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Original article

Solid-supported synthesis, molecular modeling, and biological activity of long-chain arylpiperazine derivatives with cyclic amino acid amide fragments as 5-HT$_7$ and 5-HT$_{1A}$ receptor ligands

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ABSTRACT

A 47-membered library of novel long-chain arylpiperazines, which contained cyclic amino acid amides in the terminal fragment (pyrrolidine-2-carboxamide and 1,2,3,4-tetrahydroisoquinoline-3-carboxamide), was synthesized on Rink-amide resin and biologically evaluated for binding affinity for 5-HT$_7$ and 5-HT$_{1A}$ receptors. Surprisingly, members of the designed series containing piperidine-2-carboxamide fragments underwent hydrolysis, which occurred during the acidic treatment for release from the solid-support, to their respective pipecolic acid analogs. Representative compounds from the library displayed high-to-low affinity for 5-HT$_7$ (K_i = 18–3134 nM) and 5-HT$_{1A}$ (K_i = 0.5–6307 nM) sites. The possible interactions implicated in binding of the studied compounds to the 5-HT$_7$ receptor were supported by molecular modeling. Research was also applied to support the exploration of the influence of the amide fragment, the length of alkylene spacer, and arylpiperazine substituents on the receptor's affinity and selectivity.

1. Introduction

The 5-HT$_7$ receptor (5-HT$_7$R) is the latest addition to a family of serotonin receptors that are positively coupled to adenylyl cyclase through a G$_s$ protein [1]. The distribution of 5-HT$_7$Rs in several region of the brain, such as the hippocampus, hypothalamus, or thalamus, has fortified interest in these sites as potential targets for drug development. Notably, several antidepressant and antipsychotic drugs display high affinity for 5-HT$_7$Rs, and the antagonism of 5-HT$_7$Rs produces antidepressant and pro-cognitive effects [2–4]. On the other hand, the activation of 5-HT$_7$Rs produces antinociceptive effects. Potential applications of 5-HT$_7$R agonists as co-adjuvants in opioid-mediated analgesia have been proposed and could represent a new therapeutic target for the treatment of pain [5,6].

In parallel to the acquisition of more detailed insight into the function of 5-HT$_7$R pharmacology, an academic and pharmaceutical research was directed toward the development of 5-HT$_7$R ligands [7]. Several potent 5-HT$_7$R agents belong to the group of long-chain arylpiperazines (LCAP) [8]. Unfortunately, the fact that the arylpiperazine privileged core is easily recognized by other monoaminergic receptors (e.g., 5-HT$_{1A}$, 5-HT$_{2A}$, D$_2$, D$_3$, α_1, H$_1$) limits the selectivity of the LCAPs [9,10].

Some of these obstacles were recently overcome by Leopoldo et al. [11], who reported the generation of an amide series of potent 5-HT$_7$R ligands (Fig. 1). The modification of the phenylpiperazine pharmacophore and variation in the linker length were critical to achieving 5-HT$_7$/5-HT$_{1A}$ receptor selectivity.

Inspired by these findings, we continued our search for 5-HT$_7$ ligands by designing a series of LCAPs functionalized with the

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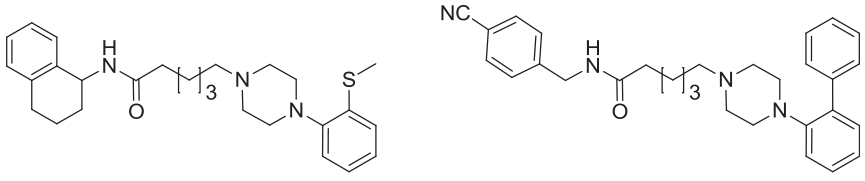


Fig. 1. LCAP examples of 5-HT$_7$R ligands.

primary amides of cyclic amino acids in the terminal fragment [12,13]. These compounds may be considered LP-44 analogs because the exocyclic amide bond was replaced with an endocyclic one and an additional primary amide group was introduced concurrently (Fig. 2). On the other hand, these studies may be regarded as an extension of our ongoing efforts toward the verification of an effect caused by an amino acid-derived terminal fragment on the affinity and selectivity of monoaminergic receptors [14]. The latter issue was extensively evaluated in a series containing *N*-acylated proline and cyclized aspartic acid moieties [15].

We present the design, which was directed by computational modeling and simulations, and solid-supported synthesis of a 47-membered library as well as the biological evaluation of selected library members for binding to 5-HT$_7$ and 5-HT$_{1A}$ receptors.

The structural modifications comprised the introduction of three cyclic amino acids to the terminal fragment, giving proline amides (Pro-amides, set **I**), piperidine-2-carboxamides (Pip-amides, set **II**), and 1,2,3,4-tetrahydroisoquinoline-3-carboxamides (Tic-amides, set **III**) as well as variation in the length of the alkylene spacer and the diversification of the aryl fragments of the N_1-piperazine moiety.

2. Results and discussion

2.1. Chemistry

Synthesis of the designed compounds was carried out on solid support according to a six-step procedure (Scheme 1). Our workflow began with the base-mediated deprotection of a commercially available Rink-amide resin, followed by coupling with Fmoc-protected L-amino acid **2**{*1–3*} (Fig. 3) with HBTU.

After the removal of the Fmoc group, the resulting secondary amines **3**{*1–3*} were reacted with ω-bromo-acyl chlorides **4**{*1–4*} (Fig. 4) to furnish the corresponding amino acid derivatives **5**{*1–3*, *1–4*}.

The resin-bound intermediates were further submitted to a nucleophile displacement with various substituted arylpiperazine derivatives **6**{*1–10*} (Fig. 5) in DMF at 75 °C for 24 h.

The final compounds with general structures **8**–**24**{*1*, *1–4*, *1–10*}, **25**–**36**{*2*, *1–3*, *1–10*} and **37**–**54**{*3*, *1–4*, *1–10*} were released from the resin via treatment with a mixture of TFA/CH_2Cl_2 (80/20, v/v). All of the compounds were purified with a Waters preparative LC/MS apparatus. The final compounds belonging to sets **I** (**8**–**24**) and **III** (**37**–**54**) were obtained in moderate yields (33–45%) and excellent purities (97–99%). In contrast, the designed pipecolic amid derivatives (set **II**), when treated with acidic medium during cleavage from solid support, underwent hydrolysis to form carboxylic acid analogs (Table 1).

Although a similar hydrolytic process was observed for an amide bond in *N*-acylated pipecolic acid derivatives on a BAL linker [16,17], no reports concerning the hydrolysis of pipecolic amides on Rink-amide resin have been disclosed. Following our previous reports, a detailed mechanism of the unexpected amide bond cleavage was proposed (Scheme 2). First, an intramolecular tetrahedral intermediate was formed; the lone pair of electrons on the nitrogen atom was no longer conjugated with the carbonyl bond in this moiety. Consequently, a nitrogen atom became a proton acceptor, allowing for the release of the cyclic structure from the support and the formation of the oxazolinium intermediate (Muchnone). The

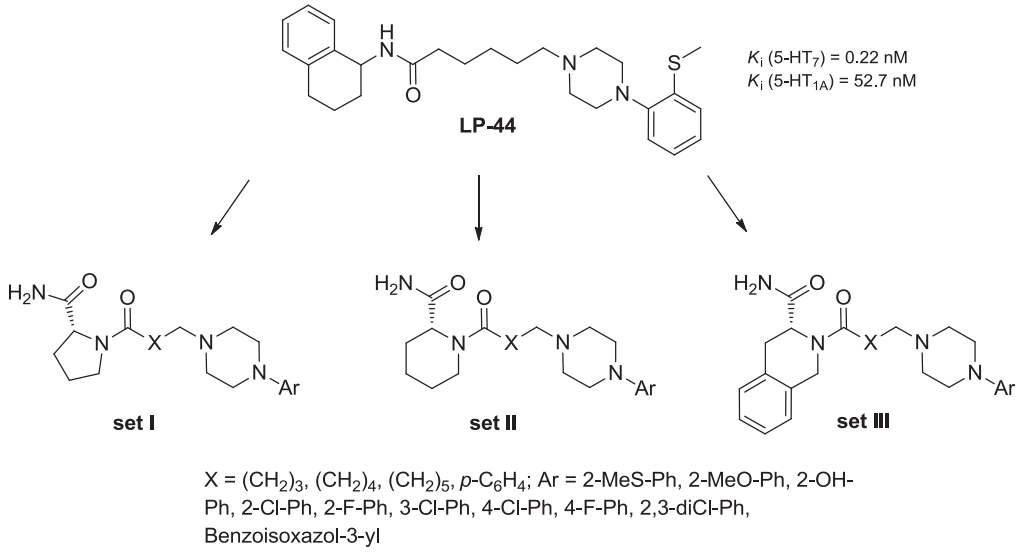
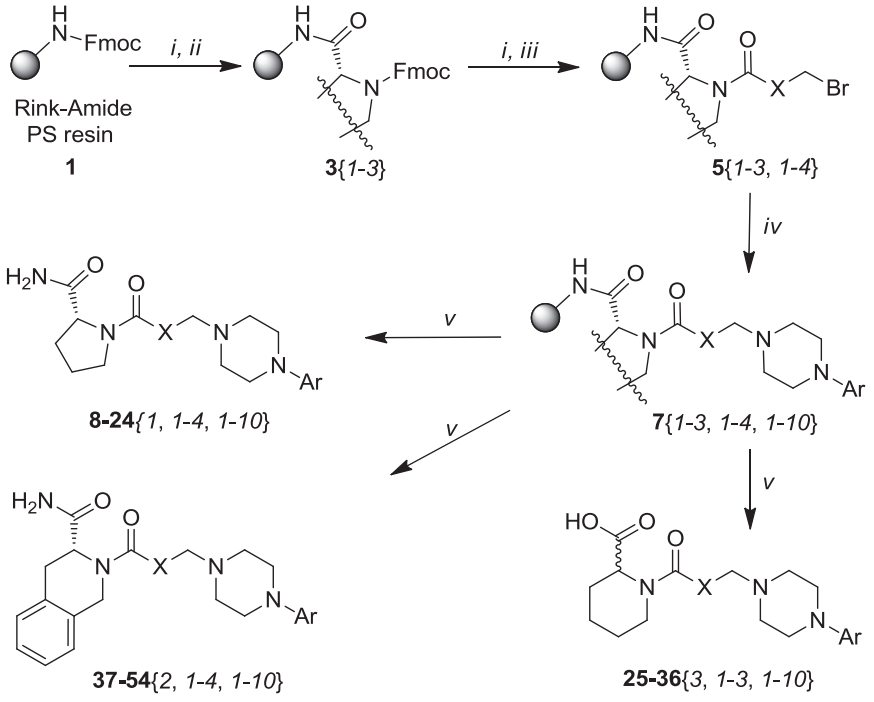


Fig. 2. General structure of the designed pyrrolidine-2-carboxamides (set **I**), piperidine-2-carboxamides (set **II**), and 1,2,3,4-tetrahydroisoquinoline-3-carboxamides (set **III**).



Scheme 1. Solid-phase synthesis of LCAP-containing cyclic amino acid amide fragments. Reagents and conditions: (*i*) piperidine/DMF (20/80, v/v), 20 min, rt; (*ii*) Fmoc-protected amino acid **2**{*1–3*}, HBTU, DIEA, DMF, 2 × 2 h, rt; (*iii*) Acyl chloride **4**{*1–4*}, DIEA, CH_2Cl_2, 4 h, rt; (*iv*) Arylpiperazine **6**{*1–10*}, DMF, 24 h, 75 °C; (*v*) TFA/CH_2Cl_2 (80/20, v/v), 2 h, rt.

latter intermediate reacted with traces of water to yield the pipecolic acid derivatives with concurrent lost of stereospecificity.

2.2. Molecular modeling

Initially, recently released crystal structures of two serotonin receptors, i.e. 5-HT_{1B} (PDB ID:4IAR) and 5-HT_{2B} (PDB ID:4IB4) complexed with ergotamine, were used as templates to generate 5-HT_7 homology models. However, docking of reference ligands of different structure, particularly various long-chain arylpiperazines, showed incoherent binding modes. Hence, six different conformations of modified rhodopsin-based 5-HT_7R homology models [18], successfully used in virtual screening of commercial databases [19] and selection of compounds to synthesis from virtual combinatorial library [20], were used to perform analysis of binding mechanism of the studied derivatives.

The structures of the synthesized molecules were prepared using LigPrep [21] and the appropriate ionization states at pH = 7.4 were assigned using Epik [22]. Docking was performed by using Glide at SP level [23]. The spatial constrain was imposed on creation of an ionic interaction between the protonated amine group of the ligand and Asp3.32 side chain. For each compound, 10 top-scored complexes were considered, of which the best one was selected by application of binding mode analysis model – SIFt-SVM, trained and used previously [20].

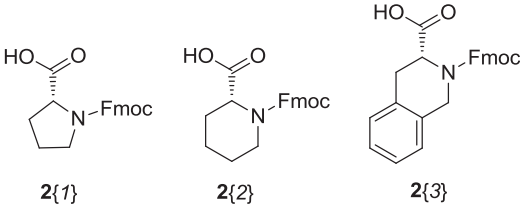


Fig. 3. Diverse Fmoc-protected amino acids, **2**{*1–3*}.

2.3. Pharmacology

2.3.1. In vitro evaluation

Radioligand binding assays were employed to determine the affinity and selectivity profiles of the synthesized compounds for human serotonin 5-HT_{1A}R and 5-HT_{7B}Rs, which are both stably expressed in HEK293 cells. This was accomplished by displacement of [^{3}H]-8-OH-DPAT (187 Ci/mmol) and [^{3}H]-5-CT (39.2 Ci/mmol) for 5-HT_{1A}R and 5-HT_7R, respectively. Each compound was tested in triplicate at 7–8 concentrations (10^{-11}–10^{-4} M) [20]. The inhibition constants (K_i) were calculated from the Cheng–Prusoff equation [24]. The results were expressed as the mean of at least two separate experiments.

2.4. Structure–activity relationship studies

A library of long-chain arylpiperazines based on LP-44 (a potent 5-HT_7R ligand) and containing terminal cyclic amino acid amides (pyrrolidine-2-carboxamide – Pro, 1,2,3,4-tetrahydroisoquinoline-3-carboxamide – Tic) was synthesized on solid-support; the members of the library were biologically evaluated for their affinity for 5-HT_7 and 5-HT_{1A} receptors. Selected library members displayed high-to-low affinity for the tested receptors, ranging from 18 to 4151 nM and from 1 to 6307 nM for 5-HT_7 and 5-HT_{1A} receptors, respectively (Table 2).

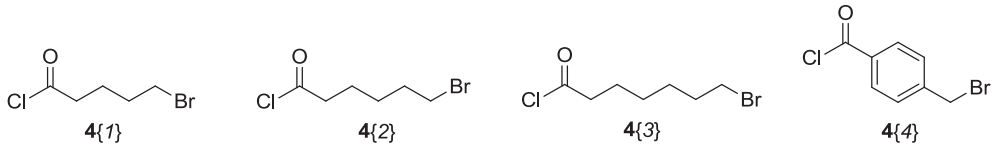


Fig. 4. Diverse alkyl chlorides, **4**{*1–4*}.

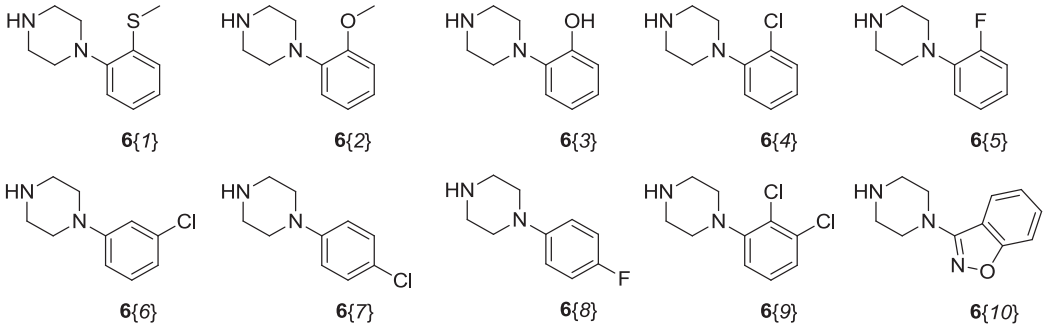
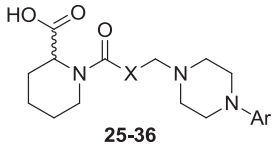


Fig. 5. Diverse secondary amines, **6**{*1–10*}.

Our primary objective was to determine the effect of replacing the 1,2,3,4-tetrahydronaphthalene system (present in LP-44) with (*S*)-pyrrolidine-2-carboxamide and (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide fragments. This modification was derived from our previous studies, which indicated that N-acylated and N-sulfonylated proline amides were moderate-to-low affinity ligands of 5-HT$_7$Rs [25]. Only L-proline, which was previously demonstrated to preferentially interact with 5-HT$_7$Rs over D-proline, was used in the current work. The results of docking of the library members **9** and **38**, as well as the reference compound LP-44, were consistent with the accumulated knowledge of ligand binding mode at 5-HT$_7$R. Besides the key ionic interaction with Asp3.32, the arylpiperazine moiety of the compounds had a specific aromatic interaction (CH–π or π–π) with the Phe6.51/Phe6.52/Trp6.48 aromatic cluster [18]. Superimposition of the docked compounds revealed the main differences between their respective interactions at the terminal fragment (Fig. 6A). In compound **38**, this fragment created a CH–π interaction (*edge-to-face*) with Phe3.28, while LP-44 formed a π–π stacking aromatic interaction with the same amino acid.

Table 1
Analytical data for the pipecolic acid derivatives **25**–**36** (set **II**).



25-36

Compd	{*BB1*} Amino acid	{*BB2*} Spacer	{*BB3*} Ar	Yield [%][a]
25	Pip	–$(CH_2)_4$–	2-OCH_3-Ph	47
26	Pip	–$(CH_2)_3$–	2-OH-Ph	32
27	Pip	–$(CH_2)_4$–	2-OH-Ph	33
28	Pip	–$(CH_2)_3$–	2-Cl-Ph	36
29	Pip	–$(CH_2)_4$–	2-Cl-Ph	32
30	Pip	–$(CH_2)_3$–	3-Cl-Ph	32
31	Pip	–$(CH_2)_4$–	3-Cl-Ph	27
32	Pip	–$(CH_2)_3$–	4-Cl	22
33	Pip	–$(CH_2)_3$–	4-F-Ph	37
34	Pip	–$(CH_2)_4$–	4-F-Ph	40
35	Pip	–$(CH_2)_3$–	2,3-diCl-Ph	33
36	Pip	–$(CH_2)_4$–	2,3-diCl-Ph	31

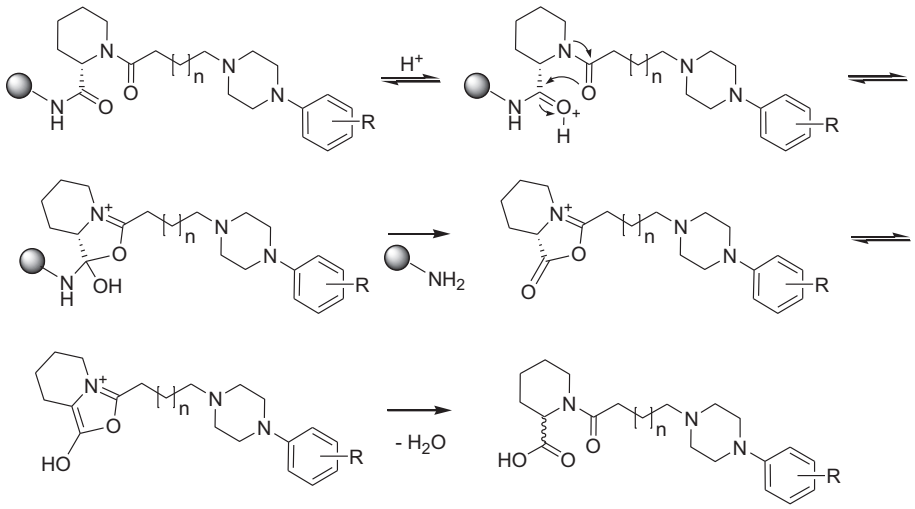
[a] Yields are based on the weight of the purified products and are calculated relative to the initial loading of the resin.

Subsequently, we investigated the contribution of the exocyclic primary amide bond in the terminal amino acid fragment to 5-HT$_7$R binding. Because it contains both H-acceptor and H-donor functionalities, the amide bond may interact with Tyr7.43 or/and Arg7.36 (with C=O function), as well as with Glu7.35 (through NH_2 function), by creating additional hydrogen bonds with the receptor (Fig. 6B). Close examination of these two orientations revealed that, for complexes of the same compound, the second orientation usually scored higher (i.e., receptor conformations and compounds). It was also observed that any interaction with Tyr7.43/Arg7.36 shifted the ligand toward the top of the binding cavity, which weakened the aromatic interaction between the aryl fragment and Phe6.51/Phe6.52/Trp6.48.

Based on SAR performed with direct Tic and Pro analogs (e.g., **23** and **53** (K_i = 50 and 22 nM, respectively) or **8** and **37** (K_i = 163 and 51 nM, respectively)), we hypothesized that the additional aromatic system in the 1,2,3,4-tetrahydroisoquinoline-3-carboxamide moiety is preferential for binding to the 5-HT$_7$R. This proposal was supported by molecular docking simulations; the Tic-amide may create CH–π or π–π interactions with Phe3.28, while the Pro fragment displayed only weak hydrophobic interactions with Phe3.28 or/and Ile 7.42 (Fig. 6C). On the other hand, the additional aromatic systems present in Tic revealed no advantage over the alicyclic proline during the interaction with 5-HT$_{1A}$Rs; Pro-amides **8**, **19**, and **21** (K_i = 47, 573 and 4 nM, respectively) displayed comparable affinities to their Tic analogs, which were **37**, **49**, and **50** (K_i = 18, 643 and 16 nM, respectively).

To determine the influence of the linker type on 5-HT$_7$R binding, compounds containing tetra-, penta- and hexa-methylene group spacers, as well as aryl fragments, were synthesized. Derivatives containing an alkylene-type linker displayed similar affinities, while the latter modification decreased the affinity for 5-HT$_7$Rs within the investigated compounds (e.g., **9** *vs* **11**, and **50** *vs* **51**). These results indicated that a spacer with limited conformational freedom is not suitable for binding the receptor pocket because the H-bond interaction with the terminal amide fragment can no longer occur. In contrast, analyzing the binding modes of the structural analogs, which differ only in the type of spacer, did not reveal any significant changes caused by using a polymethylene group spacer or aryl moiety (Fig. 6D).

The affinity for 5-HT$_{1A}$R was more susceptible to modification within the alkylene spacer relative to the affinity for 5-HT$_7$Rs.



Scheme 2. Unexpected acidolysis of an amide bond after the TFA-cleavage of the pipecolic acid derivatives (set **II**).

Elongation of the linker from four to five, or six methylene groups for both series of compounds (Pro- and Tic-amides) increased up to a 20-fold affinity for the 5-HT_{1A} sites (e.g., **8** *vs* **9**, and **10**; **20** *vs* **21**, **37** *vs* **38**, and **39**). Consequently, compounds containing an aryl fragment in the spacer displayed the lowest affinities in the series.

Because many studies have revealed the critical role of the arylpiperazine substitution pattern in determining the 5-HT_7 and 5-HT_{1A} receptor affinity, a more detailed study of the substituents was undertaken. Generally, electron-donating substituents were superior to electron-withdrawing groups for binding the above receptors. Compounds containing 2-methylthio substituents, which have a higher volume (e.g., **9**, **38**), displayed better selectivity for 5-HT_7R binding than their 2-methoxy analogs (e.g., **13**, **42**); consequently, the 2-hydroxy counterparts (**15**, **44**) were the least potent. The same relationship was observed during the SAR analysis for 5-HT_{1A}R binding. However, in contrast to LP-44, the introduction of a 2-methylthio substituent was highly preferential for the 5-HT_{1A} sites over the 5-HT_7 sites. Consequently, compound **38** displayed the highest affinity for 5-HT_{1A}Rs ($K_i \leq 1$ nM).

An interesting correlation between the binding modes and biological data for 5-HT_7R was found within the series of proline amides containing halogen substituents (Fig. 6E) in the phenylpiperazine moiety, which included **16** (2-Cl analog), **18** (3-Cl analog) and **21** (2,3-diCl analog). In addition to the previously discussed interactions, the potential for the halogenated derivatives to create additional interactions with Cys3.36 and Thr3.37 has been detected. This type of ligand-protein contact was intensively studied recently, especially in light of rational drug design [26]. The strength of these interactions depends mainly on the distance between the halogen atom and any accessible Lewis base within the binding pocket, the σ-hole angle and the type of halogen atom involved. According to these findings, a weak interaction with Cys3.36 (only one halogen bond with the distance and σ-hole equal to 3.5 Å and 145°, respectively) was observed for the 2-Cl derivative, resulting in a low affinity for 5-HT_7Rs (**16**, K_i = 1312 nM). A shift of chlorine atom from the *ortho*- to the *meta*-position allowed the generation of a strong halogen bond with Cys3.36 (distance and σ-hole were equal 3.4 Å and 136°, respectively) and a weak halogen bond (distance and σ-hole were equal 4.0 Å and 129°, respectively) with Thr3.37. Additionally, a network of halogen bonds with different strengths (distance and σ-hole vary from 3.6 to 4.6 E and 122–154°, respectively) was revealed for the 2,3-di-Cl derivative. The 2,3-di-Cl derivative **21** displayed higher affinity than both the 3-Cl derivative **18** and the *ortho*-derivative **16** (K_i = 179, 478 and 1312 nM, respectively). Interestingly, both series of 2,3-di-Cl analogs of the Pro- and Tic-amides displayed a more pronounced contribution of halogen bonds during the interactions with the 5-HT_{1A}Rs.

To extend the structural modification of the arylpiperazine moiety further, the phenyl ring was replaced with a benzisoxazole system. This modification significantly increased the affinity of both Pro-amides (**22**–**24**, K_i = 108, 50 and 101 nM, respectively) and Tic-amides (**52**–**54**, K_i = 18, 22 and 28 nM, respectively) for the 5-HT_7 receptor. On the other hand, it was found that the elongation of alkylene spacer from four to five or six decrease the selectivity over the 5-HT_{1A} sites. Molecular modeling validated the presence of an additional H-bond interaction with Ser5.42 or/and Thr3.37 (Fig. 6F).

3. Conclusions

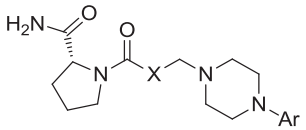
In summary, we have designed and synthesized three series of long-chain arylpiperazines, which contained cyclic amino acid amides (Pro, Pip, Tic) in the terminal fragment, on a solid-support. Surprisingly, the piperidine-2-carboxamides, after release from the Rink-amide resin, were converted into pipecolic acid analogs. Structure–activity relationship studies within the library of compounds were evaluated for 5-HT_7Rs and 5-HT_{1A}Rs and supported by molecular modeling; these studies demonstrated that the Tic-amide set favorably interacted with the 5-HT_7Rs by creating CH–π or π–π interactions with Phe3.28. Integrating an aromatic fragment was not beneficial for binding with the 5-HT_{1A} sites. In contrast to LP-44, compounds containing methylsulfanyl substituents in the *ortho*-position were highly preferential for binding with 5-HT_{1A}Rs. However, replacing the phenyl ring in the arylpiperazine moiety with a benzisoxazole system significantly increased the compound's affinity for 5-HT_7Rs because an additional H-bond with Ser5.42 or/and Thr3.37 was formed. This study permitted the identification of several compounds with significant 5-HT_7 and 5-HT_{1A} receptor affinities and provided insights into the molecular basis for investigating 5-HT_7 receptor agents.

4. Experimental protocols

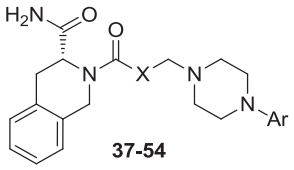
4.1. Chemistry

Solid-phase organic transformations and resin washes were carried out at ambient temperature, unless otherwise indicated. Organic solvents (from Aldrich and Fluka) were of reagent grade

Table 2
Binding affinities of selected (*S*)-pyrrolidine-2-carboxamides and (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxamides for 5-HT_7 and 5-HT_{1A}Rs.



8-24



37-54

Compd	{*BB1*} Amino acid	{*BB2*} Spacer	{*BB3*} Ar	K_i [nM][a]		Ratio K_i	
				5-HT_7	5-HT_{1A}	5-$HT_{1A}/_7$	5-$HT_7/_{1A}$
8	Pro	$-(CH_2)_3-$	2-SCH_3-Ph	163	47	<1	3
9	Pro	$-(CH_2)_4-$	2-SCH_3-Ph	153	14	<1	11
10	Pro	$-(CH_2)_5-$	2-SCH_3-Ph	495	23	<1	21
11	Pro		2-SCH_3-Ph	567	313	<1	2
12	Pro	$-(CH_2)_3-$	2-OCH_3-Ph	634	214	<1	3
13	Pro	$-(CH_2)_4-$	2-OCH_3-Ph	1199	44	<1	27
14	Pro		2-OCH_3-Ph	4151	6307	2	<1
15	Pro	$-(CH_2)_3-$	2-OH-Ph	1865	52	<1	36
16	Pro	$-(CH_2)_4-$	2-Cl-Ph	1312	232	<1	6
17	Pro	$-(CH_2)_4-$	2-F-Ph	3134	141	<1	22
18	Pro	$-(CH_2)_4-$	3-Cl-Ph	478	38	<1	13
19	Pro	$-(CH_2)_4-$	4-F-Ph	1188	573	<1	2
20	Pro	$-(CH_2)_3-$	2,3-diCl-Ph	113	83	<1	1
21	Pro	$-(CH_2)_4-$	2,3-diCl-Ph	179	4	<1	45
22	Pro	$-(CH_2)_3-$	BIO[a]	108	2908	27	<1
23	Pro	$-(CH_2)_4-$	BIO[a]	50	214	4	<1
24	Pro	$-(CH_2)_5-$	BIO[a]	101	122	1	<1
37	Tic	$-(CH_2)_3-$	2-SCH_3-Ph	51	18	<1	3
38	Tic	$-(CH_2)_4-$	2-SCH_3-Ph	78	<1	<1	>78
39	Tic	$-(CH_2)_5-$	2-SCH_3-Ph	111	2	<1	55
40	Tic		2-SCH_3-Ph	148	122	<1	1
41	Tic	$-(CH_2)_3-$	2-OCH_3-Ph	551	107	<1	5
42	Tic	$-(CH_2)_4-$	2-OCH_3-Ph	302	12	<1	25
43	Tic		2-OCH_3-Ph	579	77	<1	7
44	Tic	$-(CH_2)_4-$	2-OH-Ph	476	72	<1	7
45	Tic	$-(CH_2)_4-$	2-Cl-Ph	393	236	<1	2
46	Tic	$-(CH_2)_4-$	3-Cl-Ph	725	499	<1	1
47	Tic	$-(CH_2)_3-$	4-Cl-Ph	223	211	<1	1
48	Tic	$-(CH_2)_3-$	4-F-Ph	295	1914	6	<1
49	Tic	$-(CH_2)_4-$	4-F-Ph	107	643	6	<1
50	Tic	$-(CH_2)_4-$	2,3-diCl-Ph	95	16	<1	6
51	Tic		2,3-diCl-Ph	1196	212	<1	6
52	Tic	$-(CH_2)_3-$	BIO[b]	18	377	21	<1
53	Tic	$-(CH_2)_4-$	BIO[b]	22	230	10	<1
54	Tic	$-(CH_2)_5-$	BIO[b]	28	89	3	<1

[a] K_i values (SEM ± 22) based on three independent binding experiments.
[b] BIO = benzisoxazol-3-yl.

and were used without purification. Rink-amide-functionalized resin with 1.3 mmol/g loading was purchased from Iris Biotech (Germany). The resin-bound intermediates were air-dried after the final CH_2Cl_2 washes unless they needed to be re-weighed; in the latter case, the samples were treated for 24–36 h in a vacuum desiccator.

Analytical HPLC analyses were performed on an Alliance Waters 2695 Separations Module equipped with a Chromolith SpeedROD RP 18.5 μm column (4.6 × 50 mm). Standard conditions were as follows: eluent system A (water/0.1% TFA), system B (MeCN/0.1% TFA). A flow rate of 5 mL/min with a gradient of (0–100)% B over 3 min was used. Detection was performed with a Waters 2998 Photodiode Array Detector.

LC/MS analyses were carried out on a system consisting of a Waters Acquity UPLC coupled with a Waters TQD mass spectrometer. All of the analyses were carried out with an Acquity UPLC BEH C18, 50 × 2.1 mm column at 40 °C. A flow rate of 0.3 mL/min and a gradient of (5–95)% B over 5 min was used. The mobile phase conditions were as follows: eluent A: water/0.1% HCO_2H; eluent B: MeCN/0.1% HCO_2H. Retention times (R_t) are reported in minutes.

RP-preparative HPLC purification was performed on a Waters HPLC 4000 instrument equipped with a UV detector 486 and a Waters Delta-Pack 40 × 100 mm, 15 Å, 100 μm, reversed-phase column. Standard conditions were as follows: eluent system A (water/0.1% TFA), system B (MeCN/0.1% TFA). A flow rate of 50 mL/min and a gradient of (10–70)% B over 30 min were used with detection at 214 nm.

The resin washing steps occurred over 5 min and included three washes with DMF, one wash with MeOH to shrink the resin beads and two washes with DCM. The volume of solvent used for each wash was at least 8 times the volume of the swollen beads. (i.e., for 1 g of resin, at least 40 mL of solvent was used). Unless otherwise indicated, the yields of the final compounds were calculated based on the initial loading of the starting resins and were the overall yields of all reaction steps beginning from the resins. If the final compounds were purified, these yields included the purification yield.

^1H NMR and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz (Varian BB 200 spectrometer) using TMS (0.00 ppm) as an internal standard as well as $CDCl_3$, and DMSO-d_6 as solvents.

The *J* values are reported in Hertz (Hz), and the splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), m (multiplet), br s (broaded singlet).

Abbreviations used: **DMF, Dimethylformamide; MeOH, Methanol; DCM, Dichloromethane;** Fmoc, Chloroformic acid 9*H*-fluoren-9-ylmethyl ester; HBTU, **O-Benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate;** DIEA, *N*,*N*-Diisopropylethylamine; DMAP, 4-Dimethylaminopyridine**; AcOH, Acetic acid; TFA, Trifluoroacetic acid**.

4.2. Solid-supported library synthesis

4.2.1. Deprotection of rink amide resin and an amine group of the amino acid

The resin (2.25 g, 1.58 mmol, 0.7 mmol g^{-1}) was treated with a 20% solution of piperidine in DMF and allowed to rotate for 3 min. Subsequently, the beads were drained and washed with DMF (1 × 5 mL). The procedure described above was repeated for another 15 min. Next, the resin was drained and washed with DMF (4×), MeOH (1×), and DCM (4×) before being dried under low vacuum.

4.2.2. Preparation of solid-supported carboxamides

The amine resin was placed into 3 reactors for coupling with different Fmoc-protected amino acids. The resin was swelled in CH_2Cl_2 (10 mL) for 30 min and then washed with CH_2Cl_2–DMF (80/20, v/v). Fmoc-amino acid **2**{*1–3*} (5.4 mmol, 5 equiv), HBTU (0.88 g, 5.4 mmol, 5 equiv) in DMF (8 mL), and DIEA (1.8 mL, 10.8 mmol, 10 equiv) were then added to the resin (0.6 g, 1.08 mmol, 1.8 mmol g^{-1}). The reaction mixture was agitated with an orbital shaker for 2 h. The resin-bound amino acid was then

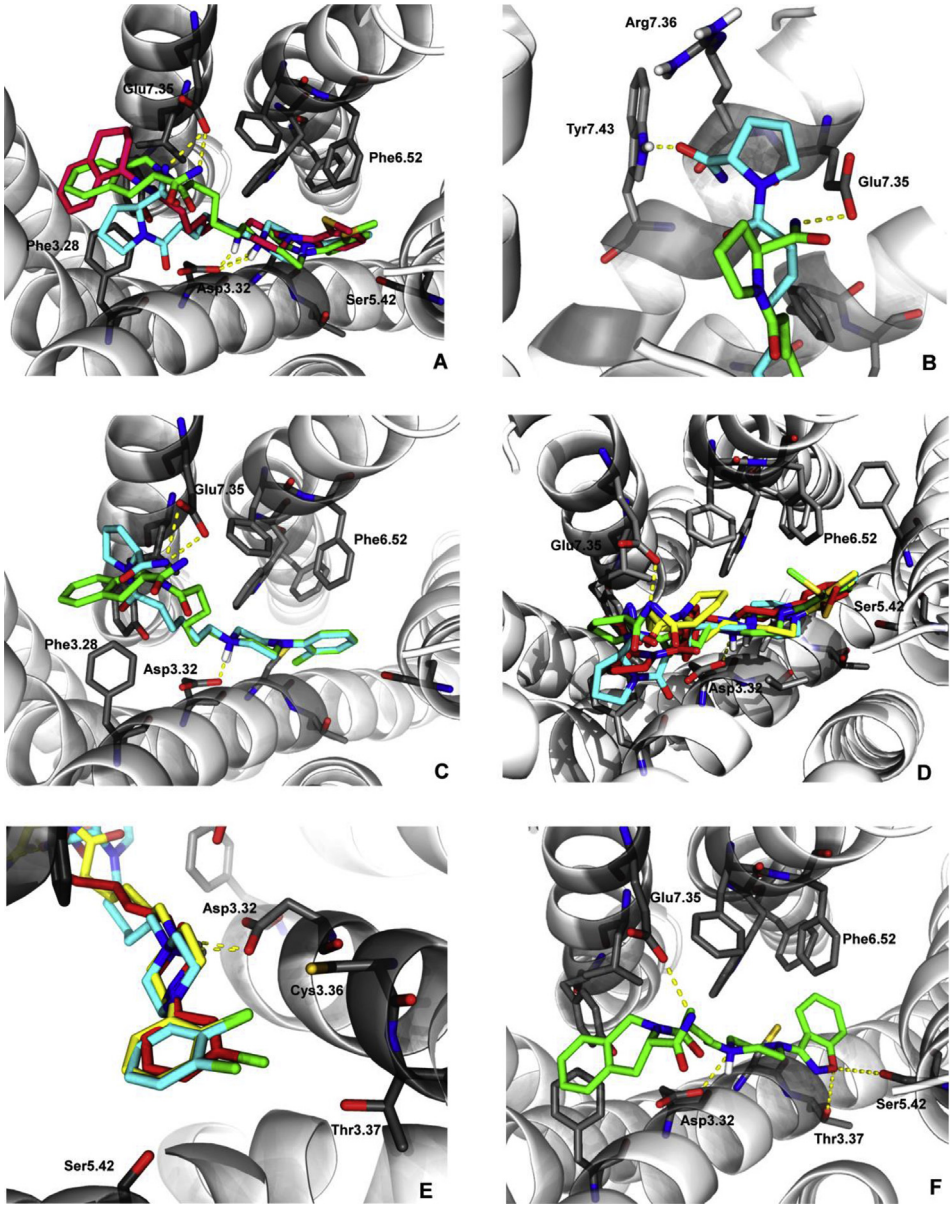


Fig. 6. (A–F). Panel illustrating molecular modeling results of the designed library for the 5-HT$_7$ receptor. (A) The comparison of the binding modes for reference ligand LP-44 (red) and the closest analogs from the Pro (cyan) and Tic (green) series. (B) The two possible amide group interactions are shown for the two complexes of compound **8**; in the first orientation, the C=O group might form an H-bond with Tyr7.43 or/and Arg7.36 (cyan), but in the second, the NH_2 can be H-bonded with Glu7.35 (green). (C) An aromatic interaction between the terminal moiety of the Tic compounds (green) with Phe3.28 may cause their activity to outperform the activity of the Pro analogs (cyan). (D) The introduction of linkers with different lengths ($(CH_2)_4$, **8** – green; $(CH_2)_5$, **9** – cyan, $(CH_2)_6$, **10** – yellow, p-C_6H_4, **11** – red) exhibited no significant changes to the binding mode. (E) The correlation between the orientation of the different analogs with the halogenated phenyl ring (i.e., **16** (yellow), **18** (red), **21** (cyan)). Their activity might be caused by the creation of halogen bonds with Cys3.36 or/and Thr3.37. (F) The most active compound in this series benzisoxazole derivative **52**, might form additional H-bond contacts with Thr3.37 or/and Ser5.42. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

drained and washed with DMF (4 × 5 mL), MeOH (1 × 5 mL), and DCM (4 × 5 mL). The procedure described above was repeated. The washed resin was dried under low vacuum for 12 h.

4.2.3. Acylation using arylacyl chlorides

The deprotected resin (140 mg, 0.08 mmol, 0.57 mmol g^{-1}) was swelled in 2 mL of DCM for 30 min; DIEA (0.08 mL, 0.45 mmol, 6 equiv) was added to the resin, followed by a solution of acyl chloride **4**{*1–4*} in 0.25 mL of DCM and DMAP (10.3 mg, 0.08 mmol, 1 equiv). The reaction mixture was allowed to rotate for 4 h. The resin was drained and washed with DMF (4×), MeOH (1×), and DCM (4×) before being allowed to dry in open air.

4.2.4. Nucleophilic displacement with arylpiperazine derivatives

The air-dried resin was placed into glass vials containing 1.5 mL of 1 M solution of secondary amine **6**{*1–10*} in DMF. The resulting reaction mixture was heated to 75 °C for 24 h. The resulting resin-bound product was drained and sequentially washed with a 10% solution of AcOH in DMF (2×), DMF (4×), MeOH (1×), and DCM (4×) before being dried in open air.

4.3. General procedure for the cleavage of the final products

Two milliliters of a TFA/DCM (80/20, v/v) mixture was added to the resin, and the cleavage reaction was carried out for 2 h.

Subsequently, the reaction was filtered and washed with a small volume of DCM; the collected filtrates were concentrated in a stream of argon to yield the target compounds.

*4.3.1. (S)-1-(5-(4-(2-(Methylthio)phenyl)piperazin-1-yl)pentanoyl) pyrrolidine-2-carboxamide (**8**)*

Yellow oil, 20 mg (36% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, $t_R = 1.02$ min. $C_{21}H_{32}N_4O_2S$, MW 404.57, Monoisotopic Mass 404.22, $[M + H]^+ = 405.5$. ^1H NMR (300 MHz, $CDCl_3$): δ 1.72–1.78 (m, 2H), 1.83–1.89 (m, 2H), 1.94–2.04 (m, 2H), 2.25–2.35 (m, 2H), 2.37–2.41 (m, 2H), 2.54 (s, 3H), 3.08–3.13 (m, 4H), 3.38–3.44 (m, 2H), 3.52–3.56 (m, 4H), 3.62–3.68 (m, 2H), 4.48–4.51 (m, 1H), 6.85–6.9 (m, 1H), 6.92–6.94 (m, 2H), 7.07–7.12 (m, 1H).

*4.3.2. (S)-1-(6-(4-(2-(Methylthio)phenyl)piperazin-1-yl)hexanoyl) pyrrolidine-2-carboxamide (**9**)*

Yellow oil, 22 mg (37% isolated yield) following preparative LC/MS purification; LC/MS purity 98%, $t_R = 1.06$ min. $C_{22}H_{34}N_4O_2S$, MW 418.6, Monoisotopic Mass 418.24, $[M + H]^+ = 419.4$. ^1H NMR (300 MHz, $CDCl_3$): δ 1.43–1.49 (m, 2H), 1.65–1.74 (m, 2H), 1.78–1.86 (m, 4H), 1.95–2.04 (m, 2H), 2.49 (s, 3H), 3.03–3.09 (m, 4H), 3.22 (t, 2H), 3.36–3.44 (m, 2H), 3.56–3.61 (m, 4H), 3.62–3.69 (m, 2H), 4.5–4.56 (m, 1H), 6.87–6.96 (m, 2H), 7.22–7.26 (m, 2H).

*4.3.3. (S)-1-(7-(4-(2-(Methylthio)phenyl)piperazin-1-yl)heptanoyl) pyrrolidine-2-carboxamide (**10**)*

Yellow oil, 25 mg (38% isolated yield) following preparative LC/MS purification; LC/MS purity 100%, $t_R = 1.16$ min. $C_{23}H_{36}N_4O_2S$, MW 432.62, Monoisotopic Mass 432.26, $[M + H]^+ = 433.3$. ^1H NMR (300 MHz, $CDCl_3$): δ 1.21–1.33 (m, 4H), 1.39–1.43 (m, 2H), 1.62–1.71 (m, 2H), 1.78–1.84 (m, 4H), 1.93–2.01 (m, 2H), 2.47 (s, 3H), 3.03–3.09 (m, 4H), 3.38–3.43 (m, 2H), 3.54–3.59 (m, 4H), 3.62–3.65 (m, 2H), 4.51–4.56 (m, 1H), 6.78–6.65 (m, 2H), 7.19–7.23 (m, 2H).

*4.3.4. (S)-1-(4-((4-(2-(Methylthio)phenyl)piperazin-1-yl)methyl) benzoyl)pyrrolidine-2-carboxamide (**11**)*

Brown oil, 19 mg (39% isolated yield) following preparative LC/MS purification; LC/MS purity 98%, $t_R = 1.1$ min. $C_{24}H_{30}N_4O_2S$, MW 438.59, Monoisotopic Mass 438.21, $[M + H]^+ = 439.7$. ^1H NMR (300 MHz, $CDCl_3$): δ 1.86–1.88 (m, 2H), 2.03–2.15 (m, 2H), 2.35–2.39 (m, 2H), 2.6 (s, 3H), 3.48–3.51 (m, 4H), 3.55–3.62 (m, 4H), 4.29 (s, 2H), 4.71–4.75 (m, 1H), 6.87–6.93 (m, 4H), 7.05–7.1 (m, 1H), 7.28 (d, 1H), 7.42 (s, 1H), 7.51 (d, 1H).

*4.3.5. (S)-1-(5-(4-(2-Methoxyphenyl)piperazin-1-yl)pentanoyl) pyrrolidine-2-carboxamide (**12**)*

Yellow oil, 19 mg (39% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, $t_R = 0.98$ min. $C_{21}H_{32}N_4O_3$, MW 388.5, Monoisotopic Mass 388.25, $[M + H]^+ = 389.5$. ^1H NMR (300 MHz, $CDCl_3$/MeOD): δ 1.7–1.76 (m, 2H), 1.84–1.91 (m, 2H), 1.93–2.02 (m, 2H), 2.27–2.35 (m, 2H), 2.39–2.44 (m, 2H), 3.08–3.13 (m, 4H), 3.38–3.44 (m, 2H), 3.5–3.56 (m, 4H), 3.62–3.68 (m, 2H), 3.86 (s, 3H), 4.48–4.5 (m, 1H), 6.87–6.9 (m, 1H), 6.92–6.94 (m, 2H), 7.05–7.1 (m, 1H).

*4.3.6. (S)-1-(5-(4-(2-Methoxyphenyl)piperazin-1-yl)hexanoyl) pyrrolidine-2-carboxamide (**13**)*

Yellow oil, 22 mg (37% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, $t_R = 1.03$ min. $C_{22}H_{34}N_4O_3$, MW 402.53, Monoisotopic Mass 402.26, $[M + H]^+ = 403.4$. ^1H NMR (300 MHz, $CDCl_3$): δ 1.41–1.49 (m, 2H), 1.63–1.73 (m, 2H), 1.76–1.86 (m, 4H), 1.95–2.02 (m, 2H), 3.04–3.09 (m, 4H), 3.22 (t, 2H), 3.38–3.46 (m, 2H), 3.56–3.61 (m, 4H), 3.62–3.68 (m, 2H), 3.92 (s, 3H), 4.49–4.53 (m, 1H), 6.87–6.96 (m, 2H), 7.23–7.26 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 23.23, 23.31, 24.83, 25.90, 28.07, 33.54, 47.45, 47.61, 52.18, 55.44, 56.95, 59.65, 111.32, 118.88, 121.23, 124.53, 138.77, 152.04, 172.73, 174.45.

*4.3.7. (S)-1-(4-((4-(2-Methoxyphenyl)piperazin-1-yl)methyl) benzoyl)pyrrolidine-2-carboxamide (**14**)*

Yellow oil, 16 mg (39% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, $t_R = 1.00$ min. $C_{24}H_{30}N_4O_3$, MW 422.52, Monoisotopic Mass 422.23, $[M + H]^+ = 423.7$. ^1H NMR (300 MHz, $CDCl_3$): δ 1.81–1.92 (m, 2H), 2.01–2.19 (m, 2H), 2.32–2.45 (m, 2H), 3.44–3.53 (m, 4H), 3.55–3.63 (m, 4H), 3.85 (s, 3H), 4.27 (s, 2H), 4.7–4.74 (m, 1H), 5.50 (br. s., 1H), 5.75 (br. s., 1H), 6.86–6.93 (m, 4H), 7.05–7.11 (m, 1H), 7.43 (s, 1H), 7.49–7.54 (m, 1H), 7.58–7.62 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 25.38, 27.68, 47.54, 50.45, 51.81, 55.42, 59.77, 60.19, 111.30, 118.93, 121.24, 124.64, 128.16, 130.43, 131.32, 137.79, 138.62, 152.03, 162.03, 162.51, 169.81, 173.49.

*4.3.8. (S)-1-(5-(4-(2-Hydroxyphenyl)piperazin-1-yl)pentanoyl) pyrrolidine-2-carboxamide (**15**)*

Colorless oil, 13 mg (31% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, $t_R = 0.88$ min. $C_{20}H_{30}N_4O_3$, MW 374.48, Monoisotopic Mass 374.23, $[M + H]^+ = 375.5$. ^1H NMR (300 MHz, $CDCl_3$): δ 1.73–1.78 (m, 2H), 1.93–1.95 (m, 2H), 1.93–2.02 (m, 2H), 2.06–2.12 (m, 2H), 2.39–2.44 (m, 2H), 3.09–3.19 (m, 4H), 3.39–3.44 (m, 2H), 3.55–3.6 (m, 4H), 3.65–3.68 (m, 2H), 4.43–4.5 (m, 1H), 6.83–6.9 (m, 2H), 7.08–7.13 (m, 2H).

*4.3.9. (S)-1-(6-(4-(2-Chlorophenyl)piperazin-1-yl)hexanoyl) pyrrolidine-2-carboxamide (**16**)*

Pale oil, 17 mg (34% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, $t_R = 1.12$ min. $C_{21}H_{31}ClN_4O_2$, MW 406.95, Monoisotopic Mass 406.21, $[M + H]^+ = 407.3$. ^1H NMR (300 MHz, $CDCl_3$): δ 1.46–1.51 (m, 2H), 1.63–1.68 (m, 2H), 1.7–1.75 (m, 2H), 1.81–1.89 (m, 2H), 1.99–2.09 (m, 2H), 2.31–2.38 (m, 2H), 3.03–3.1 (m, 4H), 3.27–3.43 (m, 2H), 3.54–3.6 (m, 4H), 3.67–3.71 (m, 2H), 4.54–4.56 (m, 1H), 7.03–7.08 (m, 2H), 7.23–7.29 (m, 1H), 7.36–7.39 (m, 1H).

*4.3.10. (S)-1-(6-(4-(2-Fluorophenyl)piperazin-1-yl)hexanoyl) pyrrolidine-2-carboxamide (**17**)*

Pale oil, 10 mg (33% isolated yield) following preparative LC/MS purification; LC/MS purity 98%, $t_R = 1.03$ min. $C_{21}H_{31}FN_4O_2$, MW 390.49, Monoisotopic Mass 390.24, $[M + H]^+ = 391.6$. ^1H NMR (300 MHz, $CDCl_3$): δ 1.48–1.55 (m, 2H), 1.62–1.68 (m, 2H), 1.72–1.75 (m, 2H), 1.81–1.89 (m, 2H), 1.99–2.06 (m, 2H), 2.3–2.38 (m, 2H), 3.03–3.14 (m, 4H), 3.27–3.46 (m, 2H), 3.54–3.6 (m, 4H), 3.67–3.73 (m, 2H), 4.54–4.56 (m, 1H), 7.02–7.08 (m, 2H), 7.22–7.29 (m, 1H), 7.36–7.39 (m, 1H).

*4.3.11. (S)-1-(6-(4-(3-chlorophenyl)piperazin-1-yl)hexanoyl) pyrrolidine-2-carboxamide (**18**)*

Pale oil, 28 mg (33% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, $t_R = 1.16$ min. $C_{21}H_{31}ClN_4O_2$, MW 406.95, Monoisotopic Mass 406.21, $[M + H]^+ = 407.5$. ^1H NMR (300 MHz, $CDCl_3$): δ 1.43–1.50 (m, 2H), 1.68–1.75 (m, 2H), 1.78–1.86 (m, 4H), 1.89–1.94 (m, 2H), 2.94–3.02 (m, 4H), 3.03–3.08 (m, 2H), 3.38–3.46 (m, 2H), 3.53–3.57 (m, 2H), 3.60–3.67 (m, 4H), 4.54–4.57 (m, 1H), 6.72–6.74 (m, 2H), 6.79 (d, 1H, $J = 7.62$ Hz), 7.24–7.30 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 20.75, 23.17, 23.83, 25.20, 25.85, 26.31, 32.67, 43.66, 47.66, 52.12, 52.31, 55.44, 57.12, 111.35, 118.89, 118.95, 121.23, 124.64, 138.58, 152.05.

*4.3.12. (S)-1-(6-(4-(4-Fluorophenyl)piperazin-1-yl)hexanoyl) pyrrolidine-2-carboxamide (**19**)*

Pale oil, 20 mg (35% isolated yield) following preparative LC/MS purification; LC/MS purity 98%, t_R = 1.03 min. $C_{21}H_{31}FN_4O_2$, MW 390.49, Monoisotopic Mass 390.24, $[M + H]^+$ = 391.4. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38–1.47 (m, 2H), 1.58–1.62 (m, 2H), 1.69–1.72 (m, 2H), 1.78–1.86 (m, 2H), 1.81–1.84 (m, 2H), 3.06–3.11 (m, 4H), 3.19–3.26 (m, 4H), 3.48–3.49 (m, 2H), 3.69–3.73 (m, 4H), 4.53–4.58 (m, 1H), 6.86–6.92 (m, 2H), 6.95–7.01 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 21.46, 23.17, 23.83, 25.24, 25.85, 26.31, 33.67, 43.66, 47.66, 52.12, 52.31, 55.75, 57.12, 111.85, 119.28, 129.35, 130.23, 145.72, 152.15.

*4.3.13. (S)-1-(5-(4-(2,3-Dichlorophenyl)piperazin-1-yl)pentanoyl) pyrrolidine-2-carboxamide (**20**)*

Pale oil, 19 mg (31% isolated yield) following preparative LC/MS purification; LC/MS purity 98%, t_R = 1.24 min. $C_{20}H_{28}Cl_2N_4O_2$, MW 427.37, Monoisotopic Mass 426.16, $[M + H]^+$ = 427.2. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.73–1.77 (m, 2H), 1.9–2.02 (m, 2H), 2.27–2.31 (m, 2H), 2.39–2.43 (m, 2H), 3.04–3.21 (m, 4H), 3.28 (t, 2H), 3.4 (t, 2H), 3.57–3.64 (m, 2H), 3.68–3.71 (m, 4H),4.51–4.54 (m, 1H), 6.98–7.01 (m, 1H), 7.16–7.28 (m, 2H).

*4.3.14. (S)-1-(6-(4-(2,3-Dichlorophenyl)piperazin-1-yl)hexanoyl) pyrrolidine-2-carboxamide (**21**)*

Pale oil, 11 mg (33% isolated yield) following preparative LC/MS purification; LC/MS purity 97%, t_R = 1.26 min. $C_{21}H_{30}Cl_2N_4O_2$, MW 441.39, Monoisotopic Mass 440.17, $[M + H]^+$ = 441.2. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43–1.5 (m, 2H), 1.67–1.74 (m, 2H), 1.78–1.86 (m, 2H), 1.93–2.02 (m, 2H), 2.27–2.38 (m, 4H), 3.00–3.11 (m, 4H), 3.27–3.43 (m, 4H), 3.54–3.61 (m, 2H), 3.67–3.71 (m, 2H)), 4.52–4.55 (m, 1H), 6.98–7.01 (m, 1H), 7.23–7.26 (m, 2H).

*4.3.15. (S)-1-(5-(4-(Benzo[d]isoxazol-3-yl)piperazin-1-yl) pentanoyl)pyrrolidine-2-carboxamide (**22**)*

Colorless Oil, 30 mg (39% isolated yield) following preparative LC/MS purification; LC/MS purity 98%, t_R = 0.98 min. $C_{21}H_{29}N_5O_3$, MW 399.49, Monoisotopic Mass 399.23, $[M + H]^+$ = 400.3. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21–1.34 (m, 2H), 1.31–1.47 (m, 4H), 1.73–1.85 (m, 2H), 1.92–1.98 (m, 2H), 2.22–2.39 (m, 2H), 3.02–3.07 (m, 4H), 3.38–3.42 (m, 2H), 3.59–3.66 (m, 4H), 4.46–4.49 (m, 1H), 7.12 (br. s, 2H), 7.24–7.29 (m, 1H), 7.46–7.56 (m, 2H), 7.65 (br. s, 1H).

*4.3.16. (S)-1-(6-(4-(Benzo[d]isoxazol-3-yl)piperazin-1-yl) hexanoyl)pyrrolidine-2-carboxamide (**23**)*

Colorless Oil, 28 mg (40% isolated yield) following preparative LC/MS purification; LC/MS purity 96%, t_R = 1.01 min. $C_{22}H_{31}N_5O_3$, MW 413.51, Monoisotopic Mass 413.24, $[M + H]^+$ = 414.5. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25–1.48 (m, 2H), 1.61–1.71 (m, 2H), 1.73–1.85 (m, 2H), 1.94–2.02 (m, 2H), 2.05–2.16 (m, 2H), 2.22–2.39 (m, 2H), 3.03–3.09 (m, 4H), 3.37–3.46 (m, 4H), 3.55–3.64 (m, 4H), 4.46–4.49 (m, 1H), 7.06 (br. s, 2H), 7.24–7.29 (m, 1H), 7.46–7.56 (m, 2H), 7.61 (br. s, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 23.92, 24.24, 25.61, 27.81, 30.22, 33.91, 47.59, 48.15, 49.78, 54.69, 56.51, 62.56, 63.90, 109.65, 116.16, 122.18, 123.80, 130.85, 161.26, 163.96, 173.51, 174.23.

*4.3.17. (S)-1-(7-(4-(Benzo[d]isoxazol-3-yl)piperazin-1-yl) heptanoyl)pyrrolidine-2-carboxamide (**24**)*

Colorless Oil, 27 mg (38% isolated yield) following preparative LC/MS purification; LC/MS purity 97%, t_R = 1.1 min. $C_{23}H_{33}N_5O_3$, MW 427.54, Monoisotopic Mass 427.26, $[M + H]^+$ = 428.4. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21–1.33 (m, 4H), 1.34–1.46 (m, 4H), 1.49–1.69 (m, 4H), 2.29–2.46 (m, 4H), 2.64 (t, J = 5.20 Hz, 4H), 3.14 (q, J = 7.30 Hz, 2H), 3.59 (t, J = 5.20 Hz, 4H), 4.58 (dd, J = 8.03, 2.00 Hz, 1H), 5.46 (br. s., 1H), 7.04 (br. s., 1H), 7.21 (ddd, J = 8.05, 6.26, 1.78 Hz, 1H), 7.41–7.48 (m, 2H), 7.69 (dt, J = 8.06, 0.91 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 22.67, 24.94, 26.51, 27.21, 28.65, 29.22, 34.51, 46.79, 47.52, 48.15, 52.51, 58.65, 59.30, 63.90, 110.45, 116.16, 122.18, 122.28, 129.50, 161.26, 163.96, 173.69, 174.52.

*4.3.18. 1-(6-(4-(2-Methoxyphenyl)piperazin-1-yl)hexanoyl)-piperidine-2-carboxylic acid (**25**)*

Yellow oil, 22 mg (47% isolated yield) following preparative LC/MS purification; LC/MS purity 100%, t_R = 1.13 min. $C_{23}H_{35}N_3O_4$, MW 417.54, Monoisotopic Mass 417.26, $[M + H]^+$ = 418.6. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39–1.41 (m, 2H), 1.43–1.45 (m, 2H), 1.62–1.64 (m, 2H), 1.66–1.7 (m, 2H), 1.77–1.8 (m, 2H), 2.26–2.41 (m, 2H), 2.44–2.51 (m, 2H), 3.04–3.14 (m, 4H), 3.2–3.25 (m, 4H), 3.5–3.54 (m, 2H), 3.69–3.76 (m, 2H), 3.85 (s, 3H), 5.28–5.3 (m, 1H), 6.87–6.9 (m, 1H), 6.93–6.97 (m, 2H), 7.05–7.11 (m, 1H), 11.1 (br s, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 20.75, 20.76, 23.17, 23.83, 25.20, 25.85, 26.31, 32.67, 43.66, 47.66, 52.12, 52.31, 55.44, 57.12, 111.35, 118.89, 118.95, 121.23, 124.64, 138.58, 152.05.

*4.3.19. 1-(5-(4-(2-Hydroxyphenyl)piperazin-1-yl)pentanoyl)-piperidine-2-carboxylic acid (**26**)*

Yellow oil, 15 mg (32% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1 min. $C_{21}H_{31}N_3O_4$, MW 389.49, Monoisotopic Mass 389.23, $[M + H]^+$ = 390.5. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39–1.48 (m, 2H), 1.58–1.61 (m, 2H), 1.64–1.71 (m, 2H), 1.74–1.77 (m, 2H), 2.28–2.38 (m, 2H), 2.43–2.51 (m, 2H), 3.07–3.12 (m, 4H), 3.19–3.27 (m, 2H), 3.49–3.61 (m, 2H), 3.69–3.74 (m, 4H), 5.23–5.24 (m, 1H), 5.53 (br s, 1H), 6.83–6.88 (m, 1H), 6.93–6.95 (m, 1H), 7.05–7.12 (m, 2H), 11.1 (br s, 1H).

*4.3.20. 1-(6-(4-(2-Hydroxyphenyl)piperazin-1-yl)hexanoyl)-piperidine-2-carboxylic acid (**27**)*

Yellow oil, 17 mg (33% isolated yield) following preparative LC/MS purification; LC/MS purity 95%, t_R = 0.98 min. $C_{22}H_{33}N_3O_4$, MW 403.52, Monoisotopic Mass 403.25, $[M + H]^+$ = 404.5. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41–1.44 (m, 2H), 1.46–1.58 (m, 2H), 1.6–1.63 (m, 2H), 1.65–1.67 (m, 2H), 1.72–1.74 (m, 2H), 1.77–1.79 (m, 2H), 2.27–2.41 (m, 2H), 2.46–2.54 (m, 2H), 3.06–3.17 (m, 4H), 3.25–3.29 (m, 2H), 3.49–3.66 (m, 2H), 3.72–3.77 (m, 4H), 5.26–5.27 (m, 1H), 5.55 (br s, 1H), 6.84–6.9 (m, 1H), 6.92–6.95 (m, 1H), 7.07–7.15 (m, 2H), 11.4 (br s, 1H).

*4.3.21. 1-(5-(4-(2-Chlorophenyl)piperazin-1-yl)pentanoyl)-piperidine-2-carboxylic acid (**28**)*

Yellow oil, 15 mg (36% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.2 min. $C_{21}H_{30}ClN_3O_3$, MW 407.93, Monoisotopic Mass 407.2, $[M + H]^+$ = 408.7. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.4–1.43 (m, 2H), 1.61–1.63 (m, 2H), 1.7–1.73 (m, 2H), 1.78–1.83 (m, 2H), 2.28–2.38 (m, 2H), 2.41–2.54 (m, 2H), 3.08–3.13 (m, 4H), 3.24–3.28 (m, 2H), 3.39–3.43 (m, 2H), 3.69–3.74 (m, 4H), 5.27–5.28 (m, 1H), 7.02–7.07 (m, 2H), 7.23–7.28 (m, 1H), 7.35–7.38 (m, 1H), 11.4 (br s, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 20.82, 21.48, 22.67, 25.15, 26.38, 31.45, 44.66, 46.50, 51.33, 51.53, 52.62, 56.61, 56.79, 113.54, 115.82, 120.13, 134.7, 137.11, 150.30, 175.64, 175.83.

*4.3.22. 1-(6-(4-(2-Chlorophenyl)piperazin-1-yl)hexanoyl)-piperidine-2-carboxylic acid (**29**)*

Pale oil, 13 mg (32% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.24 min. $C_{22}H_{32}ClN_3O_3$, MW 421.96, Monoisotopic Mass 421.21, $[M + H]^+$ = 422.3. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.4–1.43 (m, 2H), 1.46–1.56 (m, 2H), 1.61–1.63 (m, 2H), 1.7–1.73 (m, 2H), 1.78–1.83 (m, 2H), 2.28–2.38 (m, 2H), 2.41–2.54 (m, 2H), 3.08–3.13 (m, 4H), 3.24–3.28 (m, 2H), 3.39–

3.43 (m, 2H), 3.69–3.74 (m, 4H), 5.27–5.28 (m, 1H), 7.02–7.07 (m, 2H), 7.23–7.28 (m, 1H), 7.35–7.38 (m, 1H), 11.4 (br s, 1H).

*4.3.23. 1-(5-(4-(3-Chlorophenyl)piperazin-1-yl)pentanoyl)-piperidine-2-carboxylic acid (**30**)*

Pale oil, 15 mg (22% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.2 min. $C_{21}H_{30}ClN_3O_3$, MW 407.93, Monoisotopic Mass 407.2, $[M + H]^+$ = 408.7. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41–1.43 (m, 2H), 1.61–1.64 (m, 2H), 1.7–1.73 (m, 2H), 1.78–1.83 (m, 2H), 2.28–2.38 (m, 2H), 2.41–2.54 (m, 2H), 3.07–3.13 (m, 4H), 3.24–3.28 (m, 2H), 3.39–3.44 (m, 2H), 3.69–3.74 (m, 4H), 5.27–5.28 (m, 1H), 6.81–6.84 (m, 2H), 7.01–7.05 (m, 1H), 7.2–7.24 (m, 1H), 11.4 (br s, 1H).

*4.3.24. 1-(6-(4-(3-Chlorophenyl)piperazin-1-yl)hexanoyl)-piperidine-2-carboxylic acid (**31**)*

Pale oil, 13 mg (27% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.25 min. $C_{22}H_{32}ClN_3O_3$, MW 421.96, Monoisotopic Mass 421.21, $[M + H]^+$ = 422.3. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.4–1.43 (m, 2H), 1.46–1.56 (m, 2H), 1.61–1.63 (m, 2H), 1.7–1.73 (m, 2H), 1.78–1.83 (m, 2H), 2.28–2.38 (m, 2H), 2.41–2.54 (m, 2H), 3.08–3.13 (m, 4H), 3.24–3.28 (m, 2H), 3.39–3.43 (m, 2H), 3.69–3.74 (m, 4H), 5.27–5.28 (m, 1H), 6.8–6.84 (m, 2H), 7.02–7.05 (m, 1H), 7.21–7.24 (m, 1H), 11.4 (br s, 1H).

*4.3.25. 1-(5-(4-(4-Chlorophenyl)piperazin-1-yl)pentanoyl)-piperidine-2-carboxylic acid (**32**)*

Pale oil, 12 mg (22% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.21 min. $C_{21}H_{30}ClN_3O_3$, MW 407.93, Monoisotopic Mass 407.2, $[M + H]^+$ = 408.6. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39–1.43 (m, 2H), 1.59–1.63 (m, 2H), 1.71–1.73 (m, 2H), 1.81–1.85 (m, 2H), 2.27–2.31 (m, 2H), 2.41–2.54 (m, 2H), 2.98–3.02 (m, 4H), 3.06–3.1 (m, 2H), 3.23–3.26 (m, 2H), 3.58–3.73 (m, 4H), 5.23–5.25 (m, 1H), 6.83–6.87 (m, 2H), 7.21–7.27 (m, 2H), 11.2 (br s, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 20.66, 21.40, 22.98, 25.05, 26.26, 32.15, 43.66, 46.50, 51.44, 51.80, 52.40, 56.61, 56.79, 114.92, 116.98, 121.38, 130.45, 135.17, 150.50, 173.20, 173.54.

*4.3.26. 1-(5-(4-(4-Fluorophenyl)piperazin-1-yl)pentanoyl)-piperidine-2-carboxylic acid (**33**)*

Yellow oil, 20 mg (35% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.12 min. $C_{21}H_{30}FN_3O_3$, MW 391.48, Monoisotopic Mass 391.23, $[M + H]^+$ = 392.7. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37–1.43 (m, 2H), 1.59–1.66 (m, 2H), 1.7–1.75 (m, 2H), 1.81–1.85 (m, 2H), 2.28–2.31 (m, 2H), 2.41–2.54 (m, 2H), 2.98–3.04 (m, 4H), 3.06–3.11 (m, 2H), 3.23–3.26 (m, 2H), 3.6–3.73 (m, 4H), 5.23–5.25 (m, 1H), 6.83–6.87 (m, 2H), 7.22–7.27 (m, 2H), 11.2 (br s, 1H).

*4.3.27. 1-(6-(4-(4-Fluorophenyl)piperazin-1-yl)hexanoyl)-piperidine-2-carboxylic acid (**34**)*

Yellow oil, 18 mg (33% isolated yield) following preparative LC/MS purification; LC/MS purity 100%, t_R = 1.14 min. $C_{22}H_{32}FN_3O_3$, MW 405.51, Monoisotopic Mass 405.24, $[M + H]^+$ = 406.4. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37–1.43 (m, 2H), 1.45–1.49 (m, 2H), 1.59–1.68 (m, 2H), 1.72–1.75 (m, 2H), 1.83–1.87 (m, 2H), 2.28–2.31 (m, 2H), 2.44–2.54 (m, 2H), 2.95–3.03 (m, 4H), 3.06–3.11 (m, 2H), 3.23–3.26 (m, 2H), 3.62–3.73 (m, 4H), 5.23–5.26 (m, 1H), 6.83–6.87 (m, 2H), 7.23–7.27 (m, 2H), 11.5 (br s, 1H).

*4.3.28. 1-(5-(4-(2,3-Dichlorophenyl)piperazin-1-yl)pentanoyl)-piperidine-2-carboxylic acid (**35**)*

Yellow oil, 15 mg (33% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.31 min. $C_{21}H_{29}Cl_2N_3O_3$, MW 442.38, Monoisotopic Mass 441.16, $[M + H]^+$ = 442.5. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.4–1.48 (m, 2H), 1.59–1.61 (m, 2H), 1.63–1.7 (m, 2H), 1.78–1.84 (m, 2H), 2.28–2.41 (m, 2H), 2.44–2.57 (m, 2H), 3.06–3.14 (m, 4H), 3.23–3.28 (m, 2H), 3.38–3.41 (m, 2H), 3.69–3.73 (m, 4H), 5.26–5.28 (m, 1H), 6.97–7.01 (m, 1H), 7.15–7.18 (m, 1H), 7.21–7.26 (m, 1H), 11.3 (br s, 1H).

*4.3.29. 1-(6-(4-(2,3-Dichlorophenyl)piperazin-1-yl)hexanoyl)-piperidine-2-carboxylic acid (**36**)*

Yellow oil, 14 mg (31% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.35 min. $C_{22}H_{31}Cl_2N_3O_3$, MW 456.41, Monoisotopic Mass 455.17, $[M + H]^+$ = 456.4. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39–1.41 (m, 2H), 1.44–1.46 (m, 2H), 1.58–1.62 (m, 2H), 1.67–1.7 (m, 2H), 1.72–1.81 (m, 2H), 2.27–2.41 (m, 2H), 2.44–2.54 (m, 2H), 3.07–3.13 (m, 4H), 3.21–3.32 (m, 2H), 3.38–3.42 (m, 2H), 3.68–3.76 (m, 4H), 5.28–5.29 (m, 1H), 6.98–7.01 (m, 1H), 7.16–7.21 (m, 1H), 7.23–7.26 (m, 1H), 11.5 (br s, 1H).

*4.3.30. (S)-2-(5-(4-(2-(Methylthio)phenyl)piperazin-1-yl) pentanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**37**)*

Yellow oil, 20 mg (32% isolated yield) following preparative LC/MS purification; LC/MS purity 98%, t_R = 1.32 min. $C_{26}H_{34}N_4O_2S$, MW 466.64, Monoisotopic Mass 466.24, $[M + H]^+$ = 467.4. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44–1.47 (m, 2H), 1.62–1.66 (m, 2H), 2.33 (s, 3H), 2.45–2.49 (m, 4H), 2.87–2.95 (m, 2H), 3.09–3.13 (m, 2H), 3.33–3.41 (m, 6H), 4.57–4.77 (m, 2H), 4.94–4.98 (m, 1H), 7.05–7.10 (m, 4H), 7.14–7.19 (m, 4H).

*4.3.31. (S)-2-(6-(4-(2-(Methylthio)phenyl)piperazin-1-yl) hexanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**38**)*

Yellow oil, 22 mg (34% isolated yield) following preparative LC/MS purification; LC/MS purity 97%, t_R = 1.35 min. $C_{27}H_{36}N_4O_2S$, MW 480.67, Monoisotopic Mass 480.26, $[M + H]^+$ = 481.4. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.32–1.35 (m, 2H), 1.45–1.47 (m, 2H), 1.62–1.66 (m, 2H), 2.36 (s, 3H), 2.47–2.49 (m, 4H), 2.87–2.95 (m, 2H), 3.09–3.15 (m, 2H), 3.33–3.41 (m, 6H), 4.57–4.77 (m, 2H), 4.95–4.98 (m, 1H), 7.05–7.10 (m, 4H), 7.14–7.18 (m, 4H).

*4.3.32. (S)-2-(7-(4-(2-(Methylthio)phenyl)piperazin-1-yl) heptanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**39**)*

Yellow oil, 26 mg (37% isolated yield) following preparative LC/MS purification; LC/MS purity 98%, t_R = 1.41 min. $C_{28}H_{38}N_4O_2S$, MW 494.69, Monoisotopic Mass 494.27, $[M + H]^+$ = 495.5. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32–1.42 (m, 4H), 1.48–1.59 (m, 2H), 1.60–1.72 (m, 2H), 2.33–2.54 (m, 4H), 2.64–2.71 (m, 4H), 2.94–3.12 (m, 1H), 3.21–3.38 (m, 1H), 3.53–3.62 (m, 4H), 4.50–4.70 (m, 2H), 5.11 (t, J = 5.56 Hz, 1H), 5.73 (br. s., 1H), 6.35 (br. s., 1H), 7.08–7.23 (m, 5H), 7.39–7.50 (m, 2H), 7.68 (d, J = 8.08 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 24.66, 26.26, 27.12, 29.10, 30.16, 33.84, 45.99, 46.83, 47.94, 52.43, 52.93, 58.54, 110.41, 116.08, 122.19, 122.36, 125.70, 126.71, 127.67, 128.28, 129.56, 132.54, 133.71, 161.15, 163.91, 173.55, 173.91.

*4.3.33. (S)-2-(4-((4-(2-(Methylthio)phenyl)piperazin-1-yl)methyl) benzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**40**)*

Brown oil, 23 mg (35% isolated yield) following preparative LC/MS purification; LC/MS purity 97%, t_R = 1.34 min. $C_{29}H_{32}N_4O_2S$, MW 500.65, Monoisotopic Mass 500.22, $[M + H]^+$ = 501.5. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H), 2.03–2.17 (m, 2H), 2.33–2.37 (m, 2H), 3.49–3.51 (m, 4H), 3.55–3.6 (m, 4H), 4.57–4.77 (m, 2H), 4.95–4.98 (m, 1H), 7.05–7.1 (m, 4H), 7.14–7.18 (m, 4H), 7.43–7.46 (m, 2H), 7.5–7.53 (m, 2H).

*4.3.34. (S)-2-(5-(4-(2-Methoxyphenyl)piperazin-1-yl)pentanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**41**)*

Yellow oil, 15 mg (36% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.15 min. $C_{26}H_{34}N_4O_3$, MW 450.57, Monoisotopic Mass 450.26, $[M + H]^+$ = 451.3. ^1H NMR (300 MHz, $CDCl_3$): δ 1.79–1.83 (m, 2H), 1.96–2.01 (m, 2H), 2.43–2.71 (m, 2H), 3.01–3.08 (m, 4H), 3.2–3.33 (m, 2H), 3.51–3.55 (m, 4H), 3.62–3.65 (m, 2H), 3.86 (s, 3H), 4.55–4.69 (m, 2H), 5.1–5.13 (m, 1H), 6.88–7.06 (m, 4H), 7.22–7.26 (m, 4H).

*4.3.35. (S)-2-(6-(4-(2-Methoxyphenyl)piperazin-1-yl)hexanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**42**)*

Yellow oil, 20 mg (37% isolated yield) following preparative LC/MS purification; LC/MS purity 98%, t_R = 1.19 min. $C_{27}H_{36}N_4O_3$, MW 464.6, Monoisotopic Mass 464.28, $[M + H]^+$ = 465.3. ^1H NMR (300 MHz, DMSO-d_6): δ 1.21–1.24 (m, 2H), 1.28–1.35 (m, 2H), 1.52–1.56 (m, 2H), 2.47–2.49 (m, 4H), 2.71–2.75 (m, 2H), 2.93–2.95 (m, 2H), 3.01–3.11 (m, 2H), 3.35–3.59 (m, 4H), 3.75 (s, 3H), 4.57–4.77 (m, 2H), 4.94–4.98 (m, 1H), 6.85–6.95 (m, 4H), 7.14–7.20 (m, 4H).

*4.3.36. (S)-2-(4-((4-(2-Methoxyphenyl)piperazin-1-yl)methyl) benzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**43**)*

Brown oil, 27 mg (34% isolated yield) following preparative LC/MS purification; LC/MS purity 97%, t_R = 1.32 min. $C_{29}H_{32}N_4O_3$, MW 484.59, Monoisotopic Mass 484.25, $[M + H]^+$ = 485.5. ^1H NMR (300 MHz, $CDCl_3$): δ 2.03–2.17 (m, 2H), 3.49–3.51 (m, 4H), 3.55–3.6 (m, 4H), 3.33–3.37 (m, 2H), 3.85 (s, 3H), 4.57–4.77 (m, 2H), 4.95–4.98 (m, 1H), 7.05–7.1 (m, 4H), 7.14–7.18 (m, 4H), 7.43–7.46 (m, 2H), 7.5–7.53 (m, 2H).

*4.3.37. (S)-2-(6-(4-(2-Hydroxyphenyl)piperazin-1-yl)hexanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**44**)*

Colorless oil, 12 mg (34% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.14 min. $C_{26}H_{34}N_4O_3$, MW 450.57, Monoisotopic Mass 450.26, $[M + H]^+$ = 451.5. ^1H NMR (300 MHz, $CDCl_3$): δ 1.51–1.56 (m, 2H), 1.81–1.86 (m, 2H), 2.14–2.37 (m, 4H), 3.04–3.09 (m, 4H), 3.28–3.35 (m, 2H), 3.33–3.41 (m, 4H), 3.67–3.71 (m, 2H), 4.61–4.74 (m, 2H), 5.11–5.15 (m, 1H), 6.88–6.96 (m, 2H), 7.1–7.19 (m, 2H), 7.21–7.26 (m, 4H).

*4.3.38. (S)-2-(6-(4-(2-Chlorophenyl)piperazin-1-yl)hexanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**45**)*

Pale oil, 10 mg (32% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.31 min. $C_{26}H_{33}ClN_4O_2$, MW 469.02, Monoisotopic Mass 468.23, $[M + H]^+$ = 469.5. ^1H NMR (300 MHz, $CDCl_3$): δ 1.5–1.53 (m, 2H), 1.71–1.84 (m, 2H), 2.17–2.21 (m, 4H), 3.04–3.06 (m, 4H), 3.21–3.33 (m, 2H), 3.4–3.44 (m, 4H), 3.67–3.7 (m, 2H), 4.5–4.75 (m, 2H), 5.13–5.17 (m, 1H), 7.04–7.16 (m, 2H), 7.21–7.29 (m, 4H), 7.37–7.4 (m, 2H).

*4.3.39. (S)-2-(6-(4-(3-Chlorophenyl)piperazin-1-yl)hexanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**46**)*

Pale oil, 15 mg (37% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.33 min. $C_{26}H_{33}ClN_4O_2$, MW 469.02, Monoisotopic Mass 468.23, $[M + H]^+$ = 469.5. ^1H NMR (300 MHz, $CDCl_3$): δ 1.48–1.53 (m, 2H), 1.69–1.84 (m, 2H), 2.04–2.17 (m, 4H), 2.98–3.05 (m, 4H), 3.29–3.35 (m, 2H), 3.49–3.55 (m, 4H), 3.66–3.75 (m, 2H), 4.59–4.65 (m, 2H), 5.11–5.15 (m, 1H), 6.81–6.86 (m, 2H), 7.18–7.22 (m, 4H), 7.23–7.25 (m, 1H), 7.27–7.31 (m, 1H).

*4.3.40. (S)-2-(5-(4-(4-Chlorophenyl)piperazin-1-yl)pentanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**47**)*

Pale oil, 14 mg (34% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.29 min. $C_{25}H_{31}ClN_4O_2$, MW 454.99, Monoisotopic Mass 454.21, $[M + H]^+$ = 455.2. ^1H NMR (300 MHz, $CDCl_3$): δ 1.82–1.87 (m, 2H), 1.93–1.97 (m, 2H), 2.49–2.54 (m, 2H), 2.63–2.69 (m, 2H), 3.02–3.06 (m, 4H), 3.25–3.27 (m, 2H), 3.34–3.61 (m, 4H), 4.54–4.68 (m, 2H), 5.07–5.11 (m, 1H), 6.75–6.79 (m, 2H), 6.91–6.94 (m, 2H), 7.13–7.3 (m, 4H).

*4.3.41. (S)-2-(5-(4-(4-Fluorophenyl)piperazin-1-yl)pentanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**48**)*

Pale oil, 13 mg (33% isolated yield) following preparative LC/MS purification; LC/MS purity 100%, t_R = 1.19 min. $C_{25}H_{31}FN_4O_2$, MW 438.54, Monoisotopic Mass 438.24, $[M + H]^+$ = 439.5. ^1H NMR (300 MHz, $CDCl_3$): δ 1.85–1.97 (m, 4H), 2.49–2.69 (m, 2H), 3.01–3.07 (m, 4H), 3.17–3.28 (m, 2H), 3.35–3.47 (m, 4H), 3.62–3.73 (m, 2H), 4.6–4.68 (m, 2H), 5.08–5.11 (m, 1H), 6.86–6.95 (m, 2H), 6.96–7.02 (m, 2H), 7.13–7.26 (m, 4H).

*4.3.42. (S)-2-(6-(4-(4-Fluorophenyl)piperazin-1-yl)hexanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**49**)*

Pale oil, 19 mg (38% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.21 min. $C_{26}H_{33}FN_4O_2$, MW 452.56, Monoisotopic Mass 452.26, $[M + H]^+$ = 453.3. ^1H NMR (300 MHz, $CDCl_3$): δ 1.48–1.54 (m, 2H), 1.69–1.85 (m, 4H), 2.36–2.65 (m, 2H), 2.96–3.07 (m, 4H), 3.28–3.35 (m, 2H), 3.47–3.55 (m, 4H), 3.67–3.7 (m, 2H), 4.64–4.79 (m, 2H), 5.1–5.13 (m, 1H), 6.87–6.93 (m, 2H), 6.97–7.03 (m, 2H), 7.15–7.26 (m, 4H).

*4.3.43. (S)-2-(6-(4-(2,3-Dichlorophenyl)piperazin-1-yl)hexanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**50**)*

Pale oil, 29 mg (32% isolated yield) following preparative LC/MS purification; LC/MS purity 98%, t_R = 1.41 min. $C_{26}H_{32}Cl_2N_4O_2$, MW 503.46, Monoisotopic Mass 502.19, $[M + H]^+$ = 503.2. ^1H NMR (300 MHz, DMSO-d_6): δ 1.31–1.36 (m, 2H), 1.51–1.56 (m, 2H), 1.62–1.65 (m, 2H), 2.33–2.41 (m, 2H), 2.45–2.49 (m, 2H), 2.61–2.72 (m, 4H), 2.91–2.98 (m, 2H), 3.01–3.11 (m, 4H), 4.49–4.69 (m, 2H), 5.10–5.14 (m, 1H), 5.89 (s, 1H), 6.41 (s, 1H), 6.89–6.95 (m, 1H), 7.07–7.11 (m, 2H), 7.13–7.26 (m, 4H).

*4.3.44. (S)-2-(4-((4-(2,3-Dichlorophenyl)piperazin-1-yl)methyl) benzoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**51**)*

Pale oil, 16 mg (38% isolated yield) following preparative LC/MS purification; LC/MS purity 97%, t_R = 1.42 min. $C_{28}H_{28}Cl_2N_4O_2$, MW 523.45, Monoisotopic Mass 522.16, $[M + H]^+$ = 523.3. ^1H NMR (300 MHz, $CDCl_3$): δ 2.33–2.37 (m, 2H), 3.49–3.51 (m, 4H), 3.55–3.6 (m, 4H), 3.36–3.39 (m, 2H), 4.57–4.77 (m, 2H), 4.95–4.98 (m, 1H), 5.89 (s, 1H), 6.41 (s, 1H), 6.89–6.95 (m, 1H), 7.13–7.26 (m, 4H), 7.42–7.48 (m, 2H), 7.55–7.58 (m, 2H).

*4.3.45. (S)-2-(5-(4-(Benzo[d]isoxazol-3-yl)piperazin-1-yl) pentanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**52**)*

Colorless oil, 18 mg (38% isolated yield) following preparative LC/MS purification; LC/MS purity 98%, t_R = 1.16 min. $C_{26}H_{31}N_5O_3$, MW 461.56, Monoisotopic Mass 461.24, $[M + H]^+$ = 462.2. ^1H NMR (300 MHz, $CDCl_3$): δ 1.28–1.57 (m, 4H), 2.11–2.15 (m, 2H), 2.22–2.37 (m, 2H), 2.77 (t, 4H), 3.10–3.13 (m, 2H), 3.57 (t, 4H), 4.61–4.79 (m, 2H), 5.13–5.17 (m, 1H), 7.15–7.21 (m, 1H), 7.22–7.3 (m, 4H), 7.48–7.54 (m, 2H), 7.57–7.61 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 25.33, 27.12, 32.25, 33.87, 43.85, 45.99, 46.83, 48.34, 52.43, 52.98, 58.54, 111.48, 116.08, 122.19, 122.36, 125.70, 126.71, 127.67, 128.45, 129.56, 132.54, 134.31, 161.15, 163.91, 173.55, 174.12.

*4.3.46. (S)-2-(6-(4-(Benzo[d]isoxazol-3-yl)piperazin-1-yl) hexanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**53**)*

Colorless oil, 21 mg (36% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, t_R = 1.26 min. $C_{27}H_{33}N_5O_3$,

MW 475.58, Monoisotopic Mass 475.26, $[M + H]^+ = 476.4$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.39–1.47 (m, 2H), 1.53–1.58 (m, 2H), 1.67–1.77 (m, 2H), 2.39–2.44 (m, 2H), 2.47–2.54 (m, 2H), 2.63 (t, 4H), 2.98 (dd, 1H, $J_1 = 10$ Hz, $J_2 = 6$ Hz), 3.14 (dd, 1H, $J_1 = 10$ Hz,, $J_2 = 6$ Hz), 3.58 (t, 4H), 4.48–4.67 (m, 2H), 5.15–5.19 (m, 1H), 7.11 (d, 1H, $J = 6$ Hz), 7.18–7.26 (m, 4H), 7.27–7.47 (m, 2H), 7.68 (d, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 26.29, 27.12, 29.10, 32.25, 34.56, 43.85, 45.99, 46.76, 47.94, 52.43, 52.93, 58.54, 112.41, 116.08, 122.19, 122.36, 124.70, 125.73, 127.67, 128.28, 129.56, 132.54, 134.62, 161.15, 163.91, 173.55, 173.85.

4.3.47. (S)-2-(7-(4-(Benzo[d]isoxazol-3-yl)piperazin-1-yl) heptanoyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (**54**)

Colorless oil, 27 mg (37% isolated yield) following preparative LC/MS purification; LC/MS purity 99%, $t_R = 1.3$ min. $C_{28}H_{35}N_5O_3$, MW 489.61, Monoisotopic Mass 489.27, $[M + H]^+ = 490.4$.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32–1.42 (m, 4H), 1.49–1.58 (m, 2H), 1.60–1.69 (m, 2H), 2.40–2.56 (m, 4H), 2.68 (t, $J = 6.05$ Hz, 4H), 2.96–3.11 (m, 2H), 3.22–3.33 (m, 1H), 3.57 (t, $J = 6.1$ Hz, 4H), 4.64–4.70 (m, 1H), 5.06–5.13 (m, 1H), 7.10–7.22 (m, 5H), 7.40–7.49 (m, 2H), 7.68 (d, $J = 8.08$ Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 26.26, 27.12, 29.10, 30.16, 32.25, 33.90, 43.85, 45.99, 46.83, 47.94, 52.43, 52.93, 58.54, 110.41, 116.08, 122.19, 122.36, 125.70, 126.71, 127.67, 128.28, 129.56, 132.54, 133.71, 161.15, 163.91, 173.55, 173.91.

4.4. In vitro pharmacology

4.4.1. Radioligand binding assays

Cell pellets were thawed and homogenized in 20 volumes of assay buffer with an Ultra tissue homogenizer before being centrifuged twice at 35,000 g for 20 min at 4 °C; a 15 min incubation at 37 °C was carried out between rounds of centrifugation. The composition of the assay buffer was as follows: for the 5-HT_{1A}R: 50 mM Tris–HCl, 0.1 mM EDTA, 4 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbate; for the 5-HT_{7b}R: 50 mM Tris–HCl, 4 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbate.

All assays were incubated in a 200 μl total volume in a 96-well microtitre plate for 1 h at 37 °C, except for the assays for the 5-HT_{1A}R, which were incubated at room temperature for 1 h. The process of equilibration was terminated by rapid filtration through Unifilter plates with a 96-well cell harvester; the radioactivity retained on the filters was quantified with a Microbeta plate reader.

For the displacement studies, the assay samples contained radioligands: 1.5 nM [^{3}H]-8-OH-DPAT (187 Ci/mmol) for the 5-HT_{1A}R; 0.6 nM [^{3}H]-5-CT (39.2 Ci/mmol) for the 5-HT_{7b}R.

Non-specific binding was defined with 10 μM 5-HT within the 5-HT_{1A}R and 5-HT_{7b}R binding experiments. Each compound was tested in triplicate at 7–8 concentrations (10^{-11}–10^{-4} M). The inhibition constants (K_i) were calculated from the Cheng–Prusoff equation. The results were expressed as the mean of at least two separate experiments.

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Appendix A. Supplementary data

Supplementary data available: representative LC/MS, ^{1}H NMR, and ^{13}C NMR spectra studies associated with presented data is available in on-line version at http://dx.doi.org/10.1016/j.ejmech.2014.03.005.

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