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Parallel solid-phase synthesis and characterization of new sulfonamide and carboxamide proline derivatives as potential CNS agents

Paweł Zajdel,[a,b,*] Gilles Subra,[a] Andrzej J. Bojarski,[c] Beata Duszyńska,[c] Maciej Pawłowski[b] and Jean Martinez[a]

[a]*Laboratoire des Aminoacides, Peptides et Protéines (LAPP), UMR 5810, Faculté de Pharmacie, Université Montpellier I et II, 15 avenue Charles Flahault, 34060 Montpellier, France*
[b]*Department of Pharmaceutical Chemistry, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland*
[c]*Department of Medicinal Chemistry, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland*

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Abstract—A solid-phase synthesis of the 64-member library of novel sulfonamide and carboxamide proline derivatives, focused on the 5-HT_7 receptor antagonist SB-258741, was described. The final compounds were obtained in good yields and high purity upon cleavage from SynPhase Lanterns, functionalized by a BAL linker. The library representatives were screened for 5-HT_7, 5-HT_{1A} and D_2 receptors to explore the impact of a tertiary amine moiety, the length of an alkylene spacer and the aryl fragment on the receptor affinity. The preliminary biological results provided data for further investigation aimed at a search for 5-HT_7 receptor agents, and permitted the identification of several compounds with significant 5-HT_{1A} receptor affinity.

1. Introduction

The 5-HT_7 receptor (5-HT_7R) is the most recently identified subtype of the serotonin G-protein-coupled receptor (GPCR) superfamily. It shows about 36–53% homology with other human 5-HT receptors and has been found to be positively coupled to adenylate cyclase.[1] Evaluation of the expression pattern of 5-HT_7R indicates that it is present both centrally and peripherally. The prominent position of 5-HT_7 receptor in the thalamus, limbic and cortical regions of the brain, as well as high affinity for several antipsychotic and antidepressant agents suggest its involvement in such mental disorders as schizophrenia[2] and depression.[3] Furthermore, some authors have observed that 5-HT_7R may be implicated in the regulation of circadian rhythms,[4] migraine[5] and the relaxation of vascular smooth muscles.[6]

For more than 10 years since the receptor discovery, only a few selective antagonists with significant 5-HT_7 affinity have been identified, and no selective agonist has yet been available.[7–9] Although the structures of compounds active at 5-HT_7R are diversified, a relatively large group of ligands contain several common fragments, for example, an amine moiety (mostly 4-*N*-arylpiperazine, tetrahydroisoquinoline or 4-substituted tetrahydropyridine), which is connected by a different length alkyl chain (2–5 carbon atoms) to a terminal aromatic fragment.[10] Unfortunately, these structural features remain similar for other GPCR's ligands (e.g., 5-HT_{1A}, 5-HT_{2A}, D_2).

Recently, Forbes et al. reported the synthesis of aryl sulfonamide derivatives as a rich source of selective 5-HT_7 receptor agents.[11] Among them, compounds **I** (SB-258741) and **II** were classified as highly potent 5-HT_7 antagonists (Chart 1).[12]

Keywords: Solid-phase synthesis; Sulfonamide proline derivatives; 5-HT_7 receptor affinity; 5-HT_{1A} receptor ligands.
* Corresponding author. Address: Department of Pharmaceutical Chemistry, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland. Tel.: +48 12 657 05 60; fax: +48 12 657 02 62; e-mail: mfzajdel@cyf-kr.edu.pl

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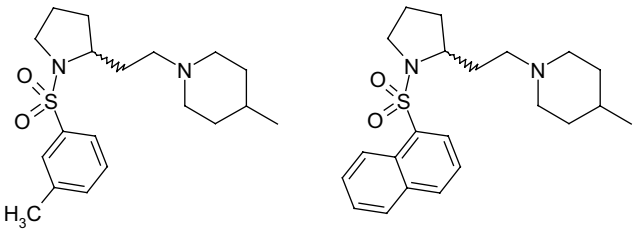


I SB-258741
5-HT$_7$ K_i = 3.2 nM

II
5-HT$_7$ K_i = 10 nM

Chart 1.

The use of solid phase enables rapid parallel synthesis of molecules without tedious and time-consuming purification. Recently, we have successfully applied this strategy for generation of focused arylpiperazine library, targeted on 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors on solid support (SynPhase Lanterns). Our original idea concerned incorporation of selected amino acid moieties (aspartic and glutamic acids, asparagine and proline) into the amide pharmacophoric group.[13]

Encouraged by the previous findings, we adopted the similar solid supported approach for the synthesis of new 5-HT$_7$ ligands. Starting from the structure of SB-258741, in which the pyrrolidine ring is connected through an ethylene chain with 4-methyltetrahydro pyridine, we designed a structurally related 64-member library of sulfonamide and carboxamide L- and D-proline derivatives.

Besides introduction of different tertiary amines and changing the number of spacer methylene groups, substituents at the proline nitrogen atom were varied to change H-bond acceptor capacity and hydrophobic property. Selected library members were screened for serotonin 5-HT$_7$ receptors affinity, and additionally, for related targets with similar substrate requirements: 5-HT$_{1A}$ and D$_2$ receptors.

2. Results

2.1. Chemistry

The library synthesis was carried out on BAL linker functionalized polyamide SynPhase Lanterns (Mimotopes, Pty). To manually manage the library construction, we chose a split-and-pool approach.[14] The lanterns were equipped with coloured cogs and spindles (corresponding to building blocks) to produce a convenient visual tagging system.[15] At each step the lanterns were manually sorted and pooled into separate vials containing the respective reagents. A library was constructed as outlined in Scheme 1.

Our workflow began upon the preparation of substituted tetrahydropyridine and piperazine alkyl-amines (Fig. 1), obtained using the procedure already described.[16] Reductive amination of the respective amines belonging to diversity reagent **IV** yielded support-bound secondary products **V**. Fmoc L- and D-proline (Fig. 2) were then coupled to a secondary amine using diisopropylcarbodiimide (DIC) in dimethylformamide (DMF) to yield products **VII**. The effectiveness of secondary amine acylation was checked by the HPLC analysis and the Chloranil test performed on a Lantern slice.[17] Next, all the lanterns were pooled together and treated with a solution of a 20% piperidine in DMF. After washing, the lanterns were sorted again and treated with appropriate acyl or sulfonyl chlorides in a basic medium (Fig. 3) to generate carboxamides and sulfonamides products **IX** and **X**, respectively. A few conditions were explored to optimize that step. We found that the best basic medium was *N*,*N*-diisopropylethylamine (DIEA) for carboxamides and a triethylamine (TEA) for sulfonamides. Additionally, DMF was the most convenient solvent for carboxamides, while the best yields of sulfonamides were obtained with dichloromethane (DCM) as a reaction medium. The final products **XI** and **XII** were cleaved from the solid support upon TFA treatment for 60 min. All the lanterns were cleaved in individual polypropylene vials. After removal of the cleavage cocktail in vacuo, the samples were solubilized in a 0.1% TFA acetonitrile/water (50:50, v/v) mixture. An aliquot of each library member was submitted to LC/MS analysis, and the remainder was lyophilized.

The final products **XI** and **XII**, presented on Scheme 1, encoded the chemical structure of the library members belonging to chemsets **1**–**9**.

The overall yields were moderate, with an average of 48% calculated on the basis of the initial loading of the lanterns. The main goal of the chemical approach applied was a quick and efficient generation of compounds of high purity, in a quantity sufficient for biological testing. That was achieved, since the LC/MS data of all the library members revealed a 90% average purity, based on the relative peak area with monitoring at 214 nm (Table 1).

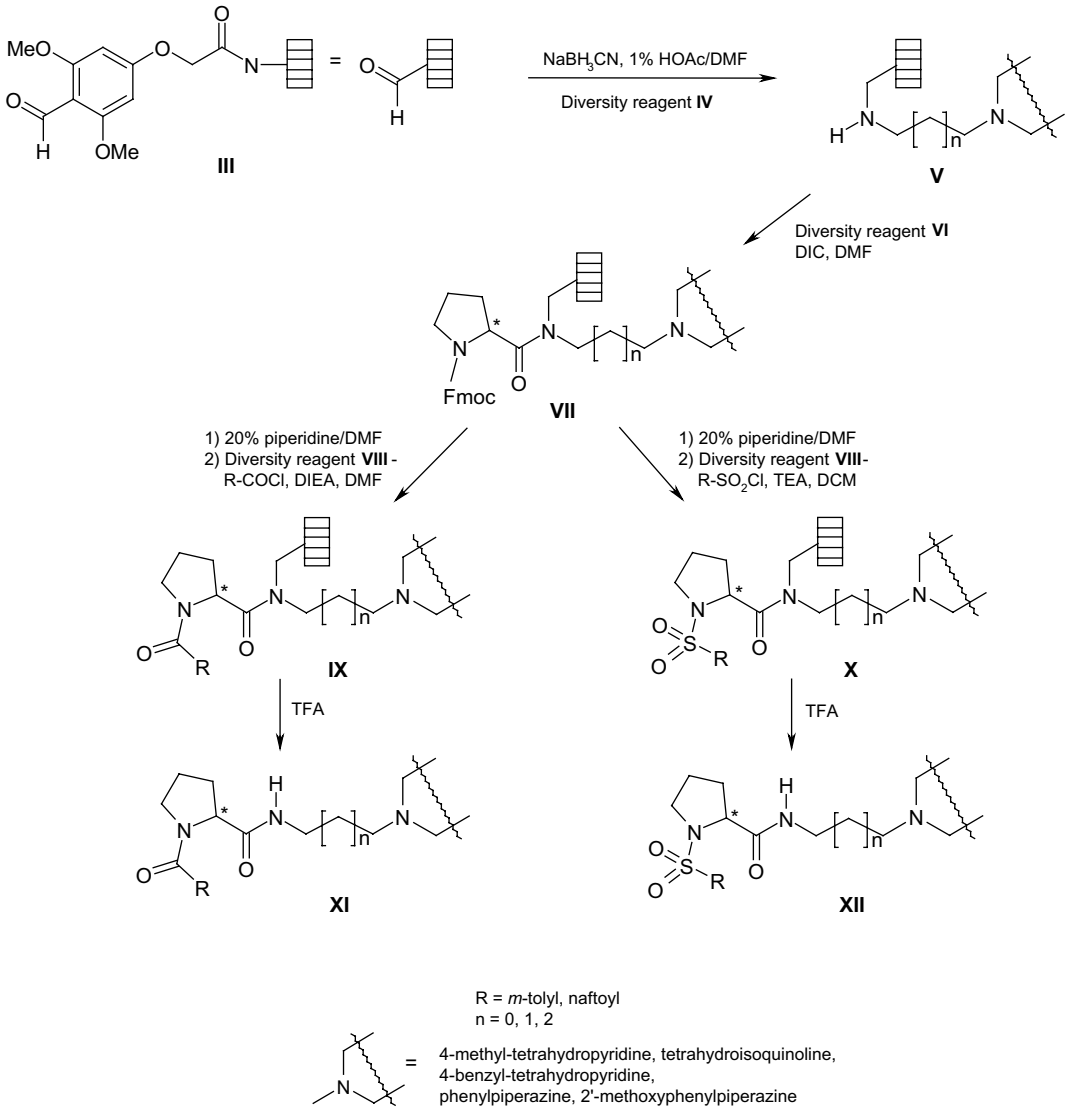
2.2. Biological evaluation

The biological characterization of the compounds was based on the screening protocol described earlier.[13] The selected library members were evaluated for their in vitro affinity at central serotonin 5-HT$_7$ receptors; additionally, the affinity of 12 compounds for D$_2$ receptors was assessed. Next, a set of 17 selected compounds were tested for their ability to bind to 5-HT$_{1A}$ receptors. The compounds selected for radioligand binding studies covered a wide range of the structural modifications applied.

The results of radioligand binding data are summarized in Tables 2 and 3.

3. Discussion

At the first stage, 56 library members belonging to chemsets **1**–**7** were synthesized and evaluated for their 5-HT$_7$ receptor affinity. All the tested compounds



Scheme 1. Solid-phase synthetic route for *N*-acyl and *N*-sulfonyl proline libraries. The final products **XI** and **XII** encode the chemical structure of chemsets **1**–**9**.

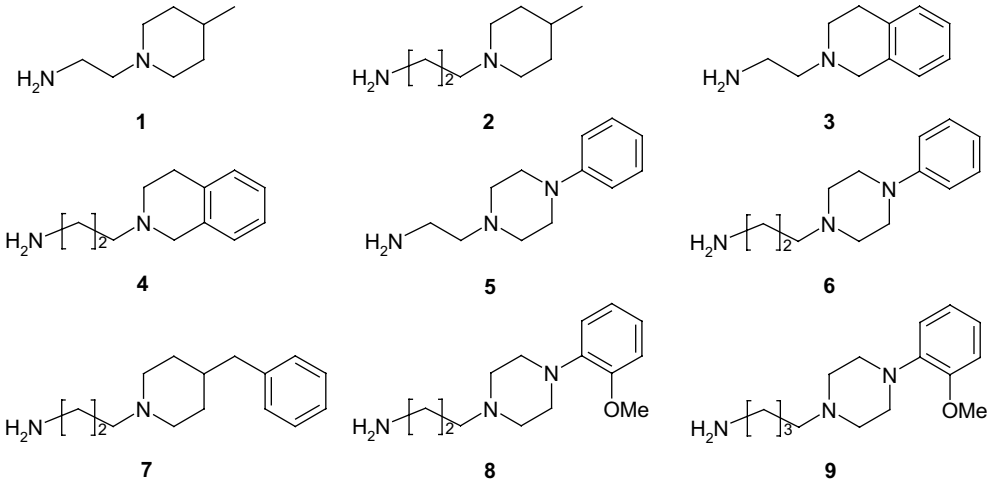


Figure 1. Diverse primary amines, **IV** {**1**–**9**}.

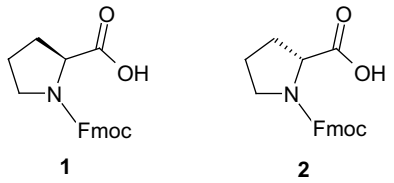


Figure 2. Diverse amino acids, **VI** {**1**–**2**}.

retained low affinity for 5-HT_7 receptors (<26% inhibition tested at 1 μM), thus it was difficult to make an inference from the structural modifications applied. It seemed that the high decrease in affinity resulted from the introduction of an additional amide bond of the proline moiety into the spacer between the pyrrolidine ring and the amine centre. Indeed, in the case of compounds **1**{1,3} and **1**{1,4}, that is, direct analogues of the model compounds **I** and **II**, the above structural modification resulted in loss of receptor affinity. Besides the low activity of the investigated series, it could be observed that, compounds with two methylene groups spacer ($n = 0$) were generally less active than their extended analogues ($n = 1$), and that the introduction of tetrahydroisoquinoline (THIQ) or phenylpiperazine (PhP) instead of 4-methylpiperidine slightly increased 5-HT_7R affinity.

Since a significant difference in 5-HT_7R affinity of the *R*-isomer ($K_i = 10$ nM) of compound **II** compared to *S*-enantiomer ($K_i = 400$ nM) was observed,[12] L- and D-proline derivatives were investigated to check whether similar stereochemical preference existed in our set of compounds. It was found that the L-enantiomer of **4**{1,1} showed higher 5-HT_7 affinity than did its D-analogue **4**{2,1}, but the opposite relations was for the L-enantiomer of **6**{1,3} and its D-counterpart **6**{2,3}

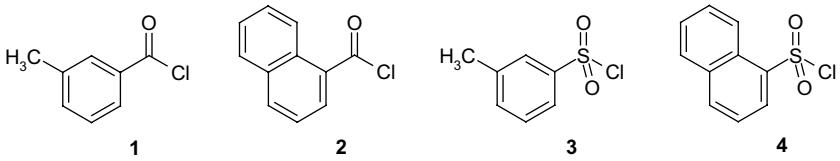


Figure 3. Diverse acid and sulfonyl chlorides, **VIII** {**1**–**4**}.

Table 1. Analytical data for chemsets **1**–**9**

Compd	Purity (%)[a]	MW calculated	$[M+H]^+$ found	Compd	Purity (%)[a]	MW calculated	$[M+H]^+$ found
1{1,1}	80	357.20	358.37	**5**{1,1}	90	420.20	421.35
1{1,2}	88	393.20	394.34	**5**{1,2}	98	456.20	457.37
1{1,3}	90	393.20	394.30	**5**{1,3}	86	456.20	457.34
1{1,4}	96	429.20	430.32	**5**{1,4}	97	492.20	493.33
1{2,1}	85	357.20	358.37	**5**{2,1}	80	420.20	421.35
1{2,2}	87	393.20	394.34	**5**{2,2}	90	456.20	457.38
1{2,3}	91	393.20	394.31	**5**{2,3}	82	456.20	457.34
2{2,4}	96	429.20	430.32	**5**{2,4}	97	492.20	493.33
2{1,1}	88	371.20	372.38	**6**{1,1}	81	434.20	435.37
2{1,2}	86	407.20	408.35	**6**{1,2}	95	470.20	471.39
2{1,3}	94	407.20	408.32	**6**{1,3}	86	470.20	471.35
2{1,4}	84	443.20	444.32	**6**{1,4}	95	506.20	507.35
2{2,1}	87	371.20	372.38	**6**{2,1}	82	434.20	435.38
2{2,2}	87	407.20	408.36	**6**{2,2}	96	470.20	471.38
2{2,3}	94	407.20	408.33	**6**{2,3}	90	470.20	471.35
2{2,4}	82	443.20	444.32	**6**{2,4}	98	506.20	507.35
3{1,1}	95	391.20	392.31	**7**{1,1}	90	447.20	448.42
3{1,2}	82	427.20	428.31	**7**{1,2}	82	483.20	484.41
3{1,3}	91	427.20	428.29	**7**{1,3}	78	483.20	484.38
3{1,4}	89	463.20	464.30	**7**{1,4}	84	519.20	520.36
3{2,1}	94	391.20	392.32	**7**{2,1}	85	447.20	448.42
3{2,2}	93	427.20	428.32	**7**{2,2}	82	483.20	484.41
3{2,3}	98	427.20	428.28	**7**{2,3}	82	483.20	484.38
3{2,4}	89	463.20	464.30	**7**{2,4}	80	519.20	520.37
4{1,1}	95	405.20	406.34	**8**{1,1}	94	464.30	465.30
4{1,2}	94	441.20	442.34	**8**{1,2}	93	500.30	501.30
4{1,3}	91	441.20	442.31	**8**{1,3}	97	500.20	501.40
4{1,4}	90	477.20	478.32	**8**{1,4}	94	536.20	537.40
4{2,1}	96	405.20	406.35	**9**{1,1}	92	478.20	479.40
4{2,2}	90	441.20	442.35	**9**{1,2}	92	514.30	515.30
4{2,3}	89	441.20	442.31	**9**{1,3}	98	514.20	515.40
4{2,4}	90	477.20	478.32	**9**{1,4}	91	550.20	551.30

[a] Based on LC using relative peak areas with monitoring at 214 nm.

Table 2. The affinity data for 5-HT_7, D_2 and 5-HT_{1A} receptors for selected library representatives of chemsets **1**–**7**

Compd	Receptor binding			Compd	Receptor binding		
	5-HT_7[a]	5-HT_{1A}[b]	D_2[a]		5-HT_7[a]	5-HT_{1A}[b]	D_2[a]
1{1,3}	0	nd	nd	**6**{1,4}	22.5	660	4.8
1{1,4}	0	nd	nd	**6**{2,1}	15.9	2580	0
2{1,3}	5.4	nd	nd	**6**{2,2}	13.9	1135	0
2{2,3}	0	nd	nd	**6**{1,3}	0	nd	nd
3{1,3}	11.7	1100	nd	**6**{2,3}	17.1	2340	0
4{1,1}	25.8	200	0	**6**{2,4}	17	255	8.9
4{2,1}	0	nd	nd	**7**{1,1}	5.9	nd	0
5{1,1}	0	1240	0	**7**{1,3}	0	nd	7.7
5{1,3}	1	nd	nd	**7**{2,3}	0	nd	nd
6{1,1}	0	640	0	**7**{1,4}	0	nd	nd

[a] % of inhibition at 1 μM; nd = not determined.
[b] Estimated K_i, nM (see Ref. 13).

Table 3. The affinity data (estimated K_i) for 5-HT_7 and 5-HT_{1A} receptors of chemsets **8** and **9**

Compd	K_i (nM)	
	5-HT_7	5-HT_{1A}
8{1,1}	615	21
8{1,2}	183	29
8{1,3}	1700	35
8{1,4}	620	42
9{1,1}	1240	57
9{1,2}	360	30
9{1,3}	837	37
9{1,4}	310	20

(Table 2). Thus, the chiral properties of the proline carbon atom affected receptor binding, however a general preference could not be strictly determined.

Of the library members discussed, those possessing THIQ and PhP moieties (a known 5-HT_{1A} pharmacophores) were further screened for their binding to 5-HT_{1A} sites. The tested representatives showed different levels of affinity, which varied from 200 nM for **4**{1,1} to 2580 nM for **6**{2,1}, and highly depended on the length of a spacer.

Recently Kikuchi et al.[18] and Perrone et al.[19] described the synthesis of 5-HT_7 receptor ligands in a group of the so-called 'long-chain' arylpiperazines—a well-known class of 5-HT_{1A} ligands. Particularly, *ortho*-methoxyphenylpiperazine (*o*-OCH_3–PhP) derivatives showed high affinity at 5-HT_7 sites. Thus an additional set of eight compounds whose amide proline centre was connected by three- and tetramethylene spacer with the *o*-OCH_3–PhP was prepared. The applied modifications increased the affinity of compounds for 5-HT_7 receptors and—as was expected—also for 5-HT_{1A} receptors. The affinity for 5-HT_7 sites varied from 180 nM (**8**{1,2}) to 1700 nM (**8**{1,3}), and ranged from 21 nM for **9**{1,4} to 57 nM for **9**{1,1} for 5-HT_{1A} receptors. A close examination of the obtained binding data revealed that the naphthoyl substituent was more preferable for the 5-HT_7 receptor than was *m*-toluoyl, while the variation in the aryl part only marginally influenced 5-HT_{1A} receptor affinity. Furthermore, both the receptor affinity values indicated no remarkable differences between the sulfonamide and the carboxamide sets.

An in vitro cross-screening towards D_2 receptors revealed that none of the tested library members displayed significant affinity.

4. Conclusions

Summing up, we developed an efficient solid supported method for the synthesis of novel sulfonamide and carboxamide proline derivatives. A 64-member library was obtained on SynPhase Lanterns, in milligram amounts, for the biological evaluation for 5-HT_7 and 5-HT_{1A} serotonin, and D_2 dopamine receptor affinities. The present study has provided initial data for further investigations concerning 5-HT_7 receptor agents. Additionally, it permitted identification of several compounds with significant affinity for 5-HT_{1A} receptors. The study showed that the receptor affinity of the investigated compounds depended on the length of the methylene spacer, the kind of an amine substituent and the size of an aromatic system connected by a sulfonamide/amide bond with the proline moiety.

5. Experimental

5.1. Materials

All the solvents were obtained from Acros, and were used without purification. PA–BAL linker polyamide SynPhase Lanterns with 18 μmol loading, differently coloured spindles and cogs were provided by Mimotopes, Pty, Clayton, Australia. The Fmoc amino acids and HBTU reagent were purchased from Senn Chemicals. Carboxylic acids and other reagents were from Aldrich and Lancaster.

The following abbreviations were used: DCM, dichloromethane; DIEA, diisopropylethylamine; DMF, dimethylformamide; HBTU, *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; TEA, triethylamine; TFA, trifluoroacetic acid. The other

abbreviations used were recommended by the IUPAC-IUB Commission.[20]

5.2. LC/MS analysis

Samples were prepared in acetonitrile/water (50:50, v/v), containing a 0.1% TFA. The LC/MS system consisted of a Waters Alliance 2690 HPLC, coupled to a Micromass (Manchester, UK) Platform II spectrometer (electrospray ionization mode, ESI+). All the analyses were carried out using a C_{18} Xterra MS, 21 × 3.0 mm column. A flow rate of 500 μL/min and a gradient of (0–100%) B over 5 min were used. Eluent A: water/0.1% TFA; eluent B: acetonitrile/0.1% TFA. Positive ion electrospray mass spectra were acquired at a solvent flow rate of 100–500 μL/min. Nitrogen was used for both the nebulizing gas and the drying gas. The data were obtained in a scan mode ranging from 400 to 1400 *m*/*z* in 0.1 s intervals; 10 scans were summed up to get the final spectrum.

5.3. Chemistry

5.3.1. Standard reductive amination protocol. The lanterns were placed in glass vials containing a suspension of natrium cyanoborohydride ([$NaBH_3CN$] = 100 mM) and the amine ([diversity reagent **IV**] = 250 mM, Fig. 1), in a 1% acetic acid in 10 mL of DMF. The reaction mixture was allowed to stand overnight at 60 °C and was then removed via a drilled adapter. The lanterns were first washed with a 10% AcOH in DMF (1 × 5 min), then using the standard washing protocol and were allowed to dry in the open air.

5.3.2. Standard washing protocol. Washing steps after reductive amination, coupling or deprotection steps, were carried out by dipping the lanterns in DMF (3 × 5 min) and DCM (3 × 5 min), respectively. A single 200 mL standard Schott flask, equipped with a drilled topper, was used. The lanterns were allowed to air-dry for 15 min after the last DCM washing.

5.3.3. Secondary amine acylation protocol. Two DMF solutions (20 mL) containing Fmoc-protected D- or L-proline and DIC, were freshly prepared in a standard Schott flask ([diversity reagent **VI**] = 200 mM, [DIC] = 100 mM), and were left for 10 min to form an active anhydride. Lanterns were then immersed in the preactivated solutions and left overnight at room temperature. The solution was decanted, and lanterns were washed following the standard washing protocol. The acylation was repeated one more time for 4 h.

5.3.4. Standard Fmoc-deprotection protocol. The Fmoc-deprotection step was carried out by immersing lanterns in a mixture of piperidine and DMF (20:80, v/v) for 60 min. A 200 mL standard flask, equipped with a drilled topper, was used. After removal of the deprotection solution, the lanterns were washed following the standard washing protocol.

5.3.5. Standard carboxamide bond formation protocol. Two DMF solutions (5 mL), containing acid chloride (Fig. 3: diversity reagent **VIII**) and DIEA, were freshly prepared in a standard Schott flask ([R–COCl] = 120 mM; [DIEA] = 240 mM). Lanterns were immersed for 3 h in the solutions at a room temperature. Solutions were decanted, and lanterns washed following the standard washing procedure.

5.3.6. Standard sulfonamide bond formation protocol. Two DCM solutions (5 mL), containing sulfonyl chloride (Fig. 3: diversity reagent **VIII**) and TEA, were freshly prepared in a standard Schott flask ([R–SO_2Cl] = 120 mM; [TEA] = 120 mM). Lanterns were immersed for 3 h in the solutions at a room temperature. Then solutions were decanted, and lanterns were washed following the standard washing procedure.

5.3.7. Cleavage protocol. TFA (500 μL) was dispensed into each 64 individual polypropylene tubes of deep 96-well plate. Cleavage was carried out for 60 min. The cleavage cocktail was removed from the tubes using a Jouan RC1010 vacuum centrifuge. Some compounds were precipitated with dry diethyl ether, centrifuged and decanted one by one. A 100 μL portion of acetonitrile/water (50:50, v/v), containing a 0.1% TFA, was poured into each tube to dissolve the sample. Then samples were frozen at −80 °C and lyophilized. The procedure was repeated twice to completely remove the remaining volatile residues.

5.4. Radioligand binding studies

In vitro affinity for native serotonin 5-HT_7, 5-HT_{1A} and D_2 receptors was determined on rat hypothalamic, hippocampal and striatal membranes, respectively. A general screening procedure was carried out according to the previously published protocol.[13] Two compound concentrations were tested: 0.1 and 1 μM, each run in triplicate. The percentage of inhibition or K_i values, estimated on the basis of three independent binding experiments, were reproducible in ±20%.

5.4.1. Serotonin 5-HT_{1A} binding assays. Radioligand studies with native 5-HT_{1A} were conducted according to the previously described method with [^{3}H]-8-OH-DPAT (170 Ci/mmol, NEN Chemicals) and 5-HT for the non-specific binding.[21]

5.4.2. Serotonin 5-HT_7 binding assays. Hypothalamic membranes were prepared according to the method described by Aguirre et al.[22] with minor modifications. In brief, the hypothalami dissected from male Wistar rats (200–250 g) were frozen at −80 °C prior to the preparation of a radioligand binding homogenate. On the day of experiment, the hypothalami were allowed to defrost, then immediately homogenized in 20 volumes of a 50 mM Tris–HCl buffer (pH 7.4 at 23 °C) and centrifuged at 48,000*g* for 10 min at 4 °C. The supernatant was removed, the resulting pellet was rehomogenized and incubated at 37 °C for 15 min to remove endogenous serotonin. After incubation, the homogenate was centrifuged twice under the same conditions as before. The final pellet was resuspended in the assay buffer (50 mM) Tris–HCl containing 0.01 mM pargyline, 4 mM $CaCl_2$ and 0.1% ascorbate. The aliquots of mem-

branes (10 mg original wet tissue weight) were incubated in the presence of 3 μM (±)-pindolol (to eliminate the binding to 5-HT_{1A} receptors) with 0.5 nM [^{3}H]-5-CT (specific activity, 34.5 Ci/mmol; NEN) and the respective concentration of the displacing drug. The non-specific binding was determined using 10 μM of serotonin. After incubation at 23 °C for 120 min, the reaction was terminated by rapid filtration through a Whatman GF/B filter.

5.4.3. Dopamine D_2 binding assays. The preparation of rat striatal membranes was conducted as previously described.[23] The final tissue concentration for D_2 receptor binding was 3 mg of the original wet weight mL^{-1}. All the assays were carried out in a 50 mM potassium phosphate buffer (pH 7.4). The radioligand used was [^{3}H]-spiperone (15.70 Ci/mmol, NEN Chemicals) in the presence of 50 nM ketanserin to prevent the radioligand binding to 5-HT_{2A} receptors. Displacement experiments were performed at a total volume of 1.2 mL. Assay tubes (in triplicate) containing 0.1 mL of 1 nM [^{3}H]-spiperone, 0.1 mL of a competing drug or 0.1 mL of the vehicle (total binding) and 1 mL of the tissue were incubated at 37 °C for 30 min. The non-specific binding was determined using 0.1 mL of 5 μM butaclamol.

Acknowledgements

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