



(51) International Patent Classification:

C07F 9/12 (2006.01) C07F 9/38 (2006.01)
C07F 9/40 (2006.01)

(21) International Application Number:

PCT/NL2024/050337

(22) International Filing Date:

26 June 2024 (26.06.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/523,289 26 June 2023 (26.06.2023) US
63/660,065 14 June 2024 (14.06.2024) US

(71) Applicants: **TECHNISCHE UNIVERSITEIT EINDHOVEN** [NL/NL]; Groene Loper 3, 5612 AE Eindhoven (NL). **ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI** [US/US]; One Gustave L Levy Place, New York, New York 10029 (US). **UNIVERSITÄT DUISBURG-ESSEN** [DE/DE]; Universitätsstrasse 2, 45141 Essen (DE).

(72) Inventors: **BRUNSVELD, Lucas**; Groene Loper 3, 5612 AE Eindhoven (NL). **OTTMANN, Christian**; Groene Loper 3, 5612 AE Eindhoven (NL). **PENNINGS, Marloes Anna Maria**; Groene Loper 3, 5612 AE Eindhoven (NL). **VISSER, Emira Josien**; Groene Loper 3, 5612 AE Eindhoven (NL). **COSSAR, Peter James**; Groene Loper 3, 5612 AE Eindhoven (NL). **KATZ, Liora S.**; One Gustave L Levy Place, New York, New York 10029 (US). **SCOTT, Donald K.**; One Gustave L Levy Place, New York, New York 10029 (US). **PLITZKO, Kathrin Felicitas**; Universitätsstrasse 2, 45141 Essen (DE). **KAISER, Markus**; Universitätsstrasse 2, 45141 Essen (DE).

(74) Agent: **ALGEMEEN OCTROOI- EN MERKENBUREAU B.V.**; P.O. Box 645, 5600 AP Eindhoven (NL).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH,

TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: SMALL-MOLECULE STABILIZERS OF THE CHREBP 14-3-3 INTERACTION

(57) Abstract: The glucose-responsive transcription factor ChREBP (Carbohydrate Response Element Binding Protein) is a key mediator in the response to glucose in pancreatic β -cells, strongly regulating glycolytic and lipogenic pathways. Several protein regulators interact with ChREBP that are involved in its activation mechanism, including 14-3-3 protein. Small-molecules are provided, which are useful as stabilizers for ChREBP 14-3-3 complexes, which were found highly useful for medical treatments, i.a. in type 1 and type 2 diabetes.

Title: Small-molecule stabilizers of the ChREBP 14-3-3 interaction

Introduction

The chronic metabolic disease of type 2 diabetes (T2D) affects more than million people worldwide, and is expected to rise to 693 million by 2045, making it one of the leading causes of global morbidity. T2D is characterized by the body's loss of control over blood sugar which results from pancreatic β -cell dysfunction leading to insulin deficiency and Insulin Resistance (IR) in target organs. This leads to various medical complications including, but not limited to, cardiovascular disease, kidney disease, cancer and impaired vision. The global epidemic of T2D is mainly driven by the rise in obesity, sedentary lifestyles and population ageing. Strong evidence shows that many cases of T2D could be prevented by maintaining a healthy lifestyle. However, the globally increasing number of patients with T2D, in combination with the progressive nature of it, demands the need for novel antidiabetic agents to arrest this process. The currently available anti-T2D drugs (e.g. metformin) aim to increase pancreatic β -cell insulin production or enhance systematic insulin sensitivity. However, to date, only a handful of protein candidates have been identified that can be targeted for treating T2D.

An emerging potential target to treat T2D is the glucose-responsive transcription factor ChREBP (Carbohydrate Response Element Binding Protein). ChREBP is a key mediator in the response to glucose in pancreatic β -cells, strongly regulating glycolytic and lipogenic pathways. Several protein regulators interact with ChREBP that are involved in its activation mechanism, including the 14-3-3 β protein. The "hub" protein 14-3-3 is involved in numerous signaling pathways, as well as in the pathophysiologic state of diabetes. ChREBP interacts with 14-3-3 via an alpha helix in its N-terminal domain (residues 117-137) and is one of the few phosphorylation-independent 14-3-3 partner proteins described. A free sulfate or phosphate ion in the 14-3-3 phospho-accepting pocket interact with both proteins (Figure 12A). Adenosine MonoPhosphate (AMP) has also been reported to bind this phospho-accepting pocket, thereby mildly stabilizing the protein complex and enhancing 14-3-3's regulation of ChREBP cytosol-nuclear trafficking (Figure 12B). The development of small molecule stabilizers of the ChREBP–14-3-3 Protein-Protein Interaction (PPI) are potentially valuable tools to suppress glucolipotoxicity in T2D by inhibiting the transcriptional activity of ChREBP.

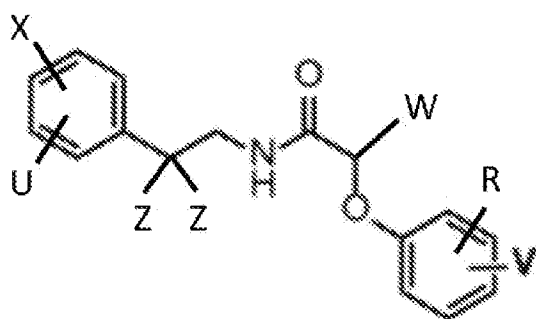
Of ChREBP two central splice isoforms exist: ChREBP- α and ChREBP- β . The ChREBP- α isoform contains the low glucose inhibitor domain (LID, including the Nuclear Export Signal (NES)) at its N-terminal region. 14-3-3 is understood to bind to an alpha-helix in this region, keeping ChREBP- α inactive by cytoplasmic retention during low glucose levels (Figure 2A). ChREBP- β lacks this N-terminal domain, and so has no steric hindrance of its activation domain, resulting in a nuclear constitutively active transcription factor. An increase in glucose levels is understood to cause ChREBP- α to disassociate from 14-3-3 and translocate to the nucleus to subsequently induce ChREBP- β transcription, ultimately resulting in glucose-stimulated β -cell proliferation to meet of the demand for insulin (Figure 2A). ChREBP- β expression is understood to be driven by Carbohydrate Response Elements (ChoREs), located near its own alternative transcription site. Under (prolonged) hyperglycemia conditions (Figure 2B) a robust positive feedback loop is understood to be triggered in which the newly produced ChREBP- β binds to its own ChoRE leading to more ChREBP- β synthesis. Overexpression of ChREBP- β is believed to give rise to glucotoxicity or glucolipotoxicity resulting in β -cell apoptosis and a reduction of β -cell mass, consequently causing a ferocious cycle with ever-increasing glucose concentrations and ever-declining β -cell function.

Description of the invention

This invention relates to small-molecule stabilizers for the ChREBP 14-3-3 complex, such as ChREBP- α 14-3-3 complexes. ChREBP- α when complexed with 14-3-3 is retained in the cytoplasm. When not in complex, it is translocated to the nucleus where ChREBP- α can induce transcription, in particular of ChREBP- β . Without being bound by theory, ChREBP- β expression, e.g. under (prolonged) hyperglycemic conditions, may give rise to glucotoxicity or glucolipotoxicity in pancreatic β -cells, which consequently may lead to cell death and reduction of the number of β -cells. As complexes formed between ChREBP and 14-3-3 play an important role in pancreatic β -cells, without being bound by theory and as briefly explained above and further explained herein, small-molecules that can stabilize the 14-3-3 and ChREBP interaction are prime candidates for the treatment of type 2 diabetes and the like, as these small molecules have the potential to improve health and/or reduce death of pancreatic β -cells.

The current invention now provides for small molecule stabilizers that, as shown in the examples herein, are not only capable of stabilizing ChREBP 14-3-3 interactions, but are also from a biological perspective safe, effective and/or selective. Such molecules may find use in particular in cellular assays, and, importantly, medical treatments. It was observed that biochemically active compounds, shown to be toxic to cells in cell culture experiments, could retain their biochemical activity and be made non-toxic by modifying the small molecules to include fluoro groups. For example, a compound such as the representative compound 2, as designated herein and as shown in the examples in Section I, which was shown to have toxic effects in cell culture, when modified to include fluoro groups, i.e. a geminal difluoro group, such modified compounds as shown herein could substantially retain or even enhance its stabilizing function while at the same time no longer be toxic in cellular assays. Moreover, as shown herein as well, these fluorinated compounds were biological active in cellular assays with regard to the feature of reducing transport of ChREBP- α to the nucleus and consequently reducing induction of transcription of ChREBP- β expression.

Hence, the current invention provides for compounds such as compound 2, and analogs thereof, which have been modified to include a fluoro group, preferably a difluoro, most preferably a geminal difluoro-group. Such novel compounds are described in the examples herein. In particular in one embodiment, such compounds are defined by formula Va:



(formula Va)

wherein at least one of Z is F, and the other Z is H, preferably wherein both of Z are F;

wherein X is selected from H, F, Br, Cl, CF₃, OCH₂Ph, and p-OCH₂Pyr (*N*-2, *N*-3 or *N*-4);

U and W are selected from F and H;

R is selected from H, CH₃ or F; and

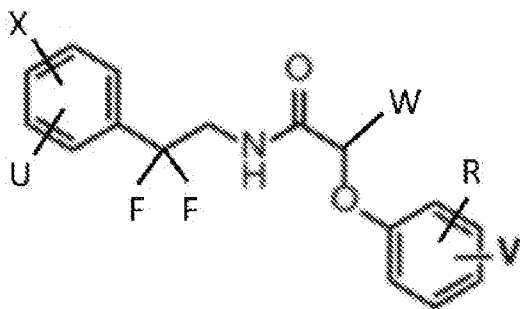
5 V is selected from PO(OH)₂ and OPO(OH)₂.

It is understood that for the X and U-groups, and for the R and V-groups as well, these groups can be positioned at the meta, ortho and para position. It is understood that in case an X group is placed at the meta position, this means that the U group is to be placed at the para or ortho position.

10 The Z groups in accordance with the invention highly preferably represent a geminal difluoro group, but in accordance with the invention a monofluoro is contemplated as well.

In another embodiment, a compound in accordance with the invention is provided in accordance with formula Vb:

15



(formula Vb)

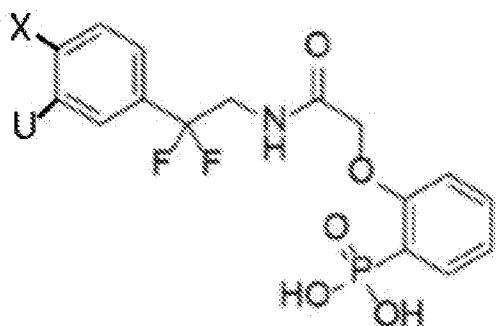
wherein X is selected from H, F, Br, Cl, CF₃, OCH₂Ph, and p-OCH₂Pyr (*N*-2, *N*-3 or *N*-4);

20 U and W are selected from F and H;

R is selected from H, CH₃ or F; and

V is selected from PO(OH)₂ and OPO(OH)₂.

In yet another embodiment, a compound is provided in accordance with the invention, which is as defined by formula VI:

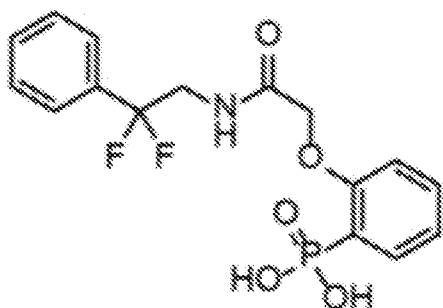


(formula VI)

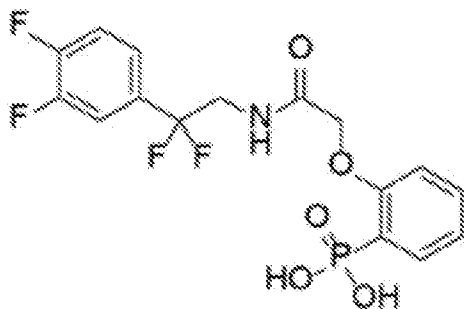
wherein X is selected from H, F, Br, Cl, CF₃, OCH₂Ph, and p-OCH₂Pyr and U is
 5 selected from F and H. It is understood that like above as explained for compounds in accordance with formula Va, instead of *geminal*-difluoro group, a monofluoro group may be contemplated.

In a further embodiment, for compounds such as defined in accordance with the invention in accordance with formula Va, Vb, VI, and VII, as described herein, at least
 10 one of X and U is F. In another further embodiment, for compounds such as defined in accordance with the invention in accordance with formula Va, Vb, VI, and VII, as described herein, both of X and U are F.

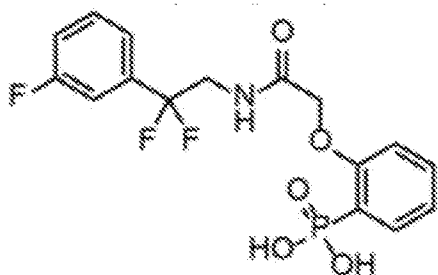
In another embodiment, the compound in accordance with the invention is



or

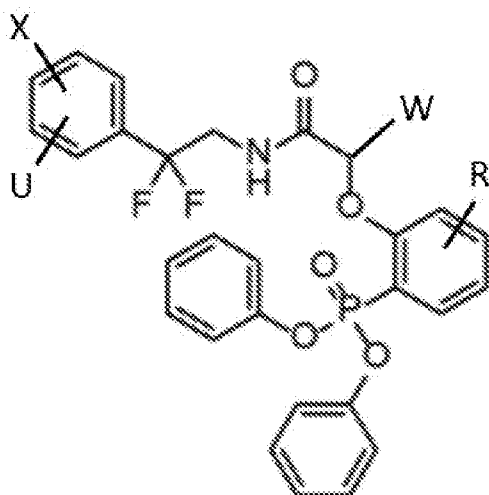


or



Compounds such as described above and disclosed herein may be highly advantageously used for the preparation of pharmaceuticals. Hence, compounds may be selected to be in the form of a pharmaceutically acceptable form. Pharmaceutically acceptable salts that may be contemplated may include e.g. salt forms of deprotonated OH groups from the phosphate group that is present, such as a sodium salt or the like. The skilled person is well capable of preparing and selecting a suitable pharmaceutical acceptable salt of the compounds in accordance with the invention.

Likewise, these compounds may be selected to be in the form of a pharmaceutically prodrug. Prodrugs are compounds that are inactive and upon conversion in the body, or in cells when tested e.g. in biologically relevant assays, become active. Hence upon conversion of the prodrug, a compound as defined in accordance with formula Va, Vb, and VI can be formed. As shown in the examples in section I herein, some prodrug forms were made and tested, e.g. compounds I, II, III and IV as depicted in Figure 21. However, some of these prodrugs were shown to induce signs of cytotoxic and some form not. Hence, the skilled person is well capable of selecting and testing appropriate pharmaceutically acceptable prodrugs that are capable of inducing the desired biological effects while at the same time are not cytotoxic. Hence, in one embodiment, the compound is in the form of formula VII, as this form of prodrug was exemplary in being both active and not cytotoxic as shown in



the examples:

(formula VII)

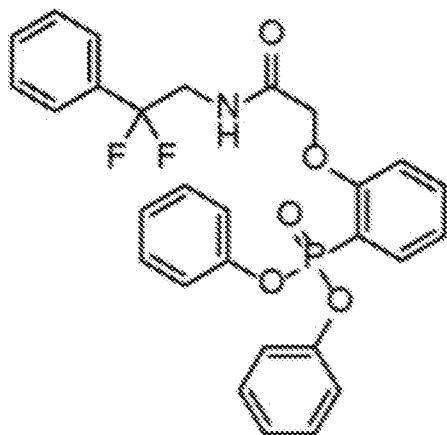
wherein X is selected from H, F, Br, Cl, CF₃, OCH₂Ph, and p-OCH₂Pyr (N-2, N-3 or N-4);

U and W are selected from F and H;

R is selected from H, CH₃ or F; and

V is selected from PO(OH)₂ and OPO(OH)₂. It is understood that like above as explained for compounds in accordance with formula Va, instead of *geminal*-difluoro group, a monofluoro group may be contemplated.

In a further embodiment, a pharmaceutically acceptable prodrug is:



5 *Use of the compounds in accordance with the invention*

As shown in the examples herein, the compounds provided in accordance with the invention may find use in interacting with a first protein and a client protein, wherein the first protein and client protein form a complex, and wherein the compound in accordance with the invention stabilizes the complex. It is understood that the compound is comprised in the complex formed between the first protein and client protein. Hence, the complex formed in the presence of the compound comprises the first protein, the client protein and the compound in accordance with the invention. The complex thus formed highly preferably and advantageously in accordance with the invention is to be more stable as compared with the complex formed in the absence of the compound. Hence, the invention provides for a use of the compound in accordance with the invention, wherein the compound is capable of interacting with a first protein and a client protein and wherein the first protein and the client protein and the compound form a complex, for stabilizing the complex. Such use as shown in the example section may be useful for example for studying the interactions between a first protein and a client protein. Such uses may include also all kinds of in vitro assays, including cellular assays, studying effects of compounds on translocation of complexes from the cytoplasm to the nucleus and/or effects on transcription, e.g. for studying effects on ChREBP- β expression induced by such compounds.

Hence, in a further embodiment, a use of the compounds in accordance with the invention is provided, wherein the first protein is a 14-3-3 protein and the client

protein is a phosphorylation-independent 14-3-3 client protein, preferably wherein the client protein is a ChREBP protein such as ChREBP- α .

As used herein, the term “ChREBP” refers to a glucose responsive-transcription element (Carbohydrate Response Element Binding Protein) which is a mediator in the response to glucose in pancreatic β -cells, strongly regulating glycolytic and lipogenic pathways. ChREBP is a phosphorylation-independent 14-3-3 partner protein. Several protein regulators interact with ChREBP that are involved in its activation mechanism, including 14-3-3 proteins, such as the 14-3-3 β protein. ChREBP interacts with 14-3-3 proteins and forms protein complexes. Adenosine MonoPhosphate (AMP) has been reported to mildly stabilize the protein complex and enhancing 14-3-3's regulation of ChREBP- α cytosol-nuclear trafficking (Figure 12B). ChREBP (e.g. uniprot reference Q9NP71) consists as different isoforms, i.a. ChREBP- α (Q9NP71-1) and ChREBP- β (Q9NP71-2). The ChREBP- α isoform contains the low glucose inhibitor domain (LID, including the Nuclear Export Signal (NES)) at its N-terminal region. 14-3-3 binds to an alpha-helix in this region, keeping ChREBP- α inactive by cytoplasm retention during low glucose levels (Figure 2A). ChREBP- β mRNA is transcribed from a ChoRE-containing promoter located upstream of the ChREBP- α transcriptional start site, in which exon 1b is spliced to exon 2, bypassing exon 1a, and retaining the remainder of the ChREBP- α exons. This results in a transcript that produces a 687-amino acid protein (as opposed to the 864-amino acids of ChREBP- α) that lacks both the nuclear export signal and the sterically-repressive low glucose inhibitory domain.

The term “14-3-3 protein” as used herein refers to a protein (or portion thereof) that is a member of the 14-3-3 protein family, including, but not limited to, various human isoforms (β , γ , ϵ , ζ , η , τ/θ and σ). The term can refer to one specific isoform or to multiple isoforms, such as the various human isoforms. 14-3-3 proteins may interact with more than 300 different partners (client proteins), including Raf kinases, heat shock proteins, oncogenes, and tumor suppressors (Mackintosh, Biochem J (2004) 381 (2): 329–342). 14-3-3 proteins are central regulators in many biological processes and pathologies. The 14-3-3 protein may be 14-3-3 σ (14-3-3sigma) (e.g., Entrez 2810, UniProt P31947, RefSeq NP_006133). The 14-3-3 protein may be 14-3-3 β (14-3-3beta) (e.g., Entrez 7529, UniProt P31946, Q4VY19, RefSeq NP_003395). The 14-3-3 protein may be 14-3-3 ϵ (14-3-3epsilon) (e.g., Entrez 7531, UniProt P62258, RefSeq NP_006752). In embodiments, the 14-3 protein may be 14-3-3 η (14-3-3eta) (e.g., Entrez 7533, UniProt Q04917, RefSeq NP_003396). The 14-3-3 protein may be 14-3-

3 γ (14-3-3 γ) (e.g., Entrez 7532, UniProt P61981, RefSeq NP_36611). The 14-3-3 protein may be 14-3-3 τ (14-3-3 τ) (e.g., Entrez 10971, UniProt P27348, RefSeq NP_006817). The 14-3-3 protein may be 14-3-3 ζ (14-3-3 ζ) (e.g., Entrez 7534, UniProt P63104, RefSeq NP_003397).

5 Hence, in one embodiment, the compounds in accordance with the invention are for use in stabilizing the formation of a complex between ChREBP and 14-3-3 protein. In another embodiment, the compounds in accordance with the invention are for use in stabilizing the formation of a complex between ChREBP- α and 14-3-3 protein. In yet another embodiment, the compounds in accordance with the invention
10 are for use in stabilizing the formation of a complex between ChREBP and 14-3-3 β protein. In yet another embodiment, the compounds in accordance with the invention are for use in stabilizing the formation of a complex between ChREBP and 14-3-3 σ protein. In still yet another embodiment, the compounds in accordance with the invention are for use in stabilizing the formation of a complex between ChREBP- α and
15 14-3-3 β . In still yet another embodiment, the compounds in accordance with the invention are for use in stabilizing the formation of a complex between ChREBP- α and 14-3-3 σ protein.

 Furthermore, as shown in the example section, the compounds in accordance with the invention in another embodiment are selective for the ChREBP and 14-3-3
20 complex. It is understood that selective in accordance with the invention means that this particular interaction between ChREBP and 14-3-3 protein, such as ChREBP- α and 14-3-3 protein, is stabilized, whereas the interaction between 14-3-3 protein and another client protein other than ChREBP are not stabilized. As shown in the examples, it was observed that compounds in accordance with the invention did not
25 apparently stabilize interactions between 14-3-3 β and client proteins such as BRAF, CRAF, ER α Exo-S, P65, Pin1 and USP8 (Figure 7B).

 It is understood that the complexes thus formed may be retained in the cytoplasm, such as shown in the examples herein, e.g. for complexes comprising ChREBP- α . Hence, the use of the compounds in accordance with the invention may
30 be for use in reducing translocation in a cell of the complex from the cytoplasm to the nucleus. As ChREBP- α is involved in transcription, which takes place in the nucleus, such use may in some embodiments include repression of ChREBP- α mediated gene expression. As in particular ChREBP- β transcription may be reduced by the

compound, in another embodiment, the compound may be for use in repression of ChREBP transcription.

As the compounds in accordance with the invention can be considered safe, effective and selective, these compounds can be considered highly suitable candidates for use in medical treatment.

In any case, without being bound by theory and/or the specific observations made and as shown in the examples, the effect of the compounds in various assays show that the compounds in accordance with the invention have an advantageous effect on parameters related to i.a. Type 2 Diabetes and appear to be both safe and selective. Hence, compounds in accordance with the invention are suitable for medical treatments, in particular of conditions such as Type 2 Diabetes and the like.

Hence, in one embodiment, a compound in accordance with the invention is provided for use in a medical treatment. In a further embodiment, such use involves the treatment of Type 2 Diabetes. In another further embodiment, such treatment involves reducing or preventing glucose toxicity. In yet another embodiment such treatment involves reducing or preventing glucolipotoxicity. Glucolipotoxicity is a phenomenon that plays a role in type 2 diabetes (T2D) and is regarded a pathogenic consequence of glucose and lipid toxicity causing additional damaging or toxic effects on the pancreatic β -cell. In still, yet another embodiment, such use involves the treatment of a condition characterized by hyperglycemia, such as a hyperglycemic condition, e.g. a prolonged hyperglycemic condition. Furthermore, as shown in the examples herein, the compounds in accordance with the invention prevent dedifferentiation of β -cells, i.e. preserve β -cell identity under conditions relevant for type 2 Diabetes. Hence, the compounds in accordance with the invention, for use in the medical treatment of type 2 Diabetes may affect β -cells in patients, allowing to preserve β -cell identity and/or prevent dedifferentiation of β -cells.

It is understood that the compounds in accordance with the invention in particular find use in the treatment of human conditions.

Further medical treatments

In addition to the highly advantageous use of the compounds in accordance with the invention in the treatment of type 2 Diabetes, the present invention provides for further

highly useful medical uses of the compounds in accordance with the invention. Such further medical uses include the treatment of type 1 Diabetes and the treatment of cancer. Furthermore, the compounds of the present invention also provide for use in altering immune function, in particular in auto-immune disease. Without being bound by theory, compounds in accordance with the invention that are selective for stabilizing the complex of 14-3-3 and ChREBP- α , and/or reduce translocation in a cell of the complex from the cytoplasm to the nucleus, and/or represses ChREBP- α mediated gene expression, and/or represses ChREBP- β transcription, in particular, compounds such as **66**, and the like, can be highly useful in these further medical treatments, such as of the diseases as described herein below.

With regard to Diabetes type 1, it is understood that this is the result of an autoimmune reaction against the β cells, which produce insulin. β cells are found in the pancreas within clusters of cells known as islets of Langerhans. The destructive mechanism involves the invasion of immune cells in the islets of Langerhans, which immune cells secrete cytokines, ultimately resulting in destruction of β cells. The present inventors have now surprisingly found that compounds in accordance with the invention, such as compound **66**, have furthermore advantageous properties that are highly useful for the treatment of Diabetes type 1, in particular for the early stages of disease wherein some β cells are still remaining.

As shown in the examples herein compounds in accordance with the invention, e.g. a stabilizing 14-3-3 ChREBP α compound such as **66**, was shown to protect β cells from cytokine induced cell death, and, in addition improved β cell function. The stabilizing compound was shown to prevent nuclear localization of ChREBP induced by cytokines. Moreover, in a mouse model for type 1 Diabetes, nuclear staining of ChREBP was already observed in the early phase of the disease in β cells, and also in CD45 $^{+}$ immune cells associated with insulinitis. Combined, this indicates that the compounds in accordance with the invention are highly useful in the treatment of type 1 diabetes as well, in particular in the early phase of the disease, wherein substantial amounts of β cells may still be present. By administering a compound in accordance with the invention to patients, β cell function can be improved, β cell killing can be reduced, and/or autoimmune function can be affected, thereby preventing or delaying onset of type 1 diabetes. It is understood that by administering a compound in accordance with the invention to patients, the effect in the treatment can comprise β cell function improvement, or, β cell killing reduction, or, affecting autoimmune

function, or, β cell function improvement and β cell killing reduction, or, β cell killing reduction and affecting autoimmune function, or, affecting autoimmune function and β cell function improvement, or, β cell killing reduction and affecting autoimmune function and β cell function improvement. Such effects are advantageous in the treatment of type 1 diabetes, and the compounds in accordance with the invention can be used in any treatment contemplated for type 1 diabetes, more preferably in the early stage of the disease.

In one embodiment, the compounds in accordance with the invention are highly useful in the treatment of type 1 Diabetes. In another embodiment the compounds in accordance with the invention are for use in the treatment of early stages of type 1 Diabetes. In another embodiment, a compound in accordance with the invention is provided for use in the treatment of patients diagnosed with type 1 diabetes. In the early phase of the disease, patients, usually after presenting an acute episode of hyperglycemia and being diagnosed with type 1 Diabetes, may enter into a period of apparent recovery for several months or more, wherein β cells die and new β cells are formed. The less β cells remain, the less insulin the pancreas makes and the more insulin need to be administered. This period is referred to as the honeymoon phase of type 1 Diabetes and can last from about 1 month up to 1 year. Hence, in particular the compounds of the present invention are for use in the treatment of type 1 Diabetes in patients in the honeymoon period of the disease. The compounds in accordance with the invention may also be used for the treatment of patients being at risk of developing type 1 Diabetes. As long as patients at risk of or diagnosed with type 1 Diabetes have β cells, such β cells can be protected by the compounds of the present invention, which may allow to delay or prevent disease progression, and such patients may benefit from treatment in accordance with the invention.

In addition, beta cell regenerative drugs are at present under development for the treatment of type 1 Diabetes. These drugs are being developed with the aim of regenerating β cells, i.e. to increase their number. The compounds in accordance with the invention may hence find further use when combining the compounds in accordance with the invention with further regenerative medicine and/or treatment. Such regenerative medicine for the treatment of type 1 diabetes include drugs that are aimed at β cell proliferation. Type 1 diabetic patients at late stages of disease, even >50 years after diagnosis, still have some insulin positive β cells. This means that regenerative treatments in such patients may advantageously include treatment with

the compounds of the present invention. Hence, the compounds in accordance with the invention may benefit type 1 Diabetes patients at any stage of disease.

As outlined above, and as shown in the examples herein, as immune cells involved in autoimmunity were shown to express ChREBP, in particular in the context of type 1 diabetes, without being bound by theory, the compounds in accordance with the invention may also be useful in autoimmune diseases in general. Immune cells of which immune cell function may in particular be modulated includes CD45+ immune cells at the site of inflammation. Hence, without being bound by theory, the compounds in accordance with the invention may also be useful in modulating immune responses, e.g. inflammation.

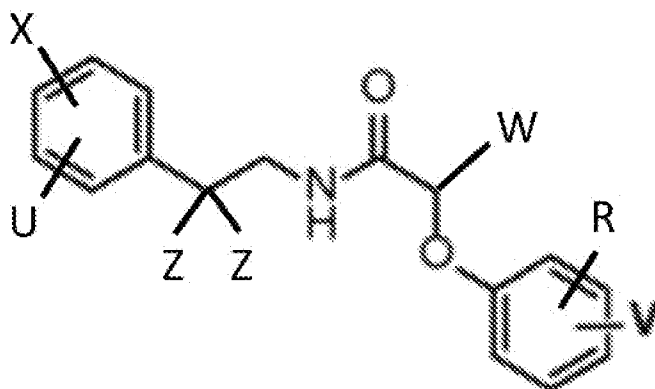
Lastly, as shown in the examples, compounds in accordance with the present invention are also useful in the treatment of cancer. As shown in the examples herein, blocking ChREBP in cancer cells was shown to block proliferation. Hence, in another embodiment, the compounds in accordance with the invention are for use in the treatment of cancer. Preferably, the treatment of cancers as contemplated herein involves the treatment of cancers that are dependent on ChREBP for proliferation. As described in the examples, such suitable cancers can include colon cancer, hepatic cell carcinoma, or insulinoma. As shown in the examples herein, dependency on ChREBP for proliferation can easily be determined in assays assessing the effect of the presence or absence of a compound such as compound 66 on proliferation and/or by staining for ChREBP as shown in the examples herein. The present invention describes numerous assays which may be adapted for use in assessing different kinds of cancer and the suitability for the compounds of the present invention for the treatment of such cancers.

Hence, cancers suitable for treatment with the compounds in accordance with the invention can easily be identified, and may not necessarily be limited to colon cancer, hepatic cell carcinoma and insulinoma. Moreover, it is understood that as many cancers at present when being treated in the clinic often involve combination treatments, i.e. wherein multiple anti-cancer drugs are combined. Hence, the compounds in accordance with the invention may find use in combination treatments of cancers as well.

Embodiments for further medical treatments

15

1. The compound as defined by formula Va:



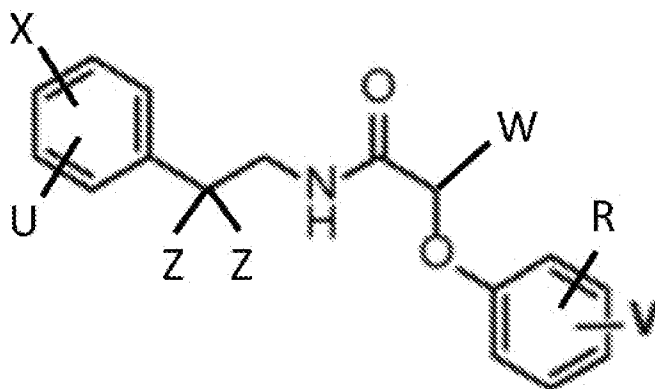
(formula Va).

- 5 wherein one of Z is F, and the other Z is H, preferably wherein both of Z are F;
 wherein X is selected from H, F, Br, Cl, CF₃, OCH₂Ph, and p-OCH₂Pyr (*N*-2, *N*-3 or *N*-4);
 U and W are each individually selected from F and H;
 R is selected from H, CH₃ or F; and
 10 V is selected from PO(OH)₂ and OPO(OH)₂, wherein the compound is for use in the treatment of type I diabetes.
2. The compound for use in accordance with embodiment 1, wherein the treatment is for preventing and/or delaying onset of type 1 diabetes.
- 15 3. The compound for use in accordance with embodiment 1 or embodiment 2, wherein the treatment is for use in the treatment of the honeymoon period of type 1 diabetes.
- 20 4. The compound for use in accordance with any of embodiments 1-3, wherein the honeymoon period is prolonged in patients in the honeymoon period of type 1 diabetes.

5. The compound for use in accordance with any of embodiments 1-4, wherein the β cell function is improved, β cell killing is reduced, and/or autoimmune function affected.

- 5 6. The compound for use in accordance with any of embodiments 1-5, wherein the treatment is combined with β cell regenerative medicine and/or treatment.

- 10 7. The compound as defined by formula Va:



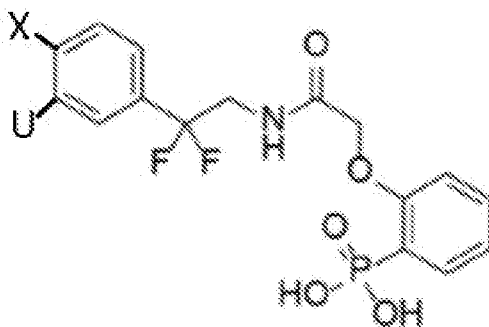
(formula Va).

- 15 wherein one of Z is F, and the other Z is H, preferably wherein both of Z are F;
 wherein X is selected from H, F, Br, Cl, CF₃, OCH₂Ph, and p-OCH₂Pyr (*N*-2, *N*-3 or *N*-4);
 U and W are selected from F and H;
 R is selected from H, CH₃ or F; and
 V is selected from PO(OH)₂ and OPO(OH)₂, wherein the compound is for use
 20 in the treatment of cancer.

8. The compound for use in accordance with embodiment 7, wherein the cancer is dependent on ChREBP for proliferation.

9. The compound for use in accordance with embodiment 7 or 8, wherein the cancer is colon cancer, hepatic cell carcinoma or insulinoma.

5 10. The compound for use in accordance with any of embodiments 1-9, wherein the compound is as defined by formula VI:



(formula VI)

10 wherein X is selected from H, F, Br, Cl, CF₃, OCH₂Ph, and p-OCH₂Pyr and U is selected from F and H.

11. The compound for use in accordance with any of embodiments 1-10, wherein at least one of X and U is F.

15

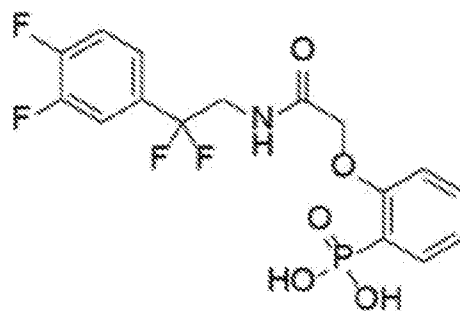
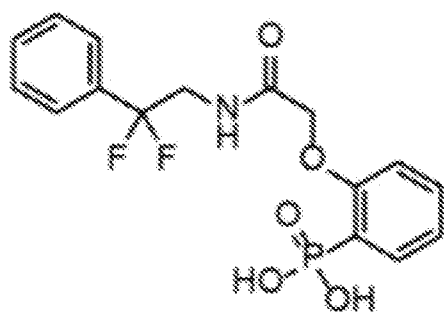
12. The compound for use in accordance with any of embodiments 1-11, wherein both of X and U are F.

20

13. The compound in accordance with any of embodiments 1-12, wherein the compound is in the form of a pharmaceutically acceptable salt.

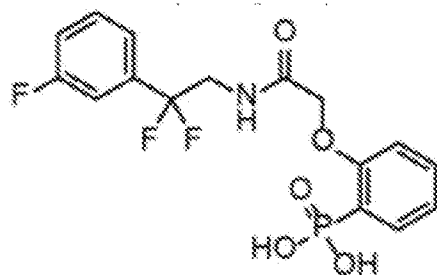
14. The compound for use in accordance with any embodiments 1-13, wherein the compound is:

18



or

or



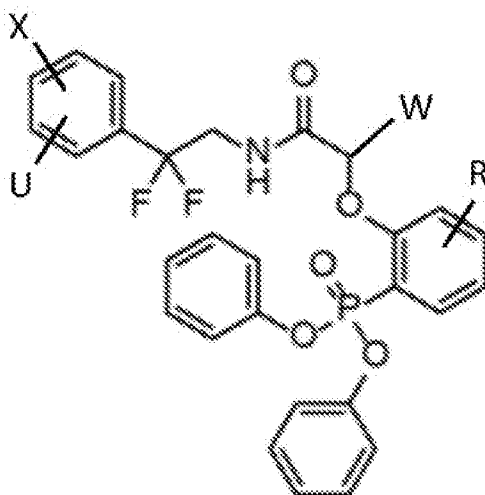
5

15. The compound for use in accordance with any of embodiments 1-14, wherein the compound is in the form of a pharmaceutically acceptable prodrug.

10

19

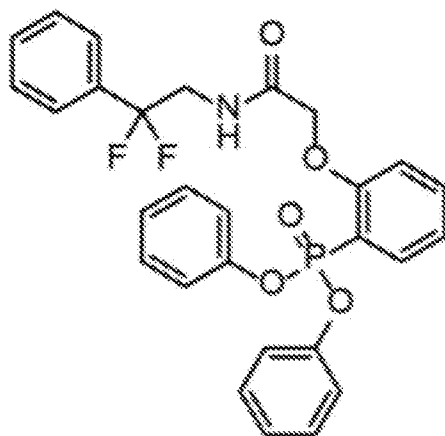
16. The compound for use in accordance with embodiment 15, wherein the compound is in the form of formula VII:



(formula VII).

5

17. The compound for use in accordance with embodiment 15 or embodiment 16 , wherein the compound is:



10

18. The compound for use in accordance with any of embodiments 1-17, wherein the compound is capable of interacting with a first protein and a client protein

and wherein the first protein and the client protein and the compound form a complex, for stabilizing the complex.

19. The compound for use in accordance with embodiment 18, wherein the first protein is the 14-3-3 protein and the client protein is a phosphorylation-independent 14-3-3 client protein, preferably ChREBP- α .

20. The compound for use in accordance with embodiment 19, wherein the compound is selective for stabilizing the complex of 14-3-3 and ChREBP- α .

21. The compound for use in accordance with embodiment 18-20, wherein the compound is capable of reducing translocation in a cell of the complex from the cytoplasm to the nucleus.

22. The compound for use in accordance with any of embodiments 19-21, wherein the compound represses ChREBP- α mediated gene expression.

23. The compound for use in accordance with any of embodiments 18-22, wherein the compound represses ChREBP- β transcription.

Figures

Figure 1: Graphical abstract of compound, i.e. stabilizer, interacting with ChREBP and the 14-3-3 protein. The middle picture depicts the crystal structure of a representative compound according to the invention (62) in complex with 14-3-3 and ChREBP. The right figure shows that the compound, ChREBP and 14-3-3, form a complex resulting in cytoplasmic retention, thereby suppressing ChREBP mediated transcription.

Figure 2: (A) Increased glucose levels activate ChREBP- α resulting in a de-association of 14-3-3, nuclear localization and induction of ChREBP- β , which is constitutively active as it is missing the LID and NES domains. A combination of metabolite-activated ChREBP- α and constitutively active ChREBP- β results in expansion of functional β -cell mass through proliferation of β -cells to meet demand for insulin. (B) Prolonged hyperglycemia gives rise to glucotoxicity and/or glucolipotoxicity, which impairs insulin production and secretion, promoting a vicious cycle with ever-increasing glucose

concentrations and ever-declining β -cell function, and eventually β -cell death. (C) Stabilizing ChREBP- α to 14-3-3 using small molecular stabilizers may prevent nuclear import of ChREBP- α , induction of ChREBP- β and thereby rescue the cells from glucotoxicity and/or glucolipotoxicity

5

Figure 3: (A) The receptor grid (black dotted box) for docking in the crystal structure of 14-3-3 β (white surface), ChREBP (red cartoon and sticks) and AMP (yellow surface) (PDB entry 5F74). (B) Molecular structures of docking hit 1 and ChREBP-14-3-3 stabilizer 2 discovered by SAR by catalogue. (C) Fluorescence Anisotropy (FA) compound titration of 2 into FITC-labeled ChREBP peptide (10 nM) and 14-3-3 β (150 nM). (D) FA 2D protein titration of 14-3-3 β in FITC-labeled ChREBP peptide (10 nM) and varied but fixed concentrations of 2 (0–500 μ M). (E) Crystal structure of compound 2 (blue sticks) in complex with 14-3-3 σ (white surface) and ChREBP (red sticks and surface). Final 2Fo-Fc electron density contoured at 1.0 σ . (F) Interactions of 2 (blue sticks) with 14-3-3 σ (white) and ChREBP (red) residues (relevant side chains are displayed in stick representation, polar contacts are shown as black dashed lines).

Figure 4: Compound 2 and two best stabilizers are highlighted. EC₅₀ in parenthesis with mean $\pm$ SD, n = 2. For FA titration graphs see Figure 16A+B. Compounds with a star (*) were also described previously in Sijbesma, E. *et al* Structure-based evolution of a promiscuous inhibitor to a selective stabilizer of protein–protein interactions. Nat. Commun. 11, 1–9 (2020)

Figure 5: (A) Crystallographic overlay of 2 (blue sticks) with 62 (yellow sticks) in complex of 14-3-3 σ (white cartoon) and ChREBP (red cartoon). (B) Interactions of 62 (yellow) with 14-3-3 (white) and ChREBP (red) (relevant side chains are displayed in stick representation, polar contacts are shown as black dashed lines). (C) Structures and bar graphs of EC₅₀ values derived from FA compound titrations, for Y=H (blue solid bars) and Y=F (yellow and diagonally striped bars). (For graphs see Figure 16A+B), for EC₅₀ values see Figure 24) (mean $\pm$ SD, n=2).

30

Figure 6: (A) Crystallographic overlay 62 (yellow) and 66 (purple) in complex with 14-3-3 σ (white cartoon) and ChREBP (red cartoon). Helix 9 of 14-3-3 σ is colored the same as the compound it's complexed with, showing a helical 'clamping' effect when 66

(purple) is present. (B) Surface representation of **66** (purple) in complex with 14-3-3 (white) and ChREBP (red), showing the distances (black dashes) of the *m*-F substitution to the residues (sticks) of 14-3-3 and ChREBP. (C) Interactions of **66** (purple) with 14-3-3 (white) and ChREBP (red) (relevant side chains are displayed in stick representation, polar contacts are shown as black dashed lines). (D) Crystallographic overlay of **62** (yellow) and **65** (green) in complex with 14-3-3 σ (white cartoon) and ChREBP (red cartoon). Helix 9 of 14-3-3 σ is colored the same as the compound it's complexed with, showing a helical 'clamping' effect when **65** (green) is present. (E) Surface representation of **65** (green) in complex with 14-3-3 (white) and ChREBP (red), showing the distances (black dashes) of the phenyl ring of **65** to the residues (sticks) of 14-3-3 and ChREBP. (F) Interactions of **65** (green) with 14-3-3 (white) and ChREBP (red) (relevant side chains are displayed in stick representation, polar contacts are shown as black dashed lines).

Figure 7: (A) Titration of 14-3-3 β to FITC labeled ChREBP peptide (10 nM) against varying fixed concentrations of **62**, **66** or **76** (0–500 μ M) (mean $\pm$ SD, n = 2, DMSO condition shown in solid black most right lines at start of arrows). (B) Apparent K_D value of the ChREBP–14-3-3 interaction (y-axis) in the presence of a range of concentrations (0–250 μ M) of **2** (blue; line with solid circles), **62** (orange; line with solid squares), **66** (purple; line with solid triangle pointing upwards) and **76** (green; line with solid triangle pointing downwards) (x-axis). Lines run in parallel with from top to bottom **2**, **66**, **76** and **66**. (C) Selectivity studies by titrating **62**, **66** or **76** to 14-3-3 β and eight different 14-3-3 interaction FITC-labeled peptides (all 10 nM) (mean $\pm$ SD, n = 2) Line showing an increase up to 150 represent ChREBP; whereas the other lines correspond with different 14-3-3 interaction FITC-labeled peptides which do not show such an increase. (D) ITC experiments of ChREBP peptide titrations (400 μ M) in 14-3-3 β (30 μ M) in the presence of DMSO or 500 μ M of **62** or **AMP**. (E) Biophysical parameters derived from ITC experiments, comparing enthalpic and entropic differences between **62** and **AMP** (measured in two independent experiments, for replicate 2 see Figure 20).

Figure 8: (A) Structures of analogs tested in cell assays in different cultured conditions. (B) Percentage (%) increase of cell number (INS-1) after 48h in culture under low glucose (blue, left bar of each set, 6 mM glucose), high glucose (yellow, middle bar of

each set, 20 mM glucose) or glucolipotoxic conditions (red, right bar of each set, 20 mM glucose + 1 mM palmitate) in the presence of DMSO (Ctrl) or 10 μ M of **IV**, **62**, or **66**. (C) percentage (%) of cell death (INS-1) after 24h in culture under low glucose (blue, left bar of each set, 6 mM glucose), high glucose (yellow, middle bar of each set, 20 mM glucose) or glucolipotoxic conditions (red, right bar of each set, 20 mM glucose + 1 mM palmitate) in the presence of DMSO (Ctrl) or 10 μ M of **IV**, **62** or **66**. (Mean $\pm$ SD, n = 3, statistical differences tested by 2way ANOVA, *p<0.05 ****p<0.001).

10 Figure 9: (A) INS-1 cells were cultured low glucose (blue, left bar of each set, 6 mM glucose), high glucose (yellow, middle bar of each set, 20 mM glucose) or glucolipotoxic conditions (red, right bar of each set, 20 mM glucose + 1 mM palmitate) for 24h, in the presence of **IV**, **62** or **66** (all 10 μ M). Cells were fixed and immuno-stained with the C-terminal antibody for ChREBP- α and ChREBP- β (left) or the N-terminal antibody for ChREBP- α (right) (mean $\pm$ SD, n=3, *p<0.05, significance test by 2way ANOVA). (B) INS-1 cells were cultured in high glucose (left, 20 mM) or glucolipotoxic conditions (right, 20 mM glucose + 1 mM palmitate) for 0 min, 30 min and 120 min, in the presence of **IV** (line with solid squares), **62** (line with solid triangle facing upward) or **66** (line with solid triangle facing downward) (all 10 μ M). Nuclear localization of ChREBP- α can be seen to rapidly (30 min.) increase and slowly decrease again (120 min.) after glucose or glucose + palmitate stimulation under normal conditions. Rapid nuclear localization of ChREBP- α is suppressed upon compound treatment after glucose stimulation (**IV**, **62**, **66**) and after glucose + palmitate stimulation (**62**, **66**). Cells were fixed and immuno-stained with the N-terminal antibody for ChREBP- α (mean $\pm$ SD, n=3, lines are meant to guide the eye).

Figure 10: INS-1 cells, that stably express luciferase under control of the Txn1p promoter, were treated for 24 hours with 10 μ M of **IV**, **62** and **66** under low glucose (blue circles, left bar of each set, 6 mM glucose) and high glucose (yellow circles, right bar of each set, 20 mM glucose) conditions followed by luciferase measurement (mean $\pm$ SD, n=3 (4 for Ctrl), 2way ANOVA, ****p<0.0001).

Figure 11: Proximity Ligation Assays (PLA) of INS-1 cells cultured under high glucose conditions (2 mM); nuclei are stained with DAPI (large grey areas) and PLA ChREBP-

α -14-3-3 co-localization signals are visualized as small dots. INS-1 cells were treated with (A) DMSO as control, or (B) 10 μ M of **62**. Results indicate direct interaction between ChREBP- α and 14-3-3 upon stabilizer treatment (right) and show increased cytoplasmic co-localization of ChREBP- α and 14-3-3 in INS-1 cells in the presence of **62** (more small dots), cultured under high glucose conditions (2 mM glucose).

Figure 12: Crystal structures of 14-3-3 β (white) in complex with ChREBP (red) and (A) SO4 (PDB ID: 4GNT), or (B) AMP (PDB ID: 5F74).

Figure 13: Molecular structure of docking hit **1** and fluorescence anisotropy (FA) data of 14-3-3 β titration to FAM-labeled ChREBP peptide (100 nM) and fixed concentrations (1, 10 and 100 μ M) of compound **1**, showing a 4-fold increase in stabilization with 100 μ M, with lines from right to left represent DMSO, 1, 10, 100 μ M.

Figure 14: Left: experimental fluorescence anisotropy data of 14-3-3 titration to FITC-labeled ChREBP peptide in the presence of several concentrations of compound **2**, up and left shifted lines represent relatively increasing concentrations of compound **2**. Right: error-landscape plot centered on the determined KDII and α factors. The contours show that there is a valley of combinations that result in relatively low mean squared error (MSE).

Figure 15: (A) side view of the 14-3-3 β dimer (white cartoon) bound by two ChREBP peptides (red cartoon) and two **2** molecules (blue sticks). (B) top view displaying the antiparallel orientation of the ChREBP helices in the 14-3-3 dimer. (C) Front view of one 14-3-3 β monomer (white surface) bound by one ChREBP peptide (red cartoon) and **2** (blue sticks). (D) close-up of the electron density of **2** solved by the co-crystallization with 14-3-3 β and ChREBP (PDB ID: 6YGJ, 'old' structure). (E) close-up of electron density of **2** solved by the soaking method into 14-3-3 σ crystals ('new' structure).

Figure 16A+B: Compounds were titrated in FITC-labeled ChREBP peptide (10 nM) with or without the presence of 14-3-3 β (150 nM). Compound numbers indicated with a star (*) are not described in Figure 4, instead their structure and EC50 value is given in Figures 23-24.

Figure 17: Molecular structure of **81** and crystal structure of **2** (blue sticks) in complex with 14-3-3 (white) and ChREBP (red), to show distances to hydrophobic roof of 14-3-3.

5

Figure 18: Crystal structures of 14-3-3 σ (white surface) in complex with ChREBP (red sticks) and stabilizer. (A) **62** as yellow sticks. (B) **65** as purple sticks. (C) **66** as green sticks. Fo-Fc electron density maps (blue mesh) are contoured at 1 σ .

10 Figure 19: Left: experimental fluorescence anisotropy data of 14-3-3 titration to FITC-labeled ChREBP peptide in the presence of several concentrations of compound **62**, **66** or **65**. Left: Depicted lines go from far left to far right corresponding with high concentration (500) going to lower concentration (0) of the compounds. Right: error-landscape plot centered on the determined KDII and α factors.

15

Figure 20: Replicate 2 of ITC experiments of ChREBP peptide titrations (400 μ M) in 14-3-3 β (30 μ M) in the presence of DMSO or 500 μ M of **62** or **AMP**.

Figure 21: Compound I, II, and III where cytotoxic, compound IV showed activity.

20

Figure 22: Crystal structure of non-natural peptide **2d** (blue sticks) in complex with 14-3-3 σ (white surface) (PDB ID: 7ZMU), overlayed with the crystal structure of compound **62** (yellow sticks) in complex with ChREBP peptide (red cartoon).

25 Figure 23: EC50 in parenthesis with mean $\pm$ SD, n = 2. For FA titration graphs see Figure 16A+B.

Figure 24: EC50 in parenthesis with mean $\pm$ SD, n = 2. For FA titration graphs see Figure 16A+B.

30

Figure 25: XRD data collection and refinement statistics for ChREBP–14-3-3 σ structures

Figure 26: 66 preserves β -cell identity in glucolipotoxicity and prevents upregulation of ChREBP β glucolipotoxicity. **a-d.** mRNA fold enrichment over control low glucose in human islets, 10 μ M of **66** was added in glucolipotoxic conditions for 24 h. Low refers to 5.5 mM glucose, High refers to 20 mM glucose; High + Pal refers to glucolipotoxicity conditions with 20 mM glucose and 500 μ M palmitate. Data are the means $\pm$ SEM, n=3-7, *p<0.05, **p<0.01.

Figure 27: 66 preserves β -cell identity in glucolipotoxicity and prevents upregulation of ChREBP β in glucolipotoxicity. **a-c** Human islets were treated with 10 μ M 66 for 24 h under various conditions. Low refers to 5.5 mM glucose, High refers to 20 mM glucose; High + pal refers to glucolipotoxicity conditions with 20 mM glucose and 500 μ M palmitate. **a-c.** Immunostaining for c-term ChREBP, PDX1, and insulin, and these stainings merged, and these stainings merged and combined with DAPI staining in dispersed islets treated for 24 h with indicated treatments. Data are the means $\pm$ SEM, n=4. Data are the means $\pm$ SEM, n=3-7, *p<0.05, **p<0.01.

Figure 28: Cytokines induce ChREBP β expression and **66** prevents nuclear accumulation of ChREBP in response to cytokines. INS-1 cells were treated for 24 h at low glucose (5.5 mM) with Cytokine mix (TNF α -100 ng/ml; IL1 β -10 ng/ml; INF γ -100 ng/ml), or without (Ctrl), indicated under the x-axis, and with 10 μ M 66 (right two bars), or without 66 (right two bars). **a,b.** Cells fixed and nuclei stained with DAPI and C-term ChREBP. In response to cytokines, marked ChREBP co-localization is apparent, while in the presence of 10 μ M **66**, ChREBP is no longer localized to the nucleus. Data are means $\pm$ SEM; n=4; **, p < 0.01; ***, P<0.005

Figure 29: **66** protects INS-1 cells from dying from cytokine induced toxicity. INS-1 cells were treated for 24 h at low glucose (5.5 mM) with Cytokine mix (TNF α -100 ng/ml; IL1 β -10 ng/ml; INF γ -100 ng/ml) or without (Ctrl), indicated under the x-axis, and with 10 μ M **66** (right two bars) and without **66** (left two bars). **A.** % change in cell numbers compared to time 0. Cell numbers assessed by Syto21 staining. **B.** % Dead cells 24 h following indicated treatment. Dead cells assessed by Yoyo3 staining. Data are means $\pm$ SEM; n=4; *, p < 0.05

Figure 30: 66 improves human islets glucose stimulated insulin secretion (GSIS) in high glucose and in cytokine induced toxicity. Human islets from cadaveric donors were treated for 24 h at low glucose (5.5 mM) (Low), high glucose (20mM) (High) or with low glucose with Cytokine mix (TNF α -100 ng/ml; IL1 β -10 ng/ml; INF γ -100 ng/ml) (Low + cyto); and with (right bar) or without (left bar) 10 μ M **66**. **a-c**. Following treatment, islets were incubated at HANKS-BSA buffer for 30 min following 30 min of low glucose, 30 min of High glucose, 10 min of KCl and determined was **a**. Stimulation Index (SI) of high versus low glucose. **b**. SI of KCl stimulation versus low glucose. **c**. Total insulin content. Data are means $\pm$ SEM; n=4; *, p < 0.05, **, p<0.01

Figure 31: hPAP scRNA-Seq dotplot of β -cell population highlighting ChREBP (gene name MLXIPL), and its homolog (MondoA, gene name MLIXP), heterodimer partner (MLX) as well as known ChREBP target genes. MLXIPL (ChREBP) expression is upregulated in beta cells of autoantibodies positive (AAB+) donors and in beta cells from Type 1 Diabetes (T1D) diabetes compared to non-diabetic (ND) donors. MLXIP, MLX and all target genes of MLXIP are upregulated in beta cells of T1D donors compared to AAB+ and to ND donors. Data obtained using the Expression Browser from <<https://doi.org/10.2337/db23-0130>>.

Figure 32: Pancreatic sections of normoglycemic NOD female mice exhibit high levels of nuclear ChREBP in β -cells and high Cytoplasmic ChREBP in CD45+ cells. Pancreatic slides from NOD mice (a) or C57BL/6 (b) mice at indicated age (4, 12 and 15 weeks) were immunostained with Insulin (left), C-term ChREBP (second from the left) recognizing both ChREBP α and β , CD45 to mark immune cells (third from the left) and DAPI (fourth from the left), and last is shown a merged image. (b) same staining as panel (a), only merged image shown Representative images from each age group are presented here. N=6-10 per age group.

Figure 33: High Cytoplasmic ChREBP α in CD45+ cells.

Pancreatic slides from NOD mice (a) or C57BL/6 (b) mice at indicated age were immunostained with Insulin (left), N-term ChREBP (second from the left) recognizing ChREBP α , CD45 to mark immune cells (third from the left) and DAPI (fourth from the left), and last is shown a merged image. (b) same staining as panel (a), only merged

image shown. Representative images from each age group are presented here. N=6-10 per age group.

Figure 34: Compound **66** attenuates glucose-stimulated proliferation of INS-1 cells.

5 INS-1 cells were cultured for 96 h with 20 mM glucose (round data points, above (ctrl high) or 20 mM glucose and **66** (triangular data points, below, (high + **66**). A much less steep slope is observed in the presence of compound **66** as compared to without compound **66**. Syto 21 was used to ascertain cell numbers. Compound **66** significantly decreased the rate of proliferation (representative trace of 3 independent
10 experiments).

Examples

SECTION I

15

EXPERIMENTAL PROCEDURES

Virtual Screening and Docking Procedures. The virtual screen was performed as described previously.¹ Briefly, the Molport Library (release date 04-08-2017) was screened for drug-like molecules by implementing it as a single workflow in KNIME
20 analytics Platform. The resulting compounds were docked using the Molecular Modeling Platform of Schrödinger into the 14-3-3 β /ChREBP/AMP complex (PDB entry 5F74, resolution 2.35 Å), generated by using the *Protein Preparation Wizard*. AMP was removed and its binding position used as a reference for ligand docking. Compound selection was prepared using *Ligprep* and docking was performed with
25 *Glide* in XP mode. Results were converted to a ranked list of docking scores containing compound SMILES using a second KNIME workflow. The top 200-ranked poses were visually inspected, and 13 compounds were selected for experimental validation. All ligands were obtained from Molport and purity was confirmed by analytical liquid chromatography coupled with mass spectrometry (LC-MS), performed on a C4 Jupiter
30 SuC4300A 150 x 2.0 mm column (using a 15 min. gradient of 5% to 100% acetonitrile in H₂O (0.1% formic acid)), connected to a ThermoFischer LCQ Fleet Ion Trap Mass Spectrometer

Purchasing of ligands. All ligands were obtained from Molport (Here, compound 1 and 2) and purity was confirmed by analytical liquid chromatography coupled with mass spectrometry (LCMS) as described previously.¹

5 **Protein expression and purification.** The 14-3-3 β FL and Δ C, and 14-3-3 σ Δ C isoform (full length and truncated C-terminus after T231 (Δ C to enhance crystallization)) containing a N-terminal His6 tag were expressed and purified using standard procedures. The 14-3-3 β full-length isoform (for fluorescence anisotropy and ITC assays) and 14-3-3 σ isoform lacking the C-terminus (amino acids 1-231; for
10 crystallography) were cloned into pPROEX HTb expression vector with a N-terminal His6 tag. Plasmids were transformed following manufacturer's instructions into RosettaTM 2(DE3)pLysS competent E. coli (Novagen). Single colonies were picked and inoculated in 30 mL precultures grown in LB media overnight at 37°C. Inoculations were then added to 1.5 L terrific broth (TB) medium. Expression was induced upon
15 reaching OD600 1.9–2.1 by adding 400 μ M IPTG. After overnight expression at 30°C, 150 rpm, cells were harvested by centrifugation at 6,500 rpm, resuspended in lysis buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 20 mM imidazole, 10% glycerol, 1 mM TCEP), and lysed by sonication. The His6-tagged protein was purified by Ni-affinity chromatography (Ni-NTA Agarose, Invitrogen) (Wash buffer 50 mM HEPES pH 7.5,
20 500 mM NaCl, 20 mM imidazole, 1 mM TCEP; Elution buffer 50 mM HEPES pH 7.5, 500 mM NaCl, 500 mM imidazole, 1 mM TCEP) and analyzed for purity by SDS-PAGE and Q-ToF LC/MS. The protein was buffer exchanged (Storage buffer 25 mM HEPES pH 7.5, 150 mM NaCl, 1 mM TCEP) and concentrated to ~67 mg/mL and aliquots flash-frozen for storage at –80°C. After Ni-affinity chromatography, the crystallography
25 construct was treated with TEV protease to cleave off the His6 tag during dialysis (25 mM HEPES, pH 7.5, 200 mM NaCl, 5% glycerol, 10 mM MgCl₂, 250 μ M TCEP) overnight at 4°C. The flow-through of a second Ni-affinity column was subjected to a final purification step by size exclusion chromatography (Superdex 75 pg 16/60 size exclusion column (GE Life Science) (SEC buffer: 25 mM HEPES pH 7.5, 100 mM NaCl,
30 10 mM MgCl₂, 250 μ M TCEP). The protein was concentrated to ~60 mg/mL, analyzed for purity by SDS-PAGE and Q-ToF LC/MS and aliquots were flash-frozen for storage at -80 °C.

Peptide Sequences. The N-terminal FITC labeled ChREBP-derived peptide (residues 117 – 142; sequence: RDKIRLNNAIWRAWYIQYVKRRKSPV-CONH₂) (SEQ ID NO.1) was synthesized via Fmoc solid phase peptide synthesis as described previously.¹ N-terminal acetylated ChREBP peptide used for crystallization was purchased from GenScript Biotech Corp.

Peptides used for selectivity studies were purchased from GenScript Biotech Corp with the following sequences: BRAF: (5-FAM-RDRSS(pS)APNVH-CONH₂) (SEQ ID NO.2), CRAF: (5- FAM-QRST(pS)TPNVH-CONH₂) (SEQ ID NO.3) , ER α : (5-FAM-AEGPFA(pT)V-COOH) (SEQ ID NO.4), EXO-S: (5-FAM-KKLMFK(pT)EGPDSD-CONH₂) (SEQ ID NO.5), P65: (5-FAM-EGRSAG(pS)IPGRRS-CONH₂) (SEQ ID NO.6), PIN1: (5- FAM-LVKHSQSRRPS(pS)WRQEK-CONH₂) (SEQ ID NO.7), USP8: (5-FAM-KLKRSY(pS)SPDITQ-CONH₂) (SEQ ID NO.8). In case of any difference between the sequences disclosed in this section and the sequence listing provided at filing, the sequences listed above prevail.

Fluorescence Anisotropy measurements. Fluorescein labeled peptides, 14-3-3 β FL protein, the compounds (100 mM stock solution in DMSO) were diluted in buffer (10 mM HEPES, pH 7.5, 150 mM NaCl, 0.1% Tween20, 1 mg/mL Bovine Serum Albumin (BSA; Sigma-Aldrich). Final DMSO in the assay was always 1%. Dilution series of 14-3-3 proteins or compounds were made in black, round-bottom 384-microwell plates (Corning) in a final sample volume of 10 μ L in duplicates. Fluorescence anisotropy values were measured using a Tecan Infinite F500 plate reader (filter set lex: 485 $\pm$ nm, lem: 535 $\pm$ 25 nm; mirror: Dichroic 510; flashes:20; integration time: 50 ms; settle time: 0 ms; gain: 55; and Z-position: calculated from well). Wells containing only FITC labeled peptide were used to set as G-factor at 35 mP. Data reported are at endpoint. EC₅₀ and KD values were obtained from fitting the data with a four-parameter logistic model (4PL) in GraphPad Prism 7 for Windows. Data was obtained and averaged based on two independent experiments.

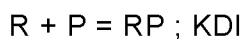
Compound Titrations were made by titrating the compound in a 3-fold dilution series (starting at 500 μ M) to a mix of FITC labelled peptide (10 nM) and 14-3-3 β (concentration at EC₂₀ value of protein-peptide complex; 150 nM for ChREBP). For the selectivity studies concentrations of 14-3-3 β were: 300 nM for ER α , 3.65 μ M for

Exo-S, 10 μ M for Pin1, 250 nM for USP8, 230 nM for B-RAF, 30 μ M for P65 and 450 nM for C-RAF. Fluorescence anisotropy measurements were performed directly.

Protein 2D titrations were made by titrating 14-3-3 β in a 2-fold dilution series (starting at 300 μ M) to a mix of FITC-labelled peptide (10 nM) against varying fixed concentrations of compound (2-fold dilution from 500 μ M), or DMSO. Fluorescence anisotropy measurements were performed directly.

Cooperativity Analysis. The cooperativity parameters for compound **2**, **62**, **66** and **65** for 14-3-3 and ChREBP were determined by using the thermodynamic equilibrium system (Geertjens et al. RSC Chem. Biol., 2023,4, 252-260). The data from 2D-titrations was provided to the model including the KDI = 1.4 μ M, 1.1 μ M, 1.8 μ M or 1.5 μ M for **2**, **62**, **66** or **62**, respectively, P_{tot} = 10 nM, and the variable concentrations of 14-3-3 and stabilizer at each data point. Fit parameters were given the following initial guess values: KDII: 5.5 μ M, α : 400.

To determine the cooperativity parameters from the 2D-titration of 14-3-3/ChREBP-peptide with **2**, **62**, **66** and **76**, we used the general framework for straightforward model construction of multi-component thermodynamic equilibrium systems as described by Geertjens et al.³⁸ This general platform generates a model to describe multi-component equilibrium systems when given a system description. In our case we gave the following system description:



with R = 14-3-3, P = ChREBP-peptide and S = stabilizer. The KDII and α are determined based on the equilibrium equations. The data from 2D-titrations was provided to the model including the KDI = 1.4 μ M, 1.1 μ M, 1.8 μ M or 1.5 μ M for **2**, **62**, **66** or **62**, respectively, P_{tot} = 10 nM, and the variable concentrations of 14-3-3 and stabilizer at each data point. Fit parameters were given the following initial guess values: KDII: 5.5 μ M, α : 400.

X-Ray crystallography data collection and refinement. The crystal structure of compound 2 in complex with 14-3-3 β and ChREBP peptide was produced and solved as described previously.¹ For the 14-3-3 σ crystal structures; a mixture of Ac-ChREBP peptide and compound (2 μ L of 5 mM stock ChREBP peptide + 0.5 μ L of 100 mM stock compound) was mixed in crystallization buffer (CB: 20 mM HEPES pH 7.5, 2 mM MgCl₂, 2 mM β ME) to a final volume of 4 μ L. This was added to preformed 14-3-3 σ /Peptide-C crystals at 4 °C that were produced as described previously.² Single crystals were fished after 7 days of incubation at 4 °C, and flash cooled in liquid nitrogen. Diffraction data was collected at either the Deutsche Elektronen Synchrotron (DESY Petra III beamline P11 proposal 11011126 and 11010888, Hamburg, Germany (crystal structures of compound 2 and 62), or at the European Synchrotron Radiation Facility (ESRF Grenoble, France, beamline ID23-2 proposal MX2268) (crystal structures of compound 66 and 65). Initial data processing was performed at DESY using XDS73 or at ESRF using DIALS74 after which pre-processed data was taken towards further scaling steps, molecular replacement and refinement.

Data was processed using the CCP4i2 suite75 (version 8.0.003). After indexing and integrating the data, scaling was done using AIMLESS76. The data was phased with MolRep77, using 4JC3 as a template. Presence of soaked ligands and ChREBP-peptide was verified by visual inspection of the Fo-Fc and 2Fo-Fc electron density maps in COOT78,79 (version 0.9.6). If electron density corresponding to ligand and peptide was present, the ChREBP peptide was built in and the structure and restraints of the ligands were generated using AceDRG80, followed by model rebuilding and refinement using REFMAC581. The PDB REDO82 server (pdb-redo.edu) was used to complete the model building and refinement. The images were created using the PyMOL Molecular Graphics System (Schrödinger LLC, version 2.2.3). For refinement statistics see Figure 25.

The structures were deposited in the protein data bank (PDB) with IDs: **8BTQ** (compound 2), **8C1Y** (compound 62), **8BWH** (compound 66) and **8BWE** (compound 65).

Synthesis of SAR library for 2. All analogs of **2** analyzed for activity and displayed in Figures 4, 23, 24 were synthesized and characterized. Detailed synthetic procedures and characterization of compounds marked with a star (*) in Figure 4 have been described previously in detail.¹

5

All the compounds as described herein throughout were synthesized following established procedures as previously described in detail (see ref. 1, i.e. Sijbesma, et al. Nat. Commun. 11, 1–9 (2020), incorporated herein by reference). Common adaptations on standard synthesis protocols were made, if necessary, depending on the availability of starting materials and/or potential requirements of alternating protecting group strategies and correlated building block coupling chemistries, and as described in Section II herein.

Generally, all compounds obtained were purified by reversed-phase high performance liquid chromatography (HPLC) and characterized by liquid chromatography mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR; 400 MHz for ¹H NMR and 100 MHz for ¹³C NMR). Compounds were prepared as 100 mM stock solutions in DMSO before use in experiments, and stored at -20 °C.

20 **Cell culture.** INS-1-derived 832/13 cells were cultured as described previously.³

Proliferation and cell death. Cellular proliferation and cell death was quantified using Real-Time Kinetic Labeling (SPARKL) technique. Syto21 marked all cells, Yoyo3 marked all dead cells.

25

Immunofluorescence. The immunofluorescence measurements were performed as described previously.⁴ Briefly, cells were plated on 12-mm laminin coated glass placed in 24-well plates. Cells were rinsed with PBS and fixed in 4% paraformaldehyde. Confocal images were acquired using the Cytation 10. Images and image analysis will be provided in the supplementary information of the original manuscript.

30

Antibodies. Anti-ChREBP C-term rabbit polyclonal at 1:250 from Novus (cat # NB400-135, AB_10002435). Anti-ChREBP N-term rabbit polyclonal at 1:250 generated by Genscript.

Luciferase Reporter. The reporter assays were done as described previously.⁵ Briefly, INS-1-derived 832/13 cells that stably express the luciferase reporter gene under control of the human Txnip promoter Cells were harvested after 24h and luciferase activity was measured using the Luciferase Reporter Assay System (Promega, Cat. #E1500) on Victor Nivo luminometer (PerkinElmer). Luciferase activity was normalized to Renilla activity.

Proximity Ligation Assay (PLA) assays were performed in INS-1-derived 832/13 cells, in the presence of DMSO or 10 μ M of compound **66**. PLA samples were prepared following manufacturer's instructions (Duolink®), using N-terminal ChREBP antibody (generated by Genscript), and *pan* 14-3-3 (sc133233).

Statistics. All studies were performed with a minimum of three independent repetition. Data represented in this study as means $\pm$ standard error of the mean (SEM). Statistical analysis was performed using Two-way ANOVA on GraphPad (Prism) V9.2.

RESULTS

In-silico screen discovers small molecular stabilizers

The two crystal structures of ChREBP–14-3-3 in the Protein Data Bank (PDB entries 4GNT and 5F74) served as entry points for the structure-based *in silico* screen for the identification of ChREBP–14-3-3 stabilizers. A phospho-binding pocket centered receptor grid was generated, using the Molecular Modeling Platform of Schrödinger, for the structure of 14-3-3 β bound to the α 2 helix of ChREBP (Figure 3A). The virtual Molport library (~6 million compounds) was filtered for drug-like compounds encompassing a phosphate- or phosphonate moiety. The resulting 471 virtual compounds were molecularly docked into the receptor grid and hits were evaluated based on their docking score. Thirteen compounds were selected for *in vitro* testing by employing fluorescence anisotropy (FA) assays.¹ Compound **1** (100 μ M) increased the binding affinity of 14-3-3 β for ChREBP in a dose-dependent fashion up to 4-fold (Figure 3B, Figure 13).¹ The attractive and synthetically accessible phosphonate-based scaffold of **1** prompted its chemical optimization to establish a SAR. An initial SAR-by-catalog study resulted in an increased PPI stabilization by **2**, with an EC₅₀ value of 37.2 ± 1.8 μ M for the ChREBP–14-3-3 β interaction as determined by FA (Figure 3C).

Considering that the characterization of complex stabilization in solution is dependent on relative concentrations of binding partners, we performed FA 2D titrations. 14-3-3 β was titrated to FAM-labelled ChREBP peptide in the presence of varied but constant concentrations of **2** (0–500 μ M), showing up to 25-fold increase in stabilization of the ChREBP–14-3-3 β interaction (Figure 3D). A thermodynamic equilibrium model was then used (Figure 14) to determine a cooperativity factor (α) of 39 for compound **2** with an intrinsic affinity for 14-3-3 β of 5.5 μ M (KDII). Of note, while the model gives these fixed values, the fitting by the model may not be optimal which is reflected by the range of possibilities in α factor and KDII values visualized by the mean squared error landscape (Figure 14).

The tertiary 14-3-3 β –ChREBP–**2** protein complex co-crystal structure was solved by X-ray crystallography at a resolution of 2.07 Å (PDB ID: 6YGJ) (Figure 15A-C).¹ The overall complex resembled the reported crystal structures for a 14-3-3 β dimer with the two antiparallel-binding ChREBP- α 2 helices. Compound **2** was positioned in the phospho-accepting pocket of 14-3-3, but the phenyl ring and linker were poorly resolved (Figure 15D). Since this co-crystallization method was laborious and could not be replicated with other stabilizers, we directed our efforts to the development of alternative crystallization approaches. Co-soaks of ChREBP-peptide and compound **2** into pre-formed 14-3-3 σ –peptide-C crystals², resulted in a displacement of peptide-C by compound **2** and ChREBP, as indicated by their high-resolution electron density at 1.60 Å (Figure 3E). This soaking and displacement approach was previously described using the low-affinity cJun-pS227 peptide, similarly resulting in robust 14-3-3–peptide crystal structures.⁶ The improved resolution of the 14-3-3–ChREBP–**2** complex resulted in an explicit density for compound **2**. This enabled the absolute positioning of the phenyl ring and linker of **2** (Figure 15E). The phosphonate group of **2** interacted with Arg128 of ChREBP and Arg56, Arg129 and Tyr130 of 14-3-3 σ (Figure 3F). Both phenyl rings of **2** made hydrophobic contacts with side chains of both proteins, as observed for the phenylphosphonate with Trp127 of ChREBP, and the second phenyl with Ile120 of ChREBP and Leu218, Ile219, and Leu222 of 14-3-3, thereby beneficially engaging the hydrophobic roof of the groove. Similar as observed for the 14-3-3 β crystal structure, an intramolecular polar interaction was detected for **2** between its amide and phosphonate, stabilizing the conformation of **2**. The improved resolution of

this novel crystal structure allowed for the detection of a water-mediated hydrogen bond between the carbonyl oxygen of **2** and the main-chain amide of Ile120 of ChREBP.

5 Focused library based on **2** establishes a crucial SAR

The stabilization mechanism of **2** was studied in more detail by synthesis of a focused library around **2** for SAR analysis. An overview of all analogs is depicted in Figure 4, where a star (*) represents the compounds that were described in our previous study.¹ Here, we built upon this SAR analysis and all compounds were screened at lower 14-3-3 β concentrations (at EC20 of ChREBP–14-3-3 β interaction) to enlarge our window for the detection of improved stabilizers. Similar trends were observed as previous with the most essential observations being: (I) the importance of the linker length of **2** (**3** – **9**), (II) substitutions at the phenylphosphonate ring moiety (**26** – **31**) were tolerated but do not lead to a significant improvement in stabilization, except for **27** which showed marginal improvement in affinity, and (III) substitutions at the N-phenylethyl ring resulted in similar or slightly improved stabilization, for which a F-substitution (**32**, **33**, **34**) or an *o*-OCH₂Ph substitution (**41**, **42**, **43**) were most beneficial while other halogens (*p*-Cl and *p*-Br) or a *p*-hydroxy substitutions were not tolerated (**35**, **36**, **37**). Further, shifting the position of the phosphonate showed that indeed the *ortho* position is most favorable (**2**, **16**, **17**). Interestingly, replacement of the phosphonate-group with a phosphate was tolerated (**18**, **19**, **20**), with even an improvement of stabilization for the *para* position (EC₅₀ = 17.4 ± 3.2 μ M). The addition of a carbon between the phosphonate and the phenyl ring to afford the benzyl phosphonic acid was not tolerated (**23**, **24**, **25**).

25

We contemplated that the double negative charge of the phosphonate group at physiological pH may be disadvantageous for cellular permeabilization. Therefore, we directed our efforts to the investigation of phosphonate-mimetics. Unfortunately replacing the phosphonate with either a boronic acid (**21**) or a sulfonamide (**22**) resulted in an inactivation. Interestingly, the addition of a pyridine group (*p*-OCH₂Pyr, **44**, **45**, **46**) largely increased the stabilization efficiency, especially for *N*-2 (**44**, EC₅₀ = 7.6 ± 0.5 μ M). Replacement of the phosphonate with a sulfonamide and incorporation of the pyridin-4-ylmethanol moiety (**81**, EC₅₀ = 68.2 ± 7.9 μ M) resulted in our first weakly active stabilizer harboring a phosphonate-mimetic (Figure 23). Notably,

sulfonamides bearing *p*-F (**78**), or OCH₂Ph (**79** and **80**) substitutions remained inactive. Phosphonates with naphthalene groups also increased the stabilization potency (**48**, **49**), whereas replacement of the phenyl ring with a benzene (**47**), indole (**51**) or purine groups (**50**, **52**, **53**) was not tolerated. Lastly, we focused on
5 modifications of the phenyl-linker. The introduction of cyclic groups was only favorable for the specific stereo centric S, R combination of the cyclopropyl ring (**54**, EC₅₀ = 17.6 ± 1.7 μM), while 194 other pairs were either not tolerated (**56**, **58**) or did not enhance the activity (**55**, **57**). Interestingly, the introduction of a *geminal*-difluoro group significantly enhanced (sixfold relative to **2**) the stabilization of the ChREBP–14-3-3
10 interaction, resulting in the best stabilization observed so far (**62**, EC₅₀ = 5.8 ± 0.4 μM). Unfortunately, this analog was not amendable for phosphonate replacement with a sulfonamide or boronic acid, since this resulted in inactivity (**82**, **92**, Figure 23). Moreover, combining the two substitutions of the best stabilizers (**44** and **62**) resulted in an analog with similar stabilization properties compared to each individual
15 substitution (**97**: EC₅₀ = 11.0 ± 1.5 μM, Figure 24).

Fluorination of compounds improves stabilization potency

The best stabilizers (**44** and **62**) were co-soaked with the ChREBP-peptide into preformed 14-3-3σ-peptide-C crystals, aiming to resolve their ChREBP–14-3-3σ–
20 stabilizer structure complex. This was successful for **62**, showing a clear density of both the ChREBP peptide and **62** (Figure 18A). Overlaying the crystal structure of **62** with **2** showed a high conformational similarity of 14-3-3σ and ChREBP peptide, with a comparable positioning of the phosphonate of **62** in the ChREBP–14-3-3 phospho-accepting pocket (Figure 5, Figure 5A). The *geminal*-difluoro group of **62** was directed
25 towards the 14-3-3 binding groove, causing an alternative bend of the linker compared to **2**. Both fluorine atoms interacted with 14-3-3σ; one contacted Lys49 directly and the other underwent water-mediated polar interactions with Asn175 (Figure 5B). These interactions align with literature showing that fluorinated small molecules can form hydrogen bonds. Additionally, due to the alternative bend of the linker, the amide of
30 **62** was now positioned to interact with Asn123 and Asn124 of ChREBP. These additional contacts with both 14-3-3 and ChREBP may explain the high increase in stabilization by only introducing the *geminal*-difluoro group. Encouraged by these observations, a focused library of fluorinated compounds was synthesized and compared to their non-fluorinated analogs (Figure 5C).

Fluorination of analogs increased their stabilization potency consistently across all library members. Even previously inactive compounds may elicit a high activity when substituted with the *geminal* -difluoro group (see e.g. *p*-Cl (**35**) and *p*-Br (**37**), as compared respectively with **63**: EC50 = 6.6 ± 0.9 μM, **64**: EC50 = 4.1 ± 0.4 μM, respectively). The fluorine functionality has a place in medicinal chemistry as reflected by a portion of drugs in the pharmaceutical pipeline containing at least one fluorine atom.⁷⁻⁸ Placing an additional fluor group at the *meta* position of the phenyl ring even further improved compound activity (**66**: EC50 = 3.8 ± 0.2 μM). Co-soaking this compound into 14-3-3σ-peptide-C crystals resulted in a clear density for the entire molecule (Figure 17B). A crystallographic overlay with **62** revealed an apparent additional conformational ‘clamping’ effect of helix-9 of 14-3-3σ in the presence of **66** (Figure 6A), causing the hydrophobic residues of 14-3-3 (Ile219, Leu218) to be closer to the phenyl ring of **66**. A more detailed analysis showed that the *meta*-fluoro group of **66** appears to be positioned at the rim of the hydrophobic interface of 14-3-3 and ChREBP, with a distance of 4.3 Å to Leu218 and 3.2 Å to Leu223 of 14-3-3, and 4.4 Å to Ile120 of ChREBP (Figure 6B). Furthermore, the fluoro groups at the linker appeared to interact with Lys49 of 14- 3-3σ, and Asn124 and Asn123 of ChREBP underwent polar contacts with the linker amide, either direct or via two water-molecules (Figure 6C). An *ortho*-fluoro substitution at the of the phenyl ring appeared less favorable (**65**, EC50 = 9.6 ± 1.1 μM) although it did result in a similar apparent ‘clamping’ effect of helix-9 of 14-3-3 (Figure 6D, Figure 17C). This *ortho* substituted fluoro was not apparently positioned at the rim of the ChREBP–14-3-3 interaction interface, instead it made a polar interaction with Asn175 of 14-3-3 (Figure 6E). Also, one of the fluoro groups at the linker of **65** contacted both Lys49 and Asn175 of 14-3-3 via a water-mediated hydrogen bond interaction. The interactions of **65** with ChREBP were analogues to **66** (Figure 6F). Additional to single fluoro substitutions at the phenyl ring of **62**, double fluoro substituted analogs were synthesized (panel IV in Figure 5C). A 2,4-fluorosubstitution resulted in the most potent stabilizer observed so far (**76**, EC50 = 3.1 ± 0.6 μM). While no crystal structure could be solved for this analog, the structure of **66** shows room for a fluoro substitution at the *para* position of its phenyl ring, explaining the high potency of **76** (Figure 6B). Likely, the electron withdrawing effects of the fluorine substitutions may be enhancing hydrophobic contacts with the hydrophobic amino acids at the roof of the 14-3-3 groove.

Fluorinated ligands show increased cooperativity and selectivity

Two-dimensional (2D) FA titrations were performed to investigate the cooperativity in ChREBP–14-3-3–stabilizer complex formation. 14-3-3 β was titrated to FITC-labeled ChREBP peptide (10 nM) in the presence of varied but constant concentrations of **62**, **66** or **76** (0–500 μ M), showing up to 138-fold, 60-fold and 111-fold increase in stabilization, respectively (Figure 7A). Fitting the change in apparent KD of the ChREBP–14-3-3 complex derived by the 2D FA titrations, over different concentration stabilizers (Figure 7B), showed that all three fluorinated compound (**62**, **66** and **76**) elicit a similar stabilizing profile with a 2.4- to 6-fold increase in stabilization compared to the defluorinated parent analog **2**. The obtained data was fitted using a thermodynamic equilibrium model³⁸ (Figure 19) to determine the cooperativity factors (α) and the intrinsic compound affinities to 14-3-3 (KDII). All three fluorinated compounds showed increased cooperativity (α = 86 (**62**), 104 (**66**) 90 (**76**)) compared to their defluorinated parent compound **2** (α = 39), while their intrinsic affinity for 14-3-3 was barely affected (KDII = 7.4 μ M, 8.2 μ M, 3.8 μ M for **62**, **66**, **76** and 5.5 μ M for **2**) (Figure 19). This indicates that the chemical compound evaluation may have mainly improved upon the interaction with ChREBP and not the background binding affinity to 14-3-3. c.

Phosphate- and phosphonate-based inhibitors can inhibit 14-3-3–client complexes in the low micromolar (IC₅₀ ~ 1-20 μ M) range. To test the specificity of the ChREBP–14-3-3 stabilizers, we selected an array of eight representative 14-3-3 client-derived peptide motifs with differentiating binding sequences and included internal binding motifs (BRAF50, CRAF51, P6552, USP853), one C-terminal binding motif (ER α 54), one special binding mode (Pin155) and another reported non-phosphorylated motif (ExoS20). Strikingly, all three compounds (**62**, **66**, **76**) displayed a high selectivity for stabilizing ChREBP, without affecting any other client peptide up to 100 μ M (Figure 7C). This data demonstrates the highly selective nature of the activity of these compounds by addressing a unique pocket only present in the ChREBP–14-3-3 complex and via a stabilization mechanism, involving a large cooperativity effect.

Lastly, ITC experiments were performed to analyze the thermodynamics of the ChREBP–14-3-3 interaction in the presence of stabilizer **62** and the natural compound

stabilizer **AMP** (Figure 7D, 6.5E). ChREBP peptide (400 μ M) was titrated to 14-3-3 β (30 μ M) with a determined KD value of $12.5 \pm 0.5 \mu$ M. The binding was enthalpically favorable ($\Delta H = -35.2 \pm 0.5 \text{ kJ.mol}^{-1}$), which compensated for the entropic cost ($\Delta S = -24.3 \pm 1.4 \text{ J/mol.K}$) probably arising from the alpha-helical formation of the ChREBP peptide upon 14-3-3 binding. Addition of **62** to the system significantly enhanced the ChREBP–14-3-3 affinity (KD = $0.2 \pm 0.03 \mu$ M) and reduced the entropic cost ($\Delta S = 3.12 \pm 11.5 \text{ J/mol.K}$), presumably by the release of water molecules from the ChREBP–14-3-3 binding pocket. **AMP** similarly increased the ChREBP–14-3-3 affinity (KD = $0.4 \pm 0.3 \mu$ M), but, in contrast to **62**, this was enthalpically driven ($\Delta H = -59.8 \pm 2.6 \text{ kJ.mol}^{-1}$) and entropically unfavorable ($\Delta S = -76.2 \pm 1.2 \text{ J/mol.K}$).

ChREBP–14-3-3 stabilizers rescue INS-1 cells from glucolipotoxicity

The stabilization of the ChREBP–14-3-3 PPI on glucotoxicity was investigated in a cellular setting using the rat insulinoma cell line, INS-1 832-13. Because of the double negative charge of phosphonates at physiological pH, they are generally recognized to have a potentially limited therapeutic impact due to their poor cellular permeability. Albeit that such challenges are more relevant for the physiological level than for certain cell lines. The protection of phosphonates as a prodrug has shown promise in drug delivery.⁹ Likewise, similar approaches may be envisioned for the advantageous compounds as described herein. Hence, four prodrugs were synthesized containing diesters and dibenzyl groups for scaffold **2** and **62**, resulting in prodrugs **I**, **II**, **III** and **IV** (Figure 21). While prodrug **I**, **II**, and **III** were cytotoxic to INS-1 cells, prodrug **IV** did not show signs of cytotoxicity at 10 μ M (data not shown). Hence, prodrug **IV** is a pharmaceutically acceptable prodrug. Analogs **2**, **62** and **66** (all 10 μ M) were also tested in INS-1 cells, despite their unprotected phosphonate group. The non-fluorinated analog **2** showed a general toxicity while the addition of the *geminal*-difluoro groups in **62** and **66** seem to counteract this (data not shown).

Non-toxic compounds **IV**, **62** and **66** (all 10 μ M) were followed up in cellular assays at low glucose- (6 mM), high glucose- (20 mM) and glucolipotoxicity conditions (20 mM glucose + 1 mM palmitate) (Figure 8A). A Single-Cell and Population-Level Analysis Using Real-Time Kinetic Labelling (SPARKL) technique was used to monitor over time cell proliferation (Figure 8B) and cell death (Figure 8C). In the control group, glucolipotoxicity caused a significant reduction of INS-1 cell proliferation (reduction of

~173%) and a significant increase in cell death (increase of ~30%), as expected. Remarkably, all three compounds (IV, **62** and **66**) were able to rescue the cells from glucolipotoxicity in a comparable manner. No significant reduction in cellular proliferation was observed in glucolipotoxicity conditions upon addition of 10 μ M of IV, **62** or **66** (Figure 8B). For the prodrug IV, there was still a small increase in cell death under glucolipotoxicity conditions (increase of ~11 %) but this was significantly lower than the control group (Figure 8C). For the unprotected analogs **62** and **66** no significant increase in cell death was observed under glucolipotoxicity conditions. Thus, all three stabilizers of the ChREBP–14-3-3 complex were able to rescue INS-1 beta cells from glucolipotoxicity, with the unprotected phosphonate analogs (**62** and **66**) showing no significant increase in cell death, and the prodrug (IV) showing a lowered but significant increase in cell death.

ChREBP–14-3-3 stabilizers reduce nuclear ChREBP levels

The nuclear localization of both ChREBP- α and ChREBP- β was analyzed by immunofluorescence, to verify if the observed effect of glucolipotoxicity prevention by the stabilizers was due to their inhibition of ChREBP nuclear localization. INS-1 cells were cultured in low glucose-, high glucose-, or glucolipotoxic conditions for 48 hours, in the presence of DMSO control, IV, **62** or **66** (all 10 μ M), followed by fixation and immunostaining with the C-terminal antibody for ChREBP, which recognizes both ChREBP- α and ChREBP- β , or the N-terminal antibody for ChREBP, specific for ChREBP- α (Figure 9A). By using the C-terminal antibody (Figure 9A left panel), under low glucose conditions, $45.8 \pm 1.5\%$ of the cells stained for nuclear ChREBP which was increased to $76.4 \pm 7.5\%$ in response to glucose and this was reduced again to $35.2 \pm 3.5\%$ under glucolipotoxicity. As such, there was no significant difference in nuclear ChREBP levels between low glucose conditions and under glucolipotoxicity. In the presence of stabilizers IV, **62** and **66**, nuclear ChREBP levels were significantly reduced under glucolipotoxicity ($35.2 \pm 3.5\%$, $24.4 \pm 2.8\%$ and $14.3 \pm 5.9\%$, respectively) compared to their low glucose conditions ($62.3 \pm 14.7\%$, $68.9 \pm 26.7\%$ and $67.8 \pm 26.7\%$, respectively) (Figure 9A, left panel). In addition, no significant increase in nuclear ChREBP levels was detected anymore upon glucose stimulation ($38.5 \pm 7.4\%$, $38.3 \pm 29.2\%$ and $36.7 \pm 18.7\%$, respectively) for all three stabilizers. These results show that stabilization of the ChREBP- α –14-3-3 complex in the cytosol, indeed results in reduced total nuclear ChREBP levels, especially under glucose

stimulation and glucolipotoxicity conditions. Since these results are obtained by using a C-terminal ChREBP antibody, this can be due to both ChREBP- α as well as ChREBP- β levels. A N-terminal ChREBP antibody, that only detects ChREBP- α , was used to distinguish between these two isoforms (Figure 9A, right panel). In all three treatment conditions (low glucose, high glucose and glucolipotoxicity), almost no ChREBP- α was present in the nucleus, nor in the control group or upon addition of the stabilizers. Thus, the effects observed by using the C-terminal antibody (Figure 9A, left panel), represents nuclear ChREBP- β levels. It has been shown that the nuclear translocation of ChREBP- α happens very rapidly, within minutes upon glucose stimulation, followed by a nuclear efflux within hours. Since the nuclear ChREBP levels shown in Figure 9A were measured after 48 hours of glucose stimulation, it may be likely that at this fixed time point ChREBP- α was already exported out of the nucleus after stimulating ChREBP- β transcription. To follow the time course of ChREBP- α nuclear localization in the presence of the stabilizers, cells were fixed and stained with the N-terminal ChREBP- α antibody after 0-, 30- and 120 minutes of glucose stimulation (Figure 9B). In the control group, ChREBP- α translocated to the nucleus within 30 minutes of glucose stimulation, and then cleared out of the nucleus within 2 hours (Figure 9B, left panel). All three stabilizers (**IV**, **62** and **66**) were able to block this nuclear translocation of ChREBP under high glucose conditions. Under glucolipotoxicity conditions (Figure 9B, right panel), ChREBP- α remained nuclear over time, which was attenuated by **66**, while for **IV** and **62** the nuclear levels remained unaffected. Thus, compound **66** showed a suppression of ChREBP- α nuclear levels after 30 minutes of glucolipotoxicity stimulation which is likely the cause of the reduced ChREBP- β nuclear levels observed after 48 hours under glucolipotoxicity conditions.

ChREBP-14-3-3 stabilizers suppress ChREBP- β transcriptional activity

Finally, a luciferase reporter assay was performed in INS-1 cells to verify if the reduction of nuclear ChREBP- β levels results in a suppression of its transcriptional activity. For this end, a cell line was generated that stably expressed luciferase under control of the human thioredoxin interaction protein (Txnip) reporter. Txnip is an important mediator of glucose toxicity and a target gene of ChREBP- β since it contains the ChoRE motifs important for ChREBP- β binding. After 24 hours of treatment with the stabilizers, these cells were harvested and firefly luciferase activity was measured and normalized to *Renilla* luciferase activity (Figure 10). All three stabilizers (**IV**, **62**

and 66) blocked the glucose response of the human Txnip promoter by 2 ± 0.2 -fold compared to the control, indicating that the transcriptional activity of ChREBP- β is indeed suppressed under high glucose conditions by our ChREBP- α -14-3-3 stabilizers.

5

Summary

Here we provided for novel and advantageous small molecule stabilizers of ChREBP – 14-3-3, such as the ChREBP α –14-3-3 complex. We implemented a structure-based *in silico* approach, based on the crystal structure of the ChREBP- α (peptide)–14-3-3–AMP complex. We reported a successful molecular docking strategy for the discovery of chemical starting points of phosphonate containing analogs to stabilize ChREBP- α 14-3-3 interaction.¹ Subsequent structure-activity relationship (SAR) optimization resulted in highly advantageous small molecule stabilizers with an increase in affinity of the ChREBP- α -14-3-3 interaction of up to 138-fold, as determined by fluorescence anisotropy. X-ray crystallography confirmed their engagement of the composite interface formed by ChREBP- α -peptide and 14-3-3.

Importantly, fluorination of the analogs could significantly enhance their stabilization efficiency. Moreover, fluorinization was shown to be important for their cellular activity and allowed to counteract general toxicity of non-fluorinated analogs. Immunofluorescence showed a retention of ChREBP- α in the cytoplasm of β -cells upon addition of the stabilizers, concomitantly suppressing its transcriptional activity and transduction of ChREBP- β , as determined by reporter assays. β -cells were shown to be rescued from glucolipotoxicity by keeping ChREBP apparently inactive at high glucose levels. Advantageously, the small molecule stabilizers thus provided are understood to be highly advantageous as a medicament and/or for use in the treatment of T2D.

REFERENCES

1. Sijbesma, E. *et al.* Structure-based evolution of a promiscuous inhibitor to a selective stabilizer of protein–protein interactions. *Nat. Commun.* **11**, 1–9 (2020).
2. Somsen, B. A. *et al.* Functional mapping of the 14-3-3 hub protein as a guide to design 14-3-3 molecular glues. *Chem. Sci.* **7112**, 13122–13131 (2022).

3. Katz, L. S. *et al.* Maladaptive positive feedback production of ChREBP- β underlies glucotoxic β -cell failure. *Nat. Commun.* **13**, (2022).
4. Mugabo, Y. *et al.* 14-3-3 ζ Constrains insulin secretion by regulating mitochondrial function in pancreatic β cells. *JCI Insight* **7**, (2022).
5. Sakiyama, H. *et al.* Regulation of nuclear import/export of carbohydrate response element-binding protein (ChREBP): Interaction of an α -helix of ChREBP with the 14-3-3 proteins and regulation by phosphorylation. *J. Biol. Chem.* **283**, 24899–24908 (2008).
6. Ballone, A., Lau, R. A., Zweipfenning, F. P. A. & Ottmann, C. A new soaking procedure for X-ray crystallographic structural determination of protein-peptide complexes. *Acta Crystallogr. Sect. F* **76**, 501–507 (2020).
7. Shah, P. & Westwell, A. D. The role of fluorine in medicinal chemistry. *J. Enzyme Inhib. Med. Chem.* **22**, 527–540 (2007).
8. Purser, S., Moore, P. R., Swallow, S. & Gouverneur, V. Fluorine in medicinal chemistry. *Chem. Soc. Rev.* **37**, 320–330 (2008).
9. Heidel, K. M. & Dowd, C. S. Phosphonate prodrugs: an overview and recent advances. *Future Med. Chem.* **11**, 1625–1643 (2019).

RESULTS II

Following the above experiments, further experiments were conducted.

We assessed the effect of stabilizers on transcription of the ChREBP splice isoforms in human islets. While ChREBP α mRNA levels remained relatively stable in all conditions tested (**Figure 26a**), ChREBP β , which contains a well characterized carbohydrate hydrate response element (ChoRE) on its promoter, was upregulated in response to high glucose, or to high glucose and high palmitate (glucolipotoxicity). Since part of the demise process in glucolipotoxicity includes β -cell de-differentiation, we looked at two β -cell identity marker genes PDX1 (**Figure 26c**) and INS (**Figure 26d**). Both were downregulated at the mRNA level in human islets exposed to glucolipotoxicity, consistent with the previously reported de-differentiation phenotype pertinent to β -cell demise. Yet, in the presence of **66** this de-differentiation was prevented (**Figure 26c-d**). Similarly, immunostaining of dispersed human islets, revealed increase in nuclear ChREBP β nuclear localization and compound **66** prevented upregulation of ChREBP β both at mRNA level (**Figure 26b**) and at protein

level (**Figure 27a-b**), indicating that inhibition of the nuclear translocation of ChREBP α by strengthening the ChREBP α /14-3-3 interaction with **66** is required for the blocking of the upregulation of glucose responsive genes such as ChREBP β . Similarly, immunostaining for PDX1 showed a marked decrease in PDX1 protein levels in glucolipotoxicity, which was rescued by **66** (**Figure 27a, 2c**).

66 and related active compounds in relation to Type 1 Diabetes

Type I diabetes results from autoimmune destruction of beta cells. Patients usually present with an acute episode of hyperglycemia followed by apparent recovery for a period of several months or more – called the honeymoon period. The destructive mechanism involves the secretion of cytokines from immune cells surrounding the islets of Langerhans (insulinitis), which include the insulin-producing beta cells. Cytokines are known to induce ChREBP, though the isoforms involved were not distinguished¹. Thus, we tested if compound **66** can block induction of ChREBP β from cytokines and if this protected beta cells from cell death. We found increased nuclear expression of ChREBP in response to TNF α , IL1 β and INF γ cytokine exposure (**Figure 28a-b**) and our 14-3-3: ChREBP α stabilizing compound, **66**, prevented nuclear localization of ChREBP in response to cytokines (**Figure 28a-b**). Moreover, addition of the cytokine mix led to massive death of INS-1 cells (**Figure 29**) and compound **66** protected INS-1 cells from cytokine-induced toxicity (**Figure 29**). Importantly, beta cell function was assessed after treatment with compound **66**. Human islets from cadaveric donors were pre-treated for 24 h with DMSO or 10 μ M of compound **66** under various conditions, including low glucose (5.5 mM), high glucose (20 mM), and high glucose with cytokine mix (TNF α , IL1 β , INF γ). Following treatment, the islets were assessed for glucose-stimulated insulin secretion (GSIS). The stimulation index (SI, insulin concentration in the media from high glucose divided by insulin concentration in the media with low glucose) was significantly higher in islets treated with compound **66** compared to untreated controls or to islets that were pre-incubated for 24 h with either high glucose or with the three cytokine mix (**Figure 30a**). These data demonstrate improved beta cell secretory function in the presence of **66**. Importantly, the SI for KCl stimulation (to determine maximal secretory capacity) versus low glucose was not significantly affected among our treatment groups (**Figure 30b**). Similarly, total insulin content was constant among our treatment groups, demonstrating that compound **66** had no effect on insulin production or turnover (**Figure 30c**).

To support the idea that ChREBP might be involved in T1D, single cell data from HPAP (human pancreas analysis program) of HIRN (human islet research network) was interrogated for *Mlxipl* (the gene name for ChREBP) and its close relative (*Mlxip*) and their target genes in subjects that were non-diabetic, auto-antibody positive, and therefore were likely to get T1D, and subjects with T1D. In **Figure 31**, *Mlxipl* is very highly expressed in auto-antibody positive subjects and *Mlxip* and its target genes are highly expressed in T1D subjects compared to non-diabetic controls. In addition, tissue sections of pancreata of female NOD (non-obese diabetic) mice up to 15 weeks of age were stained with an antibody against the C-terminus of ChREBP (recognizing both ChREBP α and ChREBP β) (**Figure 32**) or the N terminus (only recognized ChREBP α) (**Figure 33**). Female NOD mice become diabetic around week 20. In **Figure 32a**, 4-week old mice that are not diabetic, are not hyperglycemic, and did not yet show signs of insulinitis (infiltration of immune cells) nonetheless have strong nuclear staining of ChREBP, which is not present in control non-diabetic C57Bl/6 mice (**Figure 32b**). The ChREBP isoform in the nucleus is ChREBP β since all the staining of the N-terminal antibody in **Figure 33a** is cytoplasmic, as is the control C57BL/6 (**Figure 33b**). ChREBP was also found in the CD45+ immune cells associated with insulinitis seen at 15 weeks – this consistently cytoplasmic with both antibodies (**Figures 32a and 33a**) and is therefore most likely ChREBP α . ChREBP is not normally thought to be expressed in T-cells, the major cell type of insulinitis, so this finding was a surprise and forms the basis of our working hypothesis that ChREBP is important for the autoimmune function in these mice, and that treatment with **66** or related active compounds may in addition mitigate their autoimmune functions.

Together, the implications of these data are: 1) compound **66** and closely related active compounds in accordance with the invention may prevent the cytokine-mediated autoimmune destruction of beta cells, 2) that since ChREBP β is expressed before the onset of insulinitis and therefore may participate in the pathogenesis of T1D, compound **66** and closely related active compounds may be useful to prevent or delay the onset of T1D after the honeymoon period, 3) since ChREBP is expressed in the T-cells involved in autoimmune destruction of beta cells in NOD mice, and that T-cells normally do not express ChREBP, compound **66** and closely related active compounds may also alter immune function of these T-cells and therefore mitigate the autoimmune

function of immune cells in the context of T1D. Combined, this shows that compound **66** and compounds in accordance with the invention can find use in the treatment of T1D.

5 **66 and related active compounds in relation to cancer**

Compound **66** and related active compounds in accordance with the invention block ChREBP-dependent proliferation and are therefore a possible cancer therapeutic. We and others have shown that ChREBP is necessary for cellular proliferation in some cell types ²⁻⁵. In pancreatic beta cells, glucose acts as a mitogen for adaptive proliferation, and ChREBP is necessary for glucose-stimulated beta cell proliferation. In cancer, Thompson and colleagues noted the necessity of ChREBP for proliferation of HCT116 cells as early as 2009 ⁵. Recently, Benichou et al. showed that ChREBP is necessary for proliferation of hepatic cell carcinomas ². **Figure 34** shows that compound **66** blocks high glucose-mediated proliferation of INS-1 insulinoma cells. Based on these data, the literature, and our understanding of how **66** stabilizes 14-3-3:ChREBP, **66** and related active compounds in accordance with the invention may be highly useful for cancer therapy by blocking proliferation of cancer cells that are dependent on ChREBP for proliferation.

20 REFERENCES RESULTS II

- 1 Hong, K., Xu, G., Grayson, T. B. & Shalev, A. Cytokines Regulate β -Cell Thioredoxin-interacting Protein (TXNIP) via Distinct Mechanisms and Pathways. *J Biol Chem* 291, 8428-8439, (2016).
- 2 Benichou, E. et al. The transcription factor ChREBP Orchestrates liver carcinogenesis by coordinating the PI3K/AKT signaling and cancer metabolism. *Nature communications* 15, 1879, (2024).
- 3 Katz, L. S. et al. Maladaptive positive feedback production of ChREBP β underlies glucotoxic β -cell failure. *Nature communications* 13, 4423, (2022).
- 4 Metukuri, M. R. et al. ChREBP mediates glucose-stimulated pancreatic beta-cell proliferation. *Diabetes* 61, 2004-2015, (2012).
- 5 Tong, X., Zhao, F., Mancuso, A., Gruber, J. J. & Thompson, C. B. The glucose-responsive transcription factor ChREBP contributes to glucose-dependent anabolic synthesis and cell proliferation. *Proc Natl Acad Sci U S A* 106, 21660-21665, (2009).

SECTION II - Synthesis and Characterization of chemical compounds

Please note that in this Section II, synthesis of compounds is described. The compound is depicted to explicitly indicate the compound made with the synthesis. The numbering scheme in this Section II is different from the Section I above. Not every compound made in this section II was used in section I. Please note that for the preparation of compounds, the heading as listed below is subsequently followed by the number as used in the section above. For example, for compound nr. 298, i.e. KPP298, of this Section II, this corresponds with compound 62, as described in the Section I, indicated by the heading as follows:

298: *Synthesis* of 2-(2-((2,2-Difluoro-2-phenylethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP298**) **62**

General Information (chemicals, materials and instrumentation)*Reagents and dry solvents*

All reagents were purchased from ABCR, Acros Organics, Alfa Aesar, Carbolution Chemicals, Carl Roth, Fisher, Fluka, Fluorochem, Merck, Riedel de H  en, Sigma Aldrich, TCI Chemicals, Bernd Kraft or VWR Chemicals and were used without further purification. All dry solvents were purchased from the same supplier with the best quality available.

Column chromatography

Compound purification by column chromatography was achieved using glass columns filled with silica gel (particle size 35 – 70 μm , from *Acros Organics*) as stationary phase and eluent mixtures of different solvents as mobile phase. The exact ratios of the solvents are listed in the corresponding synthesis procedures.

Thin layer chromatography (TLC)

Thin layer chromatography was performed on silica coated aluminum plates (60 F₂₅₄) from *Merck*. Detection of substances was conducted with UV light (wave length 254 nm or 366 nm). The resulting R_f values including the used solvents are listed in the corresponding synthesis procedures.

Freeze-drying

Freeze-drying of the products was carried out with a lyophilizer ALPHA 2-4 LD plus (CHRIST) at an ice condenser temperature of -80 °C. The drying process is favored by a large ice surface and a low ice thickness. To obtain the largest possible ice surface, an aqueous solution of the corresponding substance was frozen in liquid nitrogen under constant rotation. The corresponding frozen compounds were then put for 24 – 72 h on the lyophilizer.

Reversed-phase liquid chromatography electrospray ionization mass spectrometry (LC-MS)

Reaction control analyses were performed on a LC-MS system from *Thermo Scientific*.

The system consisted of a Thermo Scientific Accela™ (peak detection at 210 nm) and a Thermo Scientific UltiMate™ 3000 (peak detection at 230 nm and 260 nm) equipped with an Eclipse XDB-C18 column (particle size 5 µm, from Agilent) and a *Thermo Scientific* LCQ Fleet™ ESI-MS. For analysis, a linear gradient of solvent B (0.1 % formic acid in acetonitrile) in solvent A (0.1 % formic acid in water) at flow rate of 1 mL min⁻¹ and the following gradient program: 0 min (10 % B) → 1 min (10 % B) → 10 min (100 % B) → 12 min (100 % B) → 15 min (10 % B) was used.

High-resolution mass spectrometry (HRMS)

HRMS spectra were recorded on an Exactive Plus EMR mass spectrometer from *Thermo Fisher* with an Advion TriVersa NanoMate ESI system from *Advion*.

Preparative reversed-phase high performance liquid chromatography (prep HPLC)

Compound purification by HPLC was achieved using the *Prominence UFLC* system from *Shimadzu* (peak detection at 210 nm and 254 nm). The system was equipped with a reversed-phase C18 column from *Phenomenex* (*Luna*® 5 µm C18(2), 100 x 21.20 mm). For purification a linear gradient of solvent B (0.1 % TFA in acetonitrile) in solvent A (0.1 % TFA in water) at a flow rate of 20 mL min⁻¹ was used.

Nuclear magnetic resonance spectroscopy (NMR)

Nuclear magnetic resonance (NMR) spectra were recorded on a *Bruker Avance II 400* (400 MHz for ¹H NMR and 100 MHz for ¹³C NMR) machine. As solvents deuterated

chloroform-d₁, deuterated DMSO-d₆ or deuterated methanol-d₄ were used. The chemical shifts δ are reported in parts per million (ppm). The spectra were referenced to the residual signals of undeuterated solvents (CDCl₃: δ (¹H) = 7.26 and δ (¹³C) = 77.16, DMSO: δ (¹H) = 2.50 and δ (¹³C) = 39.52, MeOD: δ (¹H) = 4.87 and δ (¹³C) = 49.00). The coupling constants J are reported in Hertz (Hz). The ¹H NMR spectral data list the chemical shifts δ , the multiplicities (s: singlet, d doublet, t: triplet, m: multiplet), the coupling constant J and the number of protons. The ¹³C NMR spectra list only the chemical shifts δ .

10 General Procedures (GP)

General Procedure A: Synthesis of the phosphate derivative

The corresponding phenol derivative (1.0 eq) was dissolved in dry DCM (0.5 mL per mmol). Tetrachlormethane (1.0 eq) and triethylamine (1.0 eq) were added. The solution was cooled to 0 °C and dimethyl phosphite (1.5 eq) was added dropwise. The resulting solution was stirred for 16 h, allowing the mixture to slowly reach room temperature. The reaction mixture was washed with 5% NaHCO₃ solution (3x), the organic phase was dried over MgSO₄ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography.

20 *General Procedure B: Synthesis of the phosphonate derivative*

Generation of LDA: Dry di-*isopropyl* amine (1.7 eq) was dissolved in dry THF (4.8 mL per mmol of the phosphate derivative) and cooled to -78 °C. *n*-BuLi (2.5 M, 1.5 eq) was added dropwise. The cooling bath was removed and the mixture was stirred for 1 h.

25 The LDA mixture was cooled to -78 °C again. The respective phosphate derivative (1.0 eq) was dissolved in dry THF (0.7 mL per mmol of the phosphate derivative), cooled to -78 °C and added to the LDA solution. The resulting mixture was stirred for 16 h, allowing the mixture to slowly reach room temperature. The reaction mixture was quenched with saturated NH₄Cl solution (0.6 mL per mmol of the phosphate derivative), the organic phase was separated and the solvent was removed under reduced pressure. The crude product was purified by column chromatography.

General Procedure C: Amide coupling with HOBt and EDC

The carboxylic acid derivative (1.0 eq) was dissolved in DCM (50 mL per mmol of the carboxylic acid derivative). EDC (4.0 eq), HOBt (4.0 eq) and DIPEA (6.0 eq) were added. The corresponding amine (2.0 eq) was added and the mixture was stirred for 16 h at room temperature. The reaction mixture was washed with 5% KHSO₄ solution (3x) and 5% NaHCO₃ solution (3x). The organic phase was dried over MgSO₄ and the solvent was removed under reduced pressure.

10 *General Procedure D: Amide coupling with bromoacetyl bromide*

The amine derivative (1.0 eq) was dissolved in dry DCM (3 – 5 mL per mmol) and cooled to 0 °C. Triethylamine (1.1 eq) and bromoacetyl bromide (1.0 – 1.2 eq) were added and the resulting mixture was stirred for 16 h, allowing the mixture to slowly reach room temperature. The reaction mixture was washed with saturated NH₄Cl solution (3x) and the organic phase was dried over MgSO₄ and the solvent was removed under reduced pressure. The crude product was purified by HPLC.

General Procedure E: Amide coupling with acid chloride

Generation of the acid chloride: The acid derivative (1.5 eq.) was dissolved in dry DCM (5 mL per mmol), oxalyl chloride (3.0 eq) and some drops of dry DMF were added. The resulting mixture was stirred for 2 h at 40 °C. After cooling down to room temperature the volatiles were removed under reduced pressure.

The resulting acid chloride was redissolved in dry DCM (5 mL per mmol amine). The amine (1.0 eq.) was dissolved in dry DCM (9 mL per mmol) and cooled to 0 °C. Triethylamine (3.0 eq.) and the acid chloride solution were added and the resulting mixture was stirred for 16 h, allowing the mixture to slowly reach room temperature. The solvent was removed under reduced pressure and the crude product was purified by HPLC.

General Procedure F: Amide coupling with isobutyl chloroformate

The acid derivative (1.0 eq.) was dissolved in dry THF (10 mL per mmol acid). *N*-Methylmorpholine (1.0 eq.) was added and the mixture was cooled to -35 °C. Isobutyl chloroformate (1.0 eq) was added and the resulting mixture was stirred for 45 min. The amine (1.0 eq.) and *N*-Methylmorpholine (1.0 eq.) were added and the resulting mixture was stirred for 16 h, allowing the mixture to slowly reach room temperature. The solvent was removed under reduced pressure and the residue was poured into ethyl acetate. The organic phase was washed with saturated NH₄Cl solution (3x), dried over MgSO₄ and the solvent was removed under reduced pressure.

General Procedure G: Williamson ether synthesis

Reaction in acetone: The respective nucleophile (1.0 eq) was dissolved in acetone (10 – 25 mL per mmol). Potassium carbonate (2.0 eq) was added and the resulting suspension was stirred for 20 min at room temperature. The respective bromide (1.0 – 1.3 eq) was added and the resulting mixture was stirred for 16 h at room temperature. The solvent was evaporated and the residue was suspended in ethyl acetate (25 mL per mmol of the phosphonate derivative). This suspension was washed with saturated NH₄Cl solution (3x) and the organic phase was dried over MgSO₄ and the solvent was removed under reduced pressure.

Reaction in DMF: The respective nucleophile (1.0 eq) was dissolved in DMF (2 – 10 mL per mmol). Potassium carbonate (2.0 eq) was added and the resulting suspension was stirred for 20 min at room temperature. The respective bromide (1.0 – 1.3 eq) was added and the resulting mixture was stirred for 16 h at room temperature. The reaction mixture was poured into water (5 mL per mL DMF) and was extracted with diethyl ether (3x). The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure.

General Procedure H: Deprotection of the phosphonates

The protected phosphonate derivative (1.0 eq) was dissolved in dry DCM (6 mL per mmol) and cooled to 0 °C. TMSBr (5.0 – 50.0 eq) was added and the resulting solution was stirred for 4 h, allowing the mixture to slowly reach room temperature. The solvent was removed under reduced pressure. The residue was re-dissolved in a mixture

MeOH/H₂O (3:1, 6 mL per mmol of the protected phosphonate derivative) and stirred for 1 h at room temperature. The solvent was removed under reduced pressure and the crude product was purified by HPLC.

5 *General Procedure J: Substitution with 2-(Boc-amino)ethyl bromide*

The heteroaromatic (1.0 eq) was dissolved in DMF (8 mL per mmol). Potassium carbonate (2.4 eq) was added and the resulting suspension was stirred for 20 min at room temperature. 2-(Boc-amino)ethyl bromide (1.0 eq) was added and the resulting mixture was stirred for 48 h at room temperature. The reaction mixture was poured
10 into water (5 mL per mL DMF) and was extracted with ethyl acetate (3x). The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure.

General Procedure K: Boc protection

15 The amine (1.0 eq.) and Di-*tert* butyl dicarbonate (1.03 eq.) was suspended in THF (4.3 mL per mmol amine) and cooled down to 0 °C. NaHCO₃ (1.11 eq.) was dissolved in H₂O (2.2 mL per mmol amine) and added to the suspension dropwise. The resulting mixture was stirred for 16 h, allowing the mixture to slowly reach room temperature. The solvent was removed under reduced pressure and the residue was
20 poured into ethyl acetate (10 mL per mmol amine). The organic phase was washed with 0.5M HCl (3x), H₂O (1x) and brine (1x), dried over MgSO₄ and the solvent was removed under reduced pressure.

General Procedure L: Boc deprotection

25 Deprotection with dioxane: The Boc protected amine (1.0 eq.) was dissolved in dioxane (0.1 – 1.0 mL), 4M HCl/dioxane was added and the reaction was stirred for 3 h at room temperature. The suspension was poured in diethyl ether and 1 M NaOH (20 mL per mmol amine) was added. The phases were separated and the aqueous phase was extracted with diethyl ether (3x). The combined organic phases
30 were dried over MgSO₄ and the solvent was removed under reduced pressure.

Deprotection with TFA: The Boc protected amine (1.0 eq.) was dissolved in DCM/TFA (1:1, 5 mL per mmol) and stirred for 16 h at room temperature. The solvent was removed under reduced pressure.

5 *General Procedure M: Henry reaction with sodium hydroxide*

The aldehyde (1.0 eq.) and nitromethane (1.1 eq.) were dissolved in ethanol (1.75 mL per mmol aldehyde) and cooled down to 0 °C. 10M NaOH (100 µL per mmol aldehyde) was added slowly and the resulting mixture was stirred for 2 h at 0 °C. The mixture was quenched with HCl/ H₂O (1:1, 5 mL per mmol aldehyde) and stirred for 1 h at 0 °C.

10 The resulting solid was filtered, washed with H₂O and dried under reduced pressure.

General Procedure N: Reduction with lithium aluminium hydride

The nitrovinyl derivative (1.0 eq.) was dissolved in dry THF (2 mL per mmol nitrovinyl derivative). Lithium aluminium hydride was suspended in dry THF (2 mL per mmol nitrovinyl derivative) and cooled down to 0 °C. The nitrovinyl derivative solution was added slowly and the resulting mixture was stirred for 20 min at 0 °C and then for 3 h at 45 °C. The mixture was then cooled down to 0 °C again and quenched with 10 % NaOH solution (1.2 mL per mmol nitrovinyl derivative). The mixture was filtered over Celite and the filter cake was washed with ethyl acetate. The crude product was dried under reduced pressure and then purified by column chromatography or HPLC.

General Procedure O: Copper mediated cross coupling

Activated copper (2.6 – 3.9 eq.) was suspended in dry DMSO or DMF (1.6 – 2.6 mL per mmol iodobenzene derivative). The iodobenzene derivative (1.0 eq.) and ethyl bromodifluoroacetate (1.0 – 1.5 eq.) were added and the reaction mixture was stirred for 16 h at 60 °C. After cooling down to room temperature the reaction mixture was poured into ice/ saturated NH₄Cl solution (1:1), the inorganic solid was filtered out and the filtrate was extracted with diethyl ether. The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography.

Copper activation: Copper was suspended in 1M HCl solution (0.4 mL per mmol copper). The suspension was stirred for 10 min at room temperature and the solvent was filtered out. The procedure was repeated one after another with H₂O, MeOH and Aceton. At the end the activated copper was dried under reduced pressure.

5

General Procedure P: Reduction with sodium boronhydride

The difluoroacetate derivative (1.0 eq.) was dissolved in MeOH (3.4 – 3.8 mL per mmol) und cooled down to 0 °C. NaBH₄ (1.0 eq.) was added and the mixture was stirred for 1 h at 0 °C. The reaction mixture was quenched with H₂O/ saturated NH₄Cl solution (1:1, 1.4 mL per mmol) and with ethyl acetate extracted. The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure.

10

General Procedure Q: Amination

The alcohol (1.0 eq.) was dissolved in dry acetonitrile (1.8 mL per mmol alcohol) and pyridine (1.6 eq.) was added. The mixture was cooled down to 0 °C and TF₂O (1.1 eq.) was added slowly. The mixture was stirred for 15 min at room temperature. NH₄OH (28 %, 1.8 mL per mmol alcohol) was added and the mixture was stirred for 16 h at room temperature. The reaction mixture was extracted with DCM, the combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure.

20

General Procedure R: Esterification with chloromethyl pivalate

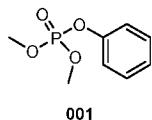
The acid (1.0 eq.) was dissolved in dry DMF (15 mL per mmol acid) and triethylamine (2.0 eq.) was added. After 10 min stirring at room temperature chloromethyl pivalate (10.0 eq.) was added and the reaction mixture was stirred for 16 h at 60 °C. After cooling down to room temperature the mixture was poured into H₂O and extracted with diethyl ether. The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure.

25

30

Synthetic Procedures and Characterization of Compounds

001: Synthesis of dimethyl phenyl phosphate (**KPP001**)

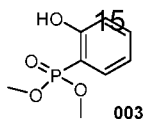


5

The phosphate **KPP001** was synthesized via GP A using phenol (9.41 g, 100 mmol), tetrachlormethane (9.6 mL, 100 mmol), triethylamine (14.9 mL, 100 mmol) and dimethyl phosphite (13.8 mL, 150 mmol) in dry DCM (50 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1 → 1:1) to obtain **KPP001** (11.82 g, 58.5 mmol, 59 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 1:1 v/v): $R_f = 0.4$. LC-MS (ESI): $t_R = 7.13$ min; $m/z = 203.03$ $[M + H]^+$. 1H NMR (400 MHz, $CDCl_3$): $\delta = 7.26 - 7.22$ (m, 2H), $7.13 - 7.06$ (m, 3H), 3.73 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 150.81, 129.95, 125.33, 120.05, 120.00, 55.01$. HRMS (ESI): $m/z = 203.0468$ calcd. for $[C_8H_{11}O_4P + H]^+$; found: 203.0462.

10

003: Synthesis of dimethyl 2-hydroxyphenylphosphonate (**KPP003**)



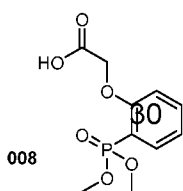
003

The phosphonate **KPP003** was synthesized via GP B using diisopropyl amine (13.8 mL, 98.5 mmol), *n*-BuLi (2.5 M, 34.8 mL, 87.8 mmol) and phosphate **KPP001** (11.80 g, 58.4 mmol) in dry THF (280 mL + 40 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP003** (9.92 g, 49.1 mmol, 84 %) as a white solid. TLC (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.4$. LC-MS (ESI): $t_R = 6.02$ min; $m/z = 202.98$ $[M + H]^+$. 1H NMR (400 MHz, $CDCl_3$): $\delta = 10.06$ (s, 1H), $7.47 - 7.43$ (m, 1H), 7.35 (ddd, $J = 14.3, 7.7, 1.7$ Hz, 1H), $6.99 - 6.90$ (m, 2H), 3.74 (d, $J = 11.6$ Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 162.77, 135.94, 132.01, 120.22, 118.29, 77.80, 77.16, 53.47$. HRMS (ESI): $m/z = 203.0468$ calcd. for $[C_8H_{11}O_4P + H]^+$; found: 203.0461.

20

25

008: Synthesis of 2-(2-(methoxyphosphono)phenoxy)acetic acid (**KPP008**)

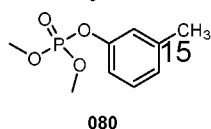


008

The phosphonate derivative **KPP008** was synthesized via GP G using phosphonate **KPP003** (1.822 g, 9.00 mmol), potassium carbonate (2.484 g, 18.0 mmol) and methyl bromoacetate (0.95 mL, 9.90 mmol) in acetone (225 mL). The crude intermediate was dissolved

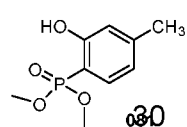
in a mixture THF/ methanol (1:1, 300 mL). Lithium hydroxide (0.647 g, 27.0 mmol, 3.0 eq) was added and the solution was stirred for 16 h at room temperature. The reaction mixture was acidified with 1M HCl solution (to a pH < 3) and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (ethyl acetate/methanol 1:1) to obtain **KPP008** (1.56 g, 6.00 mmol, 67 % over two steps) as a white solid. TLC (ethyl acetate:methanol, 1:1 v/v): $R_f = 0.5$. **LC-MS (ESI)**: $t_R = 4.92$ min; $m/z = 261.00$ $[M + H]^+$. **1H NMR** (400 MHz, $CDCl_3$): $\delta = 10.93$ (s, 1H), 7.65 – 7.59 (m, 1H), 7.55 – 7.51 (m 1H), 7.12 – 7.06 (m, 1H), 6.97 – 6.92 (m, 1H), 4.72 (d, $J = 2.6$ Hz, 2H), 3.78 (dd, $J = 11.4, 2.9$ Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$): $\delta = 169.86, 160.32, 135.53, 134.05, 122.67, 114.19, 67.73, 53.84$. **HRMS (ESI)**: $m/z = 261.0523$ calcd. for $[C_{10}H_{13}O_6P + H]^+$; found: 261.0514.

080: Synthesis of dimethyl *m*-tolyl phosphate (KPP080**)**



The phosphate **KPP080** was synthesized via GP A using *m*-cresol (2.10 mL, 20.00 mmol), tetrachlormethane (1.92 mL, 20.00 mmol), triethylamine (2.98 mL, 20.0 mmol) and dimethyl phosphite (2.77 mL, 30.00 mmol) in dry DCM (15 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1 → 1:1) to obtain **KPP080** (1.90 g, 8.79 mmol, 44 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 1:1 v/v): $R_f = 0.4$. **LC-MS (ESI)**: $t_R = 7.47$ min; $m/z = 216.99$ $[M + H]^+$. **1H NMR** (400 MHz, $CDCl_3$): $\delta = 7.16 - 7.11$ (m, 1H), 6.97 - 6.90 (m, 3H), 3.99 (d, $J = 11.5$ Hz, 6H), 2.27 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): $\delta = 150.43, 139.88, 129.29, 125.78, 120.24, 116.60, 54.65, 21.11$. **HRMS (ESI)**: $m/z = 217.0624$ calcd. for $[C_9H_{13}O_4P + H]^+$; found: 217.0621.

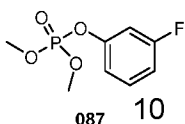
081: Synthesis of dimethyl 2-hydroxy-4-methylphenylphosphonate (KPP081**)**



The phosphonate **KPP081** was synthesized via GP B using diisopropyl amine (2.07 mL, 14.82 mmol), *n*-BuLi (2.5 M, 5.23 mL, 13.22 mmol) and phosphate **KPP080** (1.91 g, 8.79 mmol) in dry THF (45 mL + 5 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP081** (0.59 g, 2.71 mmol, 31 %) as a

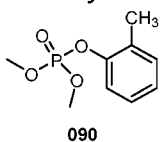
white solid. TLC (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.3$. LC-MS (ESI): $t_R = 6.84$ min; $m/z = 216.97$ $[M + H]^+$. 1H NMR (400 MHz, $CDCl_3$): $\delta = 9.90$ (s, 1H), 7.26 – 7.21 (m, 1H), 6.81 – 6.75 (m, 2H), 3.73 (d, $J = 11.5$ Hz, 6H), 2.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 162.39, 146.67, 131.35, 121.10, 117.90, 103.06, 52.87, 21.75$. HRMS (ESI): $m/z = 217.0624$ calcd. for $[C_9H_{13}O_4P + H]^+$; found: 217.0621.

087: Synthesis of 3-fluorophenyl dimethyl phosphate (KPP087)

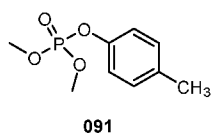


The phosphate **KPP087** was synthesized via GP A using *m*-fluorophenol (1.81 mL, 20.00 mmol), tetrachlormethane (1.92 mL, 20.00 mmol), triethylamine (2.98 mL, 20.00 mmol) and dimethyl phosphite (2.77 mL, 30.00 mmol) in dry DCM (15 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1 $\rightarrow$ 1:1) to obtain **KPP087** (1.65 g, 7.47 mmol, 34 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 1:1 v/v): $R_f = 0.4$. LC-MS (ESI): $t_R = 7.95$ min; $m/z = 220.96$ $[M + H]^+$, calcd. for $C_8H_{10}FO_4P$: 220.03. 1H NMR (400 MHz, $CDCl_3$): $\delta = 7.32 - 7.26$ (m, 1H), 7.03 – 6.95 (m, 2H), 6.91 – 6.87 (m, 1H), 3.87 – 3.84 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 164.23, 161.77, 151.46, 130.53, 115.70, 112.32, 107.88, 54.98$.

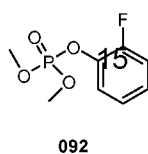
090: Synthesis of dimethyl *o*-tolyl phosphate (KPP090)



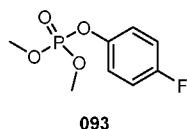
The phosphate **KPP090** was synthesized via GP A using *o*-cresol (2.08 mL, 20.00 mmol), tetrachlormethane (1.92 mL, 20.00 mmol), triethylamine (2.98 mL, 20.00 mmol) and dimethyl phosphite (2.77 mL, 30.00 mmol) in dry DCM (15 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1 $\rightarrow$ 1:1) to obtain **KPP090** (1.30 g, 6.03 mmol, 30 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 1:1 v/v): $R_f = 0.3$. LC-MS (ESI): $t_R = 7.76$ min; $m/z = 217.07$ $[M + H]^+$. 1H NMR (400 MHz, $CDCl_3$): $\delta = 7.37 - 7.26$ (m, 3H), 7.20 (t, $J = 7.4$ Hz, 1H), 3.99 (d, $J = 11.5$ Hz, 6H), 2.43 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 148.91, 131.52, 129.23, 127.19, 125.36, 119.60, 55.19, 16.25$. HRMS (ESI): $m/z = 217.0624$ calcd. for $[C_9H_{13}O_4P + H]^+$; found: 217.0622.

091: Synthesis of dimethyl p-tolyl phosphate (KPP091)

The phosphate **KPP091** was synthesized via GP A using *p*-cresol (2.10 mL, 20.00 mmol), tetrachlormethane (1.92 mL, 20.00 mmol), triethylamine (2.98 mL, 20.00 mmol) and dimethyl phosphite (2.77 mL, 30.00 mmol) in dry DCM (15 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1 → 1:1) to obtain **KPP091** (2.23 g, 10.32 mmol, 52 %) as a colorless oil. **TLC** (cyclohexane:ethyl acetate, 1:1 v/v): $R_f = 0.3$. **LC-MS (ESI)**: $t_R = 7.80$ min; $m/z = 217.07$ $[M + H]^+$. **1H NMR** (400 MHz, $CDCl_3$): $\delta = 7.09 - 7.03$ (m, 4H), 3.78 (d, $J = 11.3$ Hz, 6H), 2.26 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): $\delta = 148.37, 134.70, 130.14, 119.49, 54.81, 20.60$. **HRMS (ESI)**: $m/z = 217.0624$ calcd. for $[C_9H_{13}O_4P + H]^+$; found: 217.0623.

092: Synthesis of 2-fluorophenyl dimethyl phosphate (KPP092)

The phosphate **KPP092** was synthesized via GP A using *o*-fluorophenol (1.79 mL, 20.00 mmol), tetrachlormethane (1.92 mL, 20.00 mmol), triethylamine (2.98 mL, 20.00 mmol) and dimethyl phosphite (2.77 mL, 30.00 mmol) in dry DCM (15 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1 → 1:1) to obtain **KPP092** (1.75 g, 7.96 mmol, 40 %) as a colorless oil. **TLC** (cyclohexane:ethyl acetate, 1:1 v/v): $R_f = 0.4$. **LC-MS (ESI)**: $t_R = 7.40$ min; $m/z = 221.04$ $[M + H]^+$. **1H NMR** (400 MHz, $CDCl_3$): $\delta = 7.34 - 7.30$ (m, 1H), 7.11. - 7.06 (m, 3H), 3.88 - 3.84 (m, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$): $\delta = 154.27, 152.56, 138.44, 126.20, 124.67, 122.39, 116.92, 55.18$. **HRMS (ESI)**: $m/z = 221.0373$ calcd. for $[C_8H_9FO_4P + H]^+$; found: 221.0371.

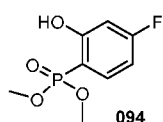
093: Synthesis of 4-fluorophenyl dimethyl phosphate (KPP093)

The phosphate **KPP093** was synthesized via GP A using *p*-fluorophenol (2.24 g, 20.00 mmol), tetrachlormethane (1.92 mL, 20.00 mmol), triethylamine (2.98 mL, 20.00 mmol) and dimethyl phosphite (2.77 mL, 30.00 mmol) in dry DCM (15 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1 → 1:1) to obtain **KPP093** (2.52 g, 11.46 mmol, 57 %) as a colorless oil. **TLC** (cyclohexane:ethyl acetate, 1:1 v/v):

$R_f = 0.3$. **LC-MS (ESI)**: $t_R = 7.39$ min; $m/z = 221.04$ $[M + H]^+$. **1H NMR** (400 MHz, $CDCl_3$): $\delta = 7.19 - 7.16$ (m, 2H), $7.04 - 7.00$ (m, 2H), $3.87 - 3.84$ (m, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$): $\delta = 161.13, 158.71, 146.66, 121.53, 116.60, 116.36, 55.15$. **HRMS (ESI)**: $m/z = 221.0373$ calcd. for $[C_8H_{10}FO_4P + H]^+$; found: 221.0369.

5

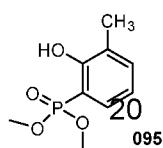
094: Synthesis of dimethyl 4-fluoro-2-hydroxyphenylphosphonate (KPP094)



The phosphonate **KPP094** was synthesized via GP B using di-*isopropyl* amine (1.78 mL, 12.69 mmol), *n*-BuLi (2.5 M, 4.45 mL, 11.21 mmol) and phosphate **KPP087** (1.64 g, 7.47 mmol) in dry THF (30 mL + 5 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP094** (0.37 g, 1.68 mmol, 23 %) as a white solid. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.5$. **LC-MS (ESI)**: $t_R = 7.50$ min; $m/z = 220.98$ $[M + H]^+$, calcd. for $C_8H_{10}FO_4P$: 220.03. **1H NMR** (400 MHz, $CDCl_3$): $\delta = 10.77$ (s, 1H), $7.44 - 7.38$ (m, 1H), $6.79 - 6.76$ (m, 1H), $6.62 - 6.56$ (m, 1H), $3.82 - 3.77$ (m, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$): $\delta = 164.15, 162.60, 136.49, 114.08, 106.47, 53.83$.

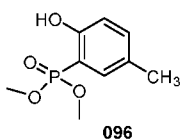
15

095: Synthesis of dimethyl 2-hydroxy-3-methylphenylphosphonate (KPP095)



The phosphonate **KPP095** was synthesized via GP B using di-*isopropyl* amine (1.42 mL, 10.17 mmol), *n*-BuLi (2.5 M, 3.59 mL, 9.07 mmol) and phosphate **KPP090** (1.31 g, 6.03 mmol) in dry THF (30 mL + 4 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP095** (0.45 g, 2.08 mmol, 34 %) as a yellow oil. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.5$. **LC-MS (ESI)**: $t_R = 7.77$ min; $m/z = 217.03$ $[M + H]^+$, calcd. for $C_9H_{13}O_4P$: 216.06. **1H NMR** (400 MHz, $CDCl_3$): $\delta = 10.02$ (s, 1H), $7.23 - 7.19$ (m, 1H), $6.89 - 6.74$ (m, 2H), 3.74 (d, $J = 11.4$ Hz, 6H), 2.34 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): $\delta = 157.59, 148.52, 131.36, 120.94, 118.06, 52.87, 21.78$.

25

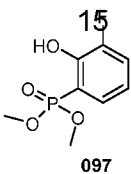
096: Synthesis of dimethyl 2-hydroxy-5-methylphenylphosphonate (**KPP096**)

The phosphonate **KPP096** was synthesized via GP B using di-isopropyl amine (2.43 mL, 17.38 mmol), *n*-BuLi (2.5 M, 6.13 mL, 15.51 mmol) and phosphate **KPP091** (2.24 g, 10.31 mmol) in dry THF (50 mL + 5 mL). The crude product was purified by column

chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP096** (1.48 g, 6.83 mmol, 66 %) as a white solid. TLC (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.3.

LC-MS (ESI): t_R = 6.84 min; m/z = 216.97 $[M + H]^+$. 1H NMR (400 MHz, $CDCl_3$): δ = 9.55 (s, 1H), 7.21 (d, J = 8.5 Hz, 1H), 7.17 – 7.07 (m, 1H), 6.89 – 6.79 (m, 1H), 3.71 (d, J = 11.6 Hz, 6H), 2.23 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 159.88, 136.62, 131.31, 129.18, 117.60, 107.53, 53.12, 20.19.

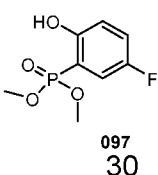
HRMS (ESI): m/z = 217.0624 calcd. for $[C_9H_{13}O_4P + H]^+$; found: 217.0621.

097: Synthesis of dimethyl 3-fluoro-2-hydroxyphenylphosphonate (**KPP097**)

The phosphonate **KPP097** was synthesized via GP B using di-isopropyl amine (1.88 mL, 13.42 mmol), *n*-BuLi (2.5 M, 4.73 mL, 11.97 mmol) and phosphate **KPP092** (1.75 g, 7.96 mmol) in dry THF (35 mL + 5 mL). The crude product was purified by column chromatography (cyclohexane/ethyl

acetate 2:1) to obtain **KPP097** (0.34 g, 1.55 mmol, 19 %) as a slightly yellow solid.

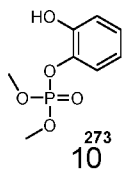
TLC (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.2. **LC-MS (ESI):** t_R = 6.23 min; m/z = 220.98 $[M + H]^+$. 1H NMR (400 MHz, $CDCl_3$): δ = 9.27 (s, 1H), 7.21 - 7.16 (m, 1H), 7.11. - 7.05 (m, 1H), 6.84. – 6.78 (m, 1H), 3.72 (d, J = 11.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 152.98, 150.57, 126.84, 121.56, 119.96, 110.96, 109.20, 53.43. **HRMS (ESI):** m/z = 221.0373 calcd. for $[C_8H_9FO_4P + H]^+$; found: 221.0361.

098: Synthesis of dimethyl 5-fluoro-2-hydroxyphenylphosphonate (**KPP098**)

The phosphonate **KPP098** was synthesized via GP B using di-isopropyl amine (2.74 mL, 19.48 mmol), *n*-BuLi (2.5 M, 6.88 mL, 17.19 mmol) and phosphate **KPP093** (2.31 g, 11.46 mmol) in dry THF (55 mL + 8 mL).

The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP098** (1.33 g, 6.03 mmol, 53 %) as a white solid. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.4. **LC-MS (ESI):**

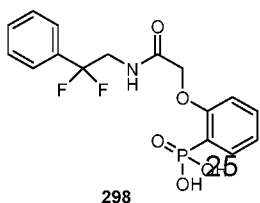
$t_R = 6.40$ min; $m/z = 220.96$ $[M + H]^+$. 1H NMR (400 MHz, $CDCl_3$): $\delta = 8.83$ (bs, 1H), 7.17 - 7.12 (m, 1H), 7.04 - 6.97 (m, 1H), 6.94 - 6.88 (m, 1H), 3.74 (d, $J = 11.5$ Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 158.80$, 157.66, 154.85, 122.96, 119.50, 116.56, 108.64, 107.08, 53.34. HRMS (ESI): $m/z = 221.0373$ calcd. for $[C_8H_{10}FO_4P + H]^+$;
 5 found: 221.068.



273: Synthesis of 2-hydroxyphenyl dimethyl phosphate (**KPP273**)

The phosphate **KPP273** was synthesized via GP A using catechol (5.51 g, 50.0 mmol), tetrachlormethane (6.90 mL, 50.0 mmol), triethylamine (7.45 mL, 50.0 mmol) and dimethyl phosphite (4.80 mL, 15.0 mmol) in dry DCM (50 mL). The crude product was purified by column chromatography (ethyl acetate) to obtain **KPP273** (7.15 g, 32.8 mmol, 66 %) as slightly brown solid. TLC (ethyl acetate, v/v): $R_f = 0.5$. LC-MS (ESI): $t_R = 6.16$ min; $m/z = 219.05$ $[M + H]^+$, calcd. for $C_8H_{11}O_5P$: 218.03. 1H NMR (400 MHz, MeOD): $\delta = 7.12$ (dt, $J = 8.1$, 1.5 Hz, 1H), 7.06 – 6.96 (m, 1H), 6.97 – 6.84 (m, 1H), 6.77 (td, $J = 7.7$, 1.6 Hz, 1H), 3.84 (d, $J = 11.5$ Hz, 6H). ^{13}C NMR (101 MHz, MeOD): $\delta = 149.66$, 139.86, 127.26, 122.29, 120.70, 118.23, 55.82.

298: Synthesis of 2-(2-((2,2-Difluoro-2-phenylethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP298**) 62

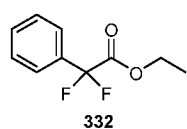


The phenylphosphonic acid derivative **KPP298** was synthesized via GP F and GP H. GP F employed phosphonate **KPP008** (260 mg, 1.00 mmol), *N*-Methylmorpholine (111 μ L, 1.00 mmol) and isobutyl chloroformate (130 μ L, 1.00 mmol) in dry THF (10 mL) as well as *N*-Methylmorpholine (111 μ L, 1.00 mmol) and 2,2-difluoro-2-phenylethanamine (157 mg, 1.00 mmol). The crude intermediate was deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and MeOH/ H_2O (3:1, 8 mL). The crude product was purified by HPLC to obtain **KPP298** (158 mg, 0.43 mmol, 43 % over two
 30 steps) as a white solid. LC-MS (ESI): $t_R = 6.11$ min; $m/z = 371.88$ $[M + H]^+$, calcd. for $C_{16}H_{16}F_2NO_5P$: 371.07. 1H NMR (400 MHz, DMSO): $\delta = 9.39$ (t, $J = 6.3$ Hz, 1H), 7.68 – 7.59 (m, 1H), 7.54 – 7.38 (m, 6H), 7.12 – 7.05 (m, 2H), 4.64 (s, 2H), 3.87 (td,

$J = 15.0, 6.3 \text{ Hz, 2H}$). $^{13}\text{C NMR}$ (101 MHz, DMSO): $\delta = 168.65, 158.78, 134.68, 133.16, 132.59, 130.30, 128.56, 125.08, 123.14, 121.19, 120.57, 113.18, 67.52, 44.09$. **HRMS (ESI)**: $m/z = 372.0807$ calcd. for $[\text{C}_{16}\text{H}_{16}\text{F}_2\text{NO}_5\text{P} + \text{H}]^+$; found: 372.0806.

5

332: Synthesis of ethyl 2,2-difluoro-2-phenylacetate (**KPP332**)

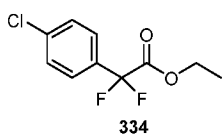


The phenylacetate derivative **KPP332** was synthesized via GP O using iodobenzene (1.12 mL, 10.00 mmol), ethyl bromodifluoroacetate (1.95 mL, 15.00 mmol) and activated copper (2.55 g, 39.00 mmol) in dry DMSO (26 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP332** (1.37 g, 6.83 mmol, 68 %) as a colorless oil. **TLC** (cyclohexane:ethyl acetate, 19:1 v/v): $R_f = 0.43$. **LC-MS (ESI)**: $t_R = 9.81 \text{ min}$; $m/z = 200.74$ $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{10}\text{H}_{10}\text{F}_2\text{O}_2$: 200.06. $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta = 7.66 - 7.59$ (m, 2H), $7.53 - 7.36$ (m, 3H), 4.28 (q, $J = 7.1 \text{ Hz}$, 2H), 1.29 (t, $J = 7.2 \text{ Hz}$, 1H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3): $\delta = 164.26, 132.89, 131.07, 128.71, 125.53, 113.49, 63.19, 13.86$.

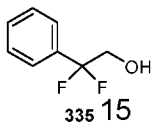
333: Synthesis of ethyl 2,2-difluoro-2-(4-fluorophenyl)acetate (**KPP333**)



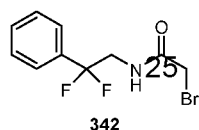
The phenylacetate derivative **KPP333** was synthesized via GP O using 4-fluoroiodobenzene (1.15 mL, 10.00 mmol), ethyl bromodifluoroacetate (1.28 mL, 10.00 mmol) and activated copper (1.65 g, 26.00 mmol) in dry DMF (15 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP333** (0.81 g, 3.71 mmol, 37 %) as a colorless oil. **TLC** (cyclohexane:ethyl acetate, 19:1 v/v): $R_f = 0.49$. **LC-MS (ESI)**: $t_R = 9.98 \text{ min}$; $m/z = 219.26$ $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{10}\text{H}_9\text{F}_3\text{O}_2$: 218.06. $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta = 7.65 - 7.55$ (m, 2H), 7.12 (t, $J = 8.6 \text{ Hz}$, 2H), 4.28 (q, $J = 7.1 \text{ Hz}$, 2H), 1.28 (t, $J = 7.2 \text{ Hz}$, 1H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3): $\delta = 165.75, 164.07, 163.11, 128.97, 127.85, 116.02, 113.15, 63.33, 13.87$.

334: Synthesis of ethyl 2,2-difluoro-2-(4-chlorophenyl)acetate (**KPP334**)

The phenylacetate derivative **KPP334** was synthesized via GP O using 4-chloriodobenzene (2.39 g, 10.00 mmol), ethyl bromodifluoroacetate (1.28 mL, 10.00 mmol) and activated copper (1.65 g, 26.00 mmol) in dry DMF (15 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP334** (1.27 g, 5.41 mmol, 54 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 19:1 v/v): R_f = 0.45. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ = 7.53 (d, J = 8.8 Hz, 2H), 7.43 (d, J = 8.8 Hz, 2H), 4.28 (q, J = 7.1 Hz, 2H), 1.28 (t, J = 7.2 Hz, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ = 170.77, 163.89, 137.42, 131.40, 129.06, 127.14, 113.05, 63.41, 13.91.

335: Synthesis of 2,2-difluoro-2-phenylethanol (**KPP335**)

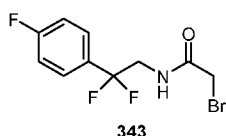
The alcohol **KPP335** was synthesized via GP P using the acetate **KPP332** (807 mg, 4.03 mmol), and NaBH_4 (152 mg, 4.03 mmol) in MeOH (14 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP335** (493 mg, 3.12 mmol, 78 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.57. LC-MS (ESI): t_R = 7.37 min; m/z = 159.62 $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_8\text{H}_8\text{F}_2\text{O}$: 158.05. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ = 7.58 – 7.50 (m, 2H), 7.49 – 7.31 (m, 3H), 3.95 (t, J = 13.5 Hz, 1H), 2.41 – 2.29 (m, 1H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ = 134.52, 130.41, 128.66, 125.59, 120.73, 66.06.

342: Synthesis of 2-bromo-N-(2,2-difluoro-2-phenylethyl)acetamide (**KPP342**)

The bromoacetamide **KPP342** was synthesized via GP Q and GP D. GP Q employed the alcohol **KPP335** (1.08 g, 6.83 mmol), pyridine (0.88 mL, 10.93 mmol) and Tf_2O (1.26 mL, 7.51 mmol) in dry acetonitrile (12.4 mL) as well as NH_4OH (28%, 12.4 mL). The crude intermediate was coupled to the amide using triethylamine (1.04 mL, 7.51 mmol) and bromoacetyl bromide (0.60 mL, 6.83 mmol) in dry DCM (34 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP342** (0.88 g, 3.16 mmol, 46 % over two steps) as a yellow solid. TLC (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.29. LC-MS (ESI): t_R = 8.11 min; m/z = 279.58 $[\text{M} + \text{H}]^+$, calcd. for

$C_{10}H_{10}BrF_2NO$: 276.99. 1H NMR (400 MHz, $CDCl_3$): δ = 7.55 – 7.39 (m, 5H), 6.86 (s, 1H), 3.94 (td, J = 14.5, 6.2 Hz, 2H), 3.87 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.85, 134.23, 130.66, 128.77, 125.32, 120.28, 46.09, 28.85.

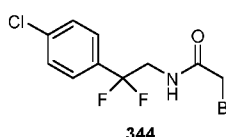
5 **343: Synthesis of 2-bromo-N-(2,2-difluoro-2-(4-fluorophenyl)ethyl)acetamide (KPP343)**



The bromoacetamide **KPP343** was synthesized via GP P, GP Q and GP D. GP P employed the acetate **KPP333** (561 mg, 2.57 mmol), and $NaBH_4$ (96 mg, 2.57 mmol) in MeOH (9 mL). The crude alcohol was converted to the amine via GP Q using pyridine (0.33 mL, 4.11 mmol) and Tf_2O (0.48 mL, 2.83 mmol) in dry acetonitrile (4.6 mL) as well as NH_4OH (28%, 4.6 mL). The crude intermediate was coupled to the amide using triethylamine (0.39 mL, 2.83 mmol) and bromoacetyl bromide (0.22 mL, 2.83 mmol) in dry DCM (13 mL). The crude product was purified by column chromatography (cyclohexane/diethyl ether 1:4) to obtain **KPP343** (200 mg, 0.67 mmol, 26 % over three steps) as a brown solid. TLC (cyclohexane:diethyl ether, 1:4 v/v): R_f = 0.38. LC-MS (ESI): t_R = 8.23 min; m/z = 295.54 $[M + H]^+$, calcd. for $C_{10}H_9BrF_3NO$: 294.98. 1H NMR (400 MHz, $CDCl_3$): δ = 7.58 – 7.42 (m, 2H), 7.13 (t, J = 8.5 Hz, 2H), 6.80 (s, 1H), 3.92 (td, J = 14.3, 6.3 Hz, 2H), 3.87 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 165.77, 130.35, 127.55, 119.97, 116.06, 115.84, 45.71, 28.84.

20

344: Synthesis of 2-bromo-N-(2,2-difluoro-2-(4-chlorophenyl)ethyl)acetamide (KPP344)

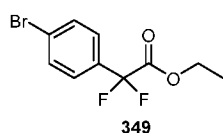


The bromoacetamide **KPP344** was synthesized via GP P, GP Q and GP D. GP P employed the acetate **KPP334** (877 mg, 3.67 mmol), and $NaBH_4$ (142 mg, 3.67 mmol) in MeOH (13 mL). The crude alcohol was converted to the amine via GP Q using pyridine (0.47 mL, 5.87 mmol) and Tf_2O (0.68 mL, 4.04 mmol) in dry acetonitrile (6.5 mL) as well as NH_4OH (28%, 6.5 mL). The crude intermediate was coupled to the amide using triethylamine (0.56 mL, 4.04 mmol) and bromoacetyl bromide (0.32 mL, 4.04 mmol) in dry DCM (18 mL). The crude product was purified by column chromatography (cyclohexane/diethyl ether 1:4) to obtain **KPP344** (216 mg, 0.69 mmol, 19 % over three steps) as an orange solid. TLC (cyclohexane:diethyl ether, 1:4 v/v): R_f = 0.43. LC-MS (ESI): t_R = 8.37 min; m/z = 311.45 $[M + H]^+$, calcd. for $C_{10}H_9BrClF_2NO$: 310.95.

30

^1H NMR (400 MHz, CDCl_3): δ = 7.50 – 7.38 (m, 4H), 6.78 (s, 1H), 3.93 (td, J = 14.3, 6.3 Hz, 2H), 3.87 (s, 2H). ^{13}C NMR (101 MHz, CDCl_3): δ = 165.76, 136.96, 132.83, 129.11, 126.82, 119.90, 45.91, 28.83.

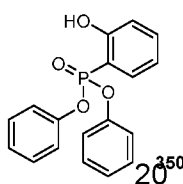
5 **349: Synthesis of ethyl 2-(4-bromophenyl)-2,2-difluoroacetate (KPP349)**



The phenylacetate derivative **KPP349** was synthesized via GP O using 4-bromoiodobenzene (2.83 g, 10.00 mmol), ethyl bromodifluoroacetate (1.95 mL, 15.00 mmol) and activated copper (2.55 g, 39.00 mmol) in dry DMSO (26 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP349** (2.38 g, 8.51 mmol, 85 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 19:1 v/v): R_f = 0.50. ^1H NMR (400 MHz, CDCl_3): δ = 7.58 (d, J = 8.7 Hz, 1H), 7.49 (d, J = 8.5 Hz, 2H), 4.30 (q, J = 7.1 Hz, 2H), 1.29 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3): δ = 163.84, 132.05, 127.25, 125.79, 113.12, 63.42, 13.94.

15

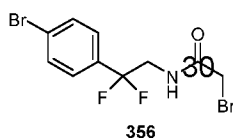
350: Synthesis of Diphenyl (2-hydroxyphenyl)phosphonate (KPP350)



The phosphonate **KPP350** was synthesized via GP B using di-*i*-propyl amine (2.39 mL, 17.0 mmol), *n*-BuLi (2.5 M, 5.95 mL, 15.0 mmol) and triphenyl phosphate (3.26 g, 10.0 mmol) in dry THF (35 mL + 15 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 4:1) to obtain **KPP350** (1.89 g, 5.80 mmol, 58 %) as a white solid. TLC (cyclohexane:ethyl acetate, 4:1 v/v): R_f = 0.43. LC-MS (ESI): t_R = 9.59 min; m/z = 327.03 $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{18}\text{H}_{15}\text{O}_4\text{P}$: 326.07. ^1H NMR (400 MHz, CDCl_3): δ = 9.89 (s, 1H), 7.65 (ddd, J = 14.9, 7.9, 1.7 Hz, 1H), 7.47 (t, J = 7.8 Hz, 1H), 7.32 (t, J = 7.8 Hz, 4H), 7.24 – 7.14 (m, 6H), 6.99 – 6.93 (m, 2H). ^{13}C NMR (101 MHz, CDCl_3): δ = 162.36, 149.88, 136.11, 131.93, 129.97, 125.68, 120.59, 119.84, 118.04, 108.39, 106.55.

25

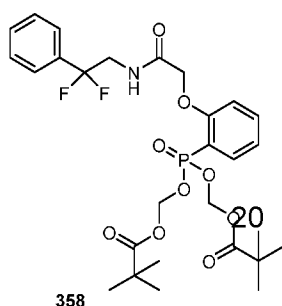
356: Synthesis of 2-bromo-N-(2-(4-bromophenyl)-2,2-difluoroethyl)acetamide (KPP356)



The bromoacetamide **KPP356** was synthesized via GP P, GP Q and GP D. GP P employed the acetate **KPP349** (2.12 g, 7.60 mmol), and NaBH_4 (0.29 g, 7.60 mmol) in MeOH (25 mL). The

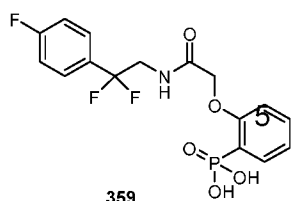
crude alcohol was converted to the amine via GP Q using pyridine (0.96 mL, 12.16 mmol) and TiF_4 (1.44 mL, 8.36 mmol) in dry acetonitrile (13 mL) as well as NH_4OH (28%, 13 mL). The crude intermediate was coupled to the amide using triethylamine (1.16 mL, 8.36 mmol) and bromoacetyl bromide (0.73 mL, 8.36 mmol) in dry DCM (18 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP356** (1.11 g, 3.10 mmol, 41 % over three steps) as an orange solid. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.43. **LC-MS (ESI)**: t_R = 9.04 min; m/z = 355.58 $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{10}\text{H}_9\text{Br}_2\text{F}_2\text{NO}$: 354.90. **^1H NMR** (400 MHz, CDCl_3): δ = 7.56 (d, J = 8.2 Hz, 2H), 7.38 (d, J = 8.5 Hz, 1H), 6.88 (s, 1H), 3.92 (td, J = 14.3, 6.4 Hz, 2H), 3.85 (s, 2H). **^{13}C NMR** (101 MHz, CDCl_3): δ = 165.95, 133.33, 132.02, 127.12, 125.20, 119.92, 45.81, 28.72.

358: Synthesis of (((2-(2-((2,2-difluor-2-phenylethyl)amino)-2-oxoethoxy)phenyl)phosphoryl)bis(oxy))bis(methylen)bis(2,2-dimethylpropanoate) (**KPP358**) III



The ester derivative **KPP358** was synthesized via GP R using the phosphonic acid derivative **KPP298** (230 mg, 0.62 mmol), triethylamine (0.17 mL, 1.24 mmol) and chloromethyl pivalate (0.90 mL, 6.20 mmol) in dry DMF (10 mL). The crude product was purified by HPLC to obtain **KPP358** (155 mg, 0.26 mmol, 42 %) as a colorless oil. **LC-MS (ESI)**: t_R = 11.00 min; m/z = 599.73 $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{28}\text{H}_{36}\text{F}_2\text{NO}_9\text{P}$: 599.21. **^1H NMR** (400 MHz, DMSO): δ = 8.47 (t, J = 6.4 Hz, 1H), 7.65 – 7.59 (m, 2H), 7.55 – 7.40 (m, 5H), 7.19 – 7.08 (m, 2H), 5.67 (d, J = 13.8 Hz, 4H), 4.67 (s, 2H), 3.98 (td, J = 14.8, 6.3 Hz, 2H), 1.07 (s, 18H). **^{13}C NMR** (101 MHz, DMSO): δ = 176.03, 167.55, 159.04, 135.48, 134.44, 133.55, 130.34, 128.54, 125.11, 121.34, 116.38, 114.49, 113.26, 81.82, 67.16, 43.90, 38.11, 26.31. **HRMS (ESI)**: m/z = 600.2169 calcd. for $[\text{C}_{28}\text{H}_{36}\text{F}_2\text{NO}_9\text{P} + \text{H}]^+$; found: 600.2166.

359: Synthesis of (2-(2-((2,2-difluoro-2-(4-fluorophenyl)ethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP359**) 67



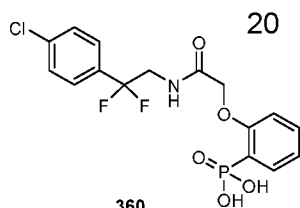
359

The phenylphosphonic acid derivative **KPP359** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (117 mg, 0.58 mmol), potassium carbonate (160 mg, 1.04 mmol) and **KPP343** (171 mg, 0.58 mmol) in DMF (6 mL). The crude intermediate was

deprotected via GP H using TMSBr (0.77 mL, 5.80 mmol) in dry DCM (5 mL) and MeOH/H₂O (3:1, 4 mL). The crude product was purified by HPLC to obtain

10 **KPP359** (92 mg, 0.24 mmol, 41 % over two steps) as a white solid. **LC-MS (ESI)**: t_R = 6.25 min; m/z = 389.84 $[M + H]^+$, calcd. for C₁₆H₁₅F₃NO₅P: 389.06. **¹H NMR** (400 MHz, DMSO): δ = 9.41 (t, J = 6.3 Hz, 1H), 7.72 – 7.57 (m, 1H), 7.52 – 7.45 (m, 3H), 7.22 (t, J = 8.8 Hz, 2H), 7.14 – 7.02 (m, 2H), 4.63 (s, 2H), 3.86 (dt, J = 14.5, 7.2 Hz, 2H). **¹³C NMR** (101 MHz, DMSO): δ = 168.58, 158.75, 133.07, 132.59, 127.76, 121.16, 115.57, 115.35, 113.24, 67.45, 43.97. **HRMS (ESI)**: m/z = 390.0713 calcd. for [C₁₆H₁₅F₃NO₅P + H]⁺; found: 390.0711.

360: Synthesis of ((2-(2-((2-(4-chlorophenyl)-2,2-difluoroethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP360**) 63



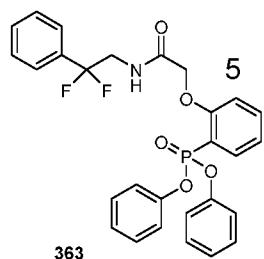
360

20 The phenylphosphonic acid derivative **KPP360** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (123 mg, 0.61 mmol), potassium carbonate (169 mg, 1.22 mmol) and **KPP344** (191 mg, 0.61 mmol) in DMF (6 mL). The crude intermediate was

25 deprotected via GP H using TMSBr (0.81 mL, 6.10 mmol) in dry DCM (5 mL) and MeOH/H₂O (3:1, 4 mL). The crude product was purified by HPLC to obtain

KPP360 (138 mg, 0.34 mmol, 56 % over two steps) as a white solid. **LC-MS (ESI)**: t_R = 6.67 min; m/z = 405.87 $[M + H]^+$, calcd. for C₁₆H₁₅ClF₂NO₅P: 405.03. **¹H NMR** (400 MHz, DMSO): δ = 9.46 (s, 1H), 7.71 – 7.58 (m, 1H), 7.53 – 7.40 (m, 5H), 7.16 – 7.06 (m, 2H), 4.65 (s, 2H), 3.88 (t, J = 12.6 Hz, 2H). **¹³C NMR** (101 MHz, DMSO): δ = 168.58, 158.85, 135.01, 133.00, 128.51, 127.23, 121.21, 113.23, 99.50, 67.43, 43.84. **HRMS (ESI)**: m/z = 406.0417 calcd. for [C₁₆H₁₅ClF₂NO₅P + H]⁺; found: 406.0417.

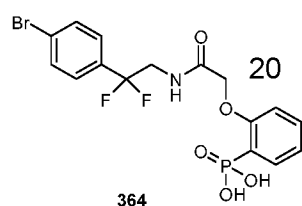
363: Synthesis of diphenyl (2-(2-((2,2-difluoro-2-phenylethyl)amino)-2-oxoethoxy)phenyl)phosphonate (**KPP363**) IV



The ester derivative **KPP363** was synthesized via GP G using phosphonate **KPP350** (245 mg, 0.75 mmol), potassium carbonate (207 mg, 1.50 mmol) and **KPP342** (209 mg, 0.75 mmol) in DMF (5 mL). The crude product was purified by HPLC to obtain **KPP363** (241 mg, 0.46 mmol, 61 %) as a colorless oil. **LC-MS (ESI)**: t_R = 10.58 min; m/z = 523.90 $[M + H]^+$,

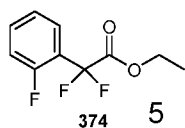
10 calcd. for $C_{28}H_{24}F_2NO_5P$: 523.14. 1H NMR (400 MHz, DMSO): δ = 8.50 (t, J = 6.3 Hz, 1H), 7.90 – 7.83 (m, 1H), 7.64 (t, J = 8.0 Hz, 1H), 7.51 – 7.33 (m, 9H), 7.28 – 7.10 (m, 8H), 4.78 (s, 2H), 3.98 (td, J = 14.9, 6.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO): δ = 167.59, 159.32, 149.98, 135.93, 134.67, 134.38, 130.34, 129.88, 128.54, 125.27, 121.44, 120.51, 120.46, 115.00, 113.23, 67.09, 43.93, 31.28. **HRMS (ESI)**:
15 m/z = 524.1433 calcd. for $[C_{28}H_{24}F_2NO_5P + H]^+$; found: 524.1422.

364: Synthesis of ((2-(2-((2-(4-bromophenyl)-2,2-difluoroethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP364**) 64



The phenylphosphonic acid derivative **KPP364** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (202 mg, 1.00 mmol), potassium carbonate (276 mg, 2.00 mmol) and **KPP356** (357 mg, 1.00 mmol) in DMF (6 mL). The crude intermediate was

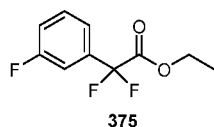
deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and
25 MeOH/H₂O (3:1, 6 mL). The crude product was purified by HPLC to obtain **KPP364** (181 mg, 0.40 mmol, 40 % over two steps) as a white solid. **LC-MS (ESI)**: t_R = 6.82 min; m/z = 449.85 $[M + H]^+$, calcd. for $C_{16}H_{15}BrF_2NO_5P$: 448.98. 1H NMR (400 MHz, DMSO): δ = 9.44 (t, J = 6.2 Hz, 1H), 7.66 – 7.58 (m, 3H), 7.50 (t, J = 7.2 Hz, 1H), 7.38 (d, J = 8.3 Hz, 2H), 7.12 – 7.06 (m, 2H), 4.64 (s, 2H), 3.88 (td, J = 14.4, 6.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO₃): δ = 168.59, 158.77, 133.82, 133.05, 132.57, 131.47, 127.47, 123.81, 121.29, 113.24, 67.44, 43.83, 31.31. **HRMS (ESI)**: m/z = 449.9912 calcd. for $[C_{16}H_{15}BrF_2NO_5P + H]^+$; found: 449.9911.

374: Synthesis of ethyl 2,2-difluoro-2-(2-fluorophenyl)acetate (**KPP374**)

The phenylacetate derivative **KPP374** was synthesized via GP O using 2-fluoriodobenzene (1.16 mL, 10.00 mmol), ethyl bromodifluoroacetate (1.95 mL, 15.00 mmol) and activated copper (2.55 g, 39.00 mmol) in dry DMSO (26 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP374** (1.80 g, 8.26 mmol, 83 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 19:1 v/v): R_f = 0.37.

LC-MS (ESI): t_R = 9.61 min; m/z = 218.58 $[M + H]^+$, calcd. for $C_{10}H_9F_3O_2$: 218.06.

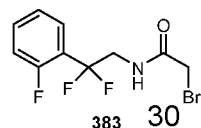
10 **1H NMR** (400 MHz, $CDCl_3$): δ = 7.66 (t, J = 7.6 Hz, 1H), 7.55 – 7.45 (m, 1H), 7.26 (d, J = 15.4 Hz, 1H), 7.19 – 7.09 (m, 1H), 4.35 (q, J = 7.2 Hz, 2H), 1.32 (t, J = 7.2 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 163.32, 158.60, 133.14, 127.17, 124.37, 120.99, 116.25, 111.76, 63.42, 13.83.

15 375: Synthesis of ethyl 2,2-difluoro-2-(3-fluorophenyl)acetate (**KPP375**)

The phenylacetate derivative **KPP375** was synthesized via GP O using 3-fluoriodobenzene (1.17 mL, 10.00 mmol), ethyl bromodifluoroacetate (1.95 mL, 15.00 mmol) and activated copper (2.55 g, 39.00 mmol) in dry DMSO (26 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP375** (10.99 g, 4.54 mmol, 45 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 19:1 v/v): R_f = 0.43. **1H NMR** (400 MHz, $CDCl_3$): δ = 7.46 – 7.38 (m, 2H), 7.31 (d, J = 9.1 Hz, 1H), 7.19 (t, J = 7.3 Hz, 1H), 4.30 (q, J = 7.1 Hz, 2H), 1.31 (t, J = 7.2 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 163.92, 161.46, 135.14, 130.62, 121.31, 118.38, 113.29, 112.74, 63.48, 13.96.

20 column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP375** (10.99 g, 4.54 mmol, 45 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 19:1 v/v): R_f = 0.43. **1H NMR** (400 MHz, $CDCl_3$): δ = 7.46 – 7.38 (m, 2H), 7.31 (d, J = 9.1 Hz, 1H), 7.19 (t, J = 7.3 Hz, 1H), 4.30 (q, J = 7.1 Hz, 2H), 1.31 (t, J = 7.2 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 163.92, 161.46, 135.14, 130.62, 121.31, 118.38, 113.29, 112.74, 63.48, 13.96.

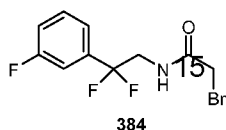
25

383: Synthesis of 2-bromo-N-(2,2-difluoro-2-(2-fluorophenyl)ethyl)acetamide (**KPP383**)

The bromoacetamide **KPP383** was synthesized via GP P, GP Q and GP D. GP P employed the acetate **KPP374** (1.45 g, 6.65 mmol), and $NaBH_4$ (0.25 g, 6.65 mmol) in MeOH (22 mL). The crude alcohol was converted to the amine via GP Q using pyridine (0.86 mL, 10.64 mmol) and

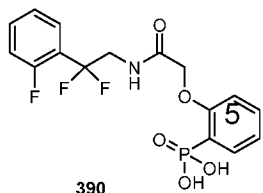
Tf₂O (1.23 mL, 7.32 mmol) in dry acetonitrile (12 mL) as well as NH₄OH (28%, 12 mL). The crude intermediate was coupled to the amide using triethylamine (1.01 mL, 7.32 mmol) and bromoacetyl bromide (0.64 mL, 7.32 mmol) in dry DCM (33 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP383** (679 mg, 2.29 mmol, 34 % over three steps) as a white solid. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.29. **LC-MS (ESI)**: t_R = 8.18 min; m/z = 295.62 [M + H]⁺, calcd. for C₁₀H₉BrF₃NO: 294.98. ¹H NMR (400 MHz, CDCl₃): δ = 7.53 (t, J = 7.6 Hz, 1H), 7.49 – 7.43 (m, 1H), 7.22 (t, J = 7.6 Hz, 1H), 7.18 – 7.12 (m, 1H), 6.82 (s, 1H), 6.24 (s, 0H), 4.07 (td, J = 14.6, 6.3 Hz, 2H), 3.85 (s, 2H). ¹³C NMR (101 MHz, CDCl₃): δ = 165.91, 158.60, 132.86, 127.50, 124.40, 121.69, 118.79, 116.85, 45.11, 28.77.

384: Synthesis of 2-bromo-N-(2,2-difluoro-2-(3-fluorophenyl)ethyl)acetamide (KPP384)



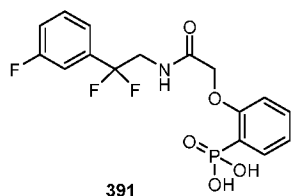
The bromoacetamide **KPP384** was synthesized via GP P, GP Q and GP D. GP P employed the acetate **KPP375** (0.73 g, 3.34 mmol), and NaBH₄ (0.13 g, 3.34 mmol) in MeOH (12 mL). The crude alcohol was converted to the amine via GP Q using pyridine (0.43 mL, 5.34 mmol) and Tf₂O (0.62 mL, 3.67 mmol) in dry acetonitrile (6 mL) as well as NH₄OH (28%, 6 mL). The crude intermediate was coupled to the amide using triethylamine (0.51 mL, 3.67 mmol) and bromoacetyl bromide (0.32 mL, 3.67 mmol) in dry DCM (17 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP384** (679 mg, 2.29 mmol, 34 % over three steps) as a white solid. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.40. **LC-MS (ESI)**: t_R = 8.27 min; m/z = 295.61 [M + H]⁺, calcd. for C₁₀H₉BrF₃NO: 294.98. ¹H NMR (400 MHz, CDCl₃): δ = 7.46 – 7.40 (m, 1H), 7.29 (d, J = 7.8 Hz, 1H), 7.24 – 7.14 (m, 2H), 6.80 (s, 1H), 3.93 (td, J = 14.4, 6.3 Hz, 2H), 3.87 (s, 2H). ¹³C NMR (101 MHz, CDCl₃): δ = 165.83, 161.49, 136.63, 130.66, 121.15, 119.50, 117.70, 113.02, 45.92, 28.77.

390: Synthesis of (2-(2-((2,2-difluoro-2-(2-fluorophenyl)ethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP390**) 65



The phenylphosphonic acid derivative **KPP390** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (202 mg, 1.00 mmol), potassium carbonate (276 mg, 2.00 mmol) and **KPP383** (296 mg, 1.00 mmol) in DMF (6 mL). The crude intermediate was deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and MeOH/H₂O (3:1, 6 mL). The crude product was purified by HPLC to obtain **KPP390** (158 mg, 0.41 mmol, 41 % over two steps) as a white solid. **LC-MS (ESI)**: t_R = 6.11 min; m/z = 389.84 [M + H]⁺, calcd. for C₁₆H₁₅F₃NO₅P: 389.06. **¹H NMR** (400 MHz, DMSO): δ = 9.46 (t, J = 6.3 Hz, 1H), 7.63 (ddd, J = 14.2, 7.7, 1.8 Hz, 1H), 7.60 – 7.44 (m, 2H), 7.38 (t, J = 7.7 Hz, 1H), 7.30 (dd, J = 11.4, 8.3 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 7.08 (td, J = 7.7, 7.3, 2.6 Hz, 2H), 4.61 (s, 2H), 3.96 (td, J = 14.8, 6.3 Hz, 2H). **¹³C NMR** (101 MHz, DMSO): δ = 168.62, 158.73, 132.51, 127.37, 124.40, 121.14, 116.36, 113.16, 67.49, 43.10. **HRMS (ESI)**: m/z = 390.0713 calcd. for [C₁₆H₁₅F₃NO₅P + H]⁺; found: 390.0712.

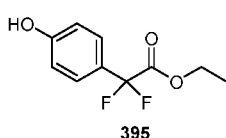
391: Synthesis of (2-(2-((2,2-difluoro-2-(3-fluorophenyl)ethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP391**) 66



The phenylphosphonic acid derivative **KPP391** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (202 mg, 1.00 mmol), potassium carbonate (276 mg, 2.00 mmol) and **KPP383** (296 mg, 1.00 mmol) in DMF (6 mL). The crude intermediate was deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and MeOH/H₂O (3:1, 6 mL). The crude product was purified by HPLC to obtain **KPP391** (111 mg, 0.28 mmol, 28 % over two steps) as a white solid. **LC-MS (ESI)**: t_R = 6.21 min; m/z = 389.91 [M + H]⁺, calcd. for C₁₆H₁₅F₃NO₅P: 389.06. **¹H NMR** (400 MHz, CDCl₃): δ = 9.42 (t, J = 6.3 Hz, 1H), 7.62 (ddd, J = 14.2, 7.4, 1.8 Hz, 1H), 7.53 – 7.42 (m, 2H), 7.37 – 7.24 (m, 3H), 7.08 (td, J = 8.1, 5.3 Hz, 2H), 4.64 (s, 2H), 3.90 (td, J = 14.5, 6.2 Hz, 2H). **¹³C NMR** (101 MHz, CDCl₃): δ = 168.64, 163.04, 160.60, 158.76, 136.97, 132.61, 132.55, 130.81, 121.18, 117.22, 113.16,

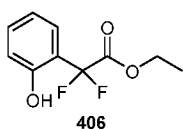
67.50, 43.48. **HRMS (ESI):** m/z = 390.0713 calcd. for $[C_{16}H_{15}F_3NO_5P + H]^+$; found: 390.0714.

5 **395: Synthesis of ethyl 2,2-difluoro-2-(4-hydroxyphenyl)acetate (KPP395)**

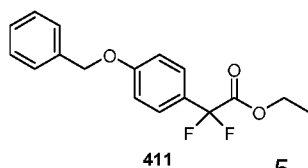


The phenylacetate derivative **KPP395** was synthesized via GP O using 4-iodophenol (2.20 g, 10.00 mmol), ethyl bromodifluoroacetate (1.95 mL, 15.00 mmol) and activated copper (2.55 g, 39.00 mmol) in dry DMSO (26 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 4:1) to obtain **KPP395** (565 mg, 2.62 mmol, 26 %) as a slightly yellow oil. **TLC** (cyclohexane:ethyl acetate, 4:1 v/v): R_f = 0.43. **1H NMR** (400 MHz, $CDCl_3$): δ = 7.46 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 4.29 (q, J = 7.1 Hz, 2H), 1.30 (t, J = 7.2 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 164.99, 158.26, 127.33, 124.86, 115.66, 113.68, 63.39, 13.95.

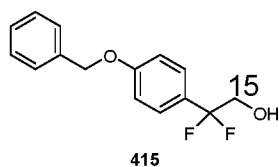
15 **406: Synthesis of ethyl 2,2-difluoro-2-(2-hydroxyphenyl)acetate (KPP406)**



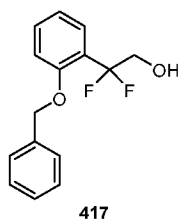
The phenylacetate derivative **KPP406** was synthesized via GP O using 2-iodophenol (22.0 g, 100.0 mmol), ethyl bromodifluoroacetate (19.5 mL, 150.0 mmol) and activated copper (25.5 g, 390.0 mmol) in dry DMSO (260 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 4:1) to obtain **KPP406** (6.73 g, 31.1 mmol, 31 %) as a yellow solid. **TLC** (cyclohexane:ethyl acetate, 4:1 v/v): R_f = 0.29. **1H NMR** (400 MHz, $CDCl_3$): δ = 7.54 (dd, J = 7.9, 1.7 Hz, 1H), 7.38 – 7.22 (m, 1H), 7.02 – 6.89 (m, 2H), 4.34 (p, J = 7.1, 6.5 Hz, 2H), 1.46 – 1.22 (m, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 165.27, 154.13, 132.64, 126.25, 120.25, 117.09, 112.84, 63.59, 13.68.

411: Synthesis of Ethyl 2-(4-(benzyloxy)phenyl)-2,2-difluoroacetate (**KPP411**)

The acetate derivative **KPP411** was synthesized via GP G using phenyl acetate **KPP395** (4.00 g, 18.50 mmol), potassium carbonate (5.11 g, 37.00 mmol) and benzylic bromide (2.42 mL, 20.35 mmol) in DMF (55 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP411** (1.70 g, 5.54 mmol, 30 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 19:1 v/v): R_f = 0.43. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ = 7.54 (d, J = 8.7 Hz, 2H), 7.47 – 7.31 (m, 5H), 7.04 (d, J = 8.6 Hz, 2H), 5.10 (s, 2H), 4.29 (q, J = 7.2 Hz, 2H), 1.31 (dt, J = 27.1, 7.2 Hz, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ = 164.53, 160.89, 136.48, 128.79, 128.31, 127.58, 127.23, 125.29, 114.99, 70.23, 63.13, 14.01.

415: Synthesis of 2-(4-(benzyloxy)phenyl)-2,2-difluoroethanol (**KPP415**)

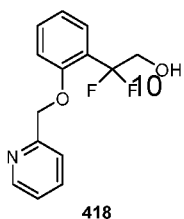
The alcohol **KPP415** was synthesized via GP P using the acetate **KPP411** (1.70 g, 5.54 mmol), and NaBH_4 (0.21 g, 5.54 mmol) in MeOH (49 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 4:1) to obtain **KPP415** (0.81 g, 3.07 mmol, 55 %) as a white solid. TLC (cyclohexane:ethyl acetate, 4:1 v/v): R_f = 0.34. LC-MS (ESI): t_R = 9.41 min; m/z = 266.04 $[\text{M} + \text{H}]^+$, 244.99 $[\text{M} - \text{H}_2\text{O}]^+$, calcd. for $\text{C}_{15}\text{H}_{14}\text{F}_2\text{O}_2$: 264.10. $^1\text{H NMR}$ (400 MHz, MeOD): δ = 7.48 – 7.40 (m, 4H), 7.38 – 7.30 (m, 3H), 7.05 (d, J = 8.6 Hz, 2H), 5.10 (s, 2H), 3.89 (t, J = 13.6 Hz, 2H). $^{13}\text{C NMR}$ (101 MHz, MeOD): δ = 161.38, 138.33, 129.52, 128.94, 128.54, 128.15, 122.29, 115.69, 70.98, 65.94.

417: Synthesis of 2-(2-(benzyloxy)phenyl)-2,2-difluoroethanol (**KPP417**)

The alcohol **KPP417** was synthesized via GP G and GP P. GP G employed acetate **KPP406** (1.62 g, 7.50 mmol), potassium carbonate (2.08 g, 15.00 mmol) and benzylic bromide (0.89 mL, 7.50 mmol) in DMF (23 mL). The crude intermediate was reduced via GP P using NaBH_4 (0.56 g, 15.00 mmol) in MeOH (25 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 4:1) to obtain **KPP417** (1.02 g, 3.87 mmol, 52 % over two steps) as a colorless oil.

TLC (cyclohexane:ethyl acetate, 4:1 v/v): $R_f = 0.40$. **LC-MS (ESI)**: $t_R = 9.33$ min; $m/z = 265.34$ $[M + H]^+$, calcd. for $C_{15}H_{14}F_2O_2$: 264.10. **1H NMR** (400 MHz, MeOD): $\delta = 7.56$ (dd, $J = 7.7, 1.8$ Hz, 1H), 7.53 – 7.21 (m, 6H), 7.14 (d, $J = 8.3$ Hz, 1H), 7.03 (t, $J = 7.6$ Hz, 1H), 5.14 (s, 2H), 4.11 (t, $J = 14.3$ Hz, 2H).
 5 **^{13}C NMR** (101 MHz, MeOD): $\delta = 157.54, 138.16, 132.78, 129.55, 129.31, 128.93, 128.68, 128.41, 127.95, 121.53, 114.26, 71.41, 64.62$.

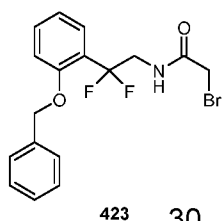
418: Synthesis of 2-(2-((pyridin-2-yl)methoxy)phenyl)-2,2-difluoroethanol (KPP418)



The alcohol **KPP418** was synthesized via GP G and GP P. GP G employed acetate **KPP406** (1.62 g, 7.50 mmol), potassium carbonate (4.15 g, 30.00 mmol) and 2-(Bromomethyl)pyridine hydrobromide (1.90 g, 7.50 mmol) in DMF (45 mL). The crude intermediate was reduced via GP P using $NaBH_4$ (0.56 g, 15.00 mmol)

in MeOH (25 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 1:2) to obtain **KPP417** (0.99 g, 3.73 mmol, 50 % over two
 15 steps) as a colorless oil. **TLC** (cyclohexane:ethyl acetate, 1:2 v/v): $R_f = 0.40$. **LC-MS (ESI)**: $t_R = 7.13$ min; $m/z = 265.92$ $[M + H]^+$, calcd. for $C_{14}H_{13}F_2NO_2$: 265.09. **1H NMR** (400 MHz, MeOD): $\delta = 8.54$ (d, $J = 4.3$ Hz, 1H), 7.88 (td, $J = 7.7, 1.8$ Hz, 1H), 7.64 (d, $J = 7.9$ Hz, 1H), 7.57 (dd, $J = 7.8, 1.8$ Hz, 1H), 7.51 – 7.33 (m, 2H), 7.13 (d, $J = 8.4$ Hz, 1H), 7.06 (t, $J = 7.6$ Hz, 1H), 5.25 (s, 2H), 4.14 (t, $J = 14.3$ Hz, 2H).
 20 **^{13}C NMR** (101 MHz, MeOD): $\delta = 157.78, 157.04, 149.81, 139.07, 132.90, 128.91, 124.46, 123.19, 122.01, 114.19, 71.54, 64.75$.

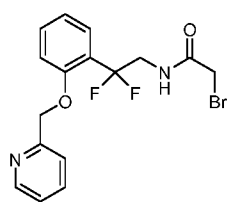
423: Synthesis of N-(2-(2-(benzyloxy)phenyl)-2,2-difluoroethyl)-2-bromoacetamide (KPP423)



The bromoacetamide **KPP423** was synthesized via GP Q and GP D. GP Q employed the alcohol **KPP417** (983 mg, 3.72 mmol), pyridine (0.48 mL, 5.95 mmol) and Tf_2O (0.69 mL, 4.09 mmol) in dry acetonitrile (7.0 mL) as well as NH_4OH (28%, 7.0 mL). The crude intermediate was coupled to the amide using triethylamine (0.57 mL, 4.09 mmol) and bromoacetyl bromide (0.36 mL, 4.09 mmol) in dry DCM (19 mL). The crude product was purified by column chromatography

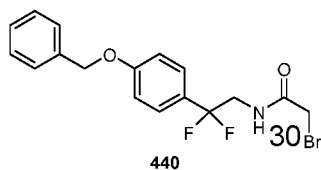
(cyclohexane/ethyl acetate 2:1) to obtain **KPP423** (512 mg, 1.33 mmol, 36 % over two steps) as a white solid. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.43$. **LC-MS (ESI)**: $t_R = 9.79$ min; $m/z = 383.69$ $[M + H]^+$, calcd. for $C_{17}H_{16}BrF_2NO_2$: 383.03. **1H NMR** (400 MHz, $CDCl_3$): $\delta = 7.53$ (dd, $J = 7.9, 1.8$ Hz, 1H), 7.51 – 7.30 (m, 6H), 7.08 – 6.96 (m, 2H), 6.71 (t, $J = 6.3$ Hz, 1H), 5.17 (s, 2H), 4.17 (td, $J = 14.4, 6.1$ Hz, 2H), 3.79 (s, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$): $\delta = 165.69, 156.19, 136.43, 132.24, 128.82, 128.22, 127.38, 120.86, 113.28, 70.77, 44.80, 28.91$.

424: Synthesis of *N*-(2-((pyridin-2-yl)methoxy)phenyl)-2,2-difluoroethyl-2-bromoacetamide (**KPP424**)



The bromoacetamide **KPP424** was synthesized via GP Q and GP D. GP Q employed the alcohol **KPP418** (954 mg, 3.60 mmol), pyridine (0.47 mL, 5.76 mmol) and Tf_2O (0.67 mL, 3.96 mmol) in dry acetonitrile (7.0 mL) as well as NH_4OH (28%, 7.0 mL). The crude intermediate was coupled to the amide using triethylamine (0.55 mL, 3.96 mmol) and bromoacetyl bromide (0.34 mL, 3.96 mmol) in dry DCM (18 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 1:4) to obtain **KPP424** (570 mg, 1.48 mmol, 41 % over two steps) as a white solid. **TLC** (cyclohexane:ethyl acetate, 1:4 v/v): $R_f = 0.30$. **LC-MS (ESI)**: $t_R = 7.66$ min; $m/z = 385.08$ $[M + H]^+$, calcd. for $C_{16}H_{15}BrF_2N_2O_2$: 384.03. **1H NMR** (400 MHz, $CDCl_3$): $\delta = 8.51$ (d, $J = 5.0$ Hz, 1H), 7.71 (td, $J = 7.7, 1.8$ Hz, 1H), 7.57 (d, $J = 7.9$ Hz, 1H), 7.48 (dd, $J = 7.7, 1.8$ Hz, 1H), 7.32 (td, $J = 7.9, 1.7$ Hz, 1H), 7.24 – 7.14 (m, 1H), 7.04 – 6.90 (m, 3H), 5.24 (s, 2H), 4.14 (td, $J = 14.6, 6.3$ Hz, 2H), 3.76 (s, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$): $\delta = 165.95, 156.26, 155.63, 148.88, 137.71, 132.33, 127.57, 123.11, 121.76, 121.14, 113.12, 70.78, 45.00, 28.85$.

440: Synthesis of 2-(4-(benzyloxy)phenyl)-2,2-difluoroethanol (**KPP440**)

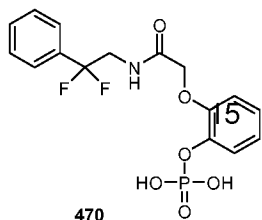


The bromoacetamide **KPP440** was synthesized via GP Q and GP D. GP Q employed the alcohol **KPP415** (751 mg, 2.84 mmol), pyridine (0.37 mL, 4.54 mmol) and Tf_2O (0.53 mL, 3.12 mmol) in dry acetonitrile (5.0 mL) as well as NH_4OH (28%, 5.0 mL). The crude intermediate was coupled to the amide using triethylamine (0.43 mL,

3.12 mmol) and bromoacetyl bromide (0.27 mL, 3.12 mmol) in dry DCM (14 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP440** (450 mg, 1.17 mmol, 41 % over two steps) as a white solid. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.37$. **LC-MS (ESI)**: $t_R = 9.76$ min; $m/z = 383.92$ $[M + H]^+$, calcd. for $C_{17}H_{16}BrF_2NO_2$: 383.03. **1H NMR** (400 MHz, $CDCl_3$): $\delta = 7.46 - 7.33$ (m, 7H), 7.03 (d, $J = 8.6$ Hz, 2H), 6.80 (t, $J = 6.2$ Hz, 1H), 5.09 (s, 2H), 3.93 (td, $J = 14.4, 6.2$ Hz, 2H), 3.87 (s, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$): $\delta = 165.90, 160.28, 136.57, 128.77, 128.27, 127.58, 126.87, 120.28, 114.99, 70.19, 46.15, 28.86$.

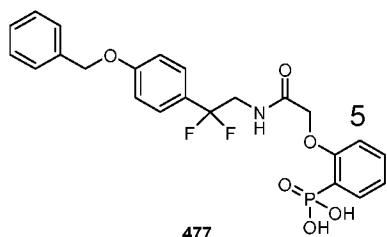
10

*470: Synthesis of 2-((2,2-difluoro-2-phenylethylcarbamoyl)methoxy)phenyl dihydrogen phosphate (**KPP470**) 72*



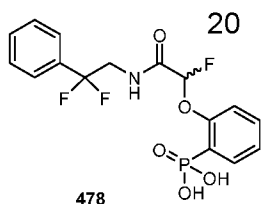
The dihydrogen phosphate derivative **KPP470** was synthesized via GP G and GP H. GP G employed phosphate **KPP273** (314 mg, 1.44 mmol), potassium carbonate (398 mg, 2.88 mmol) and **KPP342** (398 mg, 1.44 mmol) in DMF (10 mL). The crude intermediate was deprotected via GP H using TMSBr (1.90 mL, 14.40 mmol) in dry DCM (8 mL) and MeOH/H₂O (3:1, 6 mL). The crude product was purified by HPLC to obtain **KPP470** (22 mg, 0.06 mmol, 4 % over two steps) as a white solid. **LC-MS (ESI)**: $t_R = 5.86$ min; $m/z = 387.90$ $[M + H]^+$, calcd. for $C_{16}H_{16}F_2NO_6P$: 387.07. **1H NMR** (400 MHz, MeOD): $\delta = 7.38 - 7.25$ (m, 5H), 7.09 (s, 1H), 6.95 (t, $J = 7.2$ Hz, 1H), 6.85 - 6.75 (m, 2H), 4.41 (s, 2H), 3.77 (t, $J = 13.9$ Hz, 2H). **^{13}C NMR** (101 MHz, MeOD): $\delta = 163.33, 150.38, 136.26, 131.29, 129.54, 126.36, 123.20, 115.10, 101.34, 68.43$. **HRMS (ESI)**: $m/z = 388.0756$ calcd. for $[C_{16}H_{16}F_2NO_6P + H]^+$; found: 388.0753.

477: Synthesis of 2-((2-(4-(benzyloxy)phenyl)-2,2-difluoroethylcarbamoyl)methoxy)phenylphosphonic acid (**KPP477**) 70



The phenylphosphonic acid derivative **KPP477** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (202 mg, 1.00 mmol), potassium carbonate (276 mg, 2.00 mmol) and **KPP440** (384 mg, 1.00 mmol) in DMF (6 mL). The crude intermediate was deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and MeOH/H₂O (3:1, 6 mL). The crude product was purified by HPLC to obtain **KPP477** (110 mg, 0.23 mmol, 23 % over two steps) as a white solid. **LC-MS (ESI)**: t_R = 7.73 min; m/z = 477.89 [M + H]⁺, calcd. for C₂₃H₂₂F₂NO₆P: 477.12. ¹H NMR (400 MHz, DMSO): δ = 9.38 (t, J = 6.3 Hz, 1H), 7.72 – 7.60 (m, 1H), 7.58 – 7.29 (m, 8H), 7.16 – 7.00 (m, 4H), 5.13 (s, 2H), 4.65 (s, 2H), 3.86 (td, J = 14.7, 6.2 Hz, 1H). ¹³C NMR (101 MHz, DMSO): δ = 168.57, 159.59, 158.75, 136.74, 133.04, 128.50, 127.76, 126.73, 121.12, 114.63, 69.35, 67.49, 44.08. **HRMS (ESI)**: m/z = 478.1226 calcd. for [C₂₃H₂₂F₂NO₆P + H]⁺; found: 478.1223.

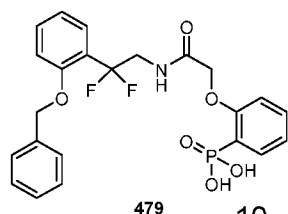
478: Synthesis of 2-((2,2-difluoro-2-phenylethylcarbamoyl)fluoromethoxy)phenylphosphonic acid (**KPP478**) 71



The phenylphosphonic acid derivative **KPP478** was synthesized via GP F and GP H. GP F employed phosphonate **KPP319** (278 mg, 1.00 mmol), *N*-Methylmorpholine (111 μ L, 1.00 mmol) and isobutyl chloroformate (130 μ L, 1.00 mmol) in dry THF (10 mL) as well as *N*-Methylmorpholine (111 μ L, 1.00 mmol) and 2,2-difluoro-2-phenylethanamine (157 mg, 1.00 mmol). The crude intermediate was deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and MeOH/H₂O (3:1, 6 mL). The crude product was purified by HPLC to obtain **KPP478** (70 mg, 18 mmol, 18 % over two steps) as a white solid. **LC-MS (ESI)**: t_R = 6.25 min; m/z = 389.99 [M + H]⁺, calcd. for C₁₆H₁₅F₃NO₅P: 389.06. ¹H NMR (400 MHz, DMSO): δ = 10.01 (t, J = 6.3 Hz, 1H), 7.71 (ddd, J = 14.0, 7.5, 1.7 Hz, 1H), 7.66 – 7.38 (m, 6H), 7.26 (tt, J = 8.2, 4.3 Hz, 2H), 6.18 (d, J = 58.9 Hz, 1H), 4.07 – 3.71 (m, 2H). ¹³C NMR (101 MHz, DMSO): δ = 164.27, 156.79, 134.63, 132.82,

130.43, 128.64, 125.21, 123.98, 120.48, 116.66, 104.70, 102.45, 99.58, 91.34, 71.13, 44.59. **HRMS (ESI):** m/z = 390.0713 calcd. for $[C_{16}H_{15}F_3NO_5P + H]^+$; found: 390.0710.

479: *Synthesis of 2-((2-(2-(benzyloxy)phenyl)-2,2-difluoroethylcarbamoyl)methoxy)phenylphosphonic acid (KPP479) 69*

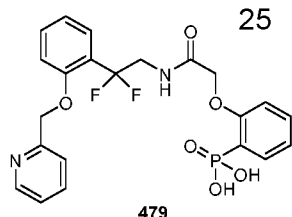


The phenylphosphonic acid derivative **KPP479** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (187 mg, 0.93 mmol), potassium carbonate (256 mg, 1.86 mmol) and **KPP423** (356 mg, 0.93 mmol) in DMF (6 mL). The crude intermediate was deprotected via GP H using TMSBr (1.22 mL, 9.30 mmol) in dry DCM (8 mL) and MeOH/H₂O (3:1, 6 mL). The crude product was purified by HPLC to obtain **KPP479** (109 mg, 0.23 mmol, 25 % over two steps) as a white solid. **LC-MS (ESI):** t_R = 7.61 min; m/z = 477.96 $[M + H]^+$, calcd. for $C_{23}H_{22}F_2NO_6P$: 477.12.

15 **¹H NMR** (400 MHz, DMSO): δ = 9.20 (t, J = 6.3 Hz, 1H), 7.70 – 7.60 (m, 1H), 7.60 – 7.53 (m, 2H), 7.52 – 7.37 (m, 4H), 7.34 – 7.30 (m, 2H), 7.19 (d, J = 8.3 Hz, 1H), 7.05 (ddt, J = 14.0, 8.3, 4.4 Hz, 2H), 6.94 (t, J = 7.5 Hz, 1H), 5.21 (s, 2H), 4.59 (s, 2H), 4.10 (td, J = 14.7, 6.2 Hz, 2H). **¹³C NMR** (101 MHz, DMSO): δ = 168.53, 158.78, 155.90, 136.83, 133.10, 132.66, 131.97, 128.56, 127.40, 123.24, 122.39, 121.25,

20 120.30, 113.48, 113.17, 69.83, 67.47, 42.64. **HRMS (ESI):** m/z = 478.1226 calcd. for $[C_{23}H_{22}F_2NO_6P + H]^+$; found: 478.1223.

480: *Synthesis of 2-((2-(2-((pyridin-2-yl)methoxy)phenyl)-2,2-difluoroethylcarbamoyl)methoxy)phenylphosphonic acid (KPP480) 97*

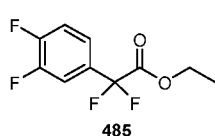


25 The phenylphosphonic acid derivative **KPP480** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (252 mg, 1.25 mmol), potassium carbonate (344 mg, 2.50 mmol) and **KPP424** (480 mg, 1.25 mmol) in DMF (7.5 mL). The crude intermediate was deprotected via GP H using TMSBr (1.64 mL, 12.45 mmol) in dry DCM (8.0 mL) and MeOH/H₂O (3:1, 6.0 mL). The crude product was purified by HPLC to obtain **KPP480** (284 mg, 0.59 mmol, 48 % over two steps) as a white solid. **LC-MS (ESI):**

30

$t_R = 5.75$ min; $m/z = 479.07$ $[M + H]^+$, calcd. for $C_{22}H_{21}F_2N_2O_6P$: 478.11. 1H NMR (400 MHz, DMSO): $\delta = 9.31$ (t, $J = 6.3$ Hz, 1H), 8.62 – 8.55 (m, 1H), 7.91 (td, $J = 7.7$, 1.8 Hz, 1H), 7.78 (d, $J = 7.8$ Hz, 1H), 7.65 (ddd, $J = 14.3$, 7.6, 1.8 Hz, 1H), 7.51 – 7.43 (m, 2H), 7.39 – 7.33 (m, 2H), 7.21 (d, $J = 8.3$ Hz, 1H), 7.07 (dd, $J = 8.7$, 6.0 Hz, 2H), 6.99 (t, $J = 7.5$ Hz, 1H), 5.29 (s, 2H), 4.63 (s, 2H), 4.12 (td, $J = 15.2$, 6.4 Hz, 2H). ^{13}C NMR (101 MHz, DMSO): $\delta = 168.59$, 158.78, 156.42, 155.53, 148.84, 137.47, 133.09, 132.67, 132.09, 126.81, 122.91, 121.35, 120.60, 113.47, 70.61, 67.51, 42.70. HRMS (ESI): $m/z = 479.1178$ calcd. for $[C_{22}H_{21}F_2N_2O_6P + H]^+$; found: 479.1169.

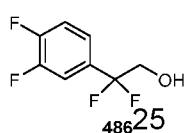
10 **485: Synthesis of ethyl 2-(3,4-difluorophenyl)-2,2-difluoroacetate (KPP485)**



The phenylacetate derivative **KPP485** was synthesized via GP O using 1,2-difluoriodobenzene (6.03 mL, 50.0 mmol), ethyl bromodifluoroacetate (9.62 mL, 75.0 mmol) and activated copper (12.75 g, 195.0 mmol) in dry DMSO (130 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP485** (9.42 g, 39.9 mmol, 80 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 19:1 v/v): $R_f = 0.44$. 1H NMR (400 MHz, $CDCl_3$): $\delta = 7.42 - 7.26$ (m, 1H), 7.31 – 7.26 (m, 1H), 7.20 – 7.11 (m, 1H), 4.22 (q, $J = 7.2$ Hz, 2H), 1.23 (dd, $J = 7.9$, 6.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 163.46$, 150.72, 148.93, 129.76, 122.25, 117.91, 115.35, 112.21, 63.43, 13.73.

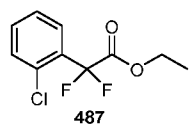
20

486: Synthesis of 2-(3,4-difluorophenyl)-2,2-difluoroethanol (KPP486)



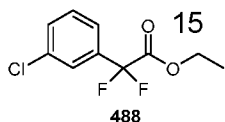
The alcohol **KPP486** was synthesized via GP P using the acetate **KPP485** (9.26 g, 39.24 mmol), and $NaBH_4$ (1.48 g, 39.24 mmol) in MeOH (135 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP486** (6.01 g, 30.95 mmol, 79 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.35$. 1H NMR (400 MHz, MeOD): $\delta = 7.49$ (ddd, $J = 11.6$, 7.8, 2.1 Hz, 1H), 7.45 – 7.30 (m, 2H), 3.95 (t, $J = 13.2$ Hz, 2H). ^{13}C NMR (101 MHz, MeOD): $\delta = 152.55$, 151.33, 133.78, 123.72, 121.15, 118.58, 116.59, 65.68.

30

487: Synthesis of ethyl 2,2-difluoro-2-(2-chlorophenyl)acetate (**KPP487**)

The phenylacetate derivative **KPP487** was synthesized via GP O using 1-chloro-2-iodobenzene (6.10 mL, 50.0 mmol), ethyl bromodifluoroacetate (9.62 mL, 75.0 mmol) and activated

5 copper (12.75 g, 195.0 mmol) in dry DMSO (130 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP487** (9.00 g, 38.4 mmol, 77 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 19:1 v/v): R_f = 0.58. LC-MS (ESI): t_R = 10.12 min; m/z = 235.07 $[M + H]^+$, calcd. for $C_{10}H_9ClF_2O_2$: 234.03. 1H NMR (400 MHz, $CDCl_3$): δ = 7.73 (d, J = 7.8 Hz, 1H), 7.45 – 7.35 (m, 3H), 4.34 (q, J = 7.3 Hz, 2H), 1.29 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 163.12, 132.17, 131.89, 131.21, 130.61, 127.31, 126.98, 112.29, 63.35, 13.72.

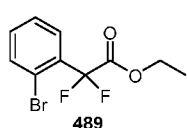
488: Synthesis of ethyl 2,2-difluoro-2-(3-chlorophenyl)acetate (**KPP488**)

The phenylacetate derivative **KPP488** was synthesized via GP O using 1-chloro-3-iodobenzene (6.22 mL, 50.0 mmol), ethyl bromodifluoroacetate (9.62 mL, 75.0 mmol) and activated

15 copper (12.75 g, 195.0 mmol) in dry DMSO (130 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP488** (6.46 g, 27.5 mmol, 55 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 19:1 v/v): R_f = 0.58. LC-MS (ESI): t_R = 10.59 min; m/z = 234.71 $[M + H]^+$, calcd. for $C_{10}H_9ClF_2O_2$: 234.03. 1H NMR (400 MHz, $CDCl_3$): δ = 7.51 (t, J = 2.0 Hz, 1H), 7.44 – 7.33 (m, 2H), 7.32 – 7.25 (m, 1H), 4.20 (q, J = 7.1 Hz, 1H), 1.21 (tt, J = 7.1, 1.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 162.75, 132.14, 131.81, 131.18, 130.57, 127.28, 126.95, 112.25, 63.32, 13.69.

489: Synthesis of ethyl 2-(2-bromophenyl)-2,2-difluoroacetate (**KPP489**)

The phenylacetate derivative **KPP489** was synthesized via GP O using 1-bromo-2-iodobenzene (6.26 mL, 50.0 mmol), ethyl bromodifluoroacetate (9.62 mL, 75.0 mmol) and activated copper (12.75 g, 195.0 mmol) in dry DMSO (130 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP489** (3.76 g, 13.5 mmol, 27 %) as a colorless oil. TLC (cyclohexane:ethyl acetate,

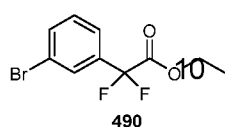


19:1 v/v): $R_f = 0.51$. **LC-MS (ESI):** $t_R = 10.21$ min;

$m/z = 278.73$ $[M + H]^+$, calcd. for $C_{10}H_9BrF_2O_2$: 277.98.

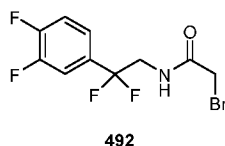
1H NMR (400 MHz, MeOD): $\delta = 7.78$ (ddd, $J = 22.0, 7.9, 1.6$ Hz, 1H), 7.72 (d, $J = 7.9$ Hz, 1H), 7.56 – 7.51 (m, 1H), 7.48 – 7.42 (m, 1H), 4.37 (q, $J = 7.1$ Hz, 2H), 1.41 – 1.22 (m, 3H). ^{13}C NMR (101 MHz, MeOD): $\delta = 164.15, 135.19, 134.04, 133.59, 128.74, 128.61, 121.16, 114.09, 64.48, 14.05$.

490: Synthesis of ethyl 2-(3-bromophenyl)-2,2-difluoroacetate (**KPP490**)



The phenylacetate derivative **KPP490** was synthesized via GP O using 1-bromo-3-iodobenzene (6.35 mL, 50.0 mmol), ethyl bromodifluoroacetate (9.62 mL, 75.0 mmol) and activated copper (12.75 g, 195.0 mmol) in dry DMSO (130 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP490** (5.75 g, 20.6 mmol, 41 %) as a colorless oil. **TLC** (cyclohexane:ethyl acetate, 19:1 v/v): $R_f = 0.58$. **LC-MS (ESI):** $t_R = 10.75$ min; $m/z = 279.23$ $[M + H]^+$, calcd. for $C_{10}H_9BrF_2O_2$: 277.98. 1H NMR (400 MHz, MeOD): $\delta = 7.78$ (s, 1H), 7.74 – 7.71 (m, 1H), 7.65 – 7.57 (m, 1H), 7.45 (t, $J = 7.9$ Hz, 1H), 4.36 (q, $J = 7.1$ Hz, 2H), 1.31 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (101 MHz, MeOD): $\delta = 164.67, 136.23, 135.37, 131.73, 129.38, 125.26, 123.57, 113.77, 64.52, 14.10$.

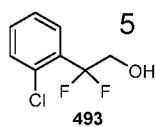
492: Synthesis of 2-bromo-N-(2-(3,4-difluorophenyl)-2,2-difluoroethyl)acetamide (**KPP492**)



The bromoacetamide **KPP492** was synthesized via GP Q and GP D. GP Q employed the alcohol **KPP486** (5.79 g, 29.86 mmol), pyridine (3.86 mL, 47.78 mmol) and Tf_2O (5.52 mL, 32.85 mmol) in dry acetonitrile (54 mL) as well as NH_4OH (28%, 54 mL). The crude intermediate was coupled to the amide using triethylamine (4.56 mL, 32.85 mmol) and bromoacetyl bromide (2.84 mL, 32.85 mmol) in dry DCM (108 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP492** (3.76 g, 11.97 mmol, 40 % over two steps) as a yellow solid. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.25$. **LC-MS (ESI):** $t_R = 8.47$ min; $m/z = 313.73$ $[M + H]^+$, calcd. for $C_{10}H_8BrF_4NO$: 312.97. 1H NMR (400 MHz, $CDCl_3$): $\delta = 7.27 - 7.17$ (m, 1H), 7.21 – 7.12 (m, 3H), 3.83 (td, $J = 14.2, 6.3$ Hz, 2H),

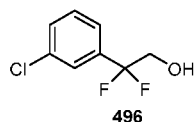
3.77 (s, 2H). ^{13}C NMR (101 MHz, CDCl_3): δ = 166.51, 151.46, 148.84, 131.30, 122.01, 119.20, 117.90, 115.28, 45.29, 28.24.

493: Synthesis of 2-(2-chlorophenyl)-2,2-difluoroethanol (**KPP493**)



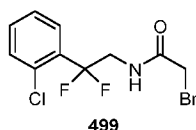
The alcohol **KPP493** was synthesized via GP P using the acetate **KPP487** (8.74 g, 37.24 mmol), and NaBH_4 (1.41 g, 37.24 mmol) in MeOH (130 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP493** (5.36 g, 27.84 mmol, 75 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.50. ^1H NMR (400 MHz, MeOD): δ = 7.66 (dd, J = 7.6, 2.0 Hz, 1H), 7.53 – 7.35 (m, 3H), 4.18 – 4.05 (m, 2H). ^{13}C NMR (101 MHz, MeOD): δ = 133.59, 132.76, 132.36, 129.93, 128.05, 121.76, 64.34, 61.61.

496: Synthesis of 2-(3-chlorophenyl)-2,2-difluoroethanol (**KPP496**)



The alcohol **KPP496** was synthesized via GP P using the acetate **KPP488** (6.23 g, 26.56 mmol), and NaBH_4 (1.00 g, 26.56 mmol) in MeOH (90 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP496** (3.48 g, 18.05 mmol, 70 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.50. ^1H NMR (400 MHz, MeOD): δ = 7.56 (t, J = 2.0 Hz, 1H), 7.50 – 7.36 (m, 3H), 3.91 (t, J = 13.4 Hz, 2H). ^{13}C NMR (101 MHz, MeOD): δ = 138.63, 135.53, 131.30, 127.15, 125.33, 121.55, 65.92, 61.67.

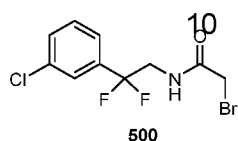
499: Synthesis of 2-bromo-N-(2,2-difluoro-2-(2-chlorophenyl)ethyl)acetamide (**KPP499**)



The bromoacetamide **KPP499** was synthesized via GP Q and GP D. GP Q employed the alcohol **KPP493** (5.25 g, 27.22 mmol), pyridine (3.52 mL, 38.33 mmol) and Tf_2O (5.04 mL, 29.94 mmol) in dry acetonitrile (48 mL) as well as NH_4OH (28%, 48 mL). The crude intermediate was coupled to the amide using triethylamine (4.16 mL, 29.94 mmol) and bromoacetyl bromide (2.60 mL, 29.94 mmol) in dry DCM (84 mL). The crude product was purified

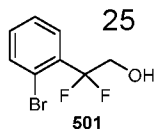
by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP499** (1.13 g, 3.60 mmol, 13 % over two steps) as a colorless solid. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.25$. **LC-MS (ESI)**: $t_R = 8.55$ min; $m/z = 311.71$ $[M + H]^+$, calcd. for $C_{10}H_9BrClF_2NO$: 310.95. **1H NMR** (400 MHz, $CDCl_3$): $\delta = 7.59$ (d, $J = 7.7$ Hz, 1H), 7.45 (d, $J = 7.9$ Hz, 1H), 7.40 (t, $J = 7.4$ Hz, 1H), 7.34 (t, $J = 7.5$ Hz, 1H), 6.78 (s, 1H), 4.15 (td, $J = 14.6, 6.3$ Hz, 2H), 3.85 (s, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$): $\delta = 165.51, 131.67, 131.27, 127.75, 126.72, 119.18, 44.16, 28.50$.

500: Synthesis of 2-bromo-N-(2,2-difluoro-2-(3-chlorophenyl)ethyl)acetamide (KPP499)



The bromoacetamide **KPP500** was synthesized via GP Q and GP D. GP Q employed the alcohol **KPP496** (3.29 g, 17.06 mmol), pyridine (2.20 mL, 27.30 mmol) and Tf_2O (3.16 mL, 18.77 mmol) in dry acetonitrile (30 mL) as well as NH_4OH (28%, 30 mL). The crude intermediate was coupled to the amide using triethylamine (2.60 mL, 18.77 mmol) and bromoacetyl bromide (1.62 mL, 18.77 mmol) in dry DCM (52 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP500** (1.03 g, 3.28 mmol, 19 % over two steps) as a yellow solid. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.25$. **LC-MS (ESI)**: $t_R = 8.80$ min; $m/z = 311.67$ $[M + H]^+$, calcd. for $C_{10}H_9BrClF_2NO$: 310.95. **1H NMR** (400 MHz, $CDCl_3$): $\delta = 7.50$ (s, 1H), 7.47 – 7.42 (m, 1H), 7.40 – 7.37 (m, 2H), 6.83 (s, 1H), 3.91 (td, $J = 14.3, 6.2$ Hz, 2H), 3.86 (s, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$): $\delta = 165.58, 135.91, 134.61, 130.59, 129.89, 125.44, 123.26, 119.20, 45.60, 28.40$.

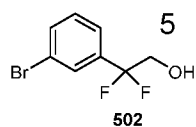
501: Synthesis of 2-(2-bromophenyl)-2,2-difluoroethanol (KPP501)



The alcohol **KPP501** was synthesized via GP P using the acetate **KPP489** (3.61 g, 12.92 mmol), and $NaBH_4$ (0.49 g, 12.92 mmol) in MeOH (45 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP501** (2.49 g, 10.52 mmol, 81 %) as a colorless oil. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.50$. **1H NMR** (400 MHz, MeOD): $\delta = 7.70$ (ddd, $J = 14.2, 8.0, 1.6$ Hz, 2H), 7.46 (t, $J = 7.6$ Hz, 1H), 7.36 (t, $J = 7.4$ Hz, 1H), 4.15 (t, $J = 14.0$ Hz, 2H).

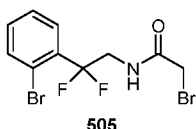
^{13}C NMR (101 MHz, MeOD): δ = 135.81, 135.09, 132.74, 130.08, 128.45, 121.74, 120.60, 64.14.

502: Synthesis of 2-(3-bromophenyl)-2,2-difluoroethanol (KPP502)

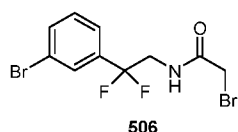


The alcohol **KPP502** was synthesized via GP **P** using the acetate **KPP490** (5.60 g, 20.06 mmol), and NaBH_4 (0.76 g, 20.06 mmol) in MeOH (70 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP502** (3.79 g, 15.97 mmol, 80 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.50. ^1H NMR (400 MHz, MeOD): δ = 7.74 (s, 1H), 7.64 (dd, J = 8.3, 2.0 Hz, 1H), 7.53 (d, J = 8.0 Hz, 1H), 7.40 (t, J = 7.9 Hz, 1H), 3.94 (t, J = 13.3 Hz, 2H), 2.04 (s, 1H), 1.26 (t, J = 7.1 Hz, 1H). ^{13}C NMR (101 MHz, MeOD): δ = 138.67, 134.13, 131.27, 129.89, 125.54, 123.22, 121.27, 65.74.

505: Synthesis of 2-bromo-N-(2-(2-bromophenyl)-2,2-difluoroethyl)acetamide (KPP505)



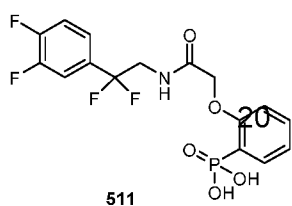
The bromoacetamide **KPP505** was synthesized via GP **Q** and GP **D**. GP **Q** employed the alcohol **KPP501** (2.34 g, 9.86 mmol), pyridine (1.27 mL, 15.77 mmol) and Tf_2O (1.82 mL, 10.85 mmol) in dry acetonitrile (17.5 mL) as well as NH_4OH (28%, 17.5 mL). The crude intermediate was coupled to the amide using triethylamine (1.37 mL, 10.85 mmol) and bromoacetyl bromide (0.94 mL, 10.85 mmol) in dry DCM (30 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP505** (2.27 g, 6.36 mmol, 65 % over two steps) as a white solid. TLC (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.25. LC-MS (ESI): t_R = 8.68 min; m/z = 355.76 $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{10}\text{H}_9\text{Br}_2\text{F}_2\text{NO}$: 354.90. ^1H NMR (400 MHz, CDCl_3): δ = 7.66 (d, J = 7.9 Hz, 1H), 7.59 (d, J = 7.7 Hz, 1H), 7.39 (t, J = 7.6 Hz, 1H), 7.31 (td, J = 7.8, 1.7 Hz, 1H), 6.83 (s, 1H), 4.18 (td, J = 14.6, 6.3 Hz, 4H), 3.89 (d, J = 6.1 Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3): δ = 171.18, 166.56, 135.43, 133.58, 132.45, 128.65, 127.94, 119.95, 44.85, 29.10.

506: Synthesis of 2-bromo-N-(2-(3-bromophenyl)-2,2-difluoroethyl)acetamide (**KPP506**)

The bromoacetamide **KPP506** was synthesized via GP Q and GP D. GP Q employed the alcohol **KPP502** (3.63 g, 15.33 mmol), pyridine (1.98 mL, 24.53 mmol) and Ti_2O (2.84 mL, 16.87 mmol) in

5 dry acetonitrile (27 mL) as well as NH_4OH (28%, 27 mL). The crude intermediate was coupled to the amide using triethylamine (2.34 mL, 16.87 mmol) and bromoacetyl bromide (1.46 mL, 16.87 mmol) in dry DCM (46 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP506** (1.55 g, 4.35 mmol, 26 % over two steps) as a yellow solid. TLC (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.25$. LC-MS (ESI): $t_R = 8.95$ min; $m/z = 355.63$ $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{10}\text{H}_9\text{Br}_2\text{F}_2\text{NO}$: 354.90. ^1H NMR (400 MHz, CDCl_3): $\delta = 7.66$ (s, 1H), 7.59 (d, $J = 8.0$ Hz, 1H), 7.43 (d, $J = 7.8$ Hz, 1H), 7.32 (t, $J = 7.9$ Hz, 1H), 6.82 (s, 1H), 3.91 (dt, $J = 14.3, 7.2$ Hz, 2H), 3.87 (s, 2H). ^{13}C NMR (101 MHz, CDCl_3): $\delta = 165.88, 136.41, 133.85, 130.43, 128.63, 124.04, 122.86, 119.41, 45.93, 28.74$.

15

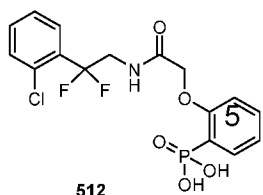
511: Synthesis of (2-(2-((2-(3,4-difluorophenyl)-2,2-difluoroethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP511**) 76

The phenylphosphonic acid derivative **KPP511** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (202 mg, 1.00 mmol), potassium carbonate (276 mg, 2.00 mmol) and **KPP492** (314 mg, 1.00 mmol) in DMF (6 mL). The crude intermediate was

deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and $\text{MeOH}/\text{H}_2\text{O}$ (3:1, 8 mL). The crude product was purified by HPLC to obtain **KPP511** (261 mg, 0.64 mmol, 64 % over two steps) as a white solid. LC-MS (ESI): $t_R = 6.43$ min; $m/z = 407.81$ $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{16}\text{H}_{14}\text{F}_4\text{NO}_5\text{P}$: 407.05. ^1H NMR (400 MHz, DMSO): $\delta = 9.40$ (t, $J = 6.4$ Hz, 1H), 7.61 (ddd, $J = 14.2, 7.5, 1.7$ Hz, 1H), 7.60 – 7.36 (m, 3H), 7.28 (ddd, $J = 8.8, 4.0, 1.9$ Hz, 1H), 7.07 (td, $J = 10.9, 9.6, 6.7$ Hz, 2H), 4.63 (s, 2H), 3.9= (td, $J = 14.1, 6.4$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO): $\delta = 168.67, 158.75, 150.46, 148.01, 133.10, 132.54, 123.17, 122.77, 122.16, 121.19, 119.73, 117.76, 117.29, 113.15, 67.45, 43.76$. HRMS (ESI): $m/z = 408.0618$ calcd. for $[\text{C}_{16}\text{H}_{14}\text{F}_4\text{NO}_5\text{P} + \text{H}]^+$; found: 408.0617.

30

512: Synthesis of (2-(2-((2-(2-chlorophenyl)-2,2-difluoroethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP512**) **93**



512

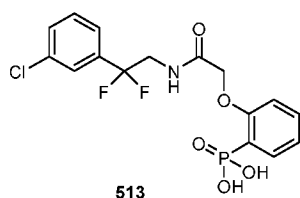
The phenylphosphonic acid derivative **KPP512** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (202 mg, 1.00 mmol), potassium carbonate (276 mg, 2.00 mmol) and **KPP499** (313 mg, 1.00 mmol) in DMF (6 mL). The crude intermediate was

deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and MeOH/H₂O (3:1, 8 mL). The crude product was purified by HPLC to obtain

10 **KPP512** (168 mg, 0.41 mmol, 41 % over two steps) as a white solid. **LC-MS (ESI)**: $t_R = 6.42$ min; $m/z = 405.85$ [M + H]⁺, calcd. for C₁₆H₁₅ClF₂NO₅P: 405.03. **¹H NMR** (400 MHz, DMSO): $\delta = 9.40$ (t, $J = 6.3$ Hz, 1H), 7.69 – 7.58 (m, 1H), 7.54 – 7.41 (m, 4H), 7.34 (t, $J = 7.5$ Hz, 1H), 7.07 (dq, $J = 8.2, 4.7$ Hz, 2H), 4.59 (s, 2H), 4.04 (td, $J = 14.6, 6.3$ Hz, 2H). **¹³C NMR** (101 MHz, DMSO): $\delta = 168.57, 158.74,$
15 133.10, 132.56, 132.09, 131.56, 131.13, 130.71, 128.13, 127.21, 123.16, 121.15, 119.80, 113.14, 67.46, 42.47. **HRMS (ESI)**: $m/z = 406.0417$ calcd. for [C₁₆H₁₅ClF₂NO₅P + H]⁺; found: 406.0415.

513: Synthesis of (2-(2-((2-(3-chlorophenyl)-2,2-difluoroethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP513**) **94**

20



513

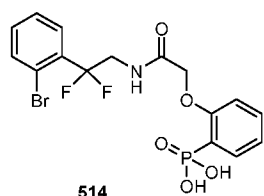
The phenylphosphonic acid derivative **KPP512** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (152 mg, 0.75 mmol), potassium carbonate (207 mg, 1.50 mmol) and **KPP500** (235 mg, 0.75 mmol) in DMF (5 mL). The crude intermediate was

deprotected via GP H using TMSBr (0.99 mL, 7.50 mmol) in dry DCM (6 mL) and MeOH/H₂O (3:1, 6 mL). The crude product was purified by HPLC to obtain

30 **KPP512** (37 mg, 0.09 mmol, 12 % over two steps) as a white solid. **LC-MS (ESI)**: $t_R = 7.45$ min; $m/z = 405.89$ [M + H]⁺, calcd. for C₁₆H₁₅ClF₂NO₅P: 405.03. **¹H NMR** (400 MHz, DMSO): $\delta = 9.45$ (t, $J = 6.3$ Hz, 1H), 7.62 (ddd, $J = 14.2, 7.4, 1.7$ Hz, 1H), 7.55 (d, $J = 8.0$ Hz, 1H), 7.52 – 7.35 (m, 4H), 7.14 – 7.03 (m, 2H), 4.63 (s, 2H), 3.89 (td, $J = 14.6, 6.3$ Hz, 2H). **¹³C NMR** (101 MHz, DMSO): $\delta = 168.85, 158.97, 139.30, 136.90, 133.46, 132.73, 130.76, 125.38, 124.31, 121.50, 113.44,$

105.43, 99.83, 67.70, 44.03. **HRMS (ESI):** $m/z = 406.0417$ calcd. for $[C_{16}H_{15}ClF_2NO_5P + H]^+$; found: 406.0415.

514: *Synthesis of (2-(2-((2-(2-bromophenyl)-2,2-difluoroethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (KPP514) 95*

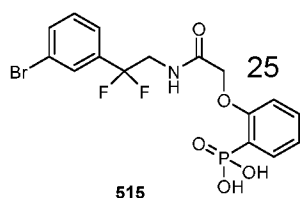


The phenylphosphonic acid derivative **KPP514** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (202 mg, 1.00 mmol), potassium carbonate (276 mg, 2.00 mmol) and **KPP505** (357 mg, 1.00 mmol) in DMF (6 mL). The crude intermediate was deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and MeOH/H₂O (3:1, 8 mL). The crude product was purified by HPLC to obtain **KPP514** (202.7 mg, 0.45 mmol, 45 % over two steps) as a white solid. **LC-MS (ESI):** $t_R = 6.49$ min; $m/z = 449.84$ $[M + H]^+$, calcd. for $C_{16}H_{15}BrF_2NO_5P$: 448.99.

15 **¹H NMR** (400 MHz, DMSO): $\delta = 9.38$ (t, $J = 6.1$ Hz, 1H), 7.98 (t, $J = 6.5$ Hz, 1H), 7.75 – 7.56 (m, 3H), 7.56 – 7.32 (m, 3H), 7.07 (dt, $J = 8.4, 6.4$ Hz, 1H), 4.59 (s, 1H), 4.05 (tt, $J = 14.8, 7.1$ Hz, 2H), 3.80 (s, 1H). **¹³C NMR** (101 MHz, DMSO): $\delta = 172.14, 168.42, 158.63, 134.46, 132.92, 132.17, 127.65, 127.52, 122.51, 121.13, 120.07, 119.10, 112.97, 67.32, 61.11, 42.15$. **HRMS (ESI):** $m/z = 449.9912$ calcd. for $[C_{16}H_{15}BrF_2NO_5P + H]^+$; found: 449.9915.

20

515: *Synthesis of (2-(2-((2-(3-bromophenyl)-2,2-difluoroethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (KPP515) 96*

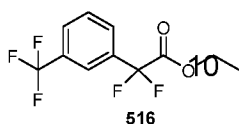


The phenylphosphonic acid derivative **KPP515** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (202 mg, 1.00 mmol), potassium carbonate (276 mg, 2.00 mmol) and **KPP506** (357 mg, 1.00 mmol) in DMF (6 mL). The crude intermediate was deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and MeOH/H₂O (3:1, 8 mL). The crude product was purified by HPLC to obtain **KPP515** (248.0 mg, 0.55 mmol, 55 % over two steps) as a white solid. **LC-MS (ESI):** $t_R = 6.72$ min; $m/z = 449.81$ $[M + H]^+$, calcd. for $C_{16}H_{15}BrF_2NO_5P$: 448.99.

30

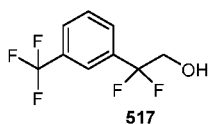
^1H NMR (400 MHz, DMSO): δ = 9.51 (t, J = 6.3 Hz, 1H), 7.74 – 7.62 (m, 3H), 7.57 – 7.33 (m, 3H), 7.06 (ddd, J = 12.7, 9.3, 6.6 Hz, 2H), 4.62 (s, 2H), 3.88 (td, J = 14.7, 6.2 Hz, 6H). ^{13}C NMR (101 MHz, DMSO): δ = 168.66, 158.76, 136.90, 133.27, 132.93, 130.77, 127.94, 124.50, 121.65, 121.24, 119.77, 117.34, 113.10, 99.54, 67.52, 43.83. HRMS (ESI): m/z = 449.9912 calcd. for $[\text{C}_{16}\text{H}_{15}\text{BrF}_2\text{NO}_5\text{P} + \text{H}]^+$; found: 449.9916.

516: Synthesis of ethyl 2,2-difluoro-2-(3-(trifluoromethyl)phenyl)acetate (**KPP516**)



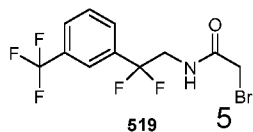
The phenylacetate derivative **KPP516** was synthesized via GP O using 3-iodobenzotrifluoride (7.21 mL, 50.0 mmol), ethyl bromodifluoroacetate (9.62 mL, 75.0 mmol) and activated copper (12.75 g, 195.0 mmol) in dry DMSO (130 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP516** (8.52 g, 31.8 mmol, 64 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 19:1 v/v): R_f = 0.50. ^1H NMR (400 MHz, CDCl_3): δ = 7.88 (s, 1H), 7.68 (dd, J = 23.3, 8.0 Hz, 2H), 7.59 (t, J = 7.9 Hz, 1H), 4.30 (q, J = 7.2 Hz, 2H), 1.29 (t, J = 7.2 Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3): δ = 163.64, 134.11, 131.64, 129.54, 129.14, 127.96, 125.05, 122.77, 112.82, 63.59, 13.79.

517: Synthesis of 2,2-difluoro-2-(3-(trifluoromethyl)phenyl)ethanol (**KPP517**)



The alcohol **KPP517** was synthesized via GP P using the acetate **KPP516** (8.28 g, 30.88 mmol), and NaBH_4 (1.17 g, 30.88 mmol) in MeOH (100 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP517** (5.09 g, 22.50 mmol, 73 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.38. ^1H NMR (400 MHz, MeOD): δ = 7.89 – 7.76 (m, 3H), 7.68 (q, J = 7.9, 7.3 Hz, 1H), 3.97 (t, J = 13.1 Hz, 4H). ^{13}C NMR (101 MHz, MeOD): δ = 138.05, 132.03, 130.60, 127.84, 126.63, 123.70, 121.59, 119.17, 65.42.

519: *Synthesis* of 2-bromo-N-(2,2-difluoro-2-(3-(trifluoromethyl)phenyl)ethyl)acetamide (**KPP519**)

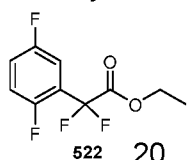


The bromoacetamide **KPP519** was synthesized via GP **Q** and GP **D**. GP **Q** employed the alcohol **KPP517** (4.94 g, 21.87 mmol), pyridine (2.82 mL, 34.99 mmol) and TiCl_4 (4.04 mL, 24.06 mmol)

in dry acetonitrile (40 mL) as well as NH_4OH (28%, 40 mL). The crude intermediate was coupled to the amide using triethylamine (3.34 mL, 24.06 mmol) and bromoacetyl bromide (2.09 mL, 24.06 mmol) in dry DCM (90 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP519** (2.89 g,

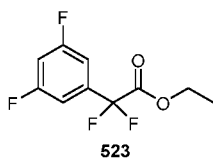
10 8.36 mmol, 38 % over two steps) as a white solid. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.20$. **LC-MS (ESI)**: $t_R = 9.14$ min; $m/z = 345.61$ $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{11}\text{H}_9\text{BrF}_5\text{NO}$: 344.98. $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta = 7.80 - 7.68$ (m, 3H), 7.60 (t, $J = 7.8$ Hz, 1H), 6.86 (s, 1H), 3.95 (td, $J = 14.2, 6.3$ Hz, 2H), 3.86 (s, 1H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3): $\delta = 166.04, 135.42, 131.25, 129.56, 128.86, 127.56,$
15 125.05, 122.49, 119.62, 45.92, 28.63.

522: *Synthesis of ethyl 2-(2,5-difluorophenyl)-2,2-difluoroacetate (**KPP522**)*



The phenylacetate derivative **KPP522** was synthesized via GP **O** using 1,4-difluoro-3-iodobenzene (6.01 mL, 50.0 mmol), ethyl bromodifluoroacetate (9.62 mL, 75.0 mmol) and activated

copper (12.75 g, 195.0 mmol) in dry DMSO (130 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP522** (7.73 g, 37.7 mmol, 65 %) as a colorless oil. **TLC** (cyclohexane:ethyl acetate, 19:1 v/v): $R_f = 0.38$. **LC-MS (ESI)**: $t_R = 10.01$ min; $m/z = 236.66$ $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{10}\text{H}_8\text{F}_4\text{O}_2$: 236.05. $^1\text{H NMR}$ (400 MHz, CDCl_3): $\delta = 7.33$ (ddd, $J = 8.4, 5.6, 3.1$ Hz, 1H), 7.22 – 7.04 (m, 2H), 4.34 (q, $J = 7.2$ Hz, 2H), 1.30 (t, $J = 7.2$ Hz, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3): $\delta = 162.77, 159.68, 157.23, 119.85, 118.01, 114.40, 110.99, 108.49, 63.66, 13.82$.

523: Synthesis of ethyl 2-(3,5-difluorophenyl)-2,2-difluoroacetate (**KPP523**)

The phenylacetate derivative **KPP523** was synthesized via GP O using 1,3-difluoro-5-iodobenzene (5.99 mL, 50.0 mmol), ethyl bromodifluoroacetate (9.62 mL, 75.0 mmol) and activated copper (12.75 g, 195.0 mmol) in dry DMSO (130 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP523** (10.29 g, 43.6 mmol, 87 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 19:1 v/v): R_f = 0.50. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ = 7.13 (qd, J = 6.4, 5.2, 3.8 Hz, 2H), 6.93 (tt, J = 8.7, 2.4 Hz, 1H), 4.30 (q, J = 7.2 Hz, 2H), 1.31 (t, J = 7.2 Hz, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ = 164.28, 163.30, 161.79, 136.27, 114.69, 112.17, 109.19, 106.74, 63.71, 13.89.

524: Synthesis of ethyl 2,2-difluoro-2-(3-(trifluoromethoxy)phenyl)acetate (**KPP524**)

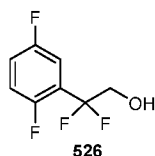
The phenylacetate derivative **KPP524** was synthesized via GP O using 3-(trifluoromethoxy) iodobenzene (7.73 mL, 50.0 mmol), ethyl bromodifluoroacetate (9.62 mL, 75.0 mmol) and activated copper (12.75 g, 195.0 mmol) in dry DMSO (130 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP524** (10.61 g, 37.4 mmol, 75 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 19:1 v/v): R_f = 0.43. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ = 7.54 – 7.45 (m, 3H), 7.39 – 7.31 (m, 1H), 4.30 (q, J = 7.2 Hz, 2H), 1.30 (t, J = 7.2 Hz, 1H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3): δ = 163.68, 149.45, 135.15, 130.50, 124.09, 121.81, 119.24, 118.64, 112.64, 63.55, 13.85.

525: Synthesis of ethyl 2-(2,6-difluorophenyl)-2,2-difluoroacetate (**KPP525**)

The phenylacetate derivative **KPP525** was synthesized via GP O using 1,3-difluoro-2-iodobenzene (6.00 mL, 50.0 mmol), ethyl bromodifluoroacetate (9.62 mL, 75.0 mmol) and activated copper (12.75 g, 195.0 mmol) in dry DMSO (130 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 19:1) to obtain **KPP525** (8.87 g, 37.55 mmol, 75 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 19:1 v/v): R_f = 0.25. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ = 7.48 – 7.41 (m, 1H), 6.97

(t, $J = 8.9$ Hz, 2H), 4.37 (q, $J = 7.1$ Hz, 2H), 1.33 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3): $\delta = 162.85, 161.79, 159.33, 133.36, 112.77, 112.51, 111.27, 100.13, 63.70, 13.84$.

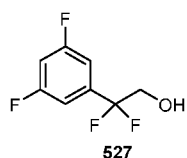
5 **526: Synthesis of 2-(2,5-difluorophenyl)-2,2-difluoroethanol (KPP526)**



The alcohol **KPP526** was synthesized via GP P using the acetate **KPP522** (7.53 g, 31.90 mmol), and NaBH_4 (1.21 g, 31.90 mmol) in MeOH (110 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain

10 **KPP526** (4.84 g, 24.94 mmol, 78 %) as a white solid. TLC (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.38$. ^1H NMR (400 MHz, DMSO): $\delta = 7.48 - 7.31$ (m, 3H), 5.74 (t, $J = 6.5$ Hz, 1H), 3.91 (tdd, $J = 14.2, 6.5, 1.2$ Hz, 2H). ^{13}C NMR (101 MHz, DMSO): $\delta = 158.96, 156.56, 123.43, 119.71, 119.16, 118.37, 115.02, 62.98$.

15 **527: Synthesis of 2-(3,5-difluorophenyl)-2,2-difluoroethanol (KPP527)**



The alcohol **KPP527** was synthesized via GP P using the acetate **KPP523** (10.11 g, 42.80 mmol), and NaBH_4 (1.61 g, 42.80 mmol) in MeOH (150 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain

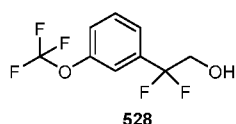
20 **KPP527** (7.57 g, 38.99 mmol, 91 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.43$. ^1H NMR (400 MHz, CDCl_3): $\delta = 7.03$ (dq, $J = 6.1, 2.3$ Hz, 2H), 6.89 (tt, $J = 8.7, 2.3$ Hz, 1H), 3.91 (ddd, $J = 14.6, 11.8, 1.4$ Hz, 2H), 3.10 – 3.04 (m, 1H). ^{13}C NMR (101 MHz, CDCl_3): $\delta = 164.24, 161.75, 138.19, 119.49, 109.42, 109.15, 105.88, 65.47$.

25

528: Synthesis of 2,2-difluoro-2-(3-(trifluoromethoxy)phenyl)ethanol (KPP528)

The alcohol **KPP528** was synthesized via GP P using the acetate **KPP524** (10.49 g, 36.90 mmol), and NaBH_4 (1.40 g, 36.90 mmol) in MeOH (125 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain

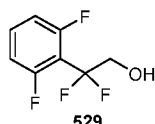
30 **KPP528** (6.45 g, 26.62 mmol, 72 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.50$. ^1H NMR (400 MHz, DMSO): $\delta = 7.69 - 7.45$ (m, 4H), 5.69



(t, $J = 6.3$ Hz, 1H), 3.89 (td, $J = 13.8, 6.3$ Hz, 2H).

^{13}C NMR (101 MHz, DMSO): $\delta = 148.21, 137.27, 130.81, 124.87, 122.76, 121.29, 120.62, 118.45, 63.59$.

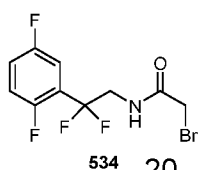
5 529: Synthesis of 2-(2,6-difluorophenyl)-2,2-difluoroethanol (**KPP529**)



The alcohol **KPP529** was synthesized via GP P using the acetate **KPP525** (8.79 g, 37.21 mmol), and NaBH_4 (1.41 g, 37.21 mmol) in MeOH (130 mL). The crude product was purified by column

chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP529** (4.32 g, 22.29 mmol, 60 %) as a colorless oil. TLC (cyclohexane:ethyl acetate, 2:1 v/v): $R_f = 0.30$. ^1H NMR (400 MHz, MeOD): $\delta = 7.54$ (tt, $J = 8.4, 6.2$ Hz, 1H), 7.13 – 7.00 (m, 2H), 4.05 (t, $J = 13.8$ Hz, 1H). ^{13}C NMR (101 MHz, MeOD): $\delta = 163.10, 160.57, 133.90, 121.10, 113.68, 113.41, 112.42, 65.39$.

15 534: Synthesis of Synthesis of 2-bromo-N-(2-(2,5-difluorophenyl)-2,2-difluoroethyl)acetamide (**KPP534**)



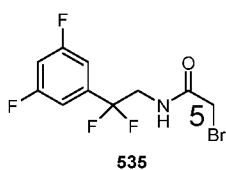
The bromoacetamide **KPP534** was synthesized via GP Q and GP D.

GP Q employed the alcohol **KPP526** (4.65 g, 23.96 mmol), pyridine (3.10 mL, 38.34 mmol) and Tf_2O (4.44 mL, 26.36 mmol) in dry acetonitrile (43 mL) as well as NH_4OH (28%, 43 mL). The crude

intermediate was coupled to the amide using triethylamine (3.66 mL, 26.36 mmol) and bromoacetyl bromide (2.28 mL, 26.36 mmol) in dry DCM (112 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 1:1) to obtain **KPP534** (3.89 g, 12.44 mmol, 52 % over two steps) as a white solid.

TLC (cyclohexane:ethyl acetate, 1:1 v/v): $R_f = 0.50$. LC-MS (ESI): $t_R = 8.58$ min; $m/z = 313.79$ $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{10}\text{H}_8\text{BrF}_4\text{NO}$: 312.97. ^1H NMR (400 MHz, DMSO): $\delta = 8.78$ (t, $J = 6.3$ Hz, 1H), 7.49 – 7.34 (m, 2H), 7.32 (ddd, $J = 8.7, 5.8, 2.9$ Hz, 1H), 3.94 (td, $J = 14.6, 6.3$ Hz, 2H), 3.82 (s, 2H). ^{13}C NMR (101 MHz, DMSO): $\delta = 166.77, 158.87, 156.47, 123.10, 119.44, 118.67, 118.42, 114.29, 43.41, 28.63$.

535: *Synthesis of Synthesis of 2-bromo-N-(2-(3,5-difluorophenyl)-2,2-difluoroethyl)acetamide (KPP535)*



The bromoacetamide **KPP535** was synthesized via GP **Q** and GP **D**.

GP **Q** employed the alcohol **KPP527** (7.46 g, 38.44 mmol), pyridine (4.96 mL, 61.52 mmol) and TiF_4 (7.12 mL, 42.30 mmol) in dry acetonitrile (68 mL) as well as NH_4OH (28%, 68 mL). The crude

intermediate was coupled to the amide using triethylamine (5.96 mL, 42.30 mmol) and bromoacetyl bromide (3.68 mL, 42.30 mmol) in dry DCM (120 mL). The crude product

was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain

10 **KPP535** (4.40 g, 14.05 mmol, 37 % over two steps) as a white solid. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.25. **LC-MS (ESI)**: t_R = 8.71 min; m/z = 313.77 $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{10}\text{H}_8\text{BrF}_4\text{NO}$: 312.97. ^1H **NMR** (400 MHz, DMSO): δ = 8.74 (t, J = 6.3 Hz, 1H), 7.38 (tt, J = 9.3, 2.4 Hz, 1H), 7.31 – 7.19 (m, 2H), 3.90 (td, J = 14.4, 6.2 Hz, 2H), 3.84 (s, 2H). ^{13}C **NMR** (101 MHz, DMSO): δ = 166.78,

15 163.49, 161.15, 138.08, 119.61, 109.48, 109.20, 106.08, 44.06, 28.69.

536: *Synthesis of Synthesis of 2-bromo-N-(2,2-difluoro-2-(3-(trifluoromethoxy)phenyl)ethyl)acetamide (KPP536)*



The bromoacetamide **KPP536** was synthesized via GP **Q** and

GP **D**. GP **Q** employed the alcohol **KPP528** (6.37 g, 26.30 mmol), pyridine (3.40 mL, 42.08 mmol) and

TiF_4 (4.88 mL, 28.92 mmol) in dry acetonitrile (48 mL) as well as NH_4OH (28%, 48 mL). The crude intermediate was coupled to the amide using

triethylamine (4.00 mL, 28.92 mmol) and bromoacetyl bromide (2.52 mL, 28.92 mmol)

in dry DCM (90 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP536** (3.08 g, 8.55 mmol, 33% over two

steps) as a white solid. **TLC** (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.25. **LC-**

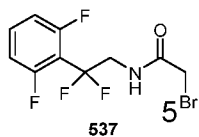
MS (ESI): t_R = 9.40 min; m/z = 361.56 $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{11}\text{H}_9\text{BrF}_5\text{NO}_2$: 360.97.

^1H **NMR** (400 MHz, DMSO): δ = 8.76 (t, J = 6.3 Hz, 1H), 7.69 – 7.45 (m, 3H),

30 3.99 – 3.80 (m, 4H). ^{13}C **NMR** (101 MHz, DMSO): δ = 166.68, 148.28, 136.76, 130.99,

124.57, 123.05, 121.29, 119.98, 118.12, 44.21, 28.67.

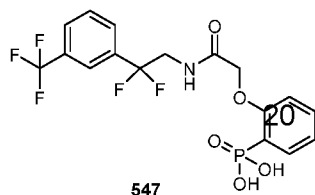
537: Synthesis of Synthesis of 2-bromo-N-(2-(2,6-difluorophenyl)-2,2-difluoroethyl)acetamide (**KPP537**)



The bromoacetamide **KPP537** was synthesized via GP Q and GP D.

GP Q employed the alcohol **KPP529** (4.19 g, 21.70 mmol), pyridine (2.80 mL, 34.72 mmol) and TiF_4 (4.02 mL, 23.88 mmol) in dry acetonitrile (39 mL) as well as NH_4OH (28%, 39 mL). The crude intermediate was coupled to the amide using triethylamine (3.32 mL, 23.88 mmol) and bromoacetyl bromide (2.08 mL, 23.88 mmol) in dry DCM (80 mL). The crude product was purified by column chromatography (cyclohexane/ethyl acetate 2:1) to obtain **KPP537** (1.65 g, 5.28 mmol, 24 % over two steps) as a white solid. TLC (cyclohexane:ethyl acetate, 2:1 v/v): R_f = 0.25. LC-MS (ESI): t_R = 8.36 min; m/z = 313.73 $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{10}\text{H}_8\text{BrF}_4\text{NO}$: 312.97. ^1H NMR (400 MHz, DMSO): δ = 8.86 (t, J = 6.4 Hz, 1H), 7.60 (ddd, J = 14.7, 8.4, 6.2 Hz, 1H), 7.18 (t, J = 9.4 Hz, 2H), 3.94 (tt, J = 17.7, 8.9 Hz, 2H), 3.82 (s, 2H). ^{13}C NMR (101 MHz, DMSO): δ = 166.80, 160.87, 158.35, 133.56, 119.18, 113.03, 112.77, 110.19, 44.10, 28.56.

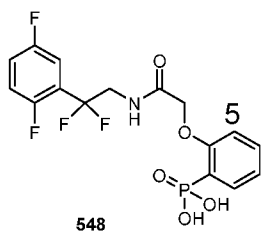
547: Synthesis of (2-(2-((2,2-difluoro-2-(3-(trifluoromethyl)phenyl)ethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP547**) 68



The phenylphosphonic acid derivative **KPP547** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (202 mg, 1.00 mmol), potassium carbonate (276 mg, 2.00 mmol) and **KPP519** (346 mg, 1.00 mmol) in DMF (6 mL). The crude intermediate was

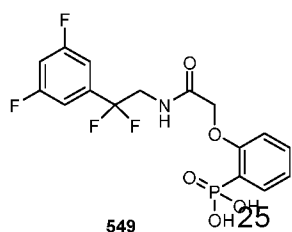
deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and MeOH/ H_2O (3:1, 8 mL). The crude product was purified by HPLC to obtain **KPP547** (194 mg, 0.44 mmol, 44 % over two steps) as a white solid. LC-MS (ESI): t_R = 7.49 min; m/z = 439.92 $[\text{M} + \text{H}]^+$, calcd. for $\text{C}_{17}\text{H}_{15}\text{F}_5\text{NO}_5\text{P}$: 439.06. ^1H NMR (400 MHz, DMSO): δ = 9.50 (t, J = 6.3 Hz, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.81 – 7.71 (m, 2H), 7.71 – 7.58 (m, 2H), 7.48 (td, J = 7.9, 1.7 Hz, 1H), 7.06 (dt, J = 10.4, 6.9 Hz, 2H), 4.60 (s, 2H), 3.96 (td, J = 14.5, 6.3 Hz, 2H). ^{13}C NMR (101 MHz, DMSO): δ = 168.67, 158.81, 135.74, 133.10, 132.55, 129.91, 129.62, 129.16, 127.18, 125.15, 123.18, 121.95, 121.32, 121.18, 113.24, 67.53, 43.83. HRMS (ESI): m/z = 440.0681 calcd. for $[\text{C}_{17}\text{H}_{15}\text{F}_5\text{NO}_5\text{P} + \text{H}]^+$; found: 440.0687.

548: Synthesis of (2-(2-((2-(2,5-difluorophenyl)-2,2-difluoroethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP548**) **74**



The phenylphosphonic acid derivative **KPP548** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (202 mg, 1.00 mmol), potassium carbonate (276 mg, 2.00 mmol) and **KPP534** (314 mg, 1.00 mmol) in DMF (6 mL). The crude intermediate was deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and MeOH/H₂O (3:1, 8 mL). The crude product was purified by HPLC to obtain **KPP548** (146 mg, 0.36 mmol, 36 % over two steps) as a white solid. **LC-MS (ESI)**: t_R = 6.89 min; m/z = 407.95 [$M + H$]⁺, calcd. for C₁₆H₁₄F₄NO₅P: 407.05. **¹H NMR** (400 MHz, DMSO): δ = 9.47 (t, J = 6.4 Hz, 1H), 7.60 (ddd, J = 14.3, 7.5, 1.7 Hz, 1H), 7.47 (td, J = 7.9, 1.7 Hz, 1H), 7.41 – 7.35 (m, 2H), 7.16 (ddd, J = 8.6, 5.7, 3.0 Hz, 1H), 7.12 – 7.02 (m, 2H), 4.62 (s, 2H), 3.96 (td, J = 14.4, 6.3 Hz, 2H). **¹³C NMR** (101 MHz, DMSO): δ = 168.74, 158.74, 156.38, 133.09, 132.49, 123.10, 121.32, 121.19, 119.61, 119.28, 118.58, 118.25, 114.41, 113.22, 67.49, 42.88. **HRMS (ESI)**: m/z = 408.0618 calcd. for [C₁₆H₁₄F₄NO₅P + H]⁺; found: 408.0586.

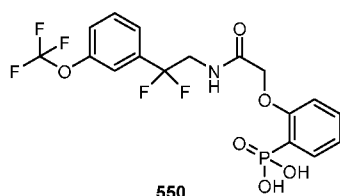
549: Synthesis of (2-(2-((2-(3,5-difluorophenyl)-2,2-difluoroethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP549**) **75**



The phenylphosphonic acid derivative **KPP549** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (202 mg, 1.00 mmol), potassium carbonate (276 mg, 2.00 mmol) and **KPP535** (314 mg, 1.00 mmol) in DMF (6 mL). The crude intermediate was deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and MeOH/H₂O (3:1, 8 mL). The crude product was purified by HPLC to obtain **KPP549** (133 mg, 0.33 mmol, 33 % over two steps) as a white solid. **LC-MS (ESI)**: t_R = 7.33 min; m/z = 407.93 [$M + H$]⁺, calcd. for C₁₆H₁₄F₄NO₅P: 407.05. **¹H NMR** (400 MHz, DMSO): δ = 9.43 (t, J = 6.3 Hz, 1H), 7.61 (ddd, J = 14.2, 7.4, 1.7 Hz, 1H), 7.48 (td, J = 7.9, 1.7 Hz, 1H), 7.38 (tt, J = 9.3, 2.4 Hz, 1H), 7.27 – 7.12 (m, 2H), 7.13 – 7.02 (m, 2H), 4.63 (s, 2H), 3.92 (td, J = 14.2, 6.3 Hz, 2H). **¹³C NMR** (101 MHz, DMSO): δ = 168.71, 163.49, 160.91, 158.74, 133.05, 132.58,

123.24, 121.31, 119.50, 113.19, 109.44, 109.16, 106.04, 67.48, 43.59. **HRMS (ESI):** $m/z = 408.0618$ calcd. for $[C_{16}H_{14}F_4NO_5P + H]^+$; found: 408.0593.

550: Synthesis of (2-(2-((2,2-difluoro-2-(3-(trifluoromethoxy)phenyl)ethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP550**) **not mentioned**

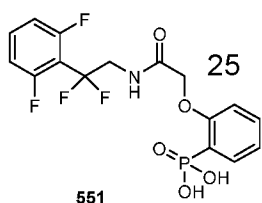


The phenylphosphonic acid derivative **KPP550** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (202 mg, 1.00 mmol), potassium carbonate (276 mg, 2.00 mmol) and **KPP536** (363 mg, 1.00 mmol) in DMF (6 mL). The crude intermediate was

deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and MeOH/H₂O (3:1, 8 mL). The crude product was purified by HPLC to obtain **KPP550** (310 mg, 0.68 mmol, 68 % over two steps) as a white solid. **LC-MS (ESI):** $t_R = 7.41$ min; $m/z = 455.89$ $[M + H]^+$, calcd. for $C_{17}H_{15}F_5NO_6P$: 455.06

¹H NMR (400 MHz, DMSO): $\delta = 9.48$ (t, $J = 6.4$ Hz, 1H), 7.69 – 7.53 (m, 2H), 7.50 – 7.38 (m, 3H), 7.41 (s, 1H), 7.07 (td, $J = 10.6, 9.3, 6.8$ Hz, 2H), 4.61 (s, 2H), 3.91 (td, $J = 14.5, 6.2$ Hz, 2H). **¹³C NMR** (101 MHz, DMSO): $\delta = 168.66, 158.81, 148.21, 136.95, 133.06, 132.63, 130.90, 124.61, 123.26, 122.99, 121.29, 119.86, 118.74, 118.17, 113.22, 67.52, 43.82$. **HRMS (ESI):** $m/z = 456.0630$ calcd. for $[C_{17}H_{15}F_5NO_6P + H]^+$; found: 456.0628.

551: Synthesis of (2-(2-((2-(2,6-difluorophenyl)-2,2-difluoroethyl)amino)-2-oxoethoxy)phenyl)phosphonic acid (**KPP551**) **73**



The phenylphosphonic acid derivative **KPP551** was synthesized via GP G and GP H. GP G employed phosphonate **KPP003** (202 mg, 1.00 mmol), potassium carbonate (276 mg, 2.00 mmol) and **KPP537** (314 mg, 1.00 mmol) in DMF (6 mL). The crude intermediate was

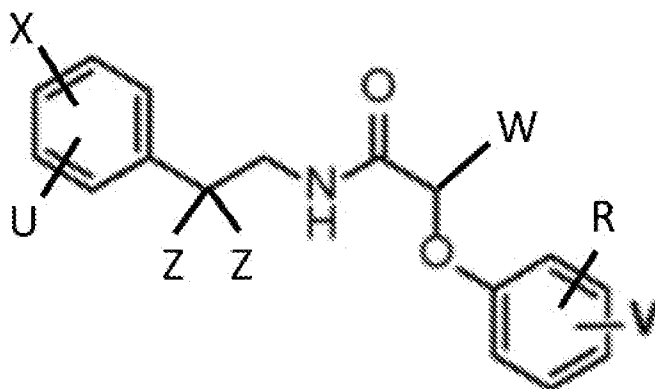
deprotected via GP H using TMSBr (1.32 mL, 10.00 mmol) in dry DCM (8 mL) and MeOH/H₂O (3:1, 8 mL). The crude product was purified by HPLC to obtain **KPP551** (60 mg, 0.15 mmol, 15 % over two steps) as a white solid. **LC-MS (ESI):** $t_R = 6.68$ min; $m/z = 407.87$ $[M + H]^+$, calcd. for $C_{16}H_{14}F_4NO_5P$: 407.05.

¹H NMR (400 MHz, DMSO): δ = 9.57 (t, J = 6.4 Hz, 1H), 7.59 (dtd, J = 15.4, 7.9, 2.0 Hz, 2H), 7.48 (td, J = 7.9, 1.7 Hz, 1H), 7.18 – 7.01 (m, 4H), 4.63 (s, 2H), 3.94 (td, J = 14.8, 6.3 Hz, 2H). ¹³C NMR (101 MHz, DMSO): δ = 168.92, 158.84, 158.28, 133.49, 133.06, 132.64, 123.24, 121.44, 121.29, 121.16, 113.21, 113.13, 113.00, 112.76, 67.58, 43.63. HRMS (ESI): m/z = 408.0618 calcd. for [C₁₆H₁₄F₄NO₅P + H]⁺; found: 408.0625.

5

CLAIMS

1. The compound as defined by formula Va:



5

(formula Va).

wherein at least one of Z is F, and the other Z is H, preferably wherein both of Z are F;

10

wherein X is selected from H, F, Br, Cl, CF₃, OCH₂Ph, and p-OCH₂Pyr (N-2, N-3 or N-4);

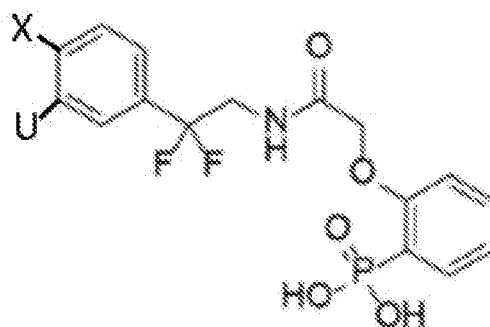
U and W are selected from F and H;

R is selected from H, CH₃ or F; and

V is selected from PO(OH)₂ and OPO(OH)₂.

15

2. The compound as defined by formula VI:

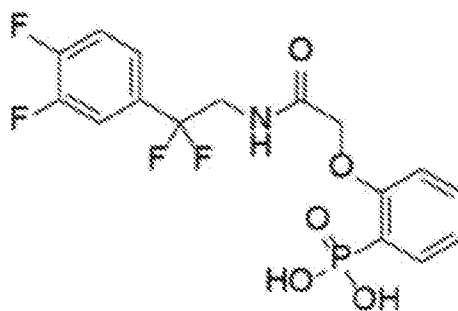
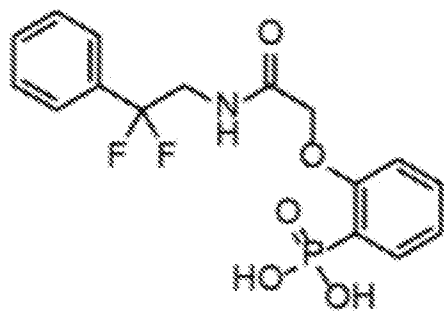


100

(formula VI)

wherein X is selected from H, F, Br, Cl, CF₃, OCH₂Ph, and p-OCH₂Pyr and
U is selected from F and H.

3. The compound in accordance with claim 1 or claim 2, wherein at least one of X and U is F.
4. The compound in accordance with claim 1 or claim 2, wherein both of X and U are F.
5. The compound in accordance with any of the previous claims, wherein the compound is in the form of a pharmaceutically acceptable salt.
6. The compound in accordance with any of the previous claims, wherein the compound is:

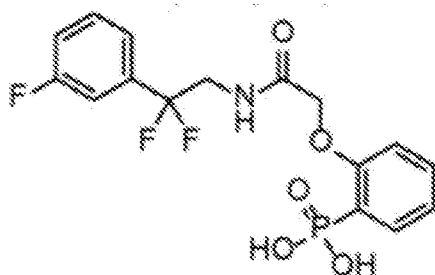


or

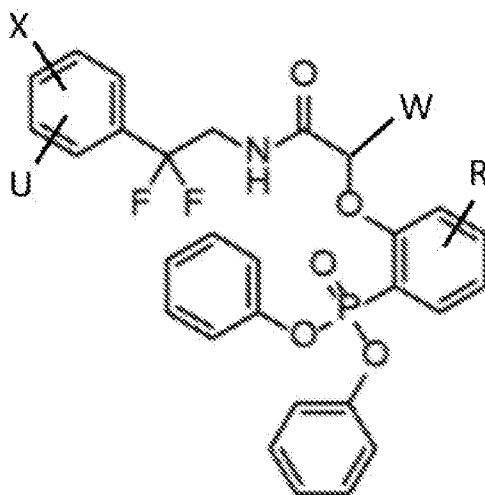
20

or

101



- 5 7. The compound in accordance with any of claims 1-6, wherein the compound is in the form of a pharmaceutically acceptable prodrug.
8. The compound in accordance with claim 7, wherein the compound is in the form of formula VII:

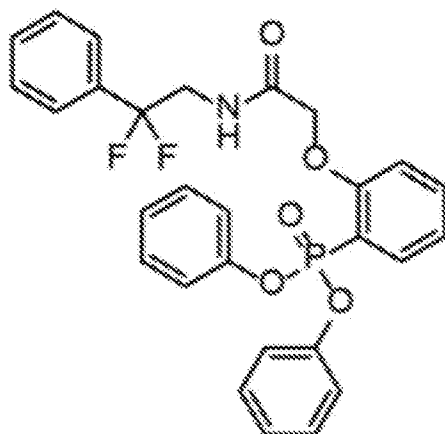


10

(formula VII).

9. The compound in accordance with claim 7 or 8, wherein the compound is:

102



10. Use of the compound according to any of the previous claims, wherein the compound is capable of interacting with a first protein and a client protein and wherein the first protein and the client protein and the compound form a complex, for stabilizing the complex.
11. The use according to claim 10, wherein the first protein is the 14-3-3 protein and the client protein is a phosphorylation-independent 14-3-3 client protein, preferably ChREBP- α .
12. The use accordance with claim 11, wherein the compound is selective for stabilizing the complex of 14-3-3 and ChREBP- α .
13. The use in accordance with claim 12, wherein the compound is for use in reducing translocation in a cell of the complex from the cytoplasm to the nucleus.
14. The use in accordance with any of claims 11-13, wherein the compound is for use in repression of ChREBP- α mediated gene expression.
15. The use in accordance with any of claims 10-14, wherein the compound is for use in repression of ChREBP- β transcription.

16. The compound according to any of the claims 1-9, wherein the compound is for use in a medical treatment.
- 5 17. The compound according to claim 16, wherein the compound is for use in the treatment of Type 2 Diabetes.
18. The compound for use in accordance with claim 16 or 17, wherein the medical treatment involves reducing or preventing glucose toxicity.
- 10 19. The compound for use in accordance with claim 16-18, wherein the medical treatment involves reducing or preventing glucolipotoxicity.
20. The compound for use in accordance with claim 16-19, for the treatment of a condition characterized by a hyperglycemic condition, such as a prolonged hyperglycemic condition.
- 15 21. The compound for use in accordance with any of claims 16, and claims 18 – 20, wherein the compound is for use in the treatment of type 1 diabetes.
- 20 22. The compound for use in accordance with claim 21, wherein the treatment is for preventing and/or delaying onset of type 1 diabetes.
23. The compound for use in accordance with any of claim 21 and claim 22, wherein the treatment is for use in the treatment of the honeymoon period of type 1 diabetes.
- 25 24. The compound for use in accordance with claim 23, wherein the honeymoon period is prolonged in patients in the honeymoon period of type 1 diabetes.
- 30 25. The compound for use in accordance with any of claims 21-24, wherein the β cell function is improved, β cell killing is reduced, and/or autoimmune function affected.

26. The compound for use in accordance with claim 16, wherein the medical treatment comprises the treatment of cancer.
27. The compound for use in accordance with claim 26, wherein the cancer is dependent on ChREBP for proliferation.
28. The compound for use in accordance with any of claim 26 or claim 27, wherein the cancer is colon cancer, hepatic cell carcinoma or insulinoma.

1/41

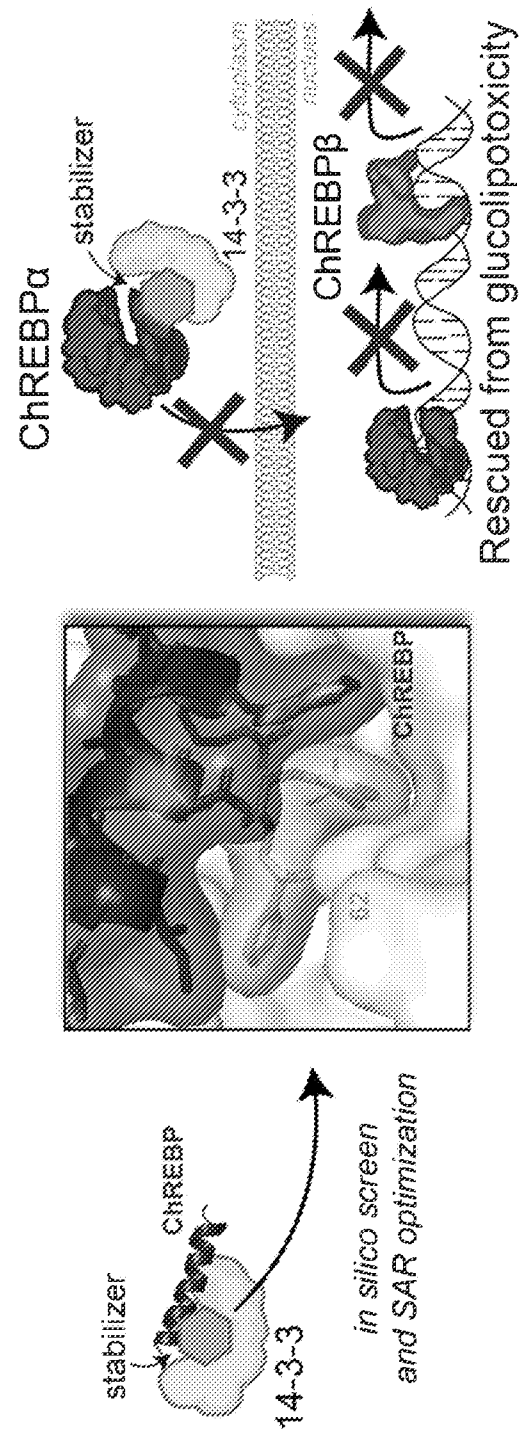


Fig. 1

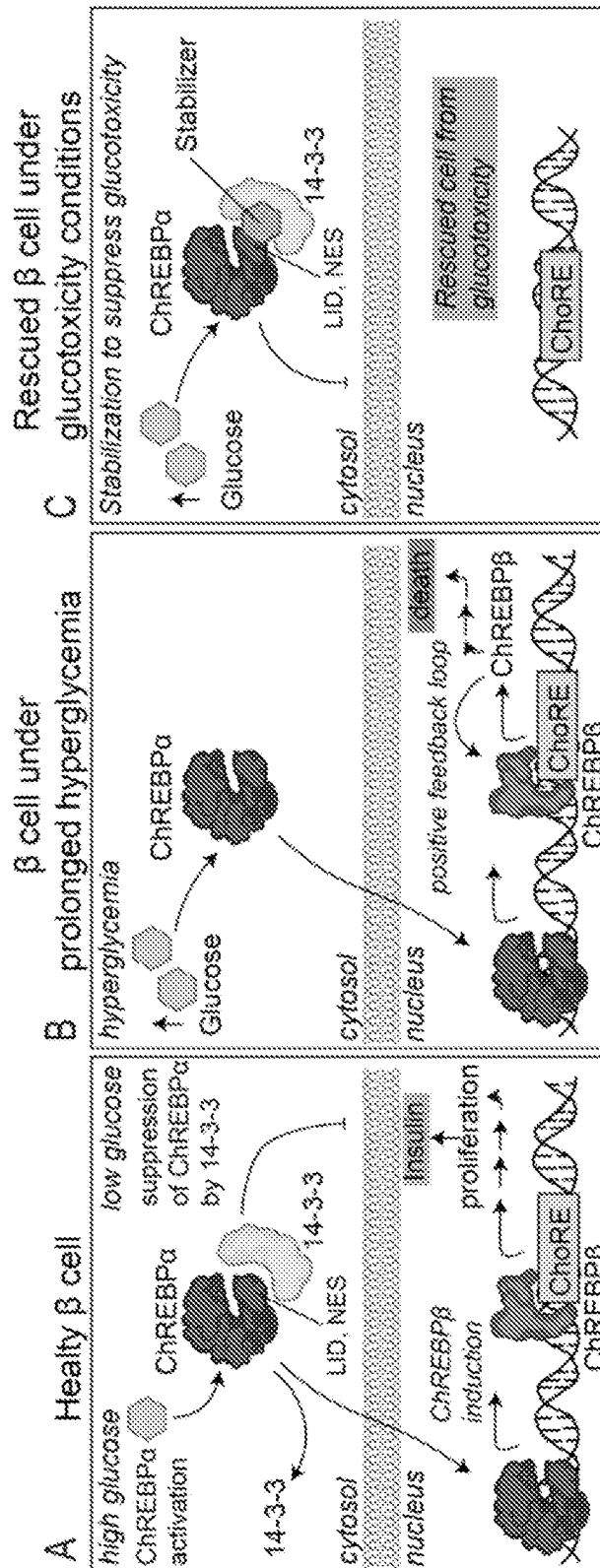


Fig. 2

3/41

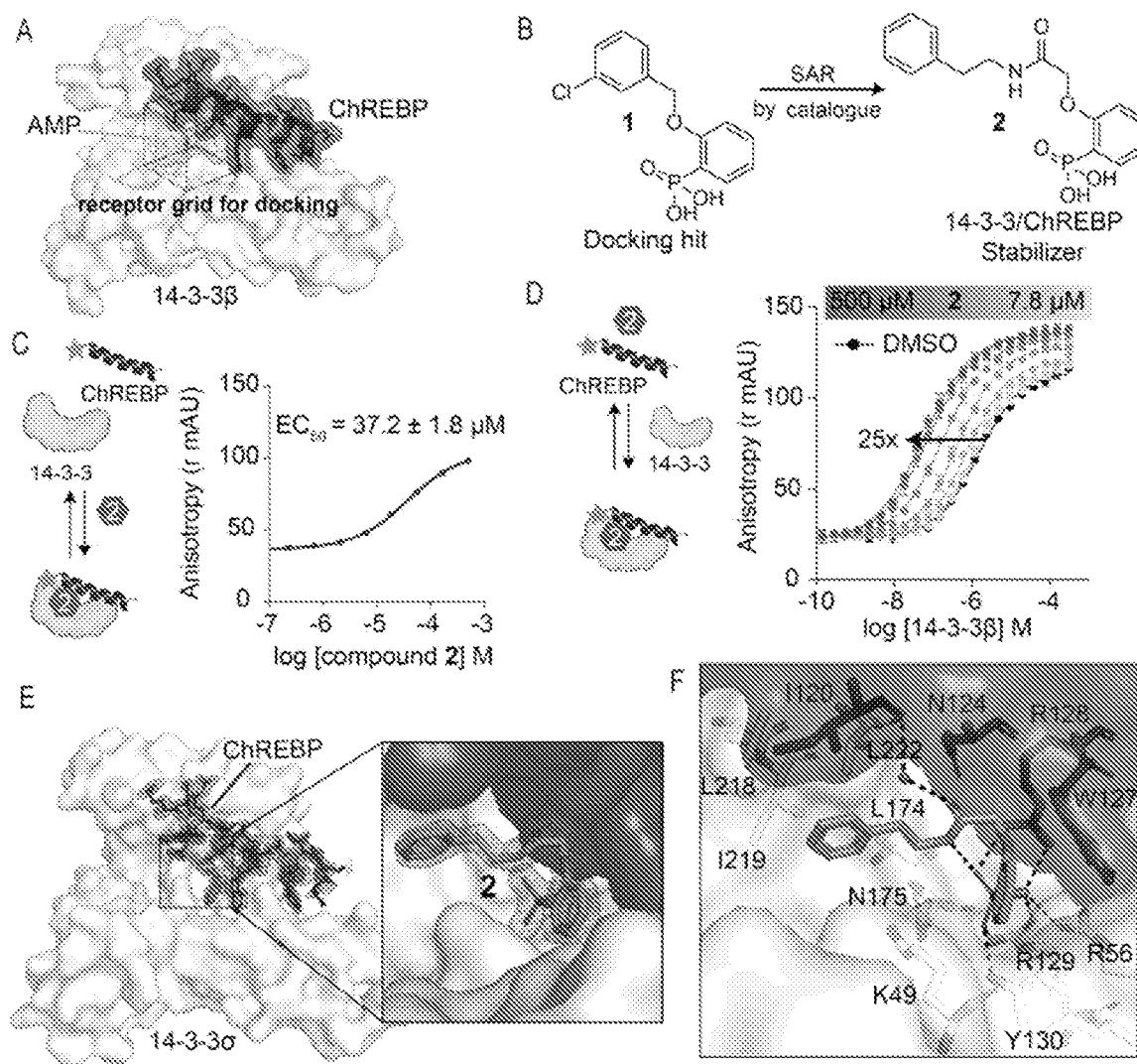


Fig. 3

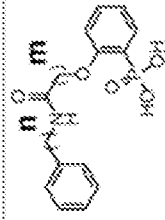
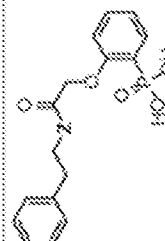
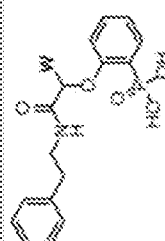
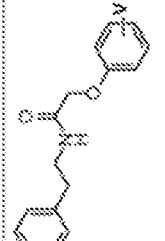
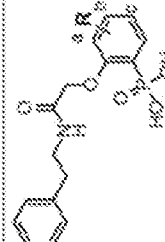
 $m=1$ 3* $n=0$ (inactive) 4* $n=1$ (17.4 ± 7.0) 2* $n=2$ (37.2 ± 1.8) 5* $n=3$ (92.3 ± 3.3) $m=2$ 6* $n=0$ (inactive) 7* $n=1$ (inactive) 8* $n=2$ (inactive) 9* $n=3$ (110 ± 48)	 10* $Z=CH_2$ (82.9 ± 9.7) 11* $Z=NMMe$ (46.1 ± 16) 12 $Z=O$ (inactive)	 13 $W=CH_3$ (inactive) 14 $W=F$ (125 ± 20) 15 $W=Ph$ (inactive)	 $V=PO(OH)_2$ $V=ortho-$ 2* o (37.2 ± 1.8) 21 $B(OH)_2$ (inactive) 16 m (123 ± 18) 22 SO_3NH_2 (inactive) 17 p (78 ± 3.5) $V=OPO(OH)_2$ $V=CH_2PO(OH)_2$ 18 o (24.3 ± 3.5) 23 o (inactive) 19 m (119 ± 43) 24 m (inactive) 20 p (17.4 ± 3.2) 25 p (inactive)	 $R=CH_3$ 26* a (54.4 ± 9.0) 27* b (27.5 ± 2.3) 28* c (66.9 ± 6.0) $R=F$ 29* a (51.9 ± 4.3) 30* b (68.1 ± 1.0) 31* c (69.4 ± 4.9)
---	---	--	--	--

Fig. 4

5/41

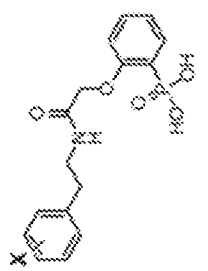
 32* <i>o</i> (25.4 ± 2.8) 33* <i>m</i> (24.1 ± 2.0) 34* <i>p</i> (20.9 ± 2.4) 35* <i>X</i> = <i>p</i>-Cl (inactive) 36* <i>X</i> = <i>p</i>-OH (inactive) 37* <i>X</i> = <i>p</i>-Br (inactive) 38* <i>o</i> (32.0 ± 3.9) 39* <i>m</i> (34.0 ± 2.8) 40* <i>p</i> (36.0 ± 2.6) 41* <i>o</i> (12.4 ± 0.5) 42* <i>m</i> (15.5 ± 1.5) 43* <i>p</i> (20.0 ± 0.9) 44 <i>N</i>-2 (7.6 ± 0.5) 45 <i>N</i>-3 (11.7 ± 1.9) 46 <i>N</i>-4 (17.5 ± 1.7)	47 (132 ± 31) 48 (16.1 ± 1.0) 49 (17.8 ± 0.9) 50 (inactive) 51 (inactive) 52 (inactive) 53 (inactive)
54 <i>S</i> Δ <i>R</i> (17.6 ± 1.7) 55 <i>R</i> Δ <i>S</i> (70.0 ± 9.9) 56 <i>R</i> Δ <i>R</i> (inactive) 57 (50.0 ± 7.6) 58 <i>R</i> Δ <i>R</i> (inactive)	59* (<i>S</i>) (25.3 ± 3.0) 60* (<i>R</i>) (35.9 ± 5.0)
61 <i>Y</i> = CH₃ (48.4 ± 7.7) 62 <i>Y</i> = F (5.8 ± 0.4)	61 <i>Y</i> = CH₃ (48.4 ± 7.7) 62 <i>Y</i> = F (5.8 ± 0.4)

Fig. 4 Continued

6/41

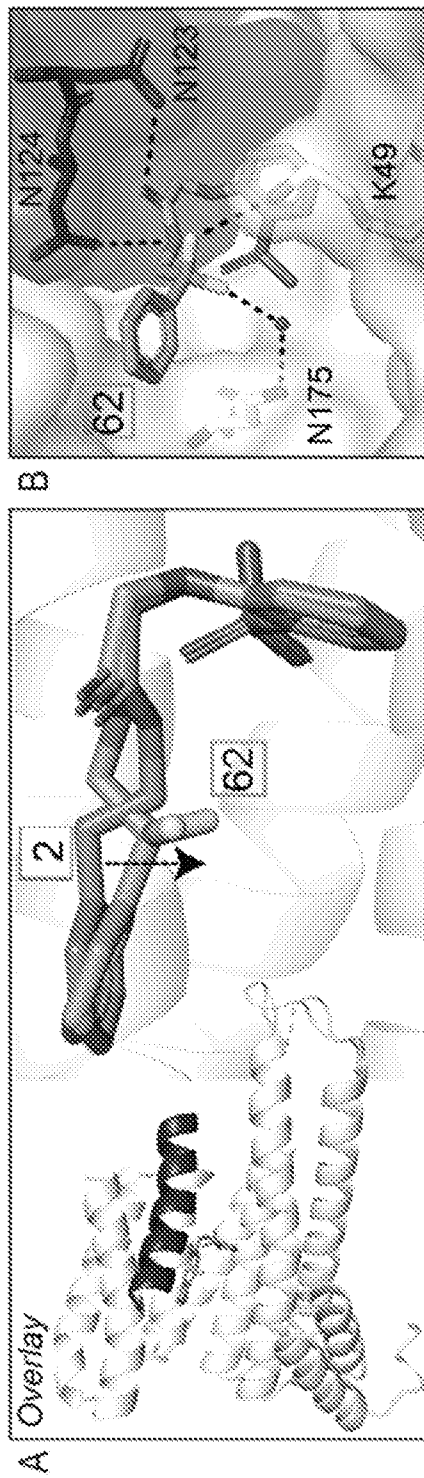


Fig. 5

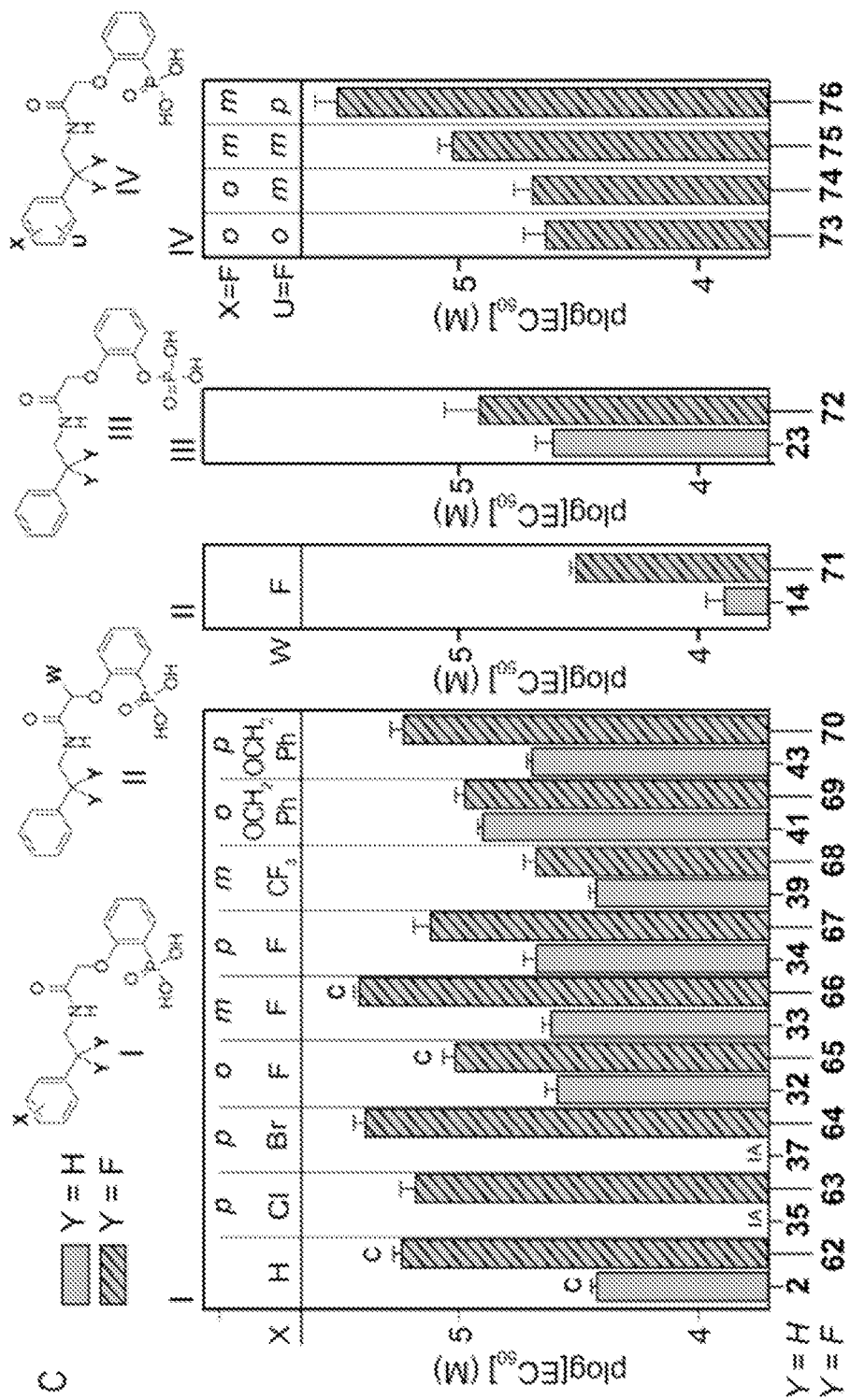


Fig. 5 Continued

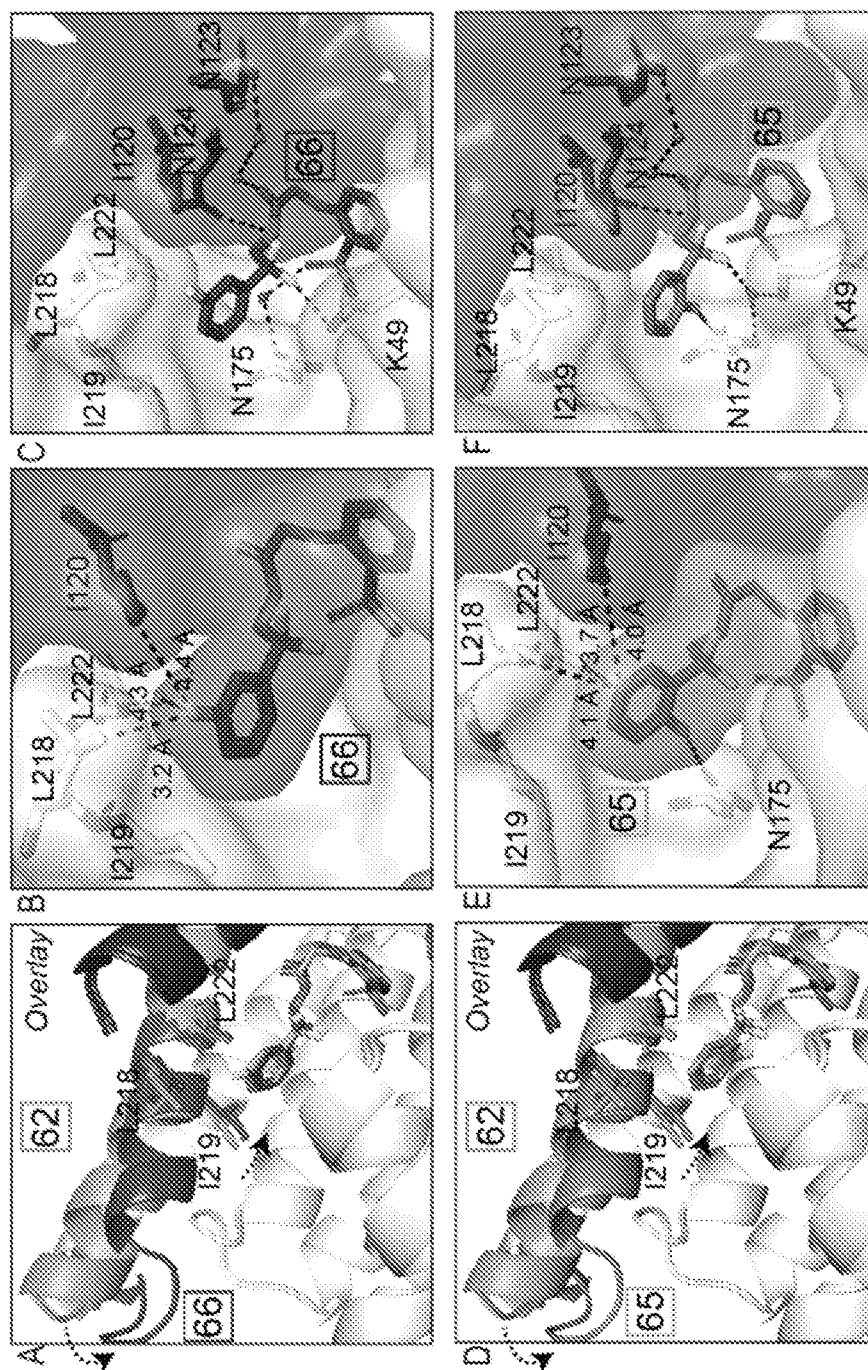


Fig. 6

9/41

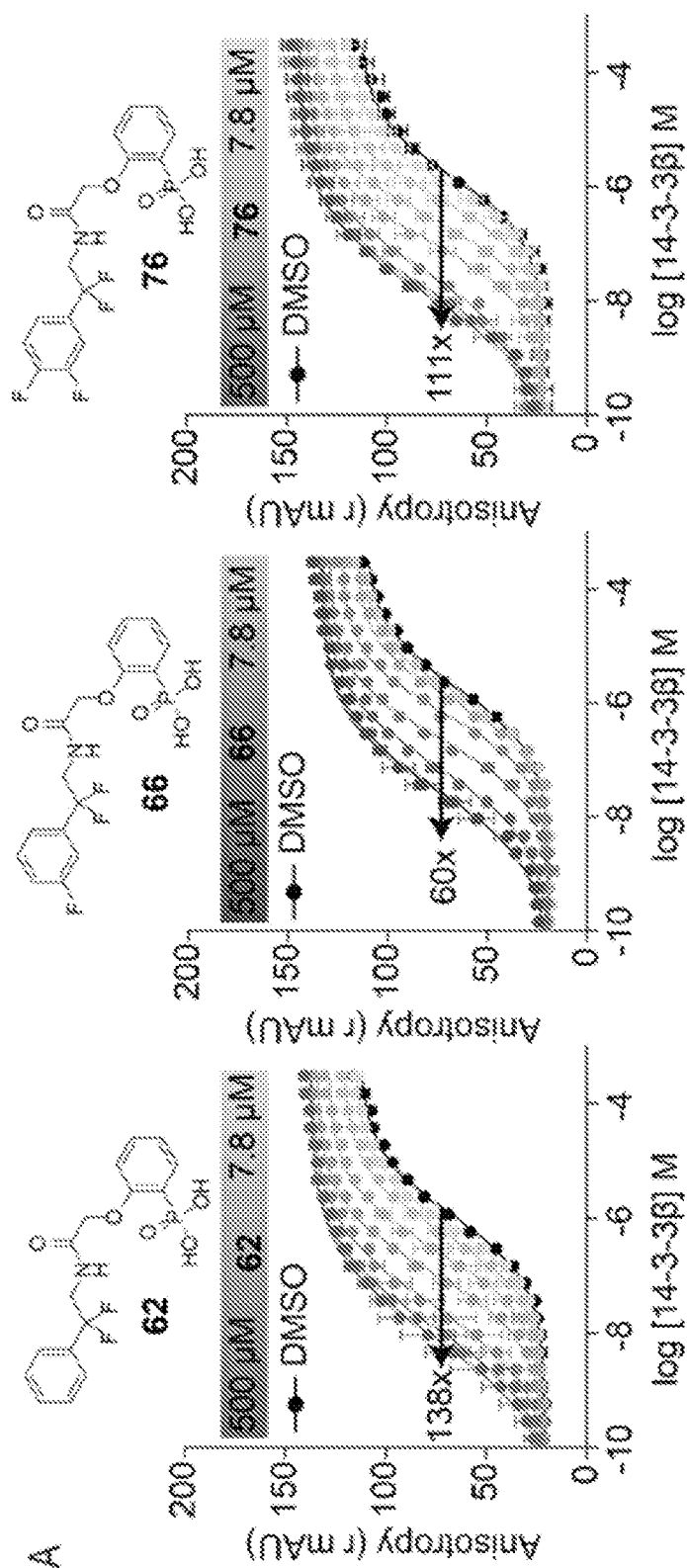


Fig. 7

10/41

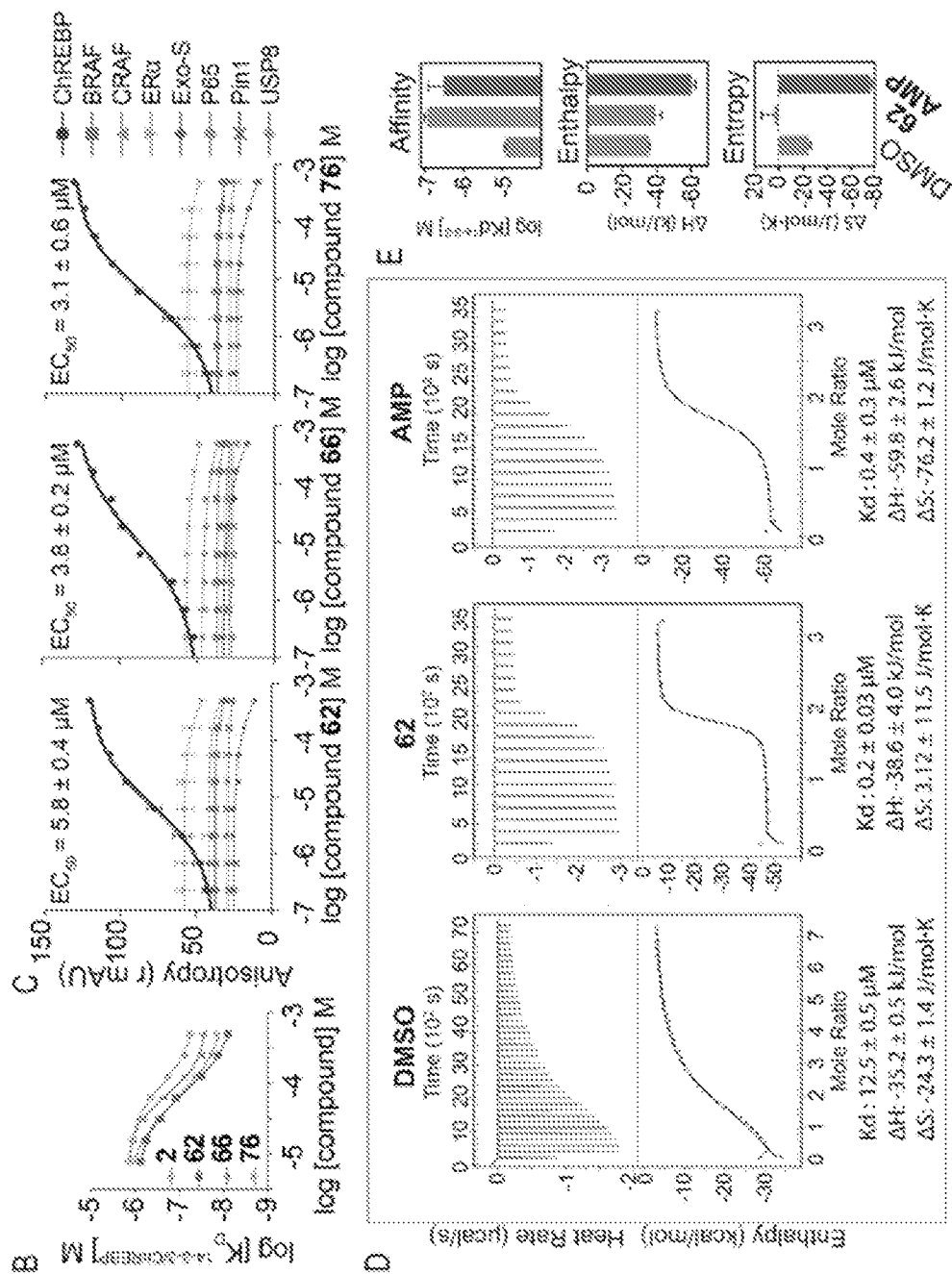


Fig. 7 Continued

11/41

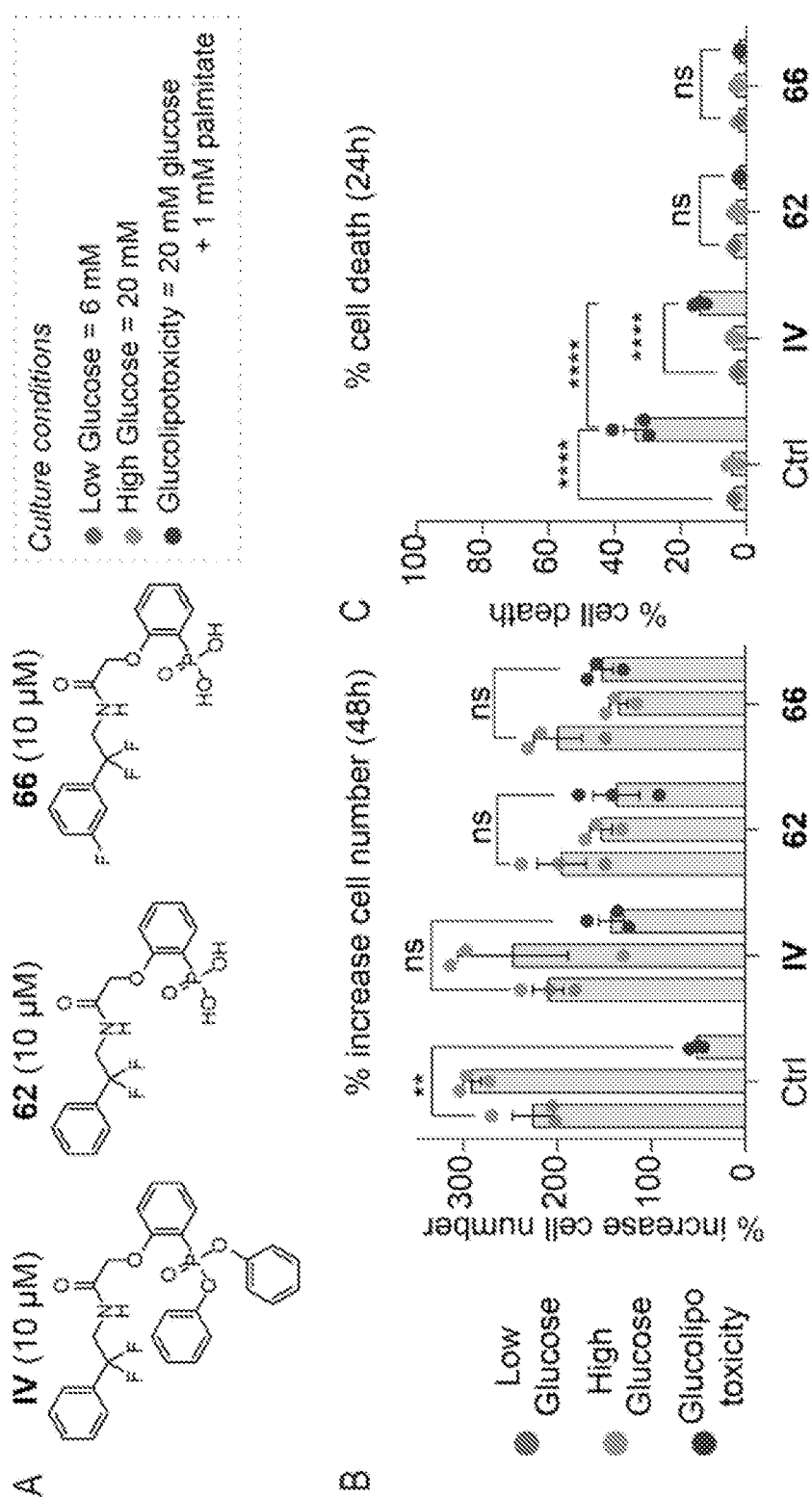


Fig. 8

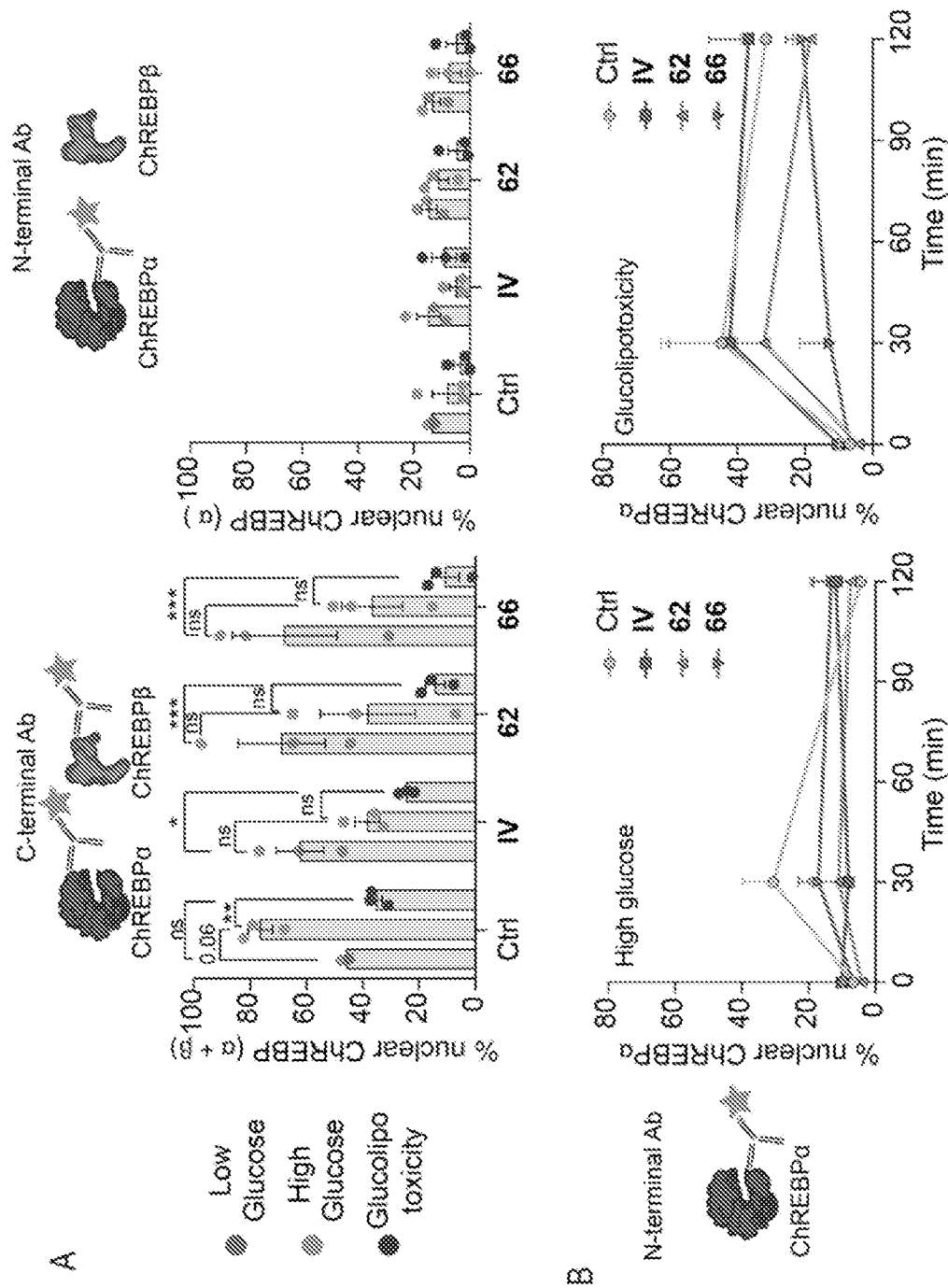


Fig. 9

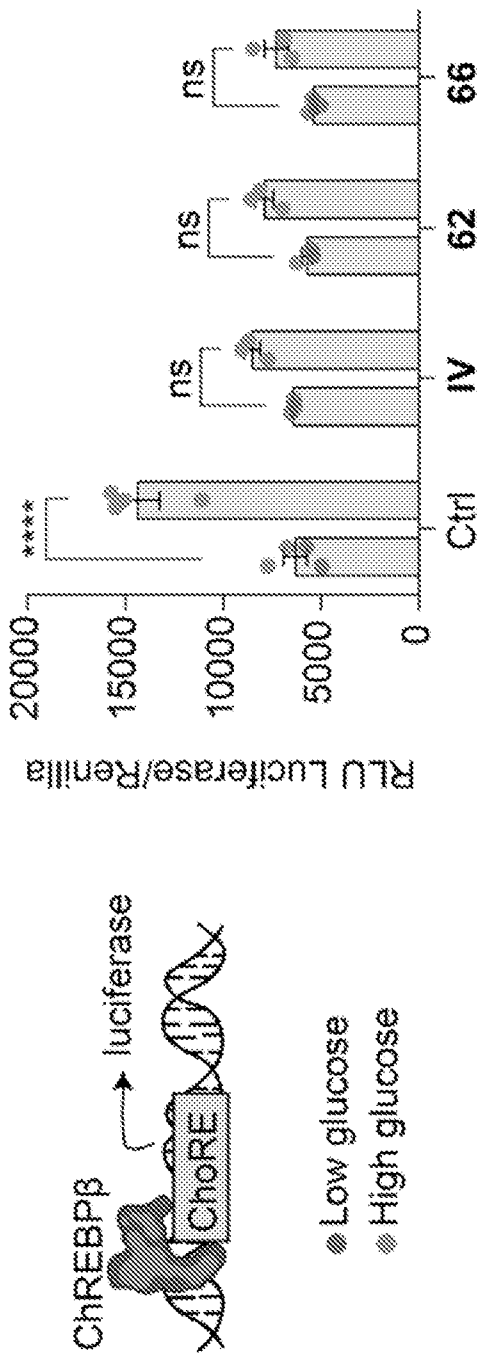


Fig. 10

14/41

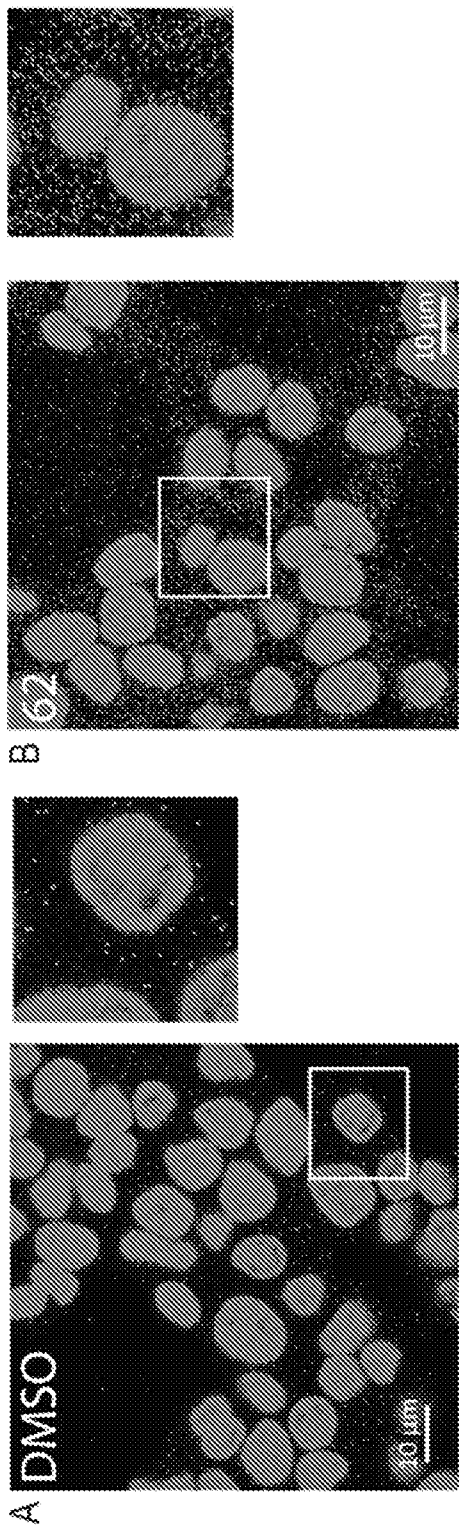


Fig. 11

15/41

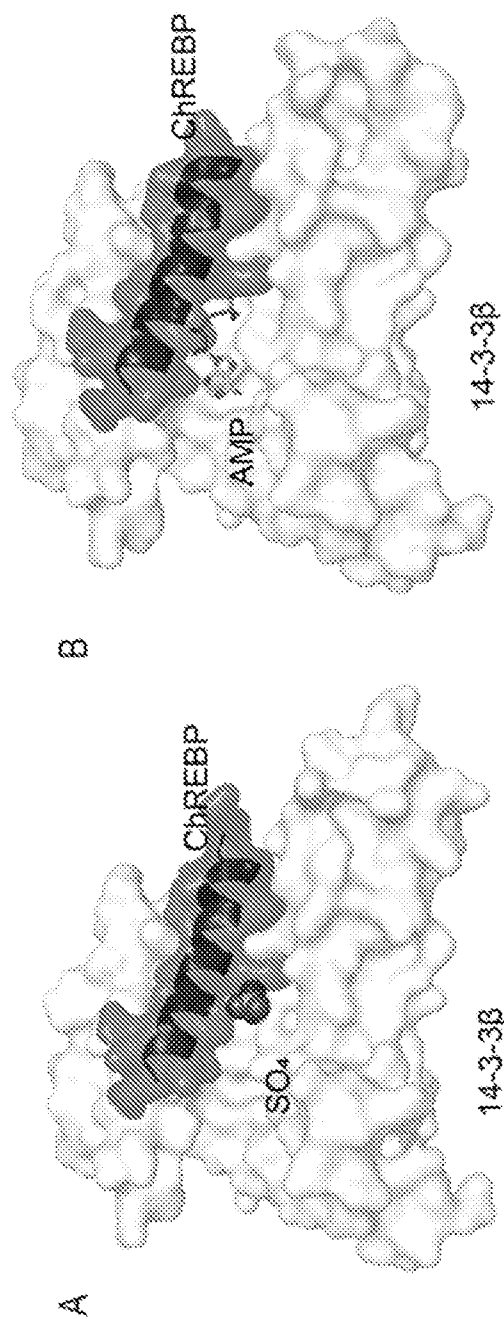


Fig. 12

16/41

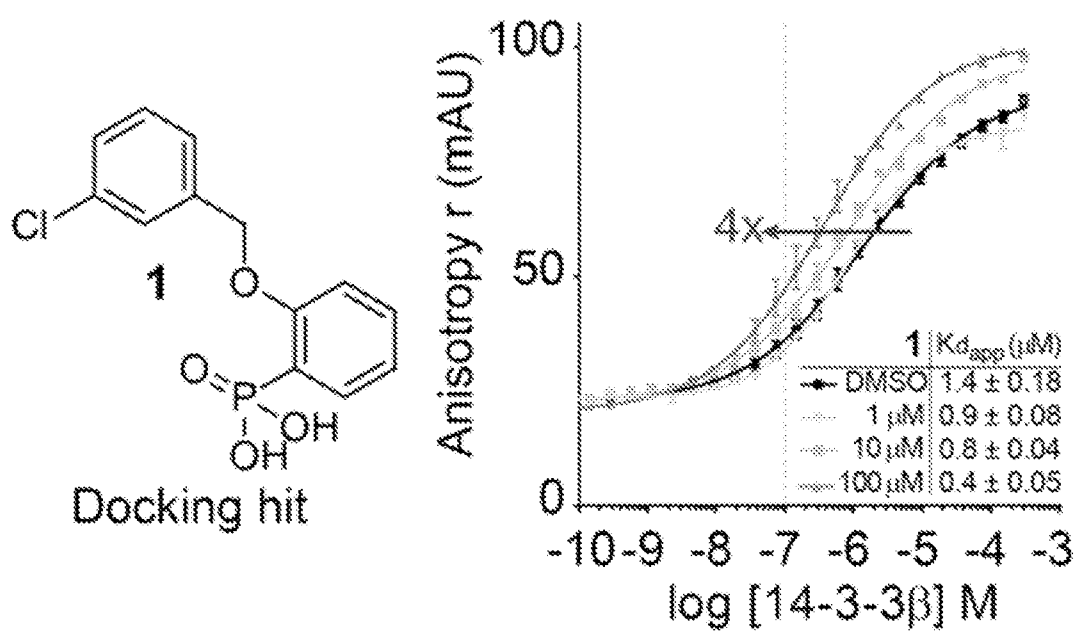


Fig. 13

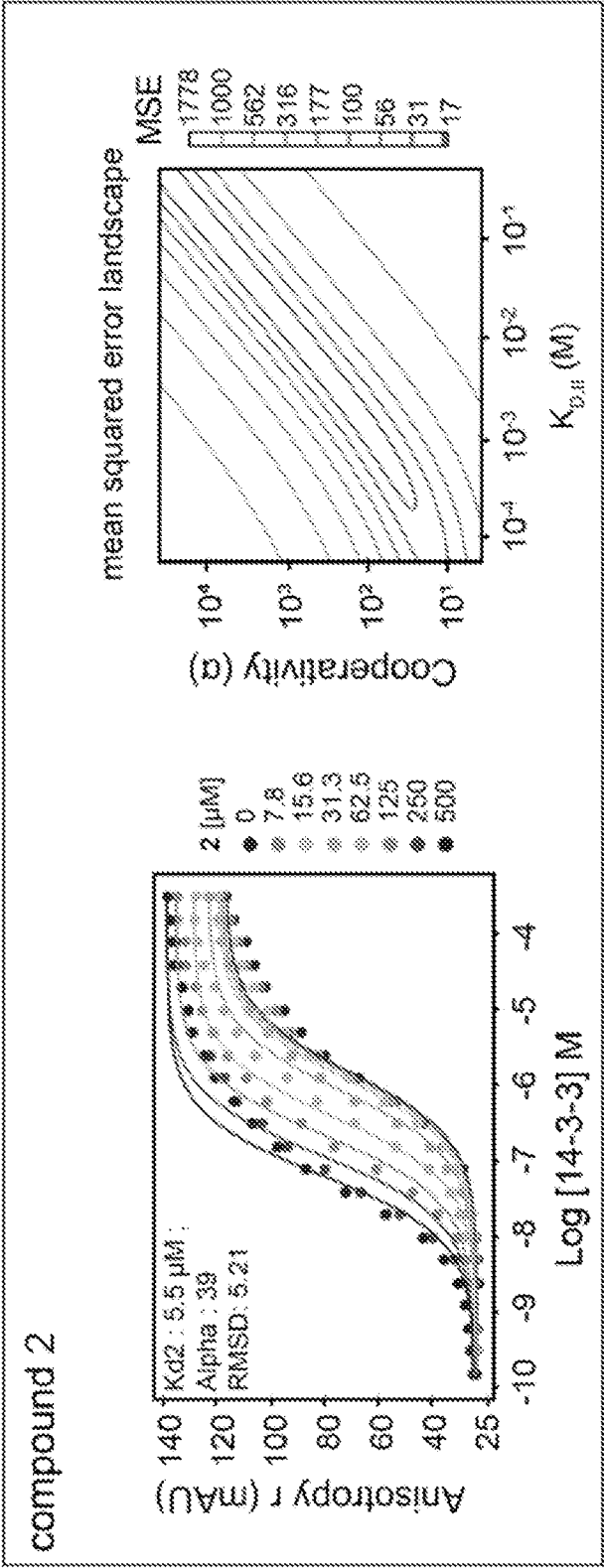


Fig. 14

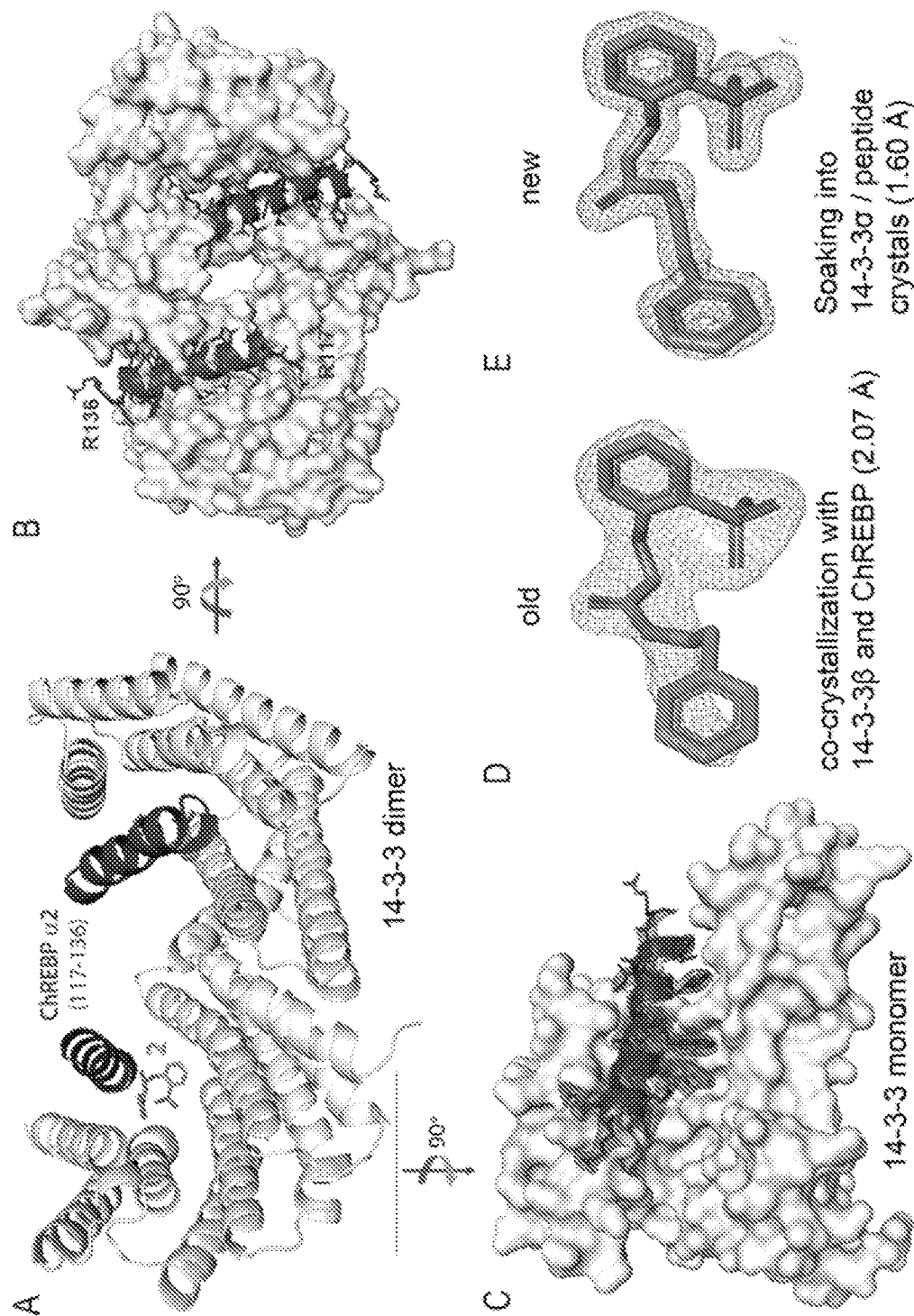


Fig. 15

19/41

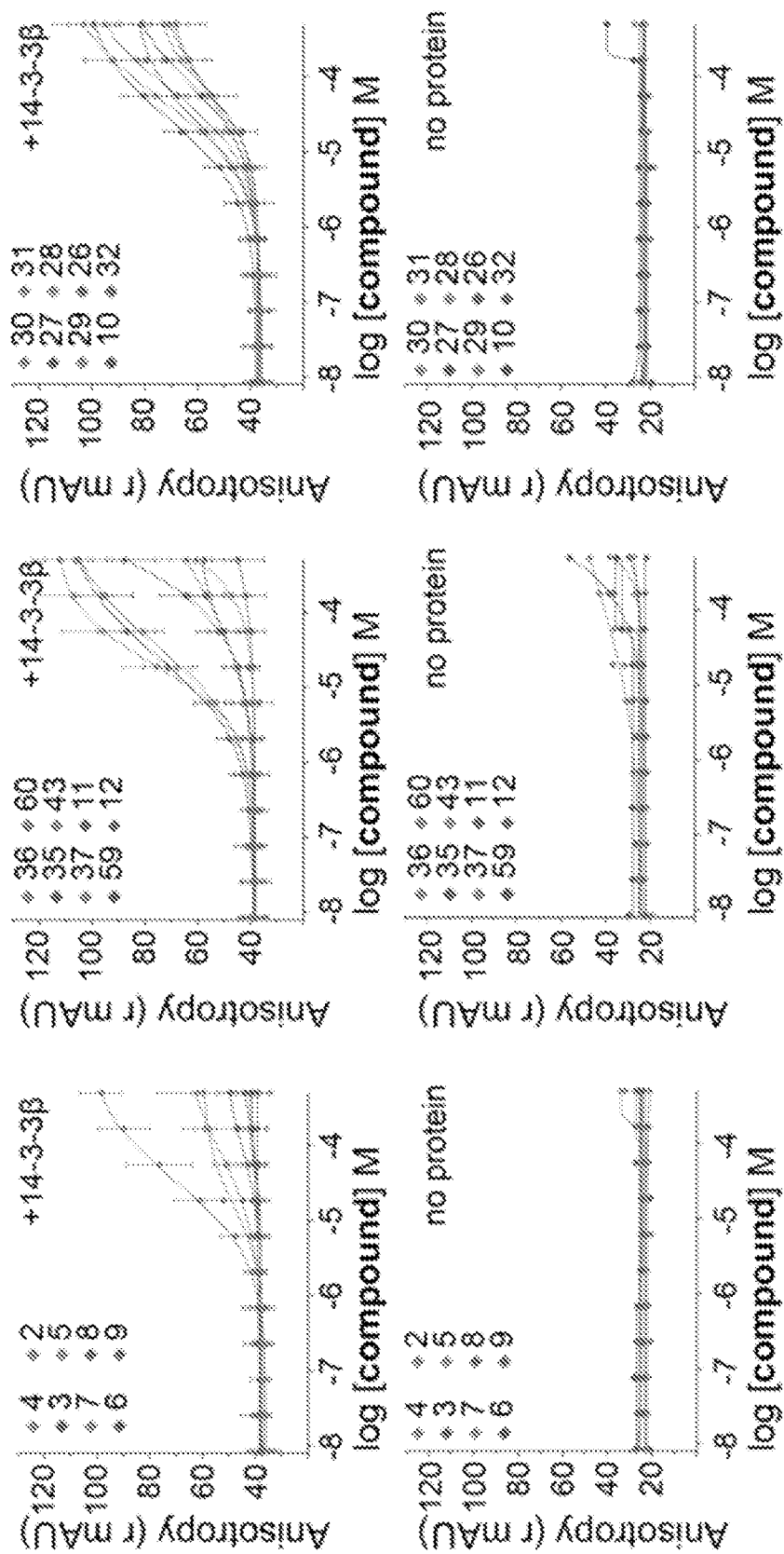


Fig. 16A

20/41

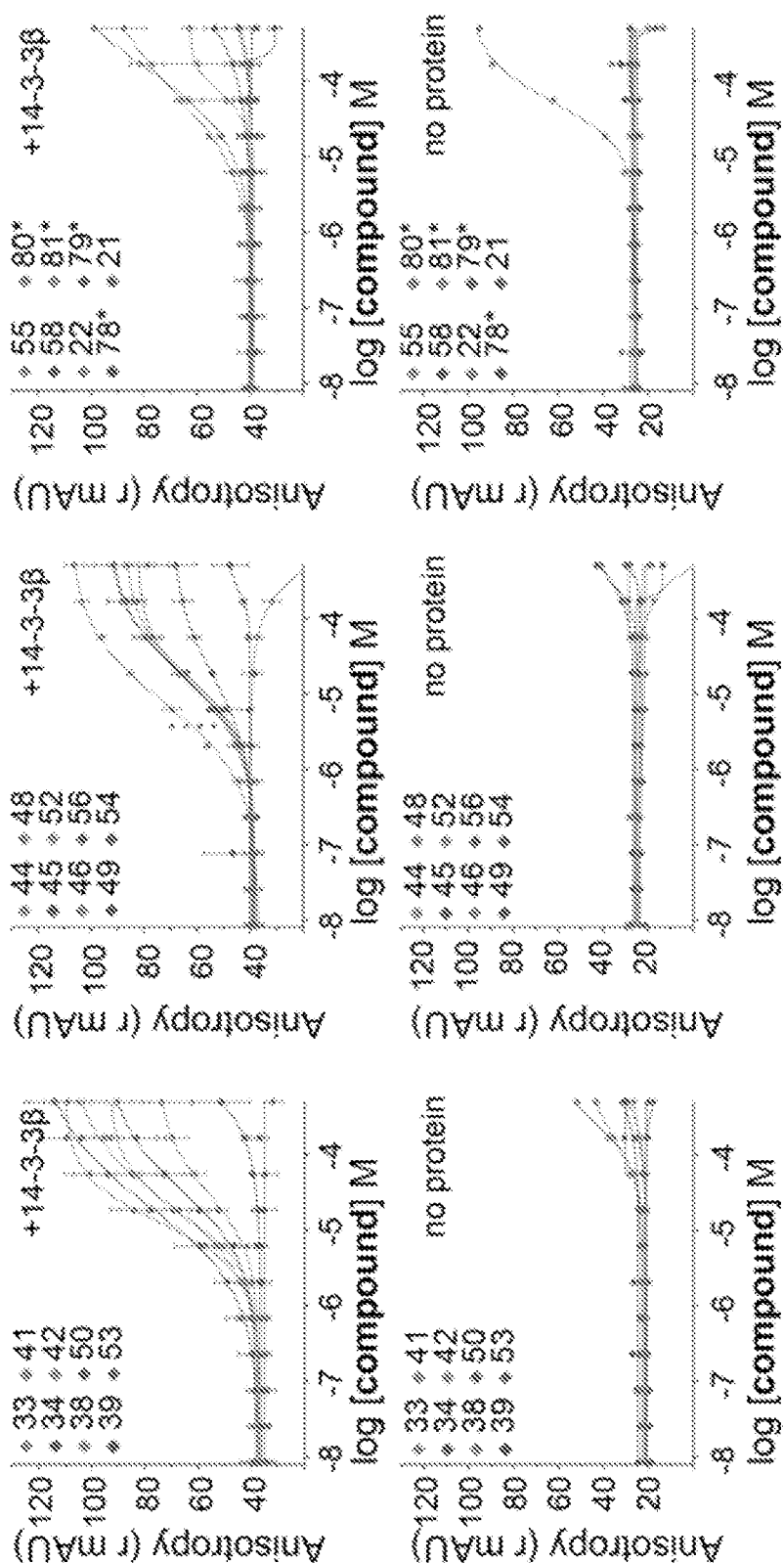


Fig. 16A Continued

21/41

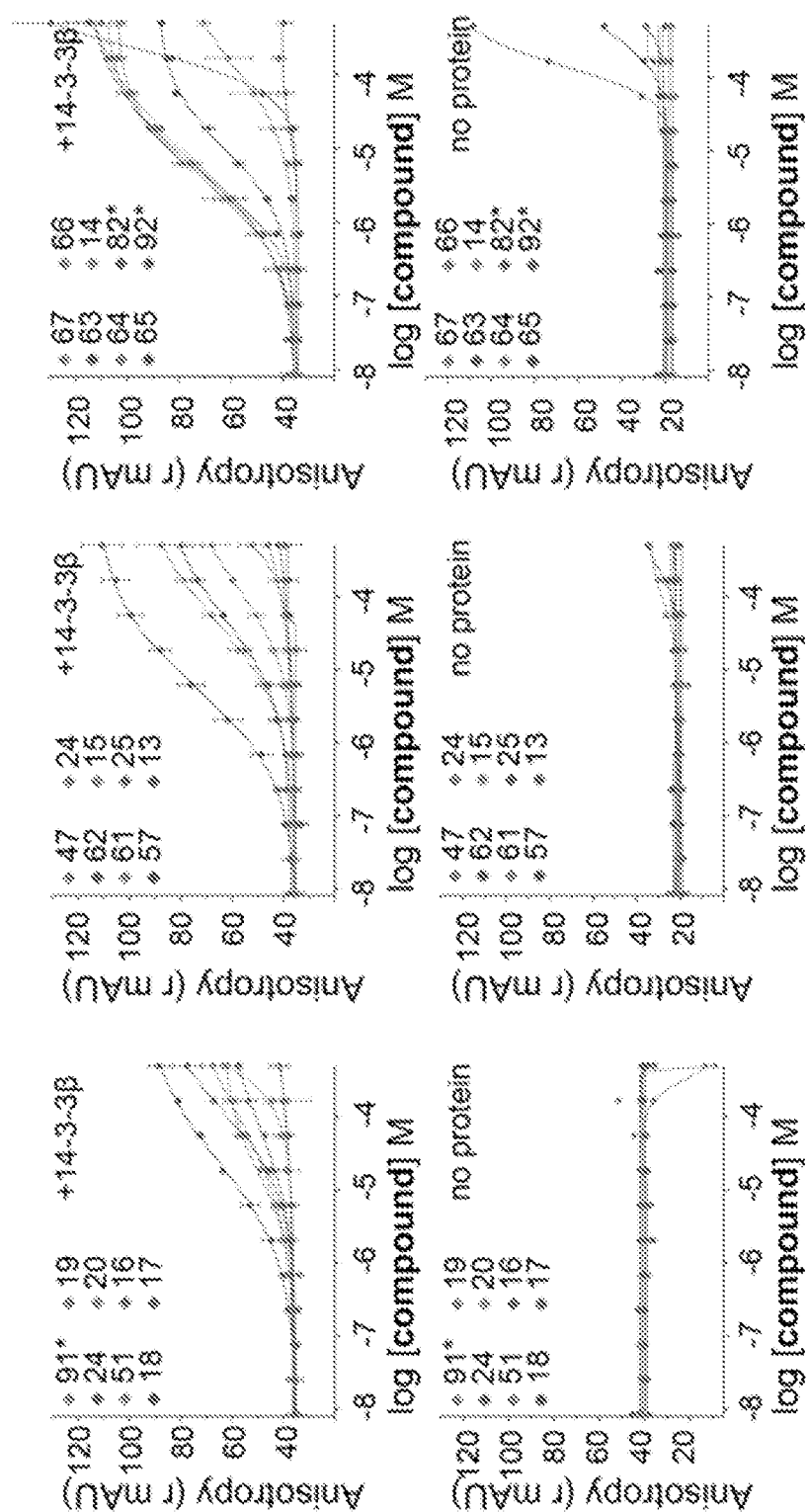


Fig. 16B

22/41

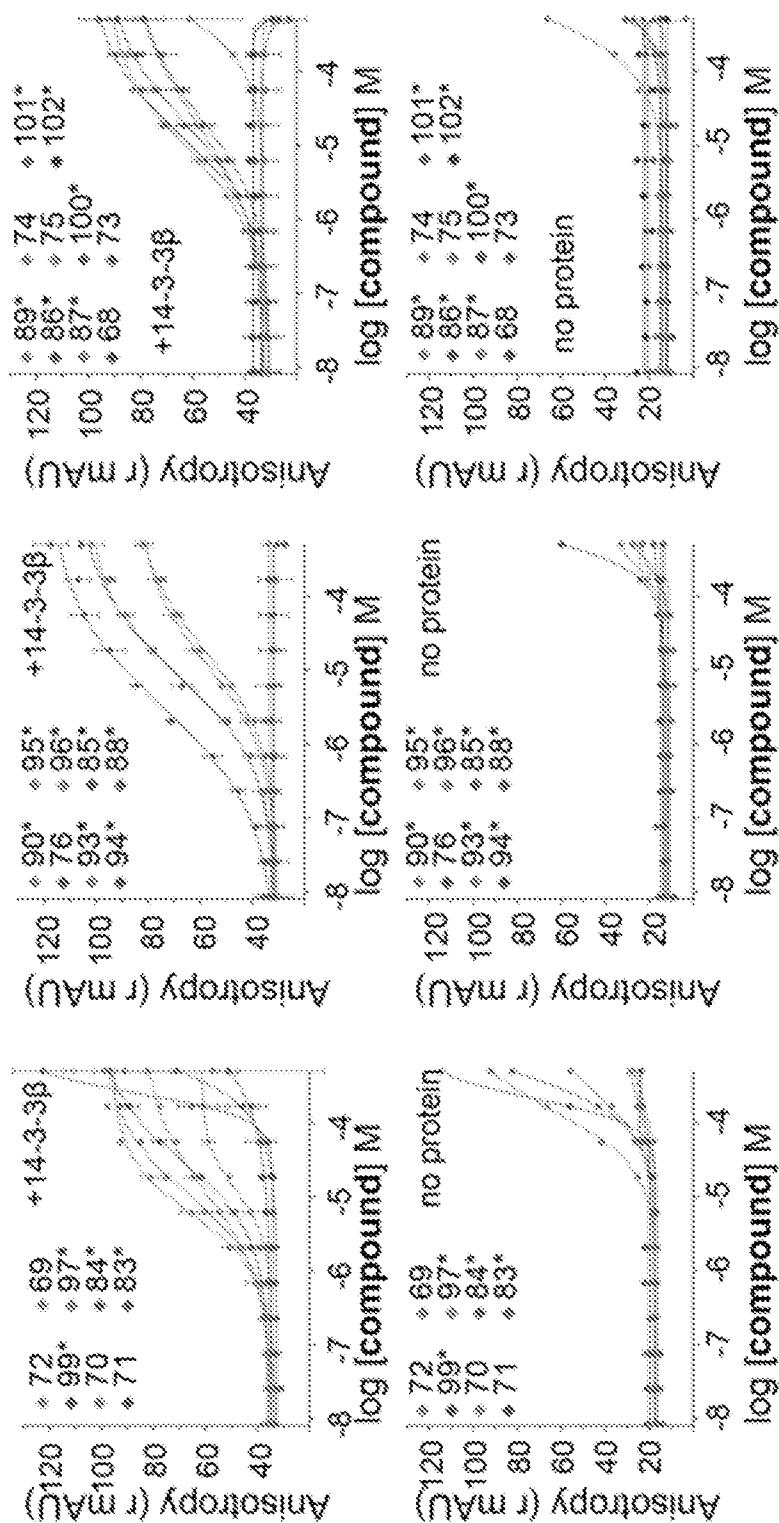


Fig. 16B Continued

23/41

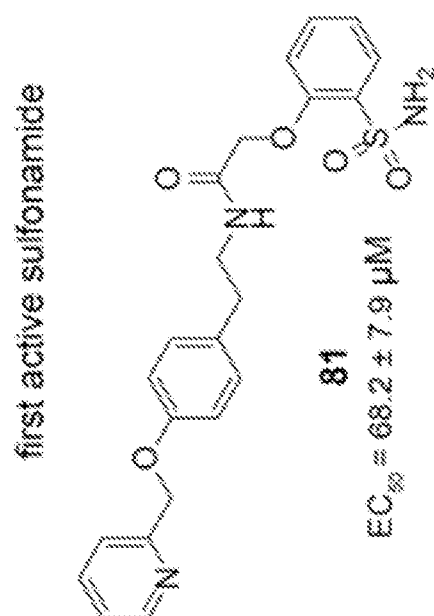
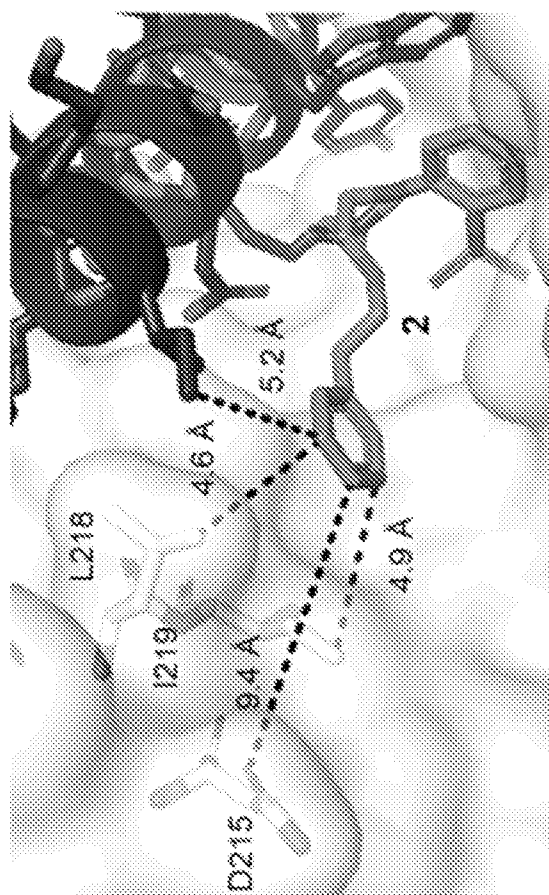


Fig. 17

24/41

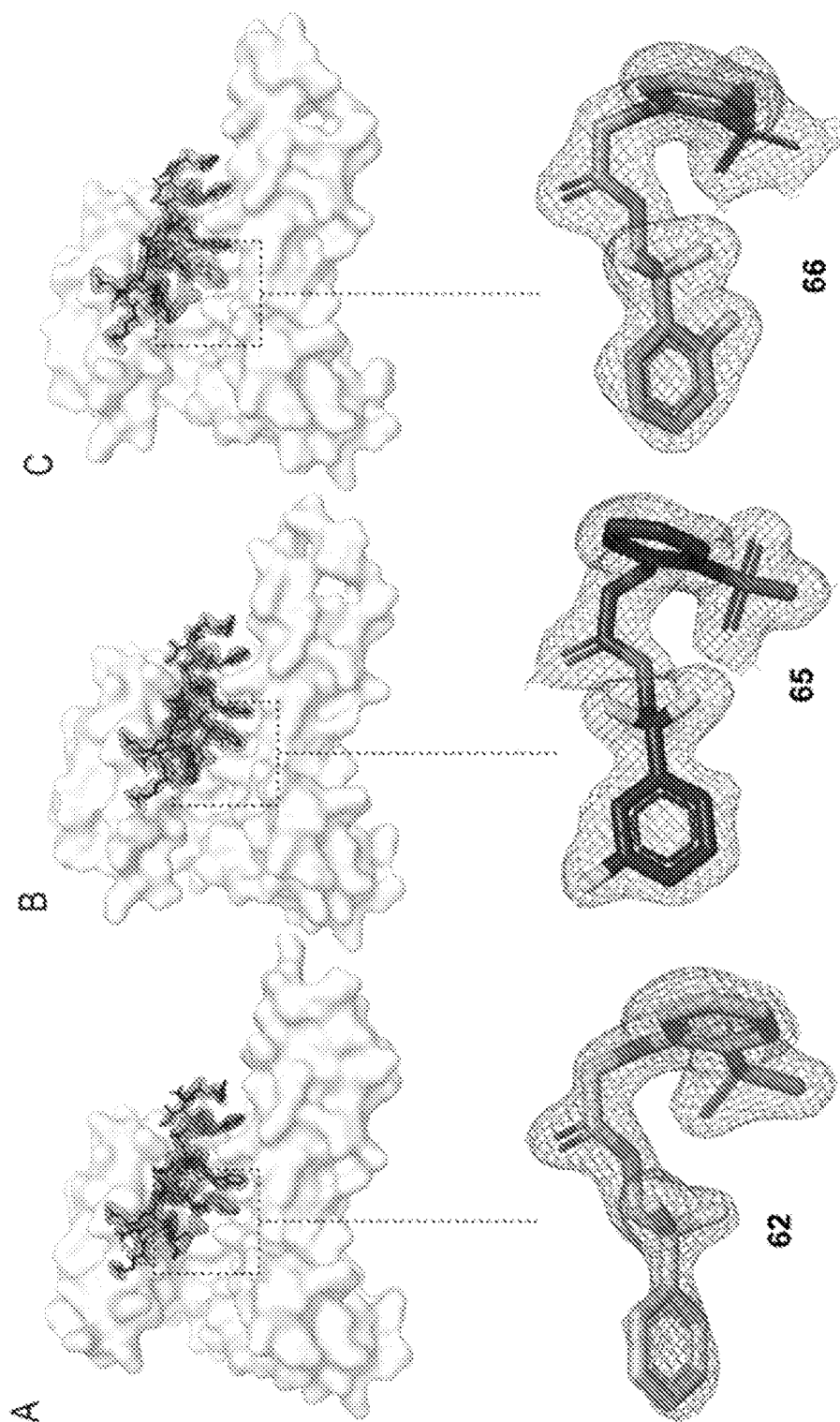


Fig. 18

25/41

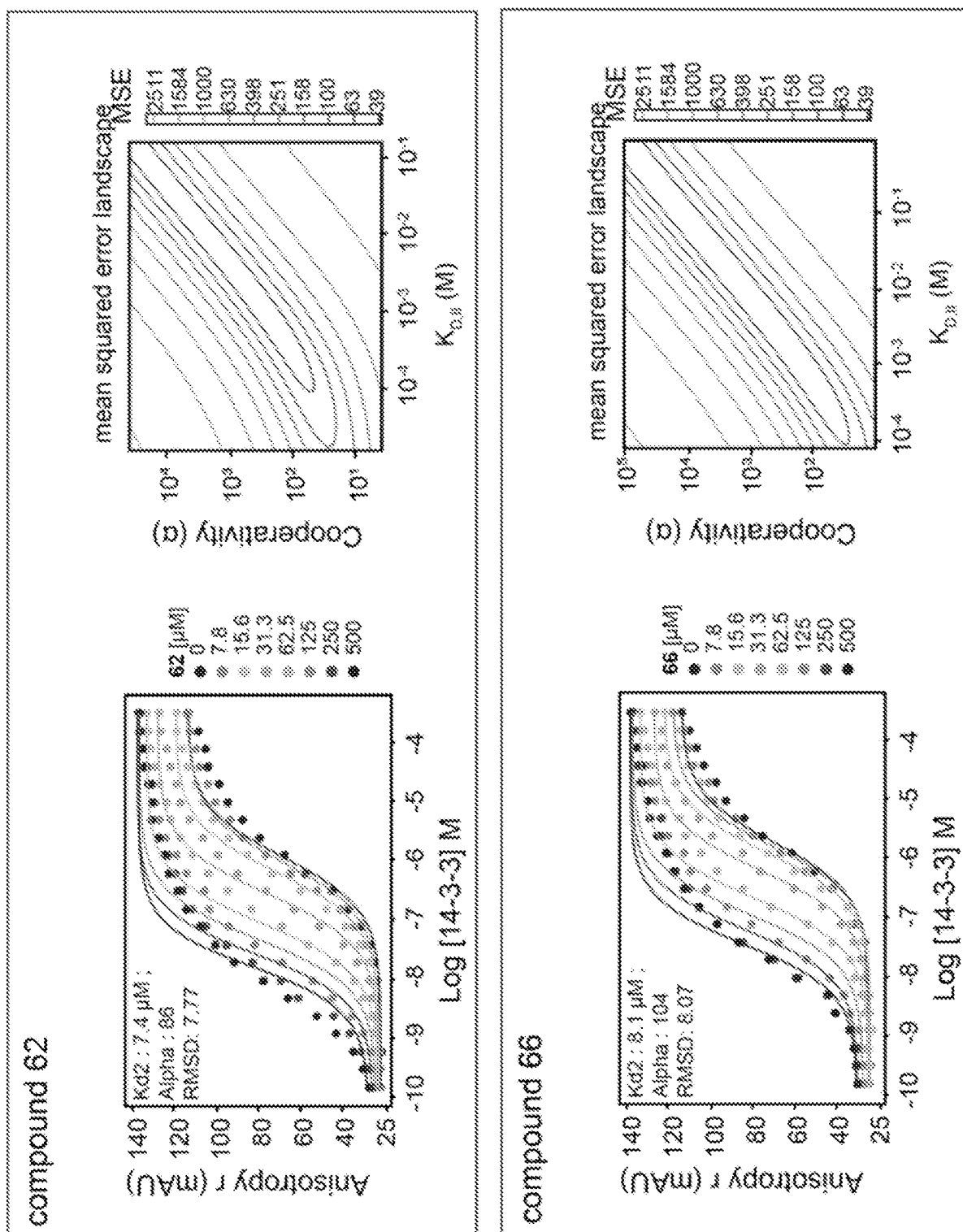


Fig. 19

26/41

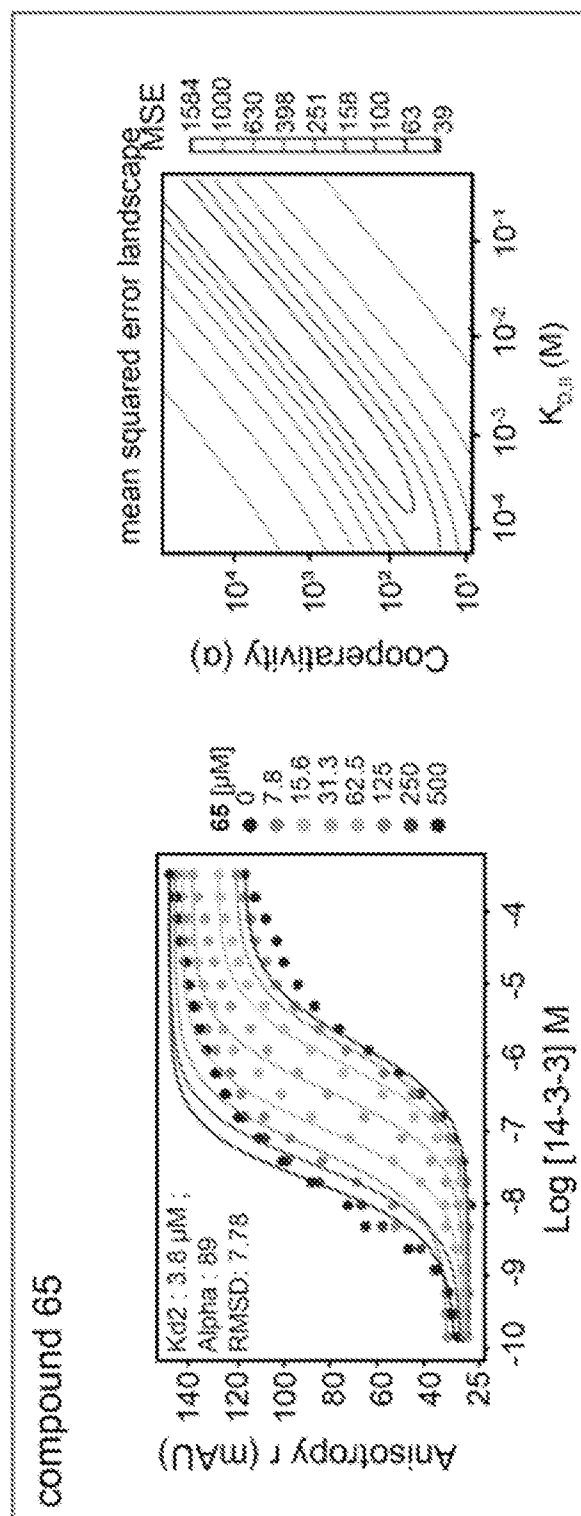


Fig. 19 Continued

27/41

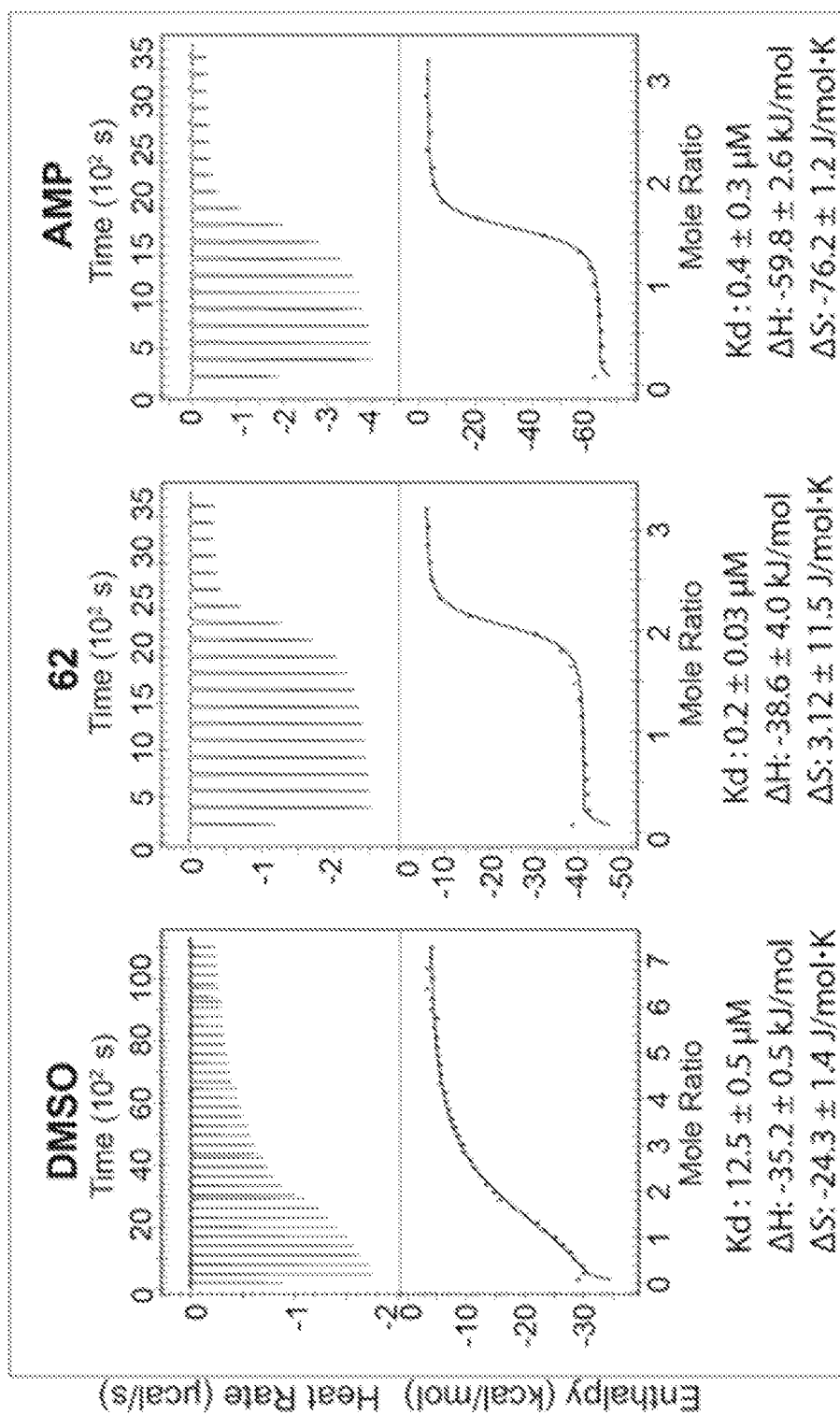


Fig. 20

28/41

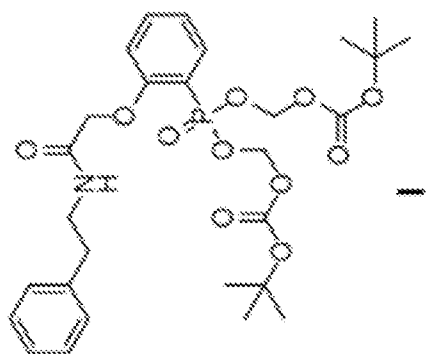
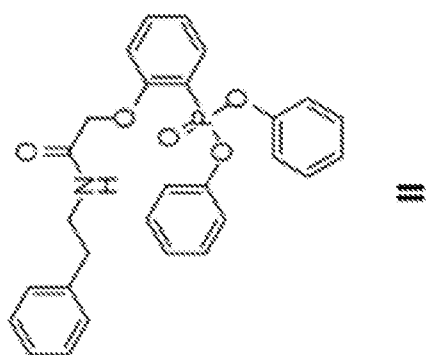
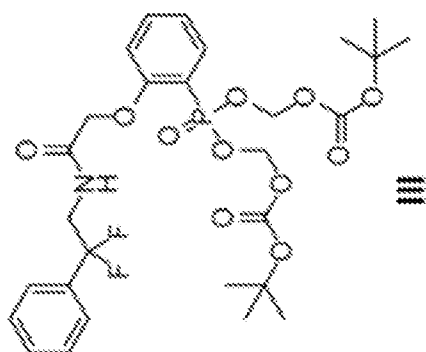
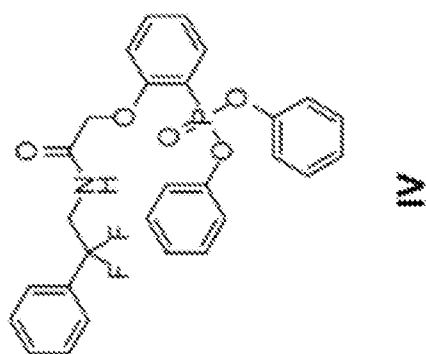


Fig. 21

29/41

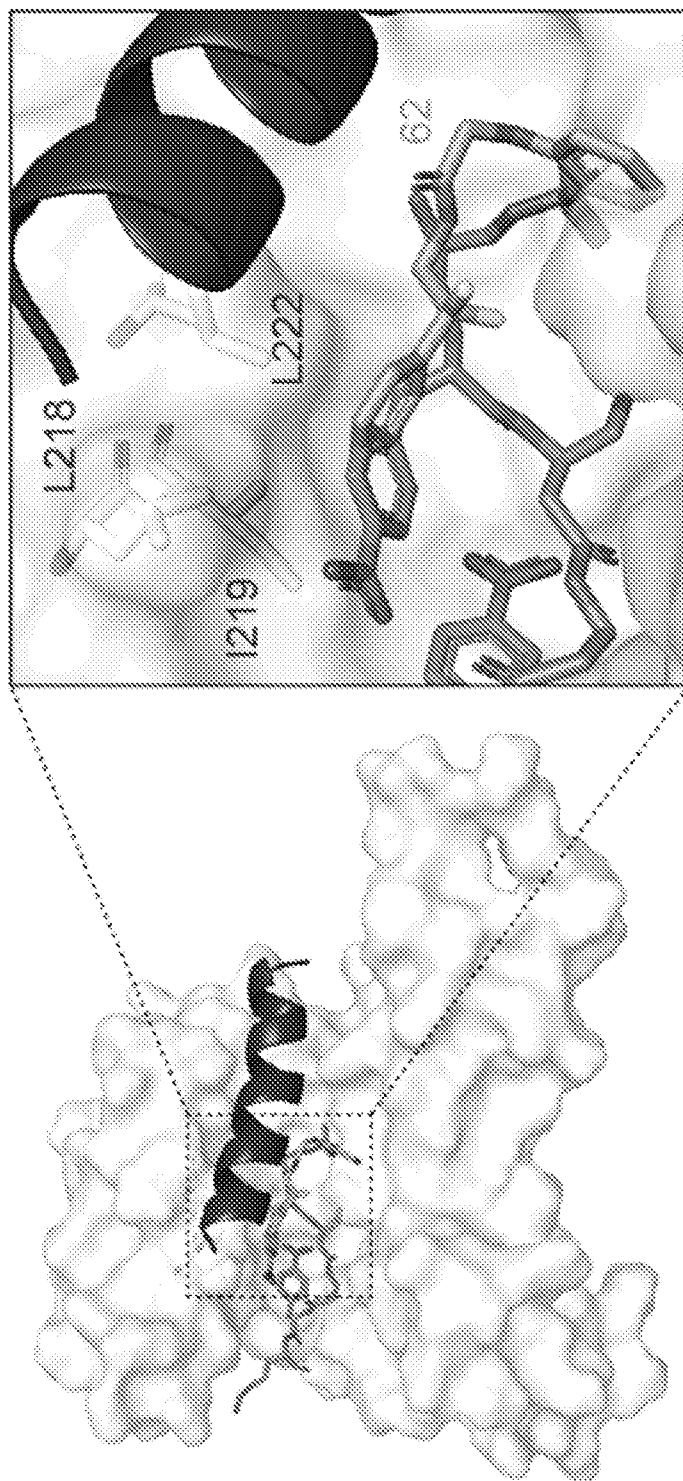


Fig. 22

30/41

 91 <i>p</i>-F (inactive)	 92 (inactive)
 87 <i>o,o</i> (inactive) 88 <i>o,m</i> (inactive) 89 <i>m,m</i> (inactive) 90 <i>m,p</i> (inactive)	X=F, U=F
 82 H (inactive) 83 <i>m</i>-F (inactive) 84 <i>p</i>-F (inactive) 85 <i>m</i>-CF₃ (inactive) 86 <i>m</i>-OCF₃ (inactive)	X=
 78 <i>p</i>-F (inactive) 79 <i>o</i>-OCH₂Ph (inactive) 80 <i>p</i>-OCH₂Ph (inactive) 81 <i>p</i>-OCH₂Pyr (N-2) (68.2 ± 7.9)	X=

Fig. 23

31/41

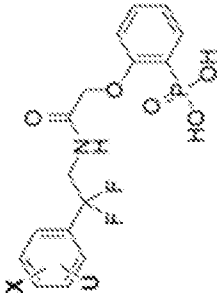
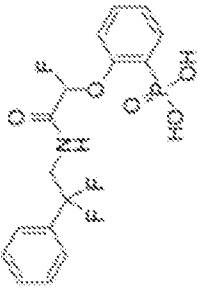
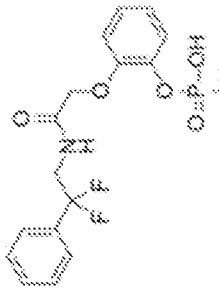
 62 X = H (5.7 ± 0.4)	 71 (30.3 ± 1.3)	X = F 65 o (9.6 ± 1.1) 66 m (3.8 ± 0.2) 67 p (7.5 ± 1.1) X = CF₃ 68 m (20.8 ± 2.3) X = OCH₂Ph 69 o (10.6 ± 1.0) 70 p (5.9 ± 0.7) 97* o-OCH₂Pyr (N-2) (11.0 ± 1.5)
X = F U = F 73 o,o (22.7 ± 4.2) 74 o,m (20.0 ± 3.2) 75 m,m (9.3 ± 1.1) 76 m,p (3.1 ± 0.6)	 72 (12.1 ± 3.4)	X = Cl 93* o (12.0 ± 1.3) 94* m (9.3 ± 1.9) 63 p (6.6 ± 0.9) X = Br 95* o (18.5 ± 3.4) 96* m (7.6 ± 0.9) 64 p (4.1 ± 0.4)

Fig. 24

32/41

14-3-3σAC/ ChREBP	2	62	66	65
PDB ID	8BTQ	8C1Y	8BWH	8BWE
Data collection				
Collection source	DESY Petra III	DESY Petra III	ESRF ID23-2	ESRF ID23-2
Collection date	04-11-2020	09-08-2020	29-01-2021	29-01-2021
Wavelength (Å)	1.033220	1.033220	8.873128	0.873128
Resolution (Å) ^a	1.60 – 45.36 (1.60 – 1.63)	1.80 – 45.47 (1.80 – 1.84)	2.00 – 46.41 (2.00 – 2.05)	2.10 – 34.64 (2.10 – 2.16)
Space group	C 2 2 2 1	C 2 2 2 1	C 2 2 2	C 2 2 2
Unit cell	81.979 111.932 62.339	81.626 111.651 62.827	63.316 147.471 76.968	63.166 147.336 77.070
Total reflections ^a	493511 (21933)	351860 (22348)	89504 (6796)	88577 (7393)
Unique reflections ^a	38129 (1837)	26659 (1571)	20127 (1550)	20407 (1695)
Redundancy ^a	12.9 (11.9)	13.2 (14.2)	4.4 (4.4)	4.3 (4.4)
Completeness (%) ^a	99.9 (99.6)	98.9 (98.6)	82.2 (85.8)	96.7 (99.2)
Average I/σ(I) ^a	26.0 (5.7)	18.8 (1.5)	9.0 (1.5)	7.2 (1.6)
Wilson B-factor (Å ²)	17.20	30.70	24.54	28.57
CC _{1/2} ^{a,b}	0.999 (0.959)	0.999 (0.635)	0.992 (0.500)	0.985 (0.613)
R _{merge} ^{a,c,e}	0.066 (0.475)	0.085 (1.854)	0.127 (0.936)	0.114 (0.717)
R _{meas} ^{a,d,e}	0.071 (0.519)	0.094 (1.994)	0.154 (1.113)	0.145 (0.894)
Refinement				
Reflections in set:	38106 (3734) /	26651 (2628) /	20125 (2070) /	20356 (2068) /
Refinement / R-free ^a	1856 (154)	1329 (125)	961 (83)	1058 (104)
Non-H atoms:	2246 / 222	2159 / 107	2180 / 124	2192 / 136
Overall / solvent				
R _{work} / R _{free} ^a	0.2027 (0.2168) / 0.2307 (0.2529)	0.2180 (0.3728) / 0.2469 (0.3932)	0.2477 (0.3136) / 0.2777 (0.3435)	0.2231 (0.2782) / 1058 (104)
RMSD from ideal geometry:				
Bond length (Å) / angles (°)	0.014 / 1.33	0.007 / 0.94	0.015 / 1.36	0.007 / 0.88
Average protein B-factor (Å ²)	16.68	30.86	19.80	39.03
Ramachandran:	98.77 / 0.00	98.80 / 0.00	96.40 / 0.40	96.80 / 0.93
Favored / outlier (%)				
Clashscore	2.50	0.99	3.45	0.49

^a Number in parentheses is for the highest resolution shell used in the refinement.

^b CC_{1/2} = Pearson's intra-dataset correlation coefficient, as described by Karplus and Diederichs.⁸³

^c R_{merge} (= R_{sym}) = $\sum_h \sum_i |I_{hi} - \langle I_h \rangle| / \sum_h \sum_i \langle I_h \rangle$, where I_{hi} is the intensity of the 1st observation of reflection h and $\langle I_h \rangle$ is the average intensity of reflection h

^d R_{meas} = $\sum_h \sqrt{(n_h/(n_h - 1)) \sum_i |I_{hi} - \langle I_h \rangle| / \sum_h \sum_i \langle I_h \rangle}$ where n_h is the number of observations of reflection h

^e Correlation of experimental intensities with intensities calculated from refined model, as described by Karplus and Diederichs.⁸³

Fig. 25

33/41

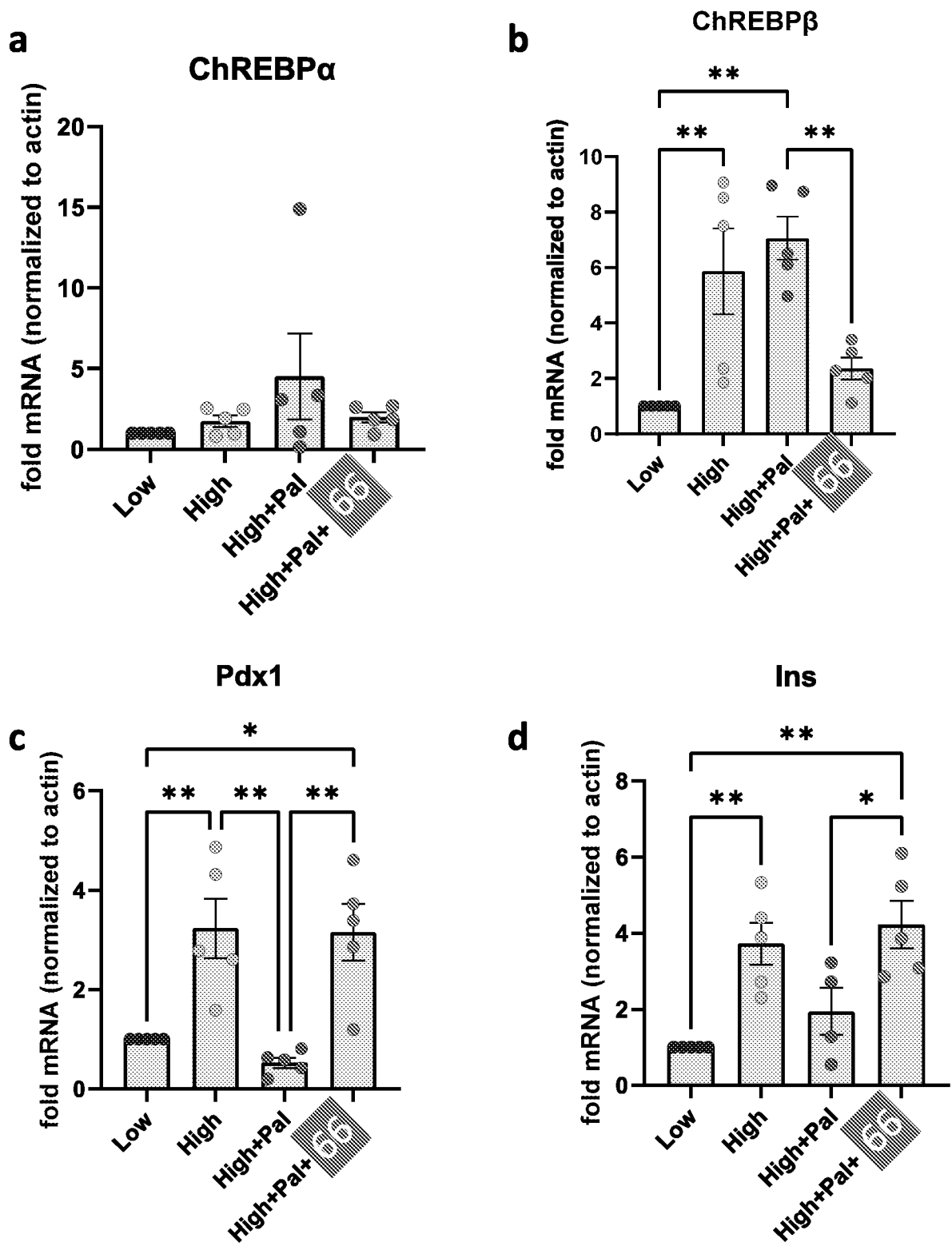


Fig. 26

34/41

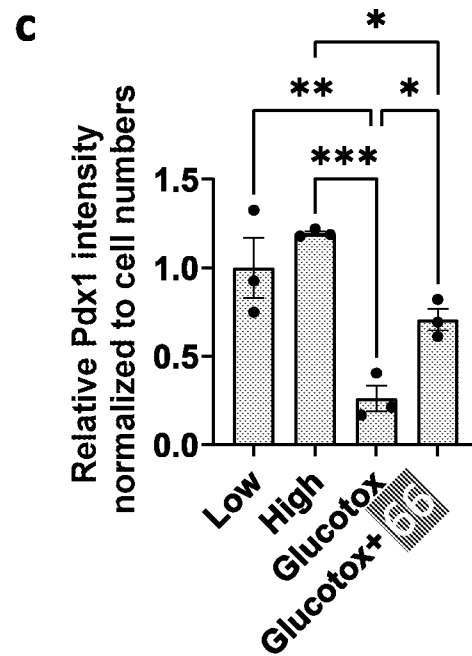
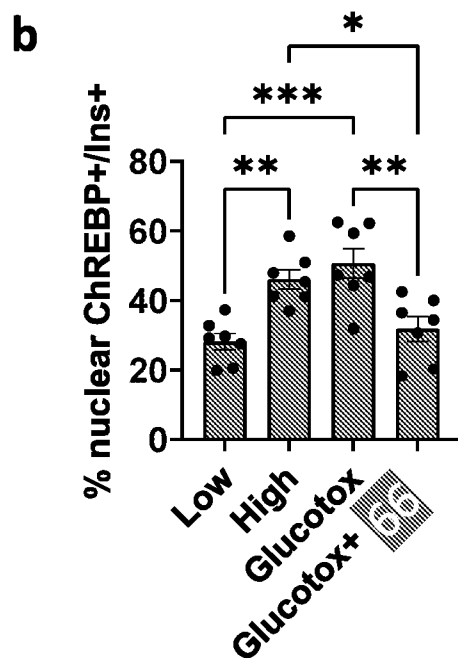
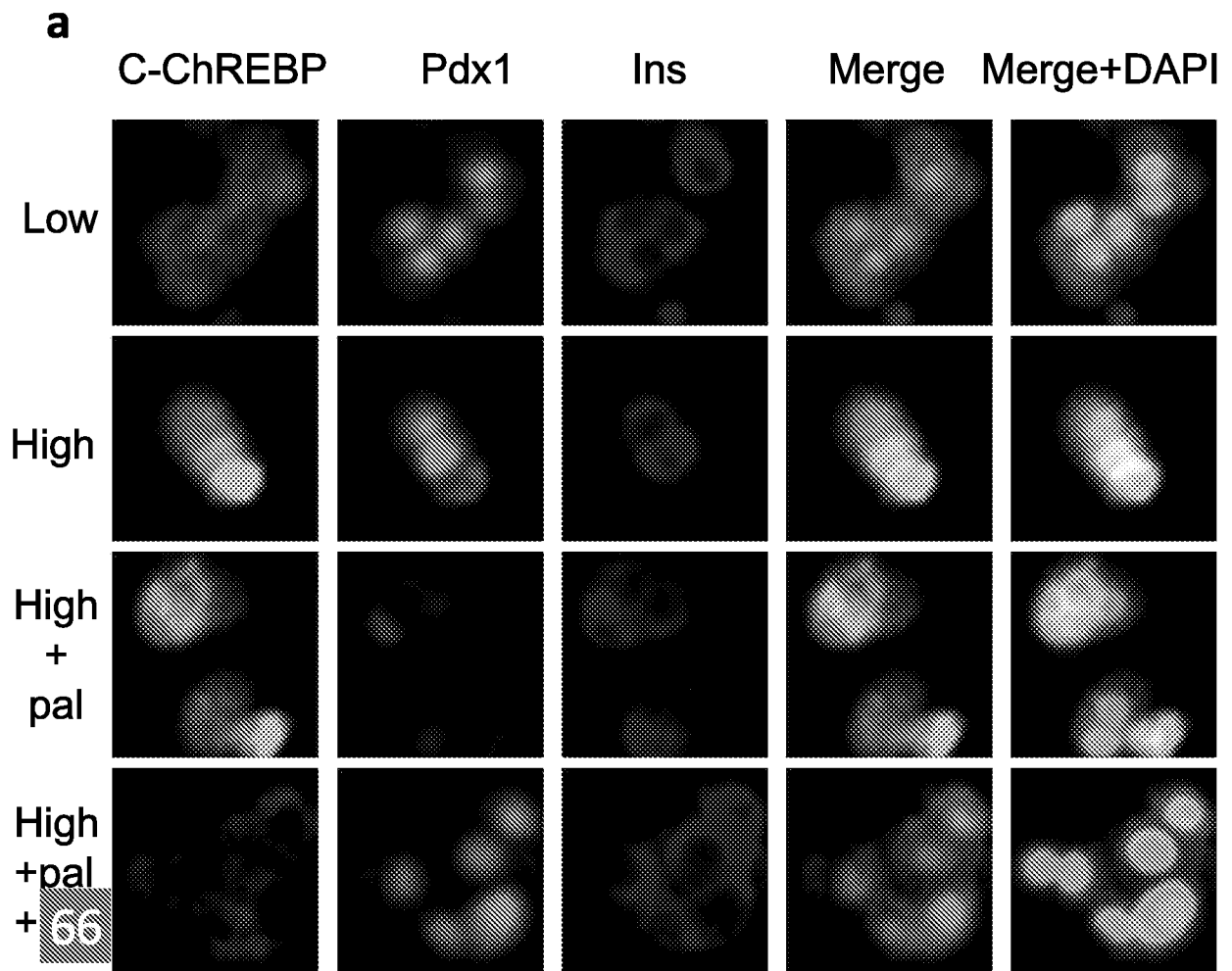


Fig. 27

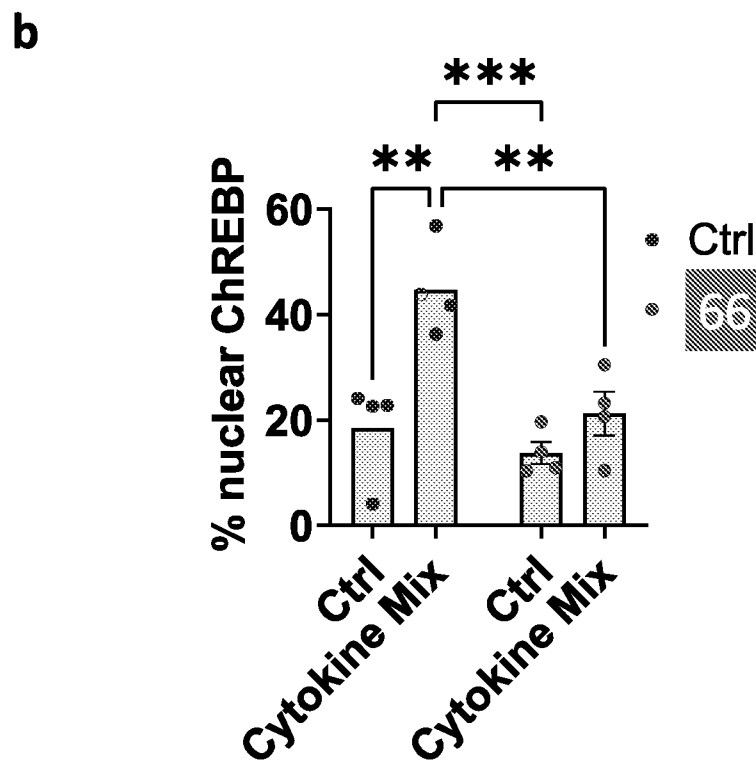
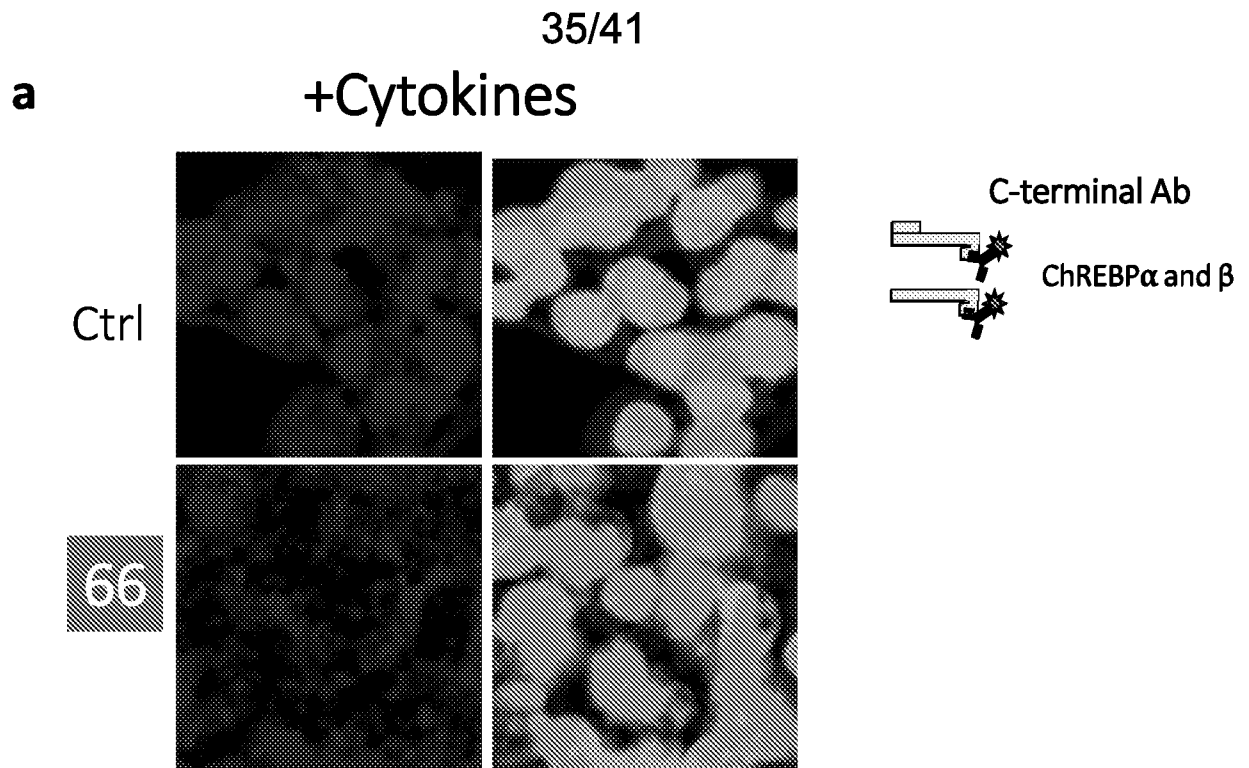


Fig. 28

36/41

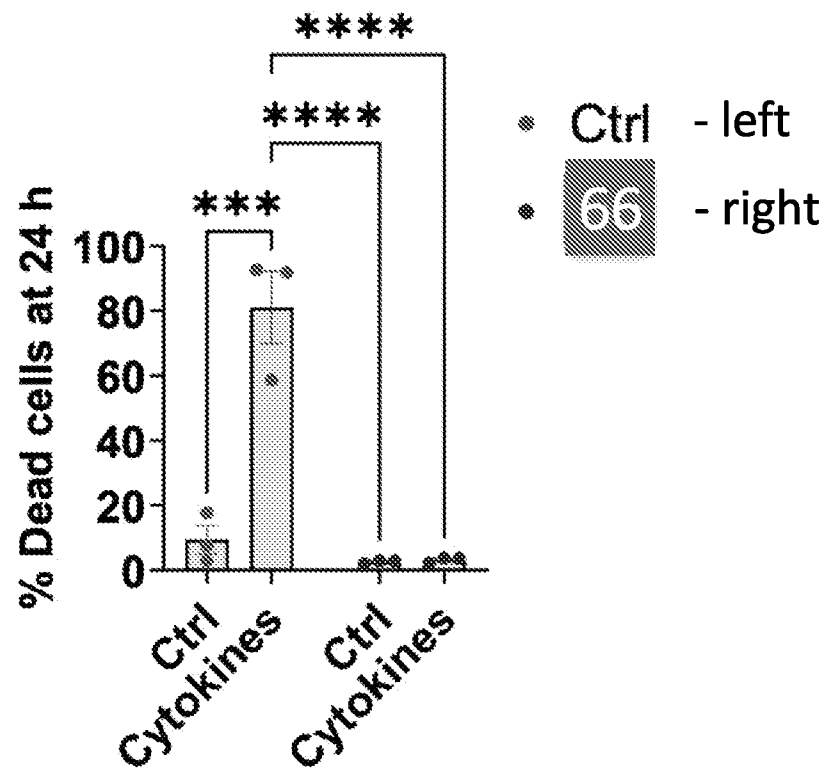
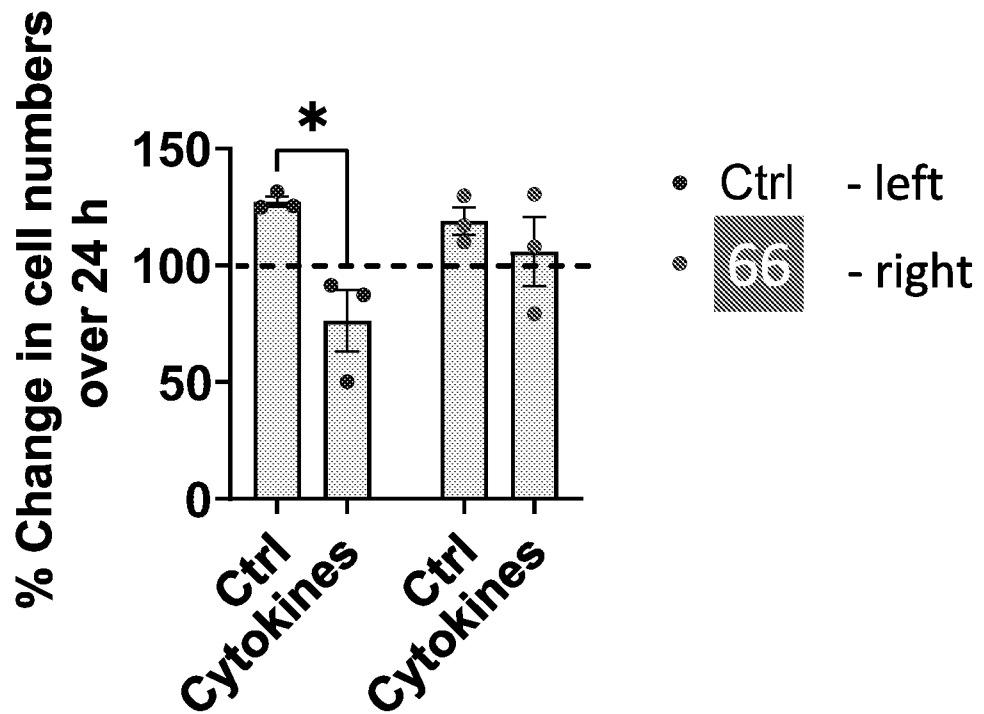


Fig. 29

37/41

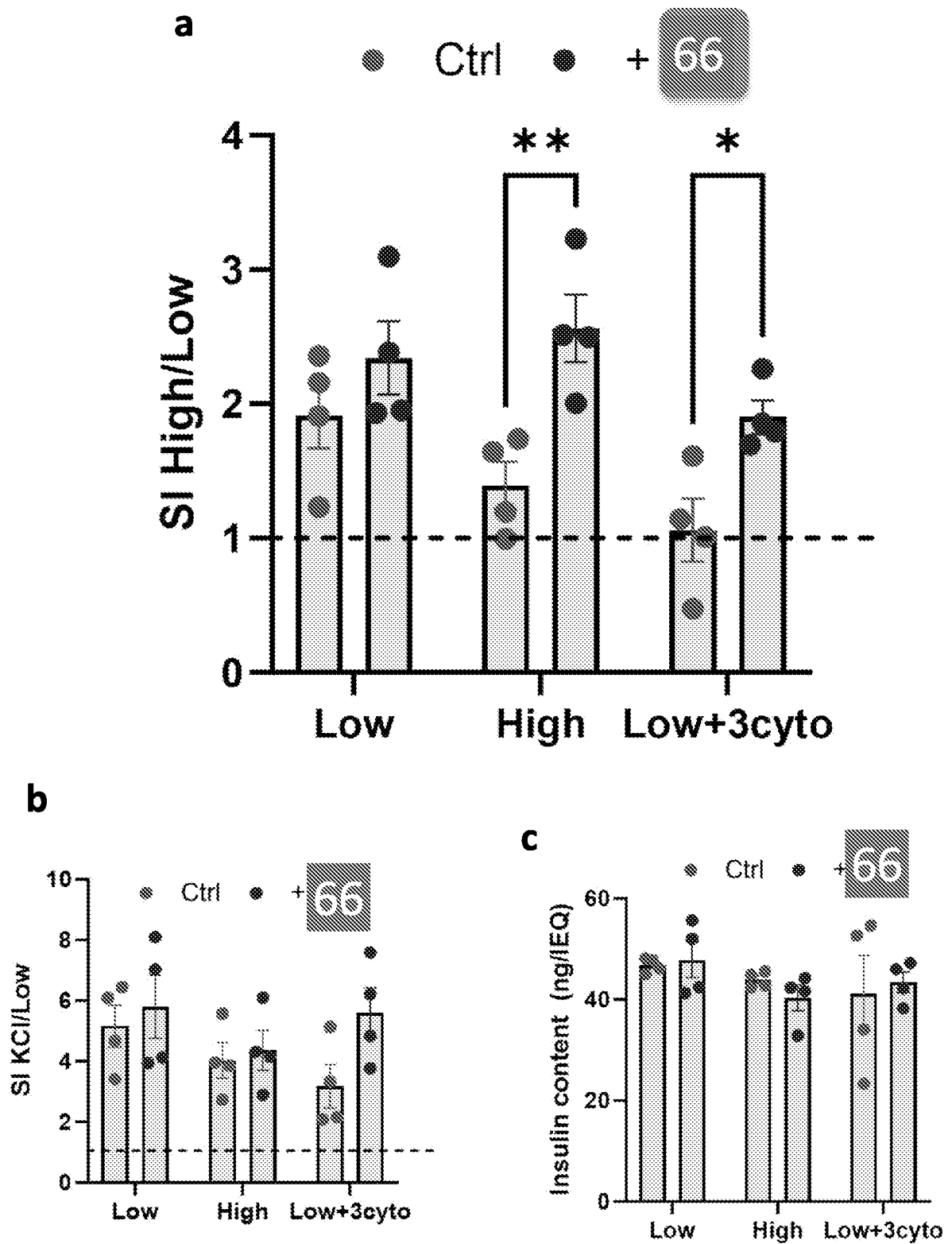


Fig. 30

38/41

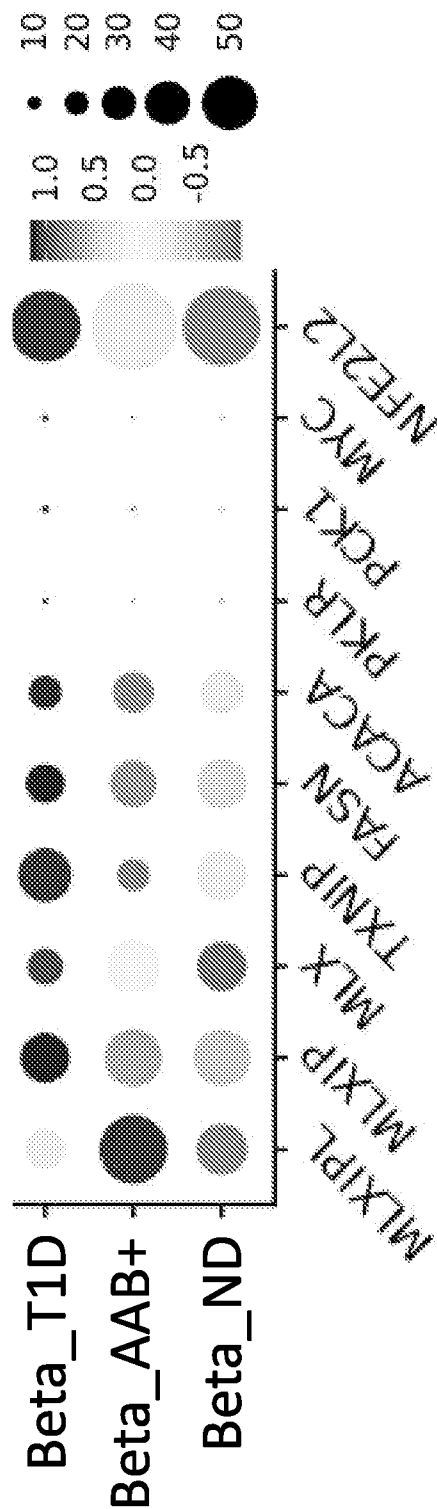


Fig. 31

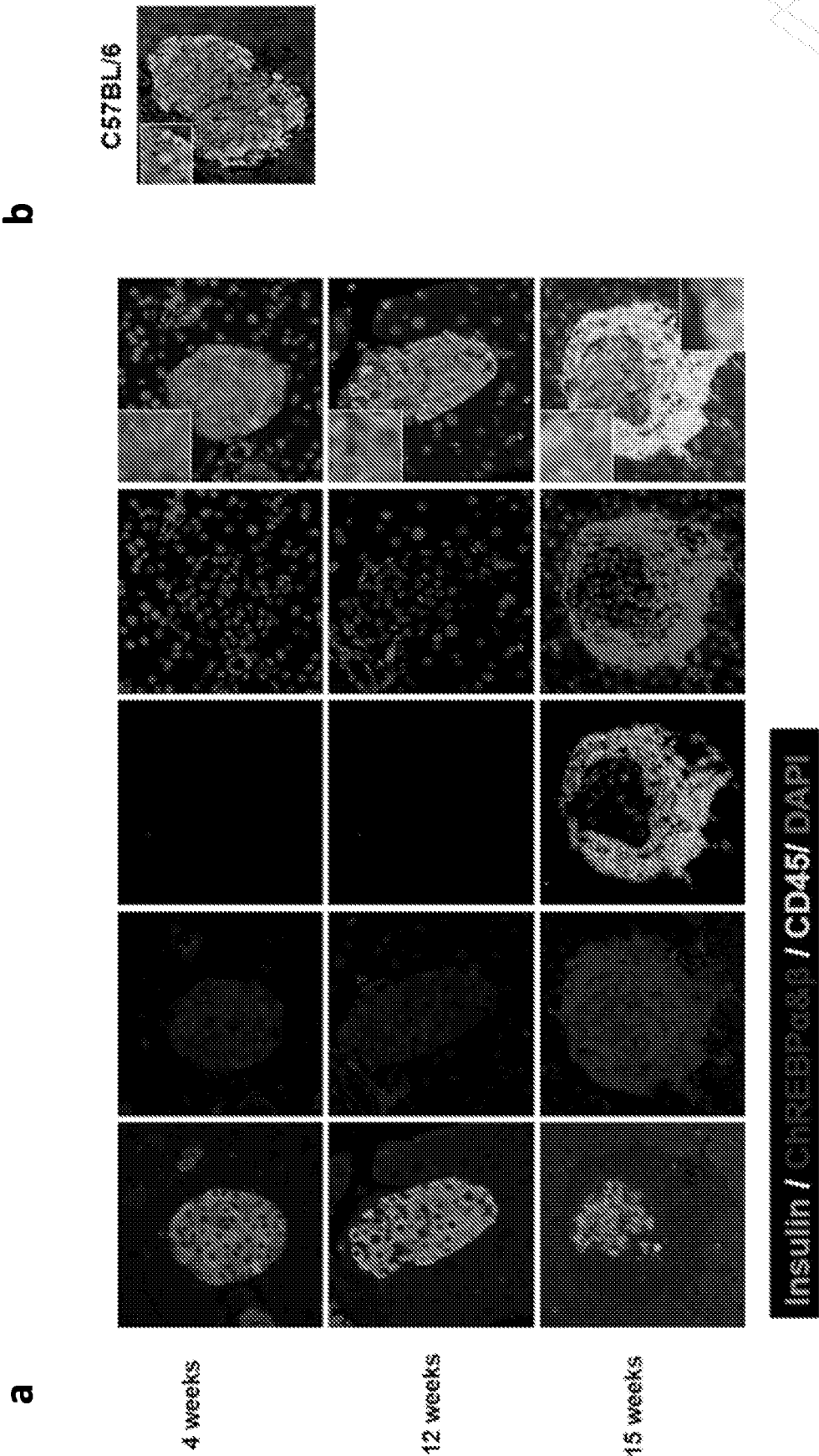
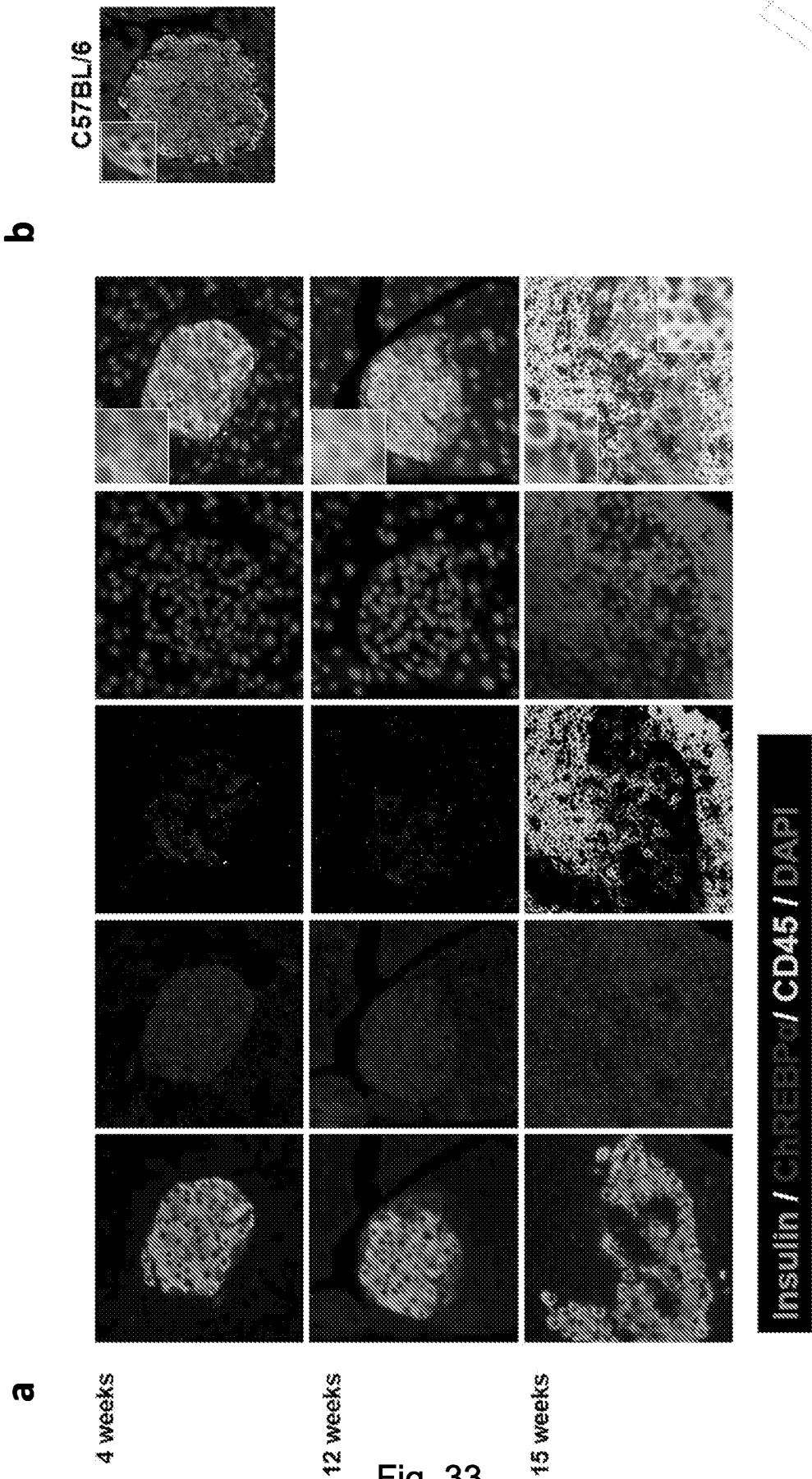


Fig. 32



41/41

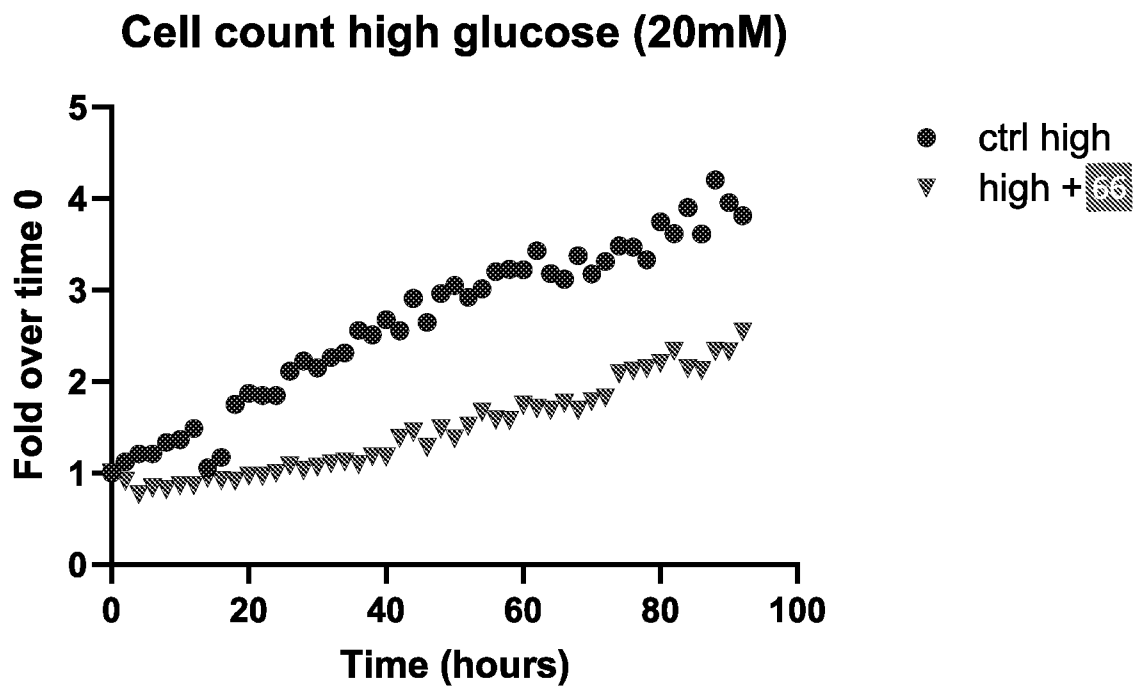


Fig. 34

INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2024/050337

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07F9/12 C07F9/40 C07F9/38
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07F

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

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

EPO-Internal**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIJBESMA ELINE ET AL: "Structure-based evolution of a promiscuous inhibitor to a selective stabilizer of protein-protein interactions", NATURE COMMUNICATIONS, vol. 11, no. 1, 7 August 2020 (2020-08-07) , XP093217411, UK ISSN: 2041-1723, DOI: 10.1038/s41467-020-17741-0 Retrieved from the Internet: URL:https://www.nature.com/articles/s41467-020-17741-0.pdf> cited in the application abstract; pages 1-2, 6 ----- -/-	1-28



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

29 October 2024

Date of mailing of the international search report

21/11/2024

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Kövecs, Monika

INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2024/050337

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THIEL PHILIPP ET AL: "Virtual screening and experimental validation reveal novel small-molecule inhibitors of 14-3-3 protein-protein interactions", CHEMICAL COMMUNICATIONS, vol. 49, no. 76, 1 January 2013 (2013-01-01), page 8468, XP093217415, UK ISSN: 1359-7345, DOI: 10.1039/c3cc44612c Retrieved from the Internet: URL:https://www.rsc.org/suppdata/cc/c3/c3cc44612c/c3cc44612c.pdf> page 8; table 51; compounds A1-A2 -----	1-28
A	CHEN SHU-YU ET AL: "What Makes a Good Protein-Protein Interaction Stabilizer: Analysis and Application of the Dual-Binding Mechanism", ACS CENTRAL SCIENCE, vol. 9, no. 5, 24 May 2023 (2023-05-24), pages 969-979, XP093217745, ISSN: 2374-7943, DOI: 10.1021/acscentsci.3c00003 Retrieved from the Internet: URL:https://pubs.acs.org/doi/pdf/10.1021/acscentsci.3c00003> pages 971-972; figure 2; compound 3 -----	1-28