDK10	CDK12	CDC13	PCTK1	PCTK2
X015788	K562	GSK3145107	dinaciclib	8.7	5.69	8.54	8.2	7.17	7.64	8.7	7.8	7.59	7.88		6.39	8
X015808	K562	GW780056		6.3	<5	5.06	5.21	5.15	6.72	6.4	7.62	8.8	5.92	5.77	6.82	6.54
X015785	A204	GW780056		6.3	<5	5.73	5.72	5.85		5.82	7.8	6.3	6.34		7.39	6.81
X015810	K562	GSK9138720	SNS-032	8.1	5.78	5.97	5.5	5.6	6.35	6.48	6.6	6.09	6.4	8.36	7	6.98
X015861	K562	GW707488		5.3	<5	5.44	5.95	5.04	5.86	6.31	6.12	5.42	5.28	5.02	6.66	6.42
X015862	K562	GW767488		5.3	<5	5.2	5.27	<5	5.85	6.02	5.89	5.22	<5	<5	6.49	6.34
X015788	K562	GW671732		5	<5	<5	5.06	5.59	6.08	<5	5.77	<5	<5	<5	5.26	5.07
X015816	A204	GW671732		5	<5	5.59	<5	6.03		5.75	6.09	5.69			5.43	5.53
X015837	K562	GW615745		4.5	<5	<5	<5	<5	5.64	<5	5.82	6.52	<5	<5	6.02	5.19
X015736	K562	GSK2606735	PD0332991	<4	<5	<5	6.25	<5	7.17	<5	<5	<5	<5	<5	5.82	5.52
X015815	K562	GW696155		<4	<5	<5	<5	<5	<5	5.21	<5	<5	<5	<5	5.62	5.48

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2017/050824

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K45/06 A61K31/426 A61K31/454 A61K31/506 A61K31/519
A61K31/7088 A61P21/00
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)
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, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/051886 A1 (SMITHKLINE BEECHAM CORP [US]; HARRIS PHILLIP ANTHONY [US]; JUNG DAVID) 26 June 2003 (2003-06-26)	38,39
A	Examples 1, 7, 26, 28, 56, 60; biological data; claims 30, 31	1-36,40

X	CHRISTIAN A. BÖSKEN ET AL: "The structure and substrate specificity of human Cdk12/Cyclin K", NATURE COMMUNICATIONS, vol. 5, 24 March 2014 (2014-03-24), XP055377119, DOI: 10.1038/ncomms4505	38
A	Fig. 6; p. 13, "Kinase inhibition assays"	1-36,39, 40

	-/--	

[X] Further documents are listed in the continuation of Box C.

[X] 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
1 June 2017	04/08/2017

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	Scheithe, Rupert

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2002/119963 A1 (SANNER MARK A [US] ET AL) 29 August 2002 (2002-08-29) paragraph 211; claims 50, 51	1-36, 38-40
A	P. PAOLETTI ET AL: "Dopaminergic and Glutamatergic Signaling Crosstalk in Huntington's Disease Neurodegeneration: The Role of p25/Cyclin-Dependent Kinase 5", JOURNAL OF NEUROSCIENCE, vol. 28, no. 40, 1 October 2008 (2008-10-01), pages 10090-10101, XP055377409, US ISSN: 0270-6474, DOI: 10.1523/JNEUROSCI.3237-08.2008 p. 10096, chapter bridging left and right col.; Fig. 7	1-36, 38-40

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2017/050824

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

38, 39(completely); 1-36, 40(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 38, 39(completely); 1-36, 40(partially)

An inhibitor for use according to claim 1, the use according to claim 34, the method according to claim 35 wherein the disorder caused by the generation of repeat expansion transcripts is myotonic dystrophy type 1 or myotonic dystrophy type 2.
A composition according to claim 38.

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2. claims: 1-36, 40(all partially)

An inhibitor for use according to claim 1, the use according to claim 34, the method according to claim 35 wherein the disorder caused by the generation of repeat expansion transcripts is fragile X associated tremor/ataxia syndrome.

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3. claims: 1-36, 40(all partially)

An inhibitor for use according to claim 1, the use according to claim 34, the method according to claim 35 wherein the disorder caused by the generation of repeat expansion transcripts is amyotrophic lateral sclerosis (ALS).

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4. claims: 1-36, 40(all partially)

An inhibitor for use according to claim 1, the use according to claim 34, the method according to claim 35 wherein the disorder caused by the generation of repeat expansion transcripts is frontotemporal dementia.

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5. claims: 1-36, 40(all partially)

An inhibitor for use according to claim 1, the use according to claim 34, the method according to claim 35 wherein the disorder caused by the generation of repeat expansion transcripts is Huntington's disease like 2 or Huntington's disease.

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6. claims: 1-36, 40(all partially)

An inhibitor for use according to claim 1, the use according to claim 34, the method according to claim 35 wherein the disorder caused by the generation of repeat expansion transcripts is spinocerebellar ataxia Types 1, 2, 3, 6, 7, 8, 10, 31, 17.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

7. claims: 1-36, 40(all partially)

An inhibitor for use according to claim 1, the use according to claim 34, the method according to claim 35 wherein the disorder caused by the generation of repeat expansion transcripts is dentatorubral-pallidoluysian atrophy.

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8. claims: 1-36, 40(all partially)

An inhibitor for use according to claim 1, the use according to claim 34, the method according to claim 35 wherein the disorder caused by the generation of repeat expansion transcripts is spinal and bulbar muscular atrophy.

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9. claim: 37

A method of screening according to claim 37.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2017/050824

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