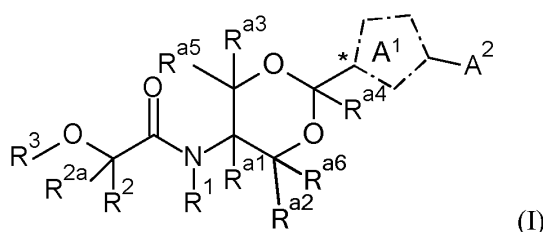


Modulators of the integrated stress response pathway

The present invention relates to compounds of formula (I)

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or pharmaceutically acceptable salts, solvates, hydrates, tautomers or stereoisomers thereof, wherein R^1 , R^2 , R^{2a} , R^3 , R^{a1} , R^{a2} , R^{a3} , R^{a4} , R^{a5} , R^{a6} , A^1 and A^2 have the meaning as indicated in the description and claims. The invention further relates to pharmaceutical compositions comprising said compounds, their use as medicament and in a method for treating and preventing of one or more diseases or disorders associated with integrated stress response.

10

The Integrated Stress Response (ISR) is a cellular stress response common to all eukaryotes (1). Dysregulation of ISR signaling has important pathological consequences linked inter alia to inflammation, viral infection, diabetes, cancer and neurodegenerative diseases.

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ISR is a common denominator of different types of cellular stresses resulting in phosphorylation of the alpha subunit of eukaryotic translation initiation factor 2 (eIF2alpha) on serine 51 leading to the suppression of normal protein synthesis and expression of stress response genes (2). In mammalian cells the phosphorylation is carried out by a family of four eIF2alpha kinases, namely: PKR-like ER kinase (PERK), double-stranded RNA-dependent protein kinase (PKR), heme-regulated eIF2alpha kinase (HRI), and general control non-derepressible 2 (GCN2), each responding to distinct environmental and physiological stresses (3).

20

25

eIF2alpha together with eIF2beta and eIF2gamma form the eIF2 complex, a key player of the initiation of normal mRNA translation (4). The eIF2 complex binds GTP and Met-tRNA_i forming a ternary complex (eIF2-GTP-Met-tRNA_i), which is recruited by ribosomes for translation initiation (5, 6).

30

eIF2B is a heterodecameric complex consisting of 5 subunits (alpha, beta, gamma, delta, epsilon) which in duplicate form a GEF-active decamer (7).

- In response to ISR activation, phosphorylated eIF2alpha inhibits the eIF2B-mediated exchange of GDP for GTP, resulting in reduced ternary complex formation and hence in the inhibition of translation of normal mRNAs characterized by ribosomes binding to the 5' AUG start codon (8). Under these conditions of reduced ternary complex abundance the translation of several specific mRNAs including the mRNA coding for the transcription factor ATF4 is activated via a mechanism involving altered translation of upstream ORFs (uORFs) (7, 9, 10). These mRNAs typically contain one or more uORFs that normally function in unstressed cells to limit the flow of ribosomes to the main coding ORF. For example, during normal conditions, uORFs in the 5' UTR of ATF occupy the ribosomes and prevent translation of the coding sequence of ATF4. However, during stress conditions, i.e. under conditions of reduced ternary complex formation, the probability for ribosomes to scan past these upstream ORFs and initiate translation at the ATF4 coding ORF is increased. ATF4 and other stress response factors expressed in this way subsequently govern the expression of an array of further stress response genes. The acute phase consists in expression of proteins that aim to restore homeostasis, while the chronic phase leads to expression of pro-apoptotic factors (1, 11, 12, 13).
- Upregulation of markers of ISR signaling has been demonstrated in a variety of conditions, among these cancer and neurodegenerative diseases. In cancer, ER stress-regulated translation increases tolerance to hypoxic conditions and promotes tumor growth (14, 15, 16), and deletion of PERK by gene targeting has been shown to slow growth of tumours derived from transformed PERK^{-/-} mouse embryonic fibroblasts (14, 17). Further, a recent report has provided proof of concept using patient derived xenograft modeling in mice for activators of eIF2B to be effective in treating a form of aggressive metastatic prostate cancer (28). Taken together, prevention of cytoprotective ISR signaling may represent an effective anti-proliferation strategy for the treatment of at least some forms of cancer.
- Further, modulation of ISR signaling could prove effective in preserving synaptic function and reducing neuronal decline, also in neurodegenerative diseases that are characterized by misfolded proteins and activation of the unfolded protein response (UPR), such as amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), Parkinson's disease (PD) and Jakob Creutzfeldt (prion) diseases (18, 19, 20). With prion disease

an example of a neurodegenerative disease exists where it has been shown that pharmacological as well as genetic inhibition of ISR signaling can normalize protein translation levels, rescue synaptic function and prevent neuronal loss (21). Specifically, reduction of levels of phosphorylated eIF2alpha by overexpression of the phosphatase controlling phosphorylated eIF2alpha levels increased survival of prion-infected mice whereas sustained eIF2alpha phosphorylation decreased survival (22).

Further, direct evidence for the importance of control of protein expression levels for proper brain function exists in the form of rare genetic diseases affecting functions of eIF2 and eIF2B. A mutation in eIF2gamma that disrupts complex integrity of eIF2 and hence results in reduced normal protein expression levels is linked to intellectual disability syndrome (ID) (23). Partial loss of function mutations in subunits of eIF2B have been shown to be causal for the rare leukodystrophy Vanishing White Matter Disease (VWMD) (24, 25). Specifically, stabilization of eIF2B partial loss of function in a VWMD mouse model by a small molecule related to ISRIB has been shown to reduce ISR markers and improve functional as well as pathological end points (26, 27).

Modulators of the eIF2 alpha pathway are described in WO 2014/144952 A2. WO 2017/193030 A1, WO 2017/193034 A1, WO 2017/193041 A1 and WO 2017/193063 A1 describe modulators of the integrated stress pathway. WO 2017/212423 A1, WO 2017/212425 A1, WO 2018/225093 A1, WO 2019/008506 A1 and WO 2019/008507 A1 describe inhibitors of the ATF4 pathway. WO 2019/032743 A1, WO 2019/046779 A1, WO 2020/167994 A1, WO 2020/168011 A1 and WO 2020/181247 A1 relate to eukaryotic initiation factor 2B modulators. In WO 2020/77217 A1 compounds, compositions, and methods useful for modulating the integrated stress response (ISR) and for treating related diseases, disorders and conditions are described.

Further documents describing modulators of the integrated stress pathway are WO 2019/090069 A1, WO 2019/090074 A1, WO 2019/090076 A1, WO 2019/090078 A1, WO 2019/090081 A1, WO 2019/090082 A1, WO 2019/090085 A1, WO 2019/090088 A1, WO 2019/090090 A1, WO 2020/223536 A1, WO 2020/223538 A1, WO 2020/252207 A1, WO 2020/216764 A1, WO 2020/216766 A1, International patent application PCT/EP2021/051697, European patent applications 20203312.2, 20203311.4 and 20203309.8. Modulators of eukaryotic initiation factors are described in WO 2019/183589 A1. WO 2019/118785 A2, WO

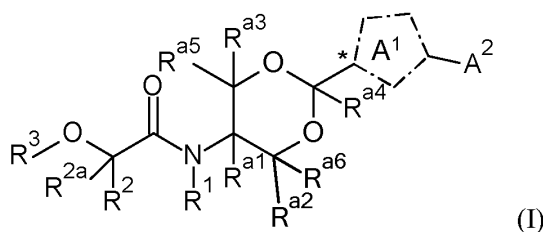
2019/236710 A1, WO 2020/176428 A1 and WO 2020/252205 A1 describe inhibitors of the integrated stress response pathway. Heteroaryl derivatives as ATF4 inhibitors are described in WO 2019/193540 A1. Bicyclic aromatic ring derivatives as ATF4 inhibitors are described in WO 2019/193541 A1. WO 2020/031107 A1 and WO 2020/012339 A1 describe inhibitors of
 5 the ATF4 pathway.

However, there is a continuing need for new compounds useful as modulators of the integrated stress response pathway with good pharmacokinetic properties.

10 Thus, an object of the present invention is to provide a new class of compounds as modulators of the integrated stress response pathway, which may be effective in the treatment of integrated stress response pathway related diseases and which may show improved pharmaceutically relevant properties including activity, solubility, selectivity, ADMET properties and/or reduced side effects.

15

Accordingly, the present invention provides a compound of formula (I)



20 or a pharmaceutically acceptable salt, solvate, hydrate, tautomer or stereoisomer thereof, wherein

R^{a1} , R^{a2} , R^{a3} , R^{a4} , R^{a5} , R^{a6} are independently selected from the group consisting of H; halogen; C_{1-4} alkyl; and A^{2a} , wherein C_{1-4} alkyl is optionally substituted with one or more substituents
 25 selected from the group consisting of halogen, OH, and O- C_{1-3} alkyl, wherein the substituents are the same or different, and
 provided that only one of R^{a1} , R^{a2} , R^{a3} , R^{a4} , R^{a5} , R^{a6} is A^{2a} ,

A¹ is C₅ cycloalkylene, C₅ cycloalkenylene, or a nitrogen ring atom containing 5-membered heterocyclene, provided that the ring atom of ring A¹ marked with an asterisk is a carbon atom, wherein A¹ is optionally substituted with one or more R⁴, which are the same or different;

- 5 each R⁴ is independently oxo (=O) where the ring is at least partially saturated, thiooxo (=S) where the ring is at least partially saturated, halogen, CN, OR⁵, or C₁₋₆ alkyl, wherein C₁₋₆ alkyl is optionally substituted with one or more halogen, which are the same or different;

- 10 R⁵ is H or C₁₋₆ alkyl, wherein C₁₋₆ alkyl is optionally substituted with one or more halogen, which are the same or different;

A² is R^{6a} or A^{2a};

- 15 R^{6a} is OR^{6a1}, SR^{6a1}, N(R^{6a1}R^{6a2}); C₁₋₆ alkyl, C₂₋₆ alkenyl or C₂₋₆ alkynyl, wherein C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl are optionally substituted with one or more substituents selected from the group consisting of halogen; OR^{6a3}; CN; and A^{2a}, wherein the substituents are the same or different;

- 20 R^{6a1}, R^{6a2} are independently selected from the group consisting of H; C₁₋₆ alkyl; C₂₋₆ alkenyl; C₂₋₆ alkynyl; and A^{2a}, wherein C₁₋₆ alkyl; C₂₋₆ alkenyl; and C₂₋₆ alkynyl are optionally substituted with one or more substituents selected from the group consisting of halogen; CN; OR^{6a3}; A^{2a}; and OA^{2a}, wherein the substituents are the same or different;

- 25 R^{6a3} is H; or C₁₋₄ alkyl, wherein C₁₋₄ alkyl is optionally substituted with one or more halogen, which are the same or different;

A^{2a} is phenyl; C₃₋₇ cycloalkyl; C₄₋₁₂ bicycloalkyl; or 3- to 7-membered heterocyclyl, wherein A^{2a} is optionally substituted with one or more R⁶, which are the same or different;

- 30 each R⁶ is independently R^{6b}; OH; OR^{6b}; halogen; or CN, wherein R^{6b} is cyclopropyl, C₁₋₆ alkyl; C₂₋₆ alkenyl; or C₂₋₆ alkynyl, wherein R^{6b} is optionally substituted with one or more halogen, which are the same or different; or
two R⁶ are joined to form together with the atoms to which they are attached a ring A^{2b};

A^{2b} is phenyl; C₃₋₇ cycloalkyl; or 3 to 7 membered heterocyclyl, wherein A^{2b} is optionally substituted with one or more R⁷, which are the same or different;

each R⁷ is independently C₁₋₆ alkyl, C₂₋₆ alkenyl or C₂₋₆ alkynyl, wherein C₁₋₆ alkyl, C₂₋₆ alkenyl,
5 and C₂₋₆ alkynyl are optionally substituted with one or more halogen, which are the same or different;

R¹ is H or C₁₋₄ alkyl, preferably H, wherein C₁₋₄ alkyl is optionally substituted with one or more halogen, which are the same or different;

10

R² is H; F; or C₁₋₄ alkyl, wherein C₁₋₄ alkyl is optionally substituted with one or more halogen, which are the same or different; and

R³ is A³, C₁₋₆ alkyl, C₂₋₆ alkenyl, or C₂₋₆ alkynyl, wherein C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl are optionally substituted with one or more R⁸, which are the same or different; or

15 R² and R³ are joined to form together with the oxygen atom and carbon atom to which they are attached a ring A^{3a}, wherein A^{3a} is a 7 to 12 membered heterobicycyl, wherein 7 to 12 membered heterobicycyl is optionally substituted with one or more R¹⁰, which are the same or different;

20 R^{2a} is H or F, preferably H;

each R⁸ is independently halogen; CN, C(O)OR⁹, OR⁹, C(O)R⁹, C(O)N(R^{9a}R^{9a}), S(O)₂N(R^{9a}R^{9a}), S(O)N(R^{9a}R^{9a}), S(O)₂R⁹, S(O)R⁹, N(R⁹)S(O)₂N(R^{9a}R^{9b}), SR⁹, N(R^{9a}R^{9a}), NO₂, OC(O)R⁹, N(R⁹)C(O)R^{9a}, N(R⁹)SO₂R^{9a}, N(R⁹)S(O)R^{9a}, N(R⁹)C(O)N(R^{9a}R^{9b}), N(R⁹)C(O)OR^{9a},
25 OC(O)N(R^{9a}R^{9a}), or A³;

R⁹, R^{9a}, R^{9b} are independently selected from the group consisting of H, C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl, wherein C₁₋₆ alkyl, C₂₋₆ alkenyl, and C₂₋₆ alkynyl are optionally substituted with one or more halogen, which are the same or different, or one OH, or one OC₁₋₄ alkyl, or
30 one A³;

each A³ is independently phenyl, naphthyl, C₃₋₇ cycloalkyl, 3 to 7 membered heterocyclyl, or 7 to 12 membered heterobicycyl, wherein A³ is optionally substituted with one or more R¹⁰, which are the same or different;

each R^{10} is independently halogen, CN, $C(O)OR^{11}$, OR^{11} , $C(O)R^{11}$, $C(O)N(R^{11}R^{11a})$, $S(O)_2N(R^{11}R^{11a})$, $S(O)N(R^{11}R^{11a})$, $S(O)_2R^{11}$, $S(O)R^{11}$, $N(R^{11})S(O)_2N(R^{11a}R^{11b})$, SR^{11} , $N(R^{11}R^{11a})$, NO_2 , $OC(O)R^{11}$, $N(R^{11})C(O)R^{11a}$, $N(R^{11})S(O)_2R^{11a}$, $N(R^{11})S(O)R^{11a}$,
 5 $N(R^{11})C(O)OR^{11a}$, $N(R^{11})C(O)N(R^{11a}R^{11b})$, $OC(O)N(R^{11}R^{11a})$, oxo (=O) where the ring is at least partially saturated, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl, wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl are optionally substituted with one or more R^{12} , which are the same or different;

10 R^{11} , R^{11a} , R^{11b} are independently selected from the group consisting of H, C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl, wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl are optionally substituted with one or more halogen, which are the same or different;

each R^{12} is independently halogen, CN, $C(O)OR^{13}$, OR^{13} , $C(O)R^{13}$, $C(O)N(R^{13}R^{13a})$,
 15 $S(O)_2N(R^{13}R^{13a})$, $S(O)N(R^{13}R^{13a})$, $S(O)_2R^{13}$, $S(O)R^{13}$, $N(R^{13})S(O)_2N(R^{13a}R^{13b})$, SR^{13} , $N(R^{13}R^{13a})$, NO_2 , $OC(O)R^{13}$, $N(R^{13})C(O)R^{13a}$, $N(R^{13})SO_2R^{13a}$, $N(R^{13})S(O)R^{13a}$, $N(R^{13})C(O)N(R^{13a}R^{13b})$, $N(R^{13})C(O)OR^{13a}$, or $OC(O)N(R^{13}R^{13a})$;

R^{13} , R^{13a} , R^{13b} are independently selected from the group consisting of H, C_{1-6} alkyl, C_{2-6}
 20 alkenyl, and C_{2-6} alkynyl, wherein C_{1-6} alkyl, C_{2-6} alkenyl, and C_{2-6} alkynyl are optionally substituted with one or more halogen, which are the same or different.

The compounds of the present invention represent 1,3-dioxane derivatives, which can be prepared according to the routes and methods described herein or elsewhere, e.g. in WO
 25 98/56788 A2.

In case a variable or substituent can be selected from a group of different variants and such variable or substituent occurs more than once the respective variants can be the same or different.

30

Within the meaning of the present invention the terms are used as follows:

The term “optionally substituted” means unsubstituted or substituted. Generally -but not limited to-, “one or more substituents” means one, two or three, preferably one or two substituents and more preferably one substituent. Generally these substituents can be the same or different.

- 5 “Alkyl” means a straight-chain or branched hydrocarbon chain. Each hydrogen of an alkyl carbon may be replaced by a substituent as further specified.

“Alkenyl” means a straight-chain or branched hydrocarbon chain that contains at least one carbon-carbon double bond. Each hydrogen of an alkenyl carbon may be replaced by a
10 substituent as further specified.

“Alkynyl” means a straight-chain or branched hydrocarbon chain that contains at least one carbon-carbon triple bond. Each hydrogen of an alkynyl carbon may be replaced by a substituent as further specified.

15

“C₁₋₄ alkyl” means an alkyl chain having 1 - 4 carbon atoms, e.g. if present at the end of a molecule: methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, or e.g. -CH₂-, -CH₂-CH₂-, -CH(CH₃)-, -CH₂-CH₂-CH₂-, -CH(C₂H₅)-, -C(CH₃)₂-, when two moieties of a molecule are linked by the alkyl group. Each hydrogen of a C₁₋₄ alkyl carbon may be replaced
20 by a substituent as further specified. The term “C₁₋₃ alkyl” is defined accordingly.

“C₁₋₆ alkyl” means an alkyl chain having 1 - 6 carbon atoms, e.g. if present at the end of a molecule: C₁₋₄ alkyl, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, n-hexyl, or e.g. -CH₂-, -CH₂-CH₂-, -CH(CH₃)-, -CH₂-CH₂-CH₂-, -CH(C₂H₅)-, -
25 C(CH₃)₂-, when two moieties of a molecule are linked by the alkyl group. Each hydrogen of a C₁₋₆ alkyl carbon may be replaced by a substituent as further specified.

“C₂₋₆ alkenyl” means an alkenyl chain having 2 to 6 carbon atoms, e.g. if present at the end of a molecule: -CH=CH₂, -CH=CH-CH₃, -CH₂-CH=CH₂, -CH=CH-CH₂-CH₃, -CH=CH-
30 CH=CH₂, or e.g. -CH=CH-, when two moieties of a molecule are linked by the alkenyl group. Each hydrogen of a C₂₋₆ alkenyl carbon may be replaced by a substituent as further specified.

“C₂₋₆ alkynyl” means an alkynyl chain having 2 to 6 carbon atoms, e.g. if present at the end of a molecule: -C≡CH, -CH₂-C≡CH, CH₂-CH₂-C≡CH, CH₂-C≡C-CH₃, or e.g. -C≡C- when two

moieties of a molecule are linked by the alkynyl group. Each hydrogen of a C₂₋₆ alkynyl carbon may be replaced by a substituent as further specified.

5 “C₃₋₇ cycloalkyl” or “C₃₋₇ cycloalkyl ring” means a cyclic alkyl chain having 3 - 7 carbon atoms, e.g. cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexenyl, cycloheptyl. Preferably, cycloalkyl refers to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, or cycloheptyl. Each hydrogen of a cycloalkyl carbon may be replaced by a substituent as further specified herein. The term “C₃₋₅ cycloalkyl” or “C₃₋₅ cycloalkyl ring” is defined accordingly.

10 “C₅ cycloalkylene” refers to a bivalent cycloalkyl with five carbon atoms, i.e. a bivalent cyclopentyl ring.

“C₅ cycloalkenylene” refers to a bivalent cycloalkenylene, i.e. a bivalent cyclopentene or cyclopentadiene.

15

“C₄₋₁₂ bicycloalkyl” or “C₄₋₁₂ bicycloalkyl ring” means a bicyclic fused, bridged or spiro alkyl chain having 4 to 12 carbon atoms, e.g. hexahydroindane, Octahydropentalen, bicycle[2.2.1]heptane or spiro(3.2)hexane. Each hydrogen of a bicycloalkyl carbon may be replaced by a substituent as further specified herein.

20

“Halogen” means fluoro, chloro, bromo or iodo. It is generally preferred that halogen is fluoro or chloro.

25 “3 to 7 membered heterocyclyl” or “3 to 7 membered heterocycle” means a ring with 3, 4, 5, 6 or 7 ring atoms that may contain up to the maximum number of double bonds (aromatic or non-aromatic ring which is fully, partially or un-saturated) wherein at least one ring atom up to 4 ring atoms are replaced by a heteroatom selected from the group consisting of sulfur (including -S(O)-, -S(O)₂-), oxygen and nitrogen (including =N(O)-) and wherein the ring is linked to the rest of the molecule via a carbon or nitrogen atom. Examples for a 3 to 7 membered heterocycle
30 are aziridine, azetidine, oxetane, thietane, furan, thiophene, pyrrole, pyrroline, imidazole, imidazoline, pyrazole, pyrazoline, oxazole, oxazoline, isoxazole, isoxazoline, thiazole, thiazoline, isothiazole, isothiazoline, thiadiazole, thiadiazoline, tetrahydrofuran, tetrahydrothiophene, pyrrolidine, imidazolidine, pyrazolidine, oxazolidine, isoxazolidine, thiazolidine, isothiazolidine, thiadiazolidine, sulfolane, pyran, dihydropyran, tetrahydropyran,

imidazolidine, pyridine, pyridazine, pyrazine, pyrimidine, piperazine, piperidine, morpholine, tetrazole, triazole, triazolidine, tetrazolidine, diazepane, azepine or homopiperazine. The term “5 to 6 membered heterocyclyl” or “5 to 6 membered heterocycle” is defined accordingly and includes 5 to 6 membered aromatic heterocyclyl or heterocycle. The term “5 membered heterocyclyl” or “5 membered heterocycle” is defined accordingly and includes 5 membered aromatic heterocyclyl or heterocycle.

The term “nitrogen ring atom containing 5-membered heterocyclene” refers to a bivalent 5-membered heterocycle, wherein at least one of the five ring atoms is a nitrogen atom and wherein the ring is linked to the rest of the molecule via a carbon or nitrogen atom.

“Saturated 4 to 7 membered heterocyclyl” or “saturated 4 to 7 membered heterocycle” means fully saturated “4 to 7 membered heterocyclyl” or “4 to 7 membered heterocycle”.

“4 to 7 membered at least partly saturated heterocyclyl” or “4 to 7 membered at least partly saturated heterocycle” means an at least partly saturated “4 to 7 membered heterocyclyl” or “4 to 7 membered heterocycle”.

“5 to 6 membered aromatic heterocyclyl” or “5 to 6 membered aromatic heterocycle” means a heterocycle derived from cyclopentadienyl or benzene, where at least one carbon atom is replaced by a heteroatom selected from the group consisting of sulfur (including -S(O)-, -S(O)₂-), oxygen and nitrogen (including =N(O)-). Examples for such heterocycles are furan, thiophene, pyrrole, imidazole, pyrazole, oxazole, isoxazole, thiazole, isothiazole, thiadiazole, triazole, tetrazole, pyridine, pyrimidine, pyridazine, pyrazine, triazine.

“5 membered aromatic heterocyclyl” or “5 membered aromatic heterocycle” means a heterocycle derived from cyclopentadienyl, where at least one carbon atom is replaced by a heteroatom selected from the group consisting of sulfur (including -S(O)-, -S(O)₂-), oxygen and nitrogen (including =N(O)-). Examples for such heterocycles are furan, thiophene, pyrrole, imidazole, pyrazole, oxazole, isoxazole, thiazole, isothiazole, thiadiazole, triazole, tetrazole.

“7 to 12 membered heterobicycyl” or “7 to 12 membered heterobicycle” means a heterocyclic system of two rings with 7 to 12 ring atoms, where at least one ring atom is shared by both rings and that may contain up to the maximum number of double bonds (aromatic or non-aromatic

ring which is fully, partially or un-saturated) wherein at least one ring atom up to 6 ring atoms are replaced by a heteroatom selected from the group consisting of sulfur (including -S(O)-, -S(O)₂-), oxygen and nitrogen (including =N(O)-) and wherein the ring is linked to the rest of the molecule via a carbon or nitrogen atom. Examples for a 7 to 12 membered heterobicycle are indole, indoline, benzofuran, benzothiophene, benzoxazole, benzisoxazole, benzothiazole, benzisothiazole, benzimidazole, benzimidazoline, quinoline, quinazoline, dihydroquinazoline, quinoline, dihydroquinoline, tetrahydroquinoline, decahydroquinoline, isoquinoline, decahydroisoquinoline, tetrahydroisoquinoline, dihydroisoquinoline, benzazepine, purine or pteridine. The term 7 to 12 membered heterobicycle also includes spiro structures of two rings like 6-oxa-2-azaspiro[3,4]octane, 2-oxa-6-azaspiro[3.3]heptan-6-yl or 2,6-diazaspiro[3.3]heptan-6-yl or bridged heterocycles like 8-aza-bicyclo[3.2.1]octane or 2,5-diazabicyclo[2.2.2]octan-2-yl or 3,8-diazabicyclo[3.2.1] octane.

“Saturated 7 to 12 membered heterobicycyl” or “saturated 7 to 12 membered heterobicycle” means fully saturated 7 to 12 membered heterobicycyl or 7 to 12 membered heterobicycle.

“7 to 12 membered at least partly saturated heterobicycyl” or “7 to 12 membered at least partly saturated heterobicycle” means an at least partly saturated “7 to 12 membered heterobicycyl” or “7 to 12 membered heterobicycle”.

20

“9 to 11 membered aromatic heterobicycyl” or “9 to 11 membered aromatic heterobicycle” means a heterocyclic system of two rings, wherein at least one ring is aromatic and wherein the heterocyclic ring system has 9 to 11 ring atoms, where two ring atoms are shared by both rings and that may contain up to the maximum number of double bonds (fully or partially aromatic) wherein at least one ring atom up to 6 ring atoms are replaced by a heteroatom selected from the group consisting of sulfur (including -S(O)-, -S(O)₂-), oxygen and nitrogen (including =N(O)-) and wherein the ring is linked to the rest of the molecule via a carbon or nitrogen atom. Examples for an 9 to 11 membered aromatic heterobicycle are indole, indoline, benzofuran, benzothiophene, benzoxazole, benzisoxazole, benzothiazole, benzisothiazole, benzimidazole, benzimidazoline, quinoline, quinazoline, dihydroquinazoline, dihydroquinoline, tetrahydroquinoline, isoquinoline, tetrahydroisoquinoline, dihydro-isoquinoline, benzazepine, purine or pteridine. The terms “9 to 10 membered aromatic heterobicycyl” or “9 to 10 membered aromatic heterobicycle” are defined accordingly.

30

Preferred compounds of formula (I) are those compounds in which one or more of the residues contained therein have the meanings given below, with all combinations of preferred substituent definitions being a subject of the present invention. With respect to all preferred compounds of the formula (I) the present invention also includes all tautomeric and stereoisomeric forms and
 5 mixtures thereof in all ratios, and their pharmaceutically acceptable salts.

In preferred embodiments of the present invention, the substituents mentioned below independently have the following meaning. Hence, one or more of these substituents can have the preferred or more preferred meanings given below.

10

Preferably, R^{a1} , R^{a2} , R^{a3} , R^{a4} , R^{a5} , R^{a6} are H.

Preferably, A^1 is a nitrogen ring atom containing 5-membered heterocyclene and wherein A^1 is optionally substituted with one or more R^4 , which are the same or different.

15

More preferably, A^1 is a nitrogen ring atom containing 5-membered heterocyclene selected from the group of bivalent heterocycles consisting of oxadiazole, imidazole, imidazolidine, pyrazole and triazole, even more preferably triazole or oxadiazole, even more preferably oxadiazole. A^1 is optionally substituted with one or more R^4 , which are the same or different.

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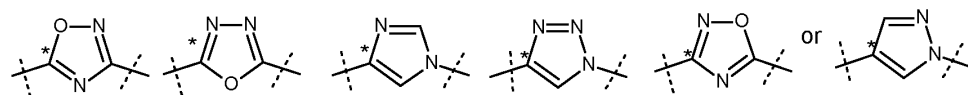
Preferably, A^1 is unsubstituted or substituted with one or two R^4 , which are the same or different, more preferably A^1 is unsubstituted.

Preferably, R^4 is independently oxo (=O) where the ring is at least partially saturated, halogen, CN, OR^5 or C_{1-6} alkyl, wherein C_{1-6} alkyl is optionally substituted with one or more halogen, which are the same or different.

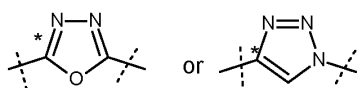
Preferably, R^4 is oxo, where the ring is at least partly saturated.

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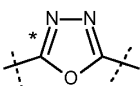
Preferably, A^1 is



More preferably, A^1 is



Even more preferably, A¹ is



5

In one embodiment A² is R^{6a}. In another embodiment A² is A^{2a}.

Preferably, R^{6a} is C₁₋₆ alkyl, optionally substituted with one or more halogen and/or one A^{2a} and/or one OR^{6a3}. More preferably, R^{6a} is C₁₋₆ alkyl, optionally substituted with one or more halogen and/or one OR^{6a3}.

Preferably, R^{6a} is OR^{6a1}.
R^{6a1} is preferably A^{2a} or C₁₋₆ alkyl, wherein C₁₋₆ alkyl is optionally substituted with one or more halogen and/or one A^{2a} and/or one OR^{6a3}. More preferably, R^{6a1} is C₁₋₆ alkyl, optionally substituted with one or more F and/or one OR^{6a3}.

Preferably, R^{6a2} is H.

20

Preferably, R^{6a} is OC₁₋₄ alkyl; OC₁₋₄ alkyl-OC₁₋₄ alkyl, wherein each C₁₋₄ alkyl is optionally substituted with one to three F; or OCH₂A^{2a}. Even more preferably, R^{6a} is O(CH₂)₃CF₃ or O(CH₂)₂OCF₃.

Preferably, A^{2a} is phenyl; C₃₋₇ cycloalkyl; or 3- to 7-membered heterocyclyl, wherein A^{2a} is optionally substituted with one or more R⁶, which are the same or different.

Preferably, A^{2a} is phenyl, or 5- to 6-membered aromatic heterocyclyl, wherein A^{2a} is optionally substituted with one or more R⁶, which are the same or different. A preferred 5- to 6-membered aromatic heterocyclyl is preferably pyridyl, pyrazinyl, pyridazinyl, pyrazolyl or 1,2,4-oxadiazolyl.

30

Preferably, A^{2a} is C₃₋₇ cycloalkyl, more preferably cyclobutyl, wherein A^{2a} is optionally substituted with one or more R⁶, which are the same or different.

5 Preferably, A^{2a} is azetidiny.

Preferably, A^{2a} is substituted with one or two R⁶, which are the same or different.

Preferably, R⁶ is independently F, Cl, CF₃, OCH₃, OCF₃, CH₃, CH₂CH₃, or cyclopropyl,
10 preferably F, Cl, CF₃, OCH₃, CH₃, CH₂CH₃, or cyclopropyl.

Even more preferably, A² is 4-chlorophenyl, 3-trifluoromethoxycyclobutyl, 3-trifluoromethoxy-azetidin-1-yl, 4,4,4-trifluorobutyl-1-oxy or 2-trifluoromethoxyethoxy.

15 Preferably, R² is H.

Preferably, R³ is A³.

Preferably, A³ is phenyl, pyridyl, pyrazinyl, pyrimidazol, cyclopropyl, cyclobutyl or
20 cyclohexyl, more preferably phenyl or pyridyl, and wherein A³ is optionally substituted with one or more R¹⁰, which are the same or different.

Preferably, A³ is substituted with one or two R¹⁰, which are the same or different.

25 Preferably, R² and R³ are joined together with the oxygen and carbon atom to which they are attached to form a dihydrobenzopyran ring, wherein the ring is optionally substituted with one or more R¹⁰, which are the same or different, preferably the ring is substituted with one or two R¹⁰.

30 Preferably, R¹⁰ is CHF₂.

Preferably, R¹⁰ is independently F, Cl, Br, CF₃, OCF₃, CH=O, CH₂OH or CH₃; preferably F, Cl, CF₃, CH=O, CH₂OH or CH₃. Even more preferably F or Cl.

Even more preferably, R³ is 3-chloro-4-difluoromethylphenyl or 3,4-dichlorophenyl.

Even more preferably, R³ is 4-chloro-3-fluorophenyl or 2-chloro-3-fluoropyridin-5-yl, even more preferably 4-chloro-3-fluorophenyl.

- 5 Compounds of the formula (I) in which some or all of the above-mentioned groups have the preferred or more preferred meanings are also an object of the present invention.

Preferred specific compounds of the present invention are selected from the group consisting of

10

2-(4-chloro-3-fluorophenoxy)-*N*-[*trans*-2-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-1,3-dioxan-5-yl]acetamide;

2-(4-chloro-3-fluorophenoxy)-*N*-[*trans*-2-{5-[*cis*-3-(trifluoromethoxy)cyclobutyl]-1,3,4-oxadiazol-2-yl}-1,3-dioxan-5-yl]acetamide;

15

2-(4-chloro-3-fluorophenoxy)-*N*-[*cis*-2-[1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl]-1,3-dioxan-5-yl]acetamide;

2-(4-chloro-3-fluorophenoxy)-*N*-[*trans*-2-[1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl]-1,3-dioxan-5-yl]acetamide;

2-(4-chloro-3-fluorophenoxy)-*N*-[*trans*-2-[5-(4,4,4-trifluorobutoxy)-1,3,4-oxadiazol-2-yl]-

20

1,3-dioxan-5-yl]acetamide;

2-(4-chloro-3-fluorophenoxy)-*N*-[*trans*-2-{5-[2-(trifluoromethoxy)ethoxy]-1,3,4-oxadiazol-2-yl}-1,3-dioxan-5-yl]acetamide;

2-(4-chloro-3-fluorophenoxy)-*N*-[*trans*-2-{5-[3-(trifluoromethoxy)azetidin-1-yl]-1,3,4-oxadiazol-2-yl}-1,3-dioxan-5-yl]acetamide;

25

2-[(6-chloro-5-fluoro-3-pyridyl)oxy]-*N*-[*trans*-2-[5-[*cis*-3-(trifluoromethoxy)cyclobutyl]-1,3,4-oxadiazol-2-yl]-1,3-dioxan-5-yl]acetamide;

2-[3-chloro-4-(difluoromethyl)phenoxy]-*N*-[*trans*-2-{5-[3-(trifluoromethoxy)azetidin-1-yl]-1,3,4-oxadiazol-2-yl}-1,3-dioxan-5-yl]acetamide; or

2-(3,4-dichlorophenoxy)-*N*-[*trans*-2-{5-[2-(trifluoromethoxy)ethoxy]-1,3,4-oxadiazol-2-yl}-

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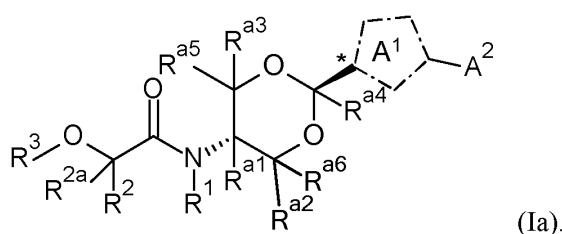
1,3-dioxan-5-yl]acetamide.

Where tautomerism, like e.g. keto-enol tautomerism, of compounds of formula (I) may occur, the individual forms, like e.g. the keto and enol form, are comprised separately and together as

mixtures in any ratio. Same applies to stereoisomers, like e.g. enantiomers, *cis/trans* isomers, conformers and the like.

Especially, when enantiomeric or diastereomeric forms are given in a compound according to
5 formula (I) each pure form separately and any mixture of at least two of the pure forms in any ratio is comprised by formula (I) and is a subject of the present invention.

A preferred compound is a compound or a pharmaceutically acceptable salt, solvate, hydrate, tautomer or stereoisomer thereof of formula (I) with a relative configuration as shown in
10 formula (Ia)



Isotopic labeled compounds of formula (I) are also within the scope of the present invention.
15 Methods for isotope labeling are known in the art. Preferred isotopes are those of the elements H, C, N, O and S. Solvates and hydrates of compounds of formula (I) are also within the scope of the present invention.

If desired, isomers can be separated by methods well known in the art, e.g. by liquid
20 chromatography. Same applies for enantiomers by using e.g. chiral stationary phases. Additionally, enantiomers may be isolated by converting them into diastereomers, i.e. coupling with an enantiomerically pure auxiliary compound, subsequent separation of the resulting diastereomers and cleavage of the auxiliary residue. Alternatively, any enantiomer of a compound of formula (I) may be obtained from stereoselective synthesis using optically pure
25 starting materials, reagents and/or catalysts.

In case the compounds according to formula (I) contain one or more acidic or basic groups, the invention also comprises their corresponding pharmaceutically or toxicologically acceptable salts, in particular their pharmaceutically utilizable salts. Thus, the compounds of the formula
30 (I) which contain acidic groups can be used according to the invention, for example, as alkali

metal salts, alkaline earth metal salts or as ammonium salts. More precise examples of such salts include sodium salts, potassium salts, calcium salts, magnesium salts or salts with ammonia or organic amines such as, for example, ethylamine, ethanolamine, triethanolamine or amino acids. Compounds of the formula (I) which contain one or more basic groups, i.e. groups which can be protonated, can be present and can be used according to the invention in the form of their addition salts with inorganic or organic acids. Examples for suitable acids include hydrogen chloride, hydrogen bromide, phosphoric acid, sulfuric acid, nitric acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acids, oxalic acid, acetic acid, tartaric acid, lactic acid, salicylic acid, benzoic acid, formic acid, propionic acid, pivalic acid, diethylacetic acid, malonic acid, succinic acid, pimelic acid, fumaric acid, maleic acid, malic acid, sulfaminic acid, phenylpropionic acid, gluconic acid, ascorbic acid, isonicotinic acid, citric acid, adipic acid, and other acids known to the person skilled in the art. If the compounds of the formula (I) simultaneously contain acidic and basic groups in the molecule, the invention also includes, in addition to the salt forms mentioned, inner salts or betaines (zwitterions). The respective salts according to the formula (I) can be obtained by customary methods which are known to the person skilled in the art like, for example by contacting these with an organic or inorganic acid or base in a solvent or dispersant, or by anion exchange or cation exchange with other salts. The present invention also includes all salts of the compounds of the formula (I) which, owing to low physiological compatibility, are not directly suitable for use in pharmaceuticals but which can be used, for example, as intermediates for chemical reactions or for the preparation of pharmaceutically acceptable salts.

As shown below compounds of the present invention are believed to be suitable for modulating the integrated stress response pathway.

The Integrated Stress Response (ISR) is a cellular stress response common to all eukaryotes (1). Dysregulation of ISR signaling has important pathological consequences linked inter alia to inflammation, viral infection, diabetes, cancer and neurodegenerative diseases.

ISR is a common denominator of different types of cellular stresses resulting in phosphorylation of the alpha subunit of eukaryotic translation initiation factor 2 (eIF2 α) on serine 51 leading to the suppression of normal protein synthesis and expression of stress response genes (2). In mammalian cells the phosphorylation is carried out by a family of four eIF2 α kinases, namely: PKR-like ER kinase (PERK), double-stranded RNA-dependent protein kinase (PKR),

heme-regulated eIF2alpha kinase (HRI), and general control non-derepressible 2 (GCN2), each responding to distinct environmental and physiological stresses (3).

5 eIF2alpha together with eIF2beta and eIF2gamma form the eIF2 complex, a key player of the initiation of normal mRNA translation (4). The eIF2 complex binds GTP and Met-tRNA_i forming a ternary complex (eIF2-GTP-Met-tRNA_i), which is recruited by ribosomes for translation initiation (5, 6).

10 eIF2B is a heterodecameric complex consisting of 5 subunits (alpha, beta, gamma, delta, epsilon) which in duplicate form a GEF-active decamer (7).

In response to ISR activation, phosphorylated eIF2alpha inhibits the eIF2B-mediated exchange of GDP for GTP, resulting in reduced ternary complex formation and hence in the inhibition of translation of normal mRNAs characterized by ribosomes binding to the 5' AUG start codon
15 (8). Under these conditions of reduced ternary complex abundance the translation of several specific mRNAs including the mRNA coding for the transcription factor ATF4 is activated via a mechanism involving altered translation of upstream ORFs (uORFs) (7, 9, 10). These mRNAs typically contain one or more uORFs that normally function in unstressed cells to limit the flow of ribosomes to the main coding ORF. For example, during normal conditions, uORFs in the 5'
20 UTR of ATF occupy the ribosomes and prevent translation of the coding sequence of ATF4. However, during stress conditions, i.e. under conditions of reduced ternary complex formation, the probability for ribosomes to scan past these upstream ORFs and initiate translation at the ATF4 coding ORF is increased. ATF4 and other stress response factors expressed in this way subsequently govern the expression of an array of further stress response genes. The acute phase
25 consists in expression of proteins that aim to restore homeostasis, while the chronic phase leads to expression of pro-apoptotic factors (1, 11, 12, 13).

Upregulation of markers of ISR signaling has been demonstrated in a variety of conditions, among these cancer and neurodegenerative diseases. In cancer, ER stress-regulated translation
30 increases tolerance to hypoxic conditions and promotes tumor growth (14, 15, 16), and deletion of PERK by gene targeting has been shown to slow growth of tumours derived from transformed PERK^{-/-} mouse embryonic fibroblasts (14, 17). Further, a recent report has provided proof of concept using patient derived xenograft modeling in mice for activators of eIF2B to be effective in treating a form of aggressive metastatic prostate cancer (28). Taken

together, prevention of cytoprotective ISR signaling may represent an effective anti-proliferation strategy for the treatment of at least some forms of cancer.

Further, modulation of ISR signaling could prove effective in preserving synaptic function and reducing neuronal decline, also in neurodegenerative diseases that are characterized by misfolded proteins and activation of the unfolded protein response (UPR), such as amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), Parkinson's disease (PD) and Jakob Creutzfeld (prion) diseases (18, 19, 20). With prion disease an example of a neurodegenerative disease exists where it has been shown that pharmacological as well as genetic inhibition of ISR signaling can normalize protein translation levels, rescue synaptic function and prevent neuronal loss (21). Specifically, reduction of levels of phosphorylated eIF2alpha by overexpression of the phosphatase controlling phosphorylated eIF2alpha levels increased survival of prion-infected mice whereas sustained eIF2alpha phosphorylation decreased survival (22).

Further, direct evidence for the importance of control of protein expression levels for proper brain function exists in the form of rare genetic diseases affecting functions of eIF2 and eIF2B. A mutation in eIF2gamma that disrupts complex integrity of eIF2 and hence results in reduced normal protein expression levels is linked to intellectual disability syndrome (ID) (23). Partial loss of function mutations in subunits of eIF2B have been shown to be causal for the rare leukodystrophy Vanishing White Matter Disease (VWMD) (24, 25). Specifically, stabilization of eIF2B partial loss of function in a VWMD mouse model by a small molecule related to ISRIB has been shown to reduce ISR markers and improve functional as well as pathological end points (26, 27).

The present invention provides compounds of the present invention in free or pharmaceutically acceptable salt form or in the form of solvates, hydrates, tautomers or stereoisomers to be used in the treatment of diseases or disorders mentioned herein.

Thus an aspect of the present invention is a compound or a pharmaceutically acceptable salt, solvate, hydrate, tautomer or stereoisomer thereof of the present invention for use as a medicament as mentioned above.

The therapeutic method described may be applied to mammals such as dogs, cats, cows, horses, rabbits, monkeys and humans. Preferably, the mammalian patient is a human patient.

Accordingly, the present invention provides a compound or a pharmaceutically acceptable salt,
5 solvate, hydrate, tautomer or stereoisomer thereof of the present invention or a pharmaceutical composition of the present invention to be used in the treatment or prevention of one or more diseases or disorders associated with integrated stress response.

A further aspect of the present invention is a compound or a pharmaceutically acceptable salt,
10 solvate, hydrate, tautomer or stereoisomer thereof of the present invention or a pharmaceutical composition of the present invention for use in a method of treating or preventing one or more disorders or diseases associated with integrated stress response.

A further aspect of the present invention is the use of a compound or a pharmaceutically
15 acceptable salt, solvate, hydrate, tautomer or stereoisomer thereof of the present invention or a pharmaceutical composition of the present invention for the manufacture of a medicament for the treatment or prophylaxis of one or more disorders or diseases associated with integrated stress response.

Yet another aspect of the present invention is a method for treating, controlling, delaying or
20 preventing in a mammalian patient in need of the treatment of one or more diseases or disorders associated with integrated stress response, wherein the method comprises administering to said patient a therapeutically effective amount of a compound or a pharmaceutically acceptable salt, solvate, hydrate, tautomer or stereoisomer thereof of the present invention or a pharmaceutical
25 composition of the present invention.

The present invention provides a compound or a pharmaceutically acceptable salt, solvate,
hydrate, tautomer or stereoisomer thereof of the present invention to be used in the treatment
or prevention of one or more diseases or disorders mentioned below.

30 A further aspect of the present invention is a compound or a pharmaceutically acceptable salt, solvate, hydrate, tautomer or stereoisomer thereof of the present invention or a pharmaceutical composition of the present invention for use in a method of treating or preventing one or more disorders or diseases mentioned below.

A further aspect of the present invention is the use of a compound or a pharmaceutically acceptable salt, solvate, hydrate, tautomer or stereoisomer thereof of the present invention or a pharmaceutical composition of the present invention for the manufacture of a medicament for the treatment or prophylaxis of one or more disorders or diseases mentioned below.

Yet another aspect of the present invention is a method for treating, controlling, delaying or preventing in a mammalian patient in need of the treatment of one or more diseases or disorders mentioned below, wherein the method comprises administering to said patient a therapeutically effective amount of a compound or a pharmaceutically acceptable salt, solvate, hydrate, tautomer or stereoisomer thereof of the present invention or a pharmaceutical composition of the present invention.

Diseases or disorders include but are not limited to leukodystrophies, intellectual disability syndrome, neurodegenerative diseases and disorders, neoplastic diseases, infectious diseases, inflammatory diseases, musculoskeletal diseases, metabolic diseases, ocular diseases as well as diseases selected from the group consisting of organ fibrosis, chronic and acute diseases of the liver, chronic and acute diseases of the lung, chronic and acute diseases of the kidney, myocardial infarction, cardiovascular disease, arrhythmias, atherosclerosis, spinal cord injury, ischemic stroke, and neuropathic pain.

Leukodystrophies

Examples of leukodystrophies include, but are not limited to, Vanishing White Matter Disease (VWMD) and childhood ataxia with CNS hypo-myelination (e.g. associated with impaired function of eIF2 or components in a signal transduction or signaling pathway including eIF2).

Intellectual disability syndrome

Intellectual disability in particular refers to a condition in which a person has certain limitations in intellectual functions like communicating, taking care of him- or herself, and/or has impaired social skills. Intellectual disability syndromes include, but are not limited to, intellectual disability conditions associated with impaired function of eIF2 or components in a signal transduction or signaling pathway including eIF2.

Neurodegenerative diseases / disorders

- Examples of neurodegenerative diseases and disorders include, but are not limited to, Alexander's disease, Alper's disease, Alzheimer's disease, Amyotrophic lateral sclerosis, Ataxia telangiectasia, Batten disease (also known as Spielmeyer-Vogt-Sjogren-Batten disease), Bovine spongiform encephalopathy (BSE), Canavan disease, Cockayne syndrome,
- 5 Corticobasal degeneration, Creutzfeldt-Jakob disease, frontotemporal dementia, Gerstmann-Straussler-Scheinker syndrome, Huntington's disease, HIV-associated dementia, Kennedy's disease, Krabbe's disease, Kuru, Lewy body dementia, Machado-Joseph disease (Spinocerebellar ataxia type 3), Multiple sclerosis, Multiple System Atrophy, Narcolepsy, Neuroborreliosis, Parkinson's disease, Pelizaeus-Merzbacher Disease, Pick's disease, Primary
- 10 lateral sclerosis, Prion diseases, Progressive supranuclear palsy, Refsum's disease, Sandhoffs disease, Schilder's disease, Subacute combined degeneration of spinal cord secondary to Pernicious Anaemia, Schizophrenia, Spinocerebellar ataxia (multiple types with varying characteristics), Spinal muscular atrophy, Steele-Richardson-Olszewski disease, Tabes dorsalis, and tauopathies.
- 15 In particular, the neurodegenerative disease or and disorder is selected from the group consisting of Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.

Neoplastic diseases

- A neoplastic disease may be understood in the broadest sense as any tissue resulting from miss-controlled cell growth. In many cases a neoplasm leads to at least bulky tissue mass optionally
- 20 innervated by blood vessels. It may or may not comprise the formation of one or more metastasis/metastases. A neoplastic disease of the present invention may be any neoplasm as classified by the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) classes C00-D48.
- 25 Exemplarily, a neoplastic disease according to the present invention may be the presence of one or more malignant neoplasm(s) (tumors) (ICD-10 classes C00-C97), may be the presence of one or more *in situ* neoplasm(s) (ICD-10 classes D00-D09), may be the presence of one or more benign neoplasm(s) (ICD-10 classes D10-D36), or may be the presence of one or more neoplasm(s) of uncertain or unknown behavior (ICD-10 classes D37-D48). Preferably, a
- 30 neoplastic disease according to the present invention refers to the presence of one or more malignant neoplasm(s), i.e., is malignant neoplasia (ICD-10 classes C00-C97).

In a more preferred embodiment, the neoplastic disease is cancer.

Cancer may be understood in the broadest sense as any malignant neoplastic disease, i.e., the presence of one or more malignant neoplasm(s) in the patient. Cancer may be solid or hematologic malignancy. Contemplated herein are without limitation leukemia, lymphoma, carcinomas and sarcomas.

- 5 In particular, neoplastic diseases, such as cancers, characterized by upregulated ISR markers are included herein.

Exemplary cancers include, but are not limited to, thyroid cancer, cancers of the endocrine system, pancreatic cancer, brain cancer (e.g. glioblastoma multiforme, glioma), breast cancer (e.g. ER positive, ER negative, chemotherapy resistant, herceptin resistant, HER2 positive,
10 doxorubicin resistant, tamoxifen resistant, ductal carcinoma, lobular carcinoma, primary, metastatic), cervix cancer, ovarian cancer, uterus cancer, colon cancer, head & neck cancer, liver cancer (e.g. hepatocellular carcinoma), kidney cancer, lung cancer (e.g. non-small cell lung carcinoma, squamous cell lung carcinoma, adenocarcinoma, large cell lung carcinoma, small cell lung carcinoma, carcinoid, sarcoma), colon cancer, esophageal cancer, stomach
15 cancer, bladder cancer, bone cancer, gastric cancer, prostate cancer and skin cancer (e.g. melanoma).

Further examples include, but are not limited to, myeloma, leukemia, mesothelioma, and sarcoma.

20

Additional examples include, but are not limited to, Medulloblastoma, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, glioma, glioblastoma multiforme, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer,
25 premalignant skin lesions, testicular cancer, lymphomas, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine or exocrine pancreas, medullary thyroid cancer, medullary thyroid carcinoma, melanoma, colorectal cancer, papillary thyroid cancer, hepatocellular carcinoma, Paget's Disease of the Nipple, Phyllodes Tumors, Lobular Carcinoma, Ductal Carcinoma, cancer of the pancreatic
30 stellate cells, and cancer of the hepatic stellate cells.

Exemplary leukemias include, but are not limited to, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute

promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemiac leukemia, basophylic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell
 5 leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocyte leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblasts leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia,
 10 plasma cell leukemia, multiple myeloma, plasmacytic leukemia, promyelocytic leukemia, Rieder cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, and undifferentiated cell leukemia.

Exemplary sarcomas include, but are not limited to, chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose
 15 sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma
 20 of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and telangiectatic sarcoma.

Exemplary melanomas include, but are not limited to, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey
 25 melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma, subungual melanoma, and superficial spreading melanoma.

Exemplary carcinomas include, but are not limited to, medullary thyroid carcinoma, familial medullary thyroid carcinoma, acinar carcinoma, acinous carcinoma, adenocystic carcinoma,
 30 adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic

carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, ductal carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniformi carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypernephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lobular carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhus carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tubular carcinoma, tuberous carcinoma, verrucous carcinoma, and carcinoma villosum.

Infectious diseases

Examples include, but are not limited to, infections caused by viruses (such as infections by HIV-1: human immunodeficiency virus type 1; IAV: influenza A virus; HCV: hepatitis C virus; DENV: dengue virus; ASFV: African swine fever virus; EBV: Epstein-Barr virus; HSV1: herpes simplex virus 1; CHIKV: chikungunya virus; HCMV: human cytomegalovirus; SARS-CoV: severe acute respiratory syndrome coronavirus; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2) and infections caused by bacteria (such as infections by *Legionella*, *Brucella*, *Simkania*, *Chlamydia*, *Helicobacter* and *Campylobacter*).

Inflammatory diseases

Examples of inflammatory diseases include, but are not limited to, postoperative cognitive dysfunction (decline in cognitive function after surgery), traumatic brain injury, arthritis, rheumatoid arthritis, psoriatic arthritis, juvenile idiopathic arthritis, multiple sclerosis, systemic lupus erythematosus (SLE), myasthenia gravis, juvenile onset diabetes, diabetes mellitus type 1, Guillain-Barre syndrome, Hashimoto's encephalitis, Hashimoto's thyroiditis, ankylosing spondylitis, psoriasis, Sjogren's syndrome, vasculitis, glomerulonephritis, auto-immune thyroiditis, Behcet's disease, Crohn's disease, ulcerative colitis, bullous pemphigoid, sarcoidosis, ichthyosis, Graves ophthalmopathy, inflammatory bowel disease, Addison's disease, Vitiligo, asthma, allergic asthma, acne vulgaris, celiac disease, chronic prostatitis, inflammatory bowel disease, pelvic inflammatory disease, reperfusion injury, sarcoidosis, transplant rejection, interstitial cystitis, atherosclerosis, and atopic dermatitis.

Musculoskeletal diseases

Examples of musculoskeletal diseases include, but are not limited to, muscular dystrophy, multiple sclerosis, Freidrich's ataxia, a muscle wasting disorder (e.g., muscle atrophy, sarcopenia, cachexia), inclusion body myopathy, progressive muscular atrophy, motor neuron disease, carpal tunnel syndrome, epicondylitis, tendinitis, back pain, muscle pain, muscle soreness, repetitive strain disorders, and paralysis.

Metabolic diseases

Examples of metabolic diseases include, but are not limited to, diabetes (in particular diabetes Type II), non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), Niemann-Pick disease, liver fibrosis, obesity, heart disease, atherosclerosis, arthritis, cystinosis, phenylketonuria, proliferative retinopathy, and Kearns-Sayre disease.

Ocular diseases

Examples of ocular diseases include, but are not limited to, edema or neovascularization for any occlusive or inflammatory retinal vascular disease, such as rubeosis irides, neovascular glaucoma, pterygium, vascularized glaucoma filtering blebs, conjunctival papilloma; choroidal neovascularization, such as neovascular age-related macular degeneration (AMD), myopia, prior uveitis, trauma, or idiopathic; macular edema, such as post surgical macular edema, macular edema secondary to uveitis including retinal and/or choroidal inflammation, macular edema secondary to diabetes, and macular edema secondary to retinovascular occlusive disease (i.e. branch and central retinal vein occlusion); retinal neovascularization due to diabetes, such

as retinal vein occlusion, uveitis, ocular ischemic syndrome from carotid artery disease, ophthalmic or retinal artery occlusion, sickle cell retinopathy, other ischemic or occlusive neovascular retinopathies, retinopathy of prematurity, or Eale's Disease; and genetic disorders, such as VonHippel-Lindau syndrome.

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Further diseases

Further diseases include, but are not limited to, organ fibrosis (such as liver fibrosis, lung fibrosis, or kidney fibrosis), chronic and acute diseases of the liver (such as fatty liver disease, or liver steatosis), chronic and acute diseases of the lung, chronic and acute diseases of the kidney, myocardial infarction, cardiovascular disease, arrhythmias, atherosclerosis, spinal cord injury, ischemic stroke, and neuropathic pain.

Yet another aspect of the present invention is a pharmaceutical composition comprising at least one compound or a pharmaceutically acceptable salt, solvate, hydrate, tautomer or stereoisomer thereof of the present invention together with a pharmaceutically acceptable carrier, optionally in combination with one or more other bioactive compounds or pharmaceutical compositions.

Preferably, the one or more bioactive compounds are modulators of the integrated stress response pathway other than compounds of formula (I).

"Pharmaceutical composition" means one or more active ingredients, and one or more inert ingredients that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing a compound of the present invention and a pharmaceutically acceptable carrier.

A pharmaceutical composition of the present invention may comprise one or more additional compounds as active ingredients like a mixture of compounds of formula (I) in the composition or other modulators of the integrated stress response pathway.

The active ingredients may be comprised in one or more different pharmaceutical compositions (combination of pharmaceutical compositions).

The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids, including inorganic bases or acids and organic bases or acids.

5

The compositions include compositions suitable for oral, rectal, topical, parenteral (including subcutaneous, intramuscular, and intravenous), ocular (ophthalmic), pulmonary (nasal or buccal inhalation), or nasal administration, although the most suitable route in any given case will depend on the nature and severity of the conditions being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well-known in the art of pharmacy.

In practical use, the compounds of formula (I) can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, *e.g.*, oral or parenteral (including intravenous). In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as powders, hard and soft capsules and tablets, with the solid oral preparations being preferred over the liquid preparations.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques. Such compositions and preparations should contain at least 0.1 percent of active compound. The percentage of active compound in these compositions may, of course, be varied and may conveniently be between about 2 percent to about 60 percent of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that an effective dosage will be obtained. The active compounds can also be administered intranasally, for example, as liquid drops or spray.

The tablets, pills, capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin. When a dosage unit form is a capsule,
5 it may contain, in addition to materials of the above type, a liquid carrier such as a fatty oil.

Various other materials may be present as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain, in addition to the active ingredient, sucrose as a sweetening agent, methyl and
10 propylparabens as preservatives, a dye and a flavoring such as cherry or orange flavor.

Compounds of formula (I) may also be administered parenterally. Solutions or suspensions of these active compounds can be prepared in water suitably mixed with a surfactant such as hydroxypropyl-cellulose. Dispersions can also be prepared in glycerol, liquid polyethylene
15 glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable
20 solutions or dispersions. In all cases, the form should be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol and liquid polyethylene glycol), suitable
25 mixtures thereof, and vegetable oils.

Any suitable route of administration may be employed for providing a mammal, especially a human, with an effective dose of a compound of the present invention. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, and the like may be employed. Dosage forms
30 include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, and the like. Preferably compounds of formula (I) are administered orally.

The effective dosage of active ingredient employed may vary depending on the particular compound employed, the mode of administration, the condition being treated and the severity

of the condition being treated. Such dosage may be ascertained readily by a person skilled in the art.

Starting materials for the synthesis of preferred embodiments of the invention may be purchased
 5 from commercially available sources such as Array, Sigma Aldrich, Acros, Fisher, Fluka, ABCR or can be synthesized using known methods by one skilled in the art.

In general, several methods are applicable to prepare compounds of the present invention. In some cases various strategies can be combined. Sequential or convergent routes may be used.
 10 Exemplary synthetic routes are described below.

Examples

I Chemical Synthesis

15

Experimental procedures:

The following Abbreviations and Acronyms are used:

	aq	aqueous
20	ACN	acetonitrile
	BF ₃ ·OEt ₂	boron trifluoride diethyl etherate
	Brine	saturated solution of NaCl in water
	Burgess reagent	1-methoxy- <i>N</i> -triethylammoniosulfonyl-methanimidate
	CDI	di-1 <i>H</i> -imidazol-1-ylmethanone
25	CSA	Camphorsulfonic acid
	CV	column volume
	δ	chemical shifts in parts per million
	DAST	<i>N,N</i> -diethylaminosulfur trifluoride
	DCM	dichloromethane
30	DMSO	dimethylsulfoxide
	DMSO- <i>d</i> ₆	deuterated dimethylsulfoxide
	DIPEA	diisopropylethylamine
	DMF	dimethyl formamide
	EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride salt
35	ELS	evaporative light scattering
	ESI+	positive ionisation mode
	ESI-	negative ionisation mode

	EtOAc	ethyl acetate
	EtOH	ethanol
	FCC	flash column chromatography
	g	gram(s)
5	HATU	1-[<i>bis</i> (dimethylamino)methylidene]-1 <i>H</i> -[1,2,3]triazolo[4,5- <i>b</i>]pyridin-1-ium 3-oxide hexafluorophosphate
	HCl	hydrochloric acid
	HOAt	1-hydroxy-7-azabenzotriazole
	HPLC	high-performance liquid chromatography
10	h	hour(s)
	H ₂	hydrogen atmosphere
	<i>J</i>	NMR coupling constant
	LC	liquid chromatography
	LCMS	liquid chromatography mass spectrometry
15	MgSO ₄	magnesium sulphate
	M	molar
	mg	milligram(s)
	MHz	megahertz(s)
	mL	millilitre (s)
20	min	minute(s)
	mm	millimetre(s)
	mM	millimolar
	mol	mole(s)
	nm	nanometre(s)
25	MW	microwave
	MTBE	methyl <i>tert</i> -butyl ether
	<i>M/Z</i>	mass to charge ratio
	N ₂	nitrogen atmosphere
	Na ₂ SO ₄	sodium sulfate
30	NaBH ₄	sodium borahydride
	NaClO ₂	sodium chlorite
	NaHCO ₃	sodium bicarbonate
	NH ₄ Cl	ammonium chloride
	NaH ₂ PO ₄	sodium dihydrogen phosphate
35	NMM	4-methylmorpholine
	NMR	nuclear magnetic resonance
	NaOCl	sodium hypochlorite
	PDA	photometric diode array
	Pd/C	Palladium on carbon

	prep.	preparative
	PTSA	<i>para</i> -toluenesulfonic acid monohydrate
	r.t.	room temperature
	RT	retention time
5	satd	saturated
	sec	second(s)
	TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxidanyl
	THF	Tetrahydrofuran
	Tol	Toluene
10	TsCl	4-toluenesulfonyl chloride
	uPLC	ultra performance liquid chromatography
	UV	ultraviolet
	μL	microlitre(s)

15 Analytical LCMS conditions are as follows:

System 1 (S1): ACIDIC IPC METHOD (MS18 and MS19)

Analytical (MET/CR/1410) HPLC-MS were performed on a Shimadzu LCMS systems using a Kinetex Core shell C18 column (2.1 mm × 50 mm, 5 μm; temperature: 40 °C) and a gradient of 5-100% B (A= 0.1% formic acid in H₂O; B= 0.1% formic acid in ACN) over 1.2 min then 100% B for 0.1 min. A second gradient of 100-5% B was then applied over 0.01 min with an injection volume of 3 μL at a flow rate of 1.2 mL/min. UV spectra were recorded at 215 nm using a SPD-M20A photo diode array detector spectrum range: 200-400 nm. Mass spectra were obtained using a 2010EV detector. Data were integrated and reported using Shimadzu LCMS-Solutions and PsiPort software.

System 2 (S2): ACIDIC IPC METHOD (MSQ2 and MSQ4):

Analytical (MET/uPLC/1704) uHPLC-MS were performed on a Waters Acquity uPLC system using a Waters UPLC® BEHTM C18 column (2.1 mm × 50 mm, 1.7 μm; temperature 40 °C) and a gradient of 5-100% B (A= 0.1% formic acid in H₂O; B= 0.1% formic acid in ACN) over 1.1 min then 100% B for 0.25 min. A second gradient of 100-5% B was then applied over 0.05 min and held for 0.1 min with an injection volume of 1 μL at a flow rate of 0.9 mL/min. UV spectra were recorded at 215 nm on a Waters Acquity PDA with a spectrum range of 200-400 nm. Mass spectra were obtained using a Waters QDa. Data were integrated and reported using Waters MassLynx and OpenLynx software.

System 3 (S3): BASIC IPC METHOD (MS16):

Analytical (MET/CR/1602) uHPLC-MS were performed on a Waters Acquity uPLC system using Waters UPLC® BEH™ C18 column (2.1 mm × 30 mm, 1.7 µm; temperature 40 °C) and a gradient of 5-100% B (A: 2 mM ammonium bicarbonate, buffered to pH 10, B: ACN) over 0.75 min, then 100% B for 0.1 min. A second gradient of 100-5% B was then applied over 0.05 min and held for 0.1 min with an injection volume of 1 µL at a flow rate of 1 mL/min. UV spectra were recorded at 215 nm on a Waters Acquity PDA with a spectrum range of 200-400 nm. Mass spectra were obtained using a Waters Quattro Premier XE. Data were integrated and reported using Waters MassLynx and OpenLynx software.

10

System 4 (S4): ACIDIC FINAL METHOD (MSQ1 and MSQ2):

Analytical (MET/uPLC/AB101) uHPLC-MS were performed on a Waters Acquity uPLC system using a Phenomenex Kinetex-XB C18 column (2.1 mm × 100 mm, 1.7 µm; temperature: 40 °C) and a gradient of 5-100% B (A = 0.1% formic acid in H₂O; B = 0.1% formic acid in ACN) over 5.3 min then 100% B for 0.5 min. A second gradient of 100-5% B was then applied over 0.02 min and held for 1.18 min with an injection volume of 1 µL at flow rate of 0.6 mL/min. UV spectra were recorded at 215 nm using a Waters Acquity PDA detector spectrum range: 200-400 nm. Mass spectra were obtained using a Waters SQD (MSQ1) or Waters Acquity QDA (MSQ2). Data were integrated and reported using Waters MassLynx and OpenLynx software.

20

System 5 (S5): ACIDIC FINAL METHOD (MS18, MS19)

Analytical (MET/CR/1416) HPLC-MS were performed on Shimadzu LCMS systems using a Waters Atlantis dC18 column (2.1 mm × 100 mm, 3 µm; temperature: 40 °C) and a gradient of 5-100% B (A = 0.1% formic acid in H₂O; B = 0.1% formic acid in ACN) over 5 min then 100% B for 0.4 min. A second gradient of 100-5% B was then applied over 0.02 min and held for 1.58 min with an injection volume of 3 µL at flow rate of 0.6 mL/min. UV spectra were recorded at 215 nm using a SPD-M20A photo diode array detector spectrum range: 200-400 nm. Mass spectra were obtained using a 2010EV detector. Data were integrated and reported using Shimadzu LCMS-Solutions and PsiPort software.

30

System 6 (S6): BASIC FINAL METHOD (MS16)

Analytical (MET/uHPLC/AB105) uPLC-MS were performed on a Waters Acquity uPLC system using a Waters UPLC® BEH™ C18 column (2.1 mm × 100 mm, 1.7 µm column;

temperature: 40 °C) and a gradient of 5-100% (A= 2 mM ammonium bicarbonate, buffered to pH 10; B = ACN) over 5.3 min then 100% B for 0.5 min. A second gradient of 100-5% B was then applied over 0.02 min and held for 1.18 min with an injection volume of 1 µL and at flow rate of 0.6 mL/min. UV spectra were recorded at 215 nm using a Waters Acquity photo diode array detector Spectrum range: 200-400 nm. Mass spectra were obtained using a Waters Quattro Premier XE mass detector. Data were integrated and reported using Waters MassLynx and OpenLynx software.

System 7 (S7): BASIC FINAL METHOD (MS10)

Analytical (MET/CR/1603) (M6) HPLC-MS were performed on a Agilent G1312A system using a Phenomenex Gemini NX C18 column (2.0 mm × 100 mm, 3 µm; temperature: 40 °C) and a gradient of 5-100% B (A= 2 mM ammonium bicarbonate, buffered to pH 10; B = ACN) over 5.5 min then 100% B for 0.4 min. A second gradient of 100-5% B was then applied over 0.02 min and held for 1.08 min with an injection volume of 3 µL and at flow rate of 0.6 mL/min. UV spectra were recorded at 215 nm using a Waters 2996 photo diode array detector spectrum range: 200-400 nm. ELS data was collected using a Water 2420 detector when reported. Mass spectra were obtained using a Waters ZQ mass detector. Data were integrated and reported using Waters MassLynx and OpenLynx software.

Purification methods are as follows:

Method 1: ACIDIC EARLY METHOD

Purifications (P1) LC were performed on a Gilson LC system using a Waters Sunfire C18 column (30 mm × 100 mm, 10 µm; temperature: r.t.) and a gradient of 10-95% B (A= 0.1% formic acid in H₂O; B= 0.1% formic acid in ACN) over 14.44 min then 95% B for 2.11 min. A second gradient of 95-10% B was then applied over 0.2 min with an injection volume of 1500 µL at flow rate of 40 mL/min. UV spectra were recorded at 215 nm using a Gilson detector.

Method 2: ACIDIC STANDARD METHOD

Purifications (P2) LC were performed on a Gilson LC system using a Waters Sunfire C18 column (30 mm × 10 mm, 10 µm; temperature: r.t.) and a gradient of 30-95% B (A= 0.1% formic acid in water; B= 0.1% formic acid in ACN) over 11.00 min then 95% B for 2.10 min. A second gradient of 95-30% B was then applied over 0.2 min with an injection volume of 1500 µL at flow rate of 40 mL/min. UV spectra were recorded at 215 nm using a Gilson detector.

Method 3: BASIC EARLY METHOD

Purifications (P3) LC were performed on a Gilson LC system using a Waters X-Bridge C18 column (30 mm × 100 mm, 10 μM; temperature: r.t.) and a gradient of 10-95% B (A= 0.2% ammonium hydroxide in H₂O; B= 0.2% ammonium hydroxide in ACN) over 14.44 min then 95% B for 2.11 min. A second gradient of 95-10% B was then applied over 0.2 min with an injection volume of 1500 μL at flow rate of 40 mL/min. UV spectra were recorded at 215 nm using a Gilson detector.

Method 4: BASIC STANDARD METHOD

Purifications (P4) LC were performed on a Gilson LC system using a Waters X-Bridge C18 column (30 mm × 10 mm, 10 μM; temperature: r.t.) and a gradient of 30-95% B (A= 0.2% ammonium hydroxide in water; B= 0.2% ammonium hydroxide in ACN) over 11.00 min then 95% B for 2.10 min. A second gradient of 95-30% B was then applied over 0.21 min with an injection volume of 1500 μL at flow rate of 40 mL/min. UV spectra were recorded at 215 nm using a Gilson detector.

Method 5: Reverse phase chromatography using acidic pH, standard elution method

Purifications by FCC on reverse phase silica (acidic pH, standard elution method) were performed on Biotage Isolera systems using the appropriate SNAP C18 cartridge and a gradient of 10% B (A= 0.1% formic acid in H₂O; B= 0.1% formic acid in ACN) over 1.7 CV then 10-100% B over 19.5 CV and 100% B for 2 CV.

Method 6: Reverse phase chromatography using neutral pH, standard elution method

Purifications by FCC on reverse phase silica (neutral pH, standard elution method) were performed on Biotage Isolera systems using the appropriate SNAP C18 cartridge and a gradient of 10% B (A= H₂O; B= ACN) over 1.7 CV then 10-100% B over 19.5 CV and 100% B for 2 CV.

NMR Conditions

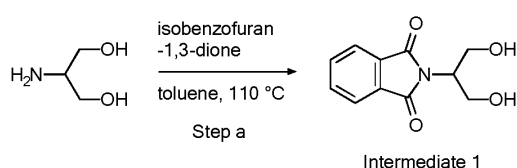
Unless otherwise stated, ¹H NMR spectra were recorded at 500 MHz, 400 MHz or 250 MHz on either a Bruker Avance III HD 500 MHz, Bruker Avance III HD 400 MHz spectrometer or Bruker Avance III HD 250 MHz spectrometer respectively. Chemical shifts, δ, are quoted in parts per million (ppm) and are referenced to the residual solvent peak. The following

abbreviations are used to denote the multiplicities and general assignments: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), ddd (doublet of doublet of doublets), dt (doublet of triplets), dq (doublet of quartets), hep (heptet), m (multiplet), pent (pentet), td (triplet of doublets), qd (quartet of doublets), app. (apparent) and br. (broad). Coupling constants, J , are quoted to the nearest 0.1 Hz.

General synthesis

All the compounds have been synthesised with a purity > 95% unless otherwise specified.

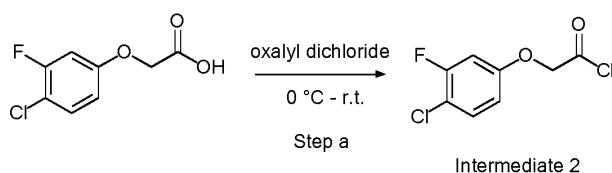
10 Scheme for route 1



Intermediate 1 (step 1.a): 2-[2-hydroxy-1-(hydroxymethyl)ethyl]isoindoline-1,3-dione

A solution of isobenzofuran-1,3-dione (854 mg, 5.8 mmol) and 2-aminopropane-1,3-diol (521 mg, 5.7 mmol) in toluene (20 mL) was heated at 110 °C for 24 h. The reaction mixture was cooled to 62 °C and treated with MTBE (20 mL), resulting in precipitation of a white powder. The suspension was stirred for 1 h, before being filtered hot to collect the precipitate. The precipitate was washed with warm MTBE (20 mL) and dried *in vacuo* to afford the title compound (786 mg, 3.45 mmol, 60% yield) as a white powder; ^1H NMR (500 MHz, DMSO- d_6) δ 7.87 – 7.81 (m, 4H), 4.89 – 4.84 (m, 2H), 4.27 – 4.20 (m, 1H), 3.83 – 3.76 (m, 2H), 3.69 – 3.62 (m, 2H); M/Z : 222 $[\text{M}+\text{H}]^+$, ESI^+ , $\text{RT} = 0.73$ (S1).

Scheme for route 2



Intermediate 2 (step 2.a): 2-(4-chloro-3-fluorophenoxy)acetyl chloride

To a solution of 2-(4-chloro-3-fluorophenoxy)acetic acid (5.16 g, 22.7 mmol) in DCM (45 mL) at 0 °C was added oxalyl dichloride (10 mL, 0.12 mol) followed by DMF (81 μL , 1.11 mmol) and the mixture was stirred at r.t. for 17 h. The reaction mixture was concentrated *in vacuo* to

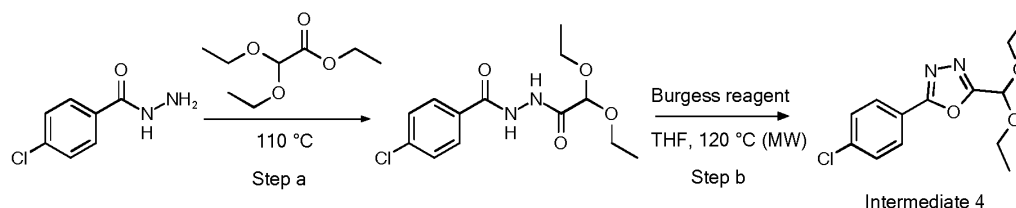
afford the title compound (90% purity, 5.30 g, 21.4 mmol, 94% yield) as an orange oil; ^1H NMR (400 MHz, chloroform-*d*) δ 7.31 (t, J = 8.6 Hz, 1H), 6.75 (dt, J = 10.2, 2.9 Hz, 1H), 6.66 (ddd, J = 8.9, 2.9, 1.2 Hz, 1H), 4.96 (s, 2H).

The intermediate in **Table 1** was synthesised according to the general route 2 as exemplified by **Intermediate 2** using the corresponding starting material.

Table 1

Intermediate	Structure	Name	Starting material	LCMS data	NMR data
3		2-(3,4-dichlorophenoxy)acetyl chloride	2-(3,4-dichlorophenoxy)acetic acid	-	^1H NMR (500 MHz, DMSO- <i>d</i> ₆) δ 7.52 (d, J = 8.9 Hz, 1H), 7.23 (d, J = 2.9 Hz, 1H), 6.95 (dd, J = 8.9, 3.0 Hz, 1H), 4.75 (s, 2H).

Scheme for route 3



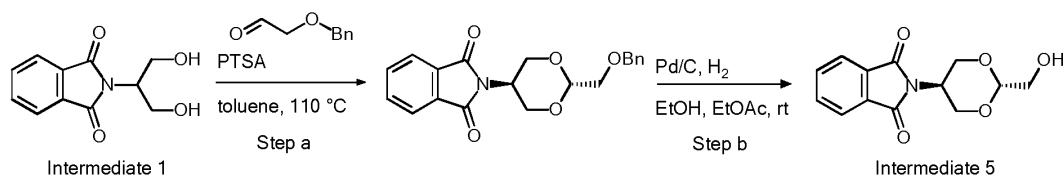
10 Step 3.a: 4-chloro-*N*'-(2,2-diethoxyacetyl)benzohydrazide

A suspension of 4-chlorobenzohydrazide (250 mg, 1.47 mmol) in ethyl 2,2-diethoxyacetate (2.7 mL, 14.7 mmol) was stirred at 110 °C for 16 h. The reaction mixture was cooled to r.t., diluted with MeOH, and the precipitate was removed by filtration. The filtrate was concentrated *in vacuo* and purified by chromatography on silica gel (0-100% EtOAc in heptane) to afford the title compound (80% purity, 135 mg, 0.36 mmol, 25% yield) as a pale yellow oil; ^1H NMR (400 MHz, chloroform-*d*) δ 9.24 (d, J = 5.3 Hz, 1H), 8.93 (d, J = 4.8 Hz, 1H), 7.81 – 7.76 (m, 2H), 7.48 – 7.41 (m, 2H) 3.82 – 3.64 (m, 4H), 1.32 – 1.24 (m, 6H); M/Z : 299, 301, $[\text{M}-\text{H}]^-$; ESI⁻, RT = 0.98 (S1).

Intermediate 4 (step 3.b): 2-(4-chlorophenyl)-5-(diethoxymethyl)-1,3,4-oxadiazole

A suspension of 4-chloro-*N*-(2,2-diethoxyacetyl)benzohydrazide (135 mg, 0.45 mmol) and methoxycarbonyl-(triethylammonio)sulfonylazanide (0.43 g, 1.80 mmol) in anhydrous THF (4.4 mL) was stirred at 120 °C for 10 min under microwave irradiation. The reaction mixture was partitioned between EtOAc and satd aq NaHCO₃ solution, and the organic layer was isolated, washed with brine, dried over MgSO₄, and concentrated *in vacuo*. The residue was purified by chromatography on silica gel (0-100% EtOAc in heptane) to afford the title compound (92% purity, 68 mg, 0.22 mmol, 49% yield) as an off-white solid; ¹H NMR (400 MHz, chloroform-*d*) δ 8.10 – 8.03 (m, 2H), 7.54 – 7.49 (m, 2H), 5.79 (s, 1H), 3.90 – 3.70 (m, 4H), 1.32 (t, *J* = 7.1, 6H); *M/Z*: 283, 285 [M+H]⁺, ESI⁺, RT = 1.21 (S1).

10 Scheme for route 4



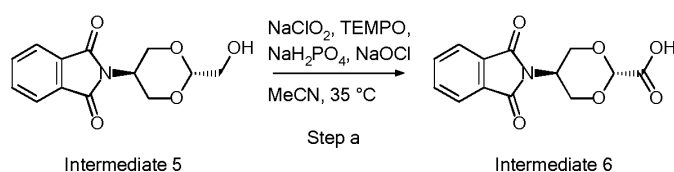
Step 4.a: 2-[*trans*-2-[(benzyloxy)methyl]-1,3-dioxan-5-yl]-2,3-dihydro-1*H*-isoindole-1,3-dione

A solution of benzyloxyacetaldehyde (0.25 mL, 1.77 mmol), PTSA (28 mg, 0.15 mmol) and 2-[2-hydroxy-1-(hydroxymethyl)ethyl]isoindoline-1,3-dione (336 mg, 1.47 mmol, Intermediate 1) in toluene (30 mL) was heated at reflux under Dean-Stark conditions for 18 h. The reaction mixture was cooled to r.t. and washed sequentially with satd aq NaHCO₃ solution (2 × 10 mL) and brine (10 mL). The organic layer was dried over Na₂SO₄, concentrated *in vacuo*, and purified by chromatography on silica gel (0-35% EtOAc in heptane) to afford the title compound (303 mg, 0.82 mmol, 55% yield) as a colourless oil; ¹H NMR (400 MHz, chloroform-*d*) δ 7.90 – 7.82 (m, 2H), 7.79 – 7.71 (m, 2H), 7.41 – 7.34 (m, 4H), 7.34 – 7.29 (m, 1H), 4.91 (t, *J* = 4.5 Hz, 1H), 4.74 – 4.66 (m, 1H), 4.64 (s, 2H), 4.52 – 4.43 (m, 2H), 4.09 (dd, *J* = 10.8, 4.9 Hz, 2H), 3.60 (d, *J* = 4.5 Hz, 2H).

25 Intermediate 5 (step 4.b): 2-[*trans*-2-(hydroxymethyl)-1,3-dioxan-5-yl]-2,3-dihydro-1*H*-isoindole-1,3-dione

A suspension of 2-[*trans*-2-[(benzyloxy)methyl]-1,3-dioxan-5-yl]-2,3-dihydro-1*H*-isoindole-1,3-dione (393 mg, 1.06 mmol) and Pd/C (10%, 112 mg, 0.11 mmol) in EtOH (10 mL) and EtOAc (6 mL) was stirred under H₂ for 5 h. The reaction mixture was purged with N₂, warmed to near reflux, and then filtered through a pad of Celite. The filtrate was concentrated *in vacuo* to afford the title compound (94% purity, 260 mg, 0.93 mmol, 88% yield) as a white solid; ¹H NMR (400 MHz, chloroform-*d*) δ 7.90 – 7.80 (m, 2H), 7.79 – 7.66 (m, 2H), 4.78 (t, *J* = 4.3 Hz, 1H), 4.69 – 4.58 (m, 1H), 4.47 (dd, *J* = 10.8 Hz, 2H), 4.07 (dd, *J* = 10.7, 4.8 Hz, 2H), 3.68 (d, *J* = 4.2 Hz, 2H), 1.92 (s, 1H).

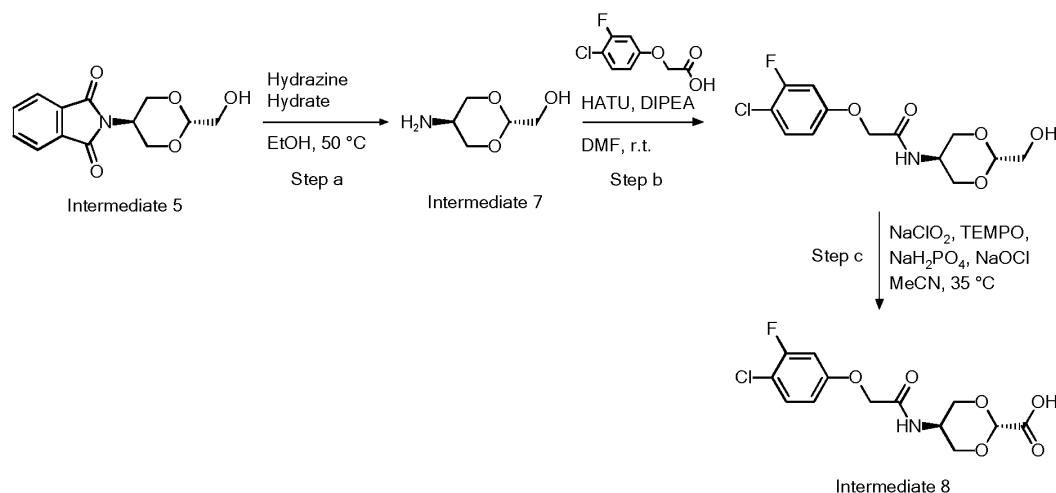
Scheme for route 5



Intermediate 6 (step 5.a): *trans*-5-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)-1,3-dioxane-2-carboxylic acid

TEMPO (0.13 g, 0.80 mmol) was added to a solution of 2-[*trans*-2-(hydroxymethyl)-1,3-dioxan-5-yl]-2,3-dihydro-1*H*-isoindole-1,3-dione (89% purity, 2.37 g, 8.01 mmol, Intermediate 5) in ACN (66 mL) and 0.67 M aq NaH₂PO₄ solution (66 mL). The reaction mixture was warmed to 35 °C. A solution of NaClO₂ (80%, 1.83 g, 16.0 mmol) in H₂O (15 mL) was added, followed by NaOCl (5.0%, 0.5 mL, 0.41 mmol). The reaction mixture was stirred at 35 °C for 20 h. Additional TEMPO (0.13 g, 0.80 mmol), NaClO₂ (80%, 1.83 g, 16.0 mmol) and NaOCl (5.0%, 0.5 mL, 0.41 mmol) was added and the reaction mixture was stirred at 35 °C for 24 h. Additional TEMPO (0.13 g, 0.80 mmol), NaClO₂ (80%, 1.83 g, 16.0 mmol) and NaOCl (5.0%, 0.5 mL, 0.41 mmol) was added and the mixture was stirred at 35 °C for 24 h. The reaction mixture was concentrated *in vacuo*. The aqueous residue was basified to pH 9 using satd aq NaHCO₃ solution, and washed with EtOAc (2 × 20 mL). The aqueous layer was cooled to 0 °C and acidified to pH 2 by the slow addition of 1 M aq HCl solution. The aqueous layer was re-extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with H₂O (50 mL), dried over Na₂SO₄ and concentrated *in vacuo* to afford the title compound (2.10 g, 7.50 mmol, 94% yield) as an off-white powder; ¹H NMR (400 MHz, methanol-*d*) δ 7.90 – 7.86 (m, 2H), 7.85 – 7.81 (m, 2H), 5.11 (s, 1H), 4.63 – 4.56 (m, 1H), 4.56 – 4.49 (m, 2H), 4.20 – 4.14 (m, 2H).

Scheme for route 6

**Intermediate 7 (step 6.a): [trans-5-amino-1,3-dioxan-2-yl]methanol**

A suspension of 2-[trans-2-(hydroxymethyl)-1,3-dioxan-5-yl]-2,3-dihydro-1*H*-isoin-1,3-dione (93% purity, 904 mg, 3.19 mmol, Intermediate 5) and hydrazine hydrate (0.6 mL, 12.8 mmol) in EtOH (49 mL) was stirred at 50 °C for 2 h. The reaction mixture was cooled to r.t. and concentrated *in vacuo*. The residue was suspended in MeOH (20 mL), resulting in precipitation of a white solid. The solid was filtered under vacuum and the filtrate was concentrated *in vacuo*. The residue was dissolved in MeOH and loaded onto an SCX cartridge (2 g). The product was washed with MeOH, then eluted with 7 M NH₃ in MeOH. The elution was collected and concentrated *in vacuo* to afford the title compound (299 mg, 2.25 mmol, 70% yield) as a viscous, yellow oil; ¹H NMR (500 MHz, DMSO-*d*₆) δ 4.76 (s, 1H), 4.34 (t, *J* = 4.7 Hz, 1H), 3.98 – 3.86 (m, 2H), 3.41 – 3.19 (m, 2H), 3.19 – 3.07 (m, 2H), 2.75 (tt, *J* = 10.2, 5.0 Hz, 1H).

Step 6.b: 2-(4-chloro-3-fluorophenoxy)-*N*-[trans-2-(hydroxymethyl)-1,3-dioxan-5-yl]acetamide

A solution of 2-(4-chloro-3-fluorophenoxy)acetic acid (459 mg, 2.3 mmol) and DIPEA (1.2 mL, 6.7 mmol) in anhydrous DMF (10 mL) was treated with HATU (807 mg, 2.1 mmol). The reaction mixture was stirred at r.t. for 10 min. [trans-5-Amino-1,3-dioxan-2-yl]methanol (299 mg, 2.25 mmol, Intermediate 7) was added and the reaction mixture was stirred at r.t. for 19 h. The reaction mixture was diluted with H₂O (30 mL) and then extracted with EtOAc (30 mL). The combined organic extracts were washed with brine (30 mL), dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. Purification by chromatography on silica gel (0-100% EtOAc in

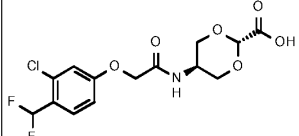
heptane) afforded the title compound (393 mg, 1.20 mmol, 54% yield) as an off-white solid; ^1H NMR (400 MHz, chloroform- d) δ 7.35 (t, J = 8.6 Hz, 1H), 6.76 (dd, J = 10.2, 2.9 Hz, 1H), 6.71 – 6.63 (m, 1H), 6.12 (d, J = 8.0 Hz, 1H), 4.61 (t, J = 4.4 Hz, 1H), 4.45 (s, 2H), 4.42 – 4.31 (m, 1H), 4.28 (dd, J = 11.2, 4.8 Hz, 2H), 3.67 (dd, J = 6.6, 4.4 Hz, 2H), 3.51 (t, J = 10.7 Hz, 2H), 2.81 (s, 4H), 1.80 (t, J = 6.6 Hz, 1H); M/Z : 320, 322 $[\text{M}+\text{H}]^+$, ESI $^+$, RT = 0.74 (S2).

Intermediate 8 (step 6.c): *trans*-5-[2-(4-chloro-3-fluorophenoxy)acetamido]-1,3-dioxane-2-carboxylic acid

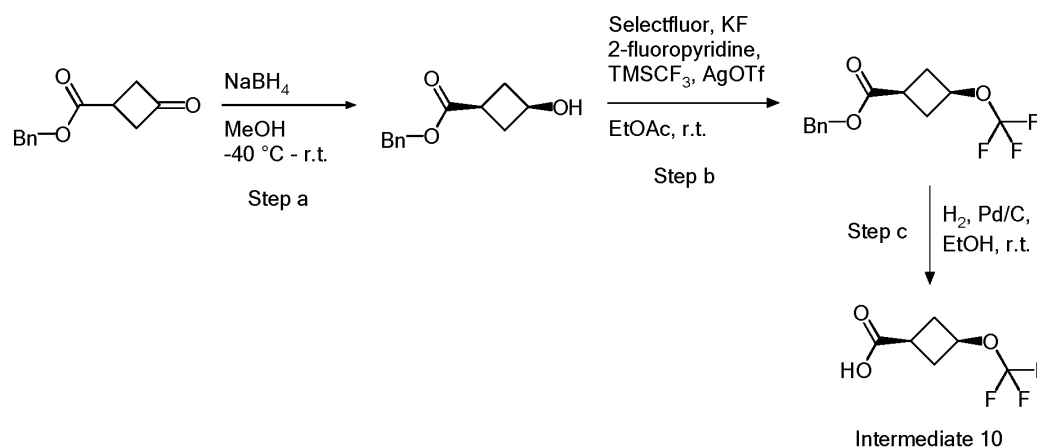
NaOCl (5%, 0.23 mL, 0.18 mmol), NaClO $_2$ (80%, 843 mg, 7.38 mmol) and TEMPO (58 mg, 0.369 mmol) were added to a solution of 2-(4-chloro-3-fluorophenoxy)-*N*-[*trans*-2-(hydroxymethyl)-1,3-dioxan-5-yl]acetamide (393 mg, 1.23 mmol) in ACN (10 mL) and NaH $_2$ PO $_4$ (0.67 M, 10 mL, 6.8 mmol) at r.t. The reaction mixture was heated at 35 °C for 22 h. The reaction mixture was cooled to r.t. and diluted with EtOAc (30 mL) and H $_2$ O (30 mL). The aqueous layer was acidified to pH 2 using 2 M aq HCl solution and extracted with EtOAc (3 $\times$ 20 mL). The combined organic layers were washed with brine (30 mL), dried over Na $_2$ SO $_4$ and concentrated *in vacuo* to afford the title compound (88% purity, 274 mg, 0.723 mmol, 59% yield) as an off-white solid; ^1H NMR (500 MHz, DMSO- d_6) δ 8.12 (d, J = 7.8 Hz, 1H), 7.49 (t, J = 8.9 Hz, 1H), 7.07 (dd, J = 11.4, 2.8 Hz, 1H), 6.85 (ddd, J = 9.0, 2.8, 1.2 Hz, 1H), 4.91 (s, 1H), 4.54 (s, 2H), 4.12 – 3.91 (m, 3H), 3.66 – 3.53 (m, 2H); M/Z : 334, 336 $[\text{M}+\text{H}]^+$, ESI $^+$, RT = 0.76 (S2).

The intermediate in **Table 2** was synthesised according to the general route 6 as exemplified by **Intermediate 8** using the corresponding starting material.

Table 2

Intermediate	Structure	Name	Starting material	LCMS data	NMR data
9		<i>trans</i> -5-{2-[3-chloro-4-(difluoromethyl)phenoxy]acetamido}-1,3-dioxane-2-carboxylic acid	2-[<i>trans</i> -2-(hydroxymethyl)-1,3-dioxan-5-yl]-2,3-dihydro-1 <i>H</i> -isoindole-1,3-dione (Intermediate 5) and 2-[3-chloro-4-(difluoromethyl)phenoxy]acetic acid (Intermediate 14)	M/Z : 366, 368 $[\text{M}+\text{H}]^+$, ESI $^+$, RT = 0.76 (S3).	^1H NMR (500 MHz, DMSO- d_6) δ 8.13 (d, J = 7.8 Hz, 1H), 7.62 (d, J = 8.8 Hz, 1H), 7.25 – 6.97 (m, 3H), 4.68 – 4.54 (m, 3H), 4.13 – 3.97 (m, 3H), 3.60 – 3.54 (m, 2H). OH not observed.

Scheme for route 7

**Step 7.a: benzyl *cis*-3-hydroxycyclobutane-1-carboxylate**

NaBH₄ (0.51 g, 13.5 mmol) was added to a solution of benzyl 3-oxocyclobutanecarboxylate (2.76 g, 13.5 mmol) in MeOH (25 mL) at $-40\text{ }^\circ\text{C}$ under N₂ and stirred for 1 h. The reaction mixture was warmed to $0\text{ }^\circ\text{C}$ and quenched with satd aq NH₄Cl solution (10 mL). The reaction mixture was warmed to r.t. and concentrated *in vacuo*. The residue was dissolved in H₂O (50 mL) and extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with brine (100 mL), dried over Na₂SO₄ and concentrated *in vacuo* to afford the title compound (2.73 g, 13.2 mmol, 98% yield) as a colourless oil; ¹H NMR (400 MHz, chloroform-*d*) δ 7.41 – 7.29 (m, 5H), 5.14 (s, 2H), 4.27 – 4.15 (m, 1H), 2.74 – 2.53 (m, 3H), 2.28 – 2.12 (m, 2H), 1.86 (d, *J* = 7.6 Hz, 1H).

Step 7.b: benzyl *cis*-3-(trifluoromethoxy)cyclobutane-1-carboxylate

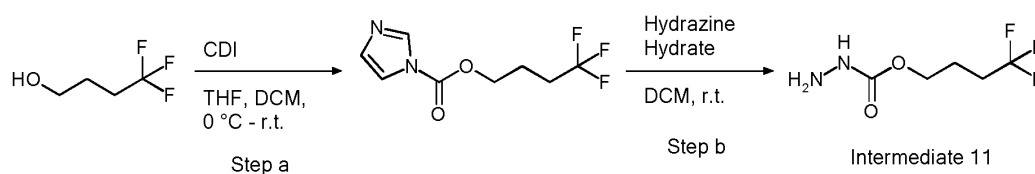
2-Fluoropyridine (3.5 mL, 40.4 mmol) and trifluoromethyltrimethylsilane (6.0 mL, 40.4 mmol) were successively added dropwise to a solution of benzyl *cis*-3-hydroxycyclobutane-1-carboxylate (2.78 g, 13.5 mmol), silver trifluoromethanesulfonate (10.43 g, 40.4 mmol), Selectfluor (7.16 g, 20.2 mmol) and potassium fluoride (3.13 g, 53.9 mmol) in EtOAc (120 mL) at r.t. under N₂ in a foil-covered flask. The reaction mixture was stirred at r.t. for 20 h before being filtered through Celite, washing with EtOAc (50 mL). The filtrate was concentrated *in vacuo* and purified by chromatography on silica gel (5-30% EtOAc in heptane) to afford the title compound (1.69 g, 6.16 mmol, 46% yield) as a colourless oil; ¹H NMR (400 MHz,

chloroform-*d*) δ 7.44 – 7.29 (m, 5H), 5.15 (s, 2H), 4.58 (p, J = 7.6 Hz, 1H), 2.83 – 2.73 (m, 1H), 2.65 (dtd, J = 10.0, 7.3, 2.6 Hz, 2H), 2.60 – 2.47 (m, 2H).

Intermediate 10 (step 7.c): *cis*-3-(trifluoromethoxy)cyclobutanecarboxylic acid

A suspension of benzyl *cis*-3-(trifluoromethoxy)cyclobutane-1-carboxylate (1.70 g, 6.20 mmol) and Pd/C (10%, 0.66 g, 0.31 mmol) in EtOH (50 mL) was stirred at r.t. under H₂ for 18 h. The reaction mixture was purged with N₂, then filtered through Celite, washing with EtOH (50 mL). The filtrate was concentrated *in vacuo* to afford the title compound (1.06 g, 5.64 mmol, 91% yield) as a pale yellow oil; ¹H NMR (400 MHz, chloroform-*d*) δ 9.11 (s, 1H), 4.60 (p, J = 7.4 Hz, 1H), 2.84 – 2.62 (m, 3H), 2.55 (q, J = 10.2, 9.5 Hz, 2H). Contains 10% of the *trans*-isomer, as observed by ¹H NMR.

Scheme for route 8



Step 8.a: 4,4,4-trifluorobutyl imidazole-1-carboxylate

A solution of 4,4,4-trifluorobutan-1-ol (3.0 g, 23.4 mmol) in DCM (50 mL) was added to a solution of CDI (5.70 g, 35.1 mmol) in THF (100 mL) at 0 °C under N₂ and stirred for 1 h. The reaction mixture was warmed to r.t. and stirred for 19 h. The reaction mixture was concentrated *in vacuo* and purified by chromatography on silica gel (5-50% EtOAc in heptane) to afford the title compound (4.92 g, 22.1 mmol, 95% yield) as a colourless oil; ¹H NMR (400 MHz, chloroform-*d*) δ 8.14 (s, 1H), 7.42 (t, J = 1.4 Hz, 1H), 7.14 – 7.04 (m, 1H), 4.50 (t, J = 6.4 Hz, 2H), 2.37 – 2.19 (m, 2H), 2.11 (dq, J = 10.3, 6.6 Hz, 2H); ¹⁹F{¹H} NMR (376 MHz, chloroform-*d*) δ -66.31 (3F, s); M/Z : 223 [M+H]⁺, ESI⁺, RT = 0.73 (S2).

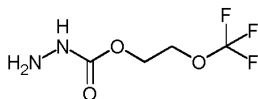
Intermediate 11 (step 8.b): 4,4,4-trifluorobutyl *N*-aminocarbamate

A solution of 4,4,4-trifluorobutyl imidazole-1-carboxylate (4.92 g, 22.1 mmol) and hydrazine hydrate (4.4 mL, 88.6 mmol) in DCM (70 mL) was stirred at r.t. for 1.5 h. Isopropanol (15 mL) was added and the organic layer was washed with H₂O (100 mL), satd aq NaHCO₃ solution (100 mL), brine (100 mL), dried over Na₂SO₄, and concentrated *in vacuo* to afford the title compound (2.54 g, 13.7 mmol, 62% yield) as a colourless oil; ¹H NMR (400 MHz, DMSO-*d*₆)

δ 8.15 (s, 1H), 4.15 – 3.89 (m, 4H), 2.40 – 2.15 (m, 2H), 1.84 – 1.67 (m, 2H); $^{19}\text{F}\{^1\text{H}\}$ NMR (376 MHz, chloroform-*d*) δ -64.89 (3F, s).

The following intermediates were prepared using route 8.

Intermediate 12: 2-(trifluoromethoxy)ethyl *N*-aminocarbamate

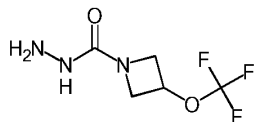


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Intermediate 12

^1H NMR (400 MHz, DMSO-*d*₆) δ 8.30 (s, 1H), 4.23 (s, 4H), 4.07 (s, 2H); $^{19}\text{F}\{^1\text{H}\}$ NMR (376 MHz, DMSO-*d*₆) δ -58.99 (3F, s).

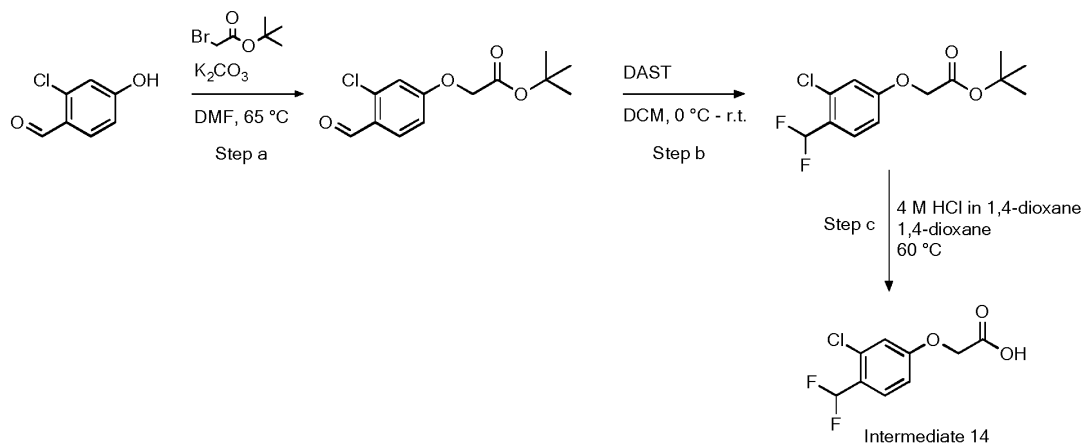
Intermediate 13: 3-(trifluoromethoxy)azetidine-1-carbohydrazide



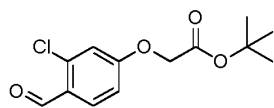
Intermediate 13

10 ^1H NMR (400 MHz, DMSO-*d*₆) δ 9.92 (s, 2H), 9.45 (s, 1H), 5.22 (tt, J = 6.9, 4.0 Hz, 1H), 4.33 (dd, J = 10.1, 6.7 Hz, 2H), 4.01 (dd, J = 10.1, 3.8 Hz, 2H); $^{19}\text{F}\{^1\text{H}\}$ NMR (376 MHz, chloroform-*d*) δ -58.43 (3F, s).

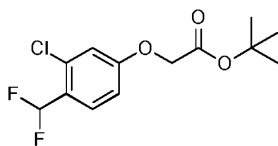
Scheme for route 9



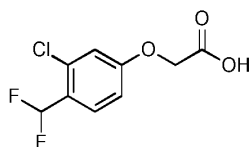
15

Step 9.a: *tert*-butyl 2-(3-chloro-4-formylphenoxy)acetate

To a solution of 2-chloro-4-hydroxybenzaldehyde (1.50 g, 9.58 mmol) in anhydrous DMF (15 mL) was added *tert*-butyl bromoacetate (1.6 mL, 10.5 mmol) followed by K₂CO₃ (2.65 g, 19.2 mmol) and the mixture was stirred at 65 °C for 2 h. The reaction mixture was cooled to r.t. and then poured onto water (100 mL). The resultant solution was extracted with EtOAc (2 × 70 mL), and the combined organic extracts were washed with brine (100 mL), dried over MgSO₄, and concentrated *in vacuo* to afford the title compound in quantitative yield (2.76 g, 9.89 mmol) as an off-white solid; ¹H NMR (400 MHz, chloroform-*d*) δ 10.34 (s, 1H), 7.90 (d, *J* = 8.7 Hz, 1H), 6.93 (d, *J* = 2.4 Hz, 1H), 6.88 (dd, *J* = 8.7, 2.2 Hz, 1H), 4.58 (s, 2H), 1.49 (s, 9H); *M/Z*: 271, 273 [M+H]⁺, ESI⁺, RT = 0.98 (S2).

Step 9.b: *tert*-butyl 2-[3-chloro-4-(difluoromethyl)phenoxy]acetate

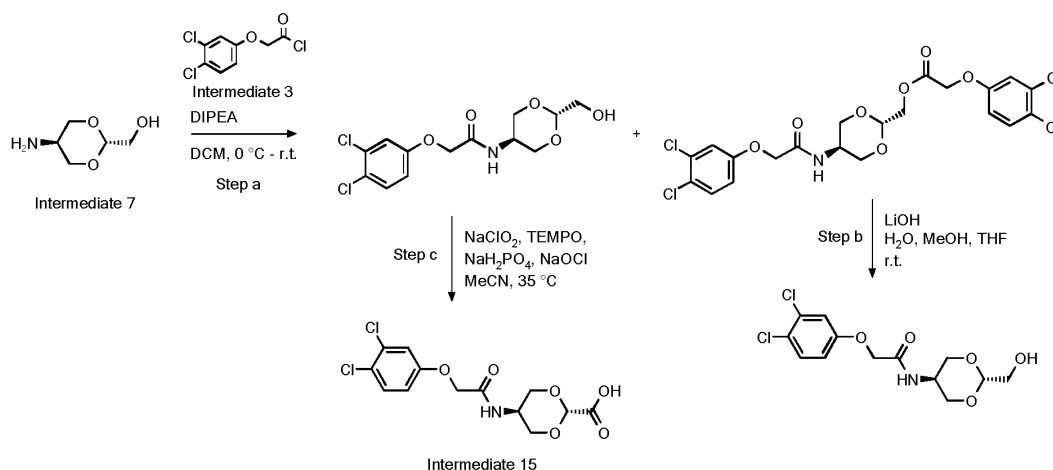
To a solution of *tert*-butyl 2-(3-chloro-4-formylphenoxy)acetate (2.76 g, 9.89 mmol) in anhydrous DCM (20 mL) at 0 °C was added DAST (2.6 mL, 19.8 mmol) dropwise and the mixture was stirred at r.t. for 3 h. Satd aq NaHCO₃ solution (50 mL) was added slowly and stirred at r.t. for 1 h. The solution was extracted with DCM (2 × 50 mL) and the combined organic extracts were washed with satd aq NaHCO₃ solution, dried over MgSO₄, and concentrated *in vacuo*. The residue was purified by chromatography on silica gel (5-40% EtOAc in heptane) to afford the title compound (2.02 g, 6.62 mmol, 67% yield) as a yellow oil; ¹H NMR (400 MHz, chloroform-*d*) δ 7.58 (d, *J* = 8.6 Hz, 1H), 7.05 – 6.73 (m, 3H), 4.53 (s, 2H), 1.49 (s, 9H); *M/Z*: 291, 293 [M-H]⁻, ESI⁻, RT = 1.06 (S2).

25 Intermediate 14 (step 9.c): 2-[3-chloro-4-(difluoromethyl)phenoxy]acetic acid

Intermediate 14

To a solution of *tert*-butyl 2-[3-chloro-4-(difluoromethyl)phenoxy]acetate (2.02 g, 6.62 mmol) in 1,4-dioxane (5 mL) was added 4 M HCl in 1,4-dioxane (17 mL, 66.2 mmol) and the mixture was stirred at 60 °C for 4 h. The reaction mixture was cooled to r.t. and concentrated *in vacuo*. H₂O was added and the resultant precipitate was filtered under vacuum, washing with H₂O. The solid was then dissolved in MeCN, filtered under vacuum and the filtrate concentrated *in vacuo* to afford the title compound (1.51 g, 6.26 mmol, 95% yield) as a cream solid; ¹H NMR (400 MHz, chloroform-*d*) δ 7.61 (d, *J* = 8.7 Hz, 1H), 7.05 – 6.74 (m, 3H), 4.73 (s, 2H); ¹⁹F NMR (376 MHz, chloroform-*d*) δ -113.69; *M/Z*: 235, 237 [*M-H*]⁺, ESI⁺, RT = 0.74 (S2).

Scheme for route 10



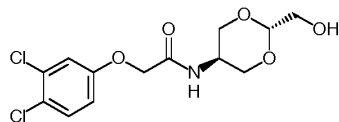
10

Step 10.a: 2-(3,4-dichlorophenoxy)-*N*-[*trans*-2-(hydroxymethyl)-1,3-dioxan-5-yl]acetamide and [*trans*-5-[2-(3,4-dichlorophenoxy)acetamido]-1,3-dioxan-2-yl]methyl 2-(3,4-dichlorophenoxy)acetate

A solution of [*trans*-5-amino-1,3-dioxan-2-yl]methanol (495 mg, 3.72 mmol, Intermediate 7) and DIPEA (3.9 mL, 22.3 mmol) in DCM (10 mL) was cooled to 0 °C and treated dropwise with a solution of 2-(3,4-dichlorophenoxy)acetyl chloride (1.87 g, 7.81 mmol, Intermediate 3) in DCM (15 mL). The mixture was allowed to warm to r.t. and stirred for 3 h. The reaction mixture was cooled to 0 °C and quenched slowly with H₂O (10 mL). The organic layer was separated and washed with satd aq NaHCO₃ solution (20 mL) and brine (20 mL), dried over Na₂SO₄, and concentrated *in vacuo*. The residue was purified by chromatography on silica gel (20-100% EtOAc in heptane) to afford the title compounds 2-(3,4-dichlorophenoxy)-*N*-[*trans*-2-(hydroxymethyl)-1,3-dioxan-5-yl]acetamide (405 mg, 1.14 mmol, 31% yield) and [*trans*-5-

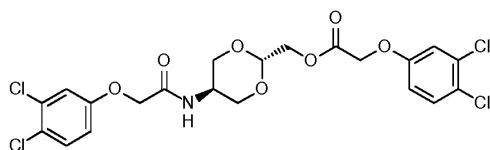
[2-(3,4-dichlorophenoxy)acetamido]-1,3-dioxan-2-yl]methyl 2-(3,4-dichlorophenoxy)acetate (92% purity, 800 mg, 1.37 mmol, 37% yield) as off-white solids.

2-(3,4-dichlorophenoxy)-N-[*trans*-2-(hydroxymethyl)-1,3-dioxan-5-yl]acetamide



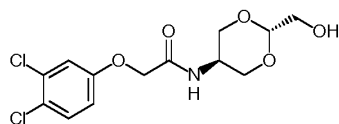
- 5 (405 mg, 1.14 mmol, 31% yield); ¹H NMR (400 MHz, chloroform-*d*) δ 7.39 (d, *J* = 8.9 Hz, 1H), 7.04 (d, *J* = 2.9 Hz, 1H), 6.78 (dd, *J* = 8.9, 2.9 Hz, 1H), 6.11 (d, *J* = 8.1 Hz, 1H), 4.60 (t, *J* = 4.4 Hz, 1H), 4.44 (s, 2H), 4.40 – 4.30 (m, 1H), 4.30 – 4.23 (m, 2H), 3.66 (dd, *J* = 6.6, 4.4 Hz, 2H), 3.54 – 3.46 (m, 2H), 1.81 (t, *J* = 6.6 Hz, 1H).

- 10 **[*trans*-5-[2-(3,4-dichlorophenoxy)acetamido]-1,3-dioxan-2-yl]methyl 2-(3,4-dichlorophenoxy)acetate**



(92% purity, 800 mg, 1.37 mmol, 37% yield); ¹H NMR (500 MHz, chloroform-*d*) δ 7.36 (m, 2H), 7.02 (m, 2H), 6.81 – 6.75 (m, 2H), 6.14 (d, *J* = 8.1 Hz, 1H), 4.71 (t, *J* = 4.4 Hz, 1H), 4.66 (s, 2H), 4.44 (s, 2H), 4.38 – 4.30 (m, 1H), 4.30 – 4.21 (m, 4H), 3.52 – 3.44 (m, 2H).

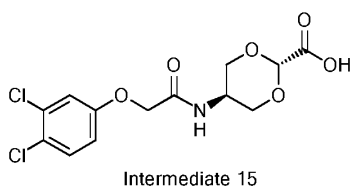
- 15 **Step 10.b: 2-(3,4-dichlorophenoxy)-N-[*trans*-2-(hydroxymethyl)-1,3-dioxan-5-yl]acetamide**



- 20 A solution of [*trans*-5-[2-(3,4-dichlorophenoxy)acetamido]-1,3-dioxan-2-yl]methyl 2-(3,4-dichlorophenoxy)acetate (92% purity, 800 mg, 1.37 mmol) in H₂O (2.8 mL), THF (2.8 mL) and MeOH (2.8 mL) was treated with LiOH (43 mg, 1.78 mmol) and stirred at r.t. for 1 h. The organic solvent was removed *in vacuo* to afford an aqueous residue which was diluted with

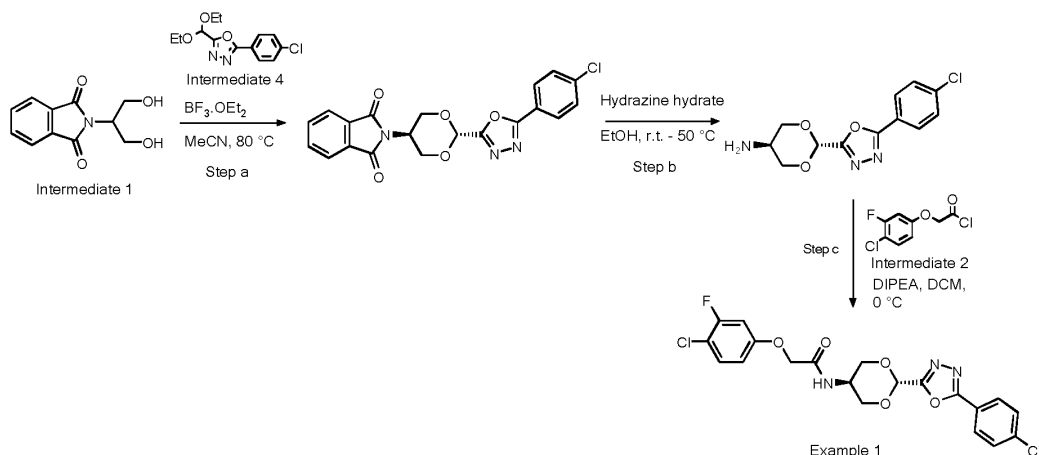
H₂O (10 mL) and extracted with EtOAc (2 × 10 mL). The combined organic extracts were washed with brine (20 mL), dried over Na₂SO₄, and concentrated *in vacuo* to afford the title compound (385 mg, 1.09 mmol, 73% yield) as an off-white solid; ¹H NMR (400 MHz, chloroform-*d*) δ 7.39 (d, *J* = 8.9 Hz, 1H), 7.05 (d, *J* = 2.9 Hz, 1H), 6.78 (dd, *J* = 8.9, 2.9 Hz, 1H), 6.11 (d, *J* = 8.2 Hz, 1H), 4.60 (t, *J* = 4.4 Hz, 1H), 4.44 (s, 2H), 4.40 – 4.31 (m, 1H), 4.30 – 4.20 (m, 2H), 3.66 (d, *J* = 4.4 Hz, 2H), 3.55 – 3.43 (m, 2H), 1.80 (s, 1H).

Intermediate 15 (step 10.c): *trans*-5-[2-(3,4-dichlorophenoxy)acetamido]-1,3-dioxane-2-carboxylic acid



NaOCl (5.0%, 0.44 mL, 0.353 mmol), NaClO₂ (80%, 1.61 g, 14.1 mmol) and TEMPO (111 mg, 0.705 mmol) were added to a solution of 2-(3,4-dichlorophenoxy)-*N*-[*trans*-2-(hydroxymethyl)-1,3-dioxan-5-yl]acetamide (790 mg, 2.35 mmol) in ACN (38 mL) and 0.67 M NaH₂PO₄ (19 mL, 12.9 mmol) at r.t. and the mixture was stirred at 35 °C for 48 h. The reaction mixture was concentrated *in vacuo* at r.t. to remove the organic solvent, and the resultant aqueous solution was basified to pH 8/9 using satd aq NaHCO₃ solution. The aqueous solution was washed with EtOAc (2 × 30 mL) and the organic extracts were discarded. The aqueous solution was then cooled to 0 °C and acidified to pH 2/3 by slow addition of 1 M aq HCl solution. The resultant solution was then extracted with EtOAc (2 × 50 mL) and the combined organic extracts were washed with H₂O (50 mL), dried over Na₂SO₄, and concentrated *in vacuo* to afford the title compound (90% purity, 760 mg, 1.95 mmol, 83% yield) as a foamy off-white solid; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.11 (d, *J* = 7.8 Hz, 1H), 7.54 (d, *J* = 8.9 Hz, 1H), 7.25 (d, *J* = 2.9 Hz, 1H), 6.98 (dd, *J* = 8.9, 2.9 Hz, 1H), 4.91 (s, 1H), 4.55 (s, 2H), 4.08 – 3.95 (m, 3H), 3.59 (m, 2H); *M/Z*: 348, 350, 352 [M-H]⁺, ESI⁺, RT = 2.61 (S4).

Scheme for route 11



Step 11.a: 2-[trans-2-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-1,3-dioxan-5-yl]isoindoline-1,3-dione

- 5 A solution of $\text{BF}_3 \cdot \text{OEt}_2$ (0.12 mL, 0.97 mmol) in anhydrous ACN (3 mL) was stirred at 80 °C. A separate solution of 2-[2-hydroxy-1-(hydroxymethyl)ethyl]isoindoline-1,3-dione (100 mg, 0.44 mmol, Intermediate 1) and 2-(4-chlorophenyl)-5-(diethoxymethyl)-1,3,4-oxadiazole (94 μL , 0.44 mmol, Intermediate 4) in ACN (2 mL) was added dropwise. The reaction mixture was stirred for 1 h, then additional $\text{BF}_3 \cdot \text{OEt}_2$ (0.12 mL, 0.97 mmol) was added and the reaction
- 10 mixture was heated at 80 °C for a further 1 h. The reaction was cooled to r.t., quenched with satd aq NaHCO_3 solution (0.1 mL) and concentrated *in vacuo*. The residue was dissolved in EtOAc (10 mL) and washed sequentially with satd aq NaHCO_3 solution (10 mL) and brine (10 mL). The organic layer was dried over anhydrous Na_2SO_4 , concentrated *in vacuo* and purified by chromatography on silica gel (10-100% EtOAc in heptane) to afford the title compound
- 15 (70% purity, 33 mg, 0.06 mmol, 13% yield) as an off-white solid, as a mixture of diastereomers; ^1H NMR (400 MHz, chloroform-*d*) δ 8.12 – 8.08 (m, 1H), 8.08 – 8.04 (m, 2H), 7.89 – 7.82 (m, 4H), 7.79 – 7.71 (m, 4H), 7.54 – 7.49 (m, 3H), 6.04 (s, 1H), 4.90 – 4.80 (m, 2H), 4.73 – 4.65 (m, 3H), 4.57 – 4.49 (m, 1H), 4.35 – 4.24 (m, 3H), 4.19 (dd, J = 11.0, 5.0 Hz, 1H), 4.12 (q, J = 7.1 Hz, 3H), 2.04 (s, 4H), 1.73–1.59 (m, 4H); M/Z : 412, 414 $[\text{M}+\text{H}]^+$, ESI $^+$, RT = 3.52, 3.67
- 20 (S4).

Step 11.b: trans-2-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-1,3-dioxan-5-amine

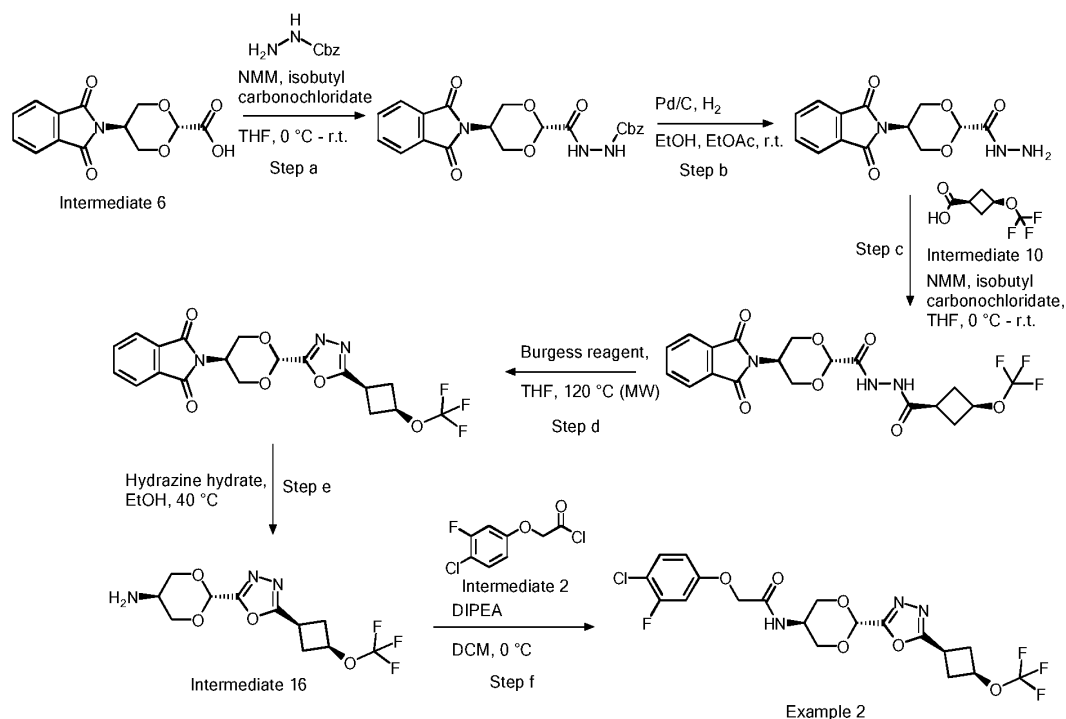
- A suspension of hydrazine hydrate (0.02 mL, 0.14 mmol) and 2-[trans-2-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-1,3-dioxan-5-yl]isoindoline-1,3-dione (33 mg, 0.06 mmol) in EtOH (0.7
- 25 mL) was stirred at r.t. for 2 h. The reaction mixture was heated at 40 °C for 25 h, then heated

at 50 °C for a further 6 h. The reaction mixture was cooled to r.t., filtered through a pad of Celite and concentrated *in vacuo* to afford the title compound (50% purity, 17 mg, 0.03 mmol, 54% yield) as a white solid; ¹H NMR (400 MHz, chloroform-*d*) δ 8.04 (m, 2H), 7.49 (m, 2H), 5.80 (s, 1H), 4.35 (dd, *J* = 11.4, 4.6 Hz, 2H), 4.09 (d, *J* = 12.0 Hz, 1H), 3.51 (t, *J* = 10.8 Hz, 2H), 3.36 – 3.25 (m, 2H); *M/Z*: 282 [M+H]⁺, ESI⁺, RT = 0.82 (S1).

Example 1 (Step 11.c): 2-(4-chloro-3-fluorophenoxy)-*N*-[*trans*-2-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-1,3-dioxan-5-yl]acetamide

A solution of *trans*-2-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-1,3-dioxan-5-amine (50%, 17 mg, 0.03 mmol) in DCM (0.5 mL) was treated with DIPEA (0.02 mL, 0.09 mmol) and cooled to 0 °C. A solution of 2-(4-chloro-3-fluorophenoxy)acetyl chloride (7.4 mg, 0.03 mmol, Intermediate 2) in DCM (0.5 mL) was added slowly. The reaction mixture was allowed to warm to r.t. and stirred for 1 h. 2-(4-chloro-3-fluorophenoxy)acetyl chloride (3.0 mg, 0.01 mmol, Intermediate 2) was added and the reaction was stirred at r.t. for 1 h. The reaction mixture was cooled to 0 °C, quenched with H₂O and concentrated *in vacuo*. The residue was purified by prep. HPLC (Method 4) to afford the title compound (5.0 mg, 0.01 mmol, 35% yield) as an off-white powder; ¹H NMR (400 MHz, chloroform-*d*) δ 8.07 – 8.01 (m, 2H), 7.54 – 7.48 (m, 2H), 7.36 (t, *J* = 8.6 Hz, 1H), 6.84 – 6.76 (m, 2H), 6.72 (ddd, *J* = 8.9, 2.9, 1.3 Hz, 1H), 6.03 (s, 1H), 4.53 – 4.44 (m, 4H), 4.39 – 4.32 (m, 1H), 3.86 (dd, *J* = 11.6, 6.6 Hz, 2H); *M/Z*: 468, 470, 472 [M+H]⁺, ESI⁺, RT = 3.65 (S4).

Scheme for route 12



Step 12.a: 2-[*trans*-2-[[[(benzyloxy)carbonyl]amino}amino]carbonyl]-1,3-dioxan-5-yl]-2,3-dihydro-1*H*-isoindole-1,3-dione

- 5 A solution of *trans*-5-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)-1,3-dioxane-2-carboxylic acid (2.07 g, 7.39 mmol, Intermediate 6) in anhydrous THF (49 mL) was treated with sequential addition of isobutyl carbonochloridate (0.9 mL, 7.02 mmol) and NMM (0.8 mL, 7.39 mmol) at 0 °C under N₂. Benzyl *N*-aminocarbamate (1.17 g, 7.02 mmol) was added and the reaction was stirred at 0 °C for 30 min, then at r.t. for 1 h. The reaction was cooled to 0 °C and quenched
- 10 with H₂O (1 mL). The resultant mixture was concentrated *in vacuo* and the residue was partitioned between EtOAc (50 mL) and H₂O (50 mL). The layers were separated and the aqueous layer was re-extracted with EtOAc (20 mL). The combined organic extracts were washed with satd aq NaHCO₃ solution (80 mL), brine (80 mL), dried over anhydrous Na₂SO₄, and concentrated *in vacuo* to afford the title compound (86%, 2.86 g, 5.78 mmol, 78% yield)
- 15 as an off-white powder; ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.16 – 9.91 (m, 1H), 9.27 (s, 1H), 7.93 – 7.82 (m, 4H), 7.44 – 7.27 (m, 5H), 5.09 (s, 2H), 5.03 (s, 1H), 4.44 – 4.28 (m, 3H), 4.22 – 4.12 (m, 2H).

Step 12.b: *trans*-5-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)-1,3-dioxane-2-carbohydrazide

A suspension of 2-[*trans*-2-[[[(benzyloxy)carbonyl]amino}amino)carbonyl]-1,3-dioxan-5-yl]-2,3-dihydro-1*H*-isoindole-1,3-dione (86% purity, 2.86 g, 5.78 mmol) and Pd/C (10%, 0.62 g, 0.578 mmol) in EtOH (160 mL) and EtOAc (50 mL) was stirred under H₂ for 3 h. Additional Pd/C (10%, 0.62 g, 0.578 mmol) was added and the reaction mixture was stirred under H₂ for a further 4 h. The reaction vessel was flushed with N₂. The reaction mixture was heated to near reflux and filtered through a pad of Celite, washing with hot EtOH. The reaction mixture was concentrated *in vacuo* and purified by chromatography on silica gel (0-7% MeOH in DCM) to afford the title compound (90% purity, 290 mg, 0.90 mmol, 15% yield) as a pale yellow powder; ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.32 (s, 1H), 7.93 – 7.79 (m, 4H), 4.93 (s, 1H), 4.41 – 4.25 (m, 5H), 4.17 – 4.08 (m, 2H).

Step 12.c: *trans*-5-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)-*N'*-[*cis*-3-(trifluoromethoxy)cyclobutanecarbonyl]-1,3-dioxane-2-carbohydrazide

A solution of *cis*-3-(trifluoromethoxy)cyclobutanecarboxylic acid (170 mg, 0.90 mmol, Intermediate 10) in anhydrous THF (7.5 mL) was treated with sequential addition of isobutyl carbonochloridate (0.11 mL, 0.85 mmol) and NMM (0.1 mL, 0.90 mmol) at 0 °C. The mixture was stirred for 15 min, then a suspension of *trans*-5-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)-1,3-dioxane-2-carbohydrazide (90% purity, 290 mg, 0.90 mmol) in anhydrous THF (5 mL) was added. The reaction was warmed to r.t. and stirred for 3 h. The reaction mixture was cooled to 0 °C and quenched by the slow addition of H₂O (5 mL). The reaction mixture was concentrated *in vacuo* and the resultant residue was partitioned between H₂O and EtOAc. The aqueous layer was re-extracted with EtOAc. The combined organic extracts were washed with satd aq NaHCO₃ solution and brine, and then concentrated *in vacuo* to afford the title compound (90% purity, 382 mg, 0.75 mmol, 84% yield) as an off-white powder; ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.04 (s, 1H), 9.96 (s, 1H), 7.88 (s, 4H), 5.04 (s, 1H), 4.89 – 4.71 (m, 1H), 4.46 – 4.26 (m, 3H), 4.26 – 4.10 (m, 2H), 2.77 – 2.63 (m, 2H), 2.36 – 2.18 (m, 4H).

Step 12.d: 2-[*trans*-2-{5-[*cis*-3-(trifluoromethoxy)cyclobutyl]-1,3,4-oxadiazol-2-yl}-1,3-dioxan-5-yl]-2,3-dihydro-1*H*-isoindole-1,3-dione

A suspension of *trans*-5-(1,3-dioxo-2,3-dihydro-1*H*-isoindol-2-yl)-*N*-[*cis*-3-(trifluoromethoxy)cyclobutanecarbonyl]-1,3-dioxane-2-carbohydrazide (85%, 365 mg, 0.68 mmol) and Burgess reagent (647 mg, 2.71 mmol) in anhydrous THF (11 mL) was irradiated at 120 °C in a microwave vial for 3 min. The reaction mixture was concentrated *in vacuo* and purified by prep. HPLC (Method 6) to afford the title compound (190 mg, 0.41 mmol, 61% yield) as an off-white solid; ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.98 – 7.73 (m, 4H), 6.09 (s, 1H), 4.97 – 4.87 (m, 1H), 4.55 – 4.39 (m, 3H), 4.29 – 4.20 (m, 2H), 3.56 – 3.46 (m, 1H), 2.92 – 2.83 (m, 2H), 2.57 – 2.51 (m, 2H).

Intermediate 16 (step 12.e): *trans*-2-{5-[*cis*-3-(trifluoromethoxy)cyclobutyl]-1,3,4-oxadiazol-2-yl}-1,3-dioxan-5-amine

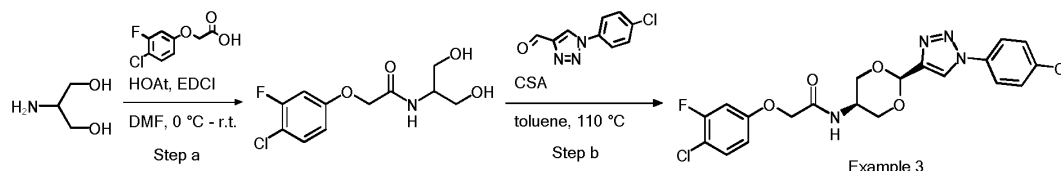
A solution of 2-[*trans*-2-{5-[*cis*-3-(trifluoromethoxy)cyclobutyl]-1,3,4-oxadiazol-2-yl}-1,3-dioxan-5-yl]-2,3-dihydro-1*H*-isoindole-1,3-dione (90% purity, 330 mg, 0.68 mmol) and hydrazine hydrate (0.13 mL, 2.70 mmol) in EtOH (7.8 mL) was heated at 40 °C for 6 h, then cooled to r.t. and stirred for 18 h. The resultant precipitate was filtered under vacuum, washing with EtOH. The combined filtrates were collected and concentrated *in vacuo* to afford the title compound (77% purity, 250 mg, 0.62 mmol, 92% yield) as an off-white solid; ¹H NMR (400 MHz, DMSO-*d*₆) δ 5.78 (s, 1H), 4.97 – 4.83 (m, 1H), 4.14 – 4.02 (m, 2H), 3.52 – 3.39 (m, 5H), 2.95 (tt, *J* = 10.2, 5.0 Hz, 1H), 2.90 – 2.80 (m, 2H), 2.55 – 2.51 (m, 2H).

Example 2 (step 12.f): 2-(4-chloro-3-fluorophenoxy)-*N*-[*trans*-2-{5-[*cis*-3-(trifluoromethoxy)cyclobutyl]-1,3,4-oxadiazol-2-yl}-1,3-dioxan-5-yl]acetamide

A solution of *trans*-2-{5-[*cis*-3-(trifluoromethoxy)cyclobutyl]-1,3,4-oxadiazol-2-yl}-1,3-dioxan-5-amine (77% purity, 255 mg, 0.64 mmol, Intermediate 16) and DIPEA (0.33 mL, 1.90 mmol) in DCM (8 mL) was cooled to 0 °C and treated with 2-(4-chloro-3-fluorophenoxy)acetyl chloride (90% purity, 173 mg, 0.70 mmol, Intermediate 2). The reaction was warmed to r.t. and stirred for 1 h. The reaction was cooled to 0 °C and quenched with H₂O (0.1 mL), then the reaction mixture was concentrated *in vacuo*. The residue was purified by prep. HPLC (Method 6) and the resulting solid was dissolved in ACN (10 mL) and H₂O (10 mL). The cloudy solution was heated to solubilise the material, then left to cool for 4 days. The resultant solid was collected and purified by prep. HPLC (Method 3) to afford the title compound (62 mg, 0.13 mmol, 20% yield) as a white solid; ¹H NMR (500 MHz, chloroform-*d*) δ 7.35 (t, *J* = 8.6 Hz, 1H), 6.79 (dd, *J* = 10.2, 2.9 Hz, 1H), 6.75 (d, *J* = 8.4 Hz, 1H), 6.71 (ddd, *J* = 8.9, 2.9, 1.2 Hz, 1H), 5.93 (s, 1H), 4.72 (p, *J* = 7.6 Hz, 1H), 4.49 (s, 2H), 4.43 (dd, *J* = 11.6, 3.8 Hz, 2H), 4.35

(dt, $J = 10.8, 7.4, 3.8$ Hz, 1H), 3.82 (dd, $J = 11.6, 7.0$ Hz, 2H), 3.38 (tt, $J = 10.1, 7.8$ Hz, 1H), 2.94 – 2.86 (m, 2H), 2.77 – 2.68 (m, 2H); M/Z : 496, 498 $[M+H]^+$, ESI⁺, RT = 3.50 (S4).

Scheme for route 13



5 Step 13.a: 2-(4-chloro-3-fluorophenoxy)-*N*-[2-hydroxy-1-(hydroxymethyl)ethyl]-acetamide

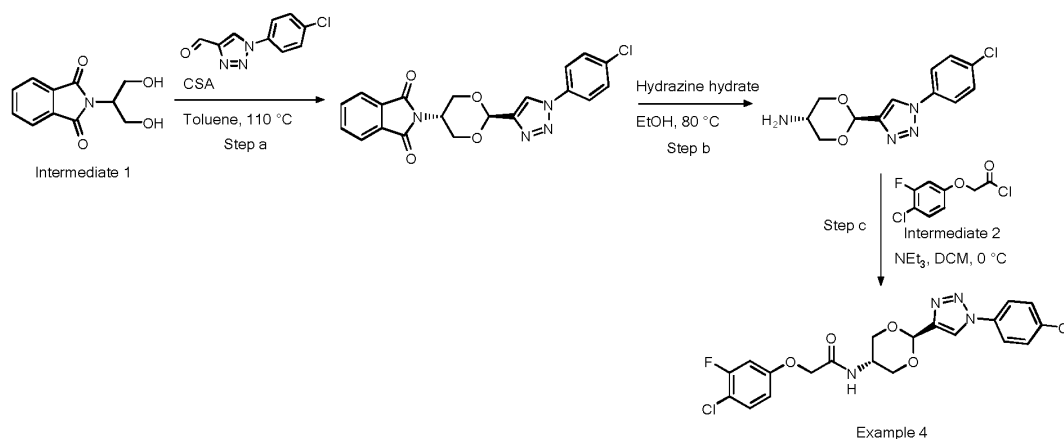
2-(4-Chloro-3-fluorophenoxy)acetic acid (200 mg, 0.98 mmol), 2-aminopropane-1,3-diol (107 mg, 1.17 mmol) and HOAt (160 mg, 1.18 mmol) were dissolved in anhydrous DMF (5 mL). EDCI (225 mg, 1.17 mmol) was then added in small portions at 0 °C. The reaction mixture was warmed to r.t. and stirred for 17 h. The reaction mixture was quenched with H₂O (20 mL) and extracted with EtOAc (2 × 30 mL). The combined organic extracts were washed with brine (20 mL), dried over MgSO₄ and concentrated *in vacuo*. Purification by chromatography on silica gel (0-100% EtOAc in heptane) afforded the title compound (278 mg, 1.0 mmol) in quantitative yield as a white powder; ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.67 (d, $J = 8.3$ Hz, 1H), 7.49 (t, $J = 8.9$ Hz, 1H), 7.08 (dd, $J = 11.4, 2.9$ Hz, 1H), 6.85 (ddd, $J = 9.0, 2.9, 1.1$ Hz, 1H), 4.74 – 4.63 (m, 2H), 4.54 (s, 2H), 3.83 – 3.74 (m, 1H), 3.46 – 3.40 (m, 4H); M/Z : 278, 280 $[M+H]^+$, ESI⁺, RT = 0.88 (S1).

Example 3 (step 13.b): 2-(4-chloro-3-fluorophenoxy)-*N*-[*cis*-2-[1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl]-1,3-dioxan-5-yl]acetamide

A suspension of 1-(4-chlorophenyl)-1*H*-1,2,3-triazole-4-carbaldehyde (50 mg, 0.241 mmol), CSA (6 mg, 0.03 mmol) and 2-(4-chloro-3-fluorophenoxy)-*N*-[2-hydroxy-1-(hydroxymethyl)ethyl]acetamide (67 mg, 0.24 mmol) in anhydrous toluene (2 mL) was heated at 110 °C for 18 h. Additional 2-(4-chloro-3-fluorophenoxy)-*N*-[2-hydroxy-1-(hydroxymethyl)ethyl]acetamide (67 mg, 0.24 mmol) and CSA (6 mg, 0.03 mmol) were added and the reaction mixture was heated at 110 °C for 5 h. The reaction mixture was cooled to r.t. and brine was added. The aqueous layer was extracted with EtOAc (2 × 50 mL), dried over MgSO₄, and concentrated *in vacuo*. Purification by chromatography on silica gel (0-100% EtOAc in heptane) followed by prep. HPLC (Method 3) afforded the title compound (10 mg,

0.02 mmol, 8.6% yield) as a white powder, as a mixture of *cis* and *trans* isomers (93:7); ^1H NMR (500 MHz, chloroform-*d*) δ 7.93 (s, 1H), 7.70 – 7.65 (m, 2H), 7.55 – 7.49 (m, 2H), 7.40 – 7.34 (m, 1H), 7.30 (t, J = 8.6 Hz, 1H), 6.78 (dd, J = 10.3, 2.9 Hz, 1H), 6.69 (dd, J = 8.9, 1.6 Hz, 1H), 5.91 (s, 1H), 4.53 (s, 2H), 4.28 – 4.15 (m, 4H), 4.11 (d, J = 8.3 Hz, 1H); M/Z : 467, 469 $[\text{M}+\text{H}]^+$, ESI^+ , RT = 3.65 (S6).

Scheme for route 14



Step 14.a: 2-[trans-2-[1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl]-1,3-dioxan-5-yl]-2,3-dihydro-1H-isoindole-1,3-dione

A suspension of 1-(4-chlorophenyl)-1H-1,2,3-triazole-4-carbaldehyde (270 mg, 1.30 mmol), CSA (32 mg, 0.14 mmol) and 2-[2-hydroxy-1-(hydroxymethyl)ethyl]isoindoline-1,3-dione, Intermediate 1 (288 mg, 1.30 mmol) in anhydrous toluene (11 mL) was heated at 110 °C for 1 h. The reaction mixture was allowed to cool to r.t. and diluted with cold DCM. The resultant precipitate was filtered under vacuum to afford the title compound (305 mg, 0.74 mmol, 57% yield) as a colourless solid; ^1H NMR (400 MHz, chloroform-*d*) δ 8.11 (s, 1H), 7.89 (dd, J = 5.5, 3.1 Hz, 2H), 7.79 (dd, J = 5.5, 3.1 Hz, 2H), 7.75 – 7.68 (m, 2H), 7.57 – 7.50 (m, 2H), 6.02 (s, 1H), 4.86 – 4.67 (m, 3H), 4.28 – 4.19 (m, 2H); M/Z : 411, 413 $[\text{M}+\text{H}]^+$, ESI^+ , RT = 1.29 (S1).

Step 14.b: trans-2-[1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl]-1,3-dioxan-5-amine

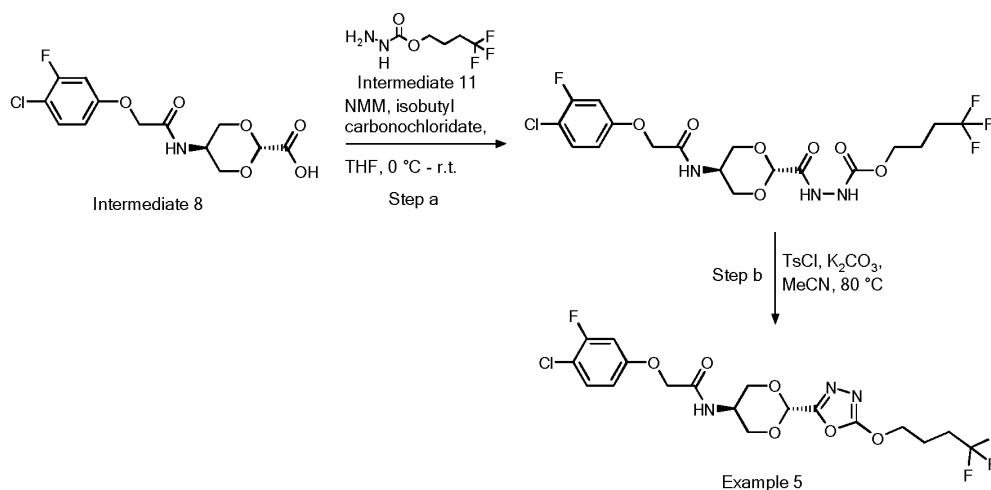
A suspension of hydrazine hydrate (0.46 mL, 3.71 mmol) and 2-[trans-2-[1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl]-1,3-dioxan-5-yl]-2,3-dihydro-1H-isoindole-1,3-dione (305 mg, 0.74 mmol) in EtOH (7.4 mL) was heated at 80 °C for 1 h. The reaction mixture was cooled to r.t. and the resulting precipitate was filtered under vacuum. The filtrate was then concentrated *in vacuo* to afford the title compound (200 mg, 0.69 mmol, 93% yield) as a colourless solid; ^1H

NMR (400 MHz, DMSO- d_6) δ 8.88 (s, 1H), 8.06 – 7.89 (m, 2H), 7.72 – 7.58 (m, 2H), 5.66 (s, 1H), 4.09 (dd, J = 11.3, 5.0 Hz, 2H), 3.52 – 3.39 (m, 2H), 2.94 (tt, J = 10.3, 5.0 Hz, 1H); M/Z : 281, 283, 285 $[M+H]^+$, ESI $^+$, RT = 0.66 (S1).

Example 4 (step 14.c): 2-(4-chloro-3-fluorophenoxy)-N-[*trans*-2-[1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl]-1,3-dioxan-5-yl]acetamide

A solution of 2-(4-chloro-3-fluorophenoxy)acetyl chloride (0.05 mL, 0.77 mmol, Intermediate 2) in DCM (1 mL) was added to a solution of *trans*-2-[1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl]-1,3-dioxan-5-amine (72 mg, 0.26 mmol) and Et₃N (0.21 mL, 1.54 mmol) in DCM (1.2 mL) at 0 °C. The reaction mixture was warmed to r.t. and stirred for 15 min. The reaction mixture was diluted with satd aq NaHCO₃, with the insoluble material isolated by filtration. The organic layer was collected, dried over Na₂SO₄ and concentrated *in vacuo*. The residual material (along with the solid isolated *via* suction filtration) was triturated with ACN to afford the title compound (87 mg, 0.18 mmol, 70% yield) as an off-white solid; ¹H NMR (400 MHz, DMSO- d_6) δ 8.94 (s, 1H), 8.17 (d, J = 7.6 Hz, 1H), 7.99 (d, J = 8.9 Hz, 2H), 7.67 (d, J = 8.9 Hz, 2H), 7.52 (t, J = 8.9 Hz, 1H), 7.10 (dd, J = 11.3, 2.8 Hz, 1H), 6.88 (dd, J = 8.9, 1.8, 1H), 5.80 (s, 1H), 4.57 (s, 2H), 4.23 – 4.08 (m, 3H), 3.85 – 3.68 (m, 2H); M/Z : 511, 513, 515 $[M+H]^+$, ESI $^+$, RT = 3.58 (S4).

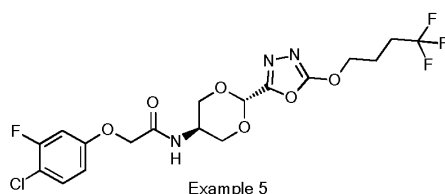
Scheme for route 15



Step 15.a: 2-(4-chloro-3-fluorophenoxy)-N-[*trans*-2-{*N'*-[(4,4,4-trifluorobutoxy)carbonyl]hydrazinecarbonyl}-1,3-dioxan-5-yl]acetamide

NMM (80 μ L, 0.7 mmol) and isobutyl carbonochloridate (90 μ L, 0.7 mmol) were added to a solution of *trans*-5-[2-(4-chloro-3-fluorophenoxy)acetamido]-1,3-dioxane-2-carboxylic acid (88%, 274 mg, 0.7 mmol, Intermediate 8) in anhydrous THF (6.5 mL) at 0 °C under N₂ and stirred for 15 min. 4,4,4-Trifluorobutyl *N*-aminocarbamate (135 mg, 0.7 mmol, Intermediate 11) was added and the reaction mixture was warmed to r.t. and stirred for 17 h. The reaction mixture was cooled to 0 °C and additional NMM (40 μ L, 0.35 mmol) and isobutyl carbonochloridate (45 μ L, 0.35 mmol) were added. The reaction mixture was warmed to r.t. and stirred for 1 h. The reaction mixture was diluted with EtOAc (30 mL) and washed with H₂O (30 mL), satd aq NaHCO₃ solution (30 mL), brine (30 mL), dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The resulting solid was triturated with ACN to afford the title compound (222 mg, 0.442 mmol, 61% yield) as a white solid; ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.92 (s, 1H), 9.25 – 8.65 (m, 1H), 8.10 (d, *J* = 7.6 Hz, 1H), 7.50 (t, *J* = 8.9 Hz, 1H), 7.08 (dd, *J* = 11.3, 2.8 Hz, 1H), 6.85 (ddd, *J* = 9.0, 2.8, 1.1 Hz, 1H), 4.89 (s, 1H), 4.54 (s, 2H), 4.14 – 3.96 (m, 5H), 3.67 – 3.51 (m, 2H), 2.39 – 2.19 (m, 2H), 1.87 – 1.61 (m, 2H); ¹⁹F{¹H} NMR (376 MHz, chloroform-*d*) δ -64.91- -65.02 (m, 3F), -113.96 (s, 1F); *M/Z*: 502, 504 [M+H]⁺, ESI⁺, RT = 0.98 (S2).

Example 5 (step 15.b): 2-(4-chloro-3-fluorophenoxy)-N-[*trans*-2-[5-(4,4,4-trifluorobutoxy)-1,3,4-oxadiazol-2-yl]-1,3-dioxan-5-yl]acetamide

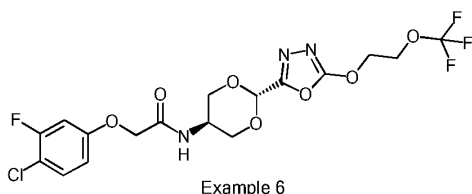


To a suspension of 2-(4-chloro-3-fluorophenoxy)-N-[*trans*-2-{*N'*-[(4,4,4-trifluorobutoxy)carbonyl]hydrazinecarbonyl}-1,3-dioxan-5-yl]acetamide (162 mg, 0.32 mmol) in ACN (5 mL) was added K₂CO₃ (223 mg, 1.6 mmol) and TsCl (154 mg, 0.81 mmol) at r.t. The resulting mixture was heated to 80 °C and stirred for 1.5 h. The reaction mixture was cooled to r.t. and diluted with DCM (30 mL), and washed with H₂O (30 mL), satd aq NaHCO₃ solution (30 mL), brine (30 mL), dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. Purification by prep. HPLC (Method 3) afforded the title compound (65 mg, 0.13 mmol, 41% yield) as a viscous, colourless gum; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.19 (d, *J* = 7.6 Hz, 1H), 7.50 (t, *J*

= 8.9 Hz, 1H), 7.08 (dd, $J = 11.3, 2.8$ Hz, 1H), 6.85 (dd, $J = 8.9, 1.8$ Hz, 1H), 5.82 (s, 1H), 4.62 – 4.45 (m, 4H), 4.20 – 3.99 (m, 3H), 3.82 – 3.67 (m, 2H), 2.10 – 1.92 (m, 2H); $^{19}\text{F}\{^1\text{H}\}$ NMR (376 MHz, chloroform- d) δ -64.89 (3F, s), -113.95 (1F, s); M/Z : 484, 486 $[\text{M}+\text{H}]^+$, ESI $^+$, RT = 3.42 (S4).

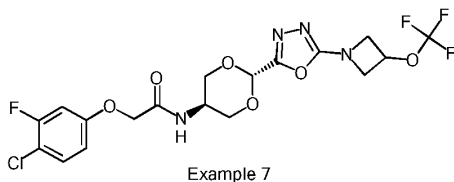
5 The following examples were prepared using route 15.

Example 6: 2-(4-chloro-3-fluorophenoxy)-*N*-[*trans*-2-{5-[2-(trifluoromethoxy)ethoxy]-1,3,4-oxadiazol-2-yl}-1,3-dioxan-5-yl]acetamide



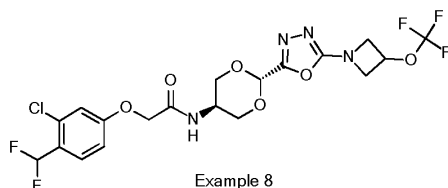
10 Using Intermediate 8 and Intermediate 12; ^1H NMR (400 MHz, DMSO- d_6) δ 8.22 (d, $J = 7.7$ Hz, 1H), 7.51 (t, $J = 8.9$ Hz, 1H), 7.08 (dd, $J = 11.4, 2.8$ Hz, 1H), 6.85 (ddd, $J = 8.9, 2.8, 1.1$ Hz, 1H), 5.84 (s, 1H), 4.79 – 4.66 (m, 2H), 4.56 (s, 2H), 4.50 – 4.42 (m, 2H), 4.22 – 4.01 (m, 3H), 3.75 (t, $J = 10.1$ Hz, 2H); M/Z : 486, 488 $[\text{M}+\text{H}]^+$, ESI $^+$, RT = 3.35 (S4).

Example 7: 2-(4-chloro-3-fluorophenoxy)-*N*-[*trans*-2-{5-[3-(trifluoromethoxy)azetidin-1-yl]-1,3,4-oxadiazol-2-yl}-1,3-dioxan-5-yl]acetamide



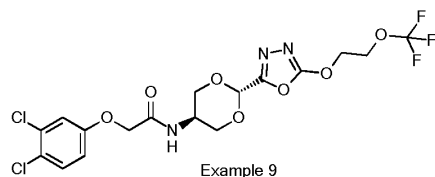
15
20 Using Intermediate 8 and Intermediate 13; ^1H NMR (400 MHz, DMSO- d_6) δ 8.18 (d, $J = 7.6$ Hz, 1H), 7.50 (t, $J = 8.9$ Hz, 1H), 7.08 (dd, $J = 11.3, 2.8$ Hz, 1H), 6.85 (ddd, $J = 9.0, 2.8, 1.2$ Hz, 1H), 5.78 (s, 1H), 5.32 (tt, $J = 7.0, 4.1$ Hz, 1H), 4.59 – 4.43 (m, 4H), 4.26 (dd, $J = 9.7, 4.0$ Hz, 2H), 4.18 – 4.01 (m, 3H), 3.74 (t, $J = 9.6$ Hz, 2H); M/Z : 497, 499 $[\text{M}+\text{H}]^+$, ESI $^+$, RT = 3.26 (S4).

Example 8: 2-[3-chloro-4-(difluoromethyl)phenoxy]-*N*-[*trans*-2-{5-[3-(trifluoromethoxy)azetidin-1-yl]-1,3,4-oxadiazol-2-yl}-1,3-dioxan-5-yl]acetamide



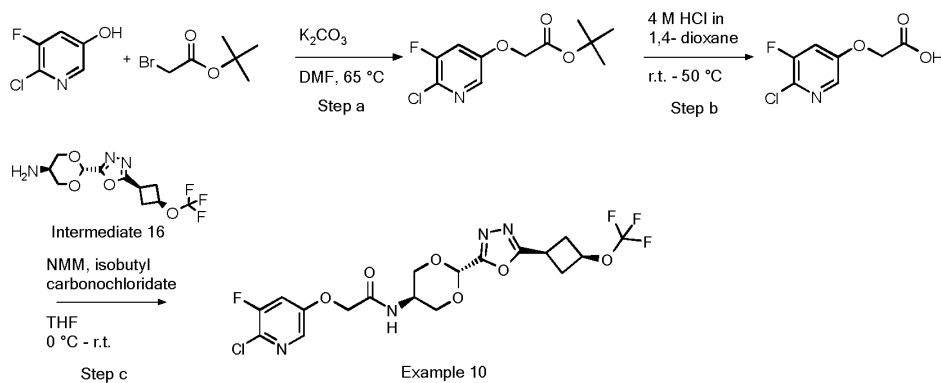
Using Intermediate 9 and Intermediate 13: ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.21 (d, $J = 7.5$ Hz, 1H), 7.63 (d, $J = 8.8$ Hz, 1H), 7.28 – 6.97 (m, 3H), 5.79 (s, 1H), 5.32 (tt, $J = 7.0, 4.1$ Hz, 1H), 4.62 (s, 2H), 4.51 (dd, $J = 9.6, 6.8$ Hz, 2H), 4.26 (dd, $J = 9.6, 4.0$ Hz, 2H), 4.18 – 4.01 (m, 3H), 3.81 – 3.67 (m, 2H); ^{19}F NMR (376 MHz, $\text{DMSO-}d_6$) δ -58.47, -112.49 (d, $J = 54.6$ Hz); M/Z : 529, 531 $[\text{M}+\text{H}]^+$, ESI^+ , $\text{RT} = 3.38$ (S4).

Example 9: 2-(3,4-dichlorophenoxy)-*N*-[*trans*-2-{5-[2-(trifluoromethoxy)ethoxy]-1,3,4-oxadiazol-2-yl}-1,3-dioxan-5-yl]acetamide



Using Intermediate 15 and Intermediate 12: ^1H NMR (500 MHz, $\text{chloroform-}d$) δ 7.40 (d, $J = 8.9$ Hz, 1H), 7.08 (d, $J = 2.9$ Hz, 1H), 6.85 – 6.79 (m, 2H), 5.83 (s, 1H), 4.77 – 4.71 (m, 2H), 4.49 (s, 2H), 4.41 (dd, $J = 11.7, 3.6$ Hz, 2H), 4.37 – 4.33 (m, 2H), 4.33 – 4.26 (m, 1H), 3.82 (dd, $J = 11.7, 6.4$ Hz, 2H); M/Z : 502, 504, 506 $[\text{M}+\text{H}]^+$, ESI^+ , $\text{RT} = 3.56$ (S4).

Scheme for route 16

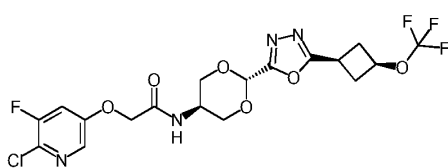


Step 16.a: *tert*-butyl 2-[(6-chloro-5-fluoropyridin-3-yl)oxy]acetate

To a solution of 6-chloro-5-fluoropyridin-3-ol (4.90 g, 33.2 mmol) in DMF (50 mL) was added *tert*-butyl 2-bromoacetate (4.5 mL, 34.9 mmol) and K₂CO₃ (13.8 g, 0.0996 mol) and the resultant mixture was stirred at 65 °C for 2 h. The reaction mixture was cooled to r.t., suspended in EtOAc (100 mL), and washed with H₂O (2 × 50 mL) and brine (50 mL). The combined organic extracts were dried over Na₂SO₄ and concentrated *in vacuo* to afford the title compound (9.00 g, 32.7 mmol, 98% yield) as a brown oil; ¹H NMR (500 MHz, chloroform-*d*) δ 7.91 (d, *J* = 2.6 Hz, 1H), 7.07 (dd, *J* = 9.1, 2.6 Hz, 1H), 4.55 (s, 2H), 1.53 – 1.39 (m, 9H); *M/Z*: 262, 264 [M+H]⁺, ESI⁺, RT = 1.00 min (S2).

Step 16.b: 2-[(6-chloro-5-fluoropyridin-3-yl)oxy]acetic acid

4 M HCl in 1,4- dioxane (25 mL, 98.0 mmol) was added to *tert*-butyl 2-[(6-chloro-5-fluoropyridin-3-yl)oxy]acetate (9.00 g, 32.7 mmol) and the resultant mixture was stirred at r.t. for 2 h. A further portion of 4 M HCl in 1,4- dioxane (25 mL, 98.0 mmol) was added and the reaction mixture was stirred at 50 °C for 5 h. The reaction mixture was concentrated *in vacuo* and then triturated using Et₂O and heptane. The resultant precipitate was filtered under vacuum to afford the title compound (6.48 g, 31.2 mmol, 96% yield) as an off white solid; ¹H NMR (500 MHz, DMSO-*d*₆) δ 13.22 (s, 1H), 8.07 (d, *J* = 2.6 Hz, 1H), 7.76 (dd, *J* = 10.4, 2.6 Hz, 1H), 4.85 (s, 2H); *M/Z*: 206, 208 [M+H]⁺, ESI⁺, RT = 0.60 min (S2).

Example 10 (step 16.c): 2-[(6-chloro-5-fluoro-3-pyridyl)oxy]-*N*-[*trans*-2-[5-[*cis*-3-(trifluoromethoxy)cyclobutyl]-1,3,4-oxadiazol-2-yl]-1,3-dioxan-5-yl]acetamide

Example 10

A solution of 2-[(6-chloro-5-fluoropyridin-3-yl)oxy]acetic acid (34 mg, 0.163 mmol) in THF (2 mL) was cooled to 0 °C and treated with isobutyl carbonochloridate (20 μL, 0.155 mmol) and NMM (18 μL, 0.163 mmol). The reaction was stirred for 15 min then a solution of *trans*-2-[5-[*cis*-3-(trifluoromethoxy)cyclobutyl]-1,3,4-oxadiazol-2-yl]-1,3-dioxan-5-amine (50 mg, 0.163 mmol, Intermediate 16, described in route 12) in THF (1 mL) was added dropwise. The reaction was stirred at r.t. for 1 h, quenched with H₂O (2 drops) and concentrated *in vacuo*.

Purification by prep. HPLC (Method 3) afforded the title compound (37 mg, 0.0745 mmol, 46% yield) as a white powder; ^1H NMR (500 MHz, chloroform-*d*) δ 8.03 (d, J = 2.6 Hz, 1H), 7.17 (dd, J = 8.8, 2.6 Hz, 1H), 6.78 (d, J = 8.4 Hz, 1H), 5.96 (s, 1H), 4.77 – 4.67 (m, 1H), 4.57 (s, 2H), 4.49 – 4.41 (m, 2H), 4.39 – 4.28 (m, 1H), 3.85 (dd, J = 11.7, 6.4 Hz, 2H), 3.44 – 3.32 (m, 1H), 2.95 – 2.84 (m, 2H), 2.78 – 2.66 (m, 2H); M/Z : 497, 499 $[\text{M}+\text{H}]^+$, ESI $^+$, RT = 3.15 (S4).

II Biological Assay

10 HEK-ATF4 High Content Imaging assay

Example compounds were tested in the HEK-ATF4 High Content Imaging assay to assess their pharmacological potency to prevent Tunicamycin induced ISR. Wild-type HEK293 cells were plated in 384-well imaging assay plates at a density of 12,000 cells per well in growth medium (containing DMEM/F12, 10% FBS, 2mM L-Glutamine, 100 U/mL Penicillin - 100 $\mu\text{g}/\text{mL}$ Streptomycin) and incubated at 37°C, 5% CO₂. 24-hrs later, the medium was changed to 50 μL assay medium per well (DMEM/F12, 0.3% FBS, 2mM L-Glutamine, 100 U/mL Penicillin - 100 $\mu\text{g}/\text{mL}$ Streptomycin). Example compounds were serially diluted in dimethyl sulfoxide (DMSO), spotted into intermediate plates and prediluted with assay medium containing 3.3 μM Tunicamycin to give an 11-fold excess of final assay concentration. In addition to the example compound testing area, the plates also contained multiples of control wells for assay normalization purposes, wells containing Tunicamycin but no example compounds (High control), as well as wells containing neither example compound nor Tunicamycin (Low control). The assay was started by transferring 5 μL from the intermediate plate into the assay plates, followed by incubation for 6 hrs at 37°C, 5% CO₂. Subsequently, cells were fixed (4% PFA in PBS, 20 min at room temperature) and submitted to indirect ATF4 immunofluorescence staining (primary antibody rabbit anti ATF4, clone D4B8, Cell Signaling Technologies; secondary antibody Alexa Fluor 488 goat anti-rabbit IgG (H+L), Thermofisher Scientific). Nuclei were stained using Hoechst dye (Thermofisher Scientific), and plates were imaged on an Opera Phenix High Content imaging platform equipped with 405nm and 488nm excitation. Finally, images were analyzed using script based algorithms. The main readout *HEK-ATF4* monitored the ATF4 signal ratio between nucleus and cytoplasm. Tunicamycin induced an increase in the overall ATF4 ratio signal, which was prevented by ISR modulating example compounds. In addition, HEK-CellCount readout was derived from counting the number of

stained nuclei corresponding to healthy cells. This readout served as an internal toxicity control. The example compounds herein did not produce significant reduction in CellCount.

Activity of the tested example compounds is provided in Table 3 as follows:

- 5 +++ = IC₅₀ 1-500 nM; ++ = IC₅₀ >500-2000 nM; + = IC₅₀ >2000-15000 nM.

Table 3

Example number	Activity
1	+++
2	+++
3	+
4	++
5	+++
6	+++
7	+++
8	+++
9	+++
10	+

10 Protocol - Measure of the Effect on hERG Channel by Tail Current Recording Using in Vitro Rapid ICE

- 15 The potency of the example compounds in inhibiting human ERG potassium channel (hERG) tail current was assessed in a recombinant HEK293 cell line stably transfected with hERG cDNA under an inducible promoter, using Rapid ICE (rapid ion channel electrophysiology) assay. Rapid ICE is an automated patch-clamp assay utilizing the QPatch HTX system (Sophion Bioscience A/S). Briefly, inducible HEK hERG cells were cultivated in minimum essential medium supplemented with 10% FBS, 1% non-essential amino acids, 1% sodium pyruvate, 2 mM l-glutamine, 15 µg/mL blasticidin, and 100 µg/mL hygromycin. hERG channel expression induction was obtained by adding 10 µg/mL tetracycline for 24, 48, or 72 h before recordings.

On the day of the experiment, cells were detached with TrypLE and prepared to be loaded on the instrument. Cells were resuspended in 7 mL of Serum-Free Media containing 25 mM Hepes and soybean trypsin inhibitor and immediately placed in the cell storage tank of the machine. The composition of the extracellular buffer was (mM): NaCl 137, KCl 4, CaCl₂ 1.8, MgCl₂ 1.0, *d*-glucose 10, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) 10, pH 7.4 with 1 M NaOH. The composition of the intracellular solution was (mM): KCl 130, MgCl₂ 1.0, ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) 5, MgATP 5, HEPES 10, pH 7.2 with 1 M KOH. The voltage protocol included the following steps: step from -80 to -50 mV for 200 ms, +20 mV for 4.8 s, step to -50 mV for 5 s, then step to the holding potential of -80 mV. Example compounds were dissolved in DMSO and diluted in extracellular buffer to achieve final test concentrations (0.3, 3, and 30 μM) in 0.3% DMSO. The voltage protocol was run and recorded continuously during the experiment. The vehicle, corresponding to 0.3% DMSO in extracellular buffer was then applied for 3 min, followed by the example compound in triplicate. The standard combined exposure time was 5 min. The average of tail current amplitude values recorded from four sequential voltage pulses was used to calculate for each cell the effect of the example compound by calculating the residual current (% control) compared with vehicle pretreatment. Data were reported as % inhibition for each concentration tested, and IC₅₀ values were estimated using QPatch software. At least two cells were tested, and even more if results diverged.

20

Table 5

Example number	hERG Activity*
2	+
5	++
6	+
7	+
8	+
9	+
10	+

*hERG Activity category: +++ = IC₅₀ 1-1000 nM; ++ = IC₅₀ >1000-5000 nM; + = IC₅₀ >5000 nM

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5

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