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Novel class of arylpiperazines containing N-acylated amino acids: Their synthesis, 5-HT_{1A}, 5-HT_{2A} receptor affinity, and in vivo pharmacological evaluation

Paweł Zajdel,[a,b,*] Gilles Subra,[a] Andrzej J. Bojarski,[c] Beata Duszyńska,[c] Ewa Tatarczyńska,[d] Agnieszka Nikiforuk,[d] Ewa Chojnacka-Wójcik,[d] 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 Medicinal 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*
[d]*Department of New Drugs Research, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-343 Kraków, Poland*

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Abstract—Novel arylpiperazines with N-acylated amino acids, selected on the basis of a preliminary screening of two libraries previously synthesized on SynPhase™ Lanterns, were prepared in solution and their affinity for 5-HT_{1A}, 5-HT_{2A}, and D_2 receptors was evaluated. The compounds bearing (3-acylamino)pyrrolidine-2,5-dione (**19**–**26**) and *N*-acylprolinamide (**29**–**34**) moieties showed high affinity for 5-HT_{1A} (K_i = 3–47 nM), high-to-low for 5-HT_{2A} (K_i = 4.2–990 nM), and low for D_2 receptors (K_i = 0.77–21.19 μM). All the new *o*-methoxy derivatives of (3-acylamino)pyrrolidine-2,5-diones tested in vivo revealed agonistic activity at postsynaptic 5-HT_{1A} receptors, while *m*-chloro derivatives were classified as antagonists of these sites; similar relations were observed for *o*-methoxy (**29**) and *m*-chlorophenylpiperazine derivatives of *N*-acylprolinamides. The reported results show that the amino acid-derived terminal fragment modified the in vivo functional profile. Finally, the selected compounds **19** and **20**, a 5-HT_{1A} partial agonist and a full agonist, respectively, and **26**, a mixed 5-HT_{1A}/5-HT_{2A} antagonist, were evaluated in preclinical animal models of depression and anxiety. The project allowed selecting the lead compound **20** which exhibited an anxiolytic-like effect in the four-plate test in mice and revealed distinct antidepressant-like effects in the forced swimming and tail suspension tests in mice.

1. Introduction

Among several innovative synthetic technologies the advent of combinatorial chemistry was one of the developments that significantly contributed to the progress in drug discovery process. By introduction of split-and-mix and parallel synthesis methods it has facilitated generation of the compound libraries and thus has offered unique opportunities for accelerating lengthy processes revolving around hit and lead identification as well as stages of lead optimization.[1,2]

Long-chain arylpiperazines have long been focusing great interest as a source of both neuropsychiatric drugs, for example, Buspirone,[3] Ziprasidone,[4] Aripiprazol,[5] and compounds with a high therapeutical potential (Adatanserin,[6] Mazapertine,[7] Flesinoxan,[8] Lecozotan,[9] Bifeprunox[10]) which—among others—exert their action via 5-HT_{1A} and 5-HT_{2A} receptors. Their diversified receptor binding profile and intrinsic activity,[11–13] depending on either the kind of substituent at the phenyl

Keywords: Long-chain arylpiperazines; Succinimides; Pyrrolidine-2,5-dione; Prolinamides; Solid-phase synthesis; 5-HT_{1A}/5-HT_{2A} receptor ligands; 5-HT_{1A} receptor agonists; Four-plate test; Forced swimming test; Tail suspension test.
* Corresponding author. Tel.: +48 12 657 05 60; fax: +48 12 657 02 62; e-mail: mfzajdel@cyf-kr.edu.pl

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ring at piperazine or the nature of an amide/imide terminal fragment, opens possibility for discovery of new potent therapeutics.[14–16]

While searching for new agents for a possible treatment of anxiety and depressive disorder, we designed a novel class of arylpiperazine derivatives containing N-acylated amino acids in the amide fragment (Fig. 1). Modifications consisted in introducing 3-aminopyrrolidine-2,5-dione (set **9**) and pyrrolidine-5-one-2-carboxamide (set **10**), which originated from the cyclization of aspartic and glutamic acid, respectively. Moreover, prolinamides (set **17**) used, as deoxo-analogs of pyroglutamyl moiety, and asparaginyl amides (set **19**) as open counterparts of set **9** were introduced. Further modifications involved diversifying the nature of an N-acyl substituent and the length of an alkyl chain connecting the amide/imide fragment to the basic nitrogen of arylpiperazine.

In the initial stage of our research project, in order to get a quick access to the above compounds, we developed a new solid-supported approach that yielded two libraries containing 72 members bearing phenylpiperazines and *m*-chlorophenylpiperazines,[17] and 60 *o*-methoxyphenylpiperazines (*o*-OCH_3-PhP).[18] Those two libraries with three points of diversity were generated by a five-step protocol using BAL linker functionalized SynPhase Lanterns[19,20] which included one-pot cleavage/cyclization for the generation of succinimide and pyroglutamyl derivatives. Of the synthesized compounds, 51 library representatives were tested in a preliminary screening for 5-HT_{1A} and 5-HT_{2A} receptors. Subsequently, structure–activity relationships within the library were analyzed by the Free-Wilson and Fujita-Ban methods.[21] In the structure of the compounds, three fragments related with the building blocks used for generating library were distinguished, and the influence of the respective substructures on receptor affinity was determined (Fig. 1). It was found that prolyl amides (set **17**), R substituents of high molecular bulk, and four-member alkyl chain connected with *o*-OCH_3-PhP had the highest incremental effect on 5-HT_{1A} affinity, while 3-aminopyrrolidine-2,5-dione (set **9**), cyclohexyl, norborn-2-ylmethyl substituents, and a 4-(3-chlorophenyl)-1-piperazinylbutyl fragment were the most beneficial to 5-HT_{2A} binding. On the basis of a preliminary screening, some potent 5-HT_{1A} and/or 5-HT_{2A} receptor ligands (with 5-HT_{1A} affinity <50 nM) were selected to be pharmacologically tested in order to determine their potential CNS activity.

In the present paper, we described the pre-final stage of our research project: the synthesis of 14 compounds bearing (3-acylamino)pyrrolidine-2,5-diones (**19–26**) and *N*-acylprolinamides (**29–34**) and containing an *o*-methoxy- and an *m*-chlorophenylpiperazine-butyl fragments; their 5-HT_{1A}, 5-HT_{2A}, dopamine D_2 receptor affinities, as well as the successive determination of their functional profile at 5-HT_{1A} (pre- and postsynaptic) and 5-HT_{2A} sites. Finally, the results of preclinical evaluation (animal models of anxiety and depression) of compounds **19** and **20**—the most interesting in terms of 5-HT_{1A} intrinsic activity—were presented.

2. Chemistry

2.1. Synthesis of *N*-((3*S*)-1-{4-[4-arylpiperazin-1-yl]butyl}pyrrolidin-2,5-dion-3-yl)cycloalkanecarboxamides (19–26)

The multi-step synthesis consisted in the parallel preparation of 4-[4-arylpiperazin-1-yl]butylamine according to the Gabriel method described elsewhere[22] and the respective N-acyl aspartic acid derivatives (Scheme 1). Esterification of 2-(9H-fluoren-9-ylmethoxycarbonyl) aspartic acid 4-*tert*-butyl ester (**1**) in a two-phase medium yielded compound **2**. After Fmoc removal, it was coupled with the respective carboxylic acids to give N-acyl aspartic acid derivatives (**3–6**) using BOP as an activating agent.[23] The latter were then subjected to catalytic hydrogenation with a 10% Pd/C, resulting in quantitative transformation into the respective free acids **7–10**. The key intermediates were coupled to an appropriate butylamine to yield the protected amido-acids **11–18**. The cyclization of the latter products was accomplished by a two-step procedure: the removal of *t*-Bu protection upon TFA treatment and the subsequent closing of amido-acids to the imides **19–26** in an acetic anhydride in the presence of sodium acetate.

2.2. Synthesis of 1-(cycloalkylcarbonyl)-*N*-{4-[4-arylpiperazin-1-yl]butyl}-L-prolinamide (29–34)

Direct coupling of *tert*-butoxycarbonyl-proline to an appropriate 4-[4-arylpiperazin-1-yl]butylamine afforded

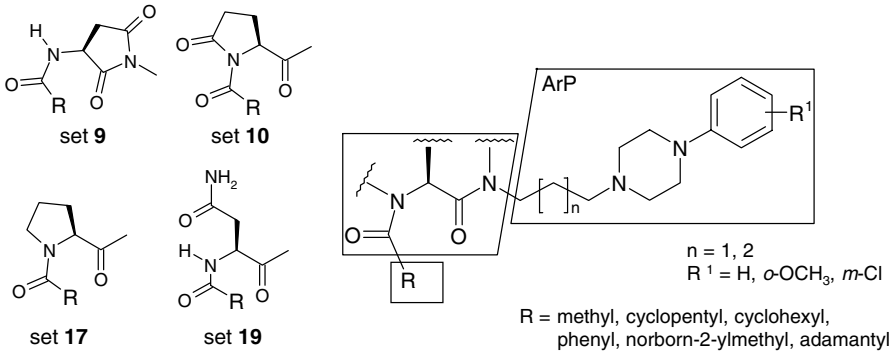
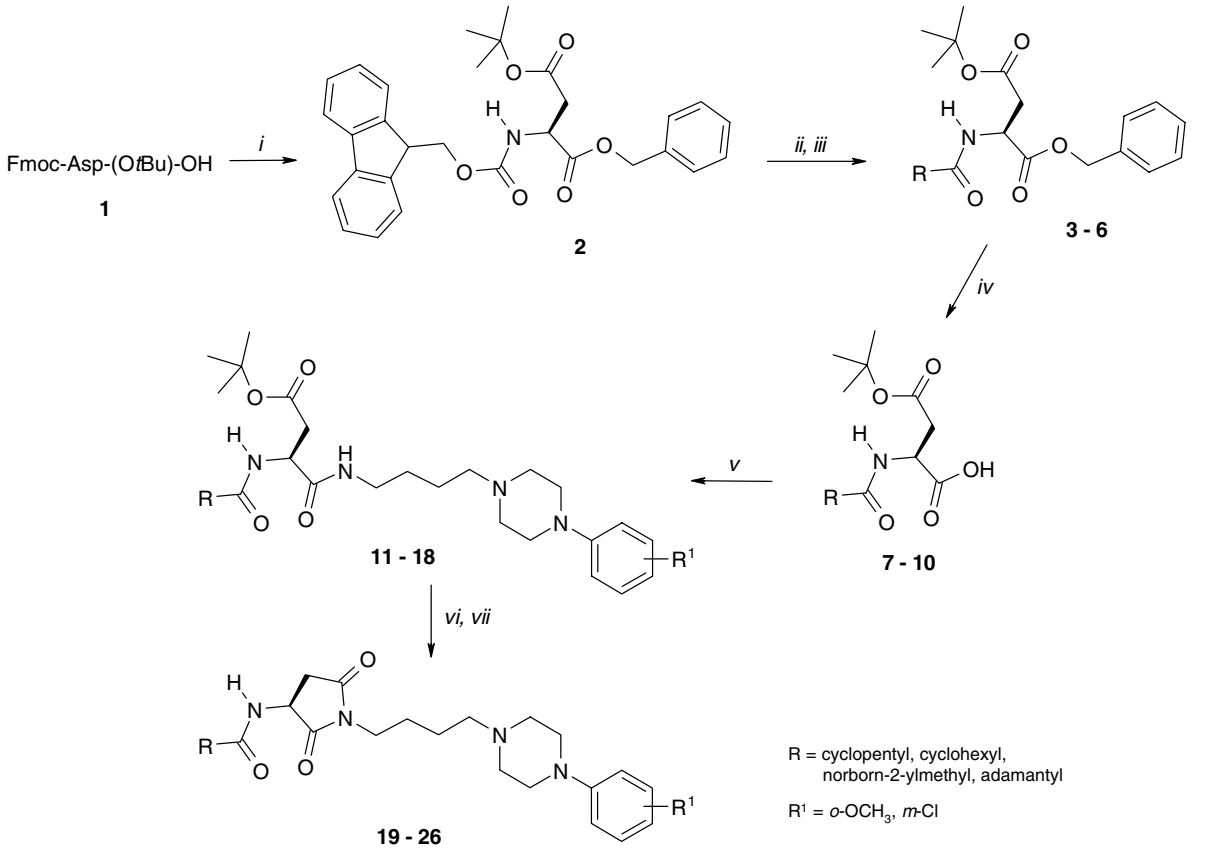


Figure 1. General structure of the investigated compounds with the marked fragments used for the Free-Wilson and the Fujita-Ban analyses.[17,18]



Scheme 1. Reagents and conditions: (i) benzyl bromide, TBAB, K_2CO_3, CH_3CN, rt, 12 h; (ii) 20% piperidine/DMF, rt, 40 min; (iii) cycloalkane carboxylic acid, BOP, TEA, CH_2Cl_2, rt, 8 h; (iv) 10% Pd/C, H_2 (gas), EtOH; (v) 4-[4-arylpiperazin-1-yl]butylamine, BOP, TEA, CH_2Cl_2, rt, 12 h; (vi) TFA, rt, 60 min; (vii) Ac_2O, AcONa, 60 °C, 2 h.

the prolyl amides **27** and **28**. After Boc removal, the latter were coupled to cycloalkane carboxylic acids to yield the final prolyl amides **29**–**34** (Scheme 2).

For pharmacological in vitro and in vivo investigations, all the final products were converted into water-soluble hydrochlorides.

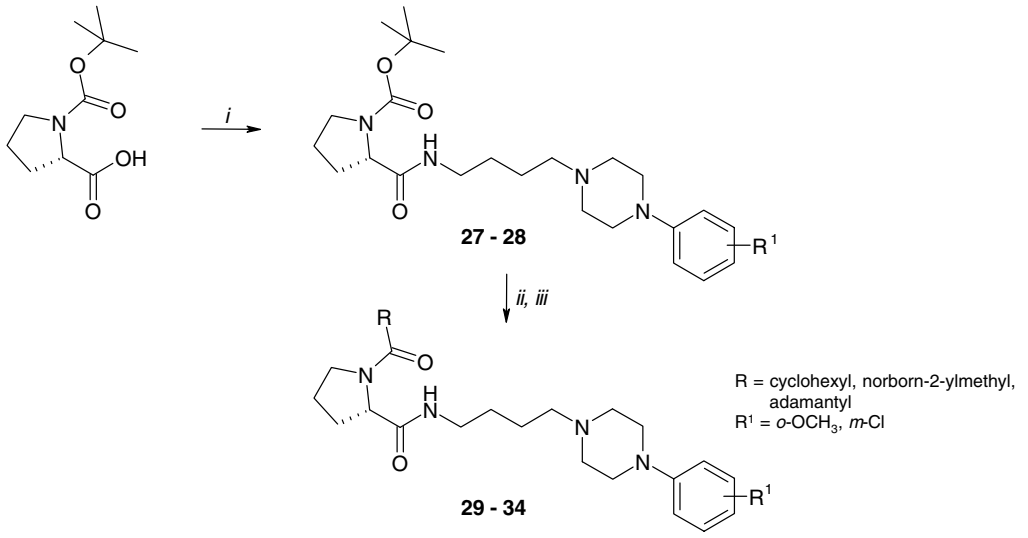
3. Pharmacology

The new compounds were tested in vitro in full displacement experiments for 5-HT_{1A} and 5-HT_{2A} receptor affinities, and according to the screening protocol for dopamine D_2 receptor affinity.

The functional activity of the investigated compounds at 5-HT_{1A} and 5-HT_{2A} receptors was tested in commonly used in vivo models. It was previously demonstrated that the hypothermia induced by the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) in mice was connected with activation of presynaptic 5-HT_{1A} receptors,[24,25] and was abolished by the selective 5-HT_{1A} receptor antagonist *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexane carboxamide (WAY 100635).[26] Thus the hypothermia produced by the tested compounds in mice (reduced by WAY 100635) was regarded as a measure of presynaptic 5-HT_{1A} receptor agonistic activity. Similarly, the ability of those compounds to inhibit the 8-OH-DPAT-induced hypothermia was taken as a measure of presynaptic 5-HT_{1A} receptor antagonistic activity. To determine the postsynaptic 5-HT_{1A} receptor agonistic effects of the tested compounds, their ability to induce lower lip retraction (LLR) in rats was tested. It is commonly accepted that the 8-OH-DPAT-induced LLR in rats depends on stimulation of postsynaptic 5-HT_{1A} receptors[27,28] and is inhibited by WAY 100635.[29] Hence the ability of the tested compounds to inhibit the 8-OH-DPAT-induced LLR was regarded as postsynaptic 5-HT_{1A} receptor antagonistic activity.

The central 5-HT_{2A} antagonistic properties of compounds showing 5-HT_{2A} receptor affinity <50 nM were assessed by their ability to antagonize head twitches induced by the 5-HT_{2A} receptor agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ((±)-DOI) in mice.[30,31]

The potential anxiolytic activity of compounds (**19**, **20**, and **26**) was evaluated by the four-plate test in mice,[32] and the antidepressant-like activity (**19** and **20**) by the forced swimming[33] and tail suspension tests in mice.[34] The effect of compounds **19** and **20** on the spontaneous locomotor activity of mice was also tested.



Scheme 2. Reagents and conditions: (i) 4-[4-arylpiperazin-1-yl]butylamine, BOP, TEA, CH_2Cl_2, rt, 12 h; (ii) TFA, rt, 60 min; (iii) cycloalkane carboxylic acid, BOP, TEA, CH_2Cl_2, rt, 12 h.

4. Results and discussion

All the compounds tested, that is, (3-acylamino)pyrrolidine-2,5-diones (**19**–**26**), and *N*-acylprolinamide derivatives (**29**–**34**), showed high affinity for 5-HT_{1A} receptors (3–47 nM), whereas their 5-HT_{2A} receptor affinities were diversified and ranged from 4.2 (**25**) to 990 nM (**29**) (Tables 1 and 2). On the basis of their binding profiles, compounds **21**, **23**–**26**, **32**, and **33** were classified as dual 5-HT_{1A}/5-HT_{2A} receptor ligands. It is worth noting that of the dual ligands only compound **21** had an *o*-OCH_3-PhP fragment; the remainder of them possessed an *m*-Cl substituent at the phenyl ring.

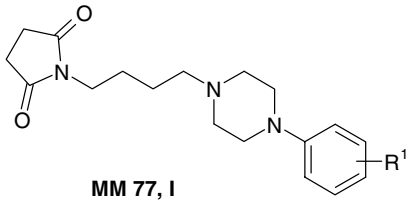
The compounds under study demonstrated weak or negligible affinity for D_2 receptor sites, which may be explained by the absence of an aromatic moiety in the amide fragment, commonly present within the structure of highly potent D_2 receptor ligands.[35,36]

As has been mentioned in the Introduction, the 5-HT receptor activity of the compounds synthesized on a solid support was determined according to the pre-screening protocol. Such quick tests of library members without their prior purification have been discussed in several papers and remain controversial.[37] Hence one of the goals of our investigation was to determine the accuracy and productiveness of the preliminary screening protocol applied to estimate the 5-HT_{1A} and 5-HT_{2A} receptor affinities of the compounds synthesized on a solid support and tested as crude products. Some problems may arise due to the fact that the analyzed samples are likely to contain impurities which do not absorb UV light (not detectable by a LC/MS analysis), and thus alter the final response, giving false biological results. In our experiments, compounds were synthesized on a solid support by a sort-and-combine approach, yielding an individual compound per vial. Although in all the cases compound purity did not exceed 95% standard, the set of library representatives tested was composed of a sufficient number of structural analogs, and the results of biological evaluation fit the general structure–activity relationships pattern. In almost all the cases the receptor affinity values obtained for crude products synthesized on a solid support were lower than the affinity data obtained in full-displacement experiments for the same pure compounds synthesized in a solution (e.g., the 5-HT_{1A} receptor affinities of *o*-OCH_3-PhP derivatives were 1.5- to 3-fold higher). It seems of great importance that for all 5-HT_{1A} receptor investigated compounds the initially determined receptor affinity rank order remained unchanged. A similar tendency was observed for compound 5-HT_{2A} receptor affinity, except for compounds **24** and **25** classified by a preliminary screening as highly potent 5-HT_{2A} receptor ligands (K_i = 2 and 1 nM, respectively), which appeared to be slightly less active in a full-displacement experiment, their K_i values being 6.5 and 4.2 nM, respectively. Nevertheless, the 5-HT_{2A} receptor affinities of **24** and **25** were still the highest in the series—only slightly lower than those reported for the reference drug (ritanserin, K_i = 1.1 nM).[17] Despite the above-listed deviations, ligand classification after full binding assays is in line with the data obtained from a preliminary screening.

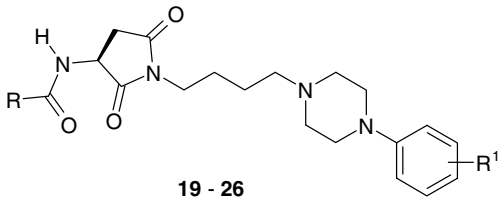
The synthesized arylpiperazines came from the introduction of N-acylated amino acid moieties into an amide fragment. Among them, (3-acylamino)pyrrolidine-2,5-diones (**19**–**26**) resulted from cyclization of N-acyl aspartic acids and may be regarded as analogs of the succinimide ligands of 5-HT_{1A} receptors, that is, **MM 77**[38] and **I**[39] (Table 1), modified by a cycloalkanecarboxamide moiety. The other set tested, that is, *N*-acylprolinamides (**29**–**34**, Table 2), may be classified as (3-acylamino)pyrrolidine-2,5-dione analogs possessing an exocyclic amide bond connected directly to an alkylen spacer.

The radioligand binding studies show that within the evaluated compounds, receptor affinity and selectivity

Table 1. The structure and binding affinity data on serotonin (5-HT_{1A}, 5-HT_{2A}) and dopaminergic D_2 receptors for (3-acylamino)pyrrolidine-2,5-dione derivatives (**19**–**26**)



MM 77, I

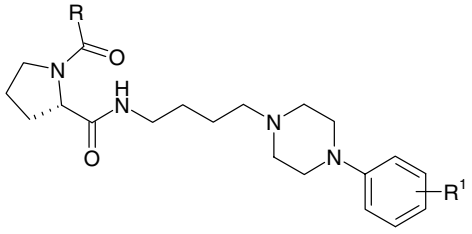


19 - 26

Compound	R	R^1	K_i (nM) ± SEM			5-HT_{1A} activity		5-HT_{2A} activity
			5-HT_{1A}	5-HT_{2A}	D_2[a]	Presynaptic	Postsynaptic	
MM 77[b]	—	*o*-OCH_3	6.4 ± 0.3	1510 ± 95	490	Non active	Antagonist	nd
I[c]	—	*m*-Cl	32 ± 2	121 ± 14	7800	Agonist	Partial agonist	nd
19	Cyclopentyl	*o*-OCH_3	23 ± 4	480 ± 38	905	Agonist	Partial agonist	nd
20	Cyclohexyl	*o*-OCH_3	19 ± 3	183 ± 12	2080	Agonist	Agonist	nd
21	Norborn-2-ylmethyl	*o*-OCH_3	9 ± 2	47 ± 6	880	Non active	Agonist	Antagonist
22	Adamantyl	*o*-OCH_3	4 ± 2	173 ± 17	1775	Non active	Partial agonist	nd
23	Cyclopentyl	*m*-Cl	28 ± 3	47 ± 8	12450	Non active	Antagonist	Antagonist
24	Cyclohexyl	*m*-Cl	26 ± 4	6.51 ± 1	2130	Non active	Antagonist	Antagonist
25	Norborn-2-ylmethyl	*m*-Cl	21 ± 4	4.2 ± 0.5	776	Non active	Antagonist	Antagonist
26	Adamantyl	*m*-Cl	47 ± 3	25 ± 2	21190	Antagonist	Antagonist	Antagonist

nd, not determined.
[a] Binding experiment run with two compound concentrations, expressed as an estimated K_i.
[b] Ref. 38.
[c] Ref. 39.

Table 2. The structure and binding affinity data on serotonin (5-HT_{1A}, 5-HT_{2A}) and dopaminergic D_2 receptors for *N*-acylprolinamides (**29**–**34**)



Compound	R	R^1	K_i (nM) ± SEM			5-HT_{1A} activity		5-HT_{2A} activity
			5-HT_{1A}	5-HT_{2A}	D_2[a]	Presynaptic	Postsynaptic	
29	Cyclohexyl	*o*-OCH_3	19 ± 3	990 ± 17	3595	Non active	Agonist	nd
30	Norborn-2-ylmethyl	*o*-OCH_3	14.5 ± 0.7	547 ± 48	1365	Agonist	Non active	nd
31	Adamantyl	*o*-OCH_3	3 ± 0.2	503 ± 8	1114	Non active	Non active	nd
32	Cyclohexyl	*m*-Cl	31 ± 4	69 ± 4	1050	Agonist	Antagonist	nd
33	Norborn-2-ylmethyl	*m*-Cl	37 ± 3	35 ± 3	1550	Agonist	Antagonist	Antagonist
34	Adamantyl	*m*-Cl	13 ± 1	140 ± 7	1780	Agonist	Antagonist	nd

nd, not determined.
[a] Binding experiment run with two compound concentrations, expressed as an estimated K_i.

depend on the nature of the amino acid moiety, as well as the size and hydrophobic properties of R substituents at the amide fragment and on arylpiperazine substitution mode (R^1). As a general trend, *N*-acylprolinamides showed preferential affinity for the 5-HT_{1A} receptors. Among them, compound **31** bearing an adamantyl moiety is characterized by the highest selectivity factor ($S_{2A/1A}$ = 167). For comparison, the highest selectivity ratio within the succinimide set (**19**–**26**) equaled 90.

Further, the 5-HT_{1A} receptor affinity of the *o*-methoxyphenylpiperazine derivatives of (3-acylamino)pyrrolidine-2,5-diones and *N*-acylprolinamides has been found to depend on the volume of a cycloalkyl substituent; the higher molecular bulk, the more potent 5-HT_{1A} ligands, that is, a change of a cyclopentyl into an adamantyl leads to a 6-fold increase in 5-HT_{1A} affinity (**19** vs **22**). Similar affinity increase (6-fold) was also observed in case of replacement of a cyclohexyl into an adamantyl (**29** vs **31**). Such a clear influence of the

kind of hydrocarbon moiety on 5-HT_{1A} affinity was not observed within *m*-chloro derivatives. Regarding 5-HT_{2A} affinity, it was found that in almost all the cases introduction of norborn-2-ylmethyl moiety, with more conformational freedom than adamantyl, gave the most potent 5-HT_{2A} receptor ligands in the series. Finally, compounds **23**–**26** and **32**–**34** bearing *m*-chloro substituent at the phenyl ring at the piperazine moiety were more potent 5-HT_{2A} receptor ligands than were *o*-methoxy counterparts (**19**–**22** and **29**–**31**). The above-quoted results confirm the general preference of a chloro substituent in the *meta* position of the phenyl ring for 5-HT_{2A} binding sites.

The results of our in vivo study demonstrate that the investigated compounds possess diversified 5-HT_{1A} receptor functional activity (Tables 1 and 2). Of the compounds tested, **19**–**23**, **29**–**34** (like 8-OH-DPAT, a 5-HT_{1A} receptor agonist) decreased body temperature in mice, whereas compounds **24**–**26** (like WAY 100635, a 5-HT_{1A} receptor antagonist) did not change it (see Supplementary Data—Table 1). The hypothermia induced by **19**, **20**, **30**, **32**, **33** or **34** (like that induced by 8-OH-DPAT) was reduced or abolished by WAY 100635 (see Supplementary Data—Table 2); therefore it seems that these compounds have features of presynaptic 5-HT_{1A} receptor agonists. At the same time, the hypothermia induced by compounds **21**, **22**, **23**, **29** or **31** (in contrast to that induced by 8-OH-DPAT) was not changed by WAY 100635 (see Supplementary Data—Table 2); thus a mechanism different from stimulation of 5-HT_{1A} receptors seems to be responsible for the hypothermic effect of these compounds. Of compounds **24**, **25**, and **26**, which did not affect mouse body temperature, only **26** (like WAY 100635) attenuated the 8-OH-DPAT-induced hypothermia in mice (see Supplementary Data—Table 3). Therefore, **26** may be classified as a presynaptic 5-HT_{1A} receptor antagonist, whereas the functional activity of **24** and **25** at those receptors was negligible in that test.

In the experiment designed to evaluate postsynaptic 5-HT_{1A} receptor activity, compounds **19**–**22** and **29**, given alone, induced LLR in rats, the latter effect being considerably weaker than that after 8-OH-DPAT administration, though the remaining compounds did not mimic the effect of 8-OH-DPAT in that test (see Supplementary Data—Table 4). The LLR induced by 8-OH-DPAT was inhibited by compounds **19**, **22**–**26**, and **32**–**34**, while WAY 100635 almost completely blocked the effect of 8-OH-DPAT (see Supplementary Data—Table 4). The above-quoted results indicated that in the LLR model in rats compounds **20**, **21**, and **29** showed features characteristic of weak agonists of postsynaptic 5-HT_{1A} receptors, compounds **19** and **22** could be classified as partial agonists, and compounds **23**–**26** and **32**–**34** behaved like antagonists of postsynaptic 5-HT_{1A} receptors. Compounds **30** and **31** were practically inactive in that model. Unexpectedly, compounds **29**–**31**, administered in a dose of 20 mg/kg, caused the death of rats.

Moreover, compounds **21**, **23**–**26**, and **33** (like ketanserin, a 5-HT_{2A} receptor antagonist) effectively inhibited the (±)-DOI-induced head twitches in mice (see Supplementary Data—Table 5); hence these compounds can also be regarded as potential 5-HT_{2A} receptor antagonists.

It was recently reported by Bojarski et al.[39] that the substitution mode in the aromatic ring of piperazine controlled the postsynaptic 5-HT_{1A} receptor functional activity of a group of aryl-4-succinimidobutylpiperazines. Among them, **MM 77**, a potent 5-HT_{1A} ligand bearing an *o*-methoxy substituent, was classified as a postsynaptic 5-HT_{1A} antagonist,[38] while its *m*-chloro analog (**I**) showed properties of a partial agonist of postsynaptic 5-HT_{1A} sites[39] (Table 1). Unlike the model compounds **MM 77**[38] and **I**,[39] in functional in vivo tests all the new *o*-methoxy (3-acylamino)pyrrolidine-2,5-diones showed properties of full (**20**, **21**) or partial (**19** and **22**) agonists at postsynaptic 5-HT_{1A} receptors, while their *m*-chloro counterparts (**23**–**26**) were classified as 5-HT_{1A} receptor postsynaptic antagonists. The above results indicate that introduction of N-acyl amino acid-derived moiety into the arylpiperazine fragment altered the in vivo functional profile compared to the parent molecules.

Of the *o*-methoxy derivatives of *N*-acylprolinamides (**29**–**34**, Table 2), compound **29** was regarded as an agonist of postsynaptic 5-HT_{1A} sites, while **30** and **31** were devoid of any postsynaptic activity. Parallel to the succinimide set (**19**–**26**, Table 1), all the *m*-chloro derivatives (**32**–**34**) were classified as postsynaptic 5-HT_{1A} antagonists.

Regarding the presynaptic 5-HT_{1A} receptor intrinsic properties, the compounds tested showed agonistic (**19**, **20**, **30**, and **32**–**34**) or antagonistic (**26**) activity or were inactive (**21**–**25**, **29**, and **31**); thus a close relationship between the chemical modifications and their presynaptic intrinsic activity could not be demonstrated. It is difficult to explain why compound **31**, which in vitro showed the highest 5-HT_{1A} receptor affinity, appeared to be inactive at both pre- and postsynaptic 5-HT_{1A} sites in functional in vivo studies. Interestingly, an earlier report showed that a compound with high 5-HT_{1A} potency in vitro was not always found to be the most potent in functional in vivo models.[40]

Unsurprisingly, all the compounds tested by the 5-HT_{2A} receptor functional model showed antagonistic properties.

The functional profile of the investigated compounds suggests that some of them may exhibit anxiolytic- and/or antidepressant-like effects. It has been demonstrated that 5-HT_{1A} receptor agonists and partial agonists exert such activity,[41–43] and mixed 5-HT_{1A}/5-HT_{2A} receptor antagonists may also be of interest as potential anxiolytics.[44] For this reason we selected compounds **19** (a partial 5-HT_{1A} receptor agonist), **20** (a full 5-HT_{1A} receptor agonist), and **26** (a 5-HT_{1A}/5-HT_{2A} receptor antagonist) for further in vivo preclinical studies.

Our results indicate that compounds **19** and **20** produce an anxiolytic-like effect in the four-plate test in mice

Table 3. Effects of compounds **19**, **20**, **26**, and diazepam in the four-plate test in mice

Treatment	Dose (mg/kg)	No. of punished crossings means ± SEM
Vehicle **19**	—	3.9 ± 0.5
	5	3.4 ± 0.4
	10	5.2 ± 0.4
	20	6.0 ± 0.5[a]
		$F(3, 36) = 7.189$
		$P < 0.001$
Vehicle **20**	—	4.0 ± 0.3
	2.5	4.7 ± 0.5
	5	6.6 ± 0.3[a]
	10	7.0 ± 0.3[a]
	20	7.5 ± 0.5[a]
		$F(4, 45) = 14.723$
		$P < 0.001$
Vehicle **26**	—	4.1 ± 1.3
	5	4.6 ± 0.5
	10	5.1 ± 0.5
	20	5.6 ± 0.6
		$F(3, 36) = 1.462$
		ns
Vehicle diazepam	—	3.5 ± 0.4
	1.25	5.5 ± 0.5
	2.5	6.8 ± 0.6[a]
	5	6.7 ± 0.6[a]
		$F(3, 36) = 9.514$
		$P < 0.001$

Compounds **19** and **20** were administered 30 min, while **26** and diazepam 60 min before the test. $n = 10$ mice per group. ns, not significant.
[a] $P < 0.01$ versus vehicle (Dunnett's test).

Table 4. Effects of compounds **19**, **20**, and imipramine in the forced swimming (A) and the tail suspension (B) tests in mice

Treatment	Dose (mg/kg)	Immobility time (s), means ± SEM	
		A	B
Vehicle **19**	—	166.0 ± 4.8	NT
	5	133.4 ±11.2	
	10	123.3 ± 15.7[a]	
	20	102.9 ± 11.5[b]	
		$F(3, 36) = 5.240$	
		$P < 0.01$	
Vehicle **20**	—	166.0 ± 4.8	178.2 ± 7.2
	2.5	131.6 ± 12.5	NT
	5	110.0 ± 9.0[b]	147.9 ± 10.6
	10	102.0 ± 12.7[b]	129.1± 6.9[b]
	20	128.1 ± 8.1[a]	116.1 ± 10.6[a]
		$F(4, 45) = 6.297$	$F(3, 36) = 8.986$
		$P < 0.001$	$P < 0.001$
Vehicle imipramine	—	167.1 ± 6.7	164.6 ± 11.0
	5	NT	147.4 ± 9.9
	10	149.1 ± 10.7[a]	102.5 ± 10.3[b]
	20	107.8 ± 12.4[b]	90.2 ± 10.7[b]
		$F(2, 27) = 8.760$	$F(3, 36) = 13.495$
		$P < 0.01$	$P < 0.001$

Compounds **19** and **20** were administered 30 min, while imipramine 60 min before the test. $n = 9$–10 mice per group. NT, not tested.
[a] $P < 0.05$.
[b] $P < 0.01$ versus vehicle (Dunnett's test).

(Table 3). It is worth noting that the effect of **20** is comparable, in terms of its potency and active doses, with that produced by diazepam (used as a reference drug), whereas the effect of **19** is found to be weaker. Compound **26** is ineffective in this test (Table 3).

The results of our successive experiments also show that—like the typical antidepressant imipramine—compounds **19** and **20** exert a distinct antidepressant-like effect in a mouse forced swimming test. It is worth noting that both these compounds in doses lower than that of imipramine shorten the immobility time of mice; in the case of **20** the minimal effective dose is 5 mg/kg (Table 4). For this reason compound **20** was additionally studied in the tail suspension test in mice. In that model, **20** used in the same doses (10–20 mg/kg) as imipramine shortened the immobility time of mice (Table 4).

The anti-immobility effect of **19** and **20** seems to be specific, since—like imipramine—neither compound changed the spontaneous locomotor activity of mice during the initial 6-min experimental sessions (i.e., at the time equal to the observation period in the forced swimming test). When locomotor activity was recorded for 30 min, **19** and imipramine reduced it, while **20** was without effect (see Supplementary Data—Table 6).

Summing up, the study presents a research project on the successful application of combinatorial chemistry techniques for the generation of compound **20** from the designed new class of arylpiperazines containing N-acylated amino acid residues targeted on 5-HT_{1A} and 5-HT_{2A} receptors. Several representatives of two libraries (132 members) were screened as crude products; then 14 derivatives were re-synthesized (**19**–**26** and **29**–**34**) and their receptor affinity was determined in full-displacement experiments. The advantage of the approach was a quick identification of potent 5-HT_{1A} receptor ligands and several dual 5-HT_{1A}/5-HT_{2A} ligands, which—evaluated further by in vivo functional and preclinical tests—allowed us to establish strong structure–activity relationships between diverse factors affecting compound activity. The results of the present preclinical study show that compound **20** (a pre- and postsynaptic 5-HT_{1A} agonist) produces potential anxiolytic/antidepressant effects in the preclinical models used. It is worth noting that its effects are comparable to those produced by standard drugs, and that active doses of **20** are devoid of a sedative effect. Further studies with this lead compound will be undertaken and reported in due course. This report also suggests that concurrent application of parallel solid-phase synthesis and screening methods may be helpful for academic and industry laboratories in discovering new lead drugs in a time- and cost-effective manner.

5. Experimental

5.1. Chemistry

All reagents and solvents were from Aldrich and Lancaster and were used without further purification.

Protected amino acids and BOP were purchased from Senn Chemicals. The purity of the products was confirmed by an ascending TLC method on pre-coated Kieselgel 60 F_{254} plates (Merck) by applying the following solvents (by volume): S_1: CH_2Cl_2/MeOH, 9:1; S_2: CH_2Cl_2/MeOH, 95:5; S_3: $CHCl_3$/acetone, 1:1; S_4: $CHCl_3$/MeOH/AcOH, 180:10:5; S_5: EtOAc/*n*-hexane, 3:7. After the development of chromatograms, the plates were inspected under UV light ($\lambda = 254$ nm); additionally, spots were visualized with ninhydrine; R_f values are given for the sake of guidance. Analytical HPLC were run on a Waters Alliance HPLC instrument, equipped with a Chromolith SpeedROD column (4.6 × 50 mm). Standard conditions were eluent system A (water/0.1% TFA), system B (acetonitrile/0.1% TFA). A flow rate of 5 mL/min and a gradient of (0–100)% B over 5 min were used, detection 214 nm. Retention times (t_R) are given in minutes. All melting points were determined with Büchi 353 capillary apparatus and remain uncorrected. ^{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, and splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), m (multiplet). ESI + mass spectra were obtained on the LC–MS system consisting of Waters Alliance 2690 HPLC coupled to a Micromass (Manchester, UK) Platform II spectrometer (an electrospray ionization mode, ESI+). All the analyses were carried out using a C18 Xterra MS, 2.1 × 30 mm column. Optical rotations ($[\alpha]_D^{20}$) for the final compounds were measured with a Jasco DIP-1000 Digital Polarimeter (5 cm cell). Elemental analyses were carried out using an Elementar Vario EL III, and were within ±0.4% of the theoretical values. Silica gel 60 230–400 mesh, purchased from Merck, was used for preparative chromatographic purification.

5.1.1. 1-Benzyl 4-*tert*-butyl *N*-(9H-fluoren-9-ylmethoxycarbonyl)-L-aspartate (2). A mixture of Fmoc-L-Asp (O*t*Bu)-OH (26.7 g, 65 mmol), *tetra*-butylammonium bromide (2.09 g, 65 mmol), and anhydrous K_2CO_3 (9.8 g, 71 mmol) in acetonitrile (160 mL) was stirred at room temperature for 20 min. Benzyl bromide (8.4 mL, 71 mmol) in acetonitrile (10 mL) was then added dropwise with intensive stirring. The mixture was stirred for 12 h at room temperature. Then the precipitate was filtered off, and a filtrate was evaporated to dryness. The resulting crude material was dissolved in EtOAc (200 mL), and the organic layer was washed with a saturated $NaHCO_3$ solution (3×100 mL), water (3×100 mL), and brine, dried over Na_2SO_4, and finally was concentrated in a vacuum. The residue was crystallized from EtOAc/*n*-hexane (2/8, v/v) to afford **2** as a white precipitate (27.8 g, 86% yield). Mp: 108–109 °C; $R_f = 0.73$ (S_5); HPLC (t_R, 3.69 min); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.39 (s, 9H, C(CH_3)$_3$), 2.73–2.80 (dd, 1H, H_β Asp, $J = 16.9$, 4.4 Hz), 2.92–2.99 (dd, 1H, H_β Asp, $J = 16.9$, 4.6 Hz), 4.19–4.43 (m, 3H, CHCH$_2$O, CHCH_2O), 4.60–4.66 (m, 1H, H_α Asp), 5.13–5.25 (m, 2H, COCH_2Ph), 5.81–5.83 (d, 1H, NH, $J = 8.6$ Hz), 7.24–7.76 (m, 13H, Ph); ESI-MS ($M+H^+$) 502.2. Anal. ($C_{30}H_{31}NO_6$) C, H, N.

5.1.2. General procedure A: preparation of 1-benzyl 4-*tert*-butyl *N*-(cycloalkylcarbonyl)-L-aspartates 3–6. 1-Benzyl 4-*tert*-butyl *N*-(9H-fluoren-9-ylmethoxycarbonyl)-L-aspartate (**2**) was stirred at a room temperature in a mixture of piperidine and dimethylformamide (DMF) (20:80, v/v) for 60 min. After evaporation of the deprotection solution, 1-benzyl 4-*tert*-butyl L-aspartate (**2a**) was characterized by LC/MS and was used without further purification. A commercially available carboxylic acid (16.5 mmol) was dissolved in 60 mL dichloromethane (CH_2Cl_2), and benzotriazol-l-yl-oxy-tris-(dimethylamino)phosphonium hexa-fluorophosphate (BOP) (7.3 g, 16.5 mmol) was added under stirring. Then triethylamine (TEA) (4.2 mL, 30 mmol) was added dropwise, followed by a solution of H-Asp (O*t*Bu)-OBzl, **2a** (4.19 g, 15 mmol) in CH_2Cl_2 (15 mL). The reaction mixture was stirred for 8 h at a room temperature. Then the solvent was evaporated and the oily residue was dissolved in EtOAc (100 mL) and washed with a saturated $NaHCO_3$ solution (3×50 mL), 1 M $KHSO_4$ solution (3×50 mL), water (3×50 mL), and brine; it was then dried over Na_2SO_4, and finally concentrated in a vacuum. The crude amides were purified by a flash chromatography. Spectral data for new intermediates **3**–**6** are presented in Supplementary Data.

5.1.3. General procedure B: preparation of 4-*tert*-butyl *N*-(cycloalkylcarbonyl)-L-aspartates 7–10. To a solution of the corresponding esters **3**–**6** (7 mmol) in methanol, 0.1 g of 10% Pd/C was added and the mixture was stirred for 4 h under atmosphere of H_2. Then it was filtered over Celite, and the filtrate was concentrated to yield the desired products as a white solid. Spectral data for new intermediates **7**–**10** are presented in Supplementary Data.

5.1.4. General procedure C: preparation of 4-*tert*-butyl *N*-(cycloalkylcarbonyl)-1-{4-[4-arylpiperazin-1-yl]butyl}-L-aspartamides 11–18. To a mixture of 4-*tert*-butyl *N*-(cycloalkylcarbonyl)-L-aspartate (4 mmol) in 50 mL CH_2Cl_2, BOP (1.94 g, 4.4 mmol) was added under stirring. Then triethylamine (1.12 mL, 8 mmol) was added dropwise followed by a solution of the respective 4-[4-arylpiperazin-1-yl]butylamine (3.6 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 6–12 h at a room temperature until the reaction was completed (TLC control). The solvent was evaporated and the oily residue was dissolved in EtOAc (100 mL). It was then washed with a saturated $NaHCO_3$ (3×50 mL), water (3×50 mL) and brine, dried over Na_2SO_4, and was finally concentrated in a vacuum. The crude amides were purified by a column chromatography (silica gel, CH_2Cl_2/MeOH, 9:1 or CH_2Cl_2/MeOH, 90:7). Spectral data for new intermediates **11**–**18** are presented in Supplementary Data.

5.1.5. General procedure D: preparation of *N*-((*S*)-1-{4-[4-arylpiperazin-1-yl]butyl}pyrrolidin-2,5-dion-3-yl)cycloalkanecarboxamides 19–26. The 4-*tert*-butyl *N*-(cycloalkylcarbonyl)-1-{4-[4-arylpiperazin-1-yl]butyl}-L-aspartamide (**11**–**18**) (2.5 mmol) was dissolved in an 8 mL mixture of TFA/CH_2Cl_2 (9/1, v/v) and was stirred at a room temperature. After complete deprotection (TLC control), an excess of TFA was removed under reduced

pressure and the residue was evaporated to dryness by repeated co-evaporation with CH_2Cl_2 and ether to yield a yellowish foam. The obtained acid was left to drying in a desiccator overnight, and was further used without analytical identification. Afterward, it was dissolved in acetic anhydride (4 mL), and sodium acetate (0.2 g, 2.5 mmol) was added. The mixture was heated at 60 °C for 2 h under stirring. When the cyclization was accomplished, the mixture was concentrated under reduced pressure and the residue was dissolved in EtOAc (50 mL). The organic phase was washed with a saturated $NaHCO_3$ (2×50 mL) and water (2×50 mL), and was dried over $MgSO_4$, and finally concentrated in a vacuum. Further purification by a column chromatography over silica gel afforded a pure product in the form of a white solid or a transparent oil.

5.1.6. *N*-((*S*)-1-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}pyrrolidin-2,5-dion-3-yl)cyclopentanecarboxamide (19). Obtained from **11** as a colorless oil (0.84 g, a 74% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 99:1); R_f = 0.61 (S_1), 0.26 (S_3); HPLC (t_R 1.90 min); $[\alpha]_D^{20} = -0.52°$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO) δ (ppm) 1.46–1.75 (m, 13H, 9H cPt, $NCH_2CH_2CH_2$), 2.06–2.48 (m, 3H, $CH_2CH_2N(CH_2)_2$, H_β imide), 2.85–2.94 (m, 5H, $N(CH_2)_2$, H_β imide), 3.31–3.39 (m, 6H, $(CH_2)_2N$, NCH_2), 3.74 (s, 3H, OCH_3), 4.29–4.36 (m, 1H, H_α imide), 6.83–6.94 (m, 4H, Ph), 8.42–8.44 (d, 1H, N*H*, *J* = 7.4 Hz); ESI-MS ($M+H^+$) 457.3. Anal. ($C_{25}H_{36}N_4O_4$) C, H, N. **19**·HCl·H_2O: mp: 181–182 °C. Anal. ($C_{21}H_{36}N_4O_4$·HCl·H_2O) C, H, N.

5.1.7. *N*-((*S*)-1-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}pyrrolidine-2,5-dion-3-yl)cyclohexanecarboxamide (20). Obtained from **12** as a white solid (0.88 g, a 76% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 9:1); mp: 113–115 °C; R_f = 0.53 (S_1), 0.28 (S_3); HPLC (t_R 2.07 min); $[\alpha]_D^{20} = -0.54°$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO) δ (ppm) 1.14–1.68 (cluster, 14H, 10H, CH_2-cHex, $NCH_2CH_2CH_2$), 2.03–2.09 (m, 1H, C*H*-cHex), 2.26–2.30 (m, 2H, $CH_2CH_2N(CH_2)_2$), 2.40–2.49 (m, 5H, $N(CH_2)_2$, H_β imide), 2.84–2.93 (m, 5H, $(CH_2)_2N$, H_β imide), 3.35–3.39 (m, 2H, NCH_2), 3.74 (s, 3H, OCH_3), 4.30–4.37 (m, 1H, H_α imide), 6.83–6.92 (m, 4H, Ph), 8.34–8.37 (d, 1H, N*H*, *J* = 7.7 Hz); ESI-MS ($M+H^+$) 471.3. Anal. ($C_{26}H_{38}N_4O_4$) C, H, N. **20**·HCl·H_2O: mp: 190–192 °C. Anal. ($C_{26}H_{38}N_4O_4$·HCl·H_2O) C, H, N.

5.1.8. *N*-((*S*)-1-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}pyrrolidin-2,5-dion-3-yl)-2-(bicyclo[2.2.1]heptan-2-yl)acetamide (21). Obtained from **13** as a white solid (0.91 g, a 74% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 9:1); mp: 110–111 °C; R_f = 0.63 (S_1), 0.34 (S_3); HPLC (t_R 2.30 min); $[\alpha]_D^{20} = -1.28°$ (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.03–1.30 (m, 6H, CH_2–Nrbo), 1.42–1.66 (m, 6H, CH_2–Nrbo, $NCH_2CH_2CH_2$), 1.83–2.10 (m, 3H, C*H*–Nrbo), 2.165–2.247 (m, 2H, CH_2CO), 2.46–2.48 (m, 2H, $CH_2CH_2N(CH_2)_2$), 2.69–2.78 (m, 5H, $N(CH_2)_2$, H_β imide), 3.04–3.13 (m, 5H, $(CH_2)_2N$, H_β imide), 3.56–3.61 (t, 2H, NCH_2, *J* = 7.0 Hz), 3.85 (s, 3H, OCH_3), 4.34–4.39 (m, 1H, H_α imide), 6.27 (m, 1H, N*H*), 6.84–7.02 (m, 4H, Ph); ESI-MS ($M+H^+$) 497.4. Anal. ($C_{28}H_{40}N_4O_4$) C, H, N. **21**·HCl·3/2 H_2O: mp: 209–211 °C. Anal. ($C_{28}H_{40}N_4O_4$·HCl·3/2 H_2O) C, H, N.

5.1.9. *N*-((*S*)-1-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}pyrrolidin-2,5-dion-yl)-1-adamantanecarboxamide (22). Obtained from **14** as a white solid (0.89 g, a 64% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 9:1); mp: 126–128 °C; R_f = 0.61 (S_1), 0.27 (S_3); HPLC (t_R 2.39 min); $[\alpha]_D^{20} = -0.56°$ (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.65–1.90 (cluster, 16H, CH_2-Ada, $NCH_2CH_2CH_2$), 2.08 (s, 3H, C*H*-Ada), 2.54–2.54 (m, 2H, $CH_2CH_2N(CH_2)_2$), 2.73–2.81 (m, 5H, $N(CH_2)_2$, H_β imide), 3.04–3.13 (q, 1H, H_β imide), 3.17 (m, 4H, $(CH_2)_2N$), 3.60–3.65 (t, 2H, NCH_2, *J* = 6.9 Hz), 3.89 (s, 3H, OCH_3), 4.34–4.41 (m, 1H, H_α imide), 6.43–6.45 (m, 1H, N*H*, *J* = 5.8 Hz), 6.87–7.06 (m, 4H, Ph); ESI-MS ($M+H^+$) 523.5. Anal. ($C_{30}H_{42}N_4O_4$) C, H, N. **22**·HCl·3/2 H_2O: mp: 222–225 °C. Anal. ($C_{30}H_{42}N_4O_4$·HCl·3/2 H_2O) C, H, N.

5.1.10. *N*-((*S*)-1-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}pyrrolidin-2,5-dion-yl)cyclopentanecarboxamide (23). Obtained from **15** as a white solid (0.77 g, a 68 % yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 9:1); mp: 145–147 °C; R_f = 0.67 (S_1), 0.43 (S_3); HPLC (t_R 2.19 min); $[\alpha]_D^{20} = -0.76°$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO) δ (ppm) 1.49–1.71 (m, 13H, 9H cPt, $NCH_2CH_2CH_2$), 2.56–2.58 (m, 2H, $CH_2CH_2N(CH_2)_2$), 2.87–2.96 (q, 1H, H_β imide), 3.02–3.22 (m, 5H, $N(CH_2)_2$, H_β imide), 3.38–3.49 (m, 4H, $(CH_2)_2N$), 3.80–3.85 (d, 2H, NCH_2), 4.31–4.37 (m, 1H, H_α imide), 6.83–7.02 (m, 3H, Ph), 7.21–7.27 (t,1H, Ph), 8.58–8.60 (d, 1H, N*H*, *J* = 7.4 Hz); ESI-MS ($M+H^+$) 461.2. Anal. ($C_{24}H_{33}ClN_4O_3$) C, H, N. **23**·HCl·H_2O: mp: 152–154 °C. Anal. ($C_{24}H_{33}ClN_4O_3$·HCl·H_2O) C, H, N.

5.1.11. *N*-((*S*)-1-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}pyrrolidin-2,5-dion-3-yl)cyclohexanecarboxamide (24). Obtained from **16** as a white solid (0.84 g, a 71% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 9:1); mp: 155–157 °C; R_f = 0.66 (S_1), 0.38 (S_3); HPLC (t_R 2.32 min); $[\alpha]_D^{20} = -0.64°$ (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.18–1.89 (cluster, 14H, 10H, CH_2-cHex, $NCH_2CH_2CH_2$), 2.09–2.20 (m, 1H, C*H*-cHex), 2.40–2.45 (m, 2H, $CH_2CH_2N(CH_2)_2$), 2.58–2.61 (m, 4H, $N(CH_2)_2$), 2.70–2.78 (dd, 1H, H_β imide, *J* = 17.9 Hz, *J* = 5.9 Hz), 3.02–3.12 (q, 1H, H_β imide), 3.18–3.22 (m, 4H, $(CH_2)_2N$), 3.56–3.60 (m, 2H, NCH_2, *J* = 6.9 Hz), 4.26–4.33 (m, 1H, H_α imide), 8.34–8.37 (d, 1H, N*H*), 6.75–6.87 (m, 3H, Ph), 7.12–7.18 (t, 1H, Ph, *J* = 8.2 Hz); ESI-MS ($M+H^+$) 475.4. Anal. ($C_{25}H_{35}ClN_4O_3$) C, H, N. **24**·HCl: mp: 169–172 °C. Anal. ($C_{25}H_{35}ClN_4O_3$·HCl) C, H, N.

5.1.12. *N*-((*S*)-1-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}pyrrolidin-2,5-dion-3-yl)-2-(bicyclo[2.2.1]heptan-2-yl)acetamide (25). Obtained from **17** as a white solid (0.84 g, a 67% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 9:1); mp: 187–189 °C; R_f = 0.67 (S_1), 0.50 (S_3); HPLC (t_R 2.55 min) $[\alpha]_D^{20} = -1.06°$ (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.04–1.15 (m, 6H, CH_2–Nrbo), 1.26–1.69 (m, 6H, CH_2–Nrbo, $NCH_2CH_2CH_2$), 1.83–2.10 (m, 3H, C*H*–Nrbo),

2.17–2.25 (m, 2H, CH_2CO), 2.44–2.46 (m, 2H, $CH_2CH_2N(CH_2)_2$), 2.61 (s, 4H, $N(CH_2)_2$), 2.72–2.79 (dd, 1H, H_β imide, $J = 17.9$ Hz), 3.03–3.12 (dd, 1H, H_β imide, $J = 17.9$ Hz), 3.19–3.23 (m, 4H, $(CH_2)_2N$), 3.56–3.61 (t, 2H, NCH_2), 4.28–4.35 (m, 1H, H_α imide), 6.17 (s, 1H, N*H*), 6.75–6.87 (m, 3H, Ph), 7.12–7.18 (t, 1H, Ph); ESI-MS ($M+H^+$) 501.2. Anal. ($C_{27}H_{37}ClN_4O_3$) C, H, N. **25**·HCl·3/2 H_2O: mp: 164–166 °C. Anal. ($C_{27}H_{37}ClN_4O_3$·HCl·3/2 H_2O) C, H, N.

5.1.13. *N*-((*S*)-1-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}pyrrolidin-2,5-dion-yl)-1-adamantanecarboxamide (26). Obtained from **18** as a white solid (0.94 g, a 72% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 9:1); mp: 201–204 °C; $R_f = 0.68$ (S_1), 0.47 (S_3); HPLC (t_R 2.64 min); $[\alpha]_D^{20} = -0.81°$ (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.56–2.05 (cluster, 16H, 12H,CH_2-Ada, $NCH_2CH_2CH_2$), 2.05 (s, 3H, C*H*-Ada), 2.41–2.46 (m, 2H, $CH_2CH_2N(CH_2)_2$), 2.60 (s, 4H, $N(CH_2)_2$), 2.69–2.77 (dd, 1H H_β imide, $J = 17.9$ Hz, $J = 5.9$ Hz), 3.00–3.09 (q, 1H, H_β imide), 3.19–3.22 (m, 4H, $(CH_2)_2N$), 3.56–3.60 (t, 2H, NCH_2, $J = 7.0$ Hz), 4.22–4.29 (m, 1H, H_α imide), 6.27–6.29 (d, 1H, N*H*, $J = 5.4$ Hz), 6.75–6.86 (m, 3H, Ph), 7.12–7.18 (t, 1H, Ph, $J = 8.1$ Hz); ESI-MS ($M+H^+$) 527.3. Anal. ($C_{29}H_{39}ClN_4O_3$) C, H, N. **26**·HCl: mp: 185–187 °C. Anal. ($C_{29}H_{39}ClN_4O_3$·HCl) C, H, N.

5.1.14. 1-(*tert*-Butoxycarbonyl)-*N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}-L-prolinamide (27). Obtained from Boc-L-proline (3.44 g, 16 mmol), 4-[4-(2-methoxyphenyl)piperazin-1-yl]butylamine (3.82 g, 14.5 mmol), and BOP (7.07 g, 16 mmol) in CH_2Cl_2 (100 mL) in the presence of TEA (4.46 mL, 32 mmol) as a yellow oil (5.7 g, a 76% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 9:1); $R_f = 0.92$ (S_1), 0.39 (S_2); HPLC (t_R 2.03 min); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.32 (s, 9H, $C(CH_3)_3$), 1.37–1.44 (m, 4H, $NHCH_2CH_2CH_2$), 1.71–1.78 (m, 3H, 1H_β Pro, 2H_γ Pro), 2.06–2.08 (m, 1H, H_β Pro), 2.52–2.69 (m, 2H, $CH_2CH_2N(CH_2)_2$), 2.99–3.12 (m, 6H, $N(CH_2)_2$, $NHCH_2$), 3.21–3.36 (m, 6H, $(CH_2)_2N$, 2H_δ Pro), 3.75 (s, 3H, OCH_3), 3.96–4.02 (m, 1H, H_α Pro), 6.82–6.96 (m, 4H, Ph), 7.77–7.83 (m, 1H, N*H*); ESI-MS ($M+H^+$) 460.97. Anal. ($C_{25}H_{40}N_4O_4$) C, H, N.

5.1.15. 1-(*tert*-Butoxycarbonyl)-*N*-{4-[4-(3-chlorophenyl)piperazin-1-yl]butyl}-L-prolinamide (28). Obtained from Boc-L-proline (3.44 g, 16 mmol), 4-[4-(3-chlorophenyl)piperazin-1-yl]butylamine (3.88 g, 14.5 mmol), and BOP (7.07 g, 16 mmol) in CH_2Cl_2 (100 mL) in the presence of TEA (4.46 mL, 32 mmol) as a white solid (5.3 g, a 72% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 9:1); mp: 132–134 °C; $R_f = 0.91$ (S_1), 0.39 (S_2); HPLC (t_R 2.32 min); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.46 (s, 9H, $C(CH_3)_3$), 1.54–1.56 (m, 4H, $NHCH_2CH_2CH_2$), 1.87–1.95 (m, 4H, 2H_β, 2H_γ Pro), 2.39–2.41 (m, 2H, $CH_2CH_2N(CH_2)_2$), 2.57–2.60 (m, 4H, $N(CH_2)_2$), 3.18–3.21 (m, 4H, $(CH_2)_2N$), 3.24–3.42 (m, 4H, $NHCH_2$, 2H_δ Pro), 4.23 (s, 1H, H_α Pro), 6.75–6.81 (m, 2H, Ph), 6.85–6.87 (t, 1H, Ph, $J = 8.0$ Hz), 7.12–7.18 (m, 1H, Ph); ESI-MS ($M+H^+$) 465.1. Anal. ($C_{24}H_{37}ClN_4O_3$) C, H, N.

5.1.16. General procedure D: 1-(cycloalkylcarbonyl)-*N*-{4-[4-arylpiperazin-1-yl]butyl}-L-prolinamides 29–34. Compound **27** or **28** was allowed to react with TFA for 60 min, and all the volatilities were removed under reduced pressure. Then deprotected compounds trifluoroacetates **27a** and **28a** (2.3 mmol) were coupled with cycloalkane carboxylic acid (2.5 mmol) using BOP (2.5 mmol) in the presence of TEA (8.5 mmol) in CH_2Cl_2 (40mL). Reaction mixture was stirred for 12 h and the solvent was removed under reduced pressure. Then the oily residue was dissolved in EtOAc, washed with a saturated $NaHCO_3$ solution, water, and brine, dried over Na_2SO_4, and finally was concentrated under vacuum. The crude residue was purified by column chromatography as detailed below to give compounds **29**–**34**.

5.1.17. 1-(Cyclohexylcarbonyl)-*N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}-L-prolinamide (29). Obtained from a trifluoroacetate of **27** (**27a**, 2.3 mmol) and cyclohexane carboxylic acid as a clear oil (0.8 g, a 74% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 9:1); $R_f = 0.73$ (S_1), 0.18 (S_2); HPLC (t_R 2.17 min); $[\alpha]_D^{20} = -4.6°$ (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.19–1.30 (m, 4H, $NHCH_2CH_2CH_2$), 1.46–1.54 (m, 5H, CH_2-cHex, H_γ Pro), 1.71–1.82 (m, 7H, CH_2-cHex, H_γ Pro), 1.95–1.98 (m, 1H, C*H*-cHex), 2.34–2.43 (m, 4H, $CH_2CH_2N(CH_2)_2$, 2H_β Pro), 2.67 (s, 4H, $N(CH_2)_2$), 3.10 (s, 4H, $(CH_2)_2N$), 3.21–3.25 (m, 2H, $NHCH_2$), 3.46–3.58 (m, 2H, H_δ Pro), 3.85 (s, 3H, OCH_3), 4.54–4.58 (dd, 1H, H_α Pro, $J = 7.95$, 1.8 Hz), 6.84–7.01 (m, 4H, Ph), 7.26–7.28 (m, 1H, N*H*); ESI-MS ($M+H^+$) 470.99. Anal. ($C_{27}H_{42}N_4O_3$) C, H, N. **29**·HCl: mp: 120–122 °C. Anal. ($C_{27}H_{43}ClN_4O_3$·HCl) C, H, N.

5.1.18. 1-((Bicyclo[2.2.1]heptan-2-yl)acetyl)-*N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}-L-prolinamide (30). Obtained from **27a** (2.3 mmol) and norbornane-2-acetic acid as a white solid (0.82 g, 72% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 99:1); mp: 153–155 °C; $R_f = 0.73$ (S_1), 0.18 (S_2); HPLC (t_R 2.45 min); $[\alpha]_D^{20} = -6.4°$ (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.00–1.21 (m, 4H, $NHCH_2CH_2CH_2$), 1.22–2.23 (m, 17H, cluster Nrbo, 2H_β Pro, 2H_γ Pro), 3.11–3.22 (m, 2H, $CH_2CH_2N(CH_2)_2$), 3.43–3.67 (m, 8H, $N(CH_2)_2$, $(CH_2)_2N$), 3.95–4.12 (m, 7H, $NHCH_2$, OCH_3, 2H_δ Pro), 4.52–4.54 (m, 1H, H_α Pro), 7.00–7.42 (m, 2H, Ph), 7.36–7.42 (m, 1H, Ph), 7.66 (m, 1H, Ph), 8.01 (s, 1H, N*H*); ESI-MS ($M+H^+$) 497.1. Anal. ($C_{29}H_{44}N_4O_3$) C, H, N. **30**·HCl: mp: 162–164 °C. Anal. ($C_{29}H_{45}ClN_4O_3$·HCl) C, H, N.

5.1.19. 1-(Adamantylcarbonyl)-*N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}-L-prolinamide (31). Obtained from **27a** (2.3 mmol) and adamantane 1-carboxylic acid as a pale white oil (0.86 g, a 72% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 99:1); $R_f = 0.76$ (S_1), 0.17 (S_2); HPLC (t_R 2.53 min); $[\alpha]_D^{20} = -5.8°$ (*c* 0.5, MeOH); ^{1}H NMR (DMSO) δ (ppm) 1.43 (m, 4H, $NHCH_2CH_2CH_2$), 1.68–1.97 (m, 19H, cluster Ada, 2H_β Pro, 2H_γ Pro), 2.30–2.32 (m, 2H, $CH_2CH_2N(CH_2)_2$), 2.51–2.52 (m, 4H, $N(CH_2)_2$), 2.96–3.13 (m, 6H, $(CH_2)_2N$, $NHCH_2$), 3.70 (s, 2H, H_δ Pro), 3.77 (s, 3H, OCH_3), 4.31 (s, 1H, H_α Pro), 6.87–6.97 (m, 4H, Ph), 7.65 (s, 1H, N*H*);

ESI-MS ($M+H^+$) 522.95. Anal. ($C_{31}H_{46}N_4O_3$) C, H, N. **31**·HCl: mp: 172–174 °C. Anal. ($C_{31}H_{47}ClN_4O_3$·HCl) C, H, N.

5.1.20. 1-(Cyclohexylcarbonyl)-*N*-{4-[4-(3-chlorophenyl)piperazin-1-yl]butyl}-L-prolinamide (32). Obtained from trifluoroacetate of **28** (**28a**, 2 mmol) and cyclohexane carboxylic acid as a transparent oil (0.78 g, a 82% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 99:1); $R_f = 0.76$ (S_1), 0.17 (S_2); HPLC (t_R 2.43 min); $[\alpha]_D^{20} = -5.6°$ (*c* 0.5, MeOH); ^1H NMR ($CDCl_3$) δ (ppm) 1.20–1.42 (m, 4H, $NHCH_2CH_2CH_2$), 1.44–1.52 (m, 4H, CH_2-cHex), 1.71–1.84 (m, 6H, CH_2-cHex), 1.931–2.25 (m, 3H, H_β, $2H_\gamma$ Pro), 2.33–2.46 (m, 4H, CH_2CH_2 $N(CH_2)_2$, H_β Pro, CH-cHex), 2.56–2.59 (m, 4H, $N(CH_2)_2$), 3.17–3.25 (m, 6H, $(CH_2)_2N$, $NHCH_2$), 3.43–3.60 (m, 2H, H_δ Pro), 4.54–4.57 (dd, 1H, H_α Pro, $J = 7.95$, 1.8 Hz), 6.75–6.85 (m, 3H, Ph), 7.12–7.17 (t, 1H, Ph, $J = 8.0$ Hz), 7.26–7.27 (m, 1H, NH, $J = 7.4$ Hz); ESI-MS ($M+H^+$) 475.15. Anal. ($C_{26}H_{39}ClN_4O_2$) C, H, N. **32**·HCl: mp: 128–130 °C. Anal. ($C_{26}H_{40}Cl_2N_4O_2$·HCl) C, H, N.

5.1.21. 1-((Bicyclo[2.2.1]heptan-2-yl)acetyl)-*N*-{4-[4-(3-chlorophenyl)piperazin-1-yl]butyl}-L-prolinamide (33). Obtained from **28a** (2 mmol) and norbornane-2-acetic acid as a colorless oil (0.68 g, a 68% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 99:1); $R_f = 0.94$ (S_1), 0.29 (S_2); HPLC (t_R 2.63 min); $[\alpha]_D^{20} = -7.4°$ (*c* 0.5, MeOH); ^1H NMR ($CDCl_3$) δ (ppm) 1.05–1.31 (m, 4H, $NHCH_2CH_2CH_2$), 1.47–1.54 (m, 8H, CH_2–Nrbo), 1.74–1.84 (m, 2H, H_γ Pro), 1.94–1.97 (m, 3H, CH–Nrbo), 2.10–2.28 (m, 3H, H_β Pro, CH_2CO), 2.41–2.47 (m, 3H, $CH_2CH_2N(CH_2)_2$, H_β Pro), 2.59–2.63 (m, 4H, $N(CH_2)_2$), 3.19–3.54 (m, 6H, $(CH_2)_2N$, $NHCH_2$), 3.39–3.56 (m, 2H, H_δ Pro), 4.55–4.57 (d, 1H, H_α Pro, $J = 7.4$ Hz), 6.75–6.87 (m, 3H, Ph), 7.12–7.18 (t, 1H, Ph, $J = 8.2$ Hz), 7.27–7.31 (d, 1H, NH); ESI-MS ($M+H^+$) 501.1. Anal. ($C_{28}H_{41}ClN_4O_2$) C, H, N. **33**·HCl: mp: 221–222 °C. Anal. ($C_{28}H_{42}Cl_2N_4O_2$·HCl) C, H, N.

5.1.22. 1-(Adamantylcarbonyl)-*N*-{4-[4-(3-chlorophenyl)piperazin-1-yl]butyl}-L-prolinamide (34). Obtained from **28a** (2 mmol) and adamantane-1-carboxylic acid as a white solid (0.81 g, a 78% yield); chromatography (SiO_2, CH_2Cl_2/MeOH, 99:1); mp: 128–130 °C; $R_f = 0.88$ (S_1), 0.38 (S_2); HPLC (t_R 2.80 min); $[\alpha]_D^{20} = -7.6°$ (*c* 0.5, MeOH); ^1H NMR ($CDCl_3$) δ (ppm) 1.42–1.56 (m, 4H, $NHCH_2CH_2CH_2$), 1.67–1.98 (m, 15H, cluster Ada, H_β, $2H_\gamma$ Pro), 2.04 (s, 3H, CH-Ada), 2.18–2.25 (m, 1H, H_β Pro), 2.39–2.43 (m, 2H, $CH_2CH_2N(CH_2)_2$), 2.57–2.61 (m, 4H, $N(CH_2)_2$), 3.18–3.27 (m, 6H, $(CH_2)_2N$, $NHCH_2$), 3.66–3.85 (m, 2H, H_δ Pro), 4.59–4.63 (m, 1H, H_α Pro, $J = 7.95$, 3.3 Hz), 6.75–6.86 (m, 3H, Ph, $J = 8.1$ Hz), 7.12–7.17 (t, 1H, Ph, $J = 8.0$ Hz); ESI-MS ($M+H^+$) 526.98. Anal. ($C_{30}H_{43}ClN_4O_2$) C, H, N. **34**·HCl: mp: 241–243 °C. Anal. ($C_{30}H_{44}Cl_2N_4O_2$·HCl) C, H, N.

5.2. In vitro studies

5.2.1. 5-HT_{1A}, 5-HT_{2A} receptor binding assays. Radioligand binding experiments were conducted for 5-HT_{1A} receptors in the hippocampus of rat brain, and for 5-HT_{2A} receptors in the cortex according to the published procedures.[45,46] The following radioligands were used: [^{3}H]-8-OH-DPAT (190 Ci/mmol, Amersham), [^{3}H]-ketanserin (60 Ci/mmol, NEN Chemicals) for 5-HT_{1A}- and 5-HT_{2A} receptors, respectively. K_i values were determined on the basis of at least three competition experiments in which 8–10 drug concentrations (10^{-10}–10^{-3} M) were run in triplicate.

5.2.2. D_2 dopaminergic receptor binding assay. Competition binding studies were performed on rat striatal membranes, prepared according to the previously published procedure. The assay was carried out in a 96-well filter plate (containing glass fiber type C, Millipore), presoaked with 100 μL of an ice-cold 50 mM potassium phosphate buffer (pH 7.4), and filtered using a Millipore Vacuum Manifold prior to sample addition. 150 μL aliquots of striatal membrane preparations, 50 μL of the radioligand ([^{3}H]-spiperone, 15.70 Ci/mmol, NEN Chemicals), and either 50 μL of the buffer (for total binding assay) or 50 μL of (±)-butaclamol (5 μM) to determine the unspecific binding, or 50 μL of the compounds to be tested, were added to each well. Additionally, to prevent [^{3}H]-spiperone binding to 5-HT_{2A} receptors, ketanserin (50 nM) was included in the assay buffer. After incubation at 37 °C for 30 min, the binding reaction was terminated by vacuum filtration and washed three times with 200 μL of the buffer. Radioactivity was determined by a liquid scintillation counting using Beckman LS 6500 apparatus.

The estimated K_i values were determined from three competition binding experiments in which 2 drug concentrations (0.1 and 1 μM), each run in triplicate, were used. The Cheng and Prusoff equation was used for K_i calculations.[47]

5.3. In vivo experiments

The experiments were performed on male Wistar rats (280–310 g), and male Albino Swiss (24–28 g) or C57BL/6J (22–23 g) mice (the latter strain was used in the tail suspension test only). The animals were kept at a room temperature of 20 ± 1 °C, and had free access to food (standard laboratory pellets, LSM) and tap water before the experiment. All the investigations were conducted in the light phase, on a natural day–night cycle (from March to July), between 9 am and 2 pm. All the experimental procedures were approved by the Local Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences in Kraków. 8-Hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT, Tocris) and (±)-2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride ((±)-DOI, Research Biochemical, Inc) were dissolved in saline, *N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY 100635, synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland) was used as aqueous solutions. The compounds under study were suspended in a 1% aqueous solution of Tween 80. 8-OH-DPAT and WAY 100635 were injected subcutaneously (sc), (±)-DOI and the tested compounds

were given intraperitoneally (ip) in a volume of 2 mL/kg (rats) and 10 mL/kg (mice). Each experimental group consisted of six to ten animals per dose and all the animals were used only once.

5.3.1. Body temperature in mice. Effects of the tested compounds given alone on the rectal body temperature in mice (measured with an Ellab thermometer) were recorded 30, 60, 90, and 120 min after their administration. In a separate experiment, the effect of WAY 100635 (0.1 mg/kg) on the hypothermia induced by compounds **19**–**23**, and **29**–**34** or 8-OH-DPAT, was tested. WAY 100635 was administered 15 min before the compounds or 8-OH-DPAT and rectal body temperature was recorded 30 and 60 min after injection of the tested compounds. In another experiment, effects of **24**–**26** or WAY 100635 (which did not change mouse body temperature) on the 8-OH-DPAT (5 mg/kg)-induced hypothermia were assessed. The tested compounds and WAY 100635 were administered at 45 and 15 min, respectively, before 8-OH-DPAT, and rectal body temperature was measured 15, 30, 45, and 60 min after 8-OH-DPAT injection. The results were expressed as a change in body temperature (Δt) with respect to the basal body temperature measured at the beginning of the experiment.

5.3.2. Lower lip retraction (LLR) in rats. LLR was assessed according to the method described by Berendsen et al.[27,28] The rats were individually placed in cages ($30 \times 25 \times 25$ cm) and were scored three times (at 15, 30, and 45 min) after administration of the tested compounds or 8-OH-DPAT as follows: 0, lower incisors not visible; 0.5, partly visible; 1, completely visible. Total maximum scores amounted to 3 for each rat. In a separate experiment, the effect of the tested compounds or WAY 100635 on the LLR induced by 8-OH-DPAT (1 mg/kg) was tested. The compounds and WAY 100635 were administered at 45 and 15 min, respectively, before 8-OH-DPAT, and the animals were scored 15, 30, and 45 min after 8-OH-DPAT administration.

5.3.3. Head twitch response in mice. To habituate mice to the experimental environment, each animal was randomly transferred to a 12 cm (diameter) $\times$ 20 cm (height) glass cage lined with sawdust at 30 min before treatment. Head twitches in mice were induced by (±)-DOI (2.5 mg/kg). Immediately after the treatment, the number of head twitches was counted throughout 20 min.[30,31] The tested compounds were administered 60 min before (±)-DOI.

5.3.4. Four-plate test in mice. The box was made of opaque plastic and was rectangular ($25 \times 18 \times 16$ cm) in shape. The floor was covered with four rectangular metal plates (11×8 cm), separated by a 4-mm gap. The plates were connected to a source of continuous current which enabled a 120 V difference of potentials between two adjacent plates for 0.5 s when the experimenter pressed the switch. Individual mice were placed gently onto the plate and were allowed to explore for 15 s. Afterward, each time a mouse passed from one plate to another, the experimenter electrified the whole floor which evoked a visible flight reaction of an animal. If the animal continued running, it received no new shocks for the following 3 s. Episodes of punished crossing were counted for 60 s.[32]

5.3.5. Forced swimming test in mice. The experiment was carried out according to the method of Porsolt et al.[33] Briefly, mice were individually placed in a glass cylinder (25 cm high, 10 cm in diameter) containing 6 cm of water maintained at 23–25 °C, and were left therein for 6 min. A mouse was regarded as immobile when it remained floating in the water, making only small movements to keep its head above it. The total duration of immobility was recorded during the last 4 min of a 6-min test session.

5.3.6. Tail suspension test in mice. The experiment was carried out on C57BL/6J mice according to the method of Steru et al.[34] The mice were individually hung by the tail using an adhesive tape placed approximately 1 cm from the tip of the tail attached to a wooden stick and hanging 75 cm above the floor. The total duration of immobility was scored manually during a 6-min test session. Immobility was defined as the absence of any limb or body movements, except for those caused by respiration.

5.3.7. Locomotor activity in mice. The spontaneous locomotor activity of mice was recorded in photoresistor actometers (24 cm in diameter) illuminated by two light beams, which were connected to a counter for the recording of light-beam interruptions. The mice were placed individually in the actometers, and the number of crossings of the light beams was counted twice: during the first 6 min, that is, at the time equal to the observation period in the forced swimming test, and during 30-min experimental sessions.

5.4. Statistics

The obtained data were analyzed by a one-way analysis of variance, followed by Dunnett's test (when only one drug was given) or by the Newman–Keuls test (when two drugs were administered). ID_{50} values were calculated by the method of Litchfield and Wilcoxon.

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Supplementary data

Spectroscopic data on intermediate compounds **3**–**18** and six tables showing the effect of the investigated compounds on the body temperature of mice, the effect of WAY 100635 on the hypothermia induced by **19**–**23** and **29**–**34** in mice, the effect of **24**–**26** on the 8-OH-DPAT-induced hypothermia in mice, the effect of the

investigated compounds on the 8-OH-DPAT-induced LLR in rats, the effect of **21**,**23**–**26**, **33**, and ketanserin on the ((±)-DOI-induced head twitch response in mice, and the effect of **19**, **20**, and imipramine on the locomotor activity of mice. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.02.018.

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