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A New Class of Arylpiperazine Derivatives: the Library Synthesis on SynPhase Lanterns and Biological Evaluation on Serotonin 5-HT_{1A} and 5-HT_{2A} Receptors

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An efficient solid-supported method for the synthesis of a new class of arylpiperazine derivatives containing amino acid residues has been developed. A 72-membered library was synthesized on SynPhase Lanterns functionalized by a BAL linker. A one-pot cleavage/cyclization step of aspartic and glutamic acid derivatives yielded succinimide- and pyroglutamyl-containing ligands (chemsets **9** and **10**). The library representatives under study showed different levels of affinity for 5-HT_{1A} and 5-HT_{2A} receptors (estimated $K_i = 24-4000$ and 1−2130 nM, respectively). Several dual 5-HT_{1A}/5-HT_{2A} ligands were found, of which two (**9**{*3,3*} and **9**{*3,5*}) displayed high 5-HT_{2A} affinity comparable to that of the reference drug ritanserin. A set of individual fragment contributions for the prediction of 5-HT_{1A} and 5-HT_{2A} affinity of all the library members were defined on the basis of the Free−Wilson analysis of 26 compounds. An alkylarylpiperazine fragment had essentially the same impact on the affinity for both receptors, whereas different terminal amide fragments were preferred by 5-HT_{1A} (chemset **17**, R^2 = adamantyl) and 5-HT_{2A} (chemset **9**, R^2 = norborn-2-ylmethyl) binding sites.

Introduction

Introduction of combinatorial approaches and developing techniques which facilitate quick screening of a vast number of synthetic analogues has had a great impact on the identification of lead structure and has contributed to the advancement of medicinal chemistry in the design of focused libraries.

Long-chain arylpiperazines (LCAPs) are an important class of molecules of considerable pharmaceutical interest. They bind to many classes of G-protein-coupled receptors (serotonin, dopamine, adrenergic) and produce a variety of pharmacological responses. In the past decade, our utmost attention was focused on 5-HT_{1A} and 5-HT_{2A} receptors,[1,2] since their role in the pathology of such mental disorders as anxiety or depression had been well-established.[3] The structure−activity relationship (SAR) studies with numerous generations of arylpiperazine derivatives showed that CNS activity and receptor affinity and selectivity depended on the *N*-1-aryl subtituent, the terminal fragment (often amide or imide), and the length of an alkyl spacer. Although many different building blocks have been introduced into the amide pharmacophoric fragment, its role in the stabilization of the ligand−receptor complex process is still unclear − in contrast to the well-known function of other ligand substructures mentioned above.[4]

As part of our ongoing efforts to discover new potent serotonin receptor ligands and in order to carry on SAR studies, we proposed incorporation of the selected amino acid moieties into the amide pharmacophoric group. It was expected that variations in the physicochemical and conformational proprieties of cyclic and side chain amino acids will influence the biological activity of compounds. To get direct and easy access to that class of molecules, we developed and optimized a new solid-supported synthetic pathway using SynPhase lanterns.[5] In the present paper, we describe the construction of a 72-member arylpiperazine library and the preliminary binding profile of 5-HT_{1A} and 5-HT_{2A} receptors.

Results and Discussion

Chemistry. A parallel solid-phase synthesis of arylpiperazine library was carried out on commercially available BAL linker-functionalized polyamide SynPhase Lanterns (Mimotopes, Pty). To quickly manage the library construction, we chose a split-and-pool approach.[6] The lanterns were attached to a transponder bearing a RF (radio frequency) tag. At each step of the synthesis, the lanterns were manually sorted using an antenna and were polled into the separate vials containing respective building blocks using TranSort software. A library of arylpiperazine derivatives was generated according to the reaction sequence presented in Schemes 1 and 2.

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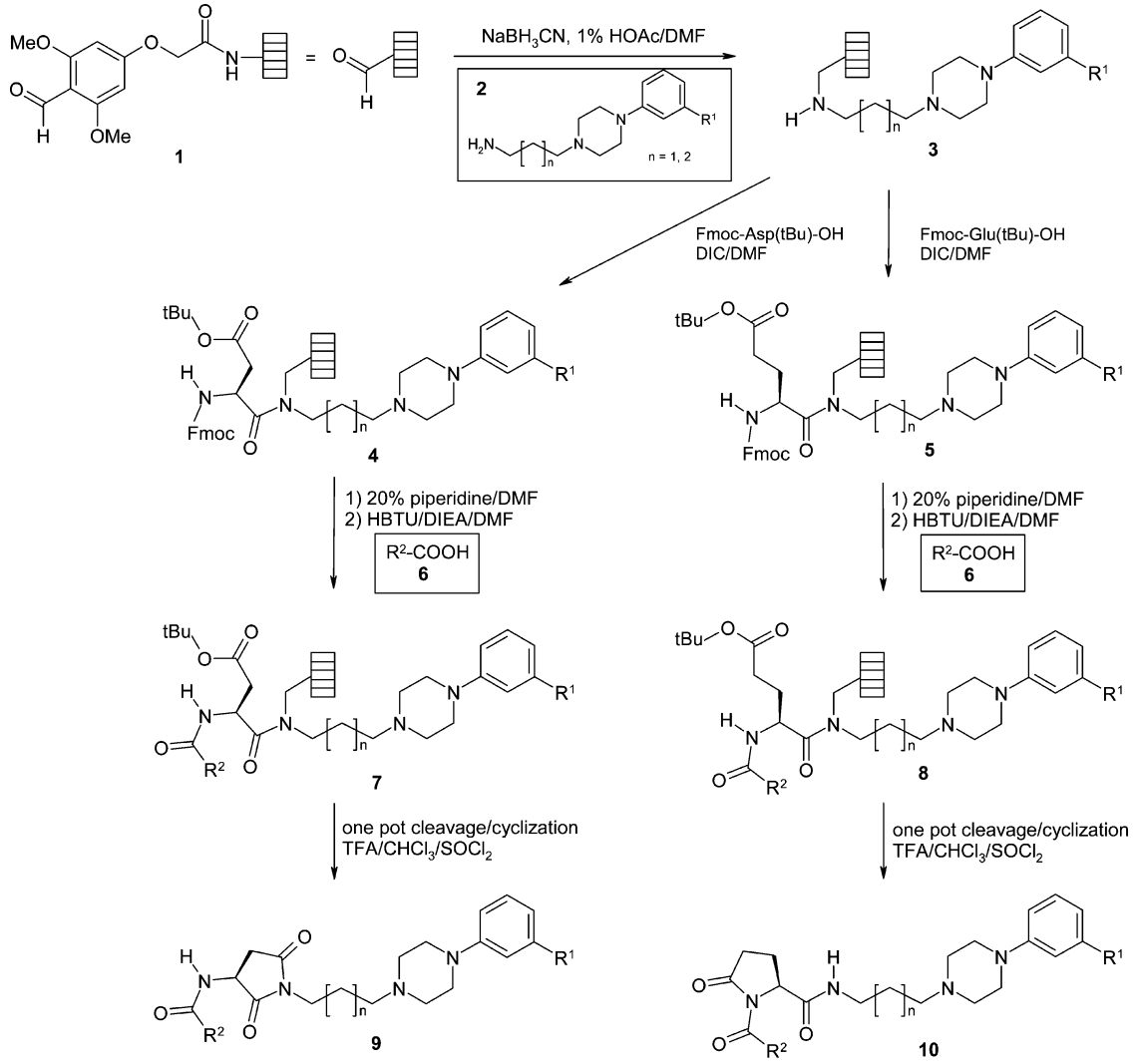
Scheme 1. Solid-Phase Synthesis Route for Chemsets **9** and **10**.



The starting material for the library synthesis was substituted arylpiperazine alkyl-amines (Figure 1), obtained using the method described elsewhere.[7] Reductive amination of the respective amines belonging to diversity reagent **2** yielded support-bound secondary products **3**. Fmoc amino acids were then selectively coupled to a secondary amine by a symmetric anhydride method using diisopropylcarbodiimide (DIC) in dimethylformamide (DMF) to obtain chemsets **4**, **5**, **11**, **12**, and **13** (Schemes 1, 2). The effectiveness of secondary amine acylation was checked by the HPLC analysis and by a colorimetric test performed on a slice of lantern (the Chloranil test).[8] Lanterns of all the chemsets were pooled together and treated with a solution of 20% piperidine in DMF. After washing, the lanterns were sorted again and were treated with six discrete protegenic carboxylic acids (Figure 2) in the presence of HBTU and DIEA in DMF solutions to form the solid-supported chemsets **7**, **8**, **14**, **15**, and **16**. Compounds of chemsets **7** and **8** were converted into chemsets **9** and **10** following a one-pot cleavage/cyclization process, which was accomplished by cleavage cocktail solution treatment. A variety of conditions were studied in order to optimize cyclization. The best yields and purities were recorded using a mixture of TFA/$CHCl_3$/$SOCl_2$ (50/50/1.5, v/v/v) at a temperature of 40 °C for 10 h. Aspartic acid derivatives were cyclized to the succinimide ring and glutamic acid ones to the pyroglutamyl cycle using conditions similar to those described by Obrecht.[9] The final chemsets **17**, **18**, and **19** were obtained by cleavage of the respective chemsets **14**, **15**, and **16** from the solid support using TFA treatment for 60 min.

All the lanterns were cleaved separately in individual glass vials in the cases of chemsets **9** and **10**, and polypropylene for chemsets **17**, **18**, and **19**. After removal of the cleavage cocktail under a nitrogen flow, the samples were solubilized in an acetonitrile/water (50:50, v/v) mixture containing a 0.1% TFA. An aliquot of each library member was submitted to LC/MS analyses, and the remainder was lyophylized.

Chromatographic and mass spectral analyses of the library members showed the success of the major transformations described. Average overall yields of the crude products in all cases were between 18 and 57% and were calculated on the basis of the initial loading of the lanterns. The LC/MS of the identified compounds of chemsets **9**, **10**, **17**, and **19** revealed an average purity exceeding 80%. Compounds bearing a proline residue, chemset **17**, were of the highest purity (>94%), whereas the lowest purity was shown for molecules with asparagine residues (compounds **19**{*1,1*} and ***19***{*1,6*}), whose purity was 51 and 43%, respectively (Table

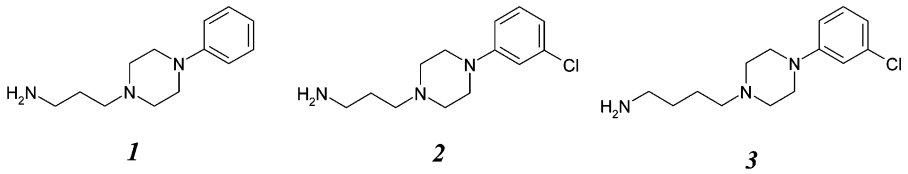


Figure 1. Diverse primary aliphatic amines, **2**{*1*−*3*}.

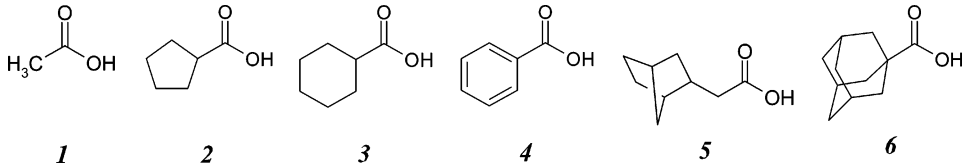
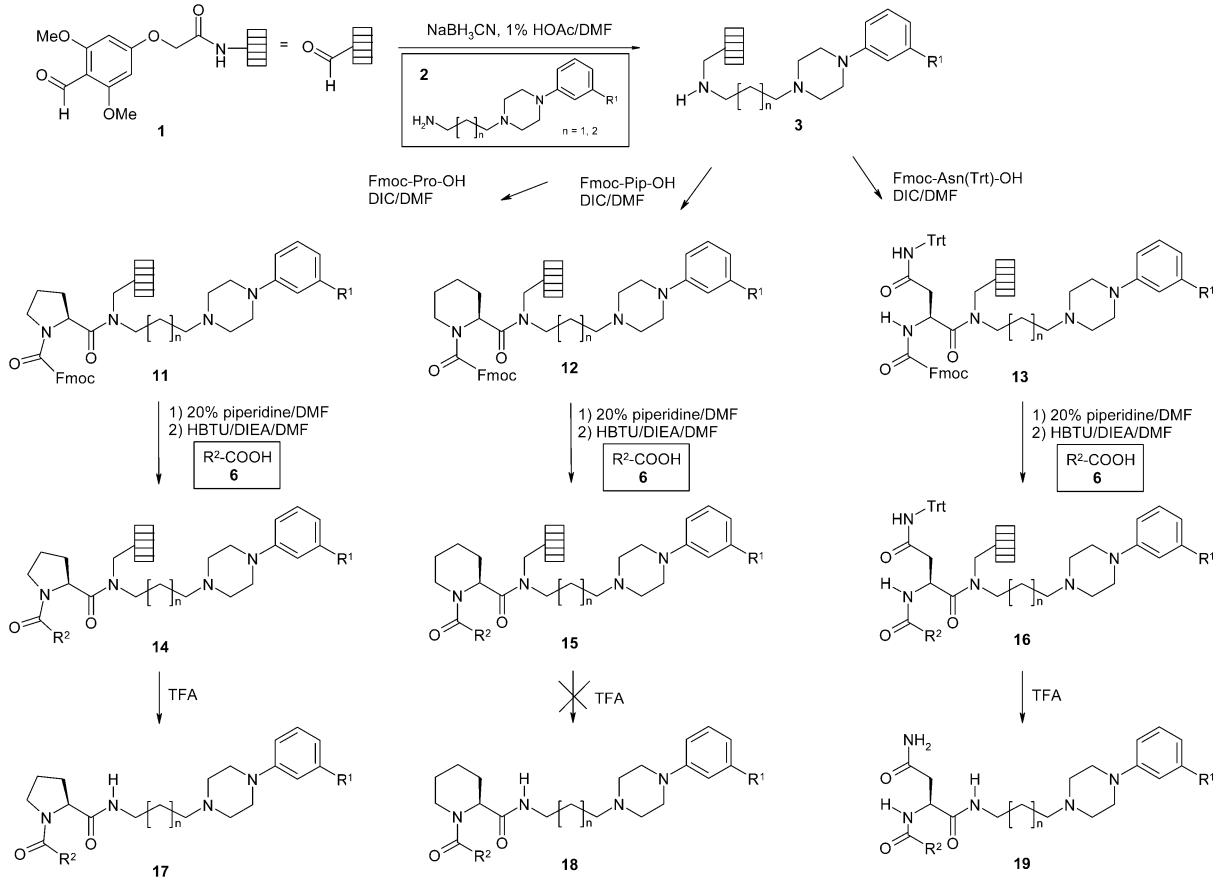


Figure 2. Diverse carboxylic acids, **6**{*1*−*6*}.

Scheme 2. Solid-Phase Synthesis Route for Chemsets **17**, **18**, **19**.



1). The LC/MS analysis of a representative library member, the cyclized succinimide derivative, ***9***{*3*,*5*}, is shown in Figure 3.

Surprisingly, the cleavage of the lantern-bound pipecolic acid derivatives, chemset **15**, did not yield the desired products of chemset **18**. Only in the case of compounds with a methyl group as a R^2 substituent, were slight traces of the final products detected by LC/MS analyses. In all the other cases, a classic cleavage procedure (TFA treatment) yielded two unexpected products corresponding to the selective hydrolysis of linker-bound amide bond, the starting arylpiperazinealkylamines, and the respective acylated pipecolic acids.

Library members **10**{*x*,*6*} containing the same adamantyl group were of low purity (≤10%). The LC/MS analysis showed that the main cleaved product was in each case an N-unacylated pyroglutamyl derivative.

Biological Evaluation. The serotonin activity of the compound library was measured using a modified prescreening protocol. A set of 26 compounds were selected for in vitro radioligand binding tests for serotonin 5-HT_{1A} and 5-HT_{2A} receptors on the basis of the purity data and in order to cover a wide range of structural modifications applied. Typically, one drug concentration is used for preliminary binding methods, and the percentage of inhibition is determined as a measure of the compound's activity. However, in an SAR analysis, this parameter is not so useful as the binding constant (K_i) obtained by a regular competition procedure which, among others, can be directly compared with the published data.

Table 1. Analitical Data for Chemsets **9**, **10** and **17**, **18**

compd	purity %[a]	MW calcd	$[M + H]^+$ found	compd	purity %[a]	MW calcd	$[M + H]^+$ found	compd	purity %[a]	MW calcd	$[M + H]^+$ found
9{*1,1*}	73	358.20	359.20	**10**{*2,1*}	79	406.70	407.12	**17**{*3,1*}	96	406.70	407.12
9{*1,2*}	85	412.20	413.19	**10**{*2,2*}	71	460.70	461.20	**17**{*3,2*}	97	460.80	461.21
9{*1,3*}	80	426.20	427.21	**10**{*2,3*}	68	474.70	475.23	**17**{*3,3*}	97	474.80	475.25
9{*1,4*}	97	420.20	421.12	**10**{*2,4*}	76	468.70	468.16	**17**{*3,4*}	98	468.70	469.18
9{*1,5*}	88	452.30	453.27	**10**{*2,5*}	77	500.80	501.25	**17**{*3,5*}	95	500.80	501.25
9{*1,6*}	83	478.30	479.35	**10**{*2,6*}	9	526.80	527.24	**17**{*3,6*}	97	526.80	527.30
9{*2,1*}	84	392.70	393.10	**10**{*3,1*}	75	420.70	421.11	**19**{*1,1*}	51	375.20	376.24
9{*2,2*}	83	446.70	447.17	**10**{*3,2*}	75	474.70	475.24	**19**{*1,2*}	69	429.30	430.25
9{*2,3*}	84	460.70	461.19	**10**{*3,3*}	74	488.80	489.25	**19**{*1,3*}	61	443.30	444.25
9{*2,4*}	97	454.70	455.13	**10**{*3,4*}	72	482.70	483.18	**19**{*1,4*}	61	437.20	438.26
9{*2,5*}	85	486.70	487.23	**10**{*3,5*}	78	514.80	515.27	**19**{*1,5*}	71	469.30	470.36
9{*2,6*}	89	512.80	513.22	**10**{*3,6*}	11	540.80	541.27	**19**{*1,6*}	43	495.30	496.40
9{*3,1*}	75	406.70	407.10	**17**{*1,1*}	93	358.20	359.19	**19**{*2,1*}	98	409.70	410.12
9{*3,2*}	80	460.70	461.19	**17**{*1,2*}	94	412.30	413.23	**19**{*2,2*}	86	463.70	464.21
9{*3,3*}	91	474.70	475.21	**17**{*1,3*}	84	426.30	427.26	**19**{*2,3*}	86	477.80	478.30
9{*3,4*}	83	468.70	469.19	**17**{*1,4*}	97	420.20	421.21	**19**{*2,4*}	80	471.70	472.17
9{*3,5*}	75	500.80	501.24	**17**{*1,5*}	95	452.30	453.29	**19**{*2,5*}	81	503.80	504.24
9{*3,6*}	77	526.80	527.26	**17**{*1,6*}	88	478.30	479.36	**19**{*2,6*}	78	529.80	530.27
10{*1,1*}	88	372.20	373.17	**17**{*2,1*}	97	392.70	393.07	**19**{*3,1*}	73	423.70	424.14
10{*1,2*}	60	426.20	427.21	**17**{*2,2*}	98	446.80	447.18	**19**{*3,2*}	79	477.80	478.24
10{*1,3*}	62	440.30	441.30	**17**{*2,3*}	97	460.80	461.20	**19**{*3,3*}	85	491.80	492.25
10{*1,4*}	72	434.20	435.23	**17**{*2,4*}	98	454.70	455.13	**19**{*3,4*}	71	485.70	486.21
10{*1,5*}	70	466.30	467.28	**17**{*2,5*}	95	486.80	487.25	**19**{*3,5*}	70	517.80	518.30
10{*1,6*}	9	492.30	493.36	**17**{*2,6*}	94	512.80	513.25	**19**{*3,6*}	87	543.80	544.29

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

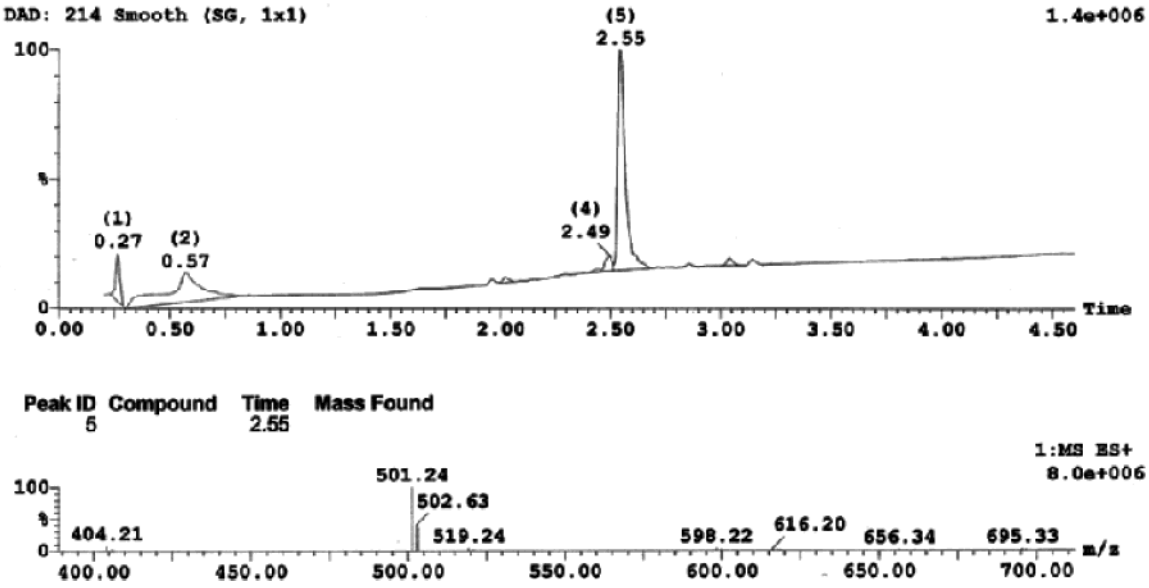


Figure 3. LC/MS analysis of the representative library member **9**{*3,5*}.

A careful analysis of the concentration−response curves generated in our laboratory for over 200 compounds indicated that an average error of the K_i values calculated from concentrations of 0.1 and 1 μM only amounted to 25%. In line with that observation, the binding to 5-HT_{1A} and 5-HT_{2A} receptors was carried out at two compound concentrations (each run in triplicate), and ligand affinity was expressed as estimated K_i values (Table 2).

At the same time, the two well-known reference serotonin drugs buspirone (a partial 5-HT_{1A} receptor agonist) and ritanserin (a 5-HT_{2A} receptor antagonist) were examined. The results obtained for those standards were consistent with our previous data[10,11] as well as with those reported in the literature.[12−16]

The compounds tested showed different levels of affinity for both receptors (estimated K_i = 24−4000 and 1−2130 nM for 5-HT_{1A} and 5-HT_{2A}, respectively); moreover, for many of them, the estimated binding constants were below 100 nM. Several compounds can be classified as dual ligands (**9**{*3,2*}, **9**{*3,3*}, **9**{*3,4*}, **9**{*3,5*}, **10**{*3,5*}, **17**{*3,3*}, **17**{*3,4*}, **19**{*3,3*}, and **19**{*3,5*}), and only one (**17**{*3,6*}) can be regarded as a selective 5-HT_{1A} agent. A qualitative data analysis indicated that affinities in each chemset strongly and systematically depended on the alkylarylpiperazine and R^2 substituents. To quantitatively determine the above relationship, we applied a Free−Wilson analysis, since this simple QSAR technique directly relates structural features to biological activity.[17,18] According to this nonparameter

Table 2. Affinity Data (Estimated K_i) for 5-HT_{1A} and 5-HT_{2A} Receptors for Library Representatives

	K_i [nM]			K_i [nM]	
compd	5-HT_{1A}	5-HT_{2A}	compd	5-HT_{1A}	5-HT_{2A}
9{*1,3*}	1430	495	**17**{*1,6*}	150	1770
9{*1,6*}	414	993	**17**{*2,1*}	580	2130
9{*2,3*}	360	92	**17**{*2,6*}	75	360
9{*2,6*}	536	589	**17**{*3,3*}	48	97
9{*3,1*}	150	434	**17**{*3,4*}	78	128
9{*3,2*}	32	64	**17**{*3,6*}	24	1950
9{*3,3*}	36	2	**19**{*1,3*}	3950	790
9{*3,4*}	41	75	**19**{*2,6*}	234	780
9{*3,5*}	30	1	**19**{*3,1*}	364	874
9{*3,6*}	54	123	**19**{*3,3*}	64	68
10{*2,3*}	1730	800	**19**{*3,5*}	64	42
10{*3,1*}	75	254	**19**{*3,6*}	52	244
10{*3,3*}	111	163	buspirone[a,b]	17	–
10{*3,5*}	100	94	ritanserin[a,b]	–	2

[a] The K_i values obtained in our laboratory were 12.3 (see ref 10) and 1.1 nM (see ref 11) for buspirone and ritanserin, respectively. [b] K_i data reported in the literature: buspirone, 9.3−29.5 nM (see refs 12, 14−16); ritanserin, 0.56 nM (see ref 13). The chemical structure of the reference compounds is presented in Chart 1.

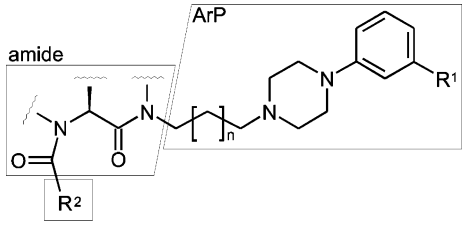
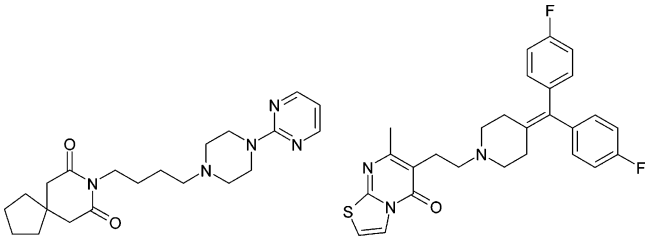


Figure 4. General structure of the investigated compounds with the marked fragments used in the Free−Wilson analysis.

Chart 1



method, compound activity in the logarithmic scale (pK_i) was expressed as a sum of the average overall μ and as

Table 3. Intercept (μ), Individual Fragment Contributions $\alpha_{x,j}$, and Statistical Data Obtained by the Free−Wilson Analysis

		$\alpha_{x,j}$, ± SE (x = amide, ArP, or R^2)	
fragment		5-HT_{1A}	5-HT_{2A}
	μ	6.85 ± 0.06	6.70 ± 0.10
amide	**9**	0.04 ± 0.05	0.42 ± 0.08
	10	−0.20 ± 0.14	−0.48 ± 0.25
	17	0.27 ± 0.11[a]	−0.16 ± 0.19
	19	−0.21 ± 0.11	−0.21 ± 0.19
ArP	**1**	−0.85 ± 0.12	−0.78 ± 0.22
	2	−0.51 ± 0.11[a]	−0.26 ± 0.19
	3	0.40 ± 0.05[a]	0.29 ± 0.09[a]
R^2	**1**	−0.34 ± 0.11	−0.58 ± 0.20
	2	0.20 ± 0.29	−0.22 ± 0.52
	3	−0.12 ± 0.09	0.35 ± 0.16[a]
	4	−0.16 ± 0.21	−0.12 ± 0.37
	5	0.11 ± 0.17	0.90 ± 0.29[a]
	6	0.26 ± 0.09[a]	−0.34 ± 0.17
no. of compds (n)		26	26
corr coeff (r)		0.929	0.877
SE of regression (s)		0.284	0.50
variance in pK_i, explained by the regression		86.2%	76.9%
calcd $F_{0.01}$, ratio		9.40	5.01

[a] $\alpha_{x,j}$ that passed the t-test at a 95% confidence level.

activity fragments contributions (α_{ArP}, α_{R2}, and α_{amide}; Figures 4 and 5, Table 3).

$$(pK_i)_j = \mu + (\alpha_{ArP})_j + (\alpha_{R2})_j + (\alpha_{amide})_j \qquad (1)$$

The statistical parameters of the obtained equations ($r = 0.93$ and 0.88, for 5-HT_{1A} and 5-HT_{2A} receptors, respectively) show that partial fragment contributions are additive, and some are significant at a 95% confidence level according to the t-test.

As can be seen from the comparison of α_{amide} parameters, different amide fragments are preferred by 5-HT_{1A} ($\alpha_{amide}17 = 0.27$) and 5-HT_{2A} ($\alpha_{amide}9 = 0.42$) receptors. Moreover, the latter are more sensitive to structural changes in that part, since the range of α_{amide} is twice as high as in the case of 5-HT_{1A} receptors (0.9 and 0.48, respectively). This observation is further confirmed by even greater differences in the ranges of α_{R2} values (a hydrocarbon substituent can be regarded as a part of a terminal amide

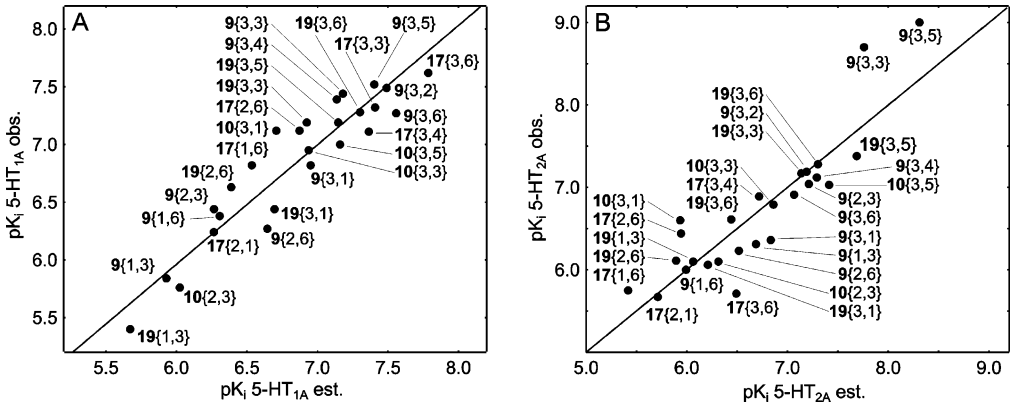


Figure 5. The observed vs calculated affinity of the investigated compounds for (A) 5-HT_{1A} and (B) 5-HT_{2A} receptors on the basis of the eq 1.

fragment). The small methyl group was marked in both cases by the most negative α_{R2} values, whereas the bulky adamantly moiety was the most beneficial for 5-HT_{1A} receptors (α_{R2}-6 = 0.26) but probably too large for 5-HT_{2A} binding sites (α_{R2}-6 = −0.34). Interestingly, a norborn-2-ylmethyl substituent—only slightly smaller, but having more conformational freedom—was found to be extremely favorable for an interaction with 5-HT_{2A} receptors; furthermore, the most active derivative **9**{*3*,*5*} displayed affinity comparable to that of the reference drug ritanserin. The alkylarylpiperazine fragment has a similar impact on the affinity for both receptors and, obviously, a tetramethylene spacer and a *m*-Cl substituent are necessary to obtain highly active ligands. These results are in line with the general view on the serotonin affinity of arylpiperazine derivatives.

Conclusions

Summing up, we developed an efficient approach to the solid-phase synthesis of arylpiperazine derivatives using parallel combinatorial chemistry, and we synthesized a focused compound library targeted on 5-HT_{1A} and 5-HT_{2A} receptors. The desired succinimide and pyroglutamyl derivatives were readily obtained from BAL linker via a one-pot cleavage/cyclization with average yield and good purity. The chemistry applied permitted introduction of diverse building blocks and, thus, extended the rational design and speeded up the search for new CNS agents.

The screening of 26 library representatives with respect to their affinity for 5-HT_{1A} and 5-HT_{2A} receptors led to the identification of several potent dual ligands. Moreover, compound **17**{*3*,*6*} was found to be a selective 5-HT_{1A} receptor agent. The successful application of a classic Free−Wilson model enabled an analysis of individual fragment contribution to the affinity of the ligands. It was found that the 4-(3-chlorophenyl)-1-piperazinylbutyl fragment was necessary for high affinity for both receptor types, whereas modifications in the terminal amide part additionally influenced 5-HT_{1A}/5-HT_{2A} selectivity.

Experimental Section

Materials. All the solvents were obtained from Acros and were used without purification. PA-BAL linker polyamide SynPhase Lanterns with 38-μmol loading, radio frequency tags and TranSort software were provided by Mimotopes, Pty, Clayton, Australia. All 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: $CHCl_3$, chloroform; DCM, dichloromethane; DIEA, diisopropylethylamine; DMF, dimethylformamide; HBTU, *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; $SOCl_2$, thionyl chloride; TFA, trifluoroacetic acid. The other abbreviations used were recommended by the IUPAC−IUB Commission (*Eur. J. Biochem.* **1984**, *138*, 9−37).

LC/MS Analysis. Samples were prepared in acetonitrile/water (50:50 v/v), containing 0.1% TFA. The LC/MS system consisted of a Waters Alliance 2690 HPLC coupled to a Micromass (Manchester, U.K.) Platform II spectrometer (electrospray ionization mode, ESI^+). All the analyses were carried out using a C18 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 to get the final spectrum.

Standard Reductive Amination Protocol. The lanterns were divided into three groups and were placed in glass vials containing a suspension of natrium cyanoborohydride ([$NaHB_3CN$] = 100 mM) and the amine ([diversity reagent **2**] = 250 mM, Figure 1,) in 1% acetic acid in 30 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 10% AcOH in DMF (1 × 5 min) then with the standard washing protocol, and afterward were allowed to dry in the open air.

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.

Secondary Amine Acylation Protocol. Five DMF solutions (20 mL), each containing a Fmoc-protected amino acid and DIC, were freshly prepared in a standard Schott flask before acylation ([Fmoc-AA−OH] = 200 mM, [DIC] = 100 mM), and were left for 10 min to form an active anhydride. Then the lanterns were immersed in a preactivated solution and left overnight at room temperature. The solution was decanted, and the lanterns were washed following the standard washing protocol. The acylation was repeated one time more for 4 h.

Standard Fmoc-Deprotection Protocol. The Fmoc-deprotection step was carried out by immersing the 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.

Standard Coupling Protocol. Six DMF solutions (25 mL), each containing carboxylic acid (Figure 2, diversity reagent **6**), HBTU, and DIEA, were freshly prepared in a standard Schott flask before coupling ([R^2−COOH] = 120 mM; [HBTU] = 120 mM; [DIEA] = 240 mM). The lanterns were immersed for 2 h in the coupling solution at room temperature. The solution was decanted, and the lanterns were washed following the standard washing procedure. The procedure described above was repeated.

Cleavage/Cyclization Protocol. Chemsets **7** and **8** were placed in glass vials containing a 1-mL mixture of TFA/$CHCl_3$/$SOCl_2$ (50/50/1.5, v/v/v). The reaction was allowed to stand for 10 h at 40 °C. Afterward, the reaction solution was removed using a Jouan RC1010 vacuum centrifuge. A 100-μL portion of acetonitrile/water (50:50, v/v) containing 0.1% TFA was poured into each tube to dissolve the samples. The samples were then frozen at −80 °C and lyophilized.

That procedure was repeated twice to completely remove the remaining volatile residues.

Cleavage Protocol. A 500-μL portion of the TFA was dispensed into 36 individual polypropylene tubes of a 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 0.1% TFA was poured into each tube to dissolve the sample. Then the samples were frozen at −80 °C and lyophilized. That procedure was repeated twice to completely remove the remaining volatile residues.

Radioligand Binding Studies. The selected compounds were tested without further purification. The in vitro affinity for native serotonin 5-HT_{1A} and 5-HT_{2A} receptors was determined by inhibiting [^{3}H]-8−OH−DPAT (170 Ci/mmol; NEN Chemicals) and [^{3}H]-ketanserin (88 Ci/mmol; NEN Chemicals) binding to rat hippocampal and cortical membranes, respectively. Membrane preparation and a general assay procedure were carried out according to the previously published protocols.[19] Two compound concentrations were tested: 0.1 and 1 μM, each run in triplicate. The K_i values, estimated on the basis of three independent binding experiments, were reproducible in ± 20%.

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References and Notes

(1) Byrtus, H.; Pawłowski, M.; Charakchieva-Minol, S.; Duszyńska, B.; Mokrosz, M. J.; Mokrosz, J. L.; Zejc, A. *Arch. Pharm. (Weinheim)* **1996**, *6*, 283−290.
(2) Pawłowski, M.; Katlabi, J.; Drabczyńska, A.; Duszyńska, B.; Charakchieva-Minol, S.; Dereń-Wesołek, A.; Tatarczyńska, E.; Chojnacka-Wójcik, E.; Mokrosz, M. J.; Bojarski, A. J. *Eur. J. Med. Chem.* **1999**, *34*, 167−175.
(3) Jones, B. J.; Blackburn, T. P. *Pharmacol. Biochem. Behav.* **2002**, *71*, 555−568.
(4) Lopez-Rodriguez, M. L.; Ayala, D.; Benhamu, B.; Morcillo, M. J.; Viso, A. *Curr. Med. Chem.* **2002**, *9*, 443−469.
(5) Bray, A. M.; Chiefari, D. S.; Valerio, R. M.; Maeji, N. J. *Tetrahedron Lett.* **1999**, *1*, 368−370.
(6) Golebiowski, A.; Klopfenstein, S. R.; Portlock, D. E. *Curr. Opin. Chem. Biol.* **2001**, *5*, 273−284.
(7) Glennon, R. A.; Naiman, N. A.; Lyon, R. A.; Titeler, M. *J. Med. Chem.* **1988**, *31*, 1968−1971.
(8) Vojkovsky, T. *Pept. Res.* **1995**, *8*, 236−237.
(9) Obrecht, D.; Bohdal, U.; Daly, J.; Lehmann, C.; Schoenholzer, P.; Mueller, K. *Tetrahedron* **1995**, *51*, 10883−10900.
(10) Mokrosz, J. L.; Dereń-Wesołek, A.; Tatarczyńska, E.; Duszyńska, B.; Bojarski, A. J.; Mokrosz, M. J.; Chojnacka-Wójcik, E. *J. Med. Chem.* **1996**, *39*, 1125−1129.
(11) Mokrosz, J. L.; Duszyńska, B.; Paluchowska, M. H.; Charakchieva-Minol, S.; Mokrosz, M. J. *Arch. Pharm. (Weinheim)* **1995**, *328*, 623−625.
(12) Piercey, M. F.; Smith, M. W.; Lum-Ragan, J. T. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 1297−1303.
(13) Hoyer, D. *Trends Pharmacol. Sci.* **1988**, *9*, 89−94.
(14) Zifa, E.; Fillion, G. *Pharmacol. Rev.* **1992**, *44*, 401−458.
(15) McCall, R. B.; Clement, M. E. *Pharmacol. Rev.* **1994**, *46*, 231−243.
(16) van Wijngaarden, I.; Tulp, M. T.; Soudijn, W. *Eur. J. Pharmacol.* **1990**, *188*, 301−312.
(17) Free, S. M.; Wilson, J. W. *J. Med. Chem.* **1964**, *53*, 395−399.
(18) Kubinyi, H. *Quant. Struct.−Act. Relat.* **1988**, *7*, 121−133.
(19) Bojarski, A. J.; Cegła, M. T.; Charakchieva-Minol, S.; Mokrosz, M. J.; Maćkowiak, M.; Mokrosz, J. L. *Pharmazie* **1993**, *48*, 289−294.

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