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Discovery of a 3,4,5-trisubstituted-1,2,4-triazole agonist with high affinity and selectivity at the somatostatin subtype-4 (sst₄) receptor†

Iman Daryaei,^{a,b} Karin Sandoval,^a Ken Witt,^a
Maria Kontoyianni^a and A. Michael Crider^{a,*}

A series of compounds containing a 1,2,4-triazole moiety were synthesized, targeting the somatostatin receptor subtype-4 (sst₄). Compounds were developed in which the Phe⁶/Phe⁷/Phe¹¹, Trp⁸, and Lys⁹ mimetic groups were interchanged at positions 3, 4, and 5 of the 1,2,4-triazole ring. The 1,2,4-triazoles containing an 2-(imidazol-4-yl)ethyl substituent at position-3 demonstrated moderate binding affinity at sst₄. 1,2,4-Triazoles containing an (indol-3-yl)methyl substituent at position-5 lacked affinity at sst₄. The 1,2,4-triazoles containing an aminopropyl group at position-4 showed enhanced binding affinity compared to the 3-position. One compound with an 3-(imidazol-4-yl)propyl group at position-4 (compound **44**) imparted high affinity and selectivity at sst₄ (sst_{2A} = >10 000 nM; sst₄ = 19 nM), acting as an agonist (EC₅₀ = 6.8 nM). Docking **44** into a model-built structure of sst₄ pointed to differences in its binding *versus* the other low-affinity compounds and was also in line with one of the two previously reported binding modes. A virtual screening (VS) experiment, employing two separate docking algorithms, was able to score **44** among the top-ranked poses. In summary, compound **44** represents a novel and promising lead structure towards the development of a clinically viable sst₄ agonist for the treatment of conditions ranging from Alzheimer's disease to chronic pain.

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Introduction

Somatostatin (somatotropin release-inhibiting factor, SRIF) is a naturally occurring polypeptide that was originally isolated from the hypothalamus.¹ Two biologically active forms exist, SRIF-14 (1, Fig. 1) and SRIF-28, derived from a 92-amino acid pro-somatostatin precursor.² Both SRIF forms demonstrate pharmacological action *via* binding to structurally-related receptors, which belong to the G-protein-coupled superfamily (G_i-coupled).³ The five receptor subtypes (sst₁–sst₅) fall into two major families, which have been cloned and characterized.⁴ Structure–activity relationship (SAR) studies on SRIF have shown that the core residues Trp⁸ and Lys⁹ of the β-turn are necessary for receptor activation and pharmacological activity.^{5,6} Furthermore, research carried out by Lewis *et al.*⁷ showed that Phe⁶, Phe⁷, and Phe¹¹ play a role in sst₂, sst₃, and sst₅ binding, whereas only Phe⁶ was involved in sst₄ binding. While the receptor signaling pathways share similarities,

difference in affinity, distribution, interactions, and trafficking enable each receptor to mediate a variety of outcomes. Given SRIF has shown to exhibit inhibitory actions on endocrine and exocrine secretions, as well as numerous modulatory effects on neuronal activity in the periphery and central nervous system (CNS),⁸ the respective receptors have become primary therapeutic targets.

Of the five receptors, sst₄ has come to the forefront as a potential mediator of CNS pathology, inflammation, and even pain mechanisms. Targeting sst₄ has additional advantages, having limited pituitary distribution and shown not to inhibit glucagon, growth hormone, or insulin secretion.⁹ Within the CNS, sst₄ has been shown to be expressed at relatively high levels in the hippocampus and neocortex,¹⁰ areas identified with memory and learning, as well as Alzheimer's disease pathology.¹¹ Recent evaluations have indeed shown sst₄ agonist action improves learning and memory in rodent models of Alzheimer's disease, which corresponded with reduced β-amyloid levels.^{12–14} Furthermore, investigations have identified sst₄ receptor agonism dose-dependently enhanced cued memory,^{15,16} and thus may have direct cognitive enhancement activity. Other research has shown sst₄ coupled to K⁺ M-channels was capable of regulating hippocampal excitability,¹⁷ with implications towards sst₄ agonist-based treatments for some forms of epilepsy. Interestingly, sst₄ agonist action

^a Department of Pharmaceutical Sciences, School of Pharmacy, Southern Illinois University Edwardsville, Edwardsville, IL, 62026-2000 USA.

E-mail: mcrider@siue.edu

^b NuvoX Pharma, 1635 18th St, Tucson, AZ, 85719 USA

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Fig. 1 Schematic depiction of the rational design of **44** from SRIF-14 peptide. Residues and corresponding substituents are colored the same.

has also been linked to peripheral anti-nociceptive and anti-inflammatory activity in association with acute and chronic pain models in rodents.¹⁸ Recent data shows SRIF released from capsaicin-sensitive receptor potential vanilloid type 1 (TRPV1)-expressing nociceptors acts on ss_{t4} , as well as ss_{t2} , to produce its anti-nociceptive effects.¹⁹ The list of applications for ss_{t4} agonist therapeutics continues to grow, ranging from mood disorders²⁰ to bladder dysfunction.²¹ Given this significant potential, our laboratory has worked to design and optimize a clinically viable ss_{t4} selective agonist.

The therapeutic use of SRIF is limited by poor bioavailability and rapid breakdown by peptidases. Although longer acting analogs such as lanreotide and octreotide have been developed, administration by the subcutaneous or intravenous routes is required.²² Recently, more stable dicarba-analogs of octreotide have been synthesized and evaluated at ss_{t2} and ss_{t5} .²³ As an extension of this work, dicarba-analogs of octreotide conjugated to 1,4,7,10-tetraazacyclodecane-1,4,7,10-tetraacetic acid (DOTA) were shown to have nanomolar affinity at ss_{t2} and ss_{t5} .²⁴ Although the development of peptide analogs of SRIF-14 remains an active area of research, our focus has long been on the design and discovery of selective, orally active, non-peptide ss_{t4} agonists.

Earlier work from our laboratory focused on a thiourea moiety in the design and discovery of non-peptide ss_{t4} agonists. Regarding the thiourea scaffold, we focused on the following three side chain mimetics: a) a heteroaromatic nucleus (Trp⁸ mimetic), b) a non-heteroaromatic group (Phe⁷ mimetic), and c) a basic moiety (Lys⁹ mimetic).^{25,26} The same reasoning directed our efforts towards the triazole derivatives. Fig. 1 specifi-

cally depicts the rationale for the design of compound **44**. The two core residues of SRIF-14 (**1**), Trp⁸ and Lys⁹, thought to be critical for binding to all ss_{t} receptors are highlighted, along with Phe⁷. Using previous structure–activity relationships, indole, dichlorobenzyl, and imidazole moieties were selectively chosen as substituents corresponding to Trp⁸, Phe^{6/7/11} and Lys⁹, respectively. The *cis*-peptide bond is replaced by a triazole for a peptidomimetic design; it also positions the aforementioned amino acid residues, mimicking the endogenous substrate optimally within the binding pocket, using the structure-based design of our earlier work.²⁷ The thiourea (NNC 26-9100, **2**, Fig. 1) was shown to have over 100-fold selectivity at ss_{t4} compared to other ss_{ts} , and in a functional assay using inhibition of forskolin-induced cyclic 3,5-adenosine monophosphate (cAMP), NNC 26-9100 was shown to be a full agonist at ss_{t4} .²⁵ Unfortunately, thioureas have been associated with adverse effects thought to result from reactive sulfur oxidation products catalyzed by cytochrome P450 and flavin monooxygenases (FMO).^{28,29} Thus, our program has been refocused towards the development of non-thioureas ss_{t4} agonists. In this regard, the 1,2,4-triazole nucleus (**3**, Fig. 2) was pursued as a principle scaffold, given it: 1) represents a highly viable scaffold that is

Fig. 2 Structures of the 3,4,5-trisubstituted-1,2,4-triazole moiety (**3**) and the 3-thio-1,2,4-triazole moiety (**4**).

readily synthesized in a variety of functional forms, 2) is a *cis*-amide bond isostere³⁰ that is found in natural peptides and is not subject to peptidase action, 3) has three points of diversity making it more easily modifiable *via* modular parallel chemistry, and 4) does not contain the potentially problematic thiourea. Moreover, the related 3-thio-1,2,4-triazole moiety (4, Fig. 2) has been previously utilized in the targeting of sst₂/sst₅ ligands,³¹ providing further validation to our approach.

Herein, our goal was to develop ligands based on the 1,2,4-triazole ring with high affinity and selectivity at sst₄. Three series of compounds were proposed in which the Phe⁶/Phe⁷/Phe¹¹, Trp⁸, and Lys⁹ mimetic groups were interchanged at positions 3, 4, and 5 of the 1,2,4-triazole ring. The choice of side chains was guided by previous SAR studies on sst₄ agonists.^{25,26,32} Using a recently generated homology model of sst₄,²⁷ docking assessments were conducted to delineate principle binding attributes of respective compounds. A VS experiment was also carried out to affirm docking findings.

Results and discussion

Chemistry

The general synthetic pathway for the synthesis of 3,4,5-trisubstituted-1,2,4-triazoles is shown in Scheme 1. This method utilizes a literature procedure in which a thioamide (I) is reacted with a hydrazide (II) to yield an intermediate acylamidrazone (III). Cyclization to the desired 3,4,5-trisubstituted-1,2,4-triazoles (IV) was facilitated by use of either mercuric acetate or silver benzoate as the thiophile.^{33,34} Compared to mercuric acetate, the use of silver benzoate as the thiophile generally decreased reaction times and afforded cleaner reaction products.

The required hydrazides (5–8, Fig. 3) were prepared from either *tert*-butoxycarbonyl (Boc) or trityl-protected methyl or ethyl esters in refluxing hydrazine hydrate. Methyl esters which were not commercially available were prepared from either amino acids or carboxylic acids using trimethylsilyl chloride (TMS-Cl).³⁵

Thioamides required for the synthesis of the 1,2,4-triazole moiety were prepared as shown in Scheme 2. Amides 11–17 were synthesized from either indoleacetic acid (9) or indole-

Fig. 3 Hydrazides 5–8 used in the synthesis of 1,2,4-triazoles.

propionic acid (10) using a mixed anhydride coupling procedure.³⁶ The 3,4-dichlorophenylacetamide derivative 25 was prepared from tryptamine and thionyl chloride in the presence of triethylamine. All amides could be thionated using Lawesson's reagent in good to excellent yields (18–24 and 26, Scheme 2).^{37,38} Since each equivalent of Lawesson's reagent produces two equivalents of active thionating agent, only 0.5–0.6 equivalent of Lawesson's reagent was required for thioamides 18–24 and 26. Due to solubility issues with amides 16 and 17, 1.0–1.1 equivalent of Lawesson's reagent was required for the synthesis of thioamides 23 and 24. Conversion of amides to thioamides results in a significant downfield shift in the ¹³C NMR spectrum. The presence of a peak in the ¹³C NMR spectrum at 200–210 ppm and the absence of the amide peak at 160–170 ppm were indicative of thioamide formation.

With the exception of 5, all required hydrazides (Fig. 3) were known compounds and were prepared by literature methods. Coupling of the appropriate hydrazide with a thioamide (Scheme 3) in tetrahydrofuran in the presence of either mercuric acetate or silver benzoate as the thiophile afforded the 3,4,5-trisubstituted-1,2,4-triazoles. Flash chromatography was required to remove the precipitated Ag or Hg from the thiophile. After cyclization, the trityl or Boc-protecting group was removed with 1 N hydrochloric acid in ethanol or methanol. The target 1,2,4-triazoles 27–44 (Scheme 3) were purified by flash chromatography, recrystallization or salt formation. Yields of the target 1,2,4-triazoles over two steps ranged from low (11%, compound 34) to good (62%, compound 44). All 1,2,4-triazoles exhibited two characteristic peaks in the ¹³C NMR spectra at 150–160 ppm corresponding to the ring carbons at positions 3 and 5.

Receptor binding assay

The direct interaction of the target 3,4,5-trisubstituted-1,2,4-triazoles with human sst_{2A} and sst₄ receptors were evaluated using *in vitro* radioligand binding displacement studies.

Scheme 1 General pathway for the synthesis of 3,4,5-trisubstituted-1,2,4-triazoles.

Scheme 2 Synthesis of amides (**11–17**, **25**) and thioamides (**18–24**, **26**). Reagents: (a) i. NNM/IBCF/THF, ii. RNH₂; (b) Lawesson's reagent/THF; (c) i. SOCl₂/CH₂Cl₂, ii. tryptamine/THF.

Membrane Target™ Systems (Perkin Elmer) for sst₄ (ES-524-M400UA) and sst_{2A} (ES-521-M400UA) were used in these studies as previously described by Wang *et al.*³⁹ The data from the competition binding studies using ¹²⁵I-Tyr-SRIF as the radioligand is summarized in Table 1. Previously reported non-peptide sst₄ agonists and octreotide are included in Table 1 for reference. Several conclusions can be drawn from the binding studies: 1) none of the target 1,2,4-triazoles exhibited any appreciable affinity for sst_{2A}, 2) 1,2,4-triazoles containing an (indol-3-yl)methyl substituent at position-5 (compounds 27–32) lacked affinity at sst₄, 3) 1,2,4-triazoles containing an 2-(imidazol-4-yl)ethyl substituent at position-3 demonstrated moderate binding affinity at sst₄ (compounds 34, 37, 39, and 41), 4) 1,2,4-triazoles containing an aminopropyl group at position-4 showed enhanced binding affinity compared to the 3-position (compare compounds 36 and 43), and 5) an 3-(imidazol-4-yl)propyl group at position-4 (compound 44) imparted high affinity and selectivity at sst₄ (sst₁ = 4750 nM; sst_{2A} = >10 000; sst₃ = 6628 nM; sst₄ = 19 nM; sst₅ = >10 000).

Given the enhanced affinity and selectivity, compound 44 was further evaluated in a functional assay based on the

forskolin-induced cAMP inhibition in recombinant Chinese hamster ovary (CHO-K1) cells expressing sst₄. Compound 44 demonstrated approximately 4-fold greater efficacy (EC₅₀ = 6.8 nM) compared to NNC 26-9100 (2, EC₅₀ = 27.91 nM) and was shown to be a full agonist in this assay.

Docking studies

Docking studies were undertaken to delineate binding modes of respective compounds (Fig. 4). The objective was two-fold: 1) predict and explain differences in activities by performing docking experiments and evaluating binding modes, and 2) carry out a VS experiment in hopes of assessing whether docking algorithms can single out the active among the top-ranked poses. Regarding the second goal, it is apparent we were pushing the ability of VS in that it has been proposed that at 2.5% fraction of an entire compound collection half of all actives are retrieved.⁴³ In other words, we were asking for a “needle in a haystack”, which is the very idea of VS, however, a higher number of actives are typically included in those experiments.

Scheme 3 Synthesis of 3,4,5-trisubstituted-1,2,4-triazoles. Reagents and conditions: (a) CH₂Cl₂, AgOBz or Hg(OAc)₂, HOAc, THF, RT; (b) 1 N HCl, EtOH, reflux.

GOLD⁴⁴ was able to explain observed activities for all molecules in line with our previous work on sst₄ binding.²⁷ Compounds 45–48 are placed in the binding pocket with favorable hydrophobic and pi-stacking interactions, but none featured all electrostatics. Specifically, the secondary amine (45) or the pyridine nitrogen (47, 48) hydrogen bond with Gln²⁴³. Aryl-aryl interactions are observed between the pyridine and Phe²³⁹ and Phe¹⁷⁵, however, no pose displays all ionic interactions involving Trp¹⁷¹, His²⁵⁸, and Asp⁹⁰. Similarly, the best pose of compound 39 has the indole N–H hydrogen bonding with Gln²⁴³ and imidazole forming a hydrogen bond with Tyr¹⁸. π – π Interactions are observed between indole and Trp¹⁷¹ and Phe²³⁹. Analysis of compound 41 reveals that the indole is interacting with Asp⁹⁰ and the triazole hydrogen bonds with the amide N–H. In compound 43, however, the indole forms a hydrogen bond with Gln²⁴³, while the primary amine points towards Asp⁹⁰. The extent of hydrophobic and pi-stacking interactions are limited in both poses. Finally, the predicted binding mode of the most active 44 reveals that imidazole forms a hydrogen bond with Gln²⁴³ and Tyr²⁴⁰, the indole N–H is positioned near Asp⁹⁰, and the backbone amide hydrogen of Trp²⁷¹ hydrogen bonds with triazole. An edge-to-face arrangement is observed between His²⁵⁸ and triazole, while the imidazole makes a π – π interaction with Phe²³⁹. The chlorine of chloro-benzyl forms a halogen bond with the carbonyl oxygen of Asp²⁵³. Table 2 shows a summary of the best

poses, respective ranking, and scoring function energies. VS with GOLD ranked the active 44 in the top 10% of the library (1005 compounds, with 996 decoys). Compound 44 was ranked 78 with GoldScore.

In regards to docking with Glide,^{45,46} the best poses of 45 and 46 form interactions with either Asp⁹⁰ or Gln²⁴³, but not with both. Specifically, the secondary amine is close to Asp⁹⁰ in 45, with Trp¹⁷¹ and Phe²³⁹ forming a hydrophobic pocket around the pyridine moiety. However, no interactions are observed with His²⁵⁸ or Gln²⁴³. A hydrogen bond interaction between the secondary amine of 46 and the carbonyl oxygen of Gln²⁴³ is predicted, while the carbonyl oxygen of the central 2-thiohydantoin ring hydrogen bonds with His²⁵⁸. The reason for the different interactions of the secondary amine is that 46 is positioned deeper in the pocket with the amino-pyridine moiety rotated away from Asp⁹⁰ and pointing towards Gln²⁴³; this also results in pyridine no longer forming π – π interactions with Trp¹⁷¹. The methoxy-substituent in 45 is exposed to the extracellular solvent while the indole groups in both compounds are facing the extracellular domain. The best poses of compounds 47 and 48 are similar to 46 in that the 2-aminopyridine forms a hydrogen bond with Gln²⁴³, and the carbonyl oxygen of the 2-thiohydantoin interacts with the backbone amide nitrogen of Trp¹⁷¹. It should be noted that neither the indole nor the dichlorophenyl and naphthalene groups form any interactions. The predicted binding mode of

Table 1 *In vitro* binding of 1,2,4-triazoles at sst_{2A} and sst₄ receptors^a

Compound	sst _{2A} ^b (nM)	sst ₄ ^c (nM)
27	>10 000	>10 000
28	>10 000	>10 000
29	—	>10 000
30	—	>10 000
31	—	>10 000
32	>10 000	>10 000
33	>10 000	<10 000
34	—	4471
35	>10 000	>10 000
36	>10 000	>10 000
37	>10 000	1426
38	>10 000	>10 000
39	>10 000	2032
40	—	>10 000
41	>10 000	4803
42	—	>10 000
43	>10 000	2380
44	>10 000	19
NNC 26-9100	2621	148
	621 ^d	6 ^d
J-2156	>5000 ^e	1.2 ^e
L-803087	4720 ^f	0.7 ^f
Octreotide	0.4–2.1 ^g	66–2100 ^g
SRIF-28	0.68	2.41

^a Competitive radioligand binding experiments were performed using Membrane Target Systems (Perkin Elmer, Boston, MA) for human sst₄ (ES-524-M400UA) and sst_{2A} (ES-521-M400UA) receptors as previously described by Wang *et al.*³⁹ Binding assays were performed in triplicate using ¹²⁵I-Tyr-SRIF (Perkin Elmer) dissolved in 1 mM HCl. ^b Concentration of radioactivity = 0.075 nM, K_D = 0.075 nM.

^c Concentration of radioactivity = 0.2625 nM, K_D = 1.05 nM.

^d Ankersen *et al.*²⁵ ^e Engstrom *et al.*⁴⁰ ^f Rohrer *et al.*⁴¹ ^g Patel.⁴²

compound 34 reveals a hydrogen bond between the imidazole and the carbonyl oxygen of Gln²⁴³, while the Nδ nitrogen

Table 2 Best GOLD rankings and respective scoring functions

Compound	GoldScore (rank number)	ChemScore (rank number)
45	60.3 (10)	18.5 (10)
46	—	—
47	66.6 (5)	36.3 (1)
48	69.3 (4)	34.5 (2)
39	67.5 (2)	27.2 (4)
41	64.3 (10)	29.1 (2)
43	67.5 (8)	35.9 (4)
44	73.1 (1)	27.4 (4)

interacts with His²⁵⁸. The indole nitrogen forms a hydrogen bond with the backbone carbonyl oxygen of Ser⁷⁰. In contrast, the binding mode of 39 has the reverse orientation with indole forming a hydrogen bond with Asp⁹⁰, and imidazole's Nε hydrogen bonding with the backbone carbonyl oxygen of Ser⁷⁰. The benzene ring mediates a π–π stacking interaction with Trp⁷⁶, and hydrophobic interactions with Val⁸⁶ and Leu⁸⁷. The only interaction observed for compound 41 is between imidazole and Asp⁹⁰ with an overall conformation that seems strained. This in turn explains its very low affinity. A salt bridge is observed between the amine of compound 43 and Asp⁹⁰, while the indole nitrogen forms a hydrogen bond with the backbone carbonyl oxygen of Ser⁷⁰. The dichlorobenzyl forms a π–π interaction with Trp¹⁷¹ and Phe²³⁹ and the triazole hydrogen bonds with His²⁵⁸. Contrary to the above binding modes, compound 44 encompasses electrostatics and hydrophobic interactions with the amino acids we predicted in prior publication as being critical.²⁷ Specifically, the binding mode analysis of 44 reveals a hydrogen bond between the indole and Gln²⁴³, while another hydrogen bond is formed between imidazole and Asp⁹⁰. Triazole hydrogen

Fig. 4 Structures of compounds docked. ^aWang *et al.*³⁹

Fig. 5 Docking of 44 with Glide.

Table 3 Summary of rankings by Glide

Compound	Rank number	Emodel
45	2	-74.5
46	1	-83.9
47	1	-79.9
48	1	-94.2
34	1	-72.9
39	1	-63.0
41	6	-53.0
43	1	-64.0
44	3	-73.8

bonds with Trp¹⁷¹ and His²⁵⁸ and a halogen bond is formed between the *meta*-chlorophenyl and the carbonyl oxygen of Ser⁷⁰. Aryl-aryl interactions are also predicted between the indole and Trp¹⁷¹ and Phe²³⁹ (Fig. 5). In summary, all compounds with the exception of the active 44 suffer from limited interactions with the binding site residues; most noticeably hydrogen bonds with Asp⁹⁰, His²⁵⁸ and Trp¹⁷¹, along with hydrophobic interactions. Furthermore and based on previous findings,²⁷ we believe that 44 binds similarly to binding mode II agonists. A summary of the rankings for each of the compounds docked is presented in Table 3. The VS experiment with Glide did not dock all compounds in the library. However, compound 44 was ranked at 132 of a total of 969 successful dockings.

Conclusions

In this report, we describe the synthesis of eighteen 3,4,5-trisubstituted-1,2,4-triazoles and their binding affinities at sst₂ and sst₄. The triazole 44, substituted with an 3-(imidazole-4-yl)propyl group at position-4, demonstrated high affinity and selectivity at sst₄ over other sst_s with selectivities: sst₄/sst₁ = 250-fold, sst₄/sst₂ = >500-fold, sst₄/sst₃ = 350-fold, sst₄/sst₅ = >500-fold. On the basis of a functional assay to access inhibition of forskolin-induced adenosine cyclic 3',5'-monophosphate (cAMP) accumulation, the triazole 44 was shown to be a highly

selective, full agonist (EC₅₀ = 6.8 nM) at sst₄. This compound has several advantages over sst₄ agonists reported from our laboratory in that it: 1) contains a 1,2,4-triazole moiety that is a *cis*-amide bond isostere, 2) is not subject to peptidase activity, 3) exhibits high selectivity at sst₄ over other sst_s, and 4) does not contain a thiourea group that can form potentially reactive metabolites. Additionally, compound 44, exhibited greater affinity at sst₄ than the previously reported thiourea, NNC 26-9100 (2).²⁵ Docking 44 into a recently model-built sst₄ structure of sst₄ (ref. 27) confirmed that this compound forms extensive interactions with key residues such as Asp⁹⁰, Gln²⁴³, Trp¹⁷¹, and His²⁵⁸, contrary to the other synthetic targets reported herein. Continued optimization of the 1,2,4-triazole moiety towards enhancement of pharmacokinetic parameters, while maintaining affinity and selectivity at sst₄, will provide a viable therapeutic with vast potential.

Experimental

The detailed procedures for the chemical synthesis, receptor binding assays, and docking studies are described in the ESI.† All intermediates and final products were purified by recrystallization or flash chromatography and were characterized by ¹H and ¹³C NMR, HRMS, and/or elemental analyses.

Conflicts of interest

No Conflicts of Interest to report by any of the authors.

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