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Original article

Synthesis and biological evaluation of novel pyrrolidine-2,5-dione derivatives as potential antidepressant agents. Part 1

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ABSTRACT

A series of 3-(1*H*-indol-3-yl)pyrrolidine-2,5-dione derivatives was synthesized and their biological activity was evaluated. The chemical structures of the newly prepared compounds were confirmed by ^{1}H NMR, ^{13}C NMR and ESI-HRMS spectra data. All tested compounds proved to be potent 5-HT_{1A} receptor and serotonin transporter protein (SERT) ligands. Among them, compounds **15**, **18**, **19** and **30** showed significant affinity for 5-HT_{1A} and SERT. Computer docking simulations carried out for compounds **15**, **31** and **32** to models of 5-HT_{1A} receptor and SERT confirm the results of biological tests. Due to high affinity for the 5-HT_{1A} receptor and moderate affinity for SERT, compounds **31**, **32**, **35**, and **37** were evaluated for their affinity for D_{2L}, 5-HT_6, 5-HT_7 and 5-HT_{2A} receptors. *In vivo* tests, in turn, resulted in determining the functional activity of compounds **15**, **18**, **19** and **30** to the 5-HT_{1A} receptor. The results of these tests indicate that all of the ligands possess properties characteristic of 5-HT_{1A} receptor agonists.

1. Introduction

The data obtained from epidemiological research indicate that the occurrence rate of clinical depression cases in today's society stands at ca. 7%. The morbidity of this disorder in the scale of the entire population reaches 15% [1]. According to the World Health Organization, the number of depression cases is continuously on the increase and by the year 2020 it is expected to be the second most common disorder in the world, after ischemic heart disease [2].

Ever since serotonin (5-hydroxytryptamine or 5-HT) was identified as a neurotransmitter in the central nervous system, there has been considerable interest in its role in the pathomechanism of affective disorders, such as depression, anxiety or schizophrenia. What was considered a breakthrough in the pharmacotherapy of depressive disorders was the introduction of selective serotonin reuptake inhibitors (SSRIs), which assume the serotoninergic system as their molecular target [3]. In spite of this, however, the efficiency rate is not satisfying; 60–70% of patients do not experience remission, and 30–40% do not react to pharmacological treatment. This points to the need for investigating new, more efficient antidepressants [4,5]. In addition, SSRI-type medicines need to be administered for a longer period of time (2–4 weeks) before a considerable improvement of the patient's mood may be observed. This is known as the latency period [6], which is related to

Abbreviations: SERT, serotonin transporter; SAR, structure–activity relationship; 5-HT, serotonin; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; K_i, inhibitor constant; SSRIs, selective serotonin inhibitors; CNS, central nervous system; NMR, nuclear magnetic resonance; HRMS, high resolution mass spectroscopy; DMSO, dimethylsulfoxide; PPI, derivatives of 3-(piperidine-4-yl)-1*H*-indole; THPI, derivatives of 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole; HEK293, human embryonic kidney cell line 293.

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adaptation processes in the central nervous system, required for the strengthening of serotonin transmission through postsynaptic receptors [7]. There is, therefore, a motivated need for investigating new medications, which would be free of a prolonged latency period. The therapeutic effect that can be observed after the administration of SSRI-type drugs is the sum of neurochemical alterations taking place in the brain, including desensitization of 5-HT_{1A} autoreceptors, down-regulation of receptors for neurotransmitters, changes in signal transmission, neurotropism, mobilization, and an increase in neurogenesis in the hippocampus [8]. Test results point to the crucial role of desensitization of somatodendritic 5-HT_{1A} autoreceptors, located in the raphe nuclei in the brainstem, which is triggered by an increased concentration of serotonin in the synaptic cleft [2,9].

The 5-HT_{1A} receptor can function both as a presynaptic receptor (autoreceptor) and a postsynaptic receptor. As an autoreceptor, it is located in neurons and dendrites of brainstem raphe nuclei. Its stimulation results in the inhibition of endogenous serotonin secretion into the synaptic cleft, and consequently, in a decreased level of transmission in serotoninergic neurons. This helps to regulate the activity of the serotoninergic system, stimulated tonically in the brainstem. On the other hand, stimulation of 5-HT_{1A} postsynaptic receptors, located somatodenritically in nerve terminals in cortico-limbic areas of the CNS, leads to enhanced transmission in serotoninergic neurons, which inhibits other neurons located in various areas of the brain. This, in turn, regulates numerous physiological processes, including psycho-emotional, autonomic, sensory and motor processes [2,10,11].

Many 5-HT_{1A} receptor agonists have currently entered various phases of clinical trials or have already been registered as medications. Their pharmacological activity is not limited to depression therapy, but extends to other indications, such as anxiety (e.g. osemozotan – phase II), schizophrenia (e.g. bifeprunox – phase III), pain (e.g. befiradol – phase II) or neurodegenerative disorders (e.g. piclozotan – phase III) [3]. The results of clinical trials for 5-HT_{1A} receptor agonists, such as naluzotan (PRX-00023), osemozotan and vilazodone (Fig. 1) point to their antidepressant activity, as well as to the enhanced efficacy of other, simultaneously administered antidepressants [4]. A number of anxiolytics, such as buspirone, which are 5-HT_{1A} receptor agonists or partial agonists, manifest antidepressant activity that result from an interaction with 5-HT_{1A} postsynaptic receptors [12].

In 1993, a hypothesis was put forward stating that co-administration of 5-HT_{1A} receptor antagonists and SSRI-type medicines should enhance the effect of antidepressants through the acceleration of desensitization and, as a consequence, the functioning of 5-HT_{1A} autoreceptors [13]. The hypothesis was confirmed by research results. It was shown that simultaneous administration of an SSRI and a 5-HT_{1A} receptor partial antagonist, Pindolol, leads to the strengthening of antidepressant activity of the former [2]. This area of interest expanded to embrace molecules possessing affinity for both the 5-HT_{1A} receptor and the SERT transporter, i.e. dual binding compounds. This resulted in the synthesis of many ligands with that particular functional profile (e.g. Lu-AA21004 – phase III) [14–25].

Nevertheless, 5-HT_{1A} receptor antagonists/SSRI may block pre- and post-synaptic receptors non-selectively, which is an unfavorable action from the perspective of depression therapy [26]. The results of *in vivo* tests are not unanimous here, since there are indications that blocking postsynaptic receptors may not have a considerable impact on the decreased efficacy of the antidepressant activity of such compounds, but at the same time other data suggest that this might actually be the case [27,28]. For this reason, a more promising direction of drug research seems to point to dual binding compounds that are agonists of 5-the HT_{1A} receptor/SSRI. This is due to the potential of such compounds to accelerate autoreceptor desensitization (as well as down-regulation) and at the same time to directly stimulate serotoninergic neurons through postsynaptic interaction and increased concentration of endogenous serotonin in the synaptic cleft [29]. Additionally, the sensitivity of postsynaptic receptors is not reduced even after multiple exposures to 5-HT_{1A} receptor agonists/SSRI, contrary to what takes place in the case of 5-HT_{1A} receptor antagonists/SSRI [29,30]. The validity of such an approach was confirmed by the registration of vilazodone (Fig. 1), the first dual binding SSRI+ (5-HT_{1A} receptor agonist/SSRI) medication, registered in January 2011 in the USA as the antidepressant Viibryd™, the efficiency rate of which is comparable to traditionally used SSRIs [31,32]. This is the cause of our interest in this particular direction of investigating new medicines.

Our earlier research resulted in the synthesis of a series of pyrido[1,2-*c*]pyrimidine derivatives (**I**, Fig. 2), characterized by dual binding to SERT and pre- or post-synaptic 5-HT_{1A} receptor agonists [33–35]. The research aim of the current project was the synthesis of a series of pyrrolidine-2,5-dione derivatives with dual binding to the 5-HT_{1A} receptor and SERT protein. The structure of synthesized compounds **15**–**38** was based on the structure of previously described pyrido[1,2-*c*]pyrimidine derivatives, as well as on numerous other structures with a confirmed high affinity for the 5-HT_{1A} receptor and SERT (Fig. 2).

A number of modifications to the leading structure of pyrrolidine-2,5-dione derivatives were designed, largely owing to the results of the tests concerning the relationship between binding and structure for compounds with a high affinity for the 5-HT_{1A} receptor and/or SERT (Fig. 2). The molecule was divided into areas A, B and C. Areas B and C were subjected to numerous alterations.

Area A includes the cyclic imide moiety. According to the test results, the presence of this group for the purpose of obtaining a compound with a high affinity for the 5-HT_{1A} receptor and SERT is fully justified [38,40–42]. Area B consists of an alkyl linker. We have introduced a 2-, 3- or 4-carbon linker, depending on different test results concerning the optimal length of the chain for the activity of the compound. It is currently assumed that what influences the interaction between the compound and the receptor is not the hydrophobic nature of the linker, but rather the distance and spatial alignment of the molecule (flexibility or rigidity) [43,44]. Residues

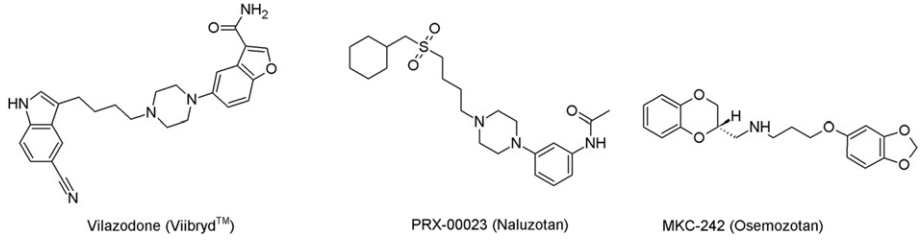


Fig. 1. 5-HT_{1A} receptor agonists.

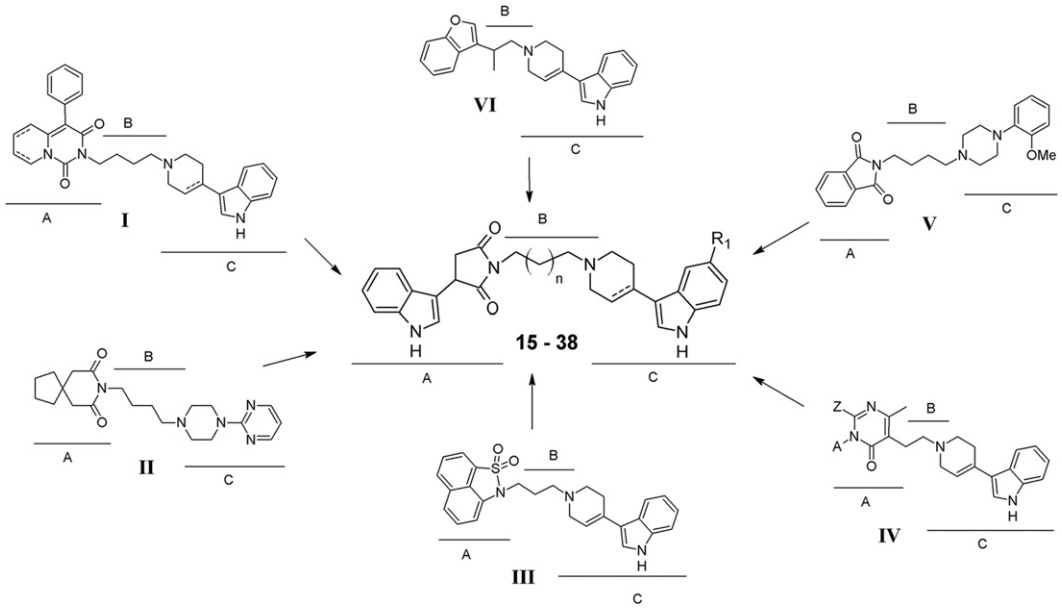


Fig. 2. Compounds with confirmed affinity for SERT and/or 5-HT_{1A} receptor, which were used for designing the leading structure. Compound **I** – pyrido[1,2-*c*]pyrimidine derivatives [34]; compound **II** – buspirone; compound **III** – naphthalenesultam derivatives [36]; compound **IV** – 1,2,3,6-tetrahydropyridine-4-yl-1*H*-indole derivatives [37]; compound **V** – NAN190 [38]; compound **VI** – 3-indoletetrahydropyridine derivatives [39], $n = 0, 1, 2$.

of 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole or 3-(piperidin-4-yl)-1*H*-indole, a pharmacophore for binding to SERT, constitutes area C. Its high affinity and key role for binding to the 5-HT_{1A} receptor and SERT has been repeatedly confirmed by test results. This emerges from its structure, which is analogous to the structure of a serotonin molecule. We have introduced a choice of different electron-acceptor functional groups at the indole 5 position = R_1, which allowed us to assess their impact on final compound binding to the receptor and the transporter [45,46].

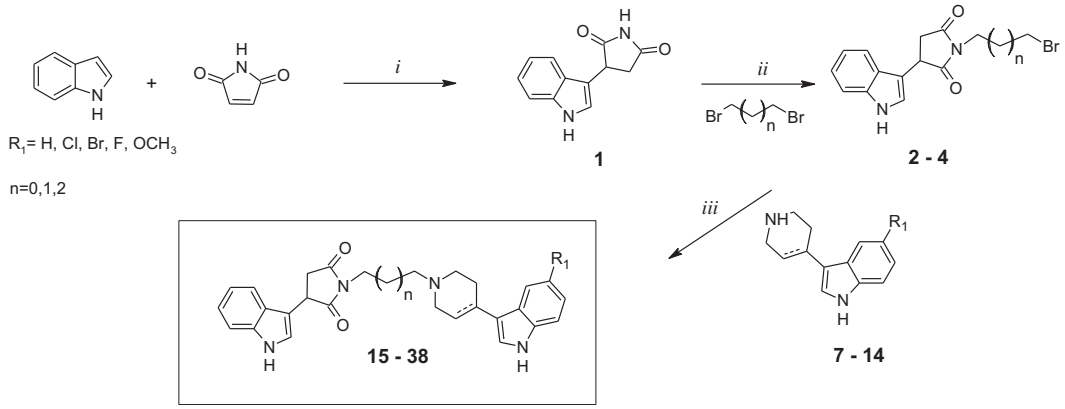
2. Results and discussion

2.1. Chemistry

Final compounds **15**–**38** were obtained via multi-step synthesis, according to Scheme 1. In order to carry out the process, three series of synthons were necessary: *N*-bromobutyl, *N*-bromopropyl and *N*-bromoethyl derivatives of 3-(1*H*-indol-3-yl)pyrrolidine-2,5-dione (**2**–**4**), derivatives of 3-(piperidine-4-yl)-1*H*-indole (**7**, **8** and **14**; PPI) and derivatives of 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (**9**–**13**; THPI).

The first stage resulted in obtaining product **1** by means of condensation of maleimide with indole in the presence of glacial acetic acid. This is a modification of the Michael addition, described by Macor et al. [47–49]. Resulting derivatives were subjected to the reaction of N-alkylation with 1,4-dibromobutane, 1,3-dibromopropane and 1,2-dibromoethane respectively, in the presence of potassium carbonate in acetone. The alkylation agent (an appropriate dibromoalkyl) was used in a large excess (5–9 mol) in order to avoid the reaction of disubstitution. Substrates **2**–**4** were obtained in high yields (Scheme 1).

Compounds **7** and **8** were obtained by means of two-stage synthesis. Condensation of *N*-benzyl-4-piperidone with 1*H*-indole and 5-methoxy-1*H*-indole, respectively, resulted in derivatives of *N*-benzyl-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (**5**–**6**), which were subsequently subjected to catalytic reduction of the double bond with hydrogen and, simultaneously, debenzylation. Derivatives of 3-(piperidin-4-yl)-1*H*-indole (**7**–**8**; PPI) were thus synthesized [51–53]. The second series of substrates (THPI) was synthesized via condensation of 4-piperidone with appropriate derivatives of 1*H*-indole (R_1 = H, Cl, Br, F, OCH_3) under anhydrous conditions in a hydrogenic atmosphere (Scheme 2). This reaction



Scheme 1. The synthesis pathways of the investigated compounds. Reagents and conditions: (i) CH_3COOH (ii) acetone, K_2CO_3 (iii) CH_3CN, K_2CO_3, KI.

resulted in obtaining compounds **9**–**13**, derivatives of 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole, as a residue, according to the described procedure [50,51]. The following stage of the synthesis involved catalytic reduction of the double bond (**13**) in the residue of 5-fluoro-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole with hydrogen in an autoclave. Compound **14** was obtained in the presence of 10% Pd/C catalyst [36].

Final ligands **15**–**38** were synthesized as a result of the condensation of compounds **7**–**14** with synthons **2**–**4** (Scheme 1), according to previously described procedures [33]. All final compounds **15**–**38**, in order to obtain analytic samples, were purified by flash or gravity chromatography using a mixture of dichloromethane and methanol in various proportions as the eluent or via crystallization from ethyl ether. The chemical structure of compounds **2**–**4** and **15**–**38** was verified by ^{1}H and ^{13}C NMR and HRMS tests. For compounds **15**–**38**, bases that were insoluble in water were obtained and, for the purpose of biological tests, were converted into hydrochlorides soluble in water after prior dissolution in an analytic amount of DMSO. Synthesis resulted in a racemic mixture of the compounds and as such was further examined.

2.2. Conformational analysis

Interpretation of the ^{1}H NMR spectra of the tested compounds showed the characteristics of conformation (see 4.1 Experimental section and Supplementary materials). The THPI series system is characterized by the dynamism of the structure and performs the fast conformational switch type chair–chair; its spatial orientation is related to the possibility of adverse interactions (due to crowding of atoms) with carbon linker protons. As far as the PPI series is concerned, the dynamism of conformational changes is much smaller than for the THPI series, which is shown as a clear distinction between the axial and equatorial protons (CaH, CbH, CcH, CdH, CeH) of the piperidine ring in a series of PPI as opposed to the protons of the THPI series. Atoms lying in the planar of the ring (E) are less shielded by the surrounding electron density than those lying above or below the cyclic structure (A); this rule is maintained in the spectra of the compounds of the PPI series. After analyzing the ^{1}H NMR spectra of the tested compounds, it can be stated that the length of the aliphatic chain is important for free rotation of the moiety of areas A and B, C (Fig. 2) relative to each other. For compounds with a four-carbon chain, the rotation of bonds in the aliphatic chain is free, which is visible in the ^{1}H NMR spectra as distinct triplets ($C1^{x}H_2$, $C4^{x}H_2$) or quintets ($C2^{x}H_2$, $C3^{x}H_2$). In terms of the derivatives with a two-carbon chain, this free rotation is stopped, therefore the protons of the aliphatic chain differentiate and give signals in the form of multiplets ($C1^{x}H_2$, $C2^{x}H_2$). These differences in the dynamism of conformational changes in the investigated compounds may also affect their affinity for different receptors and three-dimensional orientation of the molecule in the binding pocket.

2.3. Biological activity

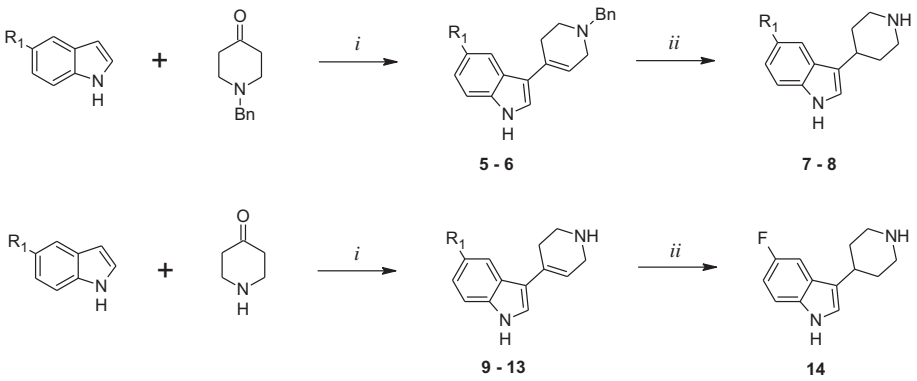
2.3.1. In vitro tests for 5-HT$_{1A}$ receptor and SERT

Competition binding studies were performed according to the previously published procedure [33] with slight modifications. Target compounds **15**–**38** were assessed for *in vitro* affinity for the 5-HT$_{1A}$ receptor and SERT by radioligand binding assays, using [^{3}H] 8-OH-DPAT and [^{3}H]citalopram, respectively, in rat brain tissues (Tables 1 and 2).

Examined compounds **15**–**38** exhibit very high, high or moderate affinity for the 5-HT$_{1A}$ receptor and SERT. The results of biological tests were divided on the basis of the degree of hydrogenation of the pyridine ring. Table 1 contains the results for compounds from the 1,2,3,6-tetrahydropyridine series (THPI), and the results for compounds with a piperidine ring (the PPI series) can be found in Table 2.

The analysis of the results of *in vitro* affinity tests for the 5-HT$_{1A}$ receptor and SERT revealed that the presence of a double bond in the pyridine ring is of significant impact. Compounds **15**–**29** (THPI series) in general manifested higher affinity for SERT than compounds **30**–**38** (PPI series), from which it can be expected that the presence of a double bond probably leads to better binding of the final compounds with the transporter. Compounds **30**–**38**, in turn, in which the pyridine ring was fully hydrogenated (PPI series) exhibited better binding to the 5-HT$_{1A}$ receptor. The differences in affinity for the 5-HT$_{1A}$ receptor might by correlated with various conformations of the indole ring and the tetrahydropyridine ring or piperidine ring; in the first case, it is coplanar conformation in another non-coplanar conformation between these two systems (rings) [54]. This fact may explain the difference in binding with the 5-HT$_{1A}$ receptor and SERT for corresponding compounds in the THPI and PPI series.

Comparing the results of tests on the R_1 fluorine substituted compounds (**18** → **31**, **23** → **34**, **28** → **37**), it can be easily noted that reduction of the double bond led to decreased affinity of these compounds for SERT, but increased the affinity for the 5-HT$_{1A}$ receptor in the case of compounds **18** → **31**, **28** → **37**. This agrees with the observations on binding described by Mewshaw et al. [55]. The presence of a double bond (THPI series) in compounds **20**, **23** and **24** with a three-carbon linker compared with their analogs **33**, **34** and **35** with a piperidine ring (PPI series) led to a significant increase in affinity for SERT.



Scheme 2. The synthesis pathway of substrates, 3-piperidyn-4-yl-1*H*-indole (PPI) and 3-(1,2,3,6-tetrahydropyridin-4-ly)-1*H*-indole (THPI) derivatives. Reagents and conditions: (i) CH_3OH, CH_3ONa (ii)10% Pd/C, CH_3OH.

Table 1
SERT and 5-HT_{1A} receptor binding affinities of 3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole derivatives (THPI).

Compound	R_1	*n*	$K_i \pm$ SEM [nM]	
			5-HT_{1A}	SERT
15	H	2	12.5 ± 1.7	11.3 ± 0.6
16	Cl	2	68.7 ± 2.3	74.4 ± 6.9
17	Br	2	59.1 ± 3.5	208.0 ± 18.4
18	F	2	17.6 ± 2.9	20.0 ± 0.7
19	OCH_3	2	3.2 ± 0.2	46.2 ± 2.5
20	H	1	161.4 ± 17.5	4.0 ± 0.1
21	Cl	1	285.2 ± 12.6	50.7 ± 5.2
22	Br	1	110.8 ± 9.2	182.0 ± 17.6
23	F	1	106.3 ± 7.2	5.2 ± 0.7
24	OCH_3	1	210.0 ± 3.0	125.0 ± 1.0
25	H	0	249.5 ± 6.8	33.8 ± 3.2
26	Cl	0	406.0 ± 38.0	96.7 ± 7.5
27	Br	0	323.8 ± 21.0	673.0 ± 63.0
28	F	0	371.7 ± 32.1	68.4 ± 3.8
29	OCH_3	0	1740.4 ± 51.0	783.5 ± 22.3
Serotonin			2.1 ± 0.2	NT
Fluoxetine			NT	12.7

NT – not tested.

Another analyzed modification of the structure was the length of the carbon chain. A two, three of four-carbon linker was introduced into the structure of pyrrolidin-2,5-dione derivatives. The results L. López-Rodríguez et al. [56] for arylpiperazines suggest that the carbon chain length has an influence on affinity for the 5-HT_{1A} receptor. It is also clear that shortening of its length (two-carbon chain) caused a decrease in binding with 5-HT_{1A} receptor, because of the bulky interaction. The optimum length of the alkyl chain for 5-HT_{1A} affinity is four carbon atoms.

A group of derivatives **15**, **16**, **18**, **19** and **30** obtained in our research showed binding to both the 5-HT_{1A} receptor and SERT. All of these derivatives contain a four-carbon linker, which leads to the conclusion that in the examined group of derivatives, this particular length is optimal for obtaining dual binding compounds. **20** and **23** demonstrate the highest binding to SERT of all THPI compounds (**20** K_i = 4.0 nM, **23** K_i = 5.2 nM). These are derivatives with a three-carbon linker, substituted in the R_1 position with –H and –F, respectively. Extension or reduction of the carbon chain by one carbon atom, resulting in compounds **15** and **18** or **25** and **28**, caused a decrease in binding to SERT. From here, it can be concluded that preserving the three-carbon length of the linker is crucial for obtaining a compound with high affinity for the transporter. The presence of $-OCH_3$ at R_1 in compounds with a two-carbon chain for THPI and PPI groups caused a decline in the affinity for SERT and the 5-HT_{1A} receptor.

Binding values show that the presence of functional groups in the R_1 position affects the affinity of synthesized compounds. In both series (THPI and PPI), derivatives substituted at R_1 exhibited higher affinity for the 5-HT_{1A} receptor. In the THPI and PPI series with a four-carbon chain, the highest affinity for the receptor was exhibited by compound **19** (K_i = 3.2 nM) and **32** (K_i = 2.2 nM) with an R_1 $-OCH_3$ functional group. This is not in accordance with our earlier observations regarding the influence of the R_1 functional group of this configuration [33]. The influence of the remaining R_1 functional groups can be arranged in descending order: –F (compounds **18** and **31**), –Br (compound **17**) and finally –Cl (compound **16**). A general remark is that a considerable reduction in binding to the 5-HT_{1A} receptor can be observed in compounds with a chlorine substituent (**16**, **21** and **26**). Lower affinity was also demonstrated by compounds with an R_1 bromine atom substitution (compounds **17**, **22** and **27**), hence the presence of those halogens (–Cl and –Br) at the R_1 position can be said to have a negative impact on binding to the 5-HT_{1A} receptor. R_1 substitution with fluorine in the THPI series compounds was beneficial for binding to SERT. These compounds (**18**, **23** and **28**) also showed very high values for binding to the 5-HT_{1A} receptor among the compounds with halogen substitution. The highest binding values for compounds with dual binding to the 5-HT_{1A} receptor and SERT were demonstrated by compounds **15** and **30**, which do not include a functional group at the 1*H*-indole 5 position (R_1 = H). This agrees with the principle that the highest degree of affinity for SERT is characteristic of compounds with R_1 = –H or –F in the THPI series in different derivatives with a similar length of carbon chain, and this is also in accordance with literature data for compounds with a substituted THPI series at R_1 [57].

Table 2
SERT and 5-HT_{1A} receptor binding affinities of 3-piperidyn-4-yl-1*H*-indole derivatives (PPI).

Compound	R_1	*n*	$K_i \pm$ SEM [nM]	
			5-HT_{1A}	SERT
30	H	2	15.7 ± 2.0	5.7 ± 0.5
31	F	2	7.5 ± 0.6	505.0 ± 49.0
32	OCH_3	2	2.2 ± 0.7	210.0 ± 18.0
33	H	1	68.5 ± 6.5	354.0 ± 27.0
34	F	1	121.6 ± 9.1	215.0 ± 21.0
35	OCH_3	1	32.6 ± 3.3	221.0 ± 19.7
36	H	0	364.4 ± 24.1	56.0 ± 5.3
37	F	0	8.1 ± 0.9	381.0 ± 36.0
38	OCH_3	0	351.7 ± 1.5	1850.0 ± 96.0

2.3.2. In vitro tests for D_{2L}, 5-HT_6, 5-HT_7 and 5-HT_{2A} receptors

Due to a high affinity for the 5-HT_{1A} receptor and moderate affinity for SERT, compounds **31**, **32**, **35**, and **37** from the PPI group were checked for inter-receptor binding, with a special focus on the D_2 receptor. This was done in order to assess the selectivity and possible improvement of their potential therapeutic effect profile.

To determine the affinity profile of the selected compounds, radioligand binding assays were performed for cloned human dopamine D_{2L} and serotonin 5-HT_6 and 5-HT_7 receptors as well as for native 5-HT_{2A} receptors. This was accomplished by assessing the displacement of [^{3}H]-raclopride, [^{3}H]-LSD and [^{3}H]-5-CT from the cloned human D_{2L}, 5-HT_6 or 5-HT_7 receptors, respectively, stably expressed in HEK293 cells and [^{3}H]-ketanserin from 5-HT_{2A} receptors from the rat cortex.

So far, the role of the dopaminergic system in the pathomechanism of depressive disorders has not triggered a lot of interest from researchers. This may change, however, as it is currently known that disturbances in dopaminergic transmission in the mesolimbic and nigrostrial systems influence the development of depression [58]. An increasing amount of interest in the area of investigation for new medications has been focused on partial agonists of D_2/5-HT_{1A} receptors [59], especially since test results indicate that postsynaptic 5-HT_{1A} receptor agonists increase the amount of DA in the mPFC (Medial Prefrontal Cortex) [60]. In addition, compounds with the structure similar to that of our final compounds manifest antagonistic activity toward the D_2 receptor [61]. The role of the 5-HT_{2A} receptor in the therapy of both schizophrenia and depression is well-documented, thus investigating its ligands appears to be a valid approach [8]. The role of other serotonin receptors such as 5-HT_6 and 5-HT_7 in the pathomechanism of CNS disorders has also been shown [8], which together with satisfactory results of therapy with multi-receptor acting profile medicines, e.g. aripiprazole [62] or olanzapine [62], encouraged us to investigate our chosen synthesized compounds from the perspective of their degree of affinity for these receptors [63].

Binding values are presented in Table 3. Compounds **31**, **32** and **37** exhibited high affinity for 5-HT_{1A} and D_2. Compound **31** is

especially interesting, as it also demonstrated promising values for binding to 5-HT_{2A} and 5-HT_6 (Table 3). The structures of compounds **31** and **37** feature a part of 5-fluoro-3-piperidin-4-yl-1*H*-indole, which most probably has a considerable impact on the bond between ligand and the D_2 receptor. There is an analogy here with derivatives of 5-fluoro-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole compounds, described by van Hes et al. [37]. All of the examined compounds **31**, **32**, **35** and **37** manifested poor affinity for 5-HT_7 receptor. The conclusion that may be drawn from preliminary *in vitro* tests for affinity for D_2, 5-HT_6, 5-HT_7 and 5-HT_{2A} is that compound **31**, belonging to the PPI group and featuring a fluorine atom at R_1, may be a good starting point for investigating new multi-receptor ligands in the therapy of such disorders as depression and/or schizophrenia. We have therefore confirmed the test results for the binding of compounds containing a 5-fluoro-3-piperidin-4-yl-1*H*-indole moiety, which demonstrate poor inter-receptor selectivity [36].

2.4. In vivo assay

Due to their very good 5-HT_{1A} receptor and SERT transporter binding properties, *in vivo* tests, i.e. the induced hypothermia and forced swimming tests, both performed in mice, were carried out for compounds **15**, **18**, **19** and **30**. This allowed us to specify their agonist–antagonist properties in reference to pre- and post-synaptic 5-HT_{1A} receptors.

Compounds **15**, **18**, **19** and **30** were tested in *in vivo* models commonly used for evaluating functional 5-HT_{1A} receptor activity. 8-OH-DPAT, a 5-HT_{1A} receptor agonist, induces hypothermia in mice, an effect mediated through the somatodendritic 5-HT_{1A} receptor [64,65]. This hypothermia is abolished by an antagonist of 5-HT_{1A} receptors, WAY 100635 [66]. All tested compounds, like 8-OH-DPAT, induced hypothermia in mice (Table 4).

However, only hypothermia induced by compounds **18** (5 mg/kg) and **19** (5 mg/kg) was attenuated by WAY 100635 (0.1 mg/kg) (Table 5). At the same time, the decrease in body temperature induced by 8-OH-DPAT (5 mg/kg) was completely blocked by WAY 100635 (0.1 mg/kg) (Table 5). Therefore, the decrease in mouse body temperature produced by **18** and **19** can be regarded as a measure of its presynaptic 5-HT_{1A} receptor agonistic activity. Because the hypothermia induced by compounds **30** and **15** was not antagonized by WAY 100635, receptors other than presynaptic 5-HT_{1A} may participate in this effect.

Furthermore, to establish if **18** and **19** produce an antidepressant-like effect mediated by postsynaptic 5-HT_{1A} receptors, mimicking the effect of 8-OH-DPAT, the Porsolt test was performed. Compounds **18** and **19** were ineffective at doses of 2.5 mg/kg and 5 mg/kg in this test (Fig. 3A and B). However, 8-OH-DPAT, at doses of 1 mg/kg and 2 mg/kg, reduced the immobility time in mice (Fig. 3C).

2.5. Molecular modeling

Three ligands, derivatives of pyrrolidine-2,5-dione, were docked to the binding site of 5-HT_{1A} receptor and SERT models to evaluate their properties as potential psychotropic agents [67]. The applied 3D model of the 5-HT_{1A} receptor was developed by Kurt Kristiansen (Department of Pharmacology, Institute of Medical Biology, University of Tromsø, Norway) on the basis of the X-ray crystal structure of the â$_2$-adrenergic receptor [68,69]. The quite recently reported X-ray structure of the human β_2-adrenergic receptor opens up new possibilities for modeling of the correct structures of the serotonin and dopamine receptors. Currently, the human β_2-adrenergic receptor is considered to be more homologous to these receptors than the more commonly and previously used bovine rhodopsin model [70].

For docking to SERT, the homologous crystal structure LeuTAa (*Aquifex aeolicus*) model of SERT was used [35].

Table 3
Binding data of the selected compounds for 5-HT and dopamine receptors.

Compound	K_i [nM] ± SEM				
	5-HT_{1A}	5-HT_{2A}	D_{2L}	5-HT_6	5-HT_7
31	7.5 ± 0.6	71 ± 9	14 ± 2	63 ± 5	196 ± 14
32	2.2 ± 0.7	498 ± 23	161 ± 14	159 ± 9	345 ± 24
35	32.6 ± 3.3	11620 ± 1750	343 ± 42	264 ± 17	589 ± 47
37	8.1 ± 0.9	384 ± 45	80 ± 6	1146 ± 152	1165 ± 98

Table 4
The effect of the tested compounds on the body temperature in mice.

Treatment	Dose (mg/kg)	Δ*t* ± SEM (°C)			
		30 min	60 min	90 min	120 min
Vehicle	–	−0.2 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.2	−0.1 ± 0.1
30	2.5	−1.3 ± 0.1[c]	−1.5 ± 0.2[c]	−1.3 ± 0.2[c]	−1.2 ± 0.2[c]
	5	−2.4 ± 0.2[c]	−2.8 ± 0.2[c]	−2.1 ± 0.1[c]	−2.1 ± 0.1[c]
Vehicle	–	−0.2 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.2	−0.1 ± 0.1
15	2.5	−0.7 ± 0.1	−0.6 ± 0.2	−0.9 ± 0.2[b]	−0.9 ± 0.2[b]
	5	−1.5 ± 0.2[c]	−1.4 ± 0.2[c]	−1.5 ± 0.3[c]	−1.4 ± 0.2[c]
Vehicle	–	−0.1 ± 0.2	−0.2 ± 0.2	−0.1 ± 0.1	−0.2 ± 0.1
18	2.5	−0.6 ± 0.1	−0.7 ± 0.2	−0.7 ± 0.1	−0.7 ± 0.1
	5	−1.6 ± 0.3[c]	−1.5 ± 0.2[c]	−1.3 ± 0.2[c]	−1.2 ± 0.3[c]
Vehicle	–	−0.2 ± 0.1	−0.2 ± 0.2	−0.1 ± 0.1	−0.1 ± 0.1
19	2.5	−0.5 ± 0.3	−0.5 ± 0.2	−0.7 ± 0.2	−0.4 ± 0.2
	5	−1.1 ± 0.1[b]	−1.1 ± 0.1[b]	−1.1 ± 0.2[b]	−0.9 ± 0.2[a]
Vehicle	–	−0.1 ± 0.2	−0.1 ± 0.1	−0.1 ± 0.3	−0.1 ± 0.2
12-OH-DPAT	5	−1.3 ± 0.1[c]	−1.3 ± 0.1[c]	−0.9 ± 0.1[b]	−0.5 ± 0.1
WAY 100635	0.1	−0.1 ± 0.2	−0.1 ± 0.2	−0.2 ± 0.1	−0.2 ± 0.2

The tested compounds were administered (*i.p.*) 30 min before the test. The absolute mean body temperatures were within a range of 37 ± 0.5 °C; n = 7–8 mice per group, [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ vs. the respective vehicle.

Table 5
The effect of WAY 100635 on hypothermia induced by the tested compounds in mice.

Compound and dose (mg/kg)	Δ*t* ± SEM (°C)	
	30 min	60 min
Vehicle + vehicle	−0.2 ± 0.1	−0.2 ± 0.1
Vehicle + **30** (2.5)	−1.3 ± 0.1[a]	−1.5 ± 0.1[a]
WAY 100635 (0.1) + **30** (2.5)	−1.0 ± 0.2[a]	−1.1 ± 0.3[a]
Vehicle + vehicle	−0.1 ± 0.2	−0.1 ± 0.1
Vehicle + **15** (5)	−1.5 ± 0.2[a]	−1.3 ± 0.1[a]
WAY 100635 (0.1) + **15** (5)	−1.2 ± 0.1[a]	−1.2 ± 0.3[a]
Vehicle + vehicle	−0.1 ± 0.1	−0.1 ± 0.2
Vehicle + **18** (5)	−1.5 ± 0.2[a]	−1.5 ± 0.2[a]
WAY 100635 (0.1) + **18** (5)	−0.5 ± 0.2[b]	−0.2 ± 0.2[b]
Vehicle + vehicle	−0.1 ± 0.1	−0.1 ± 0.1
Vehicle + **19** (5)	−1.0 ± 0.1[a]	−1.1 ± 0.2[a]
WAY 100635 (0.1) + **19** (5)	−0.4 ± 0.2[b]	−0.3 ± 0.2[b]
Vehicle + vehicle	−0.2 ± 0.2	−0.1 ± 0.2
Vehicle + 8-OH-DPAT (5)	−1.4 ± 0.1[a]	−1.0 ± 0.1[a]
WAY 100635 (0.1) + 8-OH-DPAT(5)	−0.1 ± 0.1[c]	−0.2 ± 0.1[c]

WAY 100635 was administered (*s.c.*) 15 min before the investigated compounds, n = 7–8 mice per group. The test was performed 30 min after injection of the tested compounds (*i.p.*).
The absolute mean body temperatures were within a range 37 ± 0.5 °C.
[a] $p < 0.001$ vs. respective vehicle + vehicle group.
[b] $p < 0.01$.
[c] $p < 0.001$ vs. respective vehicle + compound group.

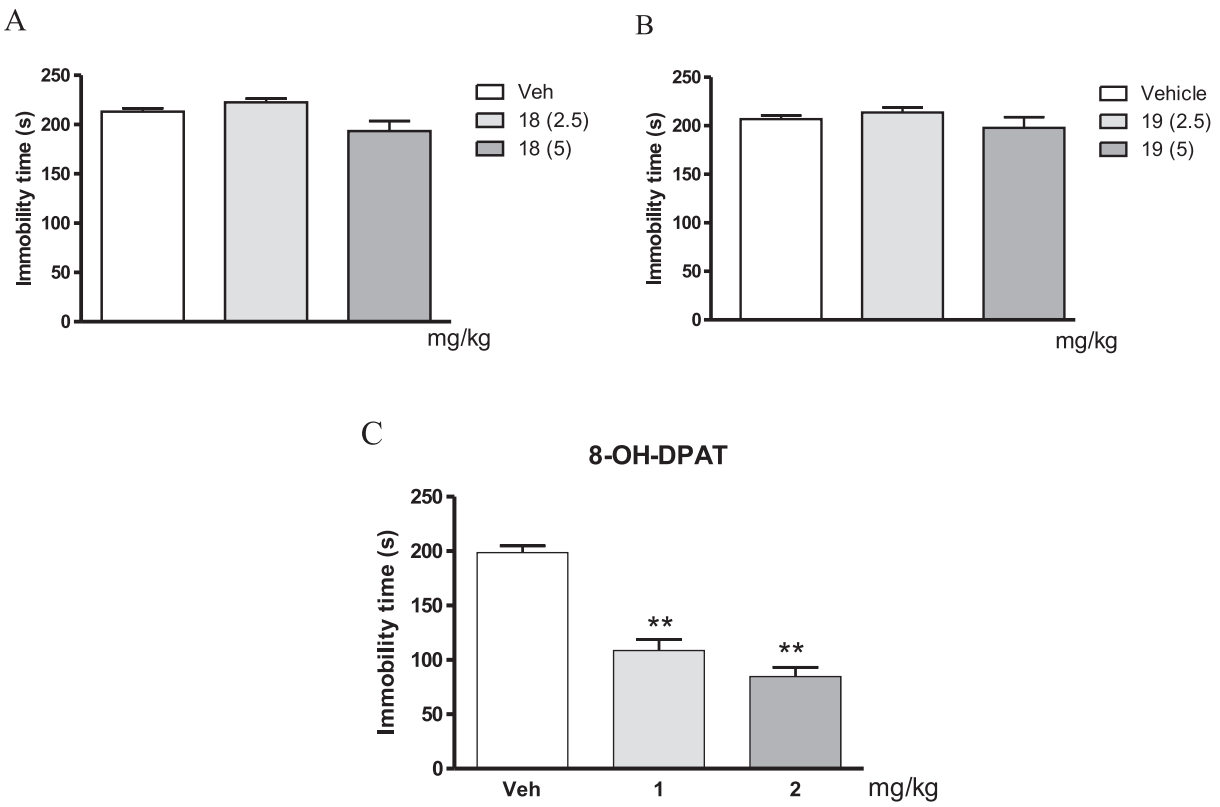


Fig. 3. Effects of compounds **18** (A), **19** (B) and 8-OH-DPAT (C) in the forced swimming test in CD-1 mice. Each bar represents the mean ± SEM of 9–10 mice. All compounds were injected 30 min before the test. $^{**}p < 0.001$ vs. the respective vehicle group (Dunnett's test).

2.5.1. Docking to the 5-HT$_{1A}$ receptor

Docking of compounds **15**, **31** and **32** to the 5HT binding site revealed (Figs. 4–6): (i) compound **15** located inside the receptor binding pocket forms three hydrogen bonds with receptor amino acids: Ala93 (1.80 Å), Asp116 (1.85 Å) and Asn386 (1.74 Å). All hydrogen bonds are strong but, specifically, the one with involving Asp116 (amino acid located in the TMH3 helix) is crucial for ligand affinity for monoamine neurotransmitter receptors. The position of compound **15** inside the receptor binding pocket is further stabilized by π–π interactions between Phe112 and the indole ring of the ligand, (ii) the protonated piperidine nitrogen atom in compound **32** interacts via a hydrogen bond with Asp116 (2.21 Å), and additionally it is involved in a π-cation interaction with Phe112 while the succinimide ring (strictly, the carbonyl oxygen atom) forms a hydrogen bond with Thr196 (2.18 Å), (iii) the protonated nitrogen atom of the piperidine ring of compound **31** interacts via a strong hydrogen bond with Asp116 (1.75 Å). As many as four successive hydrogen bonds are formed between Thr200 and the carbonyl oxygen atom at the C5 position of the pyrrolidine-2,5-dione ring (1.89 Å), Cys187 and the nitrogen atom of the indole ring bound to the succinimide moiety (2.09 Å), Trp387 and the fluorine atom (2.38 Å), and Glu97 and the fluorine atom (1.96 Å).

2.5.2. Docking to SERT

Docking of three studied ligands to the SERT binding pocket pointed out that (Figs. 7–9): (i) compound **15** forms two hydrogen bonds with its receptor amino acid vicinity. The first hydrogen bond is between Tyr95 and the nitrogen atom of the indole ring (1.83 Å) and a second, somewhat weaker one between Arg104 and the carbonyl oxygen atom at the C2 position (2.25 Å). Although the hydrogen bond at Asp98 with the ligand is considered to be a determinant of ligand affinity for SERT, the bond with Tyr95 is strong enough to bind the ligand effectively. The complex is additionally stabilized by other interactions: π–π between Phe341 and the indole ring and π–σ between Ile172 and the indole ring, (ii) the complex of compound **32** and SERT is stabilized by a relatively weak hydrogen bond formed between Asp98 and the nitrogen atom of the piperidine ring (2.11 Å). There are also three interactions of the π-cation type between the protonated piperidine nitrogen atom and Tyr95, Phe341 and Tyr176, (iii) energetic stabilization of compound **31** placed inside the SERT binding site results from three

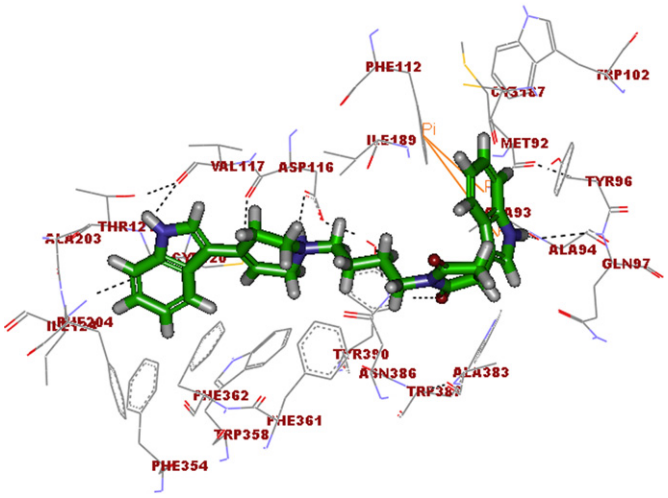


Fig. 4. Compound **15** inside 5-HT$_{1A}$ receptor binding pocket. The black dashed line denotes a hydrogen bond.

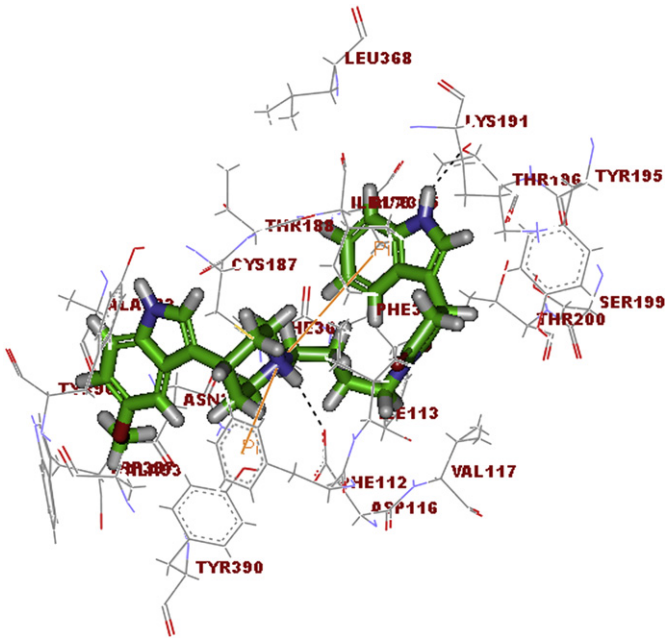


Fig. 5. Compound **32** inside 5-HT_{1A} receptor binding pocket. The black dashed line denotes a hydrogen bond.

hydrogen bonds formed between Tyr95 and the nitrogen atom of the indole ring with the fluorine substituent (2.14 Å), Arg104 and the oxygen atom at the C5 position of the pyrrolidine-2,5-dione ring (1.73 Å), and Tyr232 and the indole nitrogen atom (2.10 Å).

In all cases of the studied compounds, the differences in the ligand–receptor interaction should be accounted for by differences in two properties of the R_1 substituents i.e. electronegativity and spatial size (van der Waals radius). The first property has an impact on the distribution of electron density around the part of ligand molecule containing the given substituent and thus decides on the kind of interaction and its strength; the second factor determines

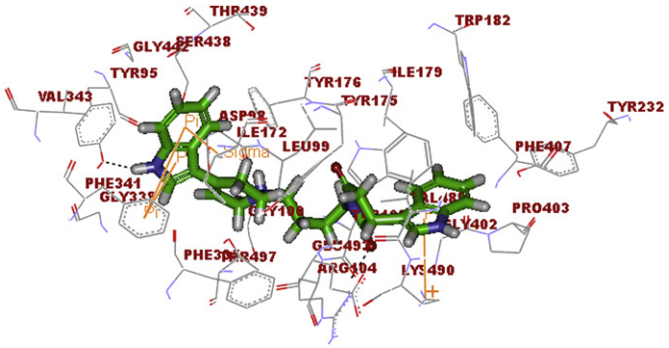


Fig. 7. Compound **15** inside SERT binding pocket. The black dashed line denotes a hydrogen bond.

steric hindrance and consequently may also force a specific conformation of the docked ligand and simultaneously its position in the receptor binding pocket. The impact of substituent electronegativity on ligand binding is explicitly notable in the case of the ligand–5-HT_{1A} receptor interaction for all three ligands studied. The electronegativity of the R_1 substituents increases in the order: $-H < -OCH_3 < -F$. Therefore, the fluorine atom in compound **31** causes the strongest local inductive effect and probably enables the piperidine hydrogen atom to form a strong hydrogen bond with receptor amino acid Asp116 (1.75 Å) as a result of a favorable spatial distribution of receptor–ligand interactions and due to the adequate position of the ligand inside the binding pocket. The same hydrogen atom in compound **32** is involved in a π-cation interaction. Yet, the piperidine hydrogen atom in compound **15** does not interact with the receptor amino acid vicinity at all. Additionally, the fluorine substituent in compound **31** indirectly forms weak hydrogen bonds with receptor amino acids Trp387 (2.38 Å) and Glu97 (1.96 Å) inside the binding pocket. A relationship between substituent electronegativity and the ligand–SERT interaction was not observed. The impact of the inductive effect of the substituents in the SERT conjugate is likely strongly dominated by steric factors

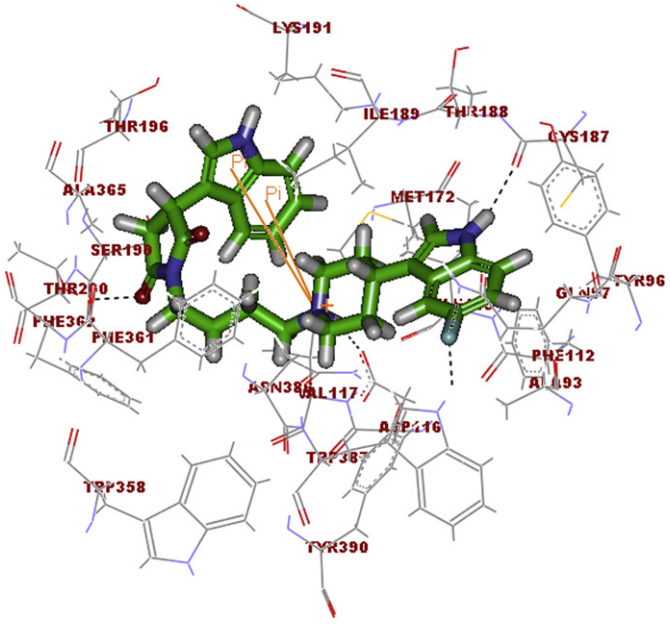


Fig. 6. Compound **31** inside 5-HT_{1A} receptor binding pocket. The black dashed line denotes a hydrogen bond.

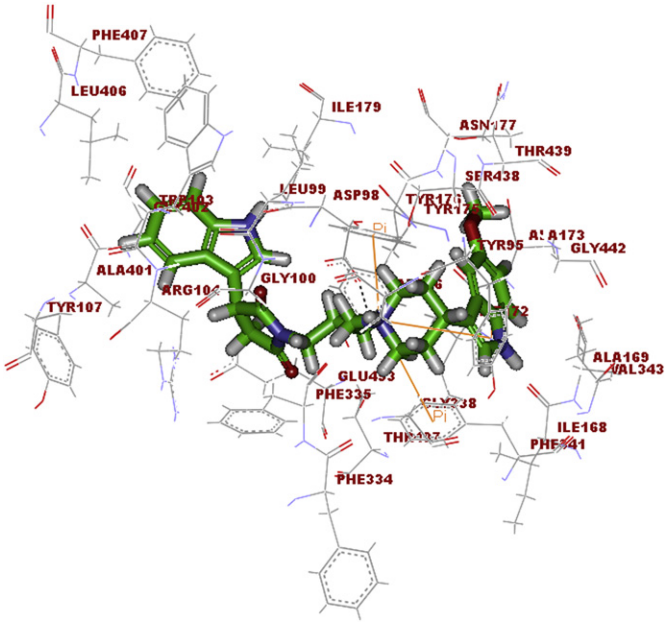


Fig. 8. Compound **32** inside SERT binding pocket. The black dashed line denotes a hydrogen bond.

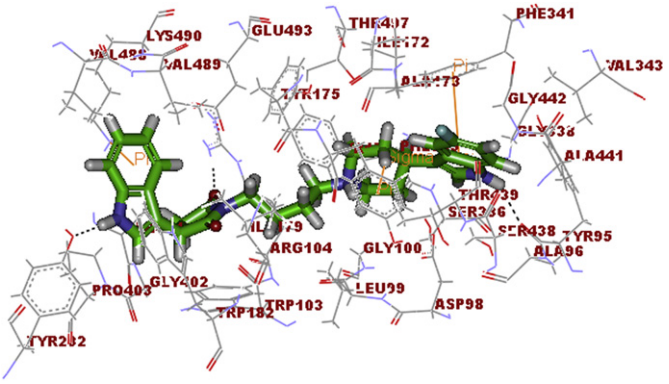


Fig. 9. Compound **31** inside SERT binding pocket. The black dashed line denotes a hydrogen bond.

determined by the spatial size of the substituents and the geometry of the SERT binding pocket. The position and conformation of ligands docked to 5-HT_{1A} and SERT result, to large degree, from the high flexibility of the four-membered hydrocarbon chain which binds the piperidine and succinimide rings. This hydrocarbon chain is long enough for the ligand be able to adjust itself to the dimensions of the binding pocket.

The conformation of the piperidine rings in all studied ligands is the "chair" one and seems to not affect the spatial position of the ligand inside the receptor binding pocket.

Referring the *in vitro* binding data to the structural properties of the studied compounds, it may be concluded that the affinity of these compounds to the 5-HT_{1A} receptor and SERT is predominantly determined by the electronegativity of the R_1 substituent and the size relationship between the ligand to the geometry of the binding pocket. All three compounds form hydrogen bonds with Asp116, of which the strongest one is formed with compound **31** (1.75 Å). This results from the highest electronegativity of the fluorine substituent in comparison to −H and $-OCH_3$. Consequently, the strong inductive effect of the −F substituent makes protonated piperidine a much more effective proton donor.

Compounds **32** and **31** are significantly less active regarding SERT than compound **15**, probably, due to the larger sizes of the $-OCH_3$ and −F substituents in comparison to −H, combined with the relatively small capacity of the SERT binding pocket. Thus, the quality of docking of the studied compounds to SERT is likely determined by steric factors.

3. Conclusions

The article describes the synthesis of a new series of 3-(1*H*-indol-3-yl)pyrrolidine-2,5-dione derivatives (**15**–**38**), compounds with dual binding to the 5-HT_{1A} receptor and SERT. Some very promising compounds were obtained, characterized by notably high binding values to both the receptor and the transporter. Compounds **15** and **30** were especially interesting and appeared to be a good starting point for further compound research in the SSRI+ group. A structure–activity relationship analysis of the *in vitro* and *in vivo* test results for compounds **15**–**38** was performed, depending on the proposed modifications of the B and C areas of the lead structure (Fig. 2). The influence of a change in chain length on binding to the 5-HT_{1A} receptor and SERT was determined. It turned out that in order to arrive at satisfying receptor and transporter binding values, a four-carbon linker is the optimal one, as introducing a two-carbon linker results in a decrease in 5-HT_{1A} receptor and SERT affinity.

As the SAR analysis of the *in vitro* test results on the 5-HT_{1A} and SERT affinity revealed, the presence of a double bond in the pyridine ring (THPI series) is of considerable importance. In general, THPI series compounds **15**–**29** exhibited a high degree of affinity for SERT, while PPI series compounds **30**–**38** demonstrated much higher affinity for the 5-HT_{1A} receptor. The analysis of the influence of the functional groups at R_1 in both THPI and PPI series leads to a conclusion that substitution at R_1 is crucial for obtaining a compound with dual binding. The best results of biological tests were obtained for compounds **15**, **18**, **19** and **30**, which at R_1 consist of R_1 = H(**15**), F(**18**), OCH_3(**19**) and H(**30**), respectively. These compounds were subjected to *in vivo* tests, which allowed us to assess their agonist–antagonist properties in relation to pre- and postsynaptic 5-HT_{1A} receptors. All tested compounds, like 8-OH-DPAT, induced hypothermia in mice, with compounds **18** and **19** being presynaptic agonists. For **15** and **30**, the *in vivo* binding assay suggested that receptors other than presynaptic 5-HT_{1A} may participate in this effect. In computer docking tests to the 5-HT_{1A} receptor and SERT, compound **15** formed very strong bonds with the most important amino acids in the active site, Asp116 in TMH3 (5-HT_{1A} receptor) and Tyr95 (SERT). It may be, therefore, concluded that compounds **15** and **30** turn out to be highly promising molecules, which will be subjected to further research on their anti-depressant activity.

The influence of modifications to the lead structure of the final compounds was also described in reference to the process of obtaining a compound with the highest degree of affinity for SERT in the described group of derivatives. The best binding was observed in the THPI series compounds, namely compounds **18**, **23** and **28** substituted with a fluorine atom at R_1, and compound **20** with R_1 = H. Preserving the 5-fluoro-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole structure for investigating new SERT ligands in this group of derivatives thus appears to be a valid approach.

What was also determined was the influence of the final compound structure modification on affinity for the 5-HT_{1A} receptor. The best receptor binding was manifested by compounds **19** and **32** with a four-carbon chain substituted with $R_1 = OCH_3$, while within the PPI series, high affinity was also exhibited by compounds **31** and **37** (two-carbon linker) substituted with a fluorine atom at the R_1 position. Compounds **31** and **37**, the structures of which include a 5-fluoro-3-piperidin-4-yl-1*H*-indole moiety, also demonstrated very good binding to D_2 and 5-HT_{2A} receptors, which is an interesting starting point for investigating new ligands that could be used in the therapy of disorders such as schizophrenia.

The test results obtained for a series of pyrrolidine-2,5-dione derivatives **15**–**38** indicate the considerable potential of this group of compounds not only as the compounds with dual binding to the 5-HT_{1A} receptor and SERT, but also as potential selective ligands with high affinity for the abovementioned protein structures. Compound **31** is potentially also a molecule with a multi-receptor profile. Further research on this group of derivatives will involve the examination of the influence of structure area A modifications, i.e. pyrrolidine-2,5-dione, on binding to the 5-HT_{1A} receptor and SERT.

4. Experimental protocols

4.1. Chemistry

4.1.1. General remarks

Reagents and solvents were purchased from common commercial suppliers were utilized without further purification, including dichloromethane. The purity (>95%) and homogeneity of the compounds were routinely confirmed by TLC on Merck plates (Kieselgel 60 F_{254}) and ^{1}H NMR spectra. Melting points (m.p.) were

determined on an Electrothermal 9100 apparatus with open capillary tubes and are uncorrected. Infrared spectra were recorded on a Shimadzu FTIR-8300 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker AVANCE DMX 500WB instrument in $CDCl_3$/DMSO-d_6 (chemical shifts are reported in δ units). Coupling constants (J) are in Hertz (Hz); the internal reference was TMS. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), bs (broad singlet), d (doublet), dd (double doublet), t (triplet), td (triple doublet), ps (pseudo triplet), 4d (quartet of doublets), m (multiplet), * – peak patterns under DMSO signal, ** – conjugation with fluorine nucleus. For the two-dimensional experiments, the pulse sequences, acquisition, and processing parameters were taken from the standard Bruker software library. ESI-HRMS spectra were obtained on a Marimer (PE Biosystems) instrument.

Flash column chromatography was carried out on Merck silica gel 60 (230–400 mesh ASTM) using dichloromethane/methanol as the solvent (99:1, 98:2, 97:3, 95:5, 90:10, 85:15 v/v). Thin layer chromatography was run on Merck silica gel (Kieselgel 60 F_{254}) plates, with mobile phases of dioxane, toluene, ethanol and 25% NH_4OH (6,0:3,2:0,5:0,2, v/v) or chloroform, methanol, diethyl ether and NH_4OH (18,0:4,0:3,6:0,4). Compounds were visualized by UV light (254 nm). Room temperature refers to 20–25 °C.

4.1.2. Procedure for the synthesis of 3-piperidin-4-yl-1H-indoles (**7**, **8**, **14**)

The starting compounds **7**, **8** and **14** were obtained according to previously described procedures [52,53].

4.1.3. Procedure for the synthesis of 5-substitued-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indoles (**9**–**13**)

The starting compounds **9**–**13** were obtained according to previously described procedures [36,50–52].

4.1.4. 3-(1H-Indol-3-yl)pyrrolidine-2,5-dione (**1**)

A mixture of indole (9.7 g 0.1 mol) and maleimide (11.7 g 0.1 mol) in acetic acid (125 ml) was refluxed on stirring for about 36 h. The completion time of reaction was assigned chromatographically (TLC). The reaction mixture was cooled, the solvent was removed under vacuo and the crude product was crystallized from ethanol to give compound (**1**) [46,48,49].

The title compound was isolated as a light brown powder. Yield: 79.58% 17.12 g, m.p. 190–191 °C (lit. 196–197 °C [47]).

4.1.5. 1-(4-Bromobutyl)-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**2**)

3-(1H-Indol-3-yl)pyrrolidine-2,5-dione (**1**) (2.14 g 0.01 mol) and 1,4-dibromobutane (0.05 mol) and K_2CO_3 (0.02 mol) and 100 ml acetone were stirred and refluxed for 4 h. Reaction time was monitored using TLC. After cooling, the mixture was filtered, and the filtrate was evaporated to dryness. The crude residue was purified by flash chromatography using CH_2Cl_2/MeOH (98:2 v/v) as an eluent. Proper fractions were identified by TLC, evaporated to dryness and crystallized from diethyl ether to afford **2**.

The title compound was isolated as a dark yellow crystals. Yield: 69.14% 2.42 g, m.p. 122–123 °C.

^{1}H NMR (500 Hz, DMSO): 4.37 (C3H, dd, $^3J_1 = 5.0$, $^3J_2 = 9.5$), 2.81 (C4H(1), dd, $^2J = 18.0$, $^3J = 5.0$), 3.24 (C4H(2), dd, $^2J = 18.0$, $^3J = 9.5$), 7.33 (C2′H, d, $^3J = 2.5$), 7.37 (C4′H, C7′H, m), 6.99 (C5′H, m, $^3J_1 = 8.0$, $^3J_2 = 7.0$, $^4J = 1.0$), 7.10 (C6′H, m, $^3J_1 = 8.5$, $^3J_2 = 7.5$, $^4J = 1.0$), 3.55 (C1^{x}H$_2$, t, $^3J = 6.5$), 1.80 (C2^{x}H$_2$, q, $^3J = 7.0$), 1.67 (C3^{x}H$_2$, q, $^3J = 7.0$), 3.49 (C4^{x}H$_2$, t, $^3J = 7.0$), 11.05 (NH, bs).

^{13}C NMR (125 Hz, DMSO): 178.3 (C2), 37.4 (C3), 35.9 (C4), 176.5 (C5), 123.4 (C2′), 110.6 (C3′), 125.8 (C3′a), 118.2 (C4′), 118.7 (C5′), 121.3 (C6′), 111.6 (C7′), 136.4 (C7′a), 34.4 (C1^x), 29.5 (C2^x), 25.9 (C3^x), 37.2 (C4^x).

HRMS (MSES+) calcd for $C_{16}H_{17}BrN_2O_2Na$, 371.0371; Found: 371.0387.

4.1.6. 1-(3-Bromopropyl)-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**3**)

3-(1H-Indol-3-yl)pyrrolidine-2,5-dione (**1**) (2.14 g 0.005 mol) and 1,3-dibromopropane (0.025 mol) and K_2CO_3 (0.01 mol) and 50 ml acetone were stirred and refluxed for 4 h. Reaction time was monitored using TLC. After cooling, the mixture was filtered, and the filtrate was evaporated to dryness. The crude residue was purified by flash chromatography using CH_2Cl_2/MeOH (98:2 v/v) as an eluent. Proper fractions were identified by TLC, evaporated to dryness to afford **3**.

The title compound was isolated as a dark yellow powder. Yield: 63.09% 1.06 g, m.p. 75–77 °C.

^{1}H NMR (500 Hz, $CDCl_3$): 4.29 (C3H, dd, $^3J_1 = 9.5$, $^3J_2 = 5.0$), 2.94 (C4H(1), dd, $^2J = 18.5$, $^3J = 5.0$), 3.27 (C4H(2), dd, $^2J = 18.5$, $^3J = 9.5$), 7.09 (C2′H, d, $^3J = 2.5$), 7.41 (C4′H, d, $^3J = 8.0$), 7.13 (C5′H, m, $^3J_1 = 8.0$, $^3J_2 = 7.0$, $^4J = 1.0$), 7.22 (C6′H, m, $^3J_1 = 8.0$, $^3J_2 = 7.5$, $^4J = 1.0$), 7.36 (C7′H, d, $^3J = 8.0$), 3.76 (C1^{x}H$_2$, t, $^3J = 7.0$), 2.23 (C2^{x}H$_2$, q, $^3J = 7.0$), 3.39 (C3^{x}H$_2$, t, $^3J = 7.0$), 8.24 (NH, bs).

^{13}C NMR (125 Hz, $CDCl_3$): 178.1 (C2), 37.9 (C3), 36.3 (C4), 176.3 (C5), 122.2 (C2′), 111.3 (C3′), 125.6 (C3′a), 118.4 (C4′), 120.2 (C5′), 122.8 (C6′), 111.7 (C7′), 136.6 (C7′a), 38.2 (C1^x), 29.8 (C2^x), 30.7 (C3^x).

HRMS (MSES+) calcd for $C_{15}H_{15}BrN_2O_2Na$, 357.0215; Found: 357.0209.

4.1.7. 1-(2-Bromoethyl)-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**4**)

3-(1H-Indol-3-yl)pyrrolidine-2,5-dione (**1**) (1.92 g 0.009 mol) and 1,2-dibromoethane (0.05 mol) and K_2CO_3 (0.02 mol) and 100 ml acetone were stirred and refluxed for 6 h. Reaction time was monitored using TLC. After cooling, the mixture was filtered, and the filtrate was evaporated to dryness. The crude residue was purified by flash chromatography using CH_2Cl_2 as an eluent. Proper fractions were identified by TLC, evaporated to dryness to afford **4**.

The title compound was isolated as a dark yellow powder. Yield: 64.36% 1.86 g, m.p. 64–67 °C.

^{1}H NMR (500 Hz, $CDCl_3$): 4.33 (C3H, dd, $^3J_1 = 9.5$, $^3J_2 = 5.0$), 2.97 (C4H(1), dd, $^2J = 18.5$, $^3J = 5.0$), 3.29 (C4H(2), dd, $^2J = 18.5$, $^3J = 9.5$), 7.12 (C2′H, d, $^3J = 2.0$), 7.48 (C4′H, d, $^3J = 8.0$), 7.14 (C5′H, m, $^3J_1 = 8.0$, $^3J_2 = 7.0$, $^4J = 1.0$), 7.22 (C6′H, m, $^3J_1 = 8.0$, $^3J_2 = 7.0$, $^4J = 1.0$), 7.37 (C7′H, dt, $^3J = 8.5$, $^4J = {}^5J = 1.0$), 4.05 (C1^{x}H$_2$, t, $^3J = 6.5$), 3.62 (C2^{x}H$_2$, td, $^2J = 1.0$, $^3J = 6.5$), 8.23 (NH, bs).

^{13}C NMR (125 Hz, $CDCl_3$): 177.8 (C2), 38.1 (C3), 36.4 (C4), 175.9 (C5), 122.3 (C2′), 111.3 (C3′), 125.6 (C3′a), 118.5 (C4′), 120.2 (C5′), 122.8 (C6′), 111.7 (C7′), 136.6 (C7′a), 40.2 (C1^x), 27.5 (C2^x).

HRMS (MSES+) calcd for $C_{14}H_{13}BrN_2O_2Na$, 343.0058; Found: 343.0063.

4.1.8. General procedure for the synthesis of derivatives 3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**15**–**38**)

Compounds **2**–**4** (0.5 mmol) and **7**–**14** (0.5 mmol) and K_2CO_3 (1 mmol), 35 ml acetonitrile, and a catalytic amount of KI were stirred and refluxed for 4–5 h. Reaction time was monitored using TLC. After cooling, the mixture was filtered, and the filtrate was evaporated to dryness. The crude residue was purified by flash chromatography using CH_2Cl_2/MeOH (98:2, 97:3, 95:5, 90:10 v/v) as an eluent. Proper fractions were identified by TLC and evaporated to dryness giving analytically pure compounds **15**–**38**.

*4.1.8.1. 1-{4-[4-(1H-Indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]butyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**15**).* The title compound was isolated as a yellow powder. Yield: 53% 0.20 g obtained from double amount of starting compounds. M.p. 82–86 °C.

^{1}H NMR (see Supplementary materials) (500 Hz, $CDCl_3$): 4.26 (C3H, 4d, $^3J_1 = 8.5$, $^3J_2 = 5.0$, $^4J = 0.5$), 2.87 (C4H (1), dd, $^2J = 18.00$,

^3J = 5.0), 3.22 (C4H(2), dd, ^2J = 18.00, ^3J = 8.5), 7.03 (C2′H, d, ^3J = 2.5), 7.45 (C4′H, dd, ^3J = 8.0, ^4J = 0.5), 7.13 (C5′H, C2″H, C5″H, m), 7.20 (C6′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.35 (C7′H, C7″H, m) 3.67 (C1^{x}H$_2$, t, ^3J = 7.0), 1.74 (C2^{x}H$_2$, q, ^3J = 7.0), 1.63 (C3^{x}H$_2$, q, ^3J = 7.0) 2.54 (C4^{x}H$_2$, t, ^3J = 7.0), 3.21 (CaH$_2$, ps), 6.16 (CbH, sp, $^3J_1$ = 5.0, $^3J_2$ = 3.5, ^4J = 2.0), 2.58 (CdH$_2$, pd), 2.72 (CeH$_2$, t, ^3J = 6.0), 7.87 (C4″H, d, ^3J = 8.0), 7.21 (C6″H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 8.38 (N1′H, bs), 8.29 (N1″H, bs).

^{13}C NMR (see Supplementary materials) (125 Hz, CDCl$_3$): 178.3 (C2), 38.9 (C3), 36.4 (C4), 176.5 (C5), 122.1 (C2′), 111.6 (C3′), 125.8 (C3′a), 118.5 (C4′), 120.1 (C5′), 122.2 (C6′), 111.6 (C7′), 136.6 (C7′a), 38.1 (C1^x), 25.8 (C2^x), 24.4 (C3^x), 57.8 (C4^x), 53.1 (Ca), 118.9 (Cb), 129.8 (Cc), 28.9 (Cd), 50.3 (Ce), 121.3 (C2″), 117.9 (C3″), 125.2 (C3″a), 120.7 (C4″), 120.0 (C5″), 122.7 (C6″), 111.3 (C7″), 136.8 (C7″a).

HRMS (MSES+) calcd for $C_{29}H_{30}N_4O_2H$, 467.2447; Found: 467.2442.

*4.1.8.2. 1-{4-[4-(5-Chloro-1H-indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]butyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**16**).* The title compound was isolated as a yellow powder. Yield: 59.9% 0.150 g, m.p. 116–122 °C (melts with decomposition).

^{1}H NMR (500 Hz, DMSO): 4.42 (C3H, 4d, $^3J_1$ = 9.5, $^3J_2$ = 4.5, ^4J = 0.5), 2.86 (C4H (1), dd, ^2J = 18.00, ^3J = 9.5), 3.23 (C4H(2), dd, ^2J = 18.00, ^3J = 4.5), 7.34 (C2′H, d, ^3J = 1.5), 7.51 (C4′H, dt, ^3J = 8.5, ^4J = ^5J = 1.0), 7.04 (C5′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.13 (C6′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.40–7.45 (C7′H, C2′H, C7″H, m), 3.59 (C1^{x}H$_2$, t, ^3J = 7.0), 1.70 (C2^{x}H$_2$, m), 1.60 (C3^{x}H$_2$, q, ^3J = 7.5), 2.52 (C4^{x}H$_2$, t, ^3J = 7.0), 3.18 (CaH$_2$, d, ^3J = 3.0), 6.13 (CbH, sp, ^3J = 3.5, ^4J = 1.5), 2.56 (CdH$_2$, m), 2.70 (CeH$_2$, t, ^3J = 5.5), 7.85 (C4″H, d, ^4J = 2.5), 7.11 (C6″H, dd, ^3J = 9.0, ^4J = 2.0), 10.24 (N1′H, bs), 10.46 (N1″H, bs).

^{13}C NMR (125 Hz, DMSO): 179.0 (C2), 39.2 (C3), 37.1 (C4), 177.1 (C5), 124.8 (C2′), 112.8 (C3′), 127.2 (C3′a), 119.6 (C4′), 120.5 (C5′), 122.7 (C6′), 112.6 (C7′), 138.0 (C7′a), 38.9 (C1^x), 26.4 (C2^x), 24.9 (C3^x), 58.4 (C4^x), 53.7 (Ca), 119.5 (Cb), 125.7 (Cc), 29.7 (Cd), 51.2 (Ce), 123.8 (C2″), 117.7 (C3″), 130.4 (C3″a), 120.0 (C4″), 127.3 (C5″), 122.5 (C6″), 113.9 (C7″), 136.7 (C7″a).

HRMS (MSES+) calcd for $C_{29}H_{29}ClN_4O_2H$, 501.2057; Found: 501.2045.

*4.1.8.3. 1-{4-[4-(5-Bromo-1H-indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]butyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**17**).* The title compound was isolated as a yellow powder. Yield: 60.5% 0.165 g, m.p. 140–144 °C.

^{1}H NMR (500 Hz, DMSO): 3.37 (C3H, dd, $^3J_1$ = 5.0, $^3J_2$ = 9.5), 2.83 (C4H(1), dd, ^2J = 18.00, ^3J = 5.0), 3.25 (C4H(2), dd, ^2J = 18.00, ^3J = 9.5), 7.34 (C2′H, d, ^3J = 2.5), 7.39 (C4′H, C7′H, m), 6.99 (C5′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.10 (C6′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 3.50 (C1^{x}H$_2$, t, ^3J = 7.0), 1.59 (C2^{x}H$_2$, q, ^3J = 7.0), 1.47 (C3^{x}H$_2$, q, ^3J = 7.0), 2.38 (C4^{x}H$_2$, t, ^3J = 7.0), 3.04 (CaH$_2$, d, ^3J = 2.5), 6.03 (CbH, t), 2.46 (CdH$_2$, ps), 2.58 (CeH$_2$, t, ^3J = 5.5), 7.43 (C2″H, d, ^4J = 2.5), 7.91 (C4″H, d, ^4J = 2.5), 7.22 (C6″H, dd, ^3J = 8.5, ^4J = 2.0), 7.35 (C7″H, d, ^3J = 8.5), 11.06 (N1′H, bs), 11.33 (N1″H, bs).

^{13}C NMR (500 Hz, DMSO): 178.3 (C2), 37.5 (C3), 35.8 (C4), 176.5 (C5), 123.3 (C2′), 110.7 (C3′), 125.7 (C3′a), 118.3 (C4′), 118.7 (C5′), 121.2 (C6′), 111.6 (C7′), 136.4 (C7′a), 38.0 (C1^x), 25.2 (C2^x), 23.8 (C3^x), 57.2 (C4^x), 52.6 (Ca), 118.3 (Cb), 126.2 (Cc), 28.5 (Cd), 50.0 (Ce), 124.2 (C2″), 111.8 (C3″), 128.9 (C3″a), 122.0 (C4″), 115.6 (C5″), 123.6 (C6″), 113.6 (C7″), 135.5 (C7″a).

HRMS (MSES+) calcd for $C_{29}H_{29}BrN_4O_2H$, 545.1552; Found: 545.1538.

*4.1.8.4. 1-{4-[4-(5-Fluoro-1H-indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]butyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**18**).* The title compound was isolated as a yellow powder. Yield: 65% 0.315 g obtained from double amount of starting compounds. M.p. 104–108 °C.

^{1}H NMR (500 Hz, CDCl$_3$): 4.28 (C3H, 4d, $^3J_1$ = 9.5, $^3J_2$ = 5.0, ^4J = 0.5), 2.89 (C4H(1), dd, ^2J = 18.5, ^3J = 5.0), 3.23 (C4H(2), dd, ^2J = 18.5, ^3J = 9.5), 7.07 (C2′H, dd, ^3J = 2.5, ^4J = 1.0), 7.45 (C4′H, 4d, ^3J = 8.0, ^4J = 2.0, ^5J = 1.0), 7.13(C5′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.22 (C6′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.5, ^4J = 1.0), 7.36 (C7′H, dt, ^3J = 8.5, ^4J = ^5J = 1.0), 3.67 (C1^{x}H$_2$, t, ^3J = 7.0), 1.72 (C2^{x}H$_2$, m), 1.62 (C3^{x}H$_2$, m), 2.52 (C4^{x}H$_2$, t, ^3J = 7.5), 3.18 (CaH$_2$, pk), 6.08 (CbH, sp, $^3J_1$ = 5.0, $^3J_2$ = 3.5, ^4J = 1.5), 2.55 (CdH$_2$, m), 2.71 (CeH$_2$, t, ^3J = 6.0), 7.15 (C2″H, d, ^3J = 2.5), 7.51 (C4″H, dd, $^3J_{H-F}$ = 10.5, ^4J = 2.5), 6.94 (C6″H, td, ^3J = 8.5, ^4J = 2.5), 7.26 (C7″H, 4d, ^3J = 9.0, $^4J_{H-F}$ = 4.5, ^5J = 1.0), 8.34 (N1′H, bs), 8.27 (N1″H, bs).

^{13}C NMR (125 Hz, CDCl$_3$): 178.2 (C2), 38.9 (C3), 36.4 (C4), 176.5 (C5), 122.1 (C2′), 111.7 (C3′), 125.8 (C3′a), 118.5 (C4′), 120.1 (C5′), 122.7 (C6′), 111.6 (C7′), 136.6 (C7′a), 38.1 (C1^x), 25.8 (C2^x), 24.4 (C3^x), 57.8 (C4^x), 53.1 (Ca), 119.2 (Cb), 129.5 (Cc), 29.0 (Cd), 50.3 (Ce), 122.9 (C2″), 118.2 (C3″, d*), 125.4 (C3″a, d*), 105.8 (C4″, d*, ^2J = 24.5), 158.1 (C5″, d*, ^1J = 234.3), 110.5 (C6″, d*, ^2J = 26.3), 111.8 (C7″, d*, ^3J = 9.5), 136.6 (C7″a).

HRMS (MSES+) calcd for $C_{29}H_{29}FN_4O_2H$, 485.2353; Found: 485.2336.

*4.1.8.5. 1-{4-[4-(5-Methoxy-1H-indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]butyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**19**).* The title compound was isolated as a yellow powder. Yield: 60.8% 0.18 g, m.p. 117–131 °C (melts with decomposition).

^{1}H NMR (500 Hz, CDCl$_3$): 4.25 (C3H, dd, $^3J_1$ = 9.5, $^3J_2$ = 5.0), 2.86 (C4H(1), dd, ^2J = 18.5, ^3J = 5.0), 3.20 (C4H(2), dd, ^2J = 18.5, ^3J = 9.5), 7.06 (C2′H, d, ^3J = 2.5), 7.44 (C4′H, d, ^3J = 8.0), 7.11 (C5′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 0.5), 7.20 (C6′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.33 (C7′H, d, ^3J = 8.5), 3.66 (C1^{x}H$_2$, t, ^3J = 7.0), 1.74 (C2^{x}H$_2$, q, ^3J = 7.0), 1.63 (C3^{x}H$_2$, q, ^3J = 7.0), 2.54 (C4^{x}H$_2$, CdH$_2$, m), 3.21 (CaH$_2$, bs), 6.08 (CbH, t), 2.72 (CeH$_2$, t, ^3J = 5.5), 7.31 (C2″H, d, ^3J = 2.0), 6.99 (C4″H, d, ^4J = 2.5), 6.86 (C6″H, dd, ^3J = 8.5, ^4J = 2.5), 7.24 (C7″H, d, ^3J = 9.0), 3.84 (OCH$_3$, s), 8.51 (N1′H, bs), 8.31 (N1″H, bs).

^{13}C NMR (125 Hz, CDCl$_3$): 178.4 (C2), 38.8 (C3), 36.4 (C4), 176.5 (C5), 122.3 (C2′), 111.5 (C3′), 125.8 (C3′a), 118.5 (C4′), 120.0 (C5′), 122.6 (C6′), 111.7 (C7′), 136.6 (C7′a), 38.1 (C1^x), 25.8 (C2^x), 24.3 (C3^x), 57.7 (C4^x), 53.0 (Ca), 118.2 (Cb), 130.0 (Cc), 28.8 (Cd), 50.4 (Ce), 122.2 (C2″), 117.5 (C3″), 125.5 (C3″a), 103.0 (C4″), 154.4 (C5″), 112.2 (C6″), 112.0 (C7″), 132.0 (C7″a), 56.1 (OCH$_3$).

HRMS (MSES+) calcd for $C_{30}H_{32}N_4O_3H$, 497.2553; Found: 497.2536.

*4.1.8.6. 1-{3-[4-(1H-Indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]propyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**20**).* The title compound was isolated as a yellow powder. Yield: 52.3% 0.13 g, m.p. 100–106 °C.

^{1}H NMR (500 Hz, CDCl$_3$): 4.27 (C3H, 4d, $^3J_1$ = 9.5, $^3J_2$ = 5.0, ^4J = 0.5), 2.88 (C4H(1), dd, ^2J = 18.0, ^3J = 5.0), 3.22 (C4H(2), dd, ^2J = 18.0, ^3J = 9.5), 7.08 (C2′H, dd, ^3J = 2.5, ^4J = 0.5), 7.38 (C4′H, C7″H), 7.08 (C5′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.21 (C6′H, m, $^3J_1$ = 8.5, $^3J_2$ = 7.0, ^4J = 1.0), 7.33 (C7′H, dt, ^3J = 8.0, ^4J = ^5J = 1.0), 3.75 (C1^{x}H$_2$, t, ^3J = 7.0), 1.96 (C2^{x}H$_2$, m), 2.58 (C3^{x}H$_2$, t, ^3J = 7.5), 3.20 (CaH$_2$, ps), 6.20 (CbH, sp, $^3J_1$ = 5.5, $^3J_2$ = 3.5, ^4J = 2.0), 2.58 (CdH$_2$, pd), 2.71 (CeH$_2$, m), 7.89 (C4″H, d, ^3J = 8.0), 7.15 (C5″H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.19 (C6″H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 8.19 (N1′H, bs), 8.13 (N1″H, bs).

^{13}C NMR (125 Hz, CDCl$_3$): 178.3 (C2), 38.1 (C3), 36.5 (C4), 176.6 (C5), 122.1 (C2′), 111.7 (C3′), 125.8 (C3′a), 118.6 (C4′), 120.0 (C5′), 122.2 (C6′), 111.5 (C7′), 136.5 (C7′a), 37.8 (C1^x), 24.8 (C2^x), 55.9 (C3^x), 53.0 (Ca), 118.9 (Cb), 129.8 (Cc), 28.9 (Cd), 50.3 (Ce), 121.3 (C2″), 117.8 (C3″), 125.2 (C3″a), 120.7 (C4″), 120.1 (C5″), 122.6 (C6″), 111.3 (C7″), 136.8 (C7″a).

HRMS (MSES+) calcd for $C_{28}H_{28}N_4O_2H$, 453.2291; Found: 453.2269.

*4.1.8.7. 1-{3-[4-(5-Chloro-1H-indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]propyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**21**).* The title compound was isolated as a yellow powder. Yield: 39.2% 0.10 g, m.p. 90–120 °C (melts with decomposition).

^{1}H NMR (500 Hz, $CDCl_3$): 4.28 (C3H, 4d, $^3J_1 = 9.5$, $^3J_2 = 5.0$, $^4J = 0.5$), 2.90 (C4H(1), dd, $^2J = 18.0$, $^3J = 5.0$), 3.24 (C4H(2), dd, $^2J = 18.0$, $^3J = 9.5$), 7.13 (C2′H, dd, $^3J = 2.5$, $^4J = 0.5$), 7.40 (C4′H, 4d, $^3J = 8.0$, $^4J = 1.5$, $^5J = 0.5$), 7.09 (C5′H, m, $^3J_1 = 8.0$, $^3J_2 = 7.5$, $^4J = 1.0$), 7.21 (C6′H, m, $^3J_1 = 8.5$, $^3J_2 = 7.0$, $^4J = 1.0$), 7.36 (C7′H, dt, $^3J = 8.0$, $^4J = {}^5J = 1.0$), 3.75 ($C1^xH_2$, t, $^3J = 7.0$), 1.96 ($C2^xH_2$, m), 2.58 ($C3^xH_2$, t, $^3J = 7.0$), 3.19 (CaH_2, pk), 6.12 (CbH, sp, $^3J_1 = 5.0$, $^3J_2 = 3.5$, $^4J = 2.0$), 2.54 (CdH_2, m), 2.70 (CeH_2, m), 7.12 (C2″H, d, $^3J = 2.5$), 7.85 (C4″H, d, $^4J = 2.0$), 7.16 (C6″H, 4d, $^3J = 8.5$, $^4J = 2.0$, $^5J = 0.5$), 7.27 (C7″H, dd, $^3J = 8.0$, $^5J = 1.0$), 8.20 (N1′H, bs), 8.13 (N1″H, bs).

^{13}C NMR (125 Hz, $CDCl_3$): 178.3 (C2), 38.2 (C3), 36.5 (C4), 176.6 (C5), 122.1 (C2′), 111.7 (C3′), 125.8 (C3′a), 118.6 (C4′), 120.1 (C5′), 122.7 (C6′), 111.6 (C7′), 136.6 (C7′a), 37.8 ($C1^x$), 24.8 ($C2^x$), 55.9 ($C3^x$), 53.0 (Ca), 119.5 (Cb), 129.3 (Cc), 29.0 (Cd), 50.2 (Ce), 122.5 (C2″), 117.7 (C3″), 128.3 (C3″a), 120.2 (C4″),126.2 (C5″), 122.5 (C6″), 112.2 (C7″), 135.1 (C7″a).

HRMS (MSES+) calcd for $C_{28}H_{27}ClN_4O_2Na$, 509.1720; Found: 509.1730.

*4.1.8.8. 1-{3-[4-(5-Bromo-1H-indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]propyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**22**).* The title compound was isolated as a yellow powder. Yield: 60.1% 0.16 g, m.p. 100–140 °C (melts with decomposition).

^{1}H NMR (500 Hz, $CDCl_3$): 4.27 (C3H, 4d, $^3J_1 = 9.5$, $^3J_2 = 5.0$, $^4J = 0.5$), 2.89 (C4H(1), dd, $^2J = 18.0$, $^3J = 5.0$), 3.22 (C4H(2), dd, $^2J = 18.0$, $^3J = 9.5$), 7.09 (C2′H, dd, $^3J = 2.5$, $^4J = 0.5$), 7.39 (C4′H, 4d, $^3J = 8.0$, $^4J = 2.0$, $^5J = 1.0$), 7.08 (C5′H, m, $^3J_1 = 8.0$, $^3J_2 = 7.0$, $^4J = 1.0$), 7.20 (C6′H, m, $^3J_1 = 8.0$, $^3J_2 = 7.0$, $^4J = 1.0$), 7.34 (C7′H, dt, $^3J = 8.5$, $^4J = {}^5J = 1.0$), 3.75 ($C1^xH_2$, t, $^3J = 7.0$), 1.94 ($C2^xH_2$, m), 2.57 ($C3^xH_2$, t, $^3J = 7.0$), 3.17 (CaH_2, pk), 6.11 (CbH, sp, $^3J_1 = 5.0$, $^3J_2 = 3.5$, $^4J = 2.0$), 2.50 (CdH_2, m), 2.67 (CeH_2, m), 7.05 (C2″H, d, $^3J = 2.5$), 8.0 (C4″H, d, $^4J = 2.0$), 7.27 (C6″H, dd, $^3J = 8.5$, $^4J = 2.0$), 7.21 (C7″H, dd, $^3J = 8.5$, $^5J = 0.5$), 8.34 (N1′H, bs), 8.21 (N1″H, bs).

^{13}C NMR (125 Hz, $CDCl_3$): 178.4 (C2), 38.2 (C3), 36.5 (C4), 176.6 (C5), 122.1 (C2′), 111.6 (C3′), 125.8 (C3′a), 118.5 (C4′), 120.1 (C5′), 122.7 (C6′), 111.6 (C7′), 136.6 (C7′a), 37.8 ($C1^x$), 24.9 ($C2^x$), 56.0 ($C3^x$), 53.1 (Ca), 119.6 (Cb), 129.3 (Cc), 29.0 (Cd), 50.2 (Ce), 122.4 (C2″), 113.4 (C3″), 126.9 (C3″a), 123.2 (C4″), 117.6 (C5″), 125.1 (C6″), 112.7 (C7″), 135.4 (C7″a).

HRMS (MSES+) calcd for $C_{28}H_{28}BrN_4O_2H$, 531.1396; Found: 531.1375.

*4.1.8.9. 1-{3-[4-(5-Fluoro-1H-indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]propyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**23**).* The title compound was isolated as a yellow powder. Yield: 67.8% 0.16 g, m.p. 90–110 °C (melts with decomposition).

^{1}H NMR (500 Hz, $CDCl_3$): 4.28 (C3H, 4d, $^3J_1 = 9.5$, $^3J_2 = 5.0$, $^4J = 0.5$), 2.90 (C4H(1), dd, $^2J = 18.0$, $^3J = 5.0$), 3.23 (C4H(2), dd, $^2J = 18.0$, $^3J = 9.5$), 7.12 (C2′H, dd, $^3J = 2.5$, $^4J = 1.0$), 7.39 (C4′H, 4d, $^3J = 8.0$), 7.09 (C5′H, m, $^3J_1 = 8.0$, $^3J_2 = 7.0$, $^4J = 1.0$), 7.20 (C6′H, m, $^3J_1 = 8.0$, $^3J_2 = 7.0$, $^4J = 1.0$), 7.35 (C7′H, dt, $^3J = 8.0$, $^4J = {}^5J = 1.0$), 3.75 ($C1^xH_2$, t, $^3J = 7.0$), 1.95 ($C2^xH_2$, m), 2.57 ($C3^xH_2$, t, $^3J = 7.0$), 3.18 (CaH_2, pk), 6.10 (CbH, sp, $^3J_1 = 5.0$, $^3J_2 = 3.5$, $^4J = 2.0$), 2.54 (CdH_2, m), 2.69 (CeH_2, t, $^3J = 6.0$), 7.13 (C2″H, d, $^3J = 2.5$), 7.53 (C4″H, dd, $^3J_{H-F} = 10.5$, $^4J = 2.5$), 6.95 (C6″H, td, $^3J = 9.0$, $^4J = 2.5$), 7.27 (C7″H, dd, $^3J = 9.0$, $^5J_{H-F} = 4.5$), 8.19 (N1′H, bs), 8.15 (N1″H, bs).

^{13}C NMR (125 Hz, $CDCl_3$): 178.3 (C2), 38.2 (C3), 36.5 (C4), 176.6 (C5), 122.1 (C2′), 111.7 (C3′), 125.8 (C3′a), 118.6 (C4′), 120.1 (C5′), 122.7 (C6′), 111.6 (C7′), 136.6 (C7′a), 37.8 ($C1^x$), 24.9 ($C2^x$), 56.0 ($C3^x$), 53.1 (Ca), 119.1 (Cb), 129.5 (Cc), 29.0 (Cd), 50.3 (Ce), 122.9 (C2″), 118.1 (C3″, d*), 125.5 (C3″a, d*), 105.8 (C4″, d*, $^2J = 24.1$), 158.2 (C5″, d*, $^1J = 234.5$), 110.6 (C6″, d*, $^2J = 26.4$), 111.8 (C7″, d*, $^3J = 9.8$), 133.3 (C7″a).

HRMS (MSES+) calcd for $C_{28}H_{27}FN_4O_2H$, 471.2196; Found: 471.2194.

*4.1.8.10. 1-{3-[4-(5-Methoxy-1H-indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]propyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**24**).* The title compound was isolated as a yellow powder. Yield: 49.6% 0.12 g, m.p. 110–117 °C (melts with decomposition).

^{1}H NMR (500 Hz, $CDCl_3$): 4.25 (C3H, dd, $^3J_1 = 9.5$, $^3J_2 = 5.0$), 2.87 (C4H(1), dd, $^2J = 18.0$, $^3J = 5.0$), 3.20 (C4H(2), dd, $^2J = 18.0$, $^3J = 9.5$), 7.03 (C2′H, d, $^3J = 2.0$), 7.38 (C4′H, d, $^3J = 8.0$), 7.07 (C5′H, m, $^3J_1 = 8.0$, $^3J_2 = 7.0$, $^4J = 1.5$), 7.08 (C6′H, m, $^3J_1 = 8.5$, $^3J_2 = 7.0$, $^4J = 1.0$), 7.31 (C7′H, d, $^3J = 8.5$), 3.74 ($C1^xH_2$, t, $^3J = 7.0$), 1.95 ($C2^xH_2$, m), 2.57 ($C3^xH_2$, t, $^3J = 7.0$), 3.18 (CaH_2, bs), 6.12 (CbH, t, $^3J = 3.5$), 2.52 (CdH_2, bs), 2.68 (CeH_2, m), 7.33 (C2″H, d, $^3J = 2.0$), 7.03 (C4″H, d, $^4J = 2.0$), 6.86 (C6″H, dd, $^3J = 8.5$, $^4J = 2.5$), 7.23 (C7″H, d, $^3J = 8.5$), 3.84 (OCH_3, s), 8.31 (N1′H, bs), 8.25 (N1″H, bs).

^{13}C NMR (125 Hz, $CDCl_3$): 178.5 (C2), 38.2 (C3), 36.5 (C4), 176.7 (C5), 122.3 (C2′), 111.5 (C3′), 125.8 (C3′a), 118.5 (C4′), 120.0 (C5′), 122.6 (C6′), 111.6 (C7′), 136.6 (C7′a), 37.8 ($C1^x$), 24.9 ($C2^x$), 56.0 ($C3^x$), 53.1 (Ca), 118.5 (Cb), 130.0 (Cc), 29.0 (Cd), 50.4 (Ce), 122.2 (C2″), 117.5 (C3″), 125.5 (C3″a), 103.0 (C4″), 154.4 (C5″), 112.2 (C6″), 112.0 (C7″), 132.0 (C7″a), 56.1 (OCH_3).

HRMS (MSES+) calcd for $C_{29}H_{30}N_4O_3H$ 483.2396; Found: 483.2378.

*4.1.8.11. 1-{2-[4-(1H-Indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]ethyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**25**).* The title compound was isolated as a yellow powder. Yield: 26.0% 0.12 g. Obtained from double amount of starting compounds. crystallization from methanol, m.p. 140–240 °C (melts with decomposition).

^{1}H NMR (500 Hz, DMSO): 4.34 (C3H, dd, $^3J_1 = 9.5$, $^3J_2 = 5.0$), 2.75 (C4H(1), dd, $^2J = 18.0$, $^3J = 5.0$), 3.22 (C4H(2), dd, $^2J = 18.0$, $^3J = 9.5$), 7.33 (C2′H, d, $^3J = 2.5$), 7.45 (C4′H, d, $^3J = 7.5$), 6.94 (C5′H, t, $^3J = 7.5$), 7.04 (C6′H, t, $^3J = 8.0$), 7.33 (C7′H, C7″H, m), 3.64 ($C1^xH_2(1)$, dt, $^2J = 13.5$, $^3J = 6.5$), 3.75 ($C1^xH_2(2)$, dt, $^2J = 13.5$, $^3J = 6.5$), 2.56–2.72 ($C2^xH_2$, CeH(1), m), 3.20 (CaH_2, pk), 6.15 (CbH, t), ~2.50* (CdH_2), 2.80 (CeH(2), m), 7.38 (C2″H, d, $^3J = 2.0$), 7.83 (C4″H, d, $^3J = 8.0$), 7.02 (C5″H, t, $^3J = 8.0$), 7.11 (C6″H, t, $^3J = 7.5$), 11.01 (N1′H, bs), 11.10 (N1″H, bs).

^{13}C NMR (125 Hz, DMSO): 179.1 (C2), 38.4 (C3), 37.0 (C4), 177.2 (C5), 124.6 (C2′), 111.6 (C3′), 125.4 (C3′a), 119.1 (C4′), 119.5 (C5′), 122.0 (C6′), 112.4 (C7′), 137.2 (C7′a), 36.5 ($C1^x$), 55.1 ($C2^x$), 53.7 (Ca), 118.1 (Cb), 126.2 (Cc), 29.2 (Cd), 50.3 (Ce), 123.5 (C2″), 116.6 (C3″), 130.4 (C3″a), 120.8 (C4″), 120.0 (C5″), 121.9 (C6″), 112.4 (C7″), 137.7 (C7″a).

HRMS (MSES+) calcd for $C_{27}H_{26}N_4ONa$, 461.1953; Found: 461.1952.

*4.1.8.12. 1-{2-[4-(5-Chloro-1H-indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]ethyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**26**).* The title compound was isolated as a yellow powder. Yield: 29.5% 0.07 g, m.p. 114–124 °C (melts with decomposition).

^{1}H NMR (500 Hz, DMSO): 4.34 (C3H, dd, $^3J_1 = 9.5$, $^3J_2 = 5.0$), 2.74 (C4H(1), dd, $^2J = 18.0$, $^3J = 5.0$), 3.22 (C4H(2), dd, $^2J = 18.0$, $^3J = 9.5$), 7.33 (C2′H, d, $^3J = 2.5$), 7.44 (C4′H, d, $^3J = 8.0$), 6.93 (C5′H, m, $^3J_1 = 8.0$, $^3J_2 = 7.0$, $^4J = 1.0$), 7.02 (C6′H, m, $^3J_1 = 8.0$, $^3J_2 = 7.0$, $^4J = 1.0$), 7.34 (C7′H, d, $^3J = 8.0$), 3.64 ($C1^xH_2(1)$, dt, $^2J = 13.5$, $^3J = 5.5$), 3.74 ($C1^xH_2(2)$, dt, $^2J = 13.5$, $^3J = 7.0$), 2.56–2.71 ($C2^xH_2$, CeH(1), m), 3.20 (CaH_2, bs), 6.10 (CbH, bs), ~2.4* (CdH_2), 2.78 (CeH(2), m), 7.47 (C2″H, d, $^3J = 2.5$), 7.81 (C4″H, d, $^4J = 2.0$), 7.11 (C6″H, dd, $^3J = 8.5$,

^4J = 2.0), 7.40 (C7″H, d, ^3J = 8.5), 11.00 (N1′H, d, ^3J = 2.5), 11.31 (N1″H, d, ^3J = 2.5).

^{13}C NMR (125 Hz, DMSO): 178.4 (C2), 37.6 (C3), 36.2 (C4), 176.5 (C5), 124.5 (C2′), 111.7 (C3′), 125.4 (C3′a), 118.1 (C4′), 118.7 (C5′), 121.2 (C6′), 110.9 (C7′), 136.5 (C7′a), 35.8 (C1^x), 54.3 (C2^x), 52.9 (Ca), 120.5 (Cb), 125.6 (Cc), 28.4 (Cd), 49.4 (Ce), 123.9 (C2″), 115.8 (C3″), 129.1 (C3″a), 118.4 (C4″),150.0 (C5″), 119.2 (C6″), 113.2 (C7″), 135.4 (C7″a).

HRMS (MSES+) calcd for $C_{27}H_{25}ClN_4O_2H$, 473.1744; Found: 473.1753.

*4.1.8.13. 1-{2-[4-(5-Bromo-1H-indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]ethyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**27**).* The title compound was isolated as a yellow powder. Yield: 58.0% 0.15 g. Obtained from double amount of starting compounds. M.p. 100–160 °C (melts with decomposition).

^{1}H NMR (500 Hz, DMSO): 4.35 (C3H, dd, $^3J_1$ = 9.5, $^3J_2$ = 5.0), 2.75 (C4H(1), dd, ^2J = 18.0, ^3J = 5.0), 3.22 (C4H(2), dd, ^2J = 18.0, ^3J = 10.0), 7.33 (C2′H, d, ^3J = 2.5), 7.45 (C4′H, d, ^3J = 8.0), 6.94 (C5′H, m, $^3J_1$ = 7.5, $^3J_2$ = 7.5, ^4J = 1.0), 7.03 (C6′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.35 (C7′H, d, ^3J = 8.0), 3.64 (C1^{x}H$_2$(1), q, ^2J = 13.5, ^3J = 6.0), 3.74 (C1^{x}H$_2$(2), q, ^2J = 13.5, ^3J = 7.0), 2.56–2.72 (C2^{x}H$_2$, CeH(1), m), 3.19 (CaH$_2$, t), 6.10 (CbH, t), 2.50 (CdH$_2$, bs), 2.80 (CeH(2), m), 7.46 (C2″H, d, ^3J = 2.0), 7.95 (C4″H, d, ^3J = 1.5), 7.23 (C6″H, dd, ^3J = 8.5, ^4J = 1.5), 7.36 (C7″H, d, ^3J = 8.5), 11.00 (N1′H, d), 11.33 (N1″H, d).

^{13}C NMR (125 Hz, DMSO): 179.1 (C2), 38.4 (C3), 37.0 (C4), 177.2 (C5), 124.6 (C2′), 112.4 (C3′), 127.1 (C3′a), 119.1 (C4′), 119.5 (C5′), 122.0 (C6′), 111.6 (C7′), 137.2 (C7′a), 36.5 (C1^x), 55.0 (C2^x), 53.6 (Ca), 118.9 (Cb), 126.2 (Cc), 29.2 (Cd), 50.1 (Ce), 125.1 (C2″), 112.7 (C3″), 127.1 (C3″a), 122.9 (C4″), 116.4 (C5″), 124.4 (C6″), 114.4 (C7″), 136.3 (C7″a).

HRMS (MSES+) calcd for $C_{27}H_{25}BrN_4O_2H$, 517.1239; Found: 517.1245.

*4.1.8.14. 1-{2-[4-(5-Fluoro-1H-indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]ethyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**28**).* The title compound was isolated as a yellow powder. Yield: 23.0% 0.105 g. Obtained from double amount of starting compounds. M.p. 80–120 °C (melts with decomposition).

^{1}H NMR (500 Hz, DMSO): 4.34 (C3H, dd, $^3J_1$ = 9.5, $^3J_2$ = 5.0), 2.75 (C4H(1), dd, ^2J = 18.0, ^3J = 5.0), 3.22 (C4H(2), dd, ^2J = 18.0, ^3J = 9.5), 7.33 (C2′H, d, ^3J = 2.5), 7.44 (C4′H, d, ^3J = 8.0), 6.93 (C5′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.02 (C6′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.35 (C7′H, d, ^3J = 8.5), 3.64 (C1^{x}H$_2$(1), dt, ^2J = 13.5, ^3J = 6.0), 3.74 (C1^{x}H$_2$(2), dt, ^2J = 13.5, ^3J = 7.0), 2.56–2.72 (C2^{x}H$_2$, CeH(1), m), 3.19 (CaH$_2$, m), 6.09 (CbH, t), ~2.5* (CdH$_2$), 2.79 (CeH(2), m), 7.46 (C2″H, d, ^3J = 3.0), 7.55 (C4″H, dd, $^3J_{H-F}$ = 11.0, ^4J = 2.5), 6.96 (C6″H, td, ^3J = 9.0, ^4J = 2.5), 7.38 (C7″H, dd, ^3J = 8.5, $^4J_{H-F}$ = 5.0), 11.00 (N1′H, d, ^3J = 2.0), 11.21 (N1″H, d, ^3J = 1.5).

^{13}C NMR (125 Hz, DMSO): 179.1 (C2), 38.4 (C3), 36.9 (C4), 177.2 (C5), 122.0 (C2′), 112.4 (C3′), 125.5 (C3′a), 119.1 (C4′), 119.5 (C5′), 124.6 (C6′), 111.6 (C7′), 137.2 (C7′a), 36.5 (C1^x), 55.1 (C2^x), 53.6 (Ca), 118.3 (Cb), 130.0 (Cc), 29.1 (Cd), 50.2 (Ce), 126.2 (C2″), 116.8 (C3″, d**, ^4J = 5.2), 125.3 (C3″a, d**, ^3J = 9.8), 105.6 (C4″, d**, ^2J = 23.9), 157.9 (C5″,d**, ^1J = 231.0), 110.0 (C6″,d**, ^2J = 26.4), 113.3 (C7″,d**, ^3J = 9.7), 134.3 (C7″a).

HRMS (MSES+) calcd for $C_{27}H_{25}FN_4O_2H$, 457.2040; Found: 457.2041.

*4.1.8.15. 1-{2-[4-(5-Methoxy-1H-indol-3-yl)-3,6-dihydro-2H-pyridin-1-yl]ethyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**29**).* The title compound was isolated as a yellow powder. Yield: 36.6% 0.09 g, m.p. 119–133 °C (melts with decomposition).

^{1}H NMR (500 Hz, acetone): 4.37 (C3H, dd, $^3J_1$ = 9.5, $^3J_2$ = 5.0), 2.79 (C4H(1), dd, ^2J = 18.0, ^3J = 5.0), 3.25 (C4H(2), dd, ^2J = 18.0, ^3J = 9.5), 7.39 (C2′H, d, ^3J = 2.0), 7.58 (C4′H, d, ^4J = 7.0), 7.03 (C5′H, C6″H, m), 7.36 (C7′H, dd, ^3J = 7.0, ^4J = 1.0), 3.73 (C1^{x}H(1), m), 3.84 (C1^{x}H(2), m), 2.65–2.76 (C2^{x}H$_2$, CeH(1), m), 3.26 (CaH$_2$, m), 6.19 (CbH, sp, ^3J = 3.5), 2.85 (CeH(2), m), 7.31 (C2″H, d, ^3J = 2.5), 7.34 (C4″, d, ^4J = 2.0), 6.80 (C6″H, dd, ^3J = 8.5, ^4J = 2.0), 7.31 (C7″H, d, ^3J = 7.5), 10.12 (N1′H, bs), 10.14 (N1″H, bs).

HRMS (MSES+) calcd for $C_{28}H_{28}N_4O_3H$ 491.2059; Found: 491.2075.

*4.1.8.16. 1-{4-[4-(1H-Indol-3-yl)piperidin-1-yl]butyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**30**).* The title compound was isolated as a yellow powder. Yield: 64.6% 0.29 g. Obtained from double amount of starting compounds. M.p. 100–105 °C.

^{1}H NMR (see Supplementary materials) (500 Hz, $CDCl_3$): 4.27 (C3H, 4d, $^3J_1$ = 10.0, $^3J_2$ = 5.0, ^4J = 0.5), 2.91 (C4H(1), dd, ^2J = 18.00, ^3J = 5.0), 3.23 (C4H(2), dd, ^2J = 18.00, ^3J = 10.0), 7.06 (C2′H, d, ^3J = 2.0), 7.45 (C4′H, d, ^3J = 8.0), 7.13 (C5′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.21 (C6′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.35 (C7′H, C7″H, m), 3.65 (C1^{x}H$_2$, t, ^3J = 7.0), 1.70 (C2^{x}H$_2$, m), 1.61 (C3^{x}H$_2$, m), 2.47 (C4^{x}H$_2$, t, ^3J = 7.5), 3.05 (CaH(E), CeH(E), m), 2.14 (CaH(A), CeH(A), m), 2.03 (CbH(E), CdH(E), m), 1.84 (CbH(A), CdH(A), m), 2.82 (CcH, tt, $^3J_1$ = 12.0, $^3J_2$ = 3.5), 6.91 (C2″H, d, ^3J = 2.0), 7.62 (C4″H, d, ^3J = 8.0), 7.09 (C5″H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.17 (C6″H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 8.49 (N1′H, bs), 8.11 (N1″H, bs).

^{13}C NMR (see Supplementary materials) (125 Hz, $CDCl_3$): 178.3 (C2), 38.1 (C3), 36.4 (C4), 176.5 (C5), 122.2 (C2′), 111.6 (C3′), 125.7 (C3′a), 118.5 (C4′), 120.1 (C5′), 122.7 (C6′), 111.2 (C7′), 136.4 (C7′a), 38.8 (C1^x), 25.8 (C2^x), 23.9 (C3^x), 58.3 (C4^x), 54.2 and 54.2 (Ca, Ce), 32.6 and 32.6 (Cb, Cd), 33.2 (Cc), 119.8 (C2″), 121.1 (C3″), 126.6 (C3″a), 119.0 (C4″), 119.1 (C5″), 121.9 (C6″), 111.2 (C7″), 136.6 (C7″a).

HRMS (MSES+) calcd for $C_{29}H_{32}N_4O_2H$, 469.2604; Found, 469.2616.

*4.1.8.17. 1-{4-[4-(5-Fluoro-1H-indol-3-yl)piperidin-1-yl]butyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**31**).* The title compound was isolated as a yellowish powder. Yield: 73.8% 0.18 g, m.p. 117–124 °C (melts with decomposition).

^{1}H NMR (500 Hz, $CDCl_3$): 4.29 (C3H, 4d, $^3J_1$ = 9.5, $^3J_2$ = 4.5, ^4J = 1.0), 2.93 (C4H(1), dd, ^2J = 18.0, ^3J = 4.5), 3.25 (C4H(2), dd, ^2J = 18.0, ^3J = 9.5), 7.11 (C2′H, dd, ^3J = 2.5, ^4J = 0.5), 7.46(4d, ^3J = 8.0, ^4J = 2.0, ^5J = 1.0), 7.13 (C5′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.22 (C6′H, m, $^3J_1$ = 8.5, $^3J_2$ = 7.0, ^4J = 1.0), 7.38 (C7′H, dt, ^3J = 8.0, ^4J = ^5J = 1.0), 3.66 (C1^{x}H$_2$, t, ^3J = 7.0), 1.71 (C2^{x}H$_2$, q, ^3J = 7.0), 1.62 (C3^{x}H$_2$, q, ^3J = 7.0), 2.48 (C4^{x}H$_2$, t, ^3J = 7.5), 3.05 (CaH(E), CeH(E), m), 2.14 (CaH(A), CeH(A), m), 1.99 (CbH(E), CdH(E), m), 1.83 (CbH(A), CdH(A), m), 2.74 (CcH, tt, $^3J_1$ = 11.5, $^3J_2$ = 3.5), 6.96 (C2″H, d, ^3J = 2.5), 7.25 (C4″H, C7″H, m), 6.92 (C6″H, td, ^3J = 9.0, ^4J = 2.5), 8.43 (N1′H, bs), 8.10 (N1″H, bs).

^{13}C NMR (125 Hz, $CDCl_3$): 178.3 (C2), 38.2 (C3), 36.3 (C4), 176.5 (C5), 122.1 (C2′), 111.8 (C3′), 132.8 (C3′a), 118.6 (C4′), 120.1 (C5′), 122.7 (C6′), 111.7 (C7′), 136.6 (C7′a), 38.7 (C1^x), 25.8 (C2^x), 23.9 (C3^x), 58.2 (C4^x), 54.1 and 54.2 (Ca, Ce), 32.4 and 32.5 (Cb, Cd), 33.1 (Cc), 121.6 (C2″), 121.2 (C3″), 126.9 (C3″a, d*, ^3J = 9.8), 103.9 (C4″, d*, ^4J = 23.7), 157.5 (C5″, d*, ^1J = 234.3), 110.3 (C6″, d*, ^2J = 26.4), 111.7 (C7″, d*, ^3J = 11.0), 136.6 (C7″a).

HRMS (MSES+) calcd for $C_{29}H_{31}FN_4O_2H$ 487.2509; Found, 487.2503.

*4.1.8.18. 1-{4-[4-(5-Methoxy-1H-indol-3-yl)piperidin-1-yl]butyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**32**).* The title compound was isolated as a yellow powder. Yield: 88.7% 0.22 g, m.p. 115–121 °C.

^{1}H NMR (500 Hz, $CDCl_3$): 4.29 (C3H, 4d, $^3J_1$ = 9.5, $^3J_2$ = 4.5, ^4J = 0.5), 2.93 (C4H(1), dd, ^2J = 18.5, ^3J = 4.5), 3.24 (C4H(2), dd, ^2J = 18.5, ^3J = 9.5), 7.08 (C2′H, d, ^3J = 1.0), 7.46 (C4′H, d, ^3J = 8.0), 7.12 (C5′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.39 (C6′H, m, $^3J_1$ = 8.0,

$^3J_2$ = 7.0, ^4J = 1.0), 7.36 (C7′H, dt, ^3J = 8.0, ^4J = ^5J = 1.0), 3.63 (C1^{x}H$_2$, t, ^3J = 7.0), 1.63 (C2^{x}H$_2$, C3^{x}H$_2$, m), 2.61 (C4^{x}H$_2$, t, ^3J = 7.5), 3.13 (CaH(E), CeH(E), m), 2.27 (CaH(A), CeH(A), m), 1.97 (CbH$_2$, CdH$_2$, m), 2.76 (CcH, m), 6.85 (C2″H, d, ^3J = 2.0), 7.02 (C4″H, d, ^4J = 2.5), 6.84 (C6″H, dd, ^3J = 9.0, ^4J = 2.5), 7.24 (C7″H, dd, ^3J = 9.0, ^5J = 0.5), 3.86 (OCH$_3$, s), 8.86 (N1′H, bs), 8.27 (N1″H, bs).

^{13}C NMR (125 Hz, CDCl$_3$): 178.5 (C2), 38.2 (C3), 36.3 (C4), 176.6 (C5), 122.3 (C2′), 111.4 (C3′), 125.8 (C3′a), 118.5 (C4′), 120.0 (C5′), 122.6 (C6′), 111.8 (C7′), 136.7 (C7′a), 38.2 (C1^x), 25.5 (C2^x), 23.1 (C3^x), 57.2 (C4^x), 53.3 and 53.5 (Ca, Ce), 31.1 (Cb, Cd), 32.5 (Cc), 121.0 (C2″), 199.5 (C3″), 126.7 (C3″a), 101.0 (C4″), 153.8 (C5″), 112.0 (C6″), 112.0 (C7″), 131.6 (C7″a), 56.1 (OCH$_3$).

HRMS (MSES+) calcd for $C_{30}H_{34}N_4O_3H$ 499.2709; Found: 499.2698.

*4.1.8.19. 1-{3-[4-(1H-Indol-3-yl)piperidin-1-yl]propyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**33**).* The title compound was isolated as a yellow powder. Yield: 65.8% 0.15 g, m.p. 111–117 °C.

^{1}H NMR (500 Hz, CDCl$_3$): 4.29 (C3H, 4d, $^3J_1$ = 10.0, $^3J_2$ = 5.0, ^4J = 0.5), 2.93 (C4H(1), dd, ^2J = 18.5, ^3J = 5.0), 3.26 (C4H(2), dd, ^2J = 18.5, ^3J = 10.0), 7.13 (C2′H, C5′H, m), 7.44 (C4′H, dd, ^3J = 8.0, ^4J = 1.0), 7.22 (C6′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.37 (C7′H, dt, ^3J = 8.0, ^4J = ^5J = 1.0), 3.70 (C1^{x}H$_2$, t, ^3J = 7.0), 1.92 (C2^{x}H$_2$, m), 2.49 (C3^{x}H$_2$, t, ^3J = 7.5), 3.05 (CaH(E), CeH(E), m), 2.12 (CaH(A), CeH(A), m), 2.04 (CbH(E), CdH(E), m), 1.80 (CbH(A), CdH(A), m), 2.82 (CcH, tt, $^3J_1$ = 12.0, $^3J_2$ = 4.0), 6.88 (C2″H, dd, ^3J = 2.5, ^4J = 0.5), 7.62 (C4″H, dd, ^3J = 8.0, ^4J = 1.0), 7.09 (C5″H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.17 (C6″H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.34 (C7″H, dt, ^3J = 8.0, ^4J = ^5J = 1.0), 8.33 (N1′H, bs), 8.03 (N1″H, bs).

^{13}C NMR (125 Hz, CDCl$_3$): 178.2 (C2), 37.6 (C3), 36.4 (C4), 176.5 (C5), 122.2 (C2′), 111.6 (C3′), 125.7 (C3′a), 118.6 (C4′), 120.1 (C5′), 122.7 (C6′), 111.7 (C7′), 136.4 (C7′a), 38.2 (C1^x), 25.1 (C2^x), 56.3 (C3^x), 54.3 (Ca, Ce), 32.8 and 32.8 (Cb, Cd), 33.3 (Cc), 119.7 (C2″), 121.2 (C3″), 126.6 (C3″a), 119.0 (C4″), 119.1 (C5″), 121.9 (C6″), 111.2 (C7″), 136.6 (C7″a).

HRMS (MSES+) calcd for $C_{28}H_{30}N_4O_2H$ 455.2447; Found: 455.2459.

*4.1.8.20. 1-{3-[4-(5-Fluoro-1H-indol-3-yl)piperidin-1-yl]propyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**34**).* The title compound was isolated as a yellowish powder. Yield: 74.5% 0.17 g, m.p. 109–116 °C (melts with decomposition).

^{1}H NMR (500 Hz, CDCl$_3$): 4.30 (C3H, dd, $^3J_1$ = 9.5, $^3J_2$ = 5.0), 2.94 (C4H(1), dd, ^3J = 18.5, ^3J = 5.0), 3.26 (C4H(2), dd, ^3J = 18.5, ^3J = 9.5), 7.15 (C2′H, d, ^3J = 2.0), 7.44 (C4′H, d, ^3J = 8.0), 7.13 (C5′H, t, ^3J = 8.0), 7.24 (C6′6, C4″H, C7″H, m), 7.38 (C7′H, d, ^3J = 8.0), 3.70 (C1^{x}H$_2$, t, ^3J = 7.0), 1.77 (C2^{x}H$_2$, m), 2.48 (C3^{x}H$_2$, t, ^3J = 7.0), 3.04 (CaH(E), CeH(E), pd), 2.08 (CaH(A), CeH(A), pt), 1.79 (CbH(E), CdH(E), pd), 1.92 (CbH(A), CdH(A), m), 2.74 (CcH, tt, $^3J_1$ = 12.0, $^3J_2$ = 3.5), 6.92 (C2″H, C6″H, m), 8.27 (N1′H, bs), 8.00 (N1″H, bs).

^{13}C NMR (125 Hz, CDCl$_3$): 178.2 (C2), 37.6 (C3), 36.4 (C4), 176.4 (C5), 122.2 (C2′), 111.8 (C3′), 132.8 (C3′a), 118.6 (C4′), 120.1 (C5′), 122.7 (C6′), 111.7 (C7′), 136.6 (C7′a), 38.2 (C1^x), 25.1 (C2^x), 56.3 (C3^x), 54.3 (Ca, Ce), 32.7 and 32.8 (Cb, Cd), 33.3 (Cc), 121.5 (C2″), 125.7 (C3″), 127.0 (C3″a, d*, ^3J = 9.3), 103.9 (C4″, d*, ^2J = 23.5), 157.5 (C5″, d*, ^1J = 234.3), 110.2 (C6″, d*, ^2J = 26.5), 111.7 (C7″, d*, ^3J = 9.7), 136.6 (C7″a).

HRMS (MSES+) calcd for $C_{28}H_{29}FN_4O_2H$ 473.2353; Found: 473.2347.

*4.1.8.21. 1-{3-[4-(5-Methoxy-1H-indol-3-yl)piperidin-1-yl]propyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**35**).* The title compound was isolated as a yellow powder. Yield: 61.7% 0.15 g, m.p. 106–111 °C.

^{1}H NMR (500 Hz, CDCl$_3$): 4.29 (C3H, 4d, $^3J_1$ = 9.5, $^3J_2$ = 5.0, ^4J = 0.5), 2.94 (C4H(1), dd, ^2J = 18.0, ^3J = 5.0), 3.26 (C4H(2), dd, ^2J = 18.0, ^3J = 9.5), 7.14 (C2′H, d, ^3J = 2.0), 7.44 (C4′H, dd, ^3J = 8.0, ^4J = 1.0), 7.22 (C6′H, m, $^3J_1$ = 8.5, $^3J_2$ = 7.0, ^4J = 1.0), 7.38 (C7′H, dt, ^3J = 8.0, ^4J = ^5J = 1.0), 3.70 (C1^{x}H$_2$, t, ^3J = 7.0), 1.93 (C2^{x}H$_2$, m), 2.49 (C3^{x}H$_2$, t, ^3J = 7.5), 3.05 (CaH(E), CeH(E), m), 2.14 (CaH(A), CeH(A), m), 2.03 (CbH(E), CdH(E), m), 1.79 (CbH(A), CdH(A), m), 2.77 (CcH, tt, $^3J_1$ = 11.5, $^3J_2$ = 3.5), 6.87 (C2″H, d, ^3J = 2.0), 7.05 (C4″H, d, ^4J = 2.5), 6.84 (C6″H dd, ^3J = 8.5, ^4J = 2.5), 7.23 (C7″H, d, ^3J = 9.0), 3.86 (OCH$_3$, s), 8.34 (N1′H, bs), 7.91 (N1″H, bs).

^{13}C NMR (125 Hz, CDCl$_3$): 178.2 (C2), 37.6 (C3), 36.4 (C4), 176.5 (C5), 122.2 (C2′), 111.6 (C3′), 125.7 (C3′a), 118.6 (C4′), 120.1 (C5′), 122.7 (C6′), 111.7 (C7′), 136.7 (C7′a), 38.2 (C1^x), 25.1 (C2^x), 56.3 (C3^x), 54.3 (Ca, Ce), 32.7 and 32.7 (Cb, Cd), 33.3 (Cc), 120.6 (C2″), 120.9 (C3″), 127.0 (C3″a), 101.2 (C4″), 153.8 (C5″), 112.0 (C6″), 111.9 (C7″), 136.7 (C7″a), 56.1 (OCH$_3$).

HRMS (MSES+) calcd for $C_{29}H_{32}N_4O_3H$ 485.2553; Found: 485.2532.

*4.1.8.22. 1-{2-[4-(1H-Indol-3-yl)piperidin-1-yl]ethyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**36**).* The title compound was isolated as a yellow powder. Yield: 45.2% 0.10 g, m.p. 97–105 °C (melts with decomposition).

^{1}H NMR (500 Hz, CDCl$_3$): 4.18 (C3H, dd, $^3J_1$ = 9.5, $^3J_2$ = 5.0), 2.89 (C4H(1), dd, ^2J = 18.5, ^3J = 5.0), 3.15 (C4H(2), dd, ^2J = 18.0, ^3J = 9.5), 7.09 (C2′H, C5′H, C5″H, m), 7.48 (C4′H, d, ^3J = 8.0), 7.17 (C6′H, C6″H), 7.32 (C7′H, C7″H, m), 3.82 (C1^{x}H$_2$, m), 2.70 (C2^{x}H$_2$, m), 3.15 (CaH(E), Ce(E), m), 2.19 (CaH(A), CeH(A), m), 2.04 (CbH(E), CdH(E), m), 1.74 (CbH(A), CdH(A), m), 2.82 (CcH, tt, $^3J_1$ = 12.0, $^3J_2$ = 3.5), 6.85 (C2″H, d, ^3J = 2.0), 7.60 (C4″H, d, ^3J = 8.0), 8.29 (N1′H, bs), 8.11 (N1″H, bs).

^{13}C NMR (125 Hz, CDCl$_3$): 178.4 (C2), 36.5 (C3), 36.4 (C4), 176.6 (C5), 122.6 (C2′), 111.8 (C3′), 125.6 (C3′a), 118.6 (C4′), 120.0 (C5′), 122.6 (C6′), 111.6 (C7′), 136.4 (C7′a), 38.2 (C1^x), 55.4 (C2^x), 54.3 and 54.4 (Ca, Ce), 32.9 and 33.0 (Cb, Cd), 33.4 (Cc), 119.7 (C2″), 121.4 (C3″), 126.7 (C3″a), 119.0 (C4″), 119.1 (C5″), 121.9 (C6″), 111.2 (C7″), 136.6 (C7″a).

HRMS (MSES+) calcd for $C_{27}H_{28}N_4O_2H$ 441.2291; Found: 441.2282.

*4.1.8.23. 1-{2-[4-(5-Fluoro-1H-indol-3-yl)piperidin-1-yl]ethyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**37**).* The title compound was isolated as a yellow powder. Yield: 60.8% 0.10 g, m.p. 108–116 °C.

^{1}H NMR (500 Hz, CDCl$_3$): 4.16 (C3H, dd, $^3J_1$ = 9.5, $^3J_2$ = 5.0), 2.87 (C4H(1), dd, ^2J = 18.5, ^3J = 5.0), 3.13 (C4H(2), dd, ^2J = 18.5, ^3J = 9.5), 7.06 (C2′H, d, ^3J = 2.5), 7.46 (C4′H, d, ^3J = 8.0), 7.09 (C5′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0 ^4J = 1.0), 7.17 (C6′H, m, $^3J_1$ = 8.0, $^3J_2$ = 7.0, ^4J = 1.0), 7.31 (C7′H, d, ^3J = 8.5), 3.82 (C1^{x}H$_2$, t, ^3J = 6.5), 2.69 (C2^{x}H$_2$, t, ^3J = 6.5), 3.16 (CaH(E), Ce(E), m), 2.17 (CaH(A), Ce(A), m), 1.98 (CbH(E), CdH(E), m), 1.69 (CbH(A), CdH(A), m), 2.72 (CcH, tt, $^3J_1$ = 12.0, $^3J_2$ = 4.0), 6.83 (C2″H, d, ^3J = 2.5), 7.19–7.23 (C4″H, C7″H, m), 6.90 (C6″H, td, ^3J = 9.0, ^4J = 2.5), 8.42 (N1′H, bs), 8.23 (N1″H, bs).

^{13}C NMR (500 Hz, CDCl$_3$): 178.5 (C2), 36.5 (C3), 36.3 (C4), 176.6 (C5), 122.6 (C2′), 111.8 (C3′), 125.6 (C3′a), 118.5 (C4′), 121.6 (C5′), 122.6 (C6′), 111.6 (C7′), 136.6 (C7′a), 38.2 (C1^x), 55.4 (C2^x), 54.2 and 54.2 (Ca, Ce), 32.7 and 32.8 (Cb, Cd), 33.3 (Cc), 120.0 (C2″), 121.4 (C3″, d*, ^4J = 4.9), 126.9 (C3″a, d*, ^3J = 9.3), 103.9 (C4″, d*, ^2J = 23.4), 157.5 (C5″, d*, ^1J = 233.9), 110.1 (C6″, d*, ^2J = 26.3), 111.7 (C7″), 132.9 (C7″a).

HRMS (MSES+) calcd for $C_{27}H_{27}FN_4O_2H$ 459.2196; Found: 459.2187.

*4.1.8.24. 1-{2-[4-(5-Methoxy-1H-indol-3-yl)piperidin-1-yl]ethyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**38**).* The title compound was isolated as a yellow powder. Yield: 42.4% 0.10 g, m.p. 111–119 °C.

^{1}H NMR (500 Hz, CDCl$_3$): 4.15 (C3H, dd, $^3J_1$ = 9.5, $^3J_2$ = 5.0), 2.86 (C4H(1), dd, ^2J = 18.5, ^3J = 5.0), 3.13 (C4H(2), dd, ^2J = 18.0, ^3J = 9.5), 7.05 (C2′H, d, ^3J = 2.5), 7.47 (C4′H, d, ^3J = 8.0), 7.09 (C5′H, m, $^3J_1$ = 7.5, $^3J_2$ = 7.0 ^4J = 1.0), 7.17 (C6′H, m, $^3J_1$ = 8.5, $^3J_2$ = 7.0 ^4J = 1.5), 7.31 (C7′H, dt, ^3J = 8.5, ^4J = ^5J = 1.0), 3.82 (C1^{x}H$_2$, t, ^3J = 6.0), 2.70 (C2^{x}H$_2$,

t, ^3J = 6.0), 3.17 (CaH(E), Ce(E), m), 2.19 (CaH(A), Ce(A), m), 2.00 (CbH(E), CdH(E), m), 1.75 (CbH(A), CdH(A), m), 2.76 (CcH, tt, $^3J_1$ = 12.0, $^3J_2$ = 3.5), 7.08 (C2″H, d, ^3J = 2.5), 6.81 (C4″H, d, ^4J = 2.5), 6.84 (C6″H, dd, ^3J = 9.0, ^4J = 2.5), 7.21 (C7″H, dd, ^3J = 8.5, ^5J = 0.5), 3.84 (OCH_3, s), 8.50 (N1′H, bs), 8.10 (N1″H, bs).

^{13}C NMR (500 Hz, $CDCl_3$): 178.5 (C2), 36.6 (C3), 36.3 (C4), 176.6 (C5), 122.5 (C2′), 111.7 (C3′), 125.6 (C3′a), 118.5 (C4′), 121.0 (C5′), 122.6 (C6′), 111.7 (C7′), 136.6 (C7′a), 38.1 ($C1^x$), 55.4 ($C2^x$), 54.3 and 54.3 (Ca, Ce), 32.8 and 32.8 (Cb, Cd), 33.5 (Cc), 120.7 (C2″), 120.0 (C3″), 127.0 (C3″a), 101.5 (C4″), 153.6 (C5″), 111.7 (C6″), 111.9 (C7″), 131.7 (C7″a), 56.1 (OCH_3).

HRMS (MSES+) calcd for $C_{28}H_{30}N_4O_3H$ 471.2396; Found: 471.2408.

4.2. Biology evaluation

4.2.1. In vitro experiment

4.2.1.1. 5-HT$_{1A}$ binding assay. [^{3}H]-8-OH-DPAT, [propyl-2,3-ring-1,2,3-3*H*], spec. act. 170.2 Ci/mmol, 1 mCi/ml (PerkinElmer) was used for labeling 5-HT$_{1A}$ receptors. Competition binding studies were performed on rat hippocampal membranes, prepared according to a previously described procedure [33] with slight modifications. In brief, the hippocampus tissue was homogenized in 20 volumes of 50 mM Tris–HCl buffer (pH 7.7 at 25 °C) using an Ultra Turrax T25B (IKA Labortechnik, USA) and then centrifuged at 32,000 *g* for 10 min. The supernatant fraction was discarded, the pellet was resuspended in the same volume of Tris–HCl buffer, and the solution was centrifuged again. Before the third centrifugation, the samples were incubated at 37 °C for 10 min. The final pellet was resuspended in Tris–HCl buffer containing 10 μM pargyline, 4 mM $CaCl_2$ and 0.1% ascorbic acid. Samples containing 240 μl of the tissue suspension (5 mg wet weight), 30 μl of 10 μM serotonin for non-specific binding, 30 μl of [^{3}H]8-OH-DPAT, and 30 μl of the analyzed compound were incubated at 37 °C for 20 min. After incubation, the reaction mixture was filtered immediately onto GF/B glass fiber filters using a 96-well FilterMate Harvester (PerkinElmer, USA). The final [^{3}H]8-OH-DPAT concentration was 1 nM, and the concentrations of the analyzed compounds ranged from 10^{-10} to 10^{-5} M.

4.2.1.2. SERT binding assay. The assay was performed according to a previously described procedure [33] with slight modifications. Rat cerebral cortex was homogenized in 30 volumes of ice-cold 50 mM Tris–HCl containing 150 mM NaCl and 5 mM KCl, pH = 7.7 at 25 °C and centrifuged at 20,000 *g* for 20 min. The supernatant was decanted and the pellet was resuspended in 30 volumes of buffer and centrifuged again. The resulting pellet was resuspended in the same quantity of the buffer and centrifuged a third time under the same conditions. [^{3}H]-Citalopram (spec. act. 85.6 Ci/mmol, PerkinElmer) was used for labeling the 5-HT-transporter. 240 μl of the tissue suspension, 30 μl of 1 nM [^{3}H]-citalopram and 30 μl of the analyzed compound or 30 μl of 1 μM imipramine (displacer) were incubated at 24 °C for 1 h. The concentrations of the analyzed compounds ranged from 10^{-10} to 10^{-5} M. Incubations were terminated by vacuum filtration over Whatman GF/B filters and washed five times with 200 μl of ice-cold buffer. Radioactivity was measured in a MicroBeta TriLux liquid scintillation counter (PerkinElmer). All assays were done in duplicate.

Radioligand binding data were analyzed using iterative curve fitting routines (GraphPAD/Prism, Version 3.0, San Diego, CA, USA). K_i values were calculated from the Cheng–Prusoff equation:

$$Ki = \frac{IC_{50}}{1 + \frac{L_O}{K_D}}$$

L_O – labeled ligand concentration
K_D – dissociation constant of labeled ligand

4.2.1.3. 5-HT$_{2A}$ binding assay. Tissue preparations (rat cortex) and competition binding experiments for 5-HT$_{2A}$ receptors were carried out according to a previously described, standard technique using 0.5 nM of [^{3}H]-ketanserin as the radioligand and 10 μM of methysergide to determine non-specific binding [71]. Following 20 min of incubation, the samples containing receptor homogenates and the investigated compound (7–9 concentrations run in triplicate) were rapidly filtered under vacuum through GF/B glass fiber filters; the filters were washed extensively with an ice cold buffer using a Brandel harvester. Bound radioactivity was measured by scintillation counting using a liquid scintillation cocktail. The inhibition constants (K_i) were calculated using the Cheng–Prusoff equation [72]. Results are expressed as the means of at least three separate experiments.

4.2.1.4. Receptor binding experiments with HEK293 cells expressing human D$_{2L}$, 5-HT$_6$ and 5-HT$_7$ receptors

4.2.1.4.1. Cell culture and preparation of cell membranes. HEK293 cells with stable expression of human dopamine D$_{2L}$ or serotonin 5-HT$_6$ and 5-HT$_{7b}$ receptors (prepared with the use of Lipofectamine 2000) were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and grown in Dulbecco's Modifier Eagle Medium containing 10% dialyzed fetal bovine serum and 500 μg/ml G418 sulfate. For membrane preparation, cells were subcultured in 10 cm diameter dishes, grown to 90% confluence, washed twice with prewarmed to 37 °C phosphate buffered saline (PBS) and pelleted by centrifugation (200 *g*) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol. Prior to membrane preparation, pellets were stored at −80 °C.

4.2.1.4.2. Radioligand binding assays. Cell pellets were thawed and homogenized in 20 volumes of assay buffer using an Ultra Turrax tissue homogenizer and centrifuged twice at 35,000 *g* for 20 min at 4 °C, with incubation for 15 min at 37 °C in between. The composition of the assay buffers was as follows: for dopamine D$_{2L}$R, 50 mM Tris–HCl, 1 mM EDTA, 4 mM $MgCl_2$, 120 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$ and 0.1% ascorbate; for 5-HT$_6$R, 50 mM Tris–HCl, 0.5 mM EDTA and 4 mM $MgCl_2$; and for 5-HT$_{7b}$R, 50 mM Tris–HCl, 4 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbate. All assays were incubated in a total volume of 200 μl in 96-well microtitre plates for 1 h at 37 °C. The process of equilibration was terminated by rapid filtration through Unifilter plates with a 96-well cell harvester and radioactivity retained on the filters was quantified on a Microbeta plate reader. For displacement studies, the assay samples contained radioligands such as [^{3}H]-raclopride (74.4 Ci/mmol) for dopamine D$_{2L}$R and 2 nM [^{3}H]-LSD (85.2 Ci/mmol for 5-HT$_6$R) or 0.6 nM [^{3}H]-5-CT (39.2 Ci/mmol) for 5-HT$_7$R.

Non-specific binding was defined with 1 μM of (+)butaclamol in the D$_{2L}$ assay, whereas 10 μM of 5-HT or 10 μM methiothepine were used in the 5-HT$_6$R or 5-HT$_7$R binding experiments, respectively.

Each compound was tested in triplicate at 7–8 concentrations (10^{-11}–10^{-4} M). The inhibition constants (K_i) were calculated from the Cheng–Prusoff equation. (B) Membrane preparation and general assay procedures for cloned receptors were adjusted to a 96-microwell format based on protocols previously described by us [73,74].

4.2.2. In vivo experiments

The experiments were performed on male CD-1 mice (24–28 g). The animals were kept at a room temperature (20–21 °C) on a natural day–night cycle (January–February) and housed under standard laboratory conditions. They had free access to food and tap water before the experiment. Each experimental group

consisted of 7–8 animals/dose, and all the animals were used only once. 8-Hydroxy-2-(di-*n*-propylamino)tetralin hydro-bromide (8-OH-DPAT, Research Biochemical Inc.) and *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexane-carboxamide trihydrochloride (WAY 100635, synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland) were used as aqueous solutions. Compounds **30**, **15**, **18**, **19** were suspended in a 1% aqueous solution of Tween 80. 8-OH-DPAT and WAY 100635 were injected subcutaneously (s.c.), **30**, **15**, **18**, **19** were given intraperitoneally (i.p.) in a volume of 10 ml/kg (mice). The obtained data were analyzed by Dunnett's test (when only one drug was given) or by the Newman–Keuls test (when two drugs were administered).

4.2.2.1. Body temperature in mice. The 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 administration. In an independent experiment, the effect of WAY 100635 (0.1 mg/kg) on the hypothermia induced by compounds **30**, **15**, **18**, **19** or 8-OH-DPAT was tested. WAY 100635 was administered 15 min before the tested compounds or 8-OH-DPAT and the rectal body temperature was recorded 30 min and 60 min after injection of the tested compounds. The results are expressed as a change in body temperature (°C) with respect to the basal body temperature, as measured at the beginning of the experiment.

4.2.2.2. Forced swimming test in mice. The experiment was carried out according to the method of Porsolt et al. (1977). Briefly, the 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 on water, making only small movements to keep its head above the surface. The total duration of immobility was measured by an experimenter during the final 4 min of a 6-min test session, after a 2-min habituation period.

4.3. Docking protocol

The starting conformations of compounds **15**, **31** and **32** in the right docking procedure were lowest-energy ones founded by the molecular mechanic Monte Carlo method. Docking was done using Schrödinger Fit Docking software [67]. Prior to the right docking procedure, the Protein Preparation Wizard function was used to correct common problems such as missing hydrogen atoms, incomplete side chains and loops, ambiguous protonation states and flipped residues which can occur in the model structures. The priority binding sites inside the binding pocket were Asp116 and Asp98 for the 5-HT_{1A} receptor and SERT, respectively. The docking procedure was carried out using the extra precision (XP) option with simultaneous fitting of the binding pocket. The particular ligand-5-HT_{1A} and ligand-SERT interactions were developed by Discovery Studio Client v2.5.5.9350 software [75].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.02.033.

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