Available online at www.sciencedirect.com

ScienceDirect

European Journal of Medicinal Chemistry 44 (2009) 800–808

EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY

http://www.elsevier.com/locate/ejmech

Original article

The influence of an ethylene spacer on the 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor affinity of arylpiperazine derivatives of amides with *N*-acylated amino acids and 3-differently substituted pyrrolidine-2,5-diones

Paweł Zajdel [a,*], Gilles Subra [b], Pascal Verdie [b], Andrzej J. Bojarski [c], Beata Duszyńska [c], Katarzyna Basista [a], Jolanta Obniska [a], Jean Martinez [b], Maciej Pawłowski [a]

[a] *Department of Medicinal Chemistry, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland*
[b] *Équipe Aminoacides Peptides et Protéines, Institut des Biomolécules Max Mousseron IBMM, UMR CNRS 5247, Faculté de Pharmacie, Université Montpellier I et II, 15 Avenue Charles Flahault, 34060 Montpellier, France*
[c] *Department of Medicinal Chemistry, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland*

Received 11 March 2008; received in revised form 21 May 2008; accepted 22 May 2008
Available online 7 July 2008

Abstract

A library of ethylene analogs of the previously described arylpiperazines with *N*-acylated amino acids was synthesized on SynPhase™ Lanterns and the library representatives were evaluated for their 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor affinities. The obtained results were compared with those reported for compounds containing propylene and a butylene spacer and they revealed that 5-HT$_{1A}$ receptor affinity decreased proportionally with the length of the alkyl chain. Furthermore, the synthesized 3-cycloalkyl derivatives containing two methylene group spacers (**20**, **21**) showed that the 3-position of pyrrolidine-2,5-dione preferred substituents of hydrophobic character.

Keywords: Long-chain arylpiperazines; Succinimides; Spirosuccinimides; Pyrrolidine-2,5-dione; 2-Azaspiro[4.5]decane-1,3-dione; Pyrrolidine-5-one-carboxamide; Prolinamides; Solid-phase synthesis; 5-HT$_{1A}$/5-HT$_{2A}$ receptor ligands

1. Introduction

Combinatorial chemistry has become an integral part of drug discovery projects in many pharmaceutical companies in the mid-1990s. Its introduction was a consequence of a growing number of new pharmacological targets, and at the same time a response to the rising demand for novel hit identification. Combinatorial chemistry combined with solid-phase methodologies allowed quick generation of a vast number of closely related compounds. A high complexity of first-generation combinatorial libraries, followed by tedious deconvolution and re-synthesis of individual library members resulted in the identification of the so-called "false positives" and contributed to the high attrition rate of several research projects. Since then, a general premise of combinatorial chemistry has evolved by changing into the preparation of biological target-oriented and rationally designed compound libraries [1]. Nowadays there is a demand for a discrete compound library of high diversity. Moreover, to provide reproducibility of biological data, library members are often purified using automated or semi-automated chromatography systems. Recent bibliography surveys of combinatorial library synthesis reveal researchers' constant interest in this technique which may ease the efforts of medicinal chemists to evaluate structure–activity relationships [2].

Apart from the increasing interest in discovering ligands acting through high-end serotonin receptor subtypes (5-HT$_6$, 5-HT$_7$), compounds modulating 5-HT$_{1A}$ and/or 5-HT$_{2A}$ receptors' activity still remain an important group with a high potential for the treatment of CNS disorders, including anxiety [3], depression, and schizophrenia [4] and they provide a prospective field for drug development [5–7].

---

* Corresponding author. Tel.: +48 12 657 05 60; fax: +48 12 657 02 62.
E-mail address: mfzajdel@cyf-kr.edu.pl (P. Zajdel).

0223-5234/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.05.021

We previously reported on the successful application of combinatorial chemistry techniques for generating the rationally designed libraries of 5-HT_{1A} and 5-HT_{2A} receptor ligands, namely arylpiperazine derivatives containing *N*-acylated amino acid fragments [8,9]. Structural modification comprised variations in the type of amide/imide moiety (3-aminopyrrolidine-2,5-dione, pyrrolidine-5-one-2-carboxamide, and asparaginyl, pipecolyl, prolyl amides), the nature of the *N*-acyl fragment (alkyl, cycloalkyl, aryl), the length of an alkyl spacer (propylene, butylene) and finally the kind of substituents at the 4-phenylpiperazine fragment (*o*-OCH_3, *m*-Cl) (Fig. 1).

The library representatives tested showed diversified affinity for 5-HT_{1A} receptors ranging from 4 nM to 4000 nM, and displayed high-to-low affinity for 5-HT_{2A} receptors (1–2130 nM) (Table 1). Structure–activity relationship studies showed that the length of a tetramethylene spacer between the amide/imide core and arylpiperazine fragment was optimal to obtain potent 5-HT_{1A} and 5-HT_{2A} receptor ligands. It was finally found that the optimized lead structure (**I–9**{*5,3*}) – pyrrolidine-2,5-dion-3-yl-cyclohexanecarboxamide, a pre- and postsynaptic 5-HT_{1A} agonists – demonstrated distinct activity in some models of anxiety and depression (Fig. 2) [10].

In the course of developing 5-HT_{1A}/5-HT_{2A} receptor ligands our research group also described some derivatives of pyrrolidine-2,5-dione with spirocycloalkyl moiety at the 3-position – 2-azaspiro[4.4]nonane- and [4.5]decane-1,3-diones [11,12]. Linkage of these imide cores using a methylene, ethylene and propylene spacer with 4-arylpiperazine moieties yielded compounds which displayed moderate-to-high affinity for 5-HT_{1A} receptors ($K_i = 3.1–370$ nM), and low-to-high affinity for 5-HT_{2A} ($K_i = 32–1370$ nM). Interestingly, the receptor affinity depended highly upon the length of an alkyl spacer, the highest affinity for both receptor subtypes being observed for ethylene analogs.

Based on the above findings, in the present research we conducted a solid-phase synthesis and preliminary biological investigation of a series of the previously described analogs of 3-amino-pyrrolidine-2,5-dione, pyrrolidine-2-oxo-carboxamide, prolinamide derivatives which contained two methylene group alkyl spacers between the imide/amide part and the basic nitrogen atom of the arylpiperazine fragment. In the second part, we discussed the influence of the kind of substituent at the 3-position of pyrrolidine-2,5-dione moiety on serotonin receptor affinity.

2. Chemistry

2.1. Library synthesis

The library synthesis was carried out on solid support – SynPhase™ Lanterns (Mimotopes, Pty). This modular type of support consists of a plastic core grafted with polyamide surface functionalized with a BAL linker [13]. To manually manage the library construction, a split-and-pool approach was chosen. The Lanterns were equipped with colored cogs and spindles (corresponding to building blocks) producing a visual tagging system. The library was constructed as outlined in Schemes 1 and 2.

The structure of the final products was encoded according to the nomenclature defining the assignment of the residues of the library members based on the American Chemical Society guidelines, where **x**{*y*,*z*} represents a compound having the general structure **x** (presented in scheme), where *y* and *z* indicate the particular residues from the separately defined list of diversity reagents **a**, and diversity reagents **b**, respectively.

The workflow began upon the preparation of substituted 4-(2-methoxyphenyl)-1-piperazinyl-ethylamine and 4-(3-chlorophenyl)-1-piperazinyl-ethylamine (diversity reagent **2**, Fig. 3), according to the procedure described elsewhere [14]. The substituted ethylamines were attached to a solid support by reductive amination to give the support-bound secondary product **3**. Lanterns were pooled together and washed with a 10% AcOH and subsequently according to the classic washing protocol. Fmoc amino acids were then selectively coupled

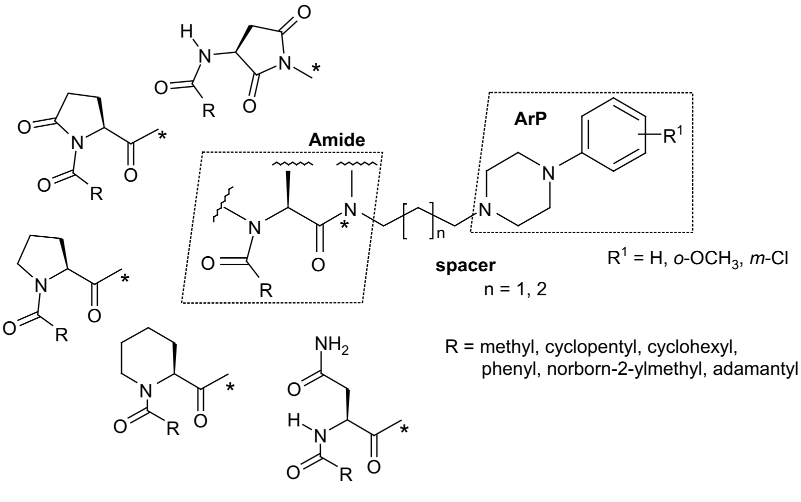


Fig. 1. General structure and chemical modifications of the investigated compounds.

Table 1
Affinity data for 5-HT_{1A} and 5-HT_{2A} receptors of the library members tested and their respective propyl and butyl analogs reported elsewhere

Compound	K_i (nM)		Compound	K_i (nM)		Compound	K_i (nM)[a]	
	5-HT_{1A}	5-HT_{2A}		5-HT_{1A}	5-HT_{2A}		5-HT_{1A}	5-HT_{2A}
9{*6,3*}	3775	1411	**9**{*2,3*}[a]	360	92	**9**{*3,3*}[c]	26 ± 4	6.51 ± 1
9{*6,4*}	10 020	1742	–	–	–	**9**{*3,4*}[a]	41	75
9{*7,1*}	7586	5678	**9**{*4,1*}[b]	480	–	**9**{*5,1*}[b]	69	–
9{*7,3*}	7440	7343	**9**{*4,3*}[b]	230	–	**9**{*5,3*}[c]	19 ± 3	183 ± 12
9{*7,5*}	497	12 417	**9**{*4,5*}[b]	40	–	**9**{*5,5*}[c]	9 ± 2	47 ± 6
9{*7,6*}	579	4799	**9**{*4,6*}[b]	63	–	**9**{*5,6*}[c]	4 ± 2	173 ± 17
10{*6,3*}	8518	3258	**10**{*2,3*}[a]	1730	800	**10**{*3,3*}[a]	111	163
10{*7,3*}	1001	14 936	–	–	–	**10**{*5,3*}[b]	17	–
10{*7,4*}	1507	8706	**10**{*4,4*}[d]	390	–	–	–	–
17{*6,4*}	1950	1302	–	–	–	**17**{*3,4*}[a]	78	128
17{*7,1*}	6600	8600	**17**{*4,1*}[d]	580	3140	–	–	–
17{*7,3*}	3553	2326	–	–	–	**17**{*5,3*}[c]	19 ± 3	990 ± 17
17{*7,6*}	350	1917	–	–	–	**17**{*5,6*}[c]	3 ± 0.2	503 ± 8
17{*6,2*}	2013	357	Clozapine[e]	–	6.05	Buspirone[e]	36	–

[a] The estimated K_i (see Ref. [8]).

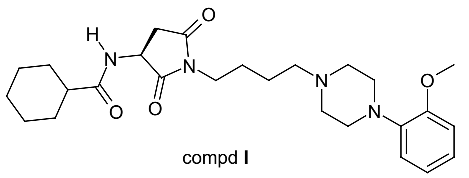
[b] The estimated K_i (see Ref. [9]).

[c] K_i from Ref. [10].

[d] Personal communication.

[e] K_i values reported in the literature: buspirone (rat hippocampus, [^{3}H]-8-OH-DPAT) 3.8–56 nM; clozapine (rat cortex, [^{3}H]-ketanserin) 2.6–12 nM (see Ref. [18]).

to a secondary amine by a symmetric anhydride method using diisopropylcarbodiimide (DIC) in dimethylformamide (DMF) to obtain chemsets **4**, **5**, and **11** (Schemes 1 and 2). The effectiveness of secondary amine acylation was checked by the HPLC analysis, and by a colorimetric test performed on a slice of the Lantern (the chloranil test). Then, 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 reacted with six discrete proteogenic carboxylic acids (Fig. 4) in the presence of HBTU and DIEA in DMF solutions, to form the solid supported chemsets **7**, **8**, and **14**. The conversion of the representatives of chemsets **7** and **8** into pyrrolidine-2,5-diones (**9**) and pyrrolidine-5-one-2-carboxamides (**10**) was achieved by the treatment with a mixture of TFA/$CHCl_3$/$SOCl_2$ (50/50/1.5, v/v/v) at a temperature of 40 °C for 10 h. The reaction was carried out in small glass vials. Finally, cleavage of the Lanterns of chemset **14** with a mixture of TFA/DCM at room temperature for 60 min afforded the desired proline amides of chemset **17**. Prolyl amides were cleaved in individual polypropylene vials. 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 an LC/MS analysis and the remainder was lyophilized.



compd **I**
K_i 5-HT_{1A} = 19 nM
K_i 5-HT_{2A} = 183 nM

Fig. 2. Chemical structure of lead structure I and its affinity for 5-HT_{1A} and 5-HT_{2A} receptors.

Library members **10**{*6,6*} and **10**{*7,6*} containing the same adamantyl group were obtained with low yields (≤5%); the main products cleaved from the support were *N*-unacylated pyroglutamyl derivatives.

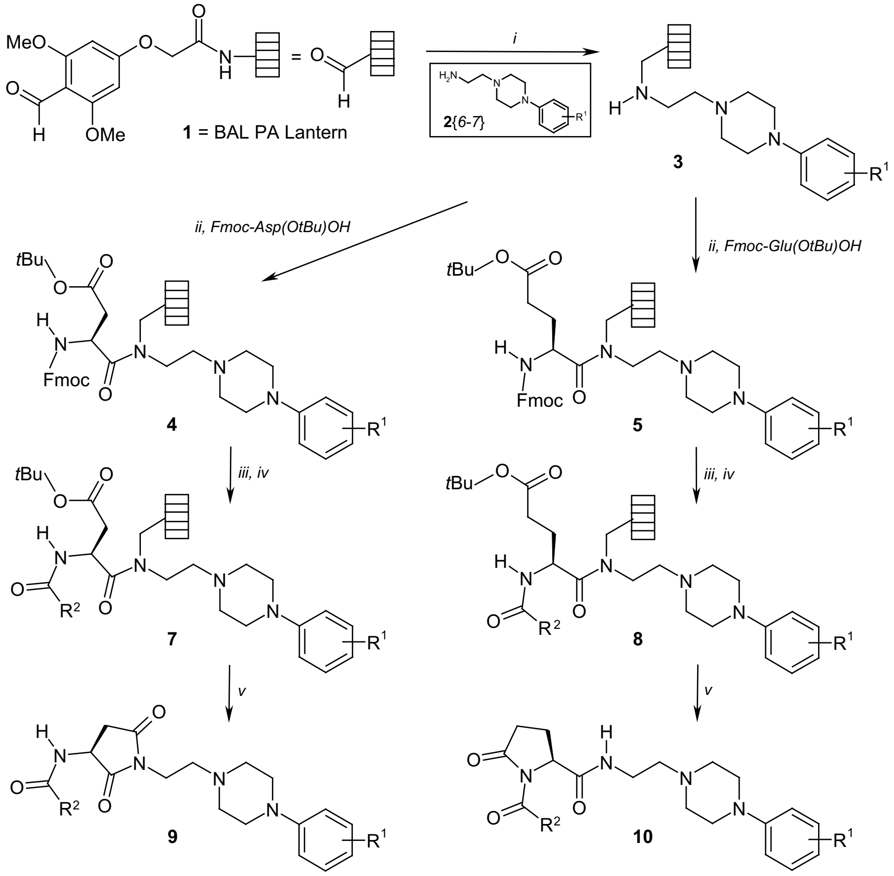
All library members were purified by using a reverse-phase preparative LC/MS ESI automated system (Waters Micromass). Then, the fractions containing the expected ions were pooled together, freeze-dried, and submitted again to LC/MS analysis. The purity percentage was based on the relative peak absorbance at 214 nm on the UV spectra (Table 2). The overall yields of the final products, calculated on the basis of the initial loading of the Lanterns, were between 26% and 49%, with purities ranging from 78% to 100%. Finally, 14 purified compounds (HPLC purity over 81%) were submitted to biological assays.

2.2. Synthesis of arylpiperazine derivatives of pyrrolidine-2,5-diones

Compounds **18–21** were synthesized according to Scheme 3. The starting 2-cyclohexane succinic acid was prepared as described elsewhere [15]. A one-pot cyclization of the succinic derivatives with the appropriate arylpiperazine–ethylamines by heating them at ca. 190–200 °C for 1.5–2 h yielded final imides.

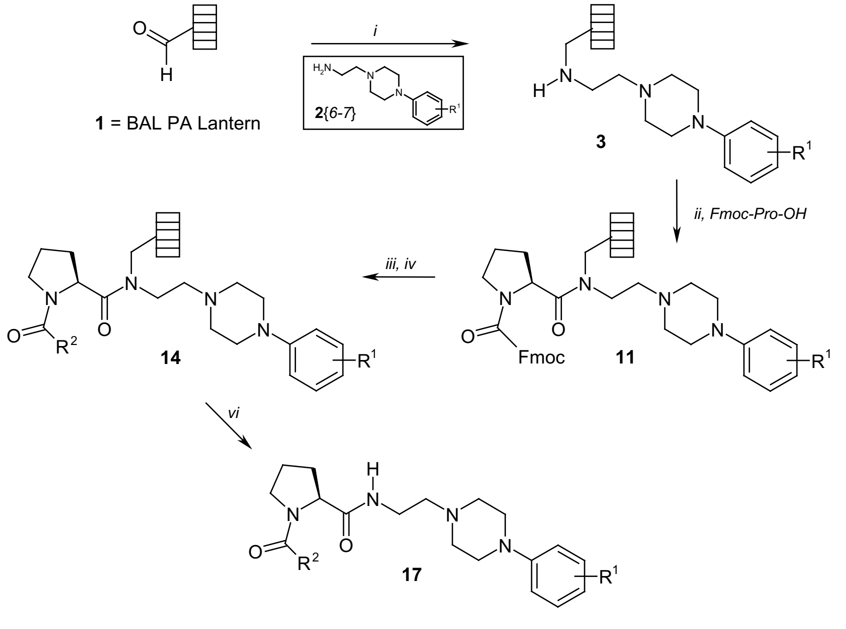
3. Biological evaluation

Biological characteristics, i.e. affinity for serotonin 5-HT_{1A} and 5-HT_{2A} receptors of the 14 selected library representatives, were measured in vitro on the basis of the screening



Scheme 1. Solid-phase synthesis routes for chemsets **9** and **10**: (*i*) diversity reagent **2**{*6–7*}, $NaBH_3CN$, 1% AcOH/DMF, 60 °C, 12 h; (*ii*) DIC, DMF, RT, 12 h; (*iii*) 20% piperidine/DMF; (*iv*) diversity reagent **6**{*1–6*}, HBTU, DIEA, DMF, RT, 2 h; (*v*) $TFA/CHCl_3/SOCl_2$, 40 °C, 10 h.

protocol described previously [8]. At the same time, the two well-known reference serotonin drugs buspirone and clozapine were examined, the obtained results being consistent with our previous data as well as with those reported in the literature (Table 1). For the four new pyrrolidine-2,5-dione derivatives, standard full binding experiments [16] were conducted and the K_i constants were calculated from IC_{50} values according to the Cheng–Prusoff equation [17] (Table 3).



Scheme 2. Solid-phase synthesis routes for chemset **17**: (*i*) Diversity reagent **2**{*6–7*}, $NaBH_3CN$, 1% AcOH/DMF, 60 °C, 12 h; (*ii*) DIC, DMF, RT, 12 h; (*iii*) 20% piperidine/DMF; (*iv*) diversity reagent **6**{*1–6*}, HBTU, DIEA, DMF, RT, 2 h; (*vi*) TFA/DCM.

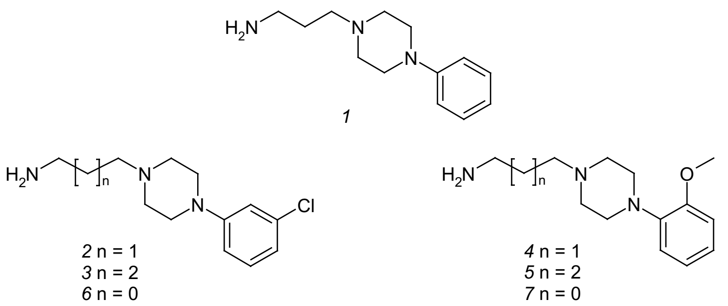


Fig. 3. Diverse primary aliphatic amines, **2**{*1–7*}.

4. Results and discussion

A 36-member library of *o*-methoxy- and *m*-chlorophenylpiperazines, connected by an ethylene spacer to *N*-acylated amino acid-derived amide fragments, was synthesized on a solid support and the 14 selected library representatives were evaluated for their 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor affinities (Table 1). The compounds were chosen for direct comparisons with the previously reported ones.

The compounds synthesized within the present project included structural analogs of the previously presented modifications in the amide fragment. The developed chemistry was amenable to the diversification of amino acid-derived small cyclic fragments: 3-acylaminopyrrolidine-2,5-dione and *N*-acyl-pyrrolidine-2-oxo-carboxamide, which resulted from the cyclization of aspartic and glutamic acid, respectively. Furthermore, prolinamides were also introduced as deoxo-analogs of pyroglutamyl derivatives.

In comparison with the previously reported libraries [8,9], the pronounced decrease in the 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor affinity of the tested compounds resulted from the shortening of the length of an alkyl spacer (Table 1). It has to be stressed that, despite their lower activity, some general and well-known trends were observed for arylpiperazine ligands. Differences in the receptor binding profile resulted mainly from the substitution pattern in the phenylpiperazine fragment (*o*-methoxy derivatives bind preferentially to 5-HT$_{1A}$ receptors) and the molecular volume of the cycloalkyl moiety. In agreement with our previous study, the substituents with a high molecular bulk were always the most potent in the series; adamantanecarboxamides had up to 10–12-fold higher affinity for 5-HT$_{1A}$ sites than cyclohexanecarboxamides.

The study showed that the length of an alkyl chain played an important role in the affinity for 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors (Table 1). However, these findings disagree with the results obtained previously with a group of azaspiranes [11]. Among others, compounds containing an ethylene spacer were always more potent ligands than their propylene analogs. Thus in the following phase of experimentation, we focused our attention on evaluating the role of the kind of substituent in the 3-position of pyrrolidine-2,5-diones on 5-HT$_{1A}$ receptor affinity. To this end, two unsubstituted (**18**, **19**) and 3-cyclohexylpyrrolidine-2,5-diones (**20**, **21**), connected by two methylene alkyl spacers with *o*-methoxy and *m*-chlorophenylpiperazines, were synthesized. The affinity of **18** and **19** for 5-HT$_{1A}$ receptors was 2087 nM and 3675 nM for *o*-OCH$_3$ and *m*-Cl derivatives, respectively (Table 3). Introduction of a cycloalkyl fragment directly connected to imide yielded compounds **20** and **21** with significantly higher 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor affinity (Table 3). However, these receptor affinity values were lower than the values reported for the respective spiro analogs – compounds **22** and **23**. The difference in 5-HT$_{1A}$ receptor affinity for the arylpiperazinyl-ethyl derivatives of 3-acylamino-, 3-cyclohexyl- and unsubstituted pyrrolidine-2,5-diones, and azaspiro[4.5]decane-1,3-dione derivatives suggests that the additional amide group in chemset **9** is unfavorable for the interaction with both types of the serotonin receptors tested. It visibly stems from the shortening of the length of a linker and concurrent restriction of conformational freedom. As a consequence, chemset **9** derivatives containing a short spacer cannot occupy a suitable space in the receptor pocket to support both the H-bond interaction originating with the amide bond and the hydrophobic interactions resulting from the presence of the cycloalkyl group (R^2). It is noteworthy that the two kinds of interactions mentioned above were advantageous to the binding of compounds containing a propylene spacer or, preferably, a butylene one. As has been mentioned in Section 1, the latter displayed a significantly higher 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor affinity (Table 1).Therefore it seems that in the case of the ethylene spacer containing pyrrolidine-2,5-diones, substituents in 3-position that induce hydrophobic interactions are the only preferable ones for 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor binding. Taking account of the data presented above, the introduction of a spirocycloalkyl fragment inducing

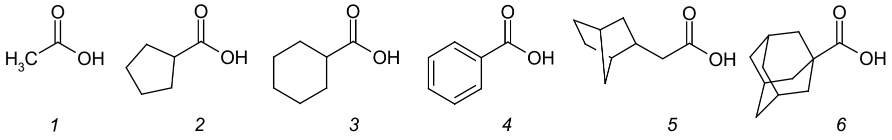


Fig. 4. Diverse carboxylic acids, **6**{*1–6*}.

Table 2
Analytical data for the library members

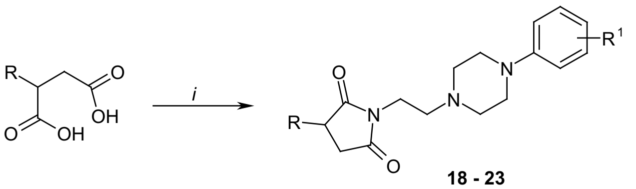
Compound	Purity %[a]	MW calc.	$[M+H]^+$ found	Compound	Purity %[a]	MW calc.	$[M+H]^+$ found
9{*6,1*}	94	378.60	379.10	**10**{*7,1*}	90	388.16	389.07
9{*6,2*}	98	432.64	433.08	**10**{*7,2*}	88	442.20	443.20
9{*6,3*}	97	446.65	447.11	**10**{*7,3*}	93	456.21	457.15
9{*6,4*}	80	440.65	441.10	**10**{*7,4*}	79	450.21	451.11
9{*6,5*}	85	472.67	473.15	**10**{*7,5*}	83	482.23	483.19
9{*6,6*}	100	498.69	499.10	**10**{*7,6*}	–	508.25	–
9{*7,1*}	86	374.15	375.10	**17**{*6,1*}	84	378.62	379.23
9{*7,2*}	92	428.19	429.00	**17**{*6,2*}	94	432.66	433.20
9{*7,3*}	96	442.20	443.30	**17**{*6,3*}	98	446.67	447.17
9{*7,4*}	95	436.20	437.14	**17**{*6,4*}	100	440.67	441.12
9{*7,5*}	88	468.22	469.15	**17**{*6,5*}	95	472.69	473.18
9{*7,6*}	100	494.24	495.11	**17**{*6,6*}	81	498.71	499.20
10{*6,1*}	100	392.61	393.00	**17**{*7,1*}	86	374.17	375.20
10{*6,2*}	82	446.65	447.11	**17**{*7,2*}	85	428.62	429.22
10{*6,3*}	78	460.66	461.15	**17**{*7,3*}	100	442.22	443.20
10{*6,4*}	93	454.66	455.05	**17**{*7,4*}	99	436.22	437.16
10{*6,5*}	79	486.68	487.13	**17**{*7,5*}	96	468.24	469.21
10{*6,6*}	–	512.70	–	**17**{*7,6*}	81	494.26	495.26

[a] Determined under monitoring HPLC at 214 nm.

orthogonal geometry of a non-pharmacophoric imide fragment is responsible for the significant enhancement of the 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor affinity of compounds containing an ethylene spacer.

5. Conclusions

Summing up, we have synthesized a 36-member library of arylpiperazines connected by an ethylene spacer with *N*-acylated amino acid-derived amide fragments. The surprisingly low affinities of the library representatives for the 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors prompted us to determine the influence of the length of an alkyl spacer and the nature of the substituent at 3-position of the pyrrolidine-2,5-dione fragment on receptor affinity. The subsequently synthesized pyrrolidine-2,5-diones containing a flexible 3-cycloalkyl fragment and displaying high 5-HT$_{1A}$/5-HT$_{2A}$ affinity supported our hypothesis that not only the length of an alkyl spacer, but above all the type of substituent at 3-position influenced 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor affinity. The results of the present study seem to suggest that in order to obtain high-affinity ligands for 5-HT$_{1A}$/5-HT$_{2A}$ receptors in a group of pyrrolidine-2,5-diones containing an ethylene spacer, the imide core should rather be substituted with a 3-cycloalkyl fragment than with a 3-carboxamido one. And last but not least, the substitution of pyrrolidin-2,5-dione with a spiro fragment seems to be the most preferential for 5-HT$_{1A}$/5-HT$_{2A}$ receptor binding.



18 - 23

Scheme 3. Synthesis of pyrrolidine-2,5-dione derivatives **18**–**23**: (*i*) diversity reagent **2**{*6*–*7*}, 180–200 °C, 3 h.

6. Experimental

6.1. Materials

All the solvents were obtained from Acros, and were used without purification. PA–BAL linker polyamide SynPhase™ Lanterns with 18 μmol loading, and colored tags were provided from Mimotopes. All the Fmoc amino acids and HBTU reagent were purchased from Senn Chemicals. All other reagents were from Aldrich.

Purity of pyrrolidin-2,5-diones was confirmed by TLC performed on Merck silica gel 60 F$_{254}$ aluminium sheets (Merck, Darmstadt, Germany), by using a mixture of DCM:methanol – S$_1$ (9:1, v/v). Spots were detected by their absorption under UV light ($\lambda = 254$ nm). The chemical structures were confirmed by elemental and spectral analyses (^{1}H NMR, MS). ^{1}H NMR spectra were obtained using a Varian BB 200 (300 MHz) spectrometer; chemical shifts (δ) are expressed in ppm downfield from the internal TMS as a reference; *J* values are in hertz (Hz), and splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), m (multiplet). Elemental analyses were carried out using an Elementar Vario EL III, and were within ±0.4% of the theoretical values.

The following abbreviations were used: CHCl$_3$ – chloroform; DCM – dichloromethane; DIC – *N*,*N*′-diisopropylcarbodiimide; 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).

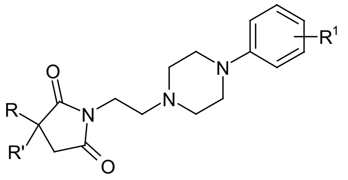
6.2. LC/MS analysis

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 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 up to get the final spectrum.

6.3. LC/MS purification

Samples were prepared in acetonitrile/water (50/50 v/v) mixture, containing 0.1% TFA. The LC/MS autopurification system consisted of a binary pump Waters 2525, an injector/

Table 3
Affinity data for 5-HT_{1A} and 5-HT_{2A} receptors of selected pyrrolidine-2,5-dione derivatives



Compound	R	R′	R^1	K_i (nM)	
				5-HT_{1A}	5-HT_{2A}
9{*7,3*}	(*S*)-Cyclohexanecarboxamide	H	*o*-OCH_3	7440[a]	7343[a]
9{*6,3*}	(*S*)-Cyclohexanecarboxamide	H	*m*-Cl	3775[a]	1411[a]
18	H	H	*o*-OCH_3	2087 ± 220	17 570 ± 1250
19	H	H	*m*-Cl	3675 ± 412	2120 ± 185
20	Cyclohexyl	H	*o*-OCH_3	24 ± 3	868 ± 73
21	Cyclohexyl	H	*m*-Cl	227 ± 15	137 ± 9
22[b]	$(CH_2)_5$		*o*-OCH_3	5.7 ± 0.8	254 ± 12
23[c]	$(CH_2)_5$		*m*-Cl	4.3 ± 0.7	15 ± 0.5

[a] The estimated K_i (see Ref. [8]).

[b] Data taken from Ref. [11].

[c] Data taken from Ref. [12].

fraction collector Waters 2676, coupled to a Waters Micromass ZQ spectrometer (electrospray ionization mode, ESI+). All the purifications were carried out using a Waters Symmetry Shield C18 19 × 100 mm, 5-μm particle size, column. A flow rate of 20 mL/min and a gradient of 0–60% B over 20 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 204 μL/min. Nitrogen was used for both the nebulizing gas and the drying gas. The data were obtained in a scan mode ranging from 100 to 1000 *m/z* in 0.1 s intervals; 10 scans were summed up to get the final spectrum. Collection control trigger was set on single protonated and diprotonated ion with an MIT (minimum intensity threshold) of 8×10^5.

6.4. Standard reductive amination protocol

The Lanterns were divided into two groups and were placed in glass vials containing a suspension of natrium cyanoborohydride ([$NaHB_3CN$] = 100 mM) and the amine ([diversity reagent **2**, **2**{*6–7*}] = 250 mM, Fig. 1), in a 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 after that allowed to dry in the open air.

6.5. 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.

6.6. Secondary amine acylation protocol

Three DMF solutions (20 mL) containing an Fmoc-protected amino acid and DIC each, 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 again for 4 h.

6.7. 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.

6.8. Standard coupling protocol

Six DMF solutions (25 mL), containing carboxylic acid (Fig. 2: diversity reagent **6**), HBTU and DIEA each, 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.

6.9. 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. Afterwards, the reaction solution was removed using a Jouan RC1010 vacuum centrifuge. A 100-μL portion of acetonitrile/water (50:50, v/v) containing a 0.1% TFA was poured into each tube to dissolve the samples. The samples were then frozen at −80 °C and lyophilized. The procedure was repeated twice to completely remove the remaining volatile residues.

6.10. Cleavage protocol

A 500 μL of the TFA was dispensed into polypropylene tubes of deep 96-well plate. Cleavage was carried out for 60 min. The cleavage cocktail was removed from the tubes under nitrogen flow. 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 the samples were frozen at −80 °C and lyophilized. The procedure was repeated twice to completely remove the remaining volatile residues.

6.11. General procedure for the preparation of pyrrolidine-2,5-diones (**18–21**)

To a suspension of 2-cyclohexyl succinic acid or succinic acid (0.005 mol) in 10 mL of water, the appropriately substituted arylpiperazine ethylamine (0.005 mol) was gradually added. The mixture was heated in an oil bath and water was simultaneously distilled off. When the water was completely removed, the reaction temperature was raised up to 180 °C and maintained for 1.5 h. The crude products were purified by column chromatography.

6.11.1. N-[2-{4-(2-Methoxyphenyl)-piperazin-1-yl}-ethyl]-pyrrolidine-2,5-dione (**18**)

Obtained from succinic acid as a white powder (64% yield); chromatography (SiO_2, CH_2Cl_2/MeOH:9/1); $R_f = 0.56$ (S_1). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.59–2.63 (t, 2H, $CH_2CH_2N(CH_2)_2$), 2.69–2.71 (m, 8H, $N(CH_2)_2$, imide), 3.03 (br, 4H, $(CH_2)_2N$), 3.67–3.71 (t, 2H, (NCH_2, $J = 6.41$ Hz)), 3.85 (s, 3H, OCH_3), 6.83–7.01 (m, 4H, Ph). ESI-MS: ($M + H^+$) 318.7; Mp: 104–105 °C. Anal. ($C_{27}H_{23}N_3O_3$) C, H, N.

6.11.2. N-[2-{4-(3-Chlorophenyl)-piperazin-1-yl}-ethyl]-pyrrolidine-2,5-dione (**19**)

Obtained from succinic acid as a white powder (67% yield); chromatography (SiO_2, CH_2Cl_2/MeOH:9/1); $R_f = 0.63$ (S_1). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.57–2.64 (m, 6H, $CH_2CH_2N(CH_2)_2$), 2.71 (s, 4H, imide), 3.11–3.15 (m, 4H, $(CH_2)_2N$), 3.66–3.70 (t, 2H, (NCH_2, $J = 6.41$ Hz)), 6.74–6.80 (m, 2H, Ph), 6.84–6.86 (m, 1H, Ph) 7.12–7.17 (m, 1H, Ph). ESI-MS: ($M + H^+$) 322.8; Mp: 115–116 °C. Anal. ($C_{16}H_{20}ClN_3O_2$) C, H, N.

6.11.3. N-[2-{4-(2-Methoxyphenyl)-piperazin-1-yl}-ethyl]-3-cyclohexyl-pyrrolidine-2,5-dione (**20**)

Obtained from 2-cyclohexyl succinic acid acid as a white powder (57% yield); chromatography (SiO_2, CH_2Cl_2/MeOH:9/1); $R_f = 0.57$ (S_1). ^{1}H NMR ($CDCl_3$) δ: 1.02–1.30 (m, 4H, Chx), 1.45 (d, 1H, Chx, $J = 11.28$ Hz), 1.65–1.78 (m, 5H, Chx), 1.90–1.97 (m, 1H, Chx), 2.48 (dd, 1H, imide, $J = 17.44$ Hz), 3.06–3.23 (m, 2H, imide), 3.46 (br s, 2H, $CH_2CH_2N(CH_2)_2$), 3.55–3.60 (m, 2H, NCH_2), 3.87–3.97 (m, 4H, $N(CH_2)_2$), 4.03 (s, 3H, OCH_3), 4.37 (br s 2H, $(CH_2)_2N$), 5.02 (t, 2H, $(CH_2)_2N$, $J = 12.05$ Hz), 7.02–7.07 (m, 2H, Ph), 7.42–7.48 (m, 1H, Ph), 8.15 (d, 1H, Ph, $J = 7.69$ Hz); **20**·HCl Mp: 208–210 °C. Anal. ($C_{23}H_{33}N_3O_3 \cdot HCl$) C, H, N.

6.11.4. N-[2-{4-(3-Chlorophenyl)-piperazin-1-yl}-ethyl]-3-cyclohexyl-pyrrolidine-2,5-dione (**21**)

Obtained from 2-cyclohexyl succinic acid as a white powder (61% yield); chromatography (SiO_2, CH_2Cl_2/MeOH:9/1); $R_f = 0.67$ (S_1). ^{1}H NMR ($CDCl_3$) δ: 0.96–1.33 (m, 4H, Chx), 1.43 (d, 1H, Chx, $J = 12.05$ Hz), 1.64–1.78 (m, 5H, Chx), 1.87–1.95 (m, 1H, Chx), 2.45 (dd, 1H, imide, $J = 18.82$ Hz), 2.91 (q, 2H, imide, $J = 8.60$ Hz), 3.05–3.23 (m, 2H, $CH_2CH_2N(CH_2)_2$), 3.29–3.31 (d, 2H, NCH_2, $J = 5.39$ Hz), 3.57–3.76 (m, 4H, $N(CH_2)_2$), 3.87–3.94 (m, 4H, $(CH_2)_2N$), 6.75–6.78 (m, 1H, Ph), 6.87–6.93 (m, 2H, Ph), 7.16–7.22 (m, 1H, Ph); **21**·HCl Mp: 240–243 °C. Anal. ($C_{22}H_{30}ClN_3O_2 \cdot HCl$) C, H, N.

6.12. Radioligand binding studies

The selected compounds were tested without further purification. 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 (67 Ci/mmol; NEN Chemicals) binding to rat hippocampal and cortical membranes, respectively. Membrane preparation and general assay procedures were carried out according to the previously published protocols [8,15]. In the screening procedure two compound concentrations were tested: 0.1 and 1 μM, whereas in full binding experiments 7–9 sample concentrations, each run in triplicate, were used to determine inhibition constant (K_i).

References

[1] R.M. Sanchez-Martin, S. Mittoo, M. Bradley, Curr. Top. Med. Chem. 4 (2004) 653–669.

[2] J.P. Kennedy, L. Williams, T.M. Bridges, R.N. Daniels, D. Weaver, C.W. Lindsley, J. Comb. Chem. 10 (2008) 345–354.

[3] B. Fulton, R.N. Brogden, CNS Drugs 7 (1997) 68–88.

[4] T. Kikuchi, K. Tottori, Y. Uwahodo, T. Hirose, T. Miwa, Y. Oshiro, S. Morita, J. Pharmacol. Exp. Ther. 274 (1995) 329–336.

[5] T. De Paulis, IDrugs 10 (2007) 193–201.

[6] O.M. Becker, D.S. Dhanoa, Y. Marantz, D. Chen, S. Shacham, S. Cheruku, A. Heifetz, P. Mohanty, M. Fichman, A. Sharadendu, R. Nudelman, M. Kauffman, S. Noiman, J. Med. Chem. 49 (2006) 3116–3135.

[7] A. Newman-Tancredi, D. Cussac, R. Depoortere, Curr. Opin. Investig. Drugs 8 (2007) 539–554.

[8] P. Zajdel, G. Subra, A.J. Bojarski, B. Duszyńska, M. Pawłowski, J. Martinez, J. Comb. Chem. 6 (2004) 761–767.

[9] P. Zajdel, G. Subra, A.J. Bojarski, B. Duszyńska, M. Pawłowski, J. Martinez, Bioorg. Med. Chem. Lett. 16 (2006) 3406–3410.

[10] P. Zajdel, G. Subra, A.J. Bojarski, B. Duszyńska, E. Tatarczyńska, A. Nikiforuk, E. Chojnacka-Wójcik, M. Pawłowski, J. Martinez, Bioorg. Med. Chem. 15 (2007) 2907−2919.

[11] J. Obniska, M. Kołaczkowski, A.J. Bojarski, B. Duszyńska, Eur. J. Med. Chem. 41 (2006) 874−881.

[12] J. Obniska, E. Tatarczyńska, A. Nikiforuk, S. Charakchieva-Minol, B. Duszyńska, Pharmacol. Rep. 58 (2006) 107−114.

[13] <www.mimotopes.com> (last accessed May, 2008).

[14] R.A. Glennon, N.A. Naiman, R.A. Lyon, M. Titeler, J. Med. Chem. 31 (1988) 1968−1971.

[15] C.A. Miller, L.M. Long, J. Am. Chem. Soc. 73 (1951) 4895−4898.

[16] A.J. Bojarski, M.T. Cegła, S. Charakiewa-Minol, M.J. Mokrosz, M. Maćkowiak, J.L. Mokrosz, Pharmazie 48 (1993) 289−294.

[17] Y. Cheng, W.H. Prusoff, Biochem. Pharmacol. 22 (1973) 3099−3108.

[18] <http://pdsp.med.unc.edu/pdsp.php> (last accessed May, 2008).