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Synthesis and Pharmacological Evaluation of Dual Acting Ligands Targeting the Adenosine A_{2A} and Dopamine D_2 Receptors for the Potential Treatment of Parkinson's Disease

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ABSTRACT: A relatively new strategy in drug discovery is the development of dual acting ligands. These molecules are potentially able to interact at two orthosteric binding sites of a heterodimer simultaneously, possibly resulting in enhanced subtype selectivity, higher affinity, enhanced or modified physiological response, and reduced reliance on multiple drug administration regimens. In this study, we have successfully synthesized a series of classical heterobivalent ligands as well as a series of more integrated and "drug-like" dual acting molecules, incorporating ropinirole as a dopamine D_2 receptor agonist and ZM 241385 as an adenosine A_{2A} receptor antagonist. The best compounds of our series maintained the potency of the original pharmacophores at both receptors (adenosine A_{2A} and dopamine D_2). In addition, the integrated dual acting ligands also showed promising results in preliminary blood-brain-barrier permeability tests, whereas the classical heterobivalent ligands are potentially more suited as pharmacological tools.

INTRODUCTION

Parkinson's disease is a progressive neurodegenerative disorder affecting about 1% of the world population over 60 years of age.[1,2] Mechanistically, current clinical therapeutics almost exclusively focus on the deficiency in dopamine production in specific parts of the brain which is associated with the primary symptoms observed in patients with Parkinson's disease.[3,4] Dopamine replacement therapies such as the pro-drug levodopa or dopamine D_2 receptor (D_2R) agonists are able to improve the quality of life enormously but are insufficient due to side effects and their inability to alter or delay the progression of the disease.[5-8] Hence, there is an urgent need for a better understanding of the disease and more effective drugs.

Animal models as well as clinical studies have given evidence that adenosine A_{2A} receptor ($A_{2A}R$) antagonists, as monotherapy and in conjunction with levodopa or D_2R agonists, are able to amplify the therapeutic effects of levodopa and reduce motor complications such as dyskinesia, on-off oscillation and wearing off effects in patients with Parkinson's disease.[9-12] Another potential benefit of $A_{2A}R$ antagonists in contrast to current drugs on the market is their proposed capability of slowing down the degeneration of dopaminergic neurons thereby delaying the onset and progression of the disease.[13,14] Therefore, $A_{2A}R$ antagonists are considered a promising new drug class that could potentially complement the dopaminergic drugs currently available for patients suffering from Parkinson's disease.

Consequently, scientists have investigated the possibility of designing a single molecule which is able to target the $A_{2A}R$ and D_2R. A strategy which gained greater attention after the discovery of $A_{2A}R$-D_2R heterodimers is the development of heterobivalent ligands; a molecule consisting of two different pharmacophores separated through a spacer which is linked to each individual drug entity (Fig. 1).[15,16] The simultaneous interactions of these molecules with both orthosteric binding sites of a D_2R-$A_{2A}R$ heterodimer has the potential to result in enhanced subtype selectivity, higher affinity, enhanced or modified physiological

response, and reduced reliance on multiple drug administration regimens. Furthermore, these heterobivalent systems can be used as pharmacological tools permitting estimations of the distance between the targets and their spatial distribution. Soriano *et al.*[17] published a series of heterobivalent ligands (XCC-PPHT-NH_2) ranging from 26 to 118 atoms spacer length to target A_{2A}R-D_2R heterodimers. The molecules are constructed of a functionalized xanthine-based adenosine A_{2A}R antagonist (XCC) and a 2-amino-5-hydroxytetralin-based dopamine D_2R agonist (PPHT-NH_2) component. In this study, higher affinity was observed for the heterobivalent ligands compared to the monovalent analog in brain striatum and cells co-expressing both receptors, whereas in cells expressing just one receptor type no change in affinity was reported. These findings were explained by the existence of A_{2A}R-D_2R heterodimers in brain striatum and simultaneous binding of the heterobivalent ligands to both receptors. Even though, such molecules are interesting pharmacological tools, their use as drugs is potentially limited due to their size.

Consequently, the design of integrated dual acting ligands (also referred to as merged or fused designed multiple ligands or twin ligands) is a more promising approach for the development of drugs for the treatment of CNS drugs such as Parkinson's disease (Fig. 1).[18] The advantages of integrated dual acting ligands over heterobivalent ligands are the reduction in molecular mass, topological polar surface area and clogP, which is equivalent to the enhancement of the "drug-like" properties including their ability to cross the blood brain barrier. Dual acting ligands for the treatment of Parkinson's disease have been reported with the aim to target the A_{2A} receptor while inhibiting MAO-B.[19,20] To the best of our knowledge, comparatively small "drug-like" molecules targeting the A_{2A} and D_2 receptors simultaneously have not formerly been reported. A related concept is the design of dual acting pro-drugs as exemplified by Gogoi *et al.*;[21] in this instance a molecule consisting of two pharmacophores is

required to cross the blood brain barrier, before it dissociates into two entities able to interact at the $A_{2A}R$ and D_2R individually.

In summary, both of these concepts provide interesting aspects that are worthwhile investigating. Heterobivalent ligands are potentially more suited as pharmacological tools to further investigate the properties of the $A_{2A}R$-D_2R heterodimers, whereas integrated dual acting ligands may find their application in drug discovery as novel drugs for the treatment of Parkinson's disease.

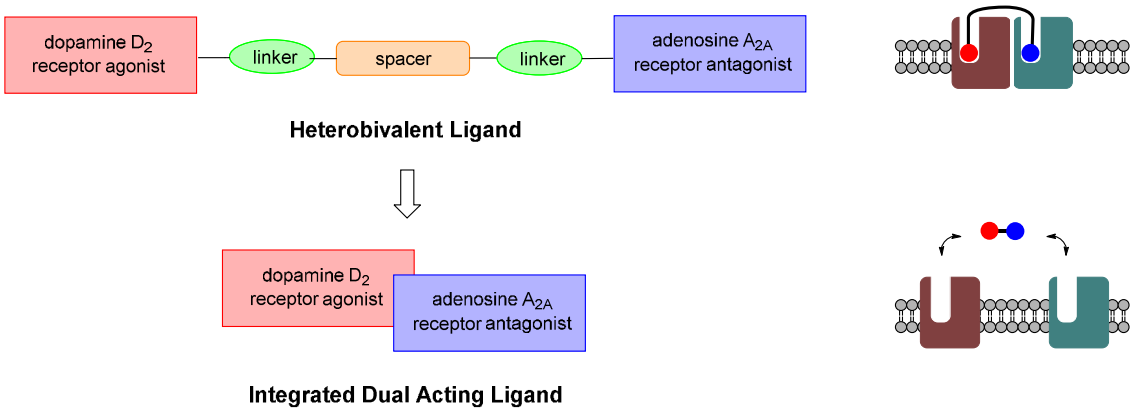


Figure 1. (left) Schematic representation of a heterobivalent ligand and integrated dual acting ligand consisting of a D_2R agonist and an $A_{2A}R$ antagonist. (right) Schematic examples of a heterobivalent ligand binding to a heterodimer and an integrated dual acting ligand acting at the two orthosteric sites of two different types of receptor monomers.

In this project the $A_{2A}R$ antagonist of choice was ZM 241385 (**1**) (Fig. 2);[22,23] the first $A_{2A}R$ antagonist where an X-ray structure of an $A_{2A}R$ antagonist in complex with the A_{2A} receptor existed,[24] which allowed good predictions of the binding mode of triazolotriazine-based analogs. The D_2R agonist of choice was the established antiparkinsonian drug ropinirole (**2**) (trade name: Requip) (Fig. 2) due to its simple structure compared to other D_2R agonist on the market.[25,26] Furthermore, its low molecular weight is beneficial for the design of dual acting ligands with more "drug-like" properties. Notably, ropinirole (**2**) is a non-ergot derived D_2R

agonist which has been shown to be superior to ergot derived first generation drugs due to reduced side-effects.[27,28] The drug is also available as an extended-release formulation permitting once-daily administration. Our group has previously published the successful development of functionalized congeners of each pharmacophore (**3**-**5**), allowing further extension into the extracellular space, whilst maintaining comparable activity to the parent molecules.[29-31] For the design of the heterobivalent ligands we have used the functionalized congeners TCAC (**3**) and OAC (**5**) as our starting point, before moving onto more "drug-like" analogs with reduced molecular mass. The synthesized dual acting ligands were tested in cell-based assays to determine their inhibitory potency at the A_{2A} receptor, and functional potency as well as the maximum response at the D_2 receptor. In addition, preliminary permeability testing was performed on selected analogs to predict their ability to cross the blood brain barrier, which is an essential attribute for a potential CNS drug.

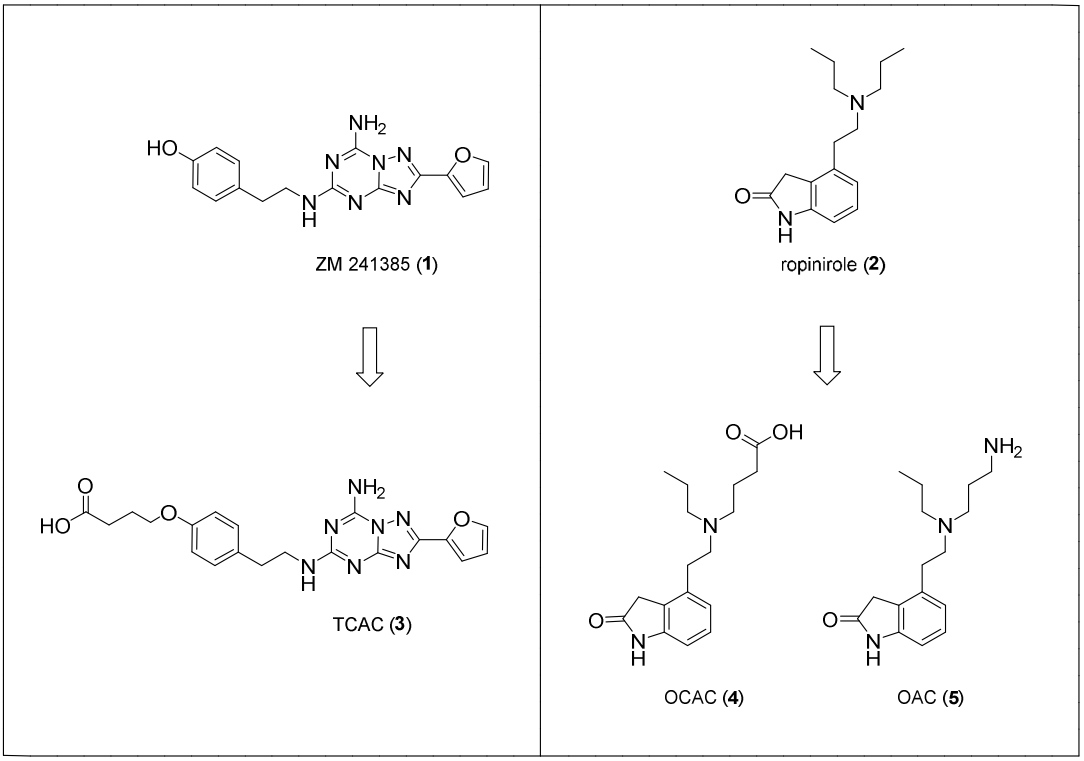


Figure 2. Chemical structures of the $A_{2A}R$ antagonist ZM 241385 (**1**) and D_2R agonist ropinirole (**2**) and their respective functionalized carboxylic acid congeners TCAC (**3**) and OCAC (**4**), as well as the functionalized amine congener OAC (**5**).

SYNTHESIS OF THE DUAL ACTING LIGANDS

We first investigated classical heterobivalent ligands, consisting of the fully furnished pharmacophores ZM 241385 (**1**) and ropinirole (**2**), separated with various lengths and types of linkers or/and spacers spanning the two moieties (Fig. 3). Secondly, we focused on more structurally integrated dual acting ligands with desirable physical properties (molecular mass, polar surface area and clogP) for the development of potential CNS drugs (Fig. 3). This was achieved by the elimination of the spacer and tyramine moiety, which according to structure-activity relationship studies[32] and the receptor bound X-ray structure[24] are non-essential for the activity of the $A_{2A}R$ antagonist ZM 241385 (**1**).

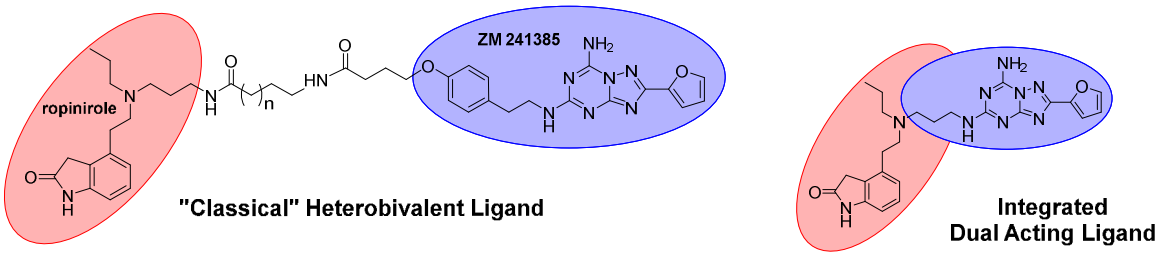


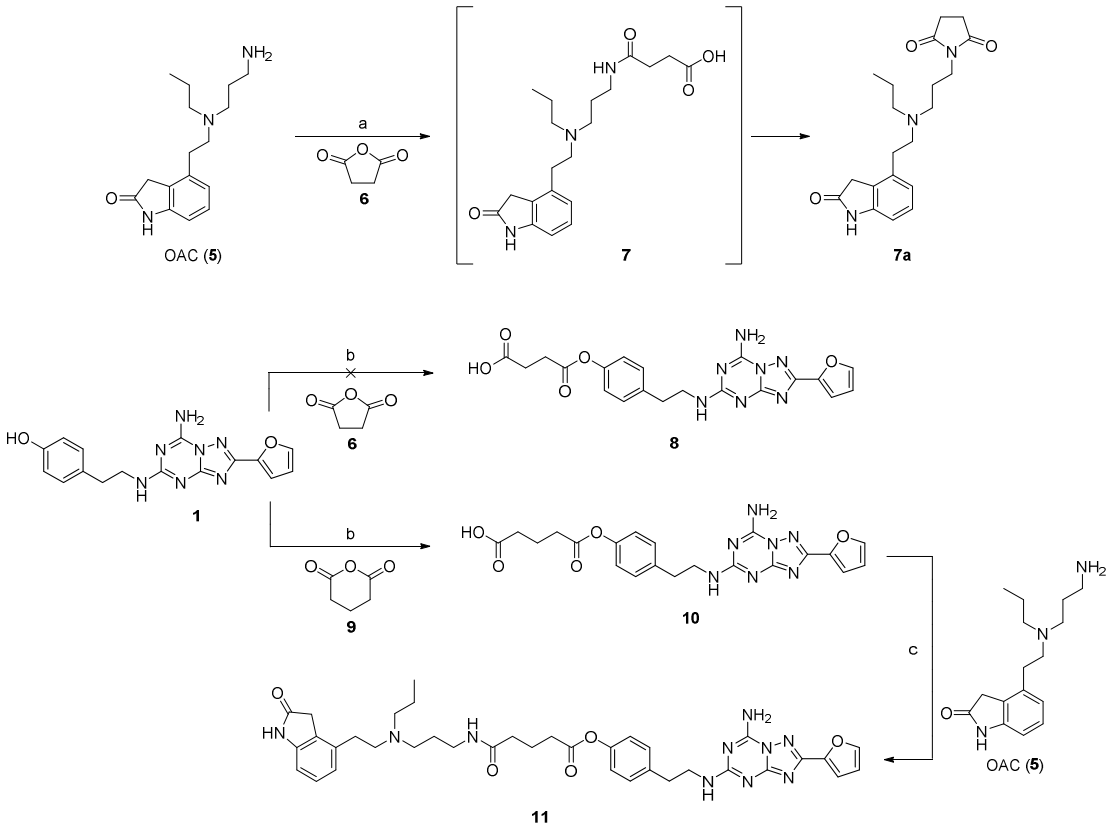
Figure 3. An example of the design principle of the "classical" heterobivalent ligands in-cooperating the fully furnished pharmacophores ZM 241385 (**1**) and ropinirole (**2**) and the integrated dual acting ligands without the tyramine moiety of the $A_{2A}R$ antagonist ZM 241385 (**1**).

Heterobivalent ligands

We first investigated ester linked heterobivalent ligands (Scheme 1) due to their simplicity and facile synthetic accessibility. The OAC (**5**) was added to succinic anhydride (**6**) in toluene and stirred at reflux. LC-MS indicated the formation of the carboxylic acid intermediate **7**, but this compound was not sufficiently stable and readily converted to the pyrrolidine-2,5-dione

byproduct **7a**. The addition of ZM 241385 (**1**) and the appropriate coupling reagents to the *in situ* formed intermediate **7** also afforded mainly the pyrrolidine-2,5-dione byproduct **7a** and not the desired heterobivalent ligand. To avoid this ring closure, the order of the reaction steps was reversed. Consequently, the cyclic anhydride was first added to the $A_{2A}R$ antagonist ZM 241385 (**1**) followed by OAC (**5**). Therefore, ZM 241385 (**1**) was reacted with succinic anhydride (**6**) or glutaric anhydride (**9**) in the presence of triethylamine in toluene at room temperature. In the case of succinic anhydride (**6**), the reaction failed whereas glutaric anhydride (**9**) furnished the desired compound **10** albeit in modest yields (28%). The carboxylic acid **10** was then converted under standard peptide coupling conditions to the heterobivalent ligand **11**. The disadvantage of this approach is the limited range of linker lengths available (larger cyclic anhydrides are not stable or commercially available) as well as the metabolic instability of ester bonds. However, this approach provided a relatively straight forward synthetic route to obtain a first dual acting ligand for preliminary *in vitro* testing as a proof-of-concept system to evaluate the overall approach.

Scheme 1. Synthesis of the ester-linked heterobivalent ligand **11**[a]



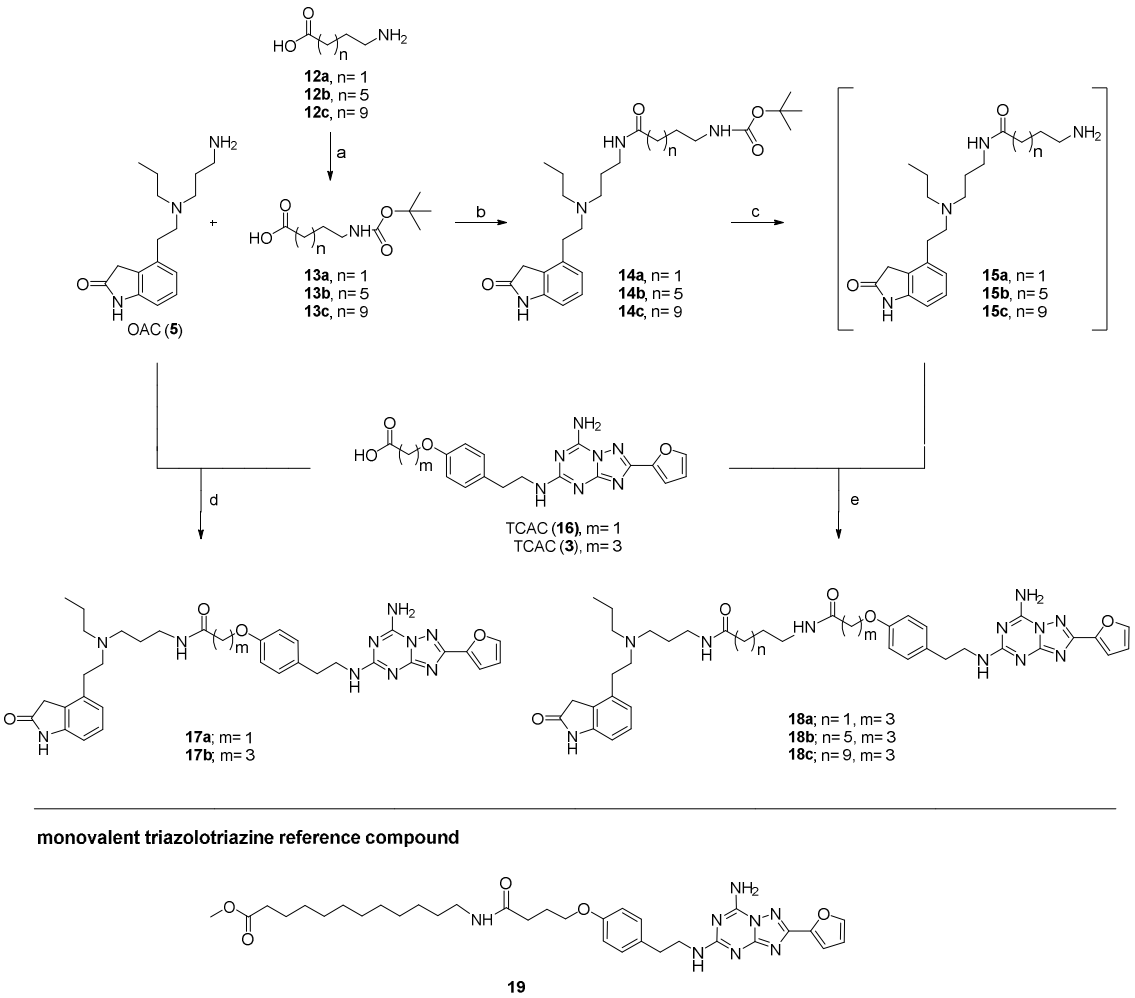
[a]Reagents and conditions: (a) toluene, 24 h, reflux, 13% (**7a**); (b) TEA, toluene, 29 h, rt, no product observed (**8**), 28% (**10**); (c) DIPEA, HCTU, DMF, 3 h, rt, 10%.

Next, we designed and synthesized heterobivalent ligands where the two pharmacophores were connected via a more hydrolytically and metabolically stable amide and ether linkage (Scheme 2). Consequently, molecules of this type provide pharmacological tools that are potentially able to interact simultaneously at both orthosteric binding sites of a D_2R-$A_{2A}R$ heterodimer. Therefore, the functionalized congeners OAC (**5**) and TCAC (**3**) as well as a shorter version of the latter (compound **16**) were either connected directly to afford compounds **17a** and **17b** or incorporated amino acid spacers of varying lengths to afford compounds **18a**-**18c**. The formation of the amide bonds was performed using standard coupling reagents and conditions. It was important to protect the amino acids **12a**-**c** to avoid undesired side products, which simplified the purification step and drastically increased the

yield. Initially benzotriazole-based coupling reagents such as BOP and HCTU were used, but the by-products of these coupling reagents were challenging to remove in the final step. The use of COMU as a coupling reagent furnished higher conversion and simplified the purification process to remove undesired by-products associated with the coupling reagent.

In addition to the heterobivalent ligands (**17a**, **17b** and **18a-c**), the monovalent analogs with the protected 12 carbon spacer attached to either the oxindole (**14c**) or triazolotriazine (**19**) congener were synthesized and tested as additional reference compounds.

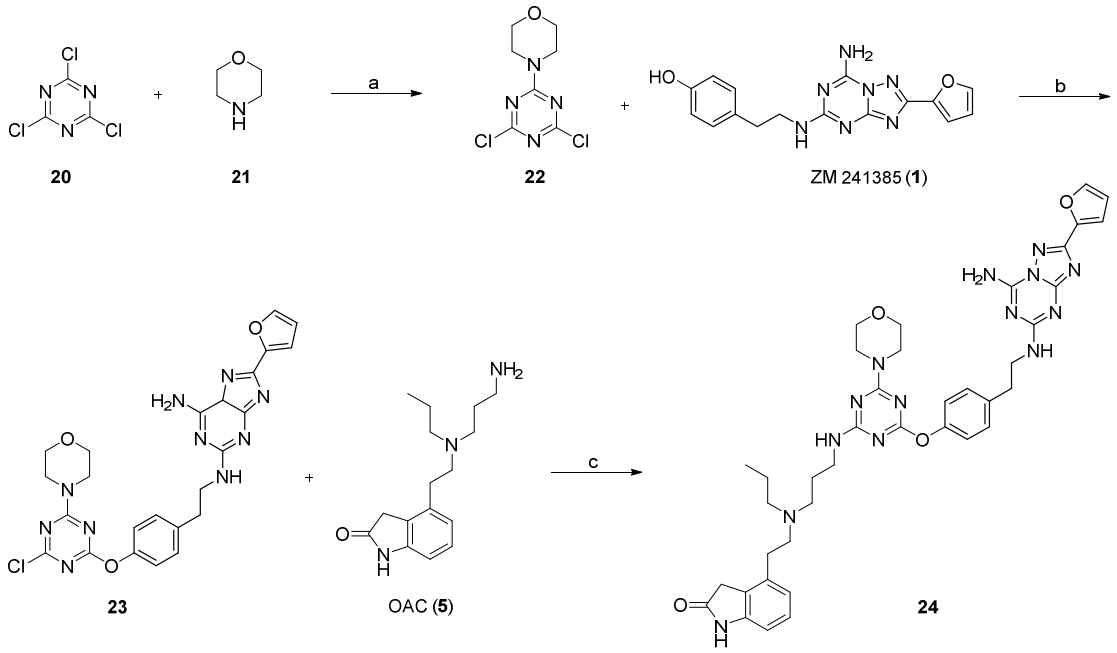
Scheme 2. Synthesis of heterobivalent ligands **17a**, **17b** and **18a-c** incorporating amide bonds and the chemical structure of the triazolotriazine monovalent ligand **19**[a]



[a]Reagents and conditions: (a) Boc anhydride, 1 M NaOH, THF, 3 h, rt, 66% (**13a**); Boc anhydride, TEA, methanol, 2 h, 60 °C, 81% (**13b**) and 68% (**13c**); (b) COMU, DIPEA, DMF, 1 h, 0 °C followed by 2 h, rt, 31% (**14a**), 23% (**14b**) and 41% (**14c**); (c) TFA, DCM, 2.5-9 h, rt, 69% (**15a**), 99% (**15b**) and 39% (**15c**); (d) BOP, DIPEA, DMF, 5 h, rt, 7% (**17a**); HCTU, DIPEA, DMF, 3 h, rt, 24% (**17b**); (e) COMU, DIPEA, DMF, 1 h, 0 °C followed by 2-9 h, rt, 17% (**18a**), 17% (**18b**) and 8% (**18c**).

Next, we investigated the possibility of incorporating a triazine spacer, which was further functionalized with a morpholine group to potentially improve the overall solubility of the heterobivalent ligand **24** (Scheme 3). The triazine linker unit has been incorporated into the heterobivalent ligands since it is a commonly used motif in drug discovery (i.e. PI3K inhibitors, dendrimers).[33-36] The starting material cyanuric chloride (**20**) is an inexpensive and commercially available compound with diverse reactivity of the three chlorine atoms allowing selective substitution. Therefore, cyanuric chloride (**20**) in acetone was added dropwise to a solution of morpholine (**21**) and triethylamine in acetone at -20 °C to afford intermediate **22** in 75% yield, which was used in the next step without further purification. ZM 241385 (**1**) was deprotonated with sodium hydride, treated with the triazine spacer **22** and the reaction mixture stirred at room temperature for two days to obtain compound **23** in 53% yield. Intermediate **23** was then further reacted with OAC (**5**) in a sealed microwave tube to furnish the heterobivalent ligand **24** in 67% yield.

Scheme 3. Synthesis of triazine spacer containing heterobivalent ligand **24**[a]

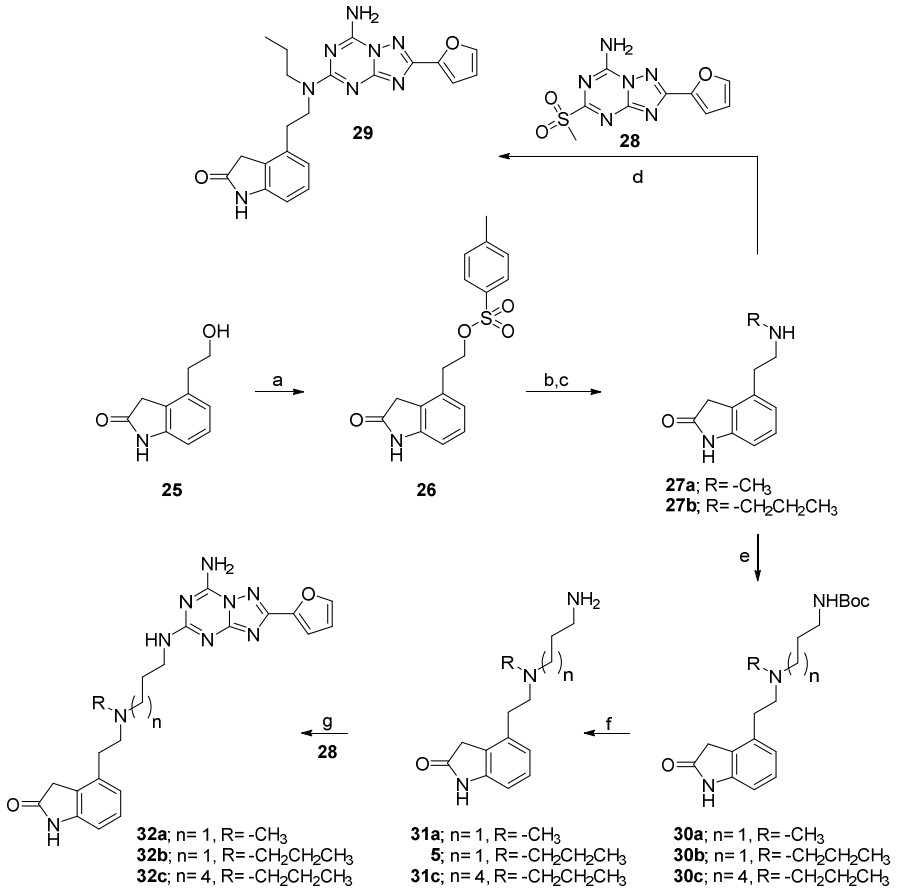


[a]Reagents and conditions: (a) TEA, acetone, 15 min, -20 °C, 75%; (b) NaH 60%, THF, 2 days, rt, 53%; (c) K_2CO_3, DMF, microwave, 15 min, 100 °C, 67%.

Integrated dual acting ligands

Unlike the synthesis of the heterobivalent ligands, the more integrated molecules did not consist of the fully furnished pharmacohore ZM 241385 (**1**). Therefore, the sulfone intermediate **28** (which does not contain the tyramine moiety of ZM 241385 (**1**)) was key to facilitate the introduction of the 2-(furan-2-yl)-5-(methylsulfonyl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine portion of the $A_{2A}R$ antagonist instead of the TCACs **3** or **16**. The synthesis of the integrated molecules **32a-c** and **29** (Scheme 4) started with commercially available 4-(2-hydroxyethyl)indolin-2-one (**25**), which was activated by conversion into the tosylated intermediate **26**, thereby allowing substitution with either methylamine or propylamine at reflux to furnish the secondary amines **27a** and **27b**. Our dual acting ligand **29** was formed by stirring intermediate **27b** and sulfone **28** in acetonitrile at room temperature for 2.5 days. In the design of compound **29** the linker between the two pharmacophores was fully removed to further reduce the molecular weight. This approach is associated with the loss of the ionizable nitrogen; as a consequence the pharmacological evaluation of this molecule will further validate the vitality of this feature for activity at the D_2 receptor. Intermediates **27a** and **27b** were also utilized for the synthesis of the target compounds **32a-c**. Boc-protected amine linkers of different lengths were incorporated into the secondary amine moieties of compound **27a** and **27b** to afford analogs **30a-c** which, after cleavage of the Boc protecting group, furnished the oxindole amine congeners **31a**, **31c** and **5**. The final step involved stirring the newly formed congeners **31a**, **31c** and **5** with sulfone **28** in acetonitrile at room temperature to afford the target compounds **32a-c** in yields of 23-65%. The dual acting ligands **32a-c** and **29** represent a small set of compounds with varying linker lengths between the two pharmacophores as well as in the length of the side chain attached to the ionizable tertiary amine of the ropinirole portion of the molecule.

Scheme 4. Synthesis of integrated dual acting ligands based on ZM 241385 (**1**) and ropinirole (**2**)[a]



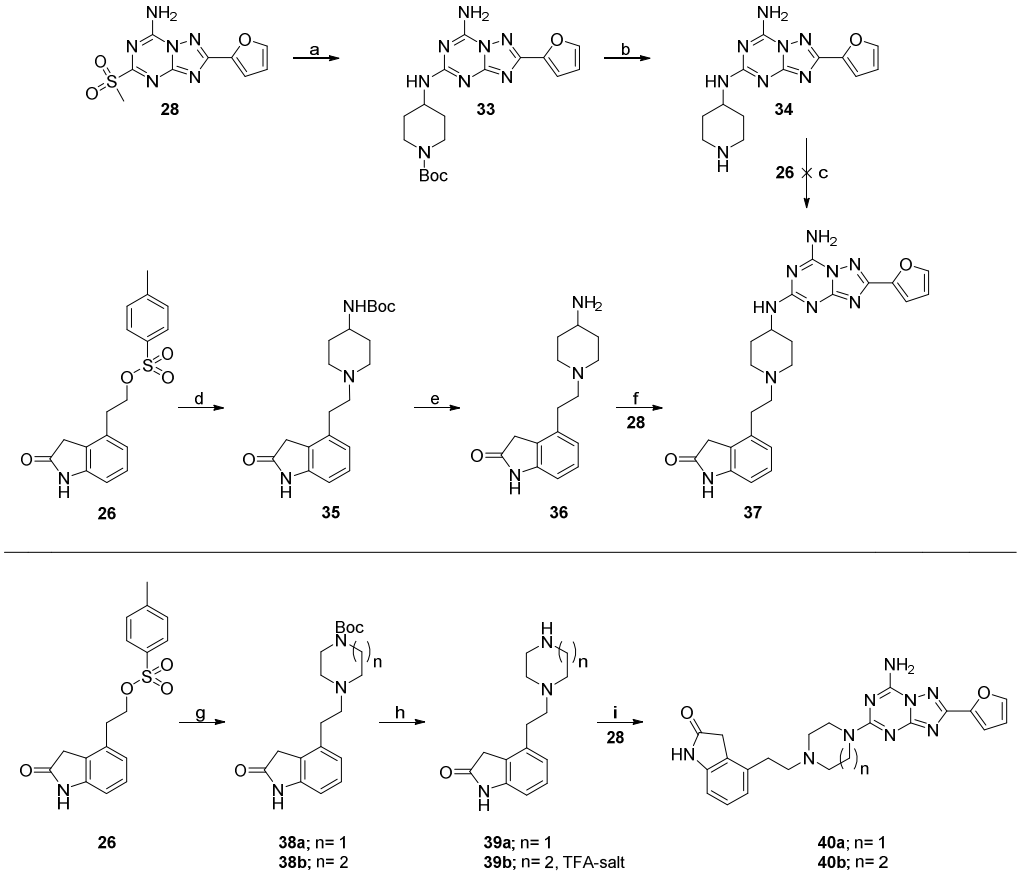
[a]Reagents and conditions: (a) *p*-toluenesulfonyl chloride, DCM, pyridine, 5-10 °C, 4 h, 80%; (b) 40% aq. methylamine solution or neat *n*-propylamine, reflux, 1.5 h, 57% and 74%, respectively; (c) 1 M NaOH, ethyl acetate or DCM, rt, 15-20 min, 54% (**27a**) and 92% (**27b**); (d) **28**, acetonitrile, rt, 2.5 days, 8%; (e) $Br(CH_2)_{n+2}NHBoc$, K_2CO_3, acetonitrile, reflux, 3-19 h, 46% (**30a**), 38% (**30b**) and 48% (**30c**); (f) TFA, DCM, rt, 1 h, 43% (**31a**), 84% (**5**) and 87% (**31c**); (g) **28**, acetonitrile, toluene, rt, 5-60 h, 23% (**32a**), 65% (**32b**) and 63% (**32c**).

Next, we looked at molecules with a cyclic linker unit between the two pharmacophores, which increases the rigidity of the molecule without interfering with the original triazolotriazine and oxindole scaffolds of ZM 241385 (**1**) and ropinirole (**2**), respectively. The idea behind this research was based on the fact that molecules with a reduced number of rotatable bonds tend to show improved penetration of the BBB compared to highly flexible

and branched compounds.[37,38] Synthesized were three variations thereof; the aminopiperidine **37**, piperazine **40a** and homopiperazine **40b** analog (Scheme 5). The first pathway for the synthesis of the dual acting ligand **37** commenced with sulfone **28** which was treated with *tert*-butyl piperidin-4-ylcarbamate in acetonitrile to afford intermediate **33** in 63%. The Boc protecting group was removed using trifluoroacetic acid followed by alkaline treatment to furnish intermediate **34** in free base form. The aminopiperidine functionalized triazolotriazine analogs **34** was then further reacted under alkaline conditions with tosylate **26** in acetonitrile at reflux, however, no conversion to molecule **37** was observed and mainly starting material was recovered. Therefore, an alternative pathway starting from tosylate **26** was investigated. Compound **26** was reacted with *tert*-butyl piperidin-4-ylcarbamate under alkaline conditions at reflux affording intermediate **35** in 34% yield. Cleavage of the Boc protecting group with TFA and treatment with 1 M aqueous sodium hydroxide furnished intermediate **36**, which was subsequently converted to the desired dual acting ligand **37** in 75% yield by adding sulfone **28** in acetonitrile at room temperature.

The second synthetic pathway was used to obtain the dual acting ligands **40a** and **40b**. The tosylate **26** was stirred under alkaline conditions with either Boc-piperazine or Boc-homopiperazine to afford intermediate **38a** and **38b**, respectively. The Boc group of compound **38a** was removed with trifluoroacetic acid followed by an alkaline treatment to afford the free amine **39a**, which was further reacted with sulfone **28** to furnish the target compound **40a** in 66% yield. Intermediate **39a** was extracted into the aqueous layer after washing with 1 M sodium hydroxide solution, therefore intermediate **39b** was isolated as the trifluoroacetate salt after the cleavage of Boc protecting group by simply evaporating the residual under reduced pressure. Consequently, the non-nucleophilic base 1,8-diazabicycloundec-7-ene (DBU) was added to intermediate **39b** and sulfone **28** in acetonitrile at room temperature to afford the target compound **40b** in low yield (5%).

Scheme 5. Synthesis of cyclic integrated dual acting ligands based on ZM 241385 (**1**) and ropinirole (**2**) [a]



[a]Reagents and conditions: (a) *tert*-butyl 4-aminopiperidine-1-carboxylate, acetonitrile, rt, 3 h, 63%; (b) TFA, DCM, rt, 3.5 h; (c) **26**, DIPEA, DBU, acetonitrile, 85 °C, 2 days, no product; (d) *tert*-butyl piperidin-4-ylcarbamate, DIPEA, acetonitrile, reflux 6 h, 34%; (e) TFA, DCM, rt, 2 h, 46%; (f) **28**, acetonitrile, DMF, rt, 22 h, 49%; (g) Boc-piperazine, DIPEA, acetonitrile, reflux, 22 h, 75% (**38a**); **26**, Boc-homopiperazine, DIPEA, acetonitrile, 23 h, reflux, 74% (**38b**); (h) TFA, DCM, rt, 5 h, followed 1 M NaOH, 71% (**39a**); TFA, DCM, rt, 3.5 h (**39b**); (i) **28**, acetonitrile, toluene, rt, 18 h, 66% (**40a**); DBU, acetonitrile, rt, 23 h, 5% (**40b**).

PHARMACOLOGICAL RESULTS

A_{2A}R inhibitory potency and D_2R functional potency

The adenosine A_{2A}R inhibitory potency (IC_{50}) of the dual acting ligands and some selected monovalent ligands was determined in a cyclic adenosine monophosphate (cAMP) assay, whereas the dopamine D_2R functional activity (EC_{50}) and efficacy (E_{max}) were determined in a [^{35}S]GTPγS assay (Table 1). The functional assays were used to compare the activity (IC_{50} and EC_{50}) of the synthesized molecules to each other as well as the parent pharmacophores ZM 241385 (**1**) and ropinirole (**2**).

The reference and parent compound ZM 241385 (**1**) inhibited 5'-*N*-ethylcarboxamidoadenosine (NECA) (500 nM) mediated cAMP accumulation with a pIC_{50} value of 7.48 ± 0.14. The attachment of the longest spacer to the triazolotriazine scaffold (compound **19**) showed a 13-fold decrease in inhibitory potency at the A_{2A} receptor; this result is in accordance with a study from our group focusing on the biological impact of extensions of selected literature A_{2A}R antagonists.[30] The reference and parent compound ropinirole (**2**) has a literature pEC_{50} value of 6.52 ± 0.02 in a [^{35}S]GTPγS assay.[39] Attachment of the 12-carbon spacer to the OAC (**5**) lead to a 3-fold increase of the functional activity at the D_2R which again is in accordance with work published earlier.[29] The triazolotriazine based monovalent **1** and **19** ligands didn't exhibit any detectable activity at the D_2 receptor, likewise the oxindole-based monovalent ligand **14c** did not show any detectable activity at the A_{2A} receptor, as expected.

The fused heterobivalent ligands **11**, **17a** and **17b** (without the incorporation of an amino acid spacer) showed very comparable activity to the initial pharmacophores. In this small group of analogs only small changes in D_2 functional potency were observed; analog **11** exhibited the lowest functional potency, being about 1.5-fold less potent than ropinirole (**2**), whereas **17b** was the most potent analog with 1.5-fold improved functional potency. Similar variations of

inhibitory potency were observed at the A_{2A} receptor ranging from 18 nM for molecules **17b** to 63 nM for **17a** in comparison to 33 nM for the parent compound **1**. The "classical" heterobivalent ligands **18a**-**18c** in general showed reduced potencies at both receptors in comparison to analogs **11**, **17a** and **17b**, with **18b** being the exception exhibiting an IC_{50} value of 41 nM. There is no obvious correlation between linker length and the observed potency values. However, analog **18c** (molecule with the longest spacer) not only showed the lowest potency at both receptors but also exhibited a significant drop in efficacy of the dopamine response possibly indicating steric clashes with the receptor.

The triazine based analog **24** exhibited a 4-fold decrease in $A_{2A}R$ inhibitory potency compared to the $A_{2A}R$ antagonist ZM 241385 (**1**) while maintaining equipotent activity compared to the D_2R agonist ropiniole (**2**). Intermediate **23** showed comparable $A_{2A}R$ inhibitory potency to final compound **24**, however failed to exhibit any functional potency at the D_2 receptor. This result was expected as the ligand lacked the D_2 pharmacophore, therefore confirming that the introduction of the oxindole scaffold is responsible for D_2 agonism of molecule **24**.

The non-cyclic integrated dual acting ligands (compounds **32a-c** and **29**) in general showed a significant drop (28-54-fold) in inhibitory potency at the A_{2A} receptor, with the exception of compound **29** that was equipotent to ZM 241385 (**1**). The decrease in antagonism of analogs **32a**-**c** can potentially be explained by the introduction of an ionizable nitrogen atom at physiological pH whereas compound **29** does not contain this feature and therefore maintained antagonism. Conversely, compound **29** didn't exhibit any detectable activity at the D_2 receptor, which may be related to the lack of the ionizable nitrogen atom whereas dual acting ligands **32a**-**c** exhibited D_2R functional potencies ranging from 38 to 389 nM. Compounds **32b** and **32c** showed a respectable 3- and 8-fold gain in D_2R functional potency, respectively, compared to the parent compound **2**. The exchange of the propyl chain of

molecule **32b** with the methyl group in analog **32a** caused a decrease in functional potency at the D_2 receptor (note **32a** was equipotent to ropinirole (**2**)) which is not unexpected as literature shows that incorporation of a propyl chain in commonly used D_2 agonist on the market improves their D_2 affinity.[40-43] The cyclic monovalent (**35**, **36**, **38a**, **38b**, **39a** and **39b**) as well as the dual acting ligands (**37**, **40a** and **40b**) did not exhibit any detectable activity at the D_2 receptor, which is possibly due to the introduction of a constrained ring system not allowing sufficient flexibility for the molecule to interact with the D_2 receptor in a favorable pose. The inhibitory potencies at the A_{2A} receptor of monovalent compounds **33** and **34** as well as the dual acting ligands **37**, **40a** and **40b** were comparable to the non-cyclic analogs **32a-c** with compound **40a** being the stand out. It is noteworthy, that the physical properties of the integrated dual acting ligands (**29**, **32a-c**, **37**, **40a** and **40b**) with topological polar surface areas and clogPs ranging from 127-140 $Å^2$ and 1.65-4.19, respectively are very respectable considering that ZM 241385 (**1**) has a polar surface area of 127 $Å^2$ and clogP of 2.82.

Table 1. Reported are the calculated molecular weight (MW), topological polar surface area (tPSA) and partition coefficient (clogP) as well as the inhibitory potency at the adenosine A_{2A} receptor and the functional potency and efficacy at the dopamine D_2 receptor of the reference compounds, selected monovalent ligands as well as dual acting molecules. E_{max} values were referenced to quinelorane at 100 μM. All the active compounds showed full inhibition in the cAMP assay. Compounds with E_{max} less than 20% were considered as inactive (pXC_{50} <4).

Compound #	MW[a] [g/mol]	tPSA[a] [Å^2]	clogP[a]	$A_{2A}R$ inhibitory potency		D_2R functional potency and efficacy		
				pIC_{50} ± SEM	IC_{50} [nM]	pEC_{50} ± SEM	EC_{50} [nM]	E_{max} [%]
ZM 241385 (**1**)	337.34	127	2.82	7.48 ± 0.14[b]	33	<4[d]	>100,000	n.d.
Ropinirole (**2**)	260.38	32	2.49	n.d.	n.d.	6.52 ± 0.02[e]	304	74 ± 1[d]
11	708.82	195	3.76	7.45 ± 0.06[c]	35	6.32 ± 0.25[d]	484	79 ± 5
14c	572.84	100	5.83	<4[d]	>100,000	6.95 ± 0.22[d]	114	66 ± 0
17a	652.75	178	3.91	7.20 ± 0.10[c]	63	6.83 ± 0.01[d]	148	88 ± 0
17b	680.80	178	4.23	7.74 ± 0.10[c]	18	6.69 ± 0.10[d]	204	95 ± 1
18a	765.92	207	3.73	6.96 ± 0.18[b]	110	6.11 ± 0.02[d]	785	68 ± 0
18b	822.03	207	5.37	7.39 ± 0.13[b]	41	6.30 ± 0.08[d]	501	77 ± 1
18c	878.14	207	7.41	5.97 ± 0.15[d]	1072	5.86 ± 0.11[d]	1396	38 ± 5
19	634.78	172	6.87	6.36 ± 0.30[d]	437	<4[d]	>100,000	n.d.
23	535.95	168	3.71	6.87 ± 0.07[b]	135	<4[d]	>100,000	n.d.
24	774.87	212	4.87	6.92 ± 0.16[b]	120	6.51 ± 0.14[d]	311	65 ± 3
29	418.46	127	3.04	7.49 ± 0.16[d]	32	<4[d]	>100,000	n.d.
32a	447.50	140	2.02	5.95 ± 0.04[d]	1122	6.41 ± 0.22[d]	389	77 ± 2
32b	475.55	140	3.04	6.04 ± 0.08[c]	912	7.06 ± 0.09[f]	88	89 ± 4
32c	517.64	140	4.19	5.75 ± 0.04[d]	1778	7.43 ± 0.09[d]	38	77 ± 0
33	400.44	137	3.00	5.76 ± 0.07[c]	1738	n.d.	n.d.	n.d.
34	300.33	119	1.53	5.07 ± 0.07[c]	8511	n.d.	n.d.	n.d.
35	359.47	71	1.90	n.d.	n.d.	<4[d]	>100,000	n.d.
36	259.35	58	1.10	n.d.	n.d.	<4[d]	>100,000	n.d.
37	459.51	140	2.27	5.82 ± 0.15[d]	1514	<4[d]	>100,000	n.d.
38a	345.44	62	1.62	n.d.	n.d.	<4[d]	>100,000	n.d.
38b	359.46	62	1.95	n.d.	n.d.	<4[d]	>100,000	n.d.
39a	245.32	44	-0.19	n.d.	n.d.	<4[d]	>100,000	n.d.
39b	259.35	44	0.09	n.d.	n.d.	<4[d]	>100,000	n.d.
40a	445.48	131	1.65	7.23 ± 0.12[c]	59	<4[d]	>100,000	n.d.
40b	459.51	131	1.98	5.83 ± 0.13[d]	1479	<4[d]	>100,000	n.d.

[a]The physical properties were calculated with the software ChemSketch from ACD Lab; [b]Data represent the mean ± SEM of three experiments performed in duplicate; [c]Data represent the mean ± SEM of four separate experiments performed in duplicate; [d]Data represent the mean ± SEM of two experiments performed in duplicate; [e]Literature value (Ghosh *et al.*)[39], ropinirole (**2**) was not tested in this assay but the potency of the reference compound quinelorane (pEC_{50} of 7.35 ± 0.14 (EC_{50} = 45 nM)) is consistent with the literature[44] thereby permitting subsequent comparisons of test compounds to ropinirole (**2**); [f]Data represent the mean ± SEM of five separate experiments performed in duplicate; n.d. = not determined.

Prediction of *in vivo* CNS penetration

Selected analogs were tested in a brain:blood partition ratio (Kbb) assay using blood and brain tissue from a Winstar Han Rat. Selection criteria were based on the inclusion of analogs with different levels of integration (MW ranging from 775 to 445 g/mol), structural diversity (cyclic and non-cyclic analog), the availability of sufficient quantities for testing (>5 mg) and the requirement of being in solid from. The percent fraction unbound in blood and brain tissue was determined which indicates the percentage amount of unbound drug available to reach the site of action (results Table 2). Compounds that exhibited <1% fraction unbound were considered to be very highly protein bound. Further determined was the *in vitro* brain:blood partition ratio (Kbb) which is a prediction of *in vivo* CNS penetration (results Table 2).[45-47] As a general rule compounds with a Kbb value >1 have a greater likelihood to be CNS penetrant, due to their higher affinity for binding to brain tissue over blood components provided the compound have a high level of membrane permeability and is not a substrate of an active efflux transporter.[45-47]

The literature $A_{2A}R$ antagonist ZM 241385 (**1**) showed a Kbb value of 0.76 and moderate protein binding. The D_2R agonist ropinirole (**2**) was not included in the testing, but given it is an established D_2R agonist for the treatment of Parkinson's disease permits that the molecule is able to cross the blood-brain barrier. Dual acting ligand **24**, although having unfavorable physical properties (MW: 775 g/mol, tPSA: 212 $Å^2$, clogP: 4.87), exhibited a Kbb value of 3.65 this value potentially is driving a higher level of CNS penetrance based on the protein binding equilibrium. Clinically used CNS drugs, for example diazepam, exhibit similar Kbb values to compound **24**, therefore indicating a realistic change of compound **24** being CNS accessible.[48] Unfortunately, compound **24** is highly protein bound implying that only a limited amount of the free drug would reach the site of action when orally administered. The higher integrated "drug-like" dual acting ligands **32b** exhibited a Kbb value of 1.29 which corresponds to higher affinity for binding to brain tissue than blood components.

Compound **32b** also exhibited low protein binding suggesting a sufficient amount of unbound drug is able to reach the site of action in an *in vivo* model. The cyclic integrated dual acting ligand **40a** exhibited promising Kbb data, but its low potency at the D_2 receptor makes it an undesirable compound for further investigations. In summary, all the designed dual acting ligands that were tested (**24**, **32b** and **40a**) exhibited promising Kbb values even though their physical properties are at the upper limit (or above for compound **24**) considering common guidelines for the design of CNS drugs. Additional studies, for example a Madin Darby canine kidney - MDR1 gene assay, will be necessary to further assess both membrane permeability and P-glycoprotein (P-gp) liability of the presented compounds. Furthermore, the initial *in vitro* permeability data will need to be confirmed by *in vivo* brain uptake studies in rodents.

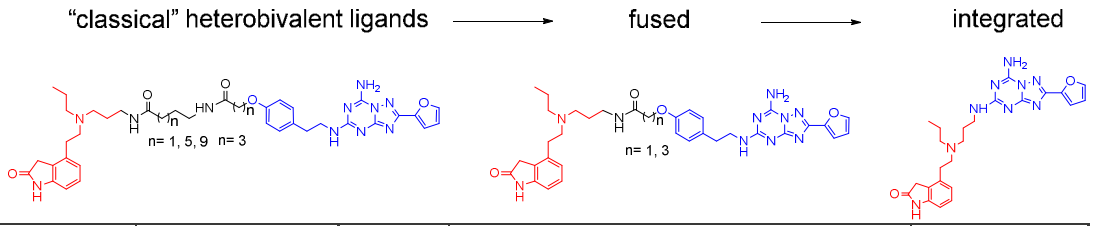
Table 2. Percent fraction unbound (Fu) in blood and brain tissue and the *in vitro* brain: blood partition ratio (Kbb) of selected analogs

Compound #	Species / Matrix	% Undiluted Fu Mean Value ± SD[a]	Kbb[a]
ZM 241385 (**1**)	Wistar Han Rat blood	6.47 ± 0.45	0.76
	Wistar Han Rat brain tissues	8.49 ± 0.23	
24	Wistar Han Rat blood	2.22 ± 0.086	3.64
	Wistar Han Rat brain tissues	0.61 ± 0.034	
32b	Wistar Han Rat blood	19.76 ± 3.16	1.29
	Wistar Han Rat brain tissues	15.33 ± 1.01	
40a	Wistar Han Rat blood	6.05 ± 0.18	1.07
	Wistar Han Rat brain tissues	5.63 ± 0.22	

[a]The experiments have been performed in triplicate.

CONCLUSION

Herein, we report the successful synthesis of a series of compounds ranging from classical heterobivalent ligands to integrated dual acting molecules based on the chemical structure of the D_2R agonist ropinirole (**1**) and the $A_{2A}R$ antagonist ZM 241385 (**2**) (Fig. 4). The most promising dual acting ligands maintained the potencies of the original pharmacophores at both receptors (adenosine A_{2A} and dopamine D_2). The results presented in this study for a set of dual acting ligands able to act at two distinct GPCRs represents a remarkable achievement considering the challenges associated with designing and synthesizing compounds to act at just one receptor system.



Synthesis:	19 steps	15 steps	10 - 12 steps
MW (g/mol):	878 - 766	709 - 652	518 - 448
tPSA:	207 - 212	195 - 178	140
cLogP:	7.4 - 3.7	4.2 - 3.8	4.2 - 2.0
pIC_{50} ($A_{2A}R$)	**7.4 - 6.0**	**7.7 - 6.9**	**6.0 - 5.8**
pEC_{50} (D_2R)	**6.3 - 5.9**	**6.8 - 6.3**	**7.4 - 6.4**

Figure 4. Overview of some of the most promising dual acting ligands analogs including chemical structures, physical structures as well as the inhibitory and functional potencies at the adenosine A_{2A} and dopamine D_2 receptors, respectively.

The "classical" heterobivalent ligands (**18a-18c**) exhibited very respectable potencies at both receptors and therefore, are useful pharmacological tools to probe the aforementioned receptor systems as monomers or potentially heterodimers, however, they are labor intensive to synthesize and have unfavorable physical properties for a potential CNS drug. Heterobivalent ligand **18c**, containing the longest spacer in our series, exhibited a substantial

drop in the inhibitory potency at the A_{2A} receptor and efficacy at the D_2 receptor. It remains open if heterobivalent ligands incorporating longer spacers would continue this trend or potentially recover the inhibitory potency at the A_{2A} receptor and efficacy at the D_2 receptor. The removal of the spacer reduced the number of synthetic steps and the molecular weight slightly while improving the functional potency at both receptors to some extent. Unlike the classical heterobivalent ligands (**18a-18c**), the analogs without a spacer (**11**, **17a** and **17b**) not only maintained the equivalent inhibitory potency of ZM 241385 (**1**) at the A_{2A} receptor but also the functional activity of ropinirole (**2**) at the D_2 receptor. The active non-cyclic integrated dual acting ligands (**32a-c**) were relatively more straightforward to synthesize from commercially available starting materials mainly due to the fact that the triazolotriazine portion was introduced via the sulfone intermediate **28**. As a result, the more labor intensive synthesis of a TCAC (compound **3** or **16**) compared to sulfone **28** was circumvented. Compounds **32a-c** exhibited EC_{50} values at the D_2 receptor ranging from equivalent to a 8-fold increased functional potency in comparison to ropinirole while the inhibitory potency at the A_{2A} receptor was still respectable but approximately 1.5 orders of magnitude weaker than ZM 241385 (**1**). Compounds **32a-c** have significantly improved physical properties, but are admittedly still at the upper limit for a potential CNS drug. Nevertheless, it should be pointed out that there are CNS drugs with similar properties on the market.[49] More importantly, initial permeability testing of selected analogs exhibited promising Kbb values indicating that these molecules might be able to cross the blood-brain barrier. Compound **24** is a very interesting molecule since it is highly potent at the A_{2A} and D_2 receptors (pIC_{50} 6.92 and pEC_{50} 6.51, respectively). It should be noted that, with a molecular weight of 775 g/mol, topological polar surface area of 212 $Å^2$ and a partition coefficient of 4.87, compound **24** does not display ideal physical properties however exhibited a promising Kbb value of 3.65.

To our knowledge, these are the first "drug-like" dual acting ligands successfully targeting the adenosine A_{2A} and dopamine D_2 receptors.[50] These dual acting ligands with relatively low

molecular weight are potential lead compounds in drug discovery. An important question that remains is if these dual acting molecules provide a benefit to patients with Parkinson's disease over the single administration of D_2R agonists in the clinic. Therefore, it would be interesting to compare the dual acting ligands **11**, **24**, **32a** and the D_2R agonist ropinirole (**2**), which all exhibit comparable functional potency and efficacy at the D_2 receptor, but differ in their inhibitory potency at the adenosine A_{2A} receptor, in an appropriate animal model. A study of this type would also provide an insight into what inhibitory potency is needed at the A_{2A} receptor to observe a beneficial therapeutic effect in an animal model. Consequently, the integrated analogs that exhibit a lower pIC_{50} value compared to ZM 241385 (**1**) are potentially as beneficial for the treatment of Parkinson's disease as molecules that have better inhibitory potency at the A_{2A} receptor (compound **24** and **11**). Future studies would include the determination of the subtype selectivity for the different adenosine and dopamine receptors, allowing a comparison of the selectivity profile of the presented dual acting ligands with the monovalent pharmacophores. This information can potentially lead to a better understanding of the properties of the different subtypes as well as improve the design of more selective D_2R and $A_{2A}R$ agonists and antagonists, respectively.

EXPERIMENTAL SECTION

General Information All reactions were stirred magnetically in oven-dried glassware. Anhydrous solvents were transferred via oven-dried syringe or cannula. Technical grade solvents used for extraction and column chromatography were distilled prior to use. Absolute solvents were used without further purification. The ropinirole hydrochloride salt was purchased from Betapharma Shanghai Co., Ltd. and 4-(2-hydroxyethyl)indolin-2-one was purchased from China Langchem Inc. and *tert*-butyl (6-bromohexyl)carbamate from Astatech Inc. All the other reagents were purchased from Merck, TCI Chemicals, Aldrich or AK Scientific in the highest available grade and used without further purification. Analytical thin layer chromatography (TLC) plates from Merck were used for reaction control (silica gel 60 on aluminum sheets). Silica gel 60 (Fluka) was used for silica gel flash chromatography. Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Bruker spectrometers Avance 400 (400 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR) at ambient temperature in the solvents indicated and referenced to tetramethylsilane (TMS). ^{13}C NMR spectra were routinely run with broadband decoupling. Distortionless enhancement by polarization transfer (DEPT) experiments were routinely used for ^{13}C NMR spectra. Chemical shifts (δ) are reported in parts per million (ppm). Coupling constants (*J*) are reported in Hertz (Hz). The following abbreviations are used: s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Characterization of known compounds has been in accordance to literature and references have been provided for relevant compounds in the Experimental Section and Supporting Information. High resolution mass spectra (HRMS) were obtained on a Waters LCT Premier XE (TOF) spectrometer fitted with an electrospray ion source. Mass signals are given in mass units per charge (*m/z*). The fragments and intensities are written in brackets. Liquid Chromatography Mass Spectra (LCMS) were measured on either two instruments. An Agilent 6100 Series Single Quad LC/MS, Agilent 1200 Series HPLC. (Pump: 1200 Series G1311A

Quaternary pump, Autosampler: 1200 Series G1329A Thermostatted Autosampler, Detector: 1200 Series G1314B Variable Wavelength Detector). Gradient takes 4 minutes to get to 100% acetonitrile; maintain for 3 minutes and a further 3 minutes to get back to the original 5% acetonitrile. An Agilent 1290 Infinity (Agilent, Palo Alto, CA); Ionisation mode: Electrospray Ionisation. Chromatographic separation was performed using an Agilent Zorbax SB-C18 Rapid Resolution HT 2.1 × 50 mm, 1.8 μm column (Agilent Technologies, Palo Alto, CA) using an acetonitrile gradient (5% to 100%) over 3.5 min at 0.5 mL/min. Solvent A = Aqueous 0.1% Formic Acid; Solvent B = Acetonitrile/0.1% Formic Acid. Preparative HPLC was performed on a Agilent 1260 infinity coupled with binary prep pump and Agilent 1260 FC-PS fraction collector. It operates on Agilent OpenLAB CDS Rev C.01.04 software. The column was an Alltima C85u 22 mm × 250 mm. Analytical HPLC was performed on an Agilent 1260 Infinity. It was operated on Agilent OpenLAB CDS Rev C.01.04 software. The column used was a Poroshell 120 SB-C18 4.6 × 100 mm, 2.7 μm. All the final compounds showed a single peak at the designated retention time and are at least 95% pure.

All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.

NMR analysis: Compound which include a triazolotriazine moiety show doubling of some signals in the ^{1}H and ^{13}C NMR spectra due to dimer formation and/ or hindered rotation. For details refer to the extensive NMR study of the adenosine $A_{2A}R$ antagonist ZM 241385 (**1**) by our group that was previously published.[23]

Synthesis

5-(4-(2-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl) phenoxy)-5-oxopentanoic acid (10)

4-(2-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenol (ZM 241385) (**1**) (50.0 mg, 148 μmol) and glutaric anhydride (**9**) (18.6 mg, 163 μmol) was suspended in toluene (4 mL). The reaction mixture was stirred at reflux for 18 h before TEA (100 μL, 717 μmol) was added. Another 5 equivalent of glutaric anhydride were added in portions while the reaction mixture was stirred for another 29 h at rt. The reaction mixture was then partitioned between ethyl acetate, toluene and brine. The organic layer was washed with water and dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The crude material was purified by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 9:1) to afford the title compound **10** (19 mg, 28%) as a white solid. ^{1}H NMR (d_6-DMSO) δ 12.17 (br s, 1H), 8.30 (2 × br s, ratio 1:6, 2H), 7.87 (m, 1H), 7.52 (2 × br t, J = 5.6 Hz, 1H), 7.29 (m, 2H), 7.11 – 6.99 (m, 3H), 6.68 (m, 1H), 3.49 (m, 2H), 2.87 (m, 2H), 2.61 (t, J = 7.4 Hz, 2H), 2.34 (t, J = 7.2 Hz, 2H), 1.85 (m, 2H). Some of the ^{13}C signal show up twice, the signal with lower intensity is marked with an asterisk (*) ^{13}C NMR (d_6-DMSO) δ 174.0 (C), 171.5 (C), 161.1 (C), 159.2 (C), 155.8 (C), 150.0 (C), 148.7 (C), 146.2 (C), 144.6 (CH), 137.1 (C), 129.6 (CH), 121.6 (CH), 111.9 (CH), 111.6 (CH), 42.7 (CH_2*), 42.1 (CH_2), 34.5 (CH_2*), 34.0 (CH_2), 32.7 (CH_2), 32.6 (CH_2), 19.8 (CH_2). LCMS: *m/z* (ESI 20 V) 452.2 (MH^+, 100).

4-(2-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenyl 5-oxo-5-((3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)amino)pentanoate (11)

4-(2-((3-Aminopropyl)(propyl)amino)ethyl)indolin-2-one (**5**) (12.2 mg, 44.3 μmol) and 5-(4-(2-((7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy)-5-oxopentanoic acid (**10**) (20.0 mg, 44.3 μmol) were dissolved DMF (4 mL) and DIPEA

(11.6 µL, 66.5 µmol) and 1-(bis(dimethylamino)methylene)-5-chloro-1*H*-benzo[*d*][1,2,3]triazole-1-ium 3-oxide hexafluorophosphate (V) (HCTU) (18.3 mg, 44.3 µmol) were added and the reaction mixture was stirred at rt for 3 h. The volatile components were removed under reduced pressure. The residue was re-dissolved in ethyl acetate and washed with 1 M aqueous potassium carbonate solution. The organic layer was dried with anhydrous sodium sulfate, filtered and evaporated to dryness. The crude material was purified by column chromatography (ethyl acetate → ethyl acetate: CH_3OH 1:1) to afford the title compound **11** (3 mg, 10%) as a purple oil. ^{1}H NMR (d_6-DMSO) δ 10.35 (br s, 1H), 8.60 – 7.95 (2 × br s, ratio 1:6, 2H), 7.85 (m, 1H), 7.83 (t, J = 5.5 Hz, 1H), 7.62 (2 × br t, ratio 1:2, J = 5.7 Hz, 1H), 7.27 (m, 2H), 7.11 – 7.01 (m, 4H), 6.76 (m, 1H), 6.68 (m, 1H), 6.64 (m, 1H), 3.49 (m, 2H), 3.43 (s, 2H), 3.06 (m, 2H), 2.86 (m, 2H), 2.58 (s, 4H), 2.54 (m, 2H), 2.44 (m, 2H), 2.37 (m, 2H), 2.16 (t, J = 7.3 Hz, 2H), 1.84 (m, 2H), 1.52 (m, 2H), 1.37 (m, 2H), 0.82 (t, J = 7.4 Hz, 3H). LCMS: *m/z* (ESI 20 V) 707.4 (MH^-, 100). HPLC: t_R 7.83 min, >95% (214 nm), >97% (254 nm). HRMS ($C_{37}H_{44}N_{10}O_5$): Calcd. 709.3574 $[M+H]^+$, Found 709.3558.

4-((*tert*-Butoxycarbonyl)amino)butanoic acid (13a)

4-Aminobutanoic acid (**12a**) (1.00 g, 9.70 mmol) was dissolved in THF (15 mL) and 1 M aqueous sodium hydroxide solution (10 mL) and di-*tert*-butyl dicarbonate (2.75 g, 12.61 mmol) were added at 0 °C before the reaction mixture was allowed to warm up to rt and stirred for another 3 h. The solvent was then evaporated under reduced pressure. The residue was taken up in water and extracted with diethyl ether. The aqueous phase was acidified with 1 M aqueous hydrochloric acid solution and quickly extracted with diethyl ether. The combined organic layers were dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. Purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 95:5) gave the title compound **13a** (1.30 g, 66%) as a colorless oil. ^{1}H NMR

($CDCl_3$) δ 10.00 (br s, 1H), 4.75 (br s, 1H), 3.19 (m, 2H), 2.40 (t, *J* = 7.2 Hz, 2H), 1.83 (p, *J* = 7.0 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 178.4 (C), 156.6 (C), 79.7 (C), 40.0 (CH_2), 31.3 (CH_2), 28.3 (CH_3), 25.1 (CH_2).

8-((*tert*-Butoxycarbonyl)amino)octanoic acid (13b)

8-Aminooctanoic acid (**12b**) (292 mg, 1.83 mmol) was suspended in CH_3OH (25 mL). TEA (365 µL, 2.75 mmol) and a solution of di-*tert*-butyl dicarbonate (800 mg, 3.67 mmol) in CH_3OH (5 mL) were added and the reaction mixture was heated up to 60 °C and stirred for 2 h before the solvent was removed under reduced pressure. The residue was taken up in $CHCl_3$ and washed quickly with 1 M aqueous hydrochloric acid solution. The organic phase was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. Purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 9:1) gave the title compound **13b** (383 mg, 81%) as a white solid; mp: 82-85 °C. ^{1}H NMR ($CDCl_3$) δ 4.56 (s, 1H), 3.10 (m, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 1.62 (m, 2H), 1.46 (m, 11H), 1.32 (m, 6H).

12-((*tert*-Butoxycarbonyl)amino)dodecanoic acid (13c)

12-Aminododecanoic acid (**12c**) (860 mg, 4.00 mmol) was suspended in CH_3OH (40 mL). TEA (796 µL, 5.99 mmol) and a solution of di-*tert*-butyl dicarbonate (1.74 g, 7.99 mmol) in CH_3OH (10 mL) were added. The reaction mixture was heated up to 60 °C and stirred for 1.5 h before the solvent was removed under reduced pressure. The residue was taken up in $CHCl_3$ and extracted quickly with 1 M aqueous hydrochloric acid solution. The organic phase was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. Purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 95:5) gave the title compound **13c** (860 mg, 68%) as a white solid; mp: 59-61 °C. ^{1}H NMR ($CDCl_3$) δ 4.53 (s, 1H), 3.10 (m, 2H), 2.34 (t, *J* = 7.4 Hz, 2H), 1.68 – 1.56 (m, 2H), 1.44 (m, 11H), 1.27 (m, 14H).

***tert*-Butyl (4-oxo-4-((3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)amino)butyl) carbamate (14a)**

(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) (299 mg, 699 μmol) was added to a mixture of 4-(2-((3-aminopropyl)(propyl)amino)ethyl)indolin-2-one (**5**) (192 mg, 699 μmol), 8-((*tert*-butoxycarbonyl)amino)butanoic acid (**13a**) (142 mg, 699 μmol) and DIPEA (51.1 mg, 699 μmol) in DMF (2 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h then for another 2 h at rt. The reaction mixture was diluted with ethyl acetate and washed with 1 M aqueous hydrochloric acid solution, saturated aqueous sodium hydrogen carbonate solution and brine. The organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. Purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 8:2) gave the title compound **14a** (10 mg, 3%) as a yellow oil. The water layer was evaporated to dryness. The residue was suspended in CH_3OH and filtered. The filtrate was evaporated to dryness and purification column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 8:1) gave the title compound **14a** (101 mg, 31%) as a yellow solid. ^{1}H NMR (d_6-Acetone) δ 9.44 (br s, 1H), 7.85 (br s, 1H), 7.19 (t, J = 7.8 Hz, 1H), 6.96 (d, J = 7.7 Hz, 1H), 6.83 (d, J = 7.7 Hz, 1H), 6.10 (br s, 1H), 3.56 (s, 2H), 3.49 (m, 2H), 3.43 – 3.30 (m, 6H), 3.17 – 3.08 (m, 4H), 2.34 (t, J = 7.2 Hz, 2H), 2.14 – 2.04 (m, 2H), 1.95 – 1.85 (m, 2H), 1.85 – 1.73 (m, 2H), 1.47 – 1.33 (m, 9H), 1.05 (t, J = 7.4 Hz, 3H). LCMS: *m/z* (ESI 20 V) 461.4 (MH^+, 100).

***tert*-Butyl (8-oxo-8-((3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)amino)octyl)carbamate (14b)**

(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) (301 mg, 702 μmol) was added to a mixture of 4-(2-((3-

aminopropyl)(propyl)amino)ethyl)indolin-2-one (**5**) (193 mg, 702 μmol), 8-((*tert*-butoxycarbonyl)amino)octanoic acid (**13b**) (182 mg, 702 μmol) and DIPEA (51.3 mg, 702 μmol) in DMF (2 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h then for another 2 h at rt. The reaction mixture was diluted with ethyl acetate and washed with 1 M aqueous hydrochloric acid solution, saturated aqueous sodium hydrogen carbonate solution and brine. The organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. Purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 8:2) gave the title compound **14b** (82 mg, 23%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 8.56 (br s, 1H), 7.11 (t, J = 7.6 Hz, 1H), 6.87 (br s, 1H), 6.82 (d, J = 7.7 Hz, 1H), 6.75 (d, J = 7.6 Hz, 1H), 4.89 (br s, 1H), 3.47 (s, 2H), 3.27 (m, 2H), 3.10 – 2.60 (m, 10H), 2.15 (m, 2H), 1.81 (m, 2H), 1.68 – 1.50 (m, 4H), 1.43 (m, 11H), 1.33 – 1.51 (m, 6H), 0.95 (t, J = 7.1 Hz, 3H). LCMS: *m/z* (ESI 20 V) 517.4 (MH^+, 100).

***tert*-Butyl (12-oxo-12-((3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)amino)dodecyl) carbamate (14c)**

(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) (260 mg, 609 μmol) was added to a mixture of 4-(2-((3-aminopropyl)(propyl)amino)ethyl)indolin-2-one (**5**) (192 mg, 609 μmol), 12-((*tert*-butoxycarbonyl)amino)dodecanoic acid (**13c**) (168 mg, 609 μmol) and DIPEA (44.5 mg, 609 μmol) in DMF (4 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h then for another 2 h at rt. The reaction mixture was diluted with ethyl acetate and washed with 1 M aqueous hydrochloric acid solution, saturated aqueous sodium hydrogen carbonate solution and brine. The organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. Purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 8:2) gave the title compound **14c** (144 mg, 41%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 8.83 (br s, 1H), 7.12 (t, J = 7.8 Hz, 1H), 6.88 (br s, 1H), 6.82 (d, J = 7.8

Hz, 1H), 6.75 (d, *J* = 7.7 Hz, 1H), 4.61 (br s, 1H), 3.47 (s, 2H), 3.30 (m, 2H), 3.08 (m, 2H), 2.82 (m, 2H), 2.75 (m, 4H), 2.62 (m, 2H), 2.13 (t, *J* = 7.6 Hz, 2H), 1.73 (m, 2H), 1.63 – 1.51 (m, 4H), 1.44 (m, 11H), 1.33 – 1.51 (m, 14H), 0.93 (t, *J* = 7.3 Hz, 3H). LCMS: *m/z* (ESI 20 V) 573.5 (MH^+, 100). HPLC: t_R 7.36 min, >99% (254 nm). HRMS ($C_{33}H_{56}N_4O_4$): Calcd. 573.4380 $[M+H]^+$, Found 573.4389.

4-Amino-*N*-(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)butanamide (15a)

Trifluoroacetic acid (1 mL) was added to solution of *tert*-butyl (4-oxo-4-((3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)amino)butyl)carbamate (**14a**) (101 mg, 217 μmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at rt for 9 h before the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and 1 M aqueous sodium hydroxide solution was added until pH-14. The aqueous layer was extracted with CH_2Cl_2, then the combined organic layers were dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The titled compound **15a** (53.9 mg, 69%) was obtained as a yellow oil. The compound **15a** was suspected to be labile and therefore immediately reacted further to furnish analog **18a** to avoid potential degradation. LCMS: *m/z* (ESI 20 V) 361.3 (MH^+, 80).

8-Amino-*N*-(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)octanamide (15b)

Trifluoroacetic acid (1 mL) was added to solution of *tert*-butyl (8-oxo-8-((3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)amino)octyl)carbamate (**14b**) (85 mg, 165 μmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at rt for 2.5 h before the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and 1 M aqueous sodium hydroxide solution was added until pH-14. The aqueous layer was extracted with CH_2Cl_2, then the combined organic layers were dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The title compound **15b** (68.0 mg, 99%)

was obtained as a yellow oil. The compound **15b** was suspected to be labile and therefore immediately reacted further to furnish analog **18b** to avoid potential degradation. LCMS: *m/z* (ESI 20 V) 417.4 (MH^+, 40).

12-Amino-*N*-(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)dodecanamide (15c)

Trifluoroacetic acid (1 mL) was added to solution of *tert*-butyl (12-oxo-12-((3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)amino)dodecyl) carbamate (**14c**) (117 mg, 204 μmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at rt for 2.5 h before the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and 1 M aqueous sodium hydroxide solution was added until pH-14. The aqueous layer was extracted with CH_2Cl_2, then the combined organic layers were dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The title compound **15c** (37.6 mg, 39%) was obtained as a yellow oil. The compound **15c** was suspected to be labile and therefore immediately reacted further to furnish analog **18c** to avoid potential degradation. LCMS: *m/z* (ESI 20 V) 473.4 (MH^+, 30).

2-(4-(2-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy)-*N*-(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)acetamide (17a)

4-(2-((3-Aminopropyl)(propyl)amino)ethyl)indolin-2-one (**5**) (48.8 mg, 177 μmol) and 2-(4-(2-((7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy)acetic acid (**16**) (70.0 mg, 177 μmol) were dissolved DMF (4 mL) and DIPEA (46.3 μL, 266 μmol) and ((1*H*-benzo[d][1,2,3]triazol-1-yl)oxy)tris(dimethylamino)phosphonium hexafluorophosphate (V) (BOP) (78.3 mg, 177 μmol) were added and the reaction mixture was stirred at rt for 5 h. The volatile compounds were removed under reduced pressure. The residue was dissolved in ethyl acetate

and washed with 1M aqueous potassium carbonate solution. The organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The crude material was purified by column chromatography (ethyl acetate → ethyl acetate: CH_3OH 7:3). The title compound **17a** (8.3 mg, 7%) was obtained as a purple oil. ^{1}H NMR (d_6-DMSO) δ 10.32 (s, 1H), 8.50 – 7.95 (2 × br s, ratio 1:6, 2H), 8.09 (t, J = 5.6 Hz, 1H), 7.87 (m, 1H), 7.55 – 7.40 (2 × br t, ratio 1:2, J = 5.7 Hz, 1H), 7.17 (m, J = 8.4 Hz, 2H), 7.10 – 7.03 (m, 2H), 6.89 (m, 2H), 6.76 (d, J = 7.7 Hz, 1H), 6.68 (m, 1H), 6.62 (d, J = 5.7 Hz, 1H), 4.42 (s, 2H), 3.50 – 3.38 (m, 4H), 3.16 (q, J = 6.6 Hz, 2H), 2.78 (m, 2H), 2.57 (m, 4H), 2.44 (t, J = 6.9 Hz, 2H), 2.34 (m, 2H), 1.57 (m, 2H), 1.38 (m, 2H), 0.82 (t, J = 7.3 Hz, 3H). Some of the ^{13}C signal show up twice, the signal with lower intensity is marked with an asterisk (*) ^{13}C NMR (d_6-DMSO) δ 176.2 (C), 167.5 (C), 161.1 (C), 159.2 (C), 156.1 (C), 155.8 (C), 150.0 (C), 146.2 (C), 144.6 (CH), 143.3 (C), 134.0 (C), 132.2 (C), 129.6 (CH), 127.4 (CH), 124.4 (C), 121.7 (CH), 114.6 (CH), 111.9 (CH), 111.6 (CH), 106.8 (CH), 67.1 (CH_2), 55.3 (CH_2), 53.5 (CH_2), 51.1 (CH_2), 42.9 (CH_2*), 42.3 (CH_2), 37.1 (CH_2), 34.5 (CH_2), 34.3 (CH_2*), 33.9 (CH_2), 29.8 (CH_2), 26.6 (CH_2), 19.9 (CH_2), 11.8 (CH_3). LCMS: *m/z* (ESI 20 V) 651.4 (MH^-, 100). HPLC: t_R 7.41 min, >99% (214 nm), >99% (254 nm). HRMS ($C_{34}H_{40}N_{10}O_4$): Calcd. 653.3312 $[M+H]^+$, Found 653.3294.

3-(4-(4-(2-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy)butanamido)-*N*-(2-(2-oxoindolin-4-yl)ethyl)-*N*-propylpropan-1-aminium 2,2,2-trifluoroacetate (17b)

4-(2-((3-Aminopropyl)(propyl)amino)ethyl)indolin-2-one (**5**) (50.1 mg, 182 µmol) and 4-(4-(2-((7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy)butanoic acid (**3**) (77.0 mg, 182 µmol) were dissolved DMF (4 mL) and DIPEA (47.5 µL, 273 µmol) and 1-(bis(dimethylamino)methylene)-5-chloro-1*H*-benzo[*d*][1,2,3]triazole-1-ium 3-oxide hexafluorophosphate (V) (HCTU) (75.2 mg, 182 µmol)

was added and the reaction mixture was stirred at rt for 3 h. The volatile compounds were removed under reduced pressure. The residue was purified by column chromatography (ethyl acetate → ethyl acetate: CH_3OH 35:65). The combined collected fractions were dried under reduced pressure and the residue was re-dissolved in ethyl acetate and washed with 1 M aqueous potassium carbonate solution. The organic layer was dried with anhydrous sodium sulfate, filtered and evaporated to dryness. Preparative HPLC was used to afford the title compound **17b** (30 mg, 24%) as a yellowish foam. Quoted NMRs are from the free base after column chromatography and washing with base. ^{1}H NMR (d_6-DMSO) δ 10.3 (s, 1H), 8.50 – 7.95 (2 × br s, ratio 1:6, 2H), 7.87 (m, 1H), 7.82 (t, J = 5.4 Hz, 1H), 7.57 – 7.42 (2 × br t, ratio 1:2, J = 5.7 Hz, 1H), 7.14 (m, 2H), 7.10 – 7.03 (m, 2H), 6.84 (m, 2H), 6.76 (d, J = 7.2 Hz, 1H), 6.67 (m, 1H), 6.64 (d, J = 7.6 Hz, 1H), 3.92 (t, J = 6.4 Hz, 2H), 3.50 – 3.37 (m, 4H), 3.06 (q, J = 6.7 Hz, 2H), 2.78 (m, 2H), 2.57 (m, 4H), 2.43 (t, J = 7.1 Hz, 2H), 2.36 (t, J = 7.8 Hz, 2H), 2.22 (t, J = 7.4 Hz, 2H), 1.92 (m, 2H), 1.53 (m, 2H), 1.38 (m, 2H), 0.81 (t, J = 7.3 Hz, 3H). Some of the ^{13}C signal show up twice, the signal with lower intensity is marked with an asterisk (*) ^{13}C NMR (d_6-DMSO) δ 176.2 (C), 171.3 (C), 161.1 (C), 159.2 (C), 156.9 (C), 155.8 (C), 150.0 (C), 146.2 (C), 144.6 (CH), 143.4 (C), 136.6 (C), 131.4 (C), 129.5 (CH), 127.4 (CH), 124.4 (C), 121.7 (CH), 114.3 (CH), 111.9 (CH), 111.6 (CH), 106.8 (CH), 66.8 (CH_2), 55.3 (CH_2), 53.6 (CH_2), 50.9 (CH_2), 42.9 (CH_2*), 42.4 (CH_2), 36.9 (CH_2), 34.5 (CH_2), 34.3 (CH_2*), 33.8 (CH_2), 31.8 (CH_2), 29.9 (CH_2), 26.9 (CH_2), 25.0 (CH_2), 20.0 (CH_2), 11.8 (CH_3). LCMS: *m/z* (ESI 20 V) 681.5 (MH^+, 100), 679.5 (MH^-, 100). HPLC: t_R 7.79 min, >99% (214 nm), >99% (254 nm). HRMS ($C_{36}H_{44}N_{10}O_4$): Calcd. 681.3625 $[M+H]^+$, Found 681.3627.

4-(4-(2-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy)-*N*-(4-oxo-4-((3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)amino)butyl)butanamide (18a)

(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) (45.0 mg, 133 μmol) was added to a mixture of 4-amino-*N*-(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)butanamide (**15a**) (47.9 mg, 133 μmol), 4-(4-(2-((7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy)butanoic acid (**3**) (45.0 mg, 106 μmol) and DIPEA (9.71 mg, 133 μmol) in DMF (2 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h then for another 9 h at rt. The reaction mixture was diluted with ethyl acetate and washed with 1 M aqueous hydrochloric acid solution, saturated aqueous sodium hydrogen carbonate solution and brine. The water layer was evaporated to dryness. The residue was taken up in CH_3OH and the suspension was filtered. The filtrate was evaporated to dryness and purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 8:2) gave the title compound **18a** (22.3 mg, 17%) as a white resin. ^{1}H NMR (d_6-DMSO) δ 10.46 (s, 1H), 8.51 – 7.95 (m, 4H), 7.87 (m, 1H), 7.57 – 7.44 (2 × br t, ratio 1:2, J = 5.7 Hz, 1H), 7.14 (m, 2H), 7.70 (m, 1H), 7.05 (m, 1H), 6.86 – 6.78 (m, 3H), 6.70 (m, 1H), 6.67 (m, 1H), 3.90 (t, J = 6.4 Hz, 2H), 3.51 (s, 2H), 3.44 (m, 2H), 3.21 – 2.56 (m, 14H), 2.22 (t, J = 7.4 Hz, 2H), 2.09 (t, J = 7.4 Hz, 2H), 1.90 (m, 2H), 1.83 – 1.44 (m, 6H), 0.87 (t, J = 7.1 Hz, 3H). LCMS: *m/z* (ESI 20 V) 766.5 (MH^+, 5). HPLC: t_R 5.25 min, 99% (214 nm), 99% (254 nm). HRMS ($C_{10}H_{51}N_{11}O_5$): Calcd. 383.7116 $[M+2H]^{2+}$, Found 383.7121; Calcd. 766.4153 $[M+H]^+$, Found 766.4141.

8-(4-(4-(2-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy) butanamido)-*N*-(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)octanamide (18b)

(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) (69.5 mg, 162 μmol) was added to a mixture of 8-amino-*N*-(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)octanamide (**15b**) (67.7 mg, 162 μmol), 4-(4-(2-((7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy)butanoic acid (55.0 mg, 130 μmol) and DIPEA (11.9 mg, 162 μmol) in DMF (2 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h then for another 4 h at rt. The reaction mixture was diluted with ethyl acetate and washed with 1 M aqueous hydrochloric acid solution, saturated aqueous sodium hydrogen carbonate solution and brine. The water layer was evaporated to dryness. The residue was taken up in CH_3OH and the suspension was filtered. The filtrate was evaporated to dryness and purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 8:2) gave the title compound **18b** (22.3 mg, 17%) as a white resin. ^{1}H NMR (d_6-DMSO) δ 10.34 (s, 1H), 8.50 – 7.95 (2 × br s, ratio 1:6, 2H), 7.87 (m, 1H), 7.82 (t, *J* = 5.5 Hz, 1H), 7.76 (br s, 1H), 7.47 (2 × br t, ratio 1:2, *J* = 5.6 Hz, 1H), 7.15 (m, 2H), 7.11 – 7.03 (m, 2H), 6.84 (m, 2H), 6.77 (m, 1H), 6.69 – 6.62 (m, 2H), 3.91 (t, *J* = 6.3 Hz, 2H), 3.45 (m, 4H), 3.10 – 2.96 (m, 4H), 2.78 (m, 2H), 2.67 – 2.29 (m, 8H), 2.21 (t, *J* = 7.4 Hz, 2H), 2.03 (t, *J* = 7.3 Hz, 2H), 1.96 – 1.83 (m, 2H), 1.61 – 1.30 (m, 8H), 1.22 (m, 6H), 0.83 (t, *J* = 7.2 Hz, 3H). LCMS: *m/z* (ESI 20 V) 822.6 (MH^+, 5). HPLC: t_R 5.71 min, 99% (214 nm), 99% (254 nm). HRMS ($C_{44}H_{59}N_{11}O_5$): Calcd. 411.7439 $[M+2H]^{2+}$, Found 411.7438; Calcd. 822.4779 $[M+H]^+$, Found 822.4774.

3-(12-(4-(4-(2-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy) butanamido)dodecanamido)-*N*-(2-(2-oxoindolin-4-yl)ethyl)-*N*-propylpropan-1-aminium 2,2,2-trifluoroacetate (18c)

(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) (40.5 mg, 94.5 μmol) was added to a mixture of 12-amino-*N*-(3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)dodecanamide (**15c**) (44.7 mg, 94.5 μmol), 4-(4-(2-((7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy)butanoic acid (**3**) (40.0 mg, 94.5 μmol) and DIPEA (6.9 mg, 94.5 μmol) in DMF (2 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h then for another 2 h at rt. The reaction mixture was diluted with ethyl acetate and washed with 1 M aqueous hydrochloric acid solution, saturated aqueous sodium hydrogen carbonate solution and brine. The organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. Purification by preparative column chromatography gave the title compound **18c** (7 mg, 8%) as a white solid. ^{1}H NMR (d_6-DMSO) δ 10.43 (s, 1H), 9.29 (br s, 1H), 8.50 – 8.00 (2 × br s, ratio 1:6, 2H), 7.96 (t, J = 5.8 Hz, 1H), 7.87 (m, 1H), 7.80 (t, J = 5.6 Hz, 1H), 7.51 (2 × br t, ratio 1:2, J = 5.6 Hz, 1H), 7.19 – 7.12 (m, 3H), 7.06 (m, 1H), 6.87 – 6.84 (m, 3H), 6.73 (m, 1H), 6.68 (m, 1H), 3.91 (t, J = 5.6 Hz, 2H), 3.55 (m, 2H), 3.40 (m, 2H), 3.25 (m, 2H), 3.16 – 3.07 (m, 6H), 3.02 (m, 2H), 2.86 (m, 2H), 2.78 (m, 2H), 2.21 (t, J = 7.5 Hz, 2H), 2.07 (t, J = 7.4 Hz, 2H), 1.91 (m, 2H), 1.79 (m, 2H), 1.66 (m, 2H), 1.48 (m, 2H), 1.36 (m, 2H), 1.23 (m, 14H), 0.93 (t, J = 7.3 Hz, 3H). LCMS: *m/z* (ESI 20 V) 878.8 (MH^+, 5). HPLC: t_R 6.50 min, >99% (214 nm), >99% (254 nm). HRMS ($C_{48}H_{67}N_{11}O_5$): Calcd. 439.7742 $[M+2H]^{2+}$, Found 439.7734; Calcd. 878.5405 $[M+H]^+$, Found 878.5363.

Methyl 12-aminododecanoate

Conc. H_2SO_4 (0.5 mL) was added to a suspension of 12-aminododecanoic acid (1.00 g, 4.64 mmol) in CH_3OH (20 mL). The reaction mixture was stirred at reflux for 24 h before the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and water was added, the white precipitate was filtered off and washed with water. The filter cake was dried under high vacuum. The title compound was obtained (750 mg, 70%) as a white solid; mp: 96-98 °C. ^{1}H NMR (d_6-DMSO) δ 3.58 (s, 3H), 2.52 – 2.48 (m, 2H), 2.29 (t, J = 7.4 Hz, 2H), 1.51 (m, 2H), 1.31 (m, 2H) 1.37 – 1.16 (m, 14H).

Methyl 12-(4-(4-(2-((7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy)butanamido)dodecanoate (19)

(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) (101 mg, 236 μmol) was added to a mixture of 4-(4-(2-((7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy)butanoic acid (**3**) (100 mg, 236 μmol), 12-((*tert*-butoxycarbonyl)amino)dodecanoic acid (54.2 mg, 236 μmol) and DIPEA (22.7 μL, 236 μmol) in DMF (2 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h then another 7 h at rt. The reaction mixture was diluted with ethyl acetate and washed with brine. The organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. Purification by column chromatography (ethyl acetate 100%) and preparative HPLC gave the title compound **19** (6 mg, 4%) as a white solid. ^{1}H NMR (d_6-DMSO) δ 8.50 – 7.95 (2 × br s, ratio 1:6, 2H), 7.87 (m, 1H), 7.80 (t, J = 5.5 Hz, 1H), 7.58 – 7.40 (2 × br t, J = 5.5 Hz, ratio 1:2, 1H), 7.15 (m, 2H), 7.06 (m, 1H), 6.84 (m, 2H), 6.68 (m, 1H), 3.91 (t, J = 6.4 Hz, 2H), 3.57 (s, 3H), 3.44 (m, 2H), 3.02 (dd, J = 12.7, 6.7 Hz, 2H), 2.78 (m, 2H), 2.27 (t, J = 7.4 Hz, 2H), 2.21 (t, J = 7.4 Hz, 2H), 1.91 (m, 2H), 1.41 (m, 2H), 1.28 (m, 2H), 1.22 (m, 14H). LCMS: *m/z* (ESI 20 V)

635.5 (MH^+, 100). HPLC: t_R 8.06 min, >99% (254 nm), >99% (214 nm). HRMS ($C_{33}H_{46}N_8O_5$): Calcd. 635.3669 $[M+H]^+$, Found 635.3650.

2-Morpholino-4,6-dichloro-1,3,5-triazine (22)

To a solution of cyanuric chloride (**20**) (5.0 g, 27 mmol) in dry acetone (50 mL) was added dropwise to a cooled solution of morpholine (**21**) (1.7 g, 19 mmol) and TEA (1.9 g, 19 mmol) in dry acetone (50 mL) at -20 °C. The resulting mixture was poured onto water and stirred for 15 min before being filtered, washed with water and cold CH_3OH then dried to yield the title compound **22** as a white solid (3.4 g, 75 %). ^{1}H NMR (CD_3OD) δ 3.90 – 3.88 (m, 4H), 3.77 – 3.74 (m, 4H). LCMS: *m/z* (ESI 20 V) 235.0 (MH^+, 100).

***N*^{5}-(4-((4-Chloro-6-morpholino-1,3,5-triazin-2-yl)oxy)phenethyl)-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazine-5,7-diamine (23)**

4-(2-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenol (ZM 241385) (1) (200 mg, 596 μmol) was dissolved in THF (100 mL). Sodium hydride (60 % suspension in mineral oil) (23.8 mg, 596 μmol) was added and the reaction mixture was stirred at rt for 30 min. Then the reaction mixture was cooled down to -78 °C and 2-morpholino-4,6-dichloro-1,3,5-triazine was added. The reaction mixture was allowed to warm up to rt and after 21 h another portion of sodium hydride (60 % suspension in mineral oil) (23.8 mg, 596 μmol) was added and the reaction mixture was stirred for an additional 26 h before ethyl acetate was added. The reaction mixture was washed with an aqueous ammonium chloride solution. The organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The crude material was purified twice by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 94:4) and (ethyl acetate: $CHCl_3$ 1:1). The title compound 23 (169 mg, 53%) was obtained as a white solid; mp: 180-183 °C. ^{1}H NMR (d_6-DMSO) δ 8.61 – 7.94 (2 × br s, ratio 1:6, 2H), 7.87 (m, 1H), 7.65 – 7.47 (2 × br

t, ratio 1:2, *J*= 5.7 Hz, 1H), 7.32 (m, 2H), 7.16 (m, 2H), 7.06 (m, 1H), 6.68 (m, 1H), 3.74 (m, 2H), 3.63 (m, 2H), 3.60 (m, 4H), 3.52 (m, 2H), 2.90 (m, 2H). Some of the ^{13}C signal show up twice, the signal with lower intensity is marked with an asterisk (*) ^{13}C NMR (d_6-DMSO) δ 170.2 (C), 164.9 (C), 161.5 (C), 161.1 (C), 159.2 (C), 155.8 (C), 150.0 (C), 149.7 (C), 146.2 (C), 144.6 (CH), 137.2 (C), 129.7 (CH), 121.3 (CH), 111.9 (CH), 111.6 (CH), 65.6 (CH_2), 65.4 (CH_2), 44.0 (CH_2), 43.8 (CH_2), 42.6 (CH_2*), 42.0 (CH_2), 34.5 (CH_2*), 34.0 (CH_2). LCMS: *m/z* (ESI 20 V) 536.2 (MH^+, 100). HPLC: t_R 9.38 min, 97% (214 nm), 95% (254 nm). HRMS ($C_{23}H_{22}ClN_{11}O_3$): Calcd. 536.1674 $[M+H]^+$, Found 536.1671.

4-(2-((3-((4-(4-(2-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)ethyl)phenoxy)-6-morpholino-1,3,5-triazin-2-yl)amino)propyl)(propyl)amino)ethyl)indolin-2-one diformic acid salt (24)

N^5-(4-((4-Chloro-6-morpholino-1,3,5-triazin-2-yl)oxy)phenethyl)-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazine-5,7-diamine (**23**) (25.9 mg, 48.1 μmol), 4-(2-((3-aminopropyl)(propyl)amino)ethyl)indolin-2-one (**5**) (13.3 mg, 48.1 μmol) and potassium carbonate (6.7 mg, 48.1 μmol) were suspended in DMF (1.5 mL) in microwave tube. The tube was sealed and the reaction mixture was stirred at 100 °C for 15 min. The solvent was removed under reduced pressure. Purification by preparative column chromatography gave the title compound **24** (25 mg, 67%) as a white solid. ^{1}H NMR (d_6-DMSO) δ 10.33 (s, 1H), 8.53 – 8.03 (m, 4H), 7.86 (m, 1H), 7.58 – 7.39 (m, 2H), 7.25 (m, 2H), 7.10 – 7.02 (m, 4H), 6.76 (m, 1H), 6.66 (m, 2H), 3.73 – 3.46 (m, 10H), 3.43 (s, 2H), 3.30 – 3.08 (m, 2H), 2.86 (m, 2H), 2.75 – 2.45 (m, 8H), 1.72 – 1.54 (m, 2H), 1.43 (m, 2H), 0.83 (t, *J* = 7.3 Hz, 3H). Some of the ^{13}C signal show up twice, the signal with lower intensity is marked with an asterisk (*) ^{13}C NMR (d_6-DMSO) δ 176.6 (C), 170.9 (C*), 170.8 (C), 167.4 (C*), 167.0 (C), 166.3 (C), 165.9 (C*), 164.1 (CH, Formic acid), 162.0 (C*), 161.6 (C), 159.7 (C), 156.6 (C*), 156.3 (C), 151.1 (C), 151.0 (C), 150.5 (C), 146.7 (C), 145.1 (CH), 143.9 (C), 136.6 (C*), 136.3 (C), 129.8

(CH), 129.6 (CH*), 128.0 (CH), 125.0 (C), 122.2 (CH), 122.1 (CH), 121.9 (CH*), 112.4 (CH), 112.1 (CH), 107.5 (CH), 66.3 (CH_2), 55.3 (CH_2), 53.5 (CH_2), 53.4 (CH_2), 51.1 (CH_2), 43.8 (CH_2), 43.2 (CH_2*), 42.6 (CH_2), 39.0 (CH_2), 38.9 (CH_2*), 34.9 (CH_2), 34.6 (CH_2*), 34.5 (CH_2), 29.7 (CH_2), 26.5 (CH_2), 26.2 (CH_2), 19.7 (CH_2), 12.1 (CH_3). LCMS: *m/z* (ESI 20 V) 775.5 (MH^+, 100). LCMS: *m/z* (ESI 20 V) 773.9 (MH^-, 100); (basic) t_R 1.17 min, 97%; (acidic) t_R 0.87 min, 98%. HRMS ($C_{39}H_{46}N_{14}O_4$): Calcd. 775.3905 $[M+H]^+$, Found 775.3894.

2-(2-Oxoindolin-4-yl)ethyl 4-methylbenzenesulfonate (26)[51]

To a cooled (5-10 °C) suspension of 4-(2-hydroxyethyl)indolin-2-one (**25**) (6.30 g, 35.6 mmol) and pyridine (14.1 g, 177.8 mmol), a solution of *p*-toluenesulfonyl chloride (8.13 g, 42.7 mmol) in CH_2Cl_2 (32 mL) was added portionwise over 30 min. The reaction mixture was stirred at 5-10 °C for 4 h. Aqueous hydrochloric acid solution (6 M, 35 mL) was added so that the temperature was maintained below 15 °C. CH_2Cl_2 was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water, then dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The organic layer was concentrated under reduced pressure until a residual volume of about 50 mL was obtained. Petroleum spirits (about 50 mL) was added to induce the product to crystallize. The suspension was filtered and the filter cake was washed with CH_2Cl_2: petroleum spirits 1:1. The filter cake was dried on the high vacuum overnight to give the title compound **26** (9.39 g, 80%) as a yellowish-white solid, mp: 128-130 °C (lit.[46] 130-131 °C). ^{1}H NMR ($CDCl_3$) δ 9.05 (s, 1H), 7.68 – 7.60 (m, 2H), 7.30 – 7.22 (m, 2H), 7.13 (t, *J* = 7.8 Hz, 1H), 6.78 (d, *J* = 2.5 Hz, 1H), 6.76 (d, *J* = 2.6 Hz, 1H), 4.23 (t, *J* = 6.7 Hz, 2H), 3.33 (s, 2H), 2.89 (t, *J* = 6.7 Hz, 2H), 2.43 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 177.4 (C), 144.9 (C), 142.6 (C), 132.8 (C), 132.6 (C), 129.8 (CH), 128.4 (CH), 127.7 (CH), 124.5 (C), 122.9 (CH), 108.5 (CH), 69.3 (CH_2), 34.9 (CH_2), 32.6 (CH_2), 21.6 (CH_3). LCMS: *m/z* (ESI 20 V) 332.2 (MH^+, 65), 663.3 ($2M-H^+$, 100).

_N_-Methyl-2-(2-oxoindolin-4-yl)ethan-1-aminium chloride

2-(2-Oxoindolin-4-yl)ethyl 4-methylbenzenesulfonate (**26**) (1.00 g, 3.02 mmol) was suspended in 40% aqueous methylamine solution (10 mL) and stirred at 50 °C for 1.5 h before the reaction mixture was cooled down to rt and ethyl acetate was added. The solution was extracted twice with 1 M aqueous potassium carbonate solution. The organic layer was then twice extracted with 1 M aqueous hydrochloric acid solution. The water layer was evaporated and the residue was suspended in CH_3OH, filtered and the filter cake was dried on the high vacuum. The title compound (393 mg, 57%) was obtained as a yellow solid. ^{1}H NMR (d_6-DMSO) δ 10.43 (s, 1H), 9.08 (br s, 2H), 7.14 (t, J = 7.8 Hz, 1H), 6.82 (d, J = 7.4 Hz, 1H), 6.73 (d, J = 7.4 Hz, 1H), 3.52 (s, 2H), 3.08 (m, 2H), 2.87 (m, 2H), 2.54 (t, J = 5.4 Hz, 3H). ^{13}C NMR (d_6-DMSO) δ 176.6 (C), 144.2 (C), 133.6 (C), 128.3 (CH), 125.5 (C), 122.0 (CH), 108.2 (CH), 48.3 (CH_2), 35.1 (CH_2), 32.7 (CH_3), 29.4 (CH_2). LCMS: *m/z* (ESI 20 V) 191.2 (MH^+, 100).

4-(2-(Propylamino)ethyl)indolin-2-one hydrochloride

2-(2-Oxoindolin-4-yl)ethyl 4-methylbenzenesulfonate (**26**) (2.00 g, 60.4 mmol) was dissolved in propylamine (3.57 g, 60.4 mmol). The reaction mixture was stirred at reflux for 1.5 h then partitioned between ethyl acetate and 1 M aqueous potassium carbonate solution. The aqueous layer was removed and the organic layer was extracted with 1 M aqueous hydrochloric acid solution. The aqueous layer was concentrated under reduced pressure and the resultant residue suspended in CH_3OH, then filtered and washed with additional CH_3OH. The filter cake was dried on high vacuum overnight affording the title compound (1.13 g, 74%) as a yellow solid. ^{1}H NMR (d_6-DMSO) δ 10.43 (s, 1H), 9.05 (br s, 2H), 7.14 (t, J = 7.7 Hz, 1H), 6.82 (d, J = 7.7 Hz, 1H), 6.73 (d, J = 7.7 Hz, 1H), 3.52 (s, 2H), 3.07 (m, 2H), 2.97 – 2.80 (m, 4H), 1.65 (m, 2H), 0.92 (t, J = 7.4 Hz, 3H). ^{13}C NMR (d_6-DMSO): δ 176.1 (C), 143.7 (C), 133.2 (C), 127.8 (CH), 124.9 (C), 121.4 (CH), 107.7 (CH), 48.2 (CH_2), 46.3 (CH_2),

34.6 (CH_2), 29.0 (CH_2), 18.9 (CH_2), 11.0 (CH_3). LCMS: *m/z* (ESI 20 V) 219.2 (MH^+, 100). HRMS ($C_{13}H_{18}N_2O$): Calcd. 219.1492 $[M+H]^+$, Found 219.1497. HPLC: t_R 7.34 min, 98% (214 nm), 97% (254 nm).

4-(2-(Methylamino)ethyl)indolin-2-one (27a)

N-Methyl-2-(2-oxoindolin-4-yl)ethan-1-aminium chloride (380 mg, 1.68 mmol) was dissolved in 1 M aqueous sodium hydroxide solution (50 mL) and ethyl acetate (50 mL) and stirred at rt for 15 min. The layers were separated and the water layer was extracted with ethyl acetate. The combined organic layers were dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The title compound **27a** (172 mg, 54%) was obtained as a purple oil. ^{1}H NMR ($CDCl_3$) δ 7.13 (t, *J* = 7.8 Hz, 1H), 6.85 (d, *J* = 7.7 Hz, 1H), 6.73 (d, *J* = 7.7 Hz, 1H), 3.67 (s, 2H), 2.86 (m, 2H), 2.76 (m, 2H), 2.45 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 178.0 (C), 143.0 (C), 136.2 (C), 128.1 (CH), 124.3 (C), 122.5 (CH), 107.9 (CH), 51.5 (CH_2), 36.2 (CH_3), 35.2 (CH_2), 33.2 (CH_2). LCMS: *m/z* (ESI 20 V) 191.2 (MH^+, 100).

4-(2-(Propylamino)ethyl)indolin-2-one (27b)

4-(2-(Propylamino)ethyl)indolin-2-one hydrochloride (1.00 g, 3.93 mmol) was dissolved in 1 M aqueous sodium hydroxide solution (100 mL) and stirred for 5 min. CH_2Cl_2 (100 mL) was added and the reaction mixture was stirred for another 15 min. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with water, dried with a phase separating funnel and the solvent was removed under reduced pressure to afford the title compound **27b** (788 mg, 92%) as a yellowish oil. ^{1}H NMR ($CDCl_3$): δ 9.08 (br s, 1H), 7.16 (t, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 8.0 Hz, 1H), 6.74 (d, *J* = 7.6 Hz, 1H), 3.49 (s, 2H), 2.89 (t, *J* = 7.2 Hz, 2H), 2.76 (t, *J* = 7.2 Hz, 2H), 2.61 (t, *J* = 7.2 Hz, 2H), 1.65 (br s, 1H), 1.49 (m, 2H), 0.90 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR ($CDCl_3$): 177.1 (C),

142.3 (C), 136.6 (C), 128.1 (CH), 124.2 (C), 122.7 (CH), 107.6 (CH), 51.8 (CH_2), 49.6 (CH_2), 35.1 (CH_2), 33.7 (CH_2), 23.2 (CH_2), 12.0 (CH_3). LCMS: *m/z* (ESI 20 V) 219.2 (MH^+, 100).

4-(2-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5 yl)(propyl)amino)ethyl)indolin-2-one (29)

4-(2-(Propylamino)ethyl)indolin-2-one (**27b**) (95 mg, 435 μmol) was added to a suspension of 2-(furan-2-yl)-5-(methylsulfonyl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (**28**) (61 mg, 218 μmol) in acetonitrile (4 mL). The reaction mixture was stirred at rt for 2.5 days before the solvent was removed under reduced pressure. Purification by column chromatography (ethyl acetate: petroleum spirits 8:2 → ethyl acetate) and preparative HPLC gave the title compound **29** (8 mg, 8%) as a white solid. ^{1}H NMR (d_6-DMSO) δ 10.35 (d, *J* = 7.6 Hz, 1H), 8.50 – 8.20 (2 × br s, ratio 1:1, 2H), 7.88 (m, 1H), 7.10 (m, 1H), 7.06 (m, 1H), 6.88 (dd, *J* = 18.8, 7.7 Hz, 1H), 6.72 – 6.64 (m, 2H), 3.71 (m, 2H), 3.53 (d, *J* = 4.4 Hz, 2H), 3.45 (m, 2H), 2.81 (m, 2H), 1.59 (m, 2H), 0.87 (q, *J* = 7.5 Hz, 3H). Some of the carbon atoms showed up twice due to rotameric forms. ^{13}C NMR (d_6-DMSO) δ 176.9 (C), 176.8 (C), 160.5 (2 × C), 156.6 (C), 156.5 (C), 150.5 (C), 146.7 (C), 145.1 (CH), 143.9 (C), 144.0 (C), 143.9 (C), 135.8 (C), 135.6 (C), 128.1 (CH), 127.9 (CH), 125.4 (C), 125.3 (C), 122.4 (CH), 122.2 (CH), 112.4 (CH), 112.2 (CH), 107.7 (CH), 107.6 (CH), 49.8 (CH_2), 49.4 (CH_2), 48.5 (CH_2), 48.1 (CH_2), 35.0 (CH_2), 34.9 (CH_2), 31.6 (CH_2), 31.1 (CH_2), 21.4 (CH_2), 21.0 (CH_2), 11.7 (CH_3). LCMS: *m/z* (ESI 20 V) 419.3 (MH^+, 100), 417.2 (MH^-, 100). HPLC: t_R 6.22 min, 99% (214 nm), >99% (254 nm). HRMS ($C_{21}H_{22}N_8O_2$): Calcd. 419.1944 $[M+H]^+$, Found 419.1935; Calcd. 441.1763 $[M+Na]^+$, Found 441.1755.

***tert*-Butyl (3-(methyl(2-(2-oxoindolin-4-yl)ethyl)amino)propyl)carbamate (30a)**

4-(2-(Methylamino)ethyl)indolin-2-one (**27a**) (577 mg, 3.03 mmol), *tert*-butyl (3-bromopropyl)carbamate (722 mg, 3.03 mmol) and potassium carbonate (461 mg, 3.34 mmol)

were dissolved in acetonitrile (25 mL). The reaction mixture was heated up to reflux and stirred for 3 h before it was cooled down to rt and the solvent was removed under reduced pressure. Purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 9:2) gave the title compound **30a** (482 mg, 46%) as a purple oil. ^{1}H NMR ($CDCl_3$) δ 9.67 (br s, 1H), 7.11 (t, *J* = 7.8 Hz, 1H), 6.81 (d, *J* = 7.7 Hz, 1H), 6.74 (d, *J* = 7.7 Hz, 1H), 5.29 (br s, 1H), 3.44 (s, 2H), 3.14 (m, 2H), 2.71 (m, 2H), 2.61 (m, 2H), 2.49 (t, *J* = 6.9 Hz, 2H), 2.31 (s, 3H), 1.66 (m, 2H), 1.42 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 177.9 (C), 156.2 (C), 142.9 (C), 136.3 (C), 128.0 (CH), 124.1 (C), 122.5 (CH), 107.9 (CH), 79.0 (C), 57.6 (CH_2), 55.5 (CH_2), 41.9 (CH_3), 39.4 (CH_2), 35.1 (CH_2), 30.7 (CH_2), 28.4 (CH_3), 27.0 (CH_2). LCMS: *m/z* (ESI 20 V) 348.3 (MH^+, 100).

***tert*-Butyl (3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl)carbamate (30b)**

4-(2-(Propylamino)ethyl)indolin-2-one (**27b**) (788 g, 3.61 mmol), *tert*-butyl (3-bromopropyl)carbamate (860 mg, 3.61 mmol) and potassium carbonate (549 mg, 3.97 mmol) were dissolved in acetonitrile (35 mL). The reaction mixture was heated to reflux and stirred for 19 h, cooled down to rt and the solvent removed under reduced pressure. Purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 9:1) gave the title compound **30b** (522 mg, 38%) as a purple oil which was immediately stored under N_2 to minimize decomposition. ^{1}H NMR (d_6-DMSO) δ 10.32 (s, 1H), 7.07 (t, *J* = 7.7 Hz, 1H), 6.77 (d, *J* = 7.1 Hz, 2H), 6.64 (d, *J* = 7.3 Hz, 1H), 3.44 (s, 2H), 2.93 (m, 2H), 2.59 (m, 4H), 2.45 (m, 2H), 2.40 (m, 2H), 1.50 (m, 2H), 1.41 (m, 2H), 1.37 (s, 9H), 0.83 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (d_6-DMSO) δ 176.2 (C), 155.5 (C), 143.4 (C), 136.5 (C), 127.4 (CH), 124.40 (C), 121.7 (CH), 106.8 (CH), 77.3 (C), 55.2 (CH_2), 53.5 (CH_2), 50.8 (CH_2), 38.3 (CH_2), 34.5 (CH_2), 29.7 (CH_2), 28.2 (CH_3), 26.9 (CH_2), 19.9 (CH_2), 11.7 (CH_3). LCMS: *m/z* (ESI 20 V) 376.5 (MH^+, 100). HRMS ($C_{21}H_{33}N_3O_3$): Calcd. 376.2595 $[M+H]^+$, Found 376.2612. HPLC: t_R 4.88 min, 97% (214 nm), 97% (254 nm).

tert-Butyl (6-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)hexyl)carbamate (30c)

4-(2-(Propylamino)ethyl)indolin-2-one (**27b**) (405 mg, 1.86 mmol), *tert*-butyl (6-bromohexyl)carbamate (520 mg, 1.86 mmol) and potassium carbonate (282 mg, 2.04 mmol) were dissolved in acetonitrile (20 mL). The reaction mixture was heated up to reflux and stirred for 18 h before it was cooled down to rt and the solvent removed under reduced pressure. Purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 9:1) gave the title compound **30c** (371 mg, 48%) as a purple oil. ^{1}H NMR ($CDCl_3$) δ 8.85 (br s, 1H), 7.16 (t, J = 7.8 Hz, 1H), 6.85 (d, J = 7.8 Hz, 1H), 6.78 (d, J = 7.7 Hz, 1H), 4.62 (br s, 1H), 3.50 (s, 2H), 3.11 (m, 2H), 2.82 (m, 4H), 2.62 (m, 4H), 1.65 – 1.45 (m, 6H), 1.48 (s, 9H), 1.38 – 1.24 (m, 4H), 0.93 (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 177.7 (C), 156.1 (C), 142.9 (C), 136.2 (C), 128.0 (CH), 124.1 (C), 122.5 (CH), 107.9 (CH), 78.9 (C), 55.7 (CH_2), 53.9 (CH_2), 53.7 (CH_2), 40.4 (CH_2), 35.2 (CH_2), 30.1 (CH_2), 30.0 (CH_2), 28.4 (CH_3), 27.03 (CH_2), 26.6 (CH_2), 26.3 (CH_2), 19.60 (CH_2), 11.8 (CH_3). LCMS: *m/z* (ESI 20 V) 418.4 (MH^+, 100).

4-(2-((3-Aminopropyl)(methyl)amino)ethyl)indolin-2-one (31a)

Trifluoroacetic acid (3 mL) was added to solution of t*ert*-butyl (3-(methyl(2-(2-oxoindolin-4-yl)ethyl)amino)propyl)carbamate (**30a**) (450 mg, 1.3 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was stirred at rt for 1 h before the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and 1 M aqueous sodium hydroxide solution was added until pH-14. The water layer was extracted with CH_2Cl_2, then the combined organic layers were dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The title compound **31a** (138 mg, 43%) was obtained as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.15 (t, J = 7.8 Hz, 1H), 6.84 (d, J = 7.8 Hz, 1H), 6.77 (d, J = 7.7 Hz, 1H), 3.50 (s, 2H), 2.84 (t, J = 6.3 Hz, 2H), 2.72 (m, 2H), 2.63 (m, 2H), 2.54 (t, J = 6.6 Hz, 2H), 2.34 (s, 3H), 1.69 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 177.7 (C), 142.8 (C), 136.5 (C), 128.1 (CH), 124.2

(C), 122.6 (CH), 108.0 (CH), 57.5 (CH_2), 56.6 (CH_2), 41.9 (CH_3), 40.7 (CH_2), 35.2 (CH_2), 30.9 (CH_2), 27.9 (CH_2). LCMS: *m/z* (ESI 20 V) 248.2 (MH^+, 100).

4-(2-((3-Aminopropyl)(propyl)amino)ethyl)indolin-2-one (5)

To a stirred solution of *tert*-butyl (3-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)propyl) carbamate (**30b**) (250 mg, 666 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was stirred at rt for 1 h before the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and 1 M aqueous sodium hydroxide solution was added until pH-14. The water layer was extracted with CH_2Cl_2, then the combined organic layers were dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The title compound **5** (154 mg, 84%) was obtained as a yellow oil. Due to instability, the title compound **5** was stored in the fridge. ^{1}H NMR ($CDCl_3$) δ 9.50 – 7.80 (br s, 1H), 7.14 (t, J = 7.7 Hz, 1H), 6.84 (d, J = 7.7 Hz, 1H), 6.71 (d, J = 7.7 Hz, 1H), 3.48 (s, 2H), 2.72 (t, J = 6.8 Hz, 2H), 2.56, (m, 4H), 2.54 (m, 2H), 2.43 (m, 2H), 1.20 – 2.20 (br s, 2H), 1.60 (m, 2H), 1.45 (m, 2H), 0.88 (t, J = 7.6 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 177.8 (C), 142.6 (C), 136.9 (C), 128.0 (CH), 124.1 (C), 122.8 (CH), 107.7 (CH), 56.0 (CH_2), 54.0 (CH_2), 52.3 (CH_2), 40.7 (CH_2), 35.1 (CH_2), 30.6 (CH_2), 29.7 (CH_2), 20.2 (CH_2), 11.9 (CH_3). LCMS: *m/z* (ESI 20 V) 276.3 (MH^+, 100). HRMS ($C_{16}H_{25}N_3O$): Calcd. 276.2070 $[M+H]^+$, Found 276.2080. HPLC: t_R 4.52 min, 95% (214 nm), 98% (254 nm).

4-(2-((6-Aminohexyl)(propyl)amino)ethyl)indolin-2-one (31c)

Trifluoroacetic acid (3 mL) was added to solution of *tert*-butyl (6-((2-(2-oxoindolin-4-yl)ethyl)(propyl)amino)hexyl)carbamate (**30c**) (371 mg, 888 μmol) in CH_2Cl_2 (20 mL). The reaction mixture was stirred at rt for 1 h before the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and 1 M aqueous sodium hydroxide solution was added until pH-14. The water layer was extracted with CH_2Cl_2, then the combined

organic layers were dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The title compound **31c** (245 mg, 87%) was obtained as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.14 (t, *J* = 7.8 Hz, 1H), 6.84 (d, *J* = 7.8 Hz, 1H), 6.76 (d, *J* = 7.6 Hz, 1H), 3.49 (s, 2H), 2.75 (m, 2H), 2.66 (m, 4H), 2.49 – 2.38 (m, 4H), 1.54 – 1.17 (m, 10H), 0.89 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 178.0 (C), 143.0 (C), 137.1 (C), 127.9 (CH), 124.2 (C), 122.6 (CH), 107.6 (CH), 56.1 (CH_2), 54.2 (CH_2), 54.1 (CH_2), 41.8 (CH_2), 35.3 (CH_2), 33.3 (CH_2), 30.8 (CH_2), 27.4 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 20.2 (CH_2), 12.0 (CH_3). LCMS: *m/z* (ESI 20 V) 318.3 (MH^+, 100).

4-(2-((3-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)propyl)(methyl)amino) ethyl)indolin-2-one (32a)

4-(2-((3-Aminopropyl)(methyl)amino)ethyl)indolin-2-one (**31a**) (138 mg, 558 µmol) was added to a suspension of 2-(furan-2-yl)-5-(methylsulfonyl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (**28**) (78 mg, 279 µmol) in acetonitrile (2 mL) and toluene (2 mL). The reaction mixture was stirred at rt for 1.5 h before another portion of 2-(furan-2-yl)-5-(methylsulfonyl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (**28**) (20 mg, 71 µmol) was added. The reaction mixture was stirred for another 16.5 h before the solvent was removed under reduced pressure. Purification by column chromatography (CH_2Cl_2: CH_3OH 8:2 → CH_2Cl_2: CH_3OH 8:4). Then the product was taken up in ethyl acetate and washed with 1M aqueous potassium carbonate solution. The organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The title compound **32a** (29 mg, 23%) was obtained as a white foam. ^{1}H NMR (d_6-DMSO) δ 10.30 (br s, 1H), 8. 50 – 7.9 (2 × br s, ratio 1:6, 2H), 7.86 (m, 1H), 7.52 – 7.37 (2 × br t, ratio 1:2, *J* = 5.6 Hz, 1H), 7.10 – 7.00 (m, 2H), 6.79 (d, *J* = 7.7 Hz, 1H), 6.68 (m, 1H), 6.63 (d, *J* = 7.5 Hz, 1H), 3.43 (s, 2H), 3.28 (m, 2H), 2.63 (m, 2H), 2.53 (m, 2H), 2.42 (m, 2H), 2.23 (s, 3H), 1.66 (m, 2H). Some of the ^{13}C signal show up twice, the signal with lower intensity is marked with an asterisk (*) ^{13}C

NMR (d_6-DMSO) δ 176.8 (C), 161.6 (C), 159.7 (C), 156.3 (C), 151.1 (C*),150.4 (C), 146.7 (C), 145.1 (CH), 143.9 (C), 136.9 (C), 127.9 (CH), 125.0 (C), 122.2 (CH), 112.4 (CH), 112.0 (CH), 107.4 (CH), 57.6 (CH_2), 55.4 (CH_2*), 55.0 (CH_2), 42.1 (CH_3), 39.5 (CH_2), 35.0 (CH_2), 30.5 (CH_2), 27.3 (CH_2*), 26.9 (CH_2). LCMS: *m/z* (ESI 20 V) 448.3 (MH^+, 100). HPLC: t_R 4.31 min, 95% (214 nm), 95% (254 nm). HRMS ($C_{22}H_{25}N_9O_2$): Calc'd. 448.2209 $[M+H]^+$, Found 448.2214.

4-(2-((3-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)propyl)(propyl)amino)ethyl)indolin-2-one (32b)

4-(2-((3-Aminopropyl)(propyl)amino)ethyl)indolin-2-one (**5**) (50 mg, 182 μmol) was added to a suspension of 2-(furan-2-yl)-5-(methylsulfonyl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (**28**) (25.4 mg, 90.8 μmol) in acetonitrile (2 mL) and toluene (2 mL). The reaction mixture was stirred at rt for 5 h before the solvent was removed under reduced pressure. Purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 7:3) gave the title compound **32b** (28 mg, 65%) as a purplish-white solid, mp: 148-150 °C. ^{1}H NMR (d_6-DMSO) δ 10.32 (s, 1H), 8.5 – 7.90 (2 × br s, ratio 1:6, 2H), 7.86 (m, 1H), 7.54 – 7.39 (2 × br t, ratio 1:2, J = 5.4 Hz, 1H), 7.10 – 7.03 (m, 2H), 6.77 (m, 1H), 6.67 (m, 1H), 6.63 (m, 1H), 3.46 (s, 2H), 3.30 (m, 2H), 2.61 (s, 4H), 2.53 (m, 2H), 2.41 (m, 2H), 1.68 (m, 2H), 1.41 (m, 2H), 0.84 (t, J = 7.3 Hz, 3H). Some of the ^{13}C signal show up twice, the signal with lower intensity is marked with an asterisk (*) ^{13}C NMR (d_6-DMSO) δ 176.2 (C), 161.1 (C), 159.2 (C), 155.8 (C), 149.9 (C), 146.2 (C), 144.5 (CH), 143.4 (C), 136.6 (C), 127.4 (C), 124.4 (CH), 121.8 (CH), 111.9 (CH), 111.5 (CH), 106.8 (CH), 55.3 (CH_2), 53.6 (CH_2), 51.1 (CH_2), 39.1 (CH_2), 34.5 (CH_2), 29.9 (CH_2), 26.8 (CH_2*), 26.4 (CH_2), 20.0 (CH_2), 11.8 (CH_3). LCMS: *m/z* (ESI 20 V) 476.3 (MH^+, 100). HPLC: t_R 6.72 min, >99% (214 nm), >99% (254 nm). HRMS ($C_{24}H_{29}N_9O_2$): Calc'd. 476.2522 $[M+H]^+$, Found 476.2518.

4-(2-((6-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)hexyl)(propyl) amino)ethyl)indolin-2-one (32c)

4-(2-((6-Aminohexyl)(propyl)amino)ethyl)indolin-2-one (**31c**) (245 mg, 772 μmol) was added to a suspension of 2-(furan-2-yl)-5-(methylsulfonyl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (**28**) (108 mg, 386 μmol) in acetonitrile (2 mL) and toluene (2 mL). The reaction mixture was stirred at rt for 1.5 h before another portion of 2-(furan-2-yl)-5-(methylsulfonyl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (**28**) (20 mg, 71 μmol) was added. The reaction mixture was stirred for another 2.5 days before the solvent was removed under reduced pressure. Purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 8:2) gave the title compound **32c** (126 mg, 63%) as a beige foam. ^{1}H NMR (d_6-DMSO) δ 10.32 (s, 1H), 8.50 – 7.9 (2 × br s, ratio 1:6, 2H), 7.86 (m, 1H), 7.52 – 7.38 (2 × br t, J =5.7 Hz, 1H), 7.07 (t, J = 7.8 Hz, 1H), 7.03 (d, J = 3.3 Hz, 1H), 6.77 (d, J = 7.7 Hz, 1H), 6.67 (dd, J = 3.2, 1.7 Hz, 1H), 6.64 (d, J = 7.7 Hz, 1H), 3.45 (s, 2H), 3.25 (m, 2H), 2.61 (m, 4H), 2.45 (m, 4H), 1.51 (m, 2H), 1.40 (m, 4H), 1.28 (m, 4H), 0.82 (t, J = 7.3 Hz, 3H). Some of the ^{13}C signal show up twice, the signal with lower intensity is marked with an asterisk (*) ^{13}C NMR (d_6-DMSO) δ 176.7 (C), 162.0 (C*), 161.6 (C), 159.7 (C), 156.6 (C*), 156.3 (C), 151.1 (C), 150.4 (C), 146.7 (C), 145.1 (CH), 143.9 (C), 125.0 (C), 127.9 (CH), 122.3 (CH), 112.4 (CH), 122.0 (CH), 107.4 (CH), 55.6 (CH_2), 54.0 (CH_2), 53.5 (CH_2), 41.4 (CH_2), 41.0 (CH_2), 35.0 (CH_2), 30.2 (CH_2), 29.7 (CH_2*), 29.3 (CH_2), 27.1 (CH_2), 26.8 (CH_2), 20.2 (CH_2), 12.2 (CH_3). LCMS: *m/z* (ESI 20 V) 518.3 (MH^+, 100). HPLC: t_R 5.08 min, 98% (214 nm), 98% (254 nm). HRMS ($C_{27}H_{35}N_9O_2$): Calcd. 518.2992 $[M+H]^+$, Found 518.2980.

***tert*-Butyl 4-((7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-yl)amino)piperidine-1-carboxylate (33)**

tert-Butyl 4-aminopiperidine-1-carboxylate (750 mg, 3.74 mmol) was added to a suspension of 2-(furan-2-yl)-5-(methylsulfonyl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (**28**)

(350 mg, 1.25 μmol) in acetonitrile (10 mL). The reaction mixture was stirred at rt for 3 h before the solvent was removed under reduced pressure. Purification by column chromatography (ethyl acetate 100%) gave the title compound **33** (316 mg, 63%) as a beige solid, mp: 220-223 °C. ^{1}H NMR (d_6-DMSO) δ 8.60 – 7.90 (2 × br s, ratio 1:5, 2H), 7.87 (m, 1H), 7.55 – 7.38 (2 × d, ratio 1:2, J = 8.0 Hz, 1H), 7.05 (m, 1H), 6.68 (m, 1H), 3.93 (m, 3H), 2.84 (m, 2H), 1.83 (m, 2H), 1.41 (s, 9H), 1.36 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 160.9 (C), 159.6 (C), 156.3 (C), 154.3 (C), 150.5 (C), 146.6 (C), 145.1 (CH), 112.4 (CH), 112.1 (CH), 79.1 (C), 48.1 (CH), 42.7 (CH_2), 31.6 (CH_2), 28.6 (CH_3). LCMS: *m/z* (ESI 20 V) 401.3 (MH^+, 100), 399.2 (MH^-, 100). HPLC: t_R 6.09 min, 97% (214 nm), 97% (254 nm). HRMS ($C_{18}H_{24}N_8O_3$): Calcd. 401.2050 $[M+H]^+$, Found 401.2050.

2-(Furan-2-yl)-N^5-(piperidin-4-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazine-5,7-diamine (34)

tert-Butyl 4-((7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)piperidine-1-carboxylate (**33**) (325 mg, 812 μmol) was dissolved in trifluoroacetic acid (1 mL) and CH_2Cl_2 (5 mL) was stirred for 2 h at rt. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and then 1M aqueous sodium hydroxide solution was added until pH-14. Isopropanol was added to get the product into the organic layer. The layers were separated and the organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The title compound **34** (114 mg, 47%) was obtained as a white solid, mp: 208 °C dec. ^{1}H NMR (d_6-DMSO) δ 8.26 (2 × br s, ratio 1:6, 2H), 7.87 (m, 1H), 7.44 (2 × d, ratio 1:2, J = 8.0 Hz, 1H), 7.04 (m, 1H), 6.68 (m, 1H), 3.77 (m, 1H), 2.96 (m, 2H), 2.50 (m, 2H), 1.78 (m, 2H), 1.34 (m, 2H). Some of the ^{13}C signal show up twice, the signal with lower intensity is marked with an asterisk (*). ^{13}C NMR (d_6-DMSO) δ 160.8 (C), 159.7 (C), 156.3 (C), 150.5 (C), 146.7 (C), 145.1 (CH), 112.4 (CH), 112.1 (CH), 49.0 (CH*),

48.8 (CH), 45.6 (CH_2), 33.5 (CH_2*), 33.0 (CH_2). LCMS: *m/z* (ESI 20 V) 301.2 (MH^+, 100), 299.2 (MH^-, 100).

tert-Butyl (1-(2-(2-oxoindolin-4-yl)ethyl)piperidin-4-yl)carbamate (35)

tert-Butyl piperidin-4-ylcarbamate (755 mg, 3.77 mmol) and diisopropylethylamine (770 µL, 4.53 mmol) was added to a solution of 2-(2-oxoindolin-4-yl)ethyl 4-methylbenzenesulfonate (**26**) (500 mg, 1.51 mmol) in acetonitrile (20 mL). The reaction mixture was stirred at reflux for 6 h before the solvent was removed under reduced pressure. The residue was taken up in CH_2Cl_2 and washed with water. The organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The crude material was purified by column chromatography (ethyl acetate 100% → ethyl acetate: CH_3OH 8:2). The title compound **35** (183 mg, 34%) was obtained as a yellow solid, mp: 192-194 °C. ^{1}H NMR ($CDCl_3$) δ 8.67 (br s, 1H), 7.15 (t, *J* = 7.8 Hz, 1H), 6.85 (d, *J* = 7.7 Hz, 1H), 6.73 (d, *J* = 7.6 Hz, 1H), 4.50 (d, *J* = 7.9 Hz, 1H), 3.48 (m, 3H), 2.92 (m, 2H), 2.74 (m, 2H), 2.57 (m, 2H), 2.16 (t, *J* = 10.9 Hz, 2H), 1.97 (m, 2H), 1.48 (m, 2H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 177.3 (C), 155.2 (C), 142.4 (C), 136.7 (C), 128.1 (CH), 124.1 (C), 122.7 (CH), 107.6 (CH), 79.3 (C), 58.6 (CH_2), 52.5 (CH_2), 47.7 (CH), 35.1 (CH_2), 32.5 (CH_2), 30.9 (CH_2), 28.4 (CH_3). LCMS: *m/z* (ESI 20 V) 360.3 (MH^+, 100). HPLC: t_R 4.92 min, 96% (214 nm), 99% (254 nm). HRMS ($C_{20}H_{29}N_3O_3$): Calcd. 360.2287 $[M+H]^+$, Found 360.2277.

4-(2-(4-Aminopiperidin-1-yl)ethyl)indolin-2-one (36)

tert-Butyl (1-(2-(2-oxoindolin-4-yl)ethyl)piperidin-4-yl)carbamate (**36**) (170 mg, 473 µmol) was dissolved in trifluoroacetic acid (3 mL) and CH_2Cl_2 (10 mL) and stirred at rt for 2 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and then 1M aqueous sodium hydroxide solution was added until pH-14. The layers were separated and the water layer was dried under reduced pressure. The residue was taken up in CH_2Cl_2 and CH_3OH and purified by

column chromatography (CH_2Cl_2 100% → CH_3OH 100%). The title compound **36** (57 mg, 46%) was obtained a as purple oil. ^{1}H NMR (d_6-DMSO) δ 10.39 (s, 1H), 7.07 (t, J = 7.7 Hz, 1H), 6.77 (d, J = 7.8 Hz, 1H), 6.64 (d, J = 7.7 Hz, 1H), 4.70 – 4.40 (br s, 2H), 3.43 (s, 2H), 3.17 (m, 1H), 2.82 (m, 2H), 2.62 (m, 2H), 2.44 (m, 2H), 1.95 (m, 2H), 1.68 (m, 2H), 1.22 (m, 2H). LCMS: *m/z* (ESI 20 V) 260.2 (MH^+, 100). HPLC: t_R 2.78 min, 83% (214 nm), >93% (254 nm). HRMS ($C_{15}H_{21}N_3O$): Calcd. 260.1763 $[M+H]^+$, Found 260.1754.

4-(2-(4-((7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)amino)piperidin-1-yl)ethyl)indolin-2-one (37)

4-(2-(4-Aminopiperidin-1-yl)ethyl)indolin-2-one (**36**) (50 mg, 193 μmol) was added to a suspension of 2-(furan-2-yl)-5-(methylsulfonyl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (**29**) (45 mg, 161 μmol) in acetonitrile (3 mL) and DMF (3 mL). The reaction mixture was stirred at rt for 22 h before the solvent was removed under reduced pressure. Purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 8:2) gave the title compound **37** (36 mg, 49%) as a white solid, mp: 267-276 °C. ^{1}H NMR (d_6-DMSO) δ 10.36 (s, 1H), 8.55 – 7.95 (2 × br s, ratio 1:5, 2H), 7.87 (m, 1H), 7.52 – 7.35 (2 × d, ratio 1:2, J = 7.4 Hz, 1H), 7.10 (t, J = 7.7 Hz, 1H), 7.05 (m, 1H), 6.80 (d, J = 7.7 Hz, 1H), 6.70 – 6.64 (m, 2H), 3.79 (m, 1H), 3.48 (s, 2H), 3.02 (m, 2H), 2.71 (m, 2H), 2.60 (m, 2H), 2.30 – 1.98 (m, 2H), 1.89 (m, 2H), 1.57 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 176.8 (C), 161.4 (C), 161.0 (C), 159.6 (C), 156.3 (C), 150.5 (C), 146.7 (C), 145.1 (CH), 143.9 (C), 128.0 (CH), 125.09 (C), 122.1 (CH), 112.4 (CH), 112.1 (CH), 107.5 (CH), 58.0 (CH_2), 52.3 (CH_2), 48.0 (CH), 34.9 (CH_2), 31.4 (CH_2), 30.2 (CH_2). LCMS: *m/z* (ESI 20 V) 460.3 (MH^+, 100). HPLC: t_R 4.43 min, 98% (214 nm), 97% (254 nm). HRMS ($C_{23}H_{26}N_9O_2$): Calcd. 460.2209 $[M+H]^+$, Found 460.2195.

***tert*-Butyl 4-(2-(2-oxoindolin-4-yl)ethyl)piperazine-1-carboxylate (38a)**

Boc-piperazine (674 mg, 3.62 mmol) and diisopropylethylamine (1.54 mL, 9.05 mmol) was added to a solution of 2-(2-oxoindolin-4-yl)ethyl 4-methylbenzenesulfonate (**26**) ((1.00 g, 3.02 mmol) in acetonitrile (40 mL). The reaction mixture was stirred at reflux for 5 h before another 1.2 equivalent of Boc-piperazine (674 mg, 3.62 mmol) was added. The reaction mixture was stirred for an additional 17 h, then solvent was removed under reduced pressure. The residue was taken up in CH_2Cl_2 and washed with water. The organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The crude material was purified by column chromatography (ethyl acetate 100% → ethyl acetate: CH_3OH 8:2) to give the title compound **38a** (778 mg, 75%) as an orange foam. ^{1}H NMR ($CDCl_3$) δ 8.74 (br s, 1H), 7.16 (t, J = 7.8 Hz, 1H), 6.86 (d, J = 7.7 Hz, 1H), 6.75 (d, J = 7.7 Hz, 1H), 3.50 – 3.42 (m, 6H), 2.75 (m, 2H), 2.60 (m, 2H), 2.48 (m, 4H), 1.47 (s, 9H). ^{13}C NMR (d_6-DMSO) δ 176.8 (C), 143.9 (C), 137.1 (C), 127.9 (CH), 125.0 (C), 122.2 (CH), 59.3 (CH_2), 54.6 (CH_2), 46.1 (CH_2), 35.0 (CH_2), 30.3 (CH_2). LCMS: *m/z* (ESI 20 V) 346.2 (MH^+, 100), 344.2 (MH^-, 100). HPLC: t_R 6.40 min, 97% (214 nm), 98% (254 nm). HRMS ($C_{19}H_{27}N_3O_3$): Calcd. 346.2131 $[M+H]^+$, Found 346.2129.

***tert*-Butyl 4-(2-(2-oxoindolin-4-yl)ethyl)-1,4-diazepane-1-carboxylate (38b)**

Boc-homopiperazine (1.45 mg, 7.24 mmol) and DIPEA (1.54 mL, 9.05 mmol) was added to a solution of 2-(2-oxoindolin-4-yl)ethyl 4-methylbenzenesulfonate (**26**) (1.00 g, 3.02 mmol) in acetonitrile (40 mL). The reaction mixture was stirred at reflux for 23 h before solvent was removed under reduced pressure. The residue was taken up in CH_2Cl_2 and washed with water, then the organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The crude material was purified by column chromatography (ethyl acetate 100% → ethyl acetate: CH_3OH 8:2) to give the title compound **38b** (799 mg, 74%) as an orange foam. ^{1}H NMR ($CDCl_3$) δ 8.95 (br d, J = 8.3 Hz, 1H), 7.15 (t, J = 7.7 Hz,

1H), 6.85 (d, J = 7.8 Hz, 1H), 6.75 (dd, J = 7.7, 2.1 Hz, 1H), 3.57 – 3.40 (m, 6H), 2.80 – 2.65 (m, 8H), 1.86 (m, 2H), 1.47 (s, 9H). Some of the carbon atoms showed up twice due to rotameric forms. ^{13}C NMR ($CDCl_3$) δ 177.51 (C), 155.7 (C), 155.5 (C), 142.5 (C), 136.7 (C), 128.1 (CH), 124.0 (C), 122.8 (CH), 107.7 (CH), 107.6 (CH), 79.4 (C), 57.9 (CH_2), 56.0 (CH_2), 55.9 (CH_2), 54.8 (CH_2), 54.6 (CH_2), 46.7 (CH_2), 46.0 (CH_2), 45.9 (CH_2), 45.0 (CH_2), 35.1 (CH_2), 31.2 (CH_2), 31.1 (CH_2), 28.5 (CH_3), 27.8 (CH_2), 27.7 (CH_2). LCMS: *m/z* (ESI 20 V) 360.3 (MH^+, 100). HPLC: t_R 6.67 min, 95% (214 nm), >99% (254 nm). HRMS ($C_{20}H_{29}N_3O_3$): Calcd. 360.2287 $[M+H]^+$, Found 360.2283.

4-(2-(Piperazin-1-yl)ethyl)indolin-2-one (39a)

tert-Butyl 4-(2-(2-oxoindolin-4-yl)ethyl)piperazine-1-carboxylate (**38a**) (740 mg, 2.14 mmol) was dissolved in trifluoroacetic acid (5 mL) and CH_2Cl_2 (10 mL) and was stirred at rt for 5 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and then 1M aqueous sodium hydroxide solution was added until pH-14. The layers were separated and the water layer was dried under reduced pressure. The residue was taken up in ethyl acetate and purified by column chromatography (ethyl acetate 100% → CH_3OH 100%). The title compound **39a** (375 mg, 71%) was obtained as an orange-yellowish solid, mp: 150-156 °C. ^{1}H NMR (d_6-DMSO) δ 10.38 (s, 1H), 7.08 (t, J = 7.7 Hz, 1H), 6.77 (d, J = 7.5 Hz, 1H), 6.65 (d, J = 7.5 Hz, 1H), 3.44 (s, 2H), 2.67 (m, 4H), 2.63 (m, 2H), 2.43 (m, 2H), 2.34 (m, 4H). ^{13}C NMR (d_6-DMSO) δ 176.8 (C), 143.9 (C), 137.1 (C), 127.9 (CH), 125.0 (C), 122.2 (CH), 107.4 (CH), 59.3 (CH_2), 54.6 (CH_2), 46.1 (CH_2), 35.0 (CH_2), 30.3 (CH_2). LCMS: *m/z* (ESI 20 V) 246.2 (MH^+, 100). HPLC: t_R 1.94 min, 95% (214 nm), >99% (254 nm). HRMS ($C_{14}H_{19}N_3O$): Calcd. 246.1606 $[M+H]^+$, Found 246.1604.

4-(2-(2-Oxoindolin-4-yl)ethyl)-1,4-diazepan-1-ium 2,2,2-trifluoroacetate (39b)

Trifluoroacetic acid (5 mL) was added to a solution of *tert*-butyl 4-(2-(2-oxoindolin-4-yl)ethyl)-1,4-diazepane-1-carboxylate (**38b**) (750 mg, 2.09 mmol) in CH_2Cl_2 (5 mL). The reaction was stirred at rt for 3.5 h before the solvents were removed under reduced pressure. The title compound **39b** (1.39 g) was obtained as a brownish oil containing residual trifluoroacetic acid. ^{1}H NMR (d_6-DMSO) δ 10.45 (s, 1H), 9.22 (br s, 2H), 7.16 (t, J = 7.8 Hz, 1H), 6.85 (d, J = 7.7 Hz, 1H), 6.74 (d, J = 7.6 Hz, 1H), 3.41 – 3.20 (m, 12H), 2.93 (m, 2H), 2.17 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 176.6 (C), 159.1 (q, $CF_3\underline{C}OO^-$, J= 37 Hz), 144.3 (C), 132.9 (C), 128.4 (CH), 125.5 (C), 122.1 (CH), 116.1 (q, J= 292 Hz, $\underline{C}F_3COO^-$), 108.4 (CH), 55.8 (CH_2), 53.2 (CH_2), 49.9 (CH_2), 44.7 (CH_2), 40.6 (CH_2), 34.9 (CH_2), 27.8 (CH_2), 21.1 (CH_2). LCMS: *m/z* (ESI 20 V) 260.2 (MH^+, 100). HPLC: t_R 3.03 min, 98% (214 nm), 98% (254 nm). HRMS ($C_{15}H_{21}N_3O$): Calcd. 260.1763 $[M+H]^+$, Found 260.1760.

4-(2-(4-(7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl)piperazin-1-yl)ethyl)indolin-2-one (40a)

4-(2-(Piperazin-1-yl)ethyl)indolin-2-one (**39a**) (325 mg, 1.32 mmol) was added to a suspension of 2-(furan-2-yl)-5-(methylsulfonyl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (**28**) (124 mg, 442 μmol) in acetonitrile (7 mL) and toluene (7 mL). The reaction mixture was stirred at rt for 18 h before the solvent was removed under reduced pressure. Purification by column chromatography (CH_2Cl_2 → CH_2Cl_2: CH_3OH 9:1) gave the title compound **40a** (130 mg, 66%) as a beige solid, mp: 280-285 °C. ^{1}H NMR (d_6-DMSO) δ 10.35 (s, 1H), 8.50 – 8.20 (2 × br s, ratio 1:1, 2H), 7.88 (dd, J = 1.7, 0.8 Hz, 1H), 7.09 (t, J = 7.8 Hz, 1H), 7.06 (dd, J = 3.4, 0.7 Hz, 1H), 6.81 (d, J = 7.5 Hz, 1H), 6.68 (dd, J = 3.4, 1.8 Hz, 1H), 6.66 (d, J = 7.6 Hz, 1H), 3.78 (m, 4H), 3.48 (s, 2H), 2.70 (m, 2H), 2.58 – 2.47 (m, 6H). ^{13}C NMR (d_6-DMSO) δ 176.8 (C), 160.3 (C), 159.6 (C), 156.7 (C), 150.7 (C), 146.6 (C), 145.2 (CH), 143.9 (C), 136.8 (C), 128.0 (CH), 125.1 (C), 122.2 (CH), 112.4 (CH), 112.2 (CH), 107.4 (CH), 58.3

(CH_2), 52.8 (CH_2), 43.9 (CH_2), 35.0 (CH_2), 30.4 (CH_2). LCMS: *m/z* (ESI 20 V) 446.3 (MH^+, 100). HPLC: t_R 6.18 min, 96% (214 nm), 99% (254 nm). HRMS ($C_{22}H_{23}N_9O_2$): Calcd. 446.2053 $[M+H]^+$, Found 446.2048.

4-(7-Amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-a][1,3,5]triazin-5-yl)-1-(2-(2-oxoindolin-4-yl)ethyl)-1,4-diazepan-1-ium 2,2,2-trifluoroacetate (40b)

1,8-Diazabicycloundec-7-ene (408 mg, 2.68 mmol) was added to a reaction mixture of 4-(2-(2-oxoindolin-4-yl)ethyl)-1,4-diazepan-1-ium 2,2,2-trifluoroacetate (**39b**) (500 mg, 1.34 mmol) and 2-(furan-2-yl)-5-(methylsulfonyl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-7-amine (**28**) (125 mg, 446 μmol) in acetonitrile (10 mL). The reaction mixture was stirred at rt for 23 h before the solvent was removed under reduced pressure. Purification by column chromatography twice (CH_2Cl_2 100% → CH_2Cl_2: CH_3OH 9:1) and ($CHCl_3$ 100% → $CHCl_3$: CH_3OH 9:1) as well as preparative HPLC gave the title compound **40b** (10 mg, 5%) as a colorless oil. ^1H NMR (d_6-DMSO) δ 10.43 (s, 1H), 9.84 – 9.67 (2 × br s, ratio 1:1, 1H), 8.65 – 8.25 (2 × br s, ratio 1:1, 2H), 7.89 (dd, *J* = 1.7, 0.8 Hz, 1H), 7.14 (t, *J* = 7.7 Hz, 1H), 7.08 (dd, *J* = 3.4, 0.8 Hz, 1H), 6.83 (d, *J* = 7.6 Hz, 1H), 6.73 (d, *J* = 7.7 Hz, 1H), 6.69 (dd, *J* = 3.4, 1.8 Hz, 1H), 4.28 (m, 2H), 3.90 – 3.55 (m, 4H), 3.55 (s, 2H), 3.32 (m, 4H), 2.93 (m, 2H), 2.18 (m, 2H). Some of the ^{13}C signal show up twice, the signal with lower intensity is marked with an asterisk (*).^{13}C NMR (d_6-DMSO) δ 176.6 (C), 160.6 (C), 158.5 (C), 156.6 (C), 150.8 (C), 146.4 (C), 145.3 (CH), 144.3 (C), 133.1 (C), 128.4 (CH), 125.5 (C), 122.2 (CH), 112.5 (CH), 112.4 (CH), 108.4 (CH), 56.0 (CH_2), 54.1 (CH_2), 53.5 (CH_2), 53.2 (CH_2*), 45.2 (CH_2), 44.9 (CH_2*), 42.2 (CH_2*), 41.9 (CH_2), 34.9 (CH_2), 27.9 (CH_2), 24.4 (CH_2), 24.0 (CH_2*). The signals for trifluoroacetate CF_3COO^- were not observed due to the low concentration. LCMS: *m/z* (ESI 20 V) 460.3 (MH^+, 100), 458.3 (MH^-, 100). HPLC: t_R 4.48 min, 98% (214 nm), 98% (254 nm). HRMS ($C_{23}H_{25}N_9O_2$): Calcd. 460.2209 $[M+H]^+$, Found 460.2202.

Adenosine A_{2A} assay

Cell culture: Chinese Hamster Ovary FlpIn (FlpIn-CHO) cells stably transfected with the human adenosine A_{2A} receptor were maintained in DMEM containing 10% FBS and 500 µg/mL hygromycin-B at 37 °C in a humidified incubator containing 5% CO_2: 95% O_2.

cAMP assay: Cells were seeded in 96-well cell culture plates at 2 x 10^4 cells per well and grown until 90% confluent. Cell were then washed with 100 µL phosphate buffered saline solution and incubated at 37 °C for 45 minutes in 80 µL stimulation buffer (140 mM NaCl, 5 mM KCl, 0.8 mM $MgSO_4 \cdot 7\,H_2O$, 0.2 mM $Na_2HPO_4 \cdot 2\,H_2O$, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2 \cdot 2\,H_2O$, 5.6 mM D-glucose, 5 mM HEPES, 0.1% BSA, 0.1 U/mL ADA and 10 µM rolipram, pH= 7.45). Cells were then stimulated for 30 minutes with 500 nM NECA in the absence or presence of increasing concentrations of compound in a final volume of 100 µL. Buffer was then removed and cells lysed with 50 µL of 100% ethanol. Following ethanol evaporation, 50 µL of detection buffer was added (0.1% BSA, 0.3% Tween-20 and 5mM HEPES, pH= 7.45) and plates agitated for 10 minutes. Lysate (10 µL) was transferred to a 384-well Optiplate™ and 1 Unit/well of AlphaScreen™ acceptor beads, AlphaScreen™ donor beads and biotinylated cAMP were added. AlphaScreen™ donor beads and biotinylated cAMP were equilibrated for 30 mins prior to addition. Plates were incubated overnight in the dark at room temperature, followed by measurement of fluorescence by an EnVision® plate reader (PerkinElmer) using AlphaScreen™ settings.

Data analysis: All data obtained were analysed in GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA). Results in the text are shown as means ± SEM, along with the number of experiments.

Dopamine D_2 assay

Cell culture: Chinese hamster ovary (CHO) cells stably expressing human D_{2L} dopamine receptors (Wilson *et al.*, 2001) were grown in Dulbecco's modified Eagle's medium

containing 5% foetal bovine serum and 400 mg/ml active geneticin (to maintain selection pressure). Cells were grown at 37 °C in a humidified atmosphere of 5% CO_2.

Membrane preparation: Membranes were prepared from CHO cells expressing D_{2L} dopamine receptors as described previously (Castro and Strange, 1993). Briefly, confluent 175 cm^2 flasks of cells were washed once with 10 ml 4-(2-hydroxyethyl)-1-piperazineethyl-sulphonic acid (HEPES) buffer (11.9 g/L HEPES, 2 ml/L ethylenediaminetetraacetic acid ((EDTA), 47 mg/L leupeptin, 25 mg/mL bacitracin; pH 7.4 using KOH). The cell pellet was resuspended in 10 volumes of buffer. Pepstatin (2×10^{-6} M) and PMSF (1 mM) were added to Waring blender and homogenized for 15 secs (full speed). Homogenate was left to stand on ice for 5 mins and homogensized for further 15 secs and let it stand for another 30 mins (500 ml blender for volumes up to 200 ml, 1 L blender for volumes 200 ml – 500 ml. Volumes larger than 500 ml are split and processed in smaller aliquots). The liquid had settled after 30 mins and was transfered to 500 ml centrifuge tubes and centrifuged at 1200 rpm, 4 °C for 10 mins. Supernatant was transferred to Beckman 70 ml ultracentrifuge tubes and centrifuged at 24,800 rpm for 36 mins. Supernatant was discarded and pellet was resuspend in approx 4 volumes of buffer using a 20ml syringe. Once the membrane pellet was an even suspension it was passed through a 0.6 x 25 mm needle and dispensed into 1 ml aliquots and stored at -80 °C until use. Protein concentration was determined using a BCA assay (determined by the method of Lowry *et al.*, 1951).

[^{35}S]GTPγS binding assays: Cell membranes (20 µg/ml) were pre-incubated for 30 min at room temperature in 20 mM HEPES buffer containing 1 µM GDP, 5 mM NaCl, 95 mM NMDG and 10 mM $MgCl_2$, pH 7.4. Also 30 µg/ml Saponin, 0.01% ploronic F-127 and 5 mg/ml of PS-WGA beads were also added before the incubation. After the addition of ligands (in duplicated, 1/3 dilution serial response) the assay was initiated by adding [^{35}S]GTPγS to give a final concentration of 500 pM. The assay was incubated for 1 hour and

then plates were centrifuged at 1200 rpm for 2 min before to read them in a ViewLux reader from PerkinElmer.

Data analysis: Results in the text are shown as means ± SEM, along with the number of experiments. Quinelorane was used as pharmaceutical standard compound as well as dopamine and preclamol in order to check the assay robustness, variability and validate the data arisen. Data from [^{35}S]GTPγS binding experiments were fitted to a sigmoidal concentration/ response curve with a Hill coefficient of one which provided the best fit to the data in all cases (P<0.05). Time course data were fitted well by mono-exponential equations from which the apparent first-order rate constant (k, min^{-1}) and maximal binding (B_{max}, fmol mg^{-1}) values could be extracted. The initial rate of [^{35}S]GTPγS binding was calculated as $k.B_{max}$ in fmol mg^{-1} min^{-1}. The maximum response values were referenced to quinelorane at 100 μM.

Kbb assay

The testing has been done by WuXi, Shanghai.

Preparation of solutions: The stock solutions in dimethyl sulfoxide were prepared to 10 mM stock concentration and 1 mM working solution and stored at 4 °C. The dialysis buffer (PBS, pH 7.4) was prepared by dissolving 1.42 g Na_2HPO_4, 0.27 g KH_2PO_4, 8.01 g NaCl and 0.20 g KCl into a final volume of 1000 mL ultra pure water, mixed, and adjust the resulting solution with 1% phosphoric acid to pH 7.4 ± 0.1. The final solutions were stored at +2-8 °C in the refrigerator within an expiration of a month after preparation.

Test system: The fresh blood and brain tissue was from a male Wistar Han Rat (WuXi 20130520). Control blood was collected fresh, diluted 1:1 (v/v) with dialysis buffer and stored at +2-8 °C refrigerator. EDTA-K_2 was used as anticoagulant in the fresh blood sample. Brains were collected and homogenized 1:2 with dialysis buffer and stored at ≤-70 °C in the freezer. The diluted blood and brain homogenates were equilibrated to room temperature before used

on the day of the experiment. HT-Dialysis plate (Model HTD 96 b, Cat# 1006) and the dialysis membrane (molecular weight cut off 12-14 kDa, Cat# 1101) were purchased from HT Dialysis LLC (Gales Ferry, CT).

Methods: The dialysis membrane was pretreated according to the manufacturer's instructions: therefore, the dialysis membrane was soaked in ultra pure water for 60 min and each strip was separated, then the membrane was soaked in 20% Ethanol for 20 min and finally in ultra pure water for 20 min. The dialysis instrument was assembled following the manufacturer's instructions. A 12-channel pipettor was used to add 150 µL of PBS to the appropriate compartment of all wells being used. The 1 mM working solutions were made by diluting stock solutions with appropriate volume of neat dimethyl sulfoxide. The final 5 µM solutions were made by diluting 3 µL of the working solutions with 597 µL of control diluted blood/brain homogenates. Each cell was loaded with 150 µL of spiked diluted blood or brain homogenates (in triplicate) to the other compartment of each well against dialysis buffer. The dialysis plate were sealed with adhesive sealing strip to the top of the block to avoid evaporation and placed it in an incubator at 37 °C for 4 h with rotating at 130 rpm (Model: VXR B, IKA). After incubation, the samples were removed from the blood or brain homogenates (5 µL) as well as the buffer (50 µL) sides to the sample collection plates. The blood or brain homogenate aliquots (5 µL) were diluted with 45 µL of diluted control blood (blood: PBS=1:1 in v:v) or control brain homogenates (brain tissue: PBS=1:2 in gram: mL) and 50 µL blank buffer to generate Donor samples; buffer samples (50 µL) were diluted with 50 µL of diluted control blood or control brain homogenates to generate Receiver samples. The diluted samples were mixed with 3 volumes of precipitation solvent (50% acetonitrile/ CH_3OH with internal standards of 200 ng/mL Labetalol plus 10 ng/ml Buspirone). The sample collection plates were shaken at 800 rpm for 10 min to mix samples and then the plate was centrifuged at 3,220 RCF for 15 min. An aliquot of supernatant (100 µL for Loperamide and test compounds, 200 µL for Naproxen) was transferred in each well of the sample

analytic plates, mixed with solution (200 µL of ultra pure water: 50% acetonitrile/ CH_3OH = 1:2 for test compounds; 500 µL for Loperamide) and replaced on the plate shaker for at least 5 mins before subjecting to LC- MS / MS analysis.

Data Analysis: MS response ratio of test compound in matrix and buffer samples was expressed using peak area ratios of analyte/internal standard. The fraction of unbound (Apparent Fu) and undiluted unbound were calculated by the following equations: Apparent fu = MS Corrected Response [Buffer] / MS Corrected Response [blood/brain]; MS Corrected Response = MS Response Ratio/ Correction Factor; Correction Factor = volume of aliquot / total volume. Blood: 5 µL, dilution 20-fold (Correction Factor = 0.05); Brain: 5 µL, dilution 20-fold (Correction Factor = 0.05); Buffer: 50 µL, dilution 2-fold (Correction Factor = 0.5). Calculation of undiluted Fu with Correction for 'Dilution Factor (D)'; % Undiluted Unbound= (1/D)/[(1/fu(apparent)-1)+1/D]*100; Blood: D = 2; Brain: D = 3.

ASSOCIATED CONTENT

Supporting Information Available: The provided document comprises the synthesis and characterization of the important intermediates ZM 241385 (**1**), TCAC (**3**), OAC (**5**), sTCAC (**16**) and sulfone **29**. This material is available free of charge via the Internet at http://pubs.acs.org

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ABBREVIATIONS

D_2R, dopamine D_2 receptor; $A_{2A}R$, adenosine A_{2A} receptor; LC-MS, liquid chromatography–mass spectrometry; BOP, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; cAMP, cyclic adenosine monophosphate; CNS, central nervous system; COMU, (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate**;** DBU, 1,8-Diazabicycloundec-7-ene; DCM, dichloromethane; DIPEA, diisopropyl ethylamine; DMF, dimethyl formamide; HCTU, 2-(6-chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate; NECA, 5'-*N*-ethylcarboxamidoadenosine or alternatively 1-(6-amino-9*H*-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide; PPHT-NH_2, 6-((4-aminophenethyl)(propyl)amino)-5,6,7,8-tetrahydronaphthalen-1-ol; TEA, triethylamine; TFA, trifluoroacetic acid; rt, room temperature; XCC, 2-(4-(2,6-dioxo-1,3-dipropyl-2,3,4,5,6,7-hexahydro-1*H*-purin-8-yl)phenoxy)acetic acid.

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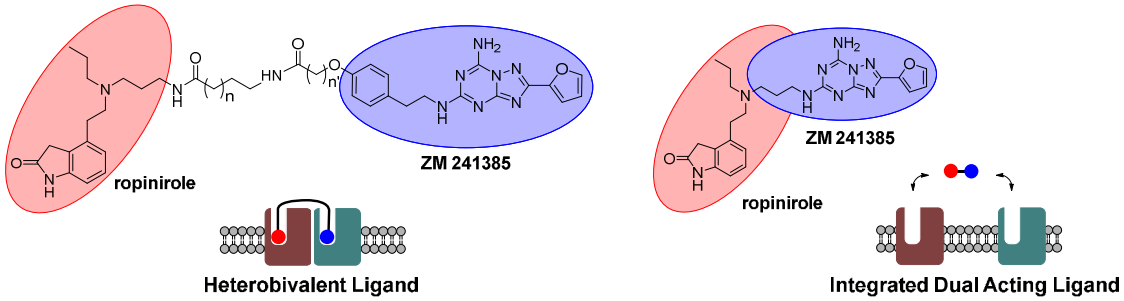

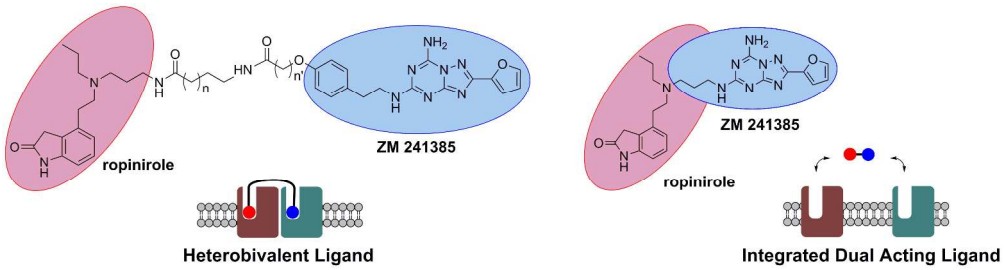

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