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Research paper

Synthesis and biological evaluation of new multi-target 3-(1*H*-indol-3-yl)pyrrolidine-2,5-dione derivatives with potential antidepressant effect

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ABSTRACT

A series of novel 3-(1*H*-indol-3-yl)pyrrolidine-2,5-dione derivatives were synthesised and evaluated for their 5-HT_{1A}/D_2/5-HT_{2A}/5-HT_6/5-HT_7 receptor affinity and serotonin reuptake inhibition. Most of the evaluated compounds displayed high affinities for 5-HT_{1A} receptors (e.g., **4c** $K_i = 2.3$ nM, **4l** $K_i = 3.2$ nM). The antidepressant activity of the selected compounds was screened *in vivo* using the forced swim test (FST). The results indicate that compound **MW005** (agonist of the pre- and postsynaptic 5-HT_{1A} receptor) exhibited promising affinities for the 5-HT_{1A}/SERT/D_2/5-HT_6/5-HT_7 receptors and showed an antidepressant-like activity in the FST model.

1. Introduction

Serotonergic transmission disorders in the central nervous system (CNS) play a very important role in the pathomechanisms of affective diseases, anxiety diseases, addictions, suicidal tendencies, migraine, bulimia and obesity, as well as sleep and memory disorders [1]. Up to now, many chemical substances with high affinity for serotonergic receptors have been described, and 5-HT_{1A} receptor ligands seem to be particularly promising [2]. Nowadays, the following partial agonists of the 5-HT_{1A} receptor are used as medications – buspirone (anxiolytic), vilazodone and vortioxetine (antidepressants), methysergide (antimigraine drug), cariprazine, aripiprazole, lurasidone and brexpiprazole (neuroleptics) (Fig. 1) – whereas many others are at various stages of clinical trials (Fig. 2) [3,4]. This shows how useful 5-HT_{1A} receptor ligands are for pharmacotherapies, especially for psychiatric illness. Currently, the studies are mostly focused on the use of 5-HT_{1A} agonists in the treatment of the most frequently diagnosed mental disease - depression, and also as atypical neuroleptics [5,6].

Depression is the leading cause of disability worldwide, affecting over 300 million people [11]. The most severe disadvantages of the most frequently used SSRI medications are their long latency period and low effectiveness (around one-third of patients do not respond to pharmacotherapy) [2]. Thus, there is an

Abbreviations: SAR, structure-activity relationship; 8-OH-DPAT, (±)-8-hydroxy-2-(di-N-propylamino)tetralin; K_i, inhibitor constant; SSRIs, selective serotonin reuptake inhibitors; hERG, Human Ether-a-go-go-related Gene; HEK293, human embryonic kidney cell line 293; FST, forced swim test; PBS, phosphate-buffered saline; P-gp, P-glycoprotein 1.

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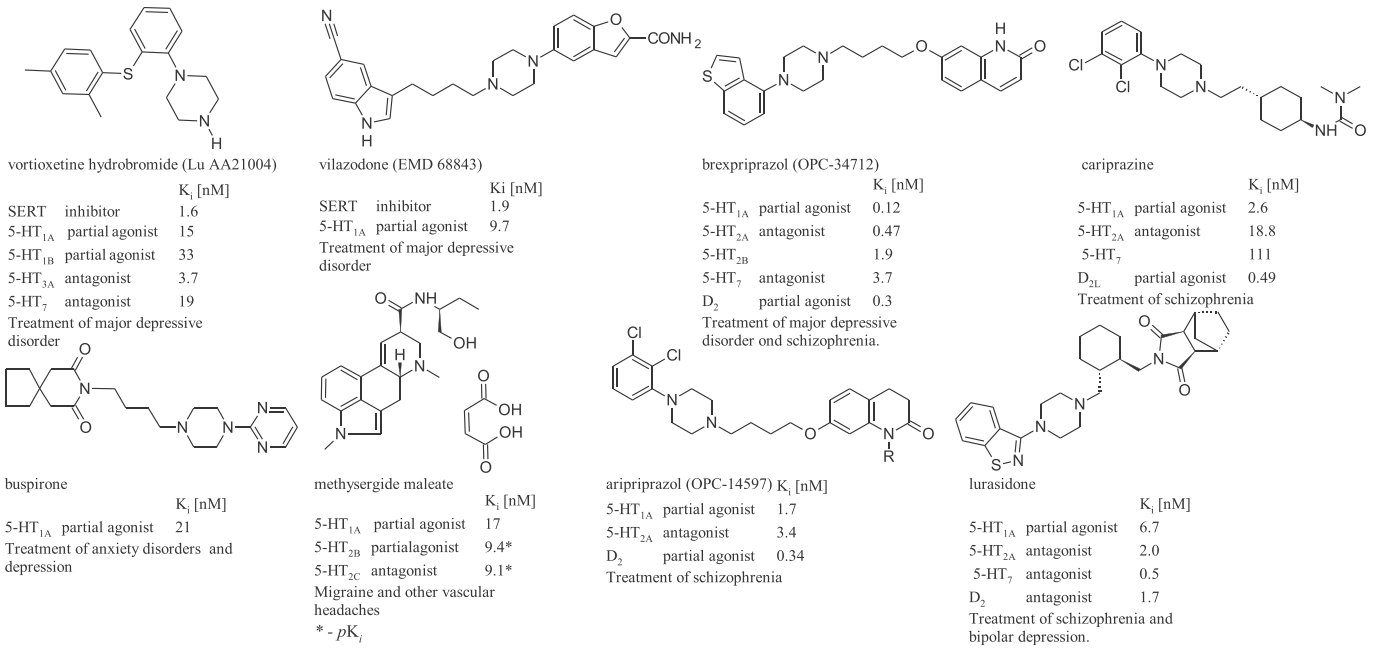


Fig. 1. Approved drugs, serotonin 1A (5-HT$_{1A}$) receptor partial agonist [7–10].

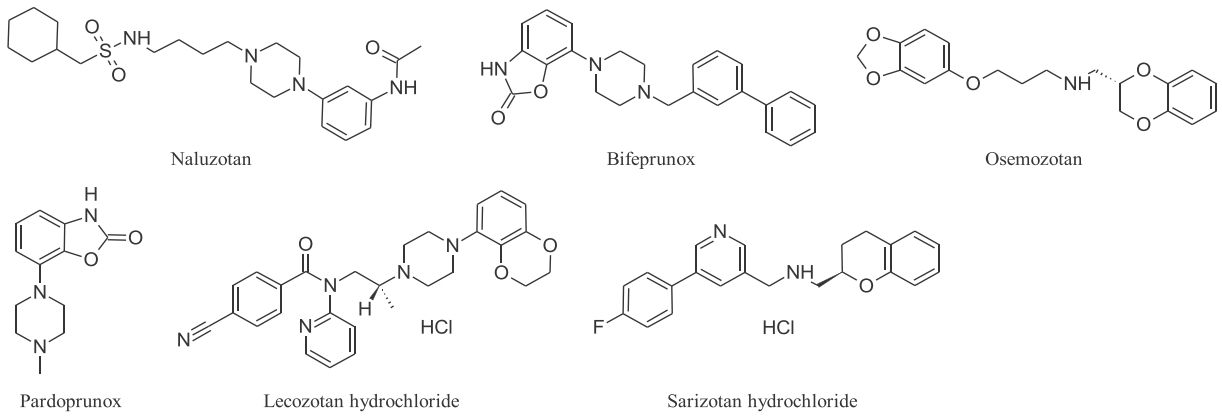


Fig. 2. The chemical structures of 5-HT$_{1A}$ receptor ligands as clinical candidates for atypical antipsychotics and antidepressants [3,4].

important need to search for new strategies for treatment of this disease. A hypothesis of shortening of the latency period of SSRI medications, involving the co-administration of substances acting as SERT inhibitors and 5-HT$_{1A}$ antagonists, has appeared and has been confirmed in pre-clinical studies. A decreased amount of endogenous serotonin released as a consequence of a single administration of SSRI medications was mostly abolished by a simultaneous administration of a 5-HT$_{1A}$ autoreceptor antagonist – either pindolol or the more selective WAY-100635 [12–15]. As has been demonstrated in further studies, a return to activity of serotonergic neurons is tightly associated with desensitisation of somatodendritic 5-HT$_{1A}$ autoreceptors [16]. Studies on the development of drugs with shorter latency periods were started on a group of 5-HT$_{1A}$R agonists. Simultaneous administration of SSRI and a 5-HT$_{1A}$ receptor agonist or a partial agonist causes an immediate increase in neurotransmission in the serotonergic system by the stimulation of postsynaptic receptors. At the same time, release of endogenous serotonin is inhibited by a negative feedback activated by the stimulation of somatodendritic autoreceptors [17]. Wilcox described the clinical action of buspirone and gepirone as partial agonists of the 5-HT$_{1A}$ receptor [18]. Simultaneous administration of buspirone and an SSRI caused a significant increase in the effectiveness of therapy, when compared to administration of the SSRI alone in patients suffering from depression, who had not previously responded to other treatments [19].

Additionally, many research results suggest, that it is implausible that a single 5-HT receptor can enhance actions of SSRI on depressive symptoms, and therefore, ligands of a single receptor are unlikely to overcome the limitations of current treatments [2]. On this basis, a novel strategy for treating depression requires searching for drugs with a polypharmacological receptor profile [20,21]. Research into new antidepressants targeting SERT/5-HT$_{1A}$/5-HT$_7$ is particularly promising, as simultaneous administration of SSRI medications and the selective 5-HT$_7$ antagonist (SB-269970) increased the SSRIs' antidepressant activity in animal models [22]. Moreover, contemporaneous research have indicated that vortioxetine has cognitive enhancement effects as only one antidepressant; this activity has been linked to the blocking of the 5-HT$_7$ receptor [21,22]. Quite recently, another serotonin receptor, 5-HT$_6$R, has begun to attract considerable interest as a valuable target

affecting learning and memory processes. In fact, 5-HT_6 receptor agents have been indicated to improve cognitive function in a statistically significant manner [23,24].

The role of the 5-HT_{2A} and D_2 receptors in the therapy of both schizophrenia and depression is well established [25–27]. An example of an antidepressant with a pharmacological profile featuring high-affinity partial agonism at 5-HT_{1A} and antagonism at D_2, 5-HT_{2A}, and 5-HT_7 receptors is lurasidone (Fig. 1), approved by the US Food and Drug Administration in June 2013 for the acute treatment of bipolar depression [28]. Therefore, ligands with polypharmacological receptor profiles and with high affinity for the 5-HT_{1A} receptor as new antidepressants deserves particular attention.

In our previous paper, we described the synthesis and biological evaluation of pyrrolidine-2,5-dione derivatives with very good binding affinities for SERT and 5-HT_{1A} receptors [29]. The SAR analysis revealed that the presence of a 1,2,3,6-tetrahydropyridine or piperidine moiety in the pharmacophore part of the molecule has a significant impact. One of the main observations was that compounds with a fully hydrogenated pyridine ring exhibited better binding to the 5-HT_{1A} receptor. Out of this series, 1-{4-[4-(5-fluoro-1*H*-indol-3-yl)piperidin-1-yl]butyl}-3-(1*H*-indol-3-yl)pyrrolidine-2,5-dione (**MW005**) [29] was identified as a potent ligand with multi-receptor profile (K_i [nM] 5-HT_{1A} = 7.5, SERT = 505, D_2 = 14, 5-HT_{2A} = 71, 5-HT_6 = 63 and 5-HT_7 = 196); therefore, it was chosen as a starting point for design of further derivatives. Hence, we designed and synthesised a series of 3-(1*H*-indol-3-yl) pyrrolidine-2,5-dione derivatives (**4a**–**r**) having a two-, three- or four-methylene spacer, methoxy or fluoro substituent in the C5 position at the 1*H*-indole ring (R_1) and a different type of substituent at 3-piperidin-4-yl-1*H*-indole in the pharmacophore part of the molecule (R_2 = –H, –F or –OCH_3).

Keeping in mind the concept of developing ligands with high affinity for 5-HT_{1A}, we conducted a new exploratory study focused on the replacement of the 3-piperidin-4-yl-1*H*-indole moiety with 3-piperidin-3-yl-1*H*-indole in compounds **6a**–**d** (Fig. 3). This residue is more similar in its structure to serotonin, and its conformational constraints aimed to increase the activity of compounds by increasing the affinity for the 5-HT_{1A} receptor [30–32].

In the current project, we focused our attention on a new series of compounds with high binding affinities for 5-HT_{1A}R and with multi-target profiles because such compounds are regarded as promising for the pharmacotherapy of depression. Therefore, the synthesised derivatives were evaluated for their 5-HT_{1A} receptor affinity and serotonin reuptake inhibition. Selected compounds were then tested for their affinity for D_2, 5-HT_{2A}, 5-HT_6, and 5-HT_7 receptors, as well as in a panel of tests comprising metabolic stability, bioavailability prediction and hERG channel modulation assays. Finally, the most promising compounds, possessing desirable receptor profiles, were subjected to *in vivo* testing in an animal behavioural model of depression.

2. Results and discussion

2.1. Chemistry

Final compounds **4a**–**r** and **6a**–**d** were obtained *via* multi-step synthesis, according to Scheme 1 and Scheme 2, respectively. In order to carry out the synthesis, series of new synthons were necessary: N-bromobutyl, N-bromopropyl and N-bromoethyl derivatives of 3-(5-methoxy-1*H*-indol-3-yl)pyrrolidine-2,5-dione (**2a**–**c**) or 3-(5-fluoro-1*H*-indol-3-yl)pyrrolidine-2,5-dione (**2d**–**f**). Other intermediates, **1a**–**b** and 1-(4-bromobutyl)-3-(1*H*-indol-3-yl)pyrrolidine-2,5-dione (**2g**), as well as 3-piperidin-4-yl-1*H*-indole (**3a**–**c**) and 3-piperidin-3-yl-1*H*-indole derivatives **(5a**–**c)** were synthesised according to previously described procedures (for more information see Supplementary data) [29,30,33–35].

Compounds **1a** and **1b** were obtained by reaction of maleimide with 5-methoxyindole or 5-fluoroindole, respectively, in glacial acetic acid (Scheme 1). This is a modification of the Michael addition, described by Macor et al. and Bergman et al. [33–35]. Macor et al. described the differences in the time and yield of this reaction as depending on the substituent in the C5 position of the 1*H*-indole (R_1): 5-methoxyindole (electron donating group) was less active and 5-fluoroindole (electron withdrawing group) was more active in the reaction [35]. Our results confirm this observation. The resulting derivatives **1a** and **1b** were subjected to N-alkylation with 1,4-dibromobutane, 1,3-dibromopropane or 1,2-dibromoethane at the N1 position, in the presence of potassium carbonate in acetone.

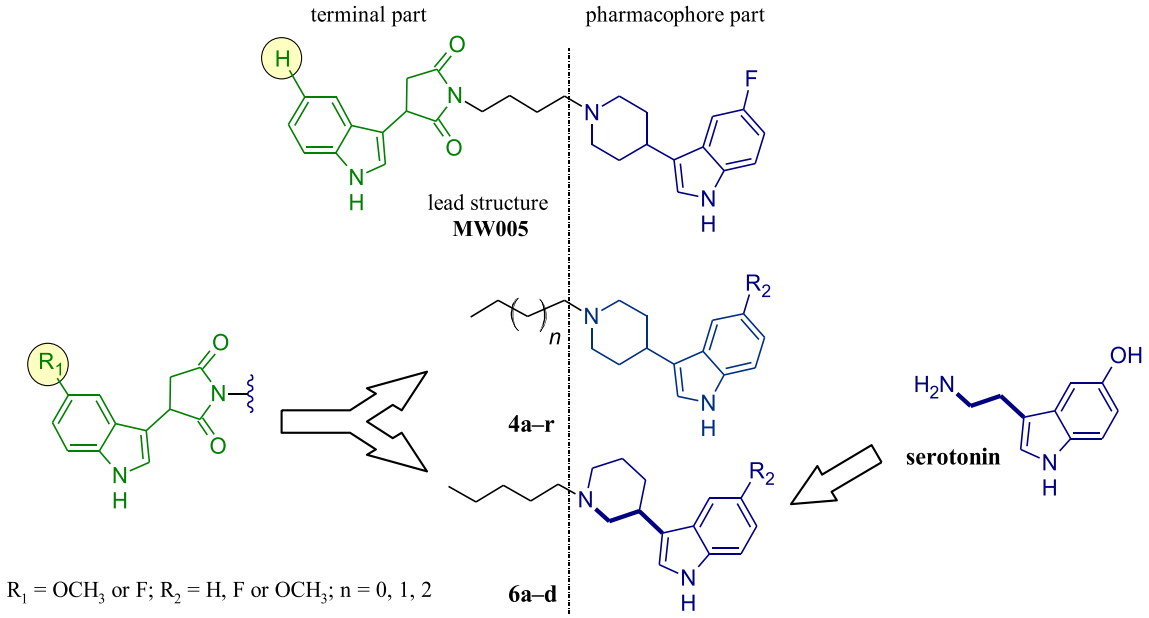
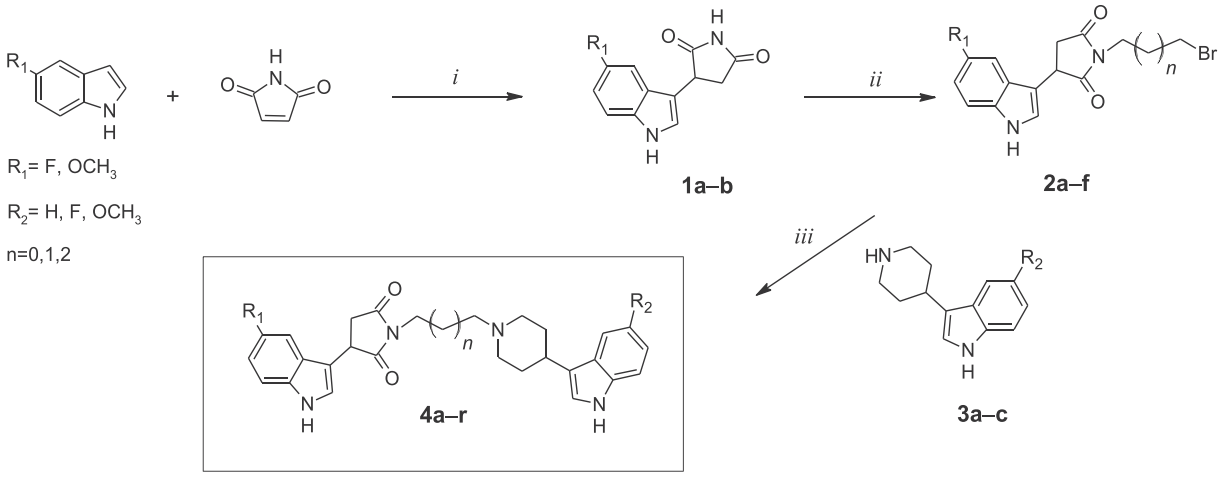
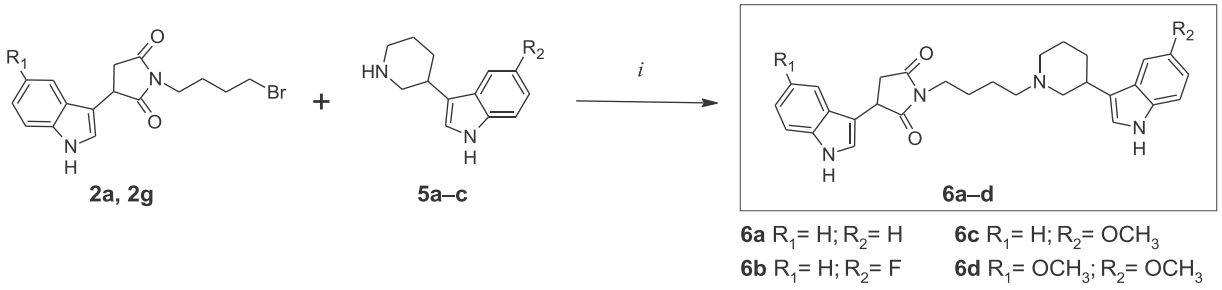


Fig. 3. Design strategy for a novel series of pyrrolidine-2,5-dione derivatives resulting from the optimisation of compound **MW005**.



Scheme 1. The synthetic pathways to the investigated 3-piperidin-4-yl-1*H*-indole derivatives **4a**–**r** (series of 3-piperidin-4-yl-1*H*-indole derivatives). Reagents and conditions: (*i*) CH_3COOH (*ii*) $BrCH_2(CH_2)_nCH_2Br$ (n = 0, 1 or 2), K_2CO_3, acetone (*iii*) K_2CO_3, $KI_{(cat.)}$, CH_3CN.



Scheme 2. The synthesis of the investigated compounds **6a**–**d** (series of 3-piperidin-3-yl-1*H*-indole derivatives). Reagents and conditions: (*i*) K_2CO_3, CH_3CN.

Intermediates **2a**–**f** were obtained in high yields (Scheme 1). Final ligands **4a**–**r** and **6a**–**d** were synthesised by N-alkylation of compounds **2a**–**f** or **2g** with synthons **3a**–**c** or **5a**–**c** (Scheme 1 and Scheme 2), according to previously described procedures [29]. To obtain analytical samples, all final compounds **4a**–**r** and **6a**–**d** were purified by column chromatography or *via* crystallisation.

2.2. In vitro receptor assay and structure–activity relationship (SAR)

Our approach focused on the development of polypharmacological agents with high affinity for 5-HT_{1A}/SERT receptors, which would possess additional receptor activity. Therefore, the synthesised compounds were initially tested for their *in vitro* affinity for the 5-HT_{1A} receptor and inhibition of SERT, using a radioligand binding assay. Next, the selected structural analogues of compound **MW005**, compounds **4a**–**r**, were tested for their D_2, 5-HT_{2A}, 5-HT_6 and 5-HT_7 receptor affinities. The results are presented in Table 1 and Table 2.

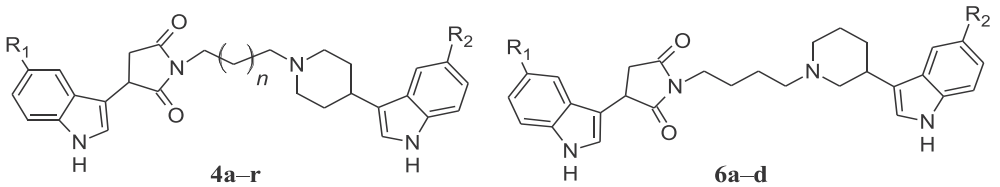
In general, all the newly synthesised compounds, excluding **4q** and **4r,** showed very high to moderate affinity for 5-HT_{1A}R (K_i = 2.3–336 nM).

Keeping in mind the results of our previous study [29], we determined the length of the alkyl linker that is optimal for 5-HT_{1A}R binding of the synthesised compounds. For this purpose, we obtained derivatives of 3-(1*H*-indol-3-yl)pyrrolidine-2,5-dione **4a**–**r** having a two-, three- or four-methylene spacer between the imide and 3-piperidin-4-yl-1*H*-indole moiety. We observed the best K_i values for **4c** (2.3 nM) and **4l** (3.2 nM) (Table 1). Generally, four methylene spacer was crucial for affinity to the 5-HT_{1A} receptor in this group of derivatives. Analogues with a shorter aliphatic chain had, on average, 30 times higher K_i values (e.g., **4r**: K_i > 5000 nM *vs* **4o**: K_i = 157.0 nM *vs* **4l**: K_i = 3.2 nM). This is in contrast with our previous results based on a similar class of compounds, which indicated good 5-HT_{1A} binding affinity of ligands with a two-carbon linker [29].

Next, we focused on the modification of the 3-(1*H*-indol-3-yl) pyrrolidine-2,5-dione moiety. We decided to leave the pyrrolidine-2,5-dione ring, because, as a cyclic imide and as a lipophilic portion of the molecule, it has an important effect on the stabilisation of the ligand–receptor complex *via* dipole–dipole and π–π interactions [5,36,37]. We synthesised compounds **4a**–**i** that possessed the methoxy group (R_1), whereas compounds **4j**–**r** had a fluorine atom as a substituent (R_1), at the C-5 position of the 1*H*-indole ring. We noticed that when comparing the compounds **4a**–**i** (R_1 = –OCH_3) to their 5-fluoro analogues **4j**–**r** (R_1 = –F), all but one (**4d** *vs* **4m**), had a higher affinity for the 5-HT_{1A} receptor.

Finally, we examined the type of substituent in the C-5 position of the 3-piperidin-4-yl-1*H*-indole (R_2) pharmacophore part of the molecule conferring optimal selectivity and affinity for 5-HT_{1A}. Following our previous studies of 3-(1*H*-indol-3-yl)pyrrolidine-2,5-dione derivatives docking to the binding site of the 5-HT_{1A} receptor, the affinity and selectivity of the investigated group of compounds were predominantly determined by the electronegativity of the R_2 substituent [29,38]. Therefore, we assumed that compounds with the –F or –OCH_3 substituent (R_2), as a result of their electronegativity and local inductive effects (in the case of fluorine), should make protonated piperidine a much more effective proton donor,

Table 1
5-HT_{1A} receptor and SERT binding affinities of 3-piperidin-4-yl-1*H*-indole derivatives **4a**–**r** and 5-HT_{1A} receptor binding affinities of 3-piperidin-3-yl-1*H*-indole derivatives **6a**–**d**.



compound	R_1	R_2	n	$K_i \pm$ SEM [nM]	
				5-HT_{1A}	SERT
4a	OCH_3	H	2	6.3 ± 0.1	705.0 ± 62.7
4b	OCH_3	F	2	4.9 ± 0.1	17.5 ± 1.2
4c	OCH_3	OCH_3	2	2.3 ± 0.8	308.0 ± 29.0
4d	OCH_3	H	1	204.1 ± 13.7	523.0 ± 33.7
4e	OCH_3	F	1	246.0 ± 8.3	30.0 ± 2.9
4f	OCH_3	OCH_3	1	68.7 ± 7.8	111.0 ± 10.3
4g	OCH_3	H	0	48.7 ± 4.2	141.8 ± 14.4
4h	OCH_3	F	0	222.0 ± 23.0	221.0 ± 14.6
4i	OCH_3	OCH_3	0	240.0 ± 22.8	63.0 ± 7.0
4j	F	H	2	13.0 ± 1.0	51.0 ± 2.1
4k	F	F	2	7.0 ± 0.3	32.4 ± 1.9
4l	F	OCH_3	2	3.2 ± 0.2	201.0 ± 7.6
4m	F	H	1	143.2 ± 12.1	150.5 ± 13.0
4n	F	F	1	336.0 ± 23.5	140.0 ± 14.0
4o	F	OCH_3	1	157.0 ± 14.7	156.0 ± 15.4
4p	F	H	0	320.0 ± 33.0	102.0 ± 7.4
4q	F	F	0	700.0 ± 71.0	314.0 ± 15.2
4r	F	OCH_3	0	>5000	1313.0 ± 150.0
6a	H	H	2	122.0 ± 11.9	NT
6b	H	F	2	183.0 ± 10.9	NT
6c	H	OCH_3	2	78.0 ± 8.0	NT
6d	OCH_3	OCH_3	2	52.0 ± 2.6	NT
serotonin				5.5 ± 0.5	NT
imipramine				NT	2.5 ± 0.3

NT - not tested.

Table 2
5-HT_{1A}, D_2, 5-HT_{2A}, 5-HT_6 and 5-HT_7 receptor binding affinities of selected 3-(1*H*-indol-3-yl)pyrrolidine-2,5-dione derivatives.

compound	R_1	R_2	n	$K_i \pm$ SEM [nM]				
				5-HT_{1A}	D_2	5-HT_{2A}	5-HT_6	5-HT_7
MW005	H	F	2	7.5 ± 0.5	14 ± 2	71 ± 9	63 ± 5	196 ± 14
MW011	H	OCH_3	2	2.2 ± 0.7	161 ± 14	498 ± 23	159 ± 9	345 ± 24
4a	OCH_3	H	2	6.3 ± 0.1	16 ± 2	31 ± 4	81 ± 6	25 ± 3
4b	OCH_3	F	2	4.9 ± 0.1	62±7[a]	118±9[a]	85 ± 11[a]	641 ± 93[a]
4c	OCH_3	OCH_3	2	2.3 ± 0.8	217 ± 13	499 ± 65	427 ± 32	320 ± 23
4j	F	H	2	13.0 ± 1.0	190 ± 16[a]	50±7[a]	29±2[a]	50±6[a]
4k	F	F	2	7.0 ± 0.3	19±2[a]	1944 ± 231[a]	301 ± 24[a]	1158 ± 175[a]
4l	F	OCH_3	2	3.2 ± 0.2	20±3[a]	209 ± 26[a]	71±8[a]	458 ± 32[a]

[a] SD.

and, as a result, able to form a strong hydrogen bond with the 5-HT_{1A} receptor binding pocket. The current results reveal that the influence of the type of R_2 substituent in the 3-(1*H*-indol-3-yl) pyrrolidine-2,5-dione derivatives on 5-HT_{1A} binding can be arranged, for the four-methylene spacer series, in descending order: OCH_3 (**4c**, **4l**) > F (**4b**, **4k**) > H (**4a**, **4j**). Surprisingly, the binding influence falls in the opposite order for the ethyl-linker series. For the three-methylene linker series, no clear influence of the R_2 substituent was observed. Nevertheless, it should be noted that the results of the binding studies did not indicate significant differences in affinity for 5-HT_{1A} arising from the type of substituent in the pharmacophore part of the molecule, particularly within the most potent four-methylene spacer series (**4a**–**c** and **4j**–**l**).

Next, we replaced the 3-piperidin-4-yl-1*H*-indole moiety with its 3-piperidin-3-yl-1*H*-indole regioisomer, which resulted in compounds **6a**–**d**. As was mentioned in the Introduction, at the initial stage of the study we checked whether series **6** shows any affinity for the 5-HT_{1A} receptor. The results for compounds (**4a**–**r**) show that a four-methylene spacer coupled with the 5-fluoro-4-piperidin-4-yl-1*H*-indole ($R_2 = -F$) or 5-methoxy-4-piperidin-4-yl-1*H*-indole ($R_2 = -OCH_3$) residue is optimal for high affinity for 5-HT_{1A}; therefore, we decided to unchanged this length of the linker. As shown in Table 1, compounds **6a**–**d** displayed good affinities for the 5-HT_{1A} receptor, with the corresponding K_i ranging from 52 nM to 183.0 nM. On the basis of these results, it can be summarised that derivatives **6** are potent 5-HT_{1A} ligands and

should be explored in the future.

We synthesised 3-(1*H*-indol-3-yl)pyrrolidine-2,5-dione derivatives in order to improve both SERT inhibition and affinity for 5-HT_{1A}. Although most of the compounds from the **4a**–**r** series display moderate SERT inhibitory activities, some of them show 5-HT_{1A}/SERT dual activities: **4b** (5-HT_{1A} $K_i = 4.9$ nM, SERT $K_i = 17.5$ nM) and **4k** (5-HT_{1A} $K_i = 7.0$ nM, SERT $K_i = 32.4$ nM). These results confirmed our previous observation that in order to achieve dual affinity for 5-HT_{1A} and SERT in this group of compounds, the introduction of a four-methylene spacer along with the 5-fluoro-3-piperidin-4-yl-1*H*-indole residue is highly recommended. This can be explained by differences in the ligand–receptor interaction depending on the R_2 substituents, that is, electronegativity and spatial size (i.e., van der Waals radius), according to the results described for reference compound **MW005** (Fig. 3) in the previous paper [29]. Moreover, the relatively small volume of the SERT binding pocket favours compounds with a −F substituent rather than those bearing the larger $-OCH_3$ group.

In order to verify the potential of the investigated group of derivatives as multi-target agents, we tested selected **MW005** analogues bearing four-methylene spacers for their binding affinities for D_2, 5-HT_{2A}, 5-HT_6 and 5-HT_7 receptors using *in vitro* assays. The results are summarised in Table 2. The affinities of the previously reported derivatives **MW005** and **MW011** are also included for comparison [29]. Compounds **4a** and **4j** displayed high potency for 5-HT_{1A}, 5-HT_{2A}, 5-HT_6 and 5-HT_7 receptors. In addition, **4a** showed a high affinity for D_2R. Compound **4j** seemed particularly promising because of its good affinity for all the tested receptors (K_i [nM]: 5-HT_{1A} = 13, SERT = 51, D_2 = 190, 5-HT_{2A} = 50, 5-HT_6 = 29 and 5-HT_7 = 50). The compounds reported by us in a previous study exhibited average to low affinities for 5-HT_7 receptors [29]. In contrast, the current results reveal that the presence of the fluoro or methoxy group (R_1) substituents increases affinity for 5-HT_7 (**4a** $K_i = 25$ nM, **4j** $K_i = 50$ nM). On the other hand, the derivatives **MW011** and **4c**, bearing the 5-methoxy-4-piperidin-4-yl-1*H*-indole ($R_2 = -OCH_3$) moiety, are clearly most selective for 5HT_{1A}R.

2.3. Metabolic stability

Because of the multireceptor profile of **MW005** (5-HT_{1A}, SERT, D_2 and 5-HT_7), its metabolic stability and *in vivo* activity was also tested. Additionally, compounds with the highest 5-HT_{1A} affinity (K_i [nM] < 15: **4a**, **4b**, **4c**, **4j**, **4k** and **4l**) were selected for a metabolic stability assay. The compounds were tested in an *in vitro* metabolic stability assay involving human liver microsomes and NADPH as a cofactor of first phase metabolism. All compounds showed rapid biotransformation with only minor differences between tested derivatives (Fig. 4).

Compounds selected for the metabolic stability test differ in substituents in R_1 (-H, $-OCH_3$, −F) and R_2 (-H, −F, $-OCH_3$) positions. Surprisingly, these structural differences have only a minor impact on their metabolic stability. It was reported that substitution of the aromatic ring by fluorine can improve stability by blocking oxidation reactions, whereas the methoxy substituent usually easily undergoes the O-dealkylation reaction decreasing stability significantly [39]. In this case, another structural element is probably responsible for its susceptibility to undergo first phase metabolism. We applied two chemoinformatic tools, namely Xenosite and SOMP - Site Of Metabolism Prediction to explore possible origins of experimental observations [39,40]. The results for **MW005** are summarised in Fig. 5. Xenosite with its human liver microsome model for CYP-mediated oxidations identified two carbon atoms in the alkyl chain as the most probable site vulnerable for biotransformation. Similarly, SOMP, equipped in several models for different CYP isoenzymes pointed out the same positions. With the SOMP algorithm atoms are ranked by deltaP value that is calculated as difference between probabilities that the atom is or is not a site of metabolism (deltaP > 0.5 are shown). The biotransformation of the above-mentioned sites of metabolism leads to N-dealkylation and, as a consequence, the degradation of a molecule into two smaller parts, which was previously reported several times for similar compounds [41,42].

2.4. In vivo assay

Next, the *in vivo* tests, i.e. the induced hypothermia and forced swim tests (FST), in mice, for the same set of compounds (**MW005**, **4a, 4b, 4c, 4j, 4k** and **4l**) were carried out. This allowed us to specify their agonist-antagonist properties in reference to pre- and postsynaptic 5-HT_{1A} receptors.

Compound 8-OH-DPAT, a 5-HT_{1A} receptor agonist, induces hypothermia in mice, an effect mediated through the somatodendritic 5-HT_{1A} receptor [43,44]. This hypothermia is abolished by WAY100635, an antagonist of 5-HT_{1A} receptors [45]. Hence the hypothermia produced by the compounds tested in mice (and reduced by WAY100635) was regarded as a measure of presynaptic 5-HT_{1A} receptor agonistic activity (Table 3 and Table 4). All tested compounds, like 8-OH-DPAT, induced hypothermia in mice, and this hypothermia (Table 3) is abolished by WAY100635 (Table 4), therefore all tested compounds were classified as presynaptic agonists.

To determine the postsynaptic 5-HT_{1A} receptor agonistic effect of the tested 5-HT_{1A} ligands, their ability to reduce immobility time in the FST was measured (Fig. 6 and Fig. 7). Only compound **MW005** shortened immobility time in the FST and decreased activity in the spontaneous locomotor activity test at 10 mg/kg dose (Fig. 7),

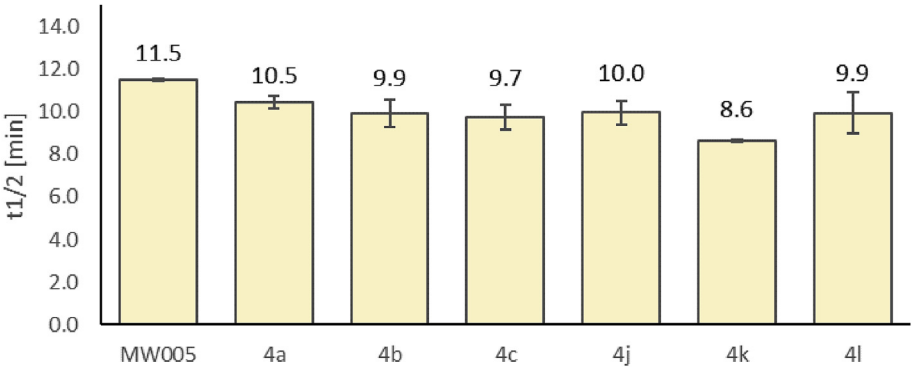


Fig. 4. Metabolic stability of selected derivatives expressed as metabolic half-life in an *in vitro* test. The box shows the average value and the whiskers represent standard deviation (n = 3).

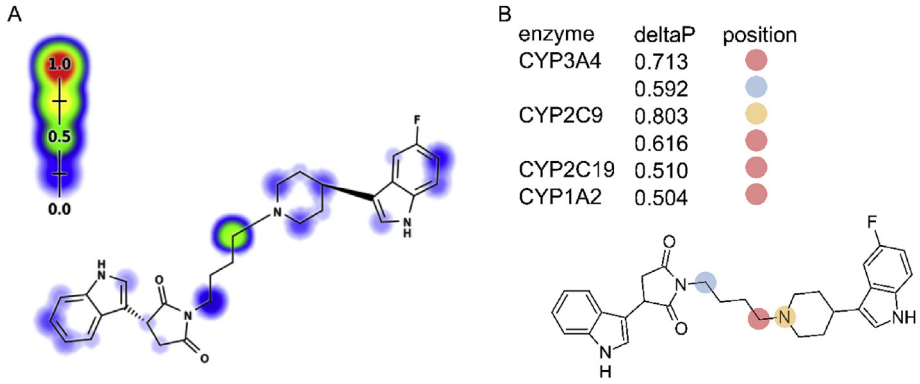


Fig. 5. Results for in silico site of metabolism prediction for **MW005**. A: Xenosite output as graphical representation with colour scale bar showing the probability of undergoing CYP-mediated oxidation (HLM Model). B: SOMP output with selected deltaP values for CYP isoforms and associated positions in a molecule. Stereochemistry of chiral centres in the molecule is forced by internal algorithm of Xenosite and cannot be changed by end-user. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

therefore it can be classified as an agonist of the pre- and postsynaptic 5-HT_{1A} receptor with antidepressant activity. The *in vivo* tests results were summarised in Table 5.

Effects of **MW005** on the immobility time in the FST in CD-1 mice. **MW005** was administered *i.p.* 30 min before the FST. Values expressed as the means ± S.E.M. were evaluated by one-way ANOVA. $^{**}P < 0.01$, vs vehicle group, n = 10; **MW005** (10 mg/kg *i.p.*) was administered 30 min before the locomotor activity test. Values are expressed as mean ± S.E.M. (n = 3–6), followed by *t*-test. $^{*}p < 0.05$ vs vehicle group.

2.5. The bioavailability prediction and cardiac toxicity in vitro assays

Concerning the results described above, early *in vitro* bioavailability assays (the Bioavailability Panel) were performed at Eurofins Pharma Discovery Services for the most promising compound **MW005**. This panel consists of the aqueous solubility, human plasma protein binding and Caco-2 permeability assays, which are used for the prediction of ADME properties, that have to be considered during decision making in the drug development process. The results are summarised in Table 6 Compound **MW005** displayed good aqueous solubility (simulated intestinal fluid (SIF) – 190.6 μM; phosphate-buffered saline, pH 7.4 (PBS) – 136.5 μM; simulated gastric fluid (SGF) – 151.0 μM), high plasma protein binding (95%) and low Caco-2 permeability ($P_{app} \leq 2.5$ – for more details see Supplementary data). Drugs that are completely absorbed in humans are characterised by permeability coefficients of $>1 \times 10^{-6}$ cm/s; >1% but <100% have permeability coefficients of $0.1–1.0 \times 10^{-6}$ cm/s, while drugs and peptides that are absorbed at <1% have permeability coefficients of $\leq 1 \times 10^{-7}$ cm/s [46]. The final efflux ratio (ER = basolateral to apical [BA]/apical to basolateral [AB] permeability) was ER ≤ 2.5, which means that compound **MW005** is not considered to be a P-gp substrate [47].

Compound **MW005** was further characterised in the hERG channel automated patch-clamp assay to verify if it has potential to trigger cardiac toxicity. Blocking the human ether-a-go-go-related gene (hERG) potassium channel causes QT interval prolongation, which is associated with potentially fatal arrhythmia, called Torsades de Pointes [48]. This study was performed at Eurofins Pharma Discovery Services. Compound **MW005** was tested at three concentrations (10.0, 1.0 and 0.1 μM) showing 96.4, 84.7 and 45.1% inhibition of tail current, respectively. This indicates that **MW005** has a high propensity for hERG channel blocking. Additionally, we determined the affinity of compound **MW005** for α_1-adrenoceptors. It was suggested that 5-HT_{1A} ligands exhibit low selectivity due to a high degree of homology (approximately 45%) between 5-HT_{1A} and α_1-adrenoceptors, and it might cause undesirable side effects such as hypotensive effects and worsened outcomes of the clinical state [49]. Compound **MW005** displayed high affinities for both the 5-HT_{1A} and α_1 receptors ($K_i = 7.5 \pm 0.5$ and $K_i = 30 \pm 3$ nM, respectively). In summary, **MW005** presented bioavailability and cardiac toxicity results that merit further optimisation of the compound to improve its permeability, oral pharmacokinetic properties and cardiac safety.

3. Conclusions

Administering of antidepressant and neuroleptic drugs with polypharmacological profiles have become a widely used therapeutic approach. In the present work, we described synthesis, receptor affinity, functional profile, metabolic stability and behavioural evaluation of a new series of 3-(1H-indol-3-yl)pyrrolidine-2,5-dione derivatives. Among them, compound **4c** exhibited the highest binding affinities at 5-HT_{1A}R ($K_i = 2.3$ nM), and compound **4b** displayed the best double binding 5-HT_{1A}/SERT activity (5-HT_{1A} $K_i = 4.9$ nM, SERT $K_i = 17.5$ nM). On the other hand, **MW005** and **4j** showed polypharmacological profiles potentially beneficial for the treatment of depression (**MW005** – K_i [nM]: 5-HT_{1A} = 7.5, SERT = 505, D_2 = 14, 5-HT_{2A} = 71, 5-HT_6 = 63, 5-HT_7 = 196; **4j** – K_i [nM]: 5-HT_{1A} = 13, SERT = 51, D_2 = 190, 5-HT_{2A} = 50, 5-HT_6 = 29, 5-HT_7 = 50). Our SAR studies revealed that A) the four-methylene spacer was crucial for affinity for the 5-HT_{1A} receptor, B) the presence of the methoxy group (R_1) substituent increased affinity for the 5-HT_{1A} receptor and C) considering the four-carbon linker series, the binding results did not indicate significant differences in affinity for 5-HT_{1A} upon varying the type of substituent in the pharmacophore part of the molecule (R_2). However, in order to obtain dual affinity for 5-HT_{1A}/SERT in this group of compounds, the introduction of a four-methylene spacer and a 5-fluoro-3-piperidin-4-yl-1*H*-indole residue was crucial. The presence of the fluoro or methoxy group (R_1) substituents increased the affinity of the compounds for 5-HT_7. These efforts led to the selection of **MW005,** which combined a multi-receptor mechanism with prominent 5-HT_{1A}R pre- and postsynaptic agonism, good metabolic stability and dose-dependent reduction of the immobility time in the FST. Nevertheless, the presented bioavailability study and the cardiac toxicity results suggested a need for further optimisation to improve oral pharmacokinetic properties and cardiac safety.

Table 3
The effect of the tested compounds: **MW005**, **4a**, **4b**, **4c**, **4j**, **4k** and **4l** on the body temperature in mice.

Treatment	Dose (mg/kg)	Δt ± SEM (^{0}C)			
		30 min	**60 min**	**90 min**	**120 min**
Vehicle	–	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
MW005	5	0.3 ± 0.1	0.4 ± 0.3	0.5 ± 0.3	0.2 ± 0.3
	10	−1.4 ± 0.1^c	−0.5 ± 0.3	0.1 ± 0.2	0.2 ± 0.2
	15	−2.0 ± 0.2^c	−0.8 ± 0.4^b	0.0 ± 0.3	0.3 ± 0.2
	20	−2.2 ± 0.1^c	−1.0 ± 0.3^c	−0.4 ± 0.2^a	−0.3 ± 0.2
		$p < 0.0001$	$p = 0.0002$	$p = 0.0496$	ns
Vehicle	–	−0.2 ± 0.0	−0.2 ± 0.1	−0.3 ± 0.1	−0.1 ± 0.2
4a	5	−0.2 ± 0.2	−0.0 ± 0.3	0.0 ± 0.2	−0.1 ± 0.1
	10	−1.3 ± 0.4^c	−0.4 ± 0.2	−0.4 ± 0.1	−0.4 ± 0.2
	20	−2.9 ± 0.1^c	−1.5 ± 0.2^c	−1.1 ± 0.2^b	−0.9 ± 0.2^b
		$p < 0.0001$	$p < 0.0001$	$p = 0.0006$	$p = 0.0084$
Vehicle	–	−0.1 ± 0.1	−0.1 ± 0.1	−0.2 ± 0.2	−0.1 ± 0.1
4b	5	−0.1 ± 0.2	0.4 ± 0.2	0.1 ± 0.2	−0.4 ± 0.2
	10	−1.3 ± 0.6^b	−0.1 ± 0.5	0.3 ± 0.3	0.4 ± 0.2
	20	−1.8 ± 0.4^c	0.5 ± 0.2	0.6 ± 0.1	0.6 ± 0.1
		$p < 0.0001$	ns	ns	$p = 0.002$
Vehicle	–	0.1 ± 0.2	0.1 ± 0.2	−0.1 ± 0.1	0.0 ± 0.2
4c	5	0.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.4 ± 0.2
	10	−0.5 ± 0.2^a	0.6 ± 0.1	0.6 ± 0.2^a	0.4 ± 0.2
	15	−0.9 ± 0.2^c	0.2 ± 0.2	0.5 ± 0.2	0.3 ± 0.2
	20	−3.1 ± 0.2^c	−1.2 ± 0.4^c	−0.6 ± 0.3	−0.5 ± 0.4
		$p < 0.0001$	$p < 0.0001$	$p = 0.0008$	$p = 0.0249$
Vehicle	–	−0.1 ± 0.1	−0.2 ± 0.1	0.1 ± 0.2	0.1 ± 0.1
4j	5	−0.4 ± 0.1	−0.2 ± 0.2	−0.2 ± 0.2	−0.3 ± 0.2
	10	−0.6 ± 0.3	0.1 ± 0.2	0.0 ± 0.2	−0.2 ± 0.2
	15	−1.8 ± 0.3^c	−0.7 ± 0.3^b	−0.4 ± 0.2	−0.4 ± 0.2
	20	−2.3 ± 0.3^c	−0.7 ± 0.3^b	−0.2 ± 0.4	−0.6 ± 0.3^a
		$p < 0.0001$	$p = 0.0011$	ns	$p = 0.0214$
Vehicle	–	−0.1 ± 0.1	0.0 ± 0.2	0.0 ± 0.2	0.1 ± 0.1
4k	5	0.4 ± 0.2^a	0.2 ± 0.2	0.4 ± 0.2	0.3 ± 0.2
	10	−1.0 ± 0.2^c	−0.1 ± 0.2	0.3 ± 0.2	0.4 ± 0.2
	20	−2.0 ± 0.2^c	−0.4 ± 0.3	0.1 ± 0.2	0.5 ± 0.2
		$p < 0.0001$	ns	ns	ns
Vehicle	–	0.1 ± 0.4	0.1 ± 0.1	−0.0 ± 0.1	0.3 ± 0.0
4l	5	0.4 ± 0.1	0.6 ± 0.1^a	0.6 ± 0.1^b	0.8 ± 0.1^a
	10	−1.1 ± 0.2^c	0.2 ± 0.2	0.2 ± 0.1	0.3 ± 0.2
	20	−3.2 ± 0.2^c	−1.4 ± 0.3^c	−0.6 ± 0.3	−0.2 ± 0.3
		$p < 0.0001$	$p < 0.0001$	$p = 0.0002$	$p = 0.0224$
Vehicle	–	0.2 ± 0.1	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2
WAY100635	0.1	0.1 ± 0.2	0.1 ± 0.2	−0.1 ± 0.2	−0.2 ± 0.1
8-OH-DPAT	5	−1.7 ± 0.2^c	−1.1 ± 0.2^c	−0.1 ± 0.1	0.3 ± 0.3
		$p < 0.0001$	$p < 0.005$	ns	ns

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

4. Experimental protocols

4.1. Chemistry

4.1.1. General remarks

All solvents and reagents were purchased from commercial sources and were used without any purification. Melting points were determined on an Electrothermal IA9200 apparatus (Cole-Parmer Ltd., Stone, Staffordshire, UK) with open capillary tubes and were uncorrected. The purity (>95%) and homogeneity of the compounds were routinely confirmed. NMR spectra were recorded on a Varian INOVA 500 MHz (Varian, Palo Alto, CA, USA) (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR), Varian Unity Plus 200 MHz (Varian, Palo Alto, CA, USA) (50 MHz for ^{13}C NMR) or Bruker AVANCE III HD 500 MHz (Bruker BioSpin, Rheinstetten, Germany) (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR) spectrometer in acetone–D_6, $CDCl_3$ or CD_3OD. Chemical shifts (δ) were expressed in parts per million (ppm) relative to tetramethylsilane used as the internal reference. The following abbreviations are used to describe peak patterns where appropriate: s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), td (triplet of doublets), pt (pseudo triplet), 4d (quartet of doublets), m (multiplet), q (quartet), qu (quintet), * - coupling with fluorine nucleus. Coupling constants (J) are in hertz (Hz).

The High-Resolution Mass Spectrometry (HRMS) was performed using Micromass LCT TOF (Waters Corporation, Milford, MA, USA) mass spectrometer equipped with ESI ionization source, TOF analyser and MCP detector. Samples as 1 mg/L concentration of tested compounds were prepared in methanol. MS detection settings were as follows: source temperature 80 °C, desolvation

Table 4
The effect of WAY100635 (0.1 mg/kg s.c.) on the hypothermia induced by compounds **MW005**, **4a, 4b, 4c, 4j, 4k and 4l**.

Treatment and dose (mg/kg)	Δt ± SEM (^{0}C)	
	30 min	**60 min**
Vehicle + vehicle	−0.0 ± 0.1	0.3 ± 0.1
Vehicle + **MW005** (10)	−1.4 ± 0.1[c]	−0.5 ± 0.3
WAY100635 (0.1) + **MW005** (10)	−0.1 ± 0.3[f]	0.7 ± 0.3
	$p < 0.0001$	ns
Vehicle	−0.0 ± 0.1	−0.1 ± 0.2
Vehicle + **4a** (10)	−1.3 ± 0.4[b]	−0.4 ± 0.2
WAY100635 + **4a** (10)	−0.4 ± 0.3[d]	−0.2 ± 0.2
	$p = 0.0036$	ns
Vehicle	0.1 ± 0.1	0.4 ± 0.0
Vehicle + **4b** (10)	−1.3 ± 0.6[b]	−0.1 ± 0.5
WAY100635 + **4b** (10)	−0.1 ± 0.1	0.4 ± 0.2
	$p = 0.0064$	ns
Vehicle	0.1 ± 0.3	0.1 ± 0.3
Vehicle + **4c** (15)	−0.9 ± 0.2[c]	0.2 ± 0.2
WAY100635 + **4c** (15)	−0.1 ± 0.2[e]	0.3 ± 0.3
	$p = 0.0006$	ns
Vehicle	−0.1 ± 0.2	−0.0 ± 0.1
Vehicle + **4j** (15)	−1.8 ± 0.3[c]	−0.7 ± 0.3
WAY100635 + **4j** (15)	−0.8 ± 0.3[e]	0.0 ± 0.2
	$p < 0.0001$	$p = 0.0308$
Vehicle	0.1 ± 0.1	0.4 ± 0.0
Vehicle + **4k** (10)	−1.0 ± 0.2[c]	−0.1 ± 0.2
WAY100635 + **4k** (10)	0.4 ± 0.2[f]	0.8 ± 0.2[b,e]
	$p < 0.0001$	$p = 0.0012$
Vehicle	0.1 ± 0.4	0.0 ± 0.2
Vehicle + **4l** (10)	−1.1 ± 0.2[c]	0.2 ± 0.2
WAY100635 + **4l** (10)	−0.4 ± 0.2[d]	0.5 ± 0.2
	$p < 0.0001$	ns

WAY100635 was administered 15 min before the tested compounds.
[a]$p < 0.05$ vs vehicle, [b]$p < 0.01$ vs vehicle, [c]$p < 0.001$ vs vehicle.
[d]$p < 0.05$ vs compound group, [e]$p < 0.01$ vs compound group, [f]$p < 0.001$ vs compound group.

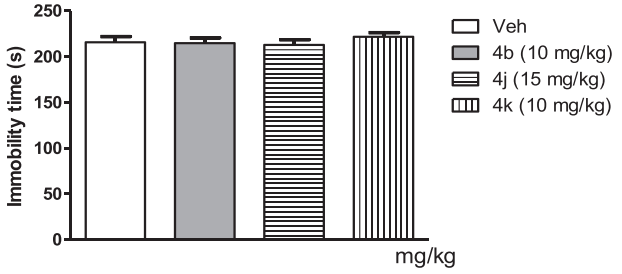


Fig. 6. Effect of compounds: **4b, 4j** and **4k** on the FST in CD-1 mice.

Table 5
Functional *in vivo* 5-HT$_{1A}$ receptor activity of the investigated compounds.

Compound	5-HT$_{1A}$ receptor functional activity	
	Presynaptic	Postsynaptic
MW005	Agonist	Agonist
4a	Agonist	
4b	Agonist	No agonists activity
4c	Agonist	
4j	Agonist	No agonists activity
4k	Agonist	No agonists activity
4l	Agonist	

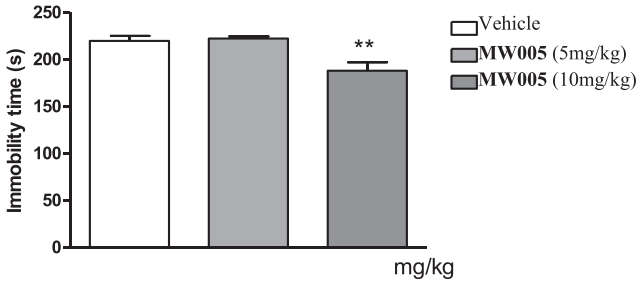
temperature 150 °C, desolvation gas flow rate 200 L/h, cone gas flow 100 L/h, capillary potential 3.50–5.00 kV, cone potential 26–50 V, extraction cone potential 4–20 V, RF Lens 260–350 V. Nitrogen was used for both nebulizing and drying gas. The data were obtained in a scan mode ranging from 20 to 2000 *m/z* in time 1.0 s intervals. Data acquisition software was MassLynx V 3.5 (Waters).

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 [*v/v*]). Thin layer chromatography was run on Merck silica gel (Kieselgel 60 F_{254}) plates (Merck, Darmstadt, Germany), 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 visualised by UV light (254 nm). Room temperature refers to 20–25 °C. Intermediate 1-(4-bromobutyl)-3-(1*H*-indol-3-yl)pyrrolidine-2,5-dione (**2g**), derivatives of 3-piperidin-4-yl-1*H*-indole (**3a**–**c**) and 3-piperidin-3-yl-1*H*-indole derivatives **(5a**–**c)**, as well as compounds **MW005** and **MW011** (Scheme 1, Scheme 2 and Table 2) were obtained following the protocol described in our previous paper (for more information see Supplementary data) [29,30]. Atom numbering, ^{1}H NMR and ^{13}C NMR spectra of all synthesised compounds is available in Supplementary data, for atom numbering see Fig. 8.

*4.1.2. General procedure for the preparation of 3-(5-substituted-1H-indol-3-yl)pyrrolidine-2,5-dione (**1a**–**b**)*

A mixture of 5-fluoro-1*H*-indole or 5-methoxy-1*H*-indole (0.10 mol) and 10% molar excess of maleimide (12.87 g, 0.11 mol) in acetic acid (125 mL) was refluxed while stirring for about 48 h and 68 h, respectively for **1a** and **1b**. The completion of the reaction was assigned chromatographically (TLC). The reaction mixture was cooled, the solvent was removed under vacuum and the crude product was crystallized as described below to give compound (**1a**–**b**) [37–39].

*4.1.2.1. 3-(5-fluoro-1H-indol-3-yl)pyrrolidine-2,5-dione (**1a**).* The crude product (brown oil) was dissolved in a few mL of cold



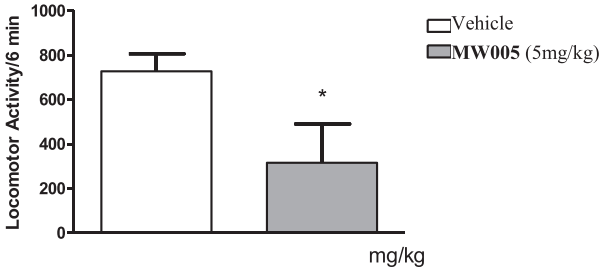


Fig. 7. Effect of **MW005** on the FST in CD-1 mice and effect of **MW005** on locomotor activity in mice.

Table 6
In vitro bioavailability data for **MW005**.

Assay		MW005 concentration	Property	Reference
Aqueous solubility	(PBS)[a]	200 μM	136.5 μM	195.4 μM (metoprolol)
	(SIF)[b]		190.6 μM	
	(SGF)[c]		151.0 μM	
Protein binding[d]		10 μM	95%	85% (warfarin)
Permeability	A-B[e]	10 μM	0.6 [10^{-6} cm/s]	0.3 [10^{-6} cm/s] (ranitidine)
	B-A[e]		1.5 [10^{-6} cm/s]	2.9 [10^{-6} cm/s] (ranitidine)

Solubility study was performed in a 200 μM; concentration.
[a] aqueous solubility at buffer pH7.4.
[b] aqueous solubility at simulated intestinal fluid.
[c] aqueous solubility in simulated gastric fluid.
[d] plasma human.
[e] Caco-2, pH 6.5/7.4.

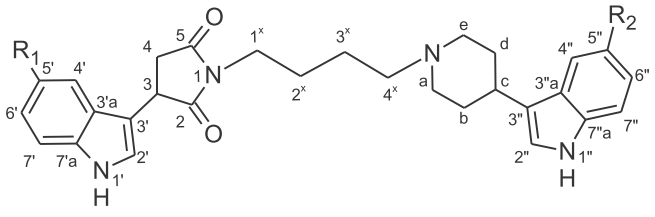


Fig. 8. General atom numbering for ^{1}H NMR and ^{13}C NMR spectra.

methanol, then dichloromethane was added to clear opacity. The mixture was left for one week at 4 °C. The precipitated solid was filtered, washed with dichloromethane and dried. The title compound was isolated as orange solid. Yield: 61% 5.28 g, m.p. 196–199 °C (lit. 190–195 °C [34]).

*4.1.2.2. 3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**1b**).* Crystallisation from ethyl acetate. The title compound was isolated as a light brown powder. Yield: 77% 6.92 g, m.p. 180–190 °C (lit. 180 °C, melts with decomposition [33]).

*4.1.3. General procedure for the preparation of 1-(4-bromoalkyl)-3-(5-substituted-1H-indol-3-yl)pyrrolidine-2,5-dione (**2a**–**f**)*

The mixture of appropriate 3-(5-substituted-1*H*-indol-3-yl) pyrrolidine-2,5-dione (**1a**–**b**) (0.01 mol) and 1,4-dibromobutane (0.05 mol), 1,3-dibromopropane (0.05 mol) or 1,2-dibromoethane (0.10 mol) and K_2CO_3 (0.02 mol) and 100 mL of acetone was stirred and refluxed for 4–6 h (14 h for 1-(2-bromoethyl)-3-(5-fluoro-1*H*-indol-3-yl)pyrrolidine-2,5-dione (**2c**)). 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*) or CH_2Cl_2 as an eluent. Proper fractions were identified by TLC and evaporated to dryness.

*4.1.3.1. 1-(4-bromobutyl)-3-(5-fluoro-1H-indol-3-yl)pyrrolidine-2,5-dione (**2a**).* The title compound was isolated as a yellow crystals. Yield: 92% (1.69 g); m.p. 108–130 °C (melts with decomposition); ESI-HRMS *m/z* calcd for $C_{16}H_{16}FBrN_2O_2Na$ $(M + Na)^+$ 389.0277, found 389.0275; ^{1}H NMR (500 MHz, acetone–D_6): δ 4.24 (C3H, 4d, $^{3}J_1 = 9.5$, $^{3}J_2 = 5.0$), 2.88 (C4H(1), dd, $^{2}J = 18.0$, $^{3}J = 5.0$), 3.25 (C4H(2), dd, $^{2}J = 18.0$, $^{3}J = 9.5$), 7.12 (C2′H, d, $^{3}J = 2.5$), 7.08 (C4′H, dd, $^{3}J_{H\text{-}F} = 9.5$, $^{4}J = 2.5$), 6.96 (C6′H, td, $^{3}J = 9.0$, $^{4}J = 2.5$), 7.26 (C7′H, dd, $^{3}J = 8.5$, $^{4}J_{H\text{-}F} = 4.5$), 3.65 (C1^{x}H$_2$, t, $^{3}J = 7.0$), 1.88 (C2^{x}H$_2$, m), 1.82 (C3^{x}H$_2$, m), 3.42 (C4^{x}H$_2$, t, $^{3}J = 6.5$), 8.32 (N1′H, bs); ^{13}C NMR (125 MHz, acetone–D_6): δ 178.0 (C2), 38.1 (C3), 36.1 (C4), 176.1 (C5), 123.8 (C2′), 111.6 (C3′, d*, $^{4}J = 4.9$), 126.0 (C3′a, d*, $^{3}J = 9.7$), 103.6 (C4′, d*, $^{2}J = 23.9$), 158.0 (C5′, d*, $^{1}J = 235.8$), 111.3 (C6′, d*, $^{2}J = 26.3$), 112.4 (C7′, d*, $^{3}J = 9.8$), 133.1 (C7′a), 32.7 (C2^{x}), 29.9 (C2^{x}), 38.0 (C3^{x}).

*4.1.3.2. 1-(3-bromopropyl)-3-(5-fluoro-1H-indol-3-yl)pyrrolidine-2,5-dione (**2b**).* The title compound was isolated as a dark yellow oil. Yield: 77% (2.18 g); ESI-HRMS *m/z* calcd for $C_{15}H_{14}FBrN_2O_2Na$ $(M + Na)^+$ 375.0120, found 375.0120; ^{1}H NMR (500 MHz, acetone–D_6): δ 4.26 (C3H, 4d, $^{3}J_1 = 9.5$, $^{3}J_2 = 5.0$, $^{4}J = 0.5$), 2.90 (C4H(1), dd, $^{2}J = 18.5$, $^{3}J = 5.0$), 3.27 (C4H(2), dd, $^{2}J = 18.5$, $^{3}J = 9.5$), 7.15 (C2′H, d, $^{3}J = 3.0$), 7.09 (C4′H, dd, $^{3}J_{H\text{-}F} = 9.0$, $^{4}J = 2.5$), 6.97 (C6′H, td, $^{3}J = 9.5$, $^{4}J = 2.5$), 7.28 (C7′H, dd, $^{3}J = 9.0$, $^{4}J_{H\text{-}F} = 4.5$), 3.77 (C1^{x}H$_2$, t, $^{3}J = 7.0$), 2.23 (C2^{x}H$_2$, q, $^{3}J = 7.0$), 3.40 (C3^{x}H$_2$, q, $^{3}J = 6.5$), 8.25 (N1′H, bs); ^{13}C NMR (125 MHz, acetone–D_6): δ 178.0 (C2), 38.0 (C3), 36.1 (C4), 176.0 (C5), 123.7 (C2′), 111.6 (C3′, d*, $^{4}J = 4.9$), 126.1 (C3′a, d*, $^{3}J = 9.8$), 103.6 (C4′, d*, $^{2}J = 23.9$), 158.0 (C5′, d*, $^{1}J = 235.9$), 111.3 (C6′, d*, $^{2}J = 26.4$), 112.4 (C7′, d*, $^{3}J = 9.3$), 133.1 (C7′a), 30.6 (C1^{x}), 29.8 (C2^{x}), 38.0 (C3^{x}).

*4.1.3.3. 1-(2-bromoethyl)-3-(5-fluoro-1H-indol-3-yl)pyrrolidine-2,5-dione (**2c**).* The title compound was isolated as a yellow oil. Yield: 50% (3.1 g); ESI-HRMS *m/z* calcd for $C_{14}H_{12}FBrN_2O_2Na$ $(M + Na)^+$ 360.9964, found 360.9977; ^{1}H NMR (500 MHz, acetone–D_6): δ 4.46 (C3H, dd, $^{3}J_1 = 9.5$, $^{3}J_2 = 5.0$), 2.90 (C4H(1), dd, $^{2}J = 18.5$, $^{3}J = 5.5$), 3.36 (C4H(2), dd, $^{2}J = 18.5$, $^{3}J = 5.5$), 7.42 (C2′H, d, $^{3}J = 2.0$), 7.31 (C4′H, 4d, $^{3}J = 8.5$, $^{4}J_{H\text{-}F} = 4.5$, $^{5}J = 0.5$), 6.94 (C6′H, td, $^{3}J_{H\text{-}F} = 9.0$, $^{4}J = 2.5$), 7.42 (C7′H, 4d, $^{3}J = 8.5$, $^{4}J = 4.5$, $^{5}J = 0.5$), 3.97 (C1^{x}H$_2$, t, $^{3}J = 7.0$), 3.69 (C2^{x}H$_2$, t, $^{3}J = 6.5$); ^{13}C NMR (125 MHz, acetone–D_6): δ 178.4 (C2), 38.6 (C3), 36.8 (C4), 158.5 (C5, d*, $^{1}J = 230.8$), 125.8 (C2'), 112.6 (C3', d*, $^{4}J = 4.9$), 127.6 (C3′a), 104.5 (C4', d*, $^{2}J = 23.4$), 113.5 (C7', d*, $^{3}J = 9.75$), 134.5 (C7′a), 40.9 (C1^{x}), 28.9 (C2^{x}).

*4.1.3.4. 1-(4-bromobutyl)-3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**2d**).* The title compound was isolated as a dark yellow crystals. Yield: 64% (1.86 g); m.p. 103–107 °C; ESI-HRMS *m/z* calcd for $C_{17}H_{19}BrN_2O_3Na$ $(M + Na)^+$ 401.0477, found 401.0473; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.26 (C3H, dd, $^{3}J_1 = 9.5$, $^{3}J_2 = 5.0$), 2.92 (C4H(1), dd, $^{2}J = 18.0$, $^{3}J = 5.0$), 3.25 (C4H(2), dd, $^{2}J = 18.0$, $^{3}J = 9.5$), 7.06 (C2′H, d, $^{3}J = 2.5$), 6.87 (C4′H, d, $^{4}J = 2.0$), 7.25 (C7′H, dd, $^{3}J = 8.5$, $^{5}J = 0.5$), 6.88 (C6′H, dd, $^{3}J = 8.5$, $^{4}J = 2.5$), 3.64 (C1^{x}H$_2$, t, $^{3}J = 7.0$), 1.88 (C2^{x}H$_2$, m), 1.82 (C3^{x}H$_2$, m), 3.42 (C4^{x}H$_2$, t, $^{3}J = 6.5$), 3.83 (OCH_3, s), 8.14 (NH, bs); ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.1 (C2), 38.0 (C3), 36.2 (C4), 176.4 (C5), 122.7 (C2′), 111.2 (C3′), 126.2 (C3′a), 100.6 (C4′), 154.4 (C5′), 112.4 (C6′), 112.8 (C7′), 131.8 (C7′a), 38.1 (C1^{x}), 29.9 (C2^{x}), 26.5 (C3^{x}), 32.7 (C4^{x}), 55.9 (OCH_3).

*4.1.3.5. 1-(3-bromopropyl)-3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**2e**).* The title compound was isolated as a dark yellow crystals. Yield: 82% (2.42 g); m.p. 141–143 °C; ESI-HRMS *m/z* calcd for $C_{16}H_{17}BrN_2O_3Na$ $(M + Na)^+$ 387.0320, found 387.0316; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.26 (C3H, dd, $^{3}J_1 = 9.5$, $^{3}J_2 = 5.0$), 2.92 (C4H(1),

dd, $^{2}J = 18.5$, $^{3}J = 5.0$), 3.26 (C4H(2), dd, $^{2}J = 18.5$, $^{3}J = 9.5$), 7.05 (C2′H, d, $^{3}J = 2.5$), 6.86 (C4′H, d, $^{4}J = 2.5$), 7.25 (C7′H, dd, $^{3}J = 8.5$, $^{5}J = 0.5$), 6.88 (C6′H, dd, $^{3}J = 8.5$, $^{4}J = 2.5$), 3.75 (C1^{x}H$_2$, t, $^{3}J = 7.0$), 2.22 (C2^{x}H$_2$, q, $^{3}J = 7.0$), 3.39 (C3^{x}H$_2$, t, $^{3}J = 7.0$), 3.83 (OCH_3, s), 8.18 (NH, bs); ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.1 (C2), 38.0 (C3), 36.2 (C4), 176.3 (C5), 122.7 (C2′), 111.0 (C3′), 126.2 (C3′a), 100.5 (C4′), 154.4 (C5′), 112.4 (C6′), 112.8 (C7′), 131.7 (C7′a), 38.1 (C1^{x}), 29.7 (C2^{x}), 30.7 (C3^{x}), 55.9 (OCH_3).

*4.1.3.6. 1-(2-bromoethyl)-3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**2f**).* The title compound was isolated as a dark yellow crystals. Yield: 55% (1.54 g); m.p. 145–148 °C; ESI-HRMS *m/z* calcd for $C_{15}H_{15}BrN_2O_3Na$ $(M + Na)^+$ 373.0164, found 373.0165; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.30 (C3H, 4d, $^{3}J_1 = 9.5$, $^{3}J_2 = 5.0$, $^{5}J = 0.5$), 2.96 (C4H(1), dd, $^{2}J = 18.5$, $^{3}J = 5.0$), 3.28 (C4H(2), dd, $^{2}J = 18.5$, $^{3}J = 9.5$), 7.09 (C2′H, d, $^{3}J = 2.5$), 6.91 (C4′H, d, $^{4}J = 2.5$), 7.25 (C7′H, dd, $^{3}J = 8.5$, $^{5}J = 0.5$), 6.89 (C6′H, dd, $^{3}J = 9.0$, $^{4}J = 2.5$), 4.04 (C1^{x}H$_2$, t, $^{3}J = 6.5$), 3.62 (C2^{x}H$_2$, t, $^{3}J = 6.5$), 3.84 (OCH_3, s), 8.14 (NH, bs); ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.7 (C2), 38.1 (C3), 36.2 (C4), 175.9 (C5), 122.9 (C2′), 110.9 (C3′), 126.2 (C3′a), 100.6 (C4′), 154.4 (C5′), 112.4 (C6′), 112.9 (C7′), 131.7 (C7′a), 40.2 (C1^{x}), 27.6 (C2^{x}), 56.0 (OCH_3).

*4.1.4. Procedure for the synthesis of 3-piperidin-4-yl-1H-indoles (**3a–c**)*

The starting compounds **3a–c** were obtained according to previously described procedures [29,50,51]. For more details see Supplementary data.

*4.1.5. General procedure for the synthesis of derivatives 3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**4a–r**)*

A mixture of appropriate derivatives of 1-(4-bromoalkyl)-3-(5-substituted-1*H*-indol-3-yl)pyrrolidine-2,5-dione (**2a–f**) (0.5 mmol), the appropriate derivatives of 5-substitued-3-(piperidin-4-yl)-1*H*-indoles (**3a–c**) (0.5 mmol), K_2CO_3 (1.0 mmol), a catalytic amount of KI and 35 mL acetonitrile was 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 **4a–r**.

*4.1.5.1. 1-{4-[4-(1H-indol-3-yl)piperidin-1-yl]butyl}-3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**4a**).* The title compound was isolated as a yellow solid. Yield: 76.6% (0.19 g); m.p. 111–117 °C; ESI-HRMS *m/z* calcd for $C_{30}H_{33}N_4O_3H$ $(M + H)^+$ 499.2709, found: 499.2703; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.21 (C3H, dd, $^{3}J_1 = 9.0$, $^{3}J_2 = 4.5$), 2.87 (C4H(1), dd, $^{2}J = 18.0$, $^{3}J = 4.5$), 3.20 (C4H(2), dd, $^{2}J = 18.0$, $^{3}J = 9.5$), 6.86 (C2′H, C6′H, m), 6.89 (C4′H, d, $^{4}J = 2.0$), 7.23 (C7′H, d, $^{3}J = 9.0$), 3.63 (C1^{x}H$_2$, t, $^{3}J = 7.0$), 1.68 (C2^{x}H$_2$, q, $^{3}J = 7.0$), 1.59 (C3^{x}H$_2$, q, $^{3}J = 7.0$), 2.45 (C4^{x}H$_2$, t, $^{3}J = 7.5$), 3.02 (CaH(E), Ce(E), m), 2.13 (CaH(A), Ce(A), m), 2.0 (CbH(E), CdH(E), m), 1.83 (CbH(A), CdH(A), m), 2.80 (CcH, tt, $^{3}J_1 = 12.0$, $^{3}J_2 = 3.5$), 6.97 (C2"H, d, $^{3}J = 2.5$), 7.60 (C4"H, d, $^{3}J = 8.0$), 7.08 (C5"H, m, $^{3}J_1 = 8.0$, $^{3}J_2 = 7.0$, $^{4}J = 1.0$), 7.16 (C6"H, m, $^{3}J_1 = 8.0$, $^{3}J_2 = 7.0$, $^{4}J = 1.0$), 7.33 (C7"H, d, $^{3}J = 8.5$), 3.82 (OCH_3, s), 8.57 (N1′H, bs), 8.25 (N1"H, bs); ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.4 (C2), 38.1 (C3), 36.2 (C4), 176.6 (C5), 122.8 (C2′), 111.1 (C3′), 126.2 (C3′a), 100.6 (C4′), 154.3 (C5′), 112.4 (C6′), 112.7 (C7′), 131.8 (C7′a), 38.6 (C1^{x}), 25.8 (C2^{x}), 23.8 (C3^{x}), 58.2 (C4^{x}), 54.1 and 54.2 (Ca, Ce), 32.4 and 32.4 (Cb, Cd), 33.1 (Cc), 119.8 (C2"), 120.8 (C3"), 126.6 (C3"a), 119.0 (C4"), 119.0 (C5"), 121.9 (C6"), 111.3 (C7"), 136.4 (C7"a), 55.9 (OCH_3).

*4.1.5.2. 1-{4-[4-(5-fluoro-1H-indol-3-yl)piperidin-1-yl]butyl}-3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**4b**).* The title compound was isolated as a yellow solid. Yield: 46.5% (0.12 g); m.p. 111–119 °C; ESI-HRMS *m/z* calcd for $C_{30}H_{32}FN_4O_3H$ $(M + H)^+$ 517.2612, found: 517.2615; ^{1}H NMR (500 MHz, acetone–D_6): δ 4.51 (C3H, dd, $^{3}J_1 = 9.5$, $^{3}J_2 = 5.0$), 2.88 (C4H(1), dd, $^{2}J = 18.0$, $^{3}J = 5.0$), 3.37 (C4H(2), dd, $^{2}J = 18.0$, $^{3}J = 9.5$), 7.35 (C2′H, d, $^{3}J = 2.5$), 7.04 (C4′H, d, $^{4}J = 2.5$), 6.80 (C6′H, dd, $^{3}J = 8.5$, $^{4}J = 2.5$), 7.33 (C7′H, dt, $^{3}J = 8.5$, $^{5}J = 0.5$), 3.61 (C1^{x}H$_2$, t, $^{3}J = 7.0$), 1.88 (C2^{x}H$_2$, m), 1.73 (C3^{x}H$_2$, q, $^{3}J = 7.5$), 3.03 (C4^{x}H$_2$, t), 3.48 (CaH(E), CeH(E), pt), 2.93 (CaH(A), CeH(A), m), 2.34 (CbH(E), CdH(E), pk), 2.14 (CbH(A), CdH(A), pd), 3.13 (CcH, m), 7.22 (C2"H, d, $^{3}J = 2.0$), 7.42 (C4"H, dd, $^{3}J_{H\text{-}F} = 10.5$, $^{4}J = 2.5$), 6.89 (C6"H, td, $^{3}J = 9.0$, $^{4}J = 2.5$), 7.39 (C7"H, 4d, $^{3}J = 8.5$, $^{4}J_{H\text{-}F} = 4.0$, $^{5}J = 0.5$), 3.80 (OCH_3, s), 10.11 (N1′H, bs), 10.17 (N1"H, bs).

*4.1.5.3. 1-{4-[4-(5-methoxy-1H-indol-3-yl)piperidin-1-yl]butyl}-3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**4c**).* The title compound was isolated as a yellow solid. Yield: 60.8% (0.16 g); m.p. 105–114 °C; ESI-HRMS *m/z* calcd for $C_{31}H_{36}N_4O_4H$ $(M + H)^+$ 529.2815, found: 529.2829; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.21 (C3H, dd, $^{3}J_1 = 9.5$, $^{3}J_2 = 5.0$), 2.87 (C4H(1), dd, $^{2}J = 18.0$, $^{3}J = 5.0$), 3.20 (C4H(2), dd, $^{2}J = 18.0$, $^{3}J = 9.5$), 7.04 (C2′H, d, $^{3}J = 2.0$), 6.85–6.89 (C4′H, C6′H, C"4H, m), 7.23 (C7′H, d, $^{3}J = 8.5$), 3.64 (C1^{x}H$_2$, t, $^{3}J = 7.5$), 1.69 (C2^{x}H$_2$, q, $^{3}J = 7.5$), 1.58 (C3^{x}H$_2$, q, $^{3}J = 7.5$), 2.43 (C4^{x}H$_2$, t, $^{3}J = 7.5$), 3.02 (CaH(E), Ce(E), m), 2.12 (CaH(A), Ce(A), m), 2.0 (CbH(E), CdH(E), m), 1.81 (CbH(A), CdH(A), m), 2.76 (CcH, tt, $^{3}J_1 = 12.0$, $^{3}J_2 = 3.5$), 6.97 (C2"H, d, $^{3}J = 2.5$), 6.83 (C6"H, dd, $^{3}J = 9.0$, $^{4}J = 2.5$), 7.21 (C7"H, d, $^{3}J = 8.5$), 3.82 (C5′-OCH_3, s), 3.84 (C5"-OCH_3, s), 8.55 (N1′H, bs), 8.11 (N1"H, bs); ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.4 (C2), 38.1 (C3), 36.2 (C4), 176.5 (C5), 122.8 (C2′), 111.1 (C3′), 126.2 (C3′a), 100.6 (C4′), 154.3 (C5′), 112.4 (C6′), 112.7 (C7′), 131.8 (C7′a), 38.8 (C1^{x}), 25.9 (C2^{x}), 24.0 (C3^{x}), 58.3 (C4^{x}), 54.3 (Ca, Ce), 32.6 (Cb, Cd), 33.3 (Cc), 120.7 (C2"), 120.8 (C3"), 126.9 (C3"a), 101.1 (C4"), 133.7 (C5"), 111.9 (C6"), 111.9 (C7"), 131.6 (C7"a), 55.9 (5′-OCH_3), 56.1 (5"-OCH_3).

*4.1.5.4. 1-{3-[4-(1H-indol-3-yl)piperidin-1-yl]propyl}-3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**4d**).* The title compound was isolated as a yellow solid. Yield: 67.0% (0.16 g); m.p. 111–116 °C; ESI-HRMS *m/z* calcd for $C_{29}H_{32}N_4O_3H$ $(M + H)^+$ 485.2553, found: 485.2569; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.25 (C3H, 4d, $^{3}J_1 = 9.0$, $^{3}J_2 = 5.0$, $^{5}J = 0.5$), 2.91 (C4H(1), dd, $^{2}J = 18.5$, $^{3}J = 5.0$), 3.25 (C4H(2), dd, $^{2}J = 18.5$, $^{3}J = 9.5$), 6.88 (C2′H, C4′H, C6′H, m), 7.26 (C7′H, d, $^{3}J = 9.5$), 3.69 (C1^{x}H$_2$, t, $^{3}J = 7.0$), 1.92 (C2^{x}H$_2$, m), 2.48 (C3^{x}H$_2$, t, $^{3}J = 7.0$), 3.04 (CaH(E), CeH(E), m), 2.13 (CaH(A), CeH(A), m), 2.03 (CbH(E), CdH(E), m), 1.81 (CbH(A), CdH(A), m), 2.82 (CcH, tt, $^{3}J_1 = 11.5$, $^{3}J_2 = 3.5$), 7.08 (C2"H, C5"H, m), 7.61 (C4"H, d, $^{3}J = 8.0$), 7.17 (C6"H, m, $^{3}J_1 = 8.0$, $^{3}J_2 = 7.0$, $^{4}J = 1.5$), 7.34 (C7"H, dt, $^{3}J = 8.0$, $^{4}J = {}^{5}J = 1.0$), 3.83 (OCH_3, s), 8.25 (N1′H, bs), 8.06 (N1"H, bs); ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.2 (C2), 37.5 (C3), 36.3 (C4), 176.5 (C5), 122.8 (C2′), 111.3 (C3′), 126.3 (C3′a), 100.7 (C4′), 154.3 (C5′), 112.4 (C6′), 112.6 (C7′), 131.8 (C7′a), 38.1 (C1^{x}), 25.1 (C2^{x}), 56.2 (C3^{x}), 54.2 (Ca, Ce), 32.7 and 32.8 (Cb, Cd), 33.3 (Cc), 119.7 (C2"), 121.1 (C3"), 126.6 (C3"a), 119.0 (C4"), 119.1 (C5"), 121.9 (C6"), 111.2 (C7"), 136.4 (C7"a), 55.9 (OCH_3).

*4.1.5.5. 1-{3-[4-(5-fluoro-1H-indol-3-yl)piperidin-1-yl]propyl}-3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**4e**).* The title compound was isolated as a yellow solid. Yield: 59.7% (0.15 g); m.p. 106–117 °C; ESI-HRMS *m/z* calcd for $C_{29}H_{31}FN_4O_3H$ $(M + H)^+$ 503.2458, found: 503.2476; ^{1}H NMR (500 MHz, acetone–D_6): δ 4.38 (C3H, 4d, $^{3}J_1 = 9.5$, $^{3}J_2 = 4.5$, $^{4}J = 0.5$), 2.85 (C4H(1), dd, $^{2}J = 18.0$, $^{3}J = 5.0$), 3.32 (C4H(2), dd, $^{2}J = 18.0$, $^{3}J = 9.5$), 7.31 (C2′H, d, $^{3}J = 2.5$), 6.99 (C4′H, d, $^{4}J = 2.0$), 6.80 (C6′H, dd, $^{3}J = 9.0$, $^{4}J = 2.5$), 7.32 (C7′H, d, $^{3}J = 8.5$), 3.64 (C1^{x}H$_2$, t, $^{3}J = 7.0$), 1.85 (C2^{x}H$_2$, m), 2.45 (C3^{x}H$_2$, t, $^{3}J = 7.0$), 3.03 (CaH(E), CeH(E), pd), 2.12 (CaH(A), CeH(A), m), 1.99

(CbH(E), CdH(E), m), 1.78 (CbH(A), CdH(A), m), 2.78 (CcH, tt, $^{3}J_{A-A}$ = 12.0, $^{3}J_{A-E}$ = 3.5), 7.15 (C2"H, d, ^{3}J = 2.5), 7.29 (C4"H, dd, $^{3}J_{H-F}$ = 12.0, ^{4}J = 3.0), 6.87 (C6"H, td, ^{3}J = 9.0, ^{4}J = 2.5), 7.35 (C7"H, 4d, ^{3}J = 9.0, $^{4}J_{H-F}$ = 4.5, ^{5}J = 0.5), 3.79 (OCH_3, s), 10.05 (N1′H, bs), 10.09 (N1"H, bs).

*4.1.5.6. 1-{3-[4-(5-methoxy-1H-indol-3-yl)piperidin-1-yl]propyl}-3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**4f**).* The title compound was isolated as a yellow solid. Yield: 93.8% (0.24 g); m.p. 100–109 °C; ESI-HRMS *m/z* calcd for $C_{30}H_{34}N_4O_4H$ (M + H)$^+$ 515.2658, found: 515.2678; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.24 (C3H, dd, $^{3}J_1$ = 9.5, $^{3}J_2$ = 5.0), 2.90 (C4H(1), dd, ^{2}J = 18.5, ^{3}J = 5.0), 3.22 (C4H(2), dd, ^{2}J = 18.5, ^{3}J = 9.5), 7.05 (C2′H, d, ^{3}J = 2.5), 6.85–6.89 (C4′H, C6′H, m), 7.23 (C7′H, dd, ^{3}J = 8.5, ^{5}J = 0.5), 3.68 (C1^{x}H$_2$, t, ^{3}J = 7.0), 1.92 (C2^{x}H$_2$, m), 2.49 (C3^{x}H$_2$, t, ^{3}J = 7.5), 3.04 (CaH(E), Ce(E), m), 2.14 (CaH(A), Ce(A), m), 2.0 (CbH(E), CdH(E), m), 1.79 (CbH(A), CdH(A), m), 2.75 (CcH, tt, $^{3}J_1$ = 11.5, $^{3}J_2$ = 3.5), 7.03 (C2"H, d, ^{3}J = 2.5), 6.81–6.84 (C4"H, C6"H, m), 7.21 (C7"H, d, ^{3}J = 8.5), 3.81 (C5′-OCH_3, s), 3.84 (C5"-OCH_3, s), 8.44 (N1′H, d, ^{3}J = 1.0), 8.08 (N1"H, bs); ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.3 (C2), 37.4 (C3), 36.3 (C4), 176.5 (C5), 122.9 (C2′), 111.2 (C3′), 126.3 (C3′a), 100.7 (C4′), 154.3 (C5′), 112.4 (C6′), 112.5 (C7′), 131.8 (C7′a), 38.1 (C1^x), 24.9 (C2^x), 56.1 (C3^x, 5"-OCH_3), 54.2(Ca, Ce) 32.4 and 32.5 (Cb, Cd), 33.2 (Cc), 120.7 (C2"), 120.6 (C3"), 126.9 (C3"a), 101.1 (C4"), 153.7 (C5"), 111.9 (C6", C7"), 131.6 (C7"a), 55.9 (5′-OCH_3).

*4.1.5.7. 1-{2-[4-(1H-indol-3-yl)piperidin-1-yl]ethyl}-3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**4g**).* The title compound was isolated as a light brown solid. Yield: 41.7% (0.10 g); m.p. 104–108 °C; ESI-HRMS *m/z* calcd for $C_{28}H_{30}N_4O_3H$ (M + H)$^+$ 471.2389, found: 471.2396; ^{1}H NMR (500 MHz, acetone–D_6): δ 4.36 (C3H, 4d, $^{3}J_1$ = 9,5, $^{3}J_2$ = 4.5, ^{4}J = 0.5), 2.81 (C4H(1), dd, ^{2}J = 18.0, ^{3}J = 5.0), 3.30 (C4H(2), dd, ^{2}J = 18.0, ^{3}J = 9.5), 7.04 (C2′H, C4′H, m), 6.81 (C6′H, dd, ^{3}J = 8.5, ^{4}J = 2.5), 7.31 (C7′H, dd, ^{3}J = 9.0, ^{5}J = 0.5), 3.72 (C1^{x}H$_2$, t, ^{3}J = 7.0) 2.61 (C2^{x}H$_2$, t, ^{3}J = 6.5), 3.10 (CaH(E), CeH(E), m), 2.18 (CaH(A), CeH(A), m), 1.98 (CbH(E), CdH(E), m), 1.73 (CbH(A), CdH(A), m), 2.80 (CcH, m*), 7.32 (C2"H, d, ^{3}J = 2.5), 7.58 (C4"H, dd, ^{3}J = 7.5, ^{4}J = 1.0), 6.98 (C5"H, m, $^{3}J_1$ = 7.5, $^{3}J_2$ = 7.0, ^{4}J = 1.0), 7.07 (C6"H, m, $^{3}J_1$ = 8.0, $^{3}J_2$ = 7.0, ^{4}J = 1.0), 7.36 (C7"H, dt, ^{3}J = 8.0, ^{4}J = ^{5}J = 1.0), 3.79 (OCH_3, s), 9.92 (N1′H, bs), 10.09 (N1"H, bs).

*4.1.5.8. 1-{2-[4-(5-fluoro-1H-indol-3-yl)piperidin-1-yl]ethyl}-3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**4h**).* The title compound was isolated as a light brown solid. Yield: 57.2% (0.14 g); m.p. 203–204 °C; ESI-HRMS *m/z* calcd for $C_{28}H_{29}FN_4O_3Na$ (M + Na)$^+$ 511.2132, found: 511.2121; ^{1}H NMR (500 MHz, acetone–D_6): δ 4.38 (C3H, dd, $^{3}J_1$ = 9.0, $^{3}J_2$ = 4.5), 2.81 (C4H(1), dd, ^{2}J = 18.0, ^{3}J = 4.5), 3.32 (C4H(2), dd, ^{2}J = 18.0, ^{3}J = 9.5), 7.33 (C2′H, d, ^{3}J = 2.5), 7.04 (C4′H, d, ^{4}J = 2.5), 6.87 (C6′H, td, $^{3}J_{H-F}$ = 2.5, ^{3}J = 9.0), 7.28 (C7′H, dd, ^{3}J = 10.0, ^{4}J = 2.0), 3.72 (C1^{x}H$_2$, t, ^{3}J = 6.5), 2.61 (C2^{x}H$_2$, ps), 3.09 (CaH(E), CeH(E), m), 2.19 (CaH(A), CeH(A), m), 1.97 (CbH(E), CdH(E), m), 1.70 (CbH(A), CdH(A), m), 2.77 (CcH, m*), 7.13 (C2"H, pd, ^{3}J = 2.0), 7.26 (C4"H, d, $^{3}J_{H-F}$ = 9.0), 6.80 (C6"H, dd, ^{3}J = 9.0, ^{4}J = 2.5), 7.35 (C7"H, dd, ^{3}J = 9.0, $^{4}J_{H-F}$ = 4.5), 3.79 (OCH_3', s), 10.03 (N1′H, bs), 10.08 (N1"H, bs); ^{13}C NMR (125 MHz, acetone–D_6): δ 178.8 (C2), 38.8 (C3), 37.3 (C4), 177.0 (C5), 123.2 (C2'), 112.8 (C3'), 127.7 (C3′a), 101.6 (C4'), 155.0 (C5'), 112.6 (C6') 113.1 (C7'), 133.0 (C7′a), 36.9 (C1^x), 56.2 (C2^x), 55.0 (Ca), 33.9 (Cb), 34.3 (Cc), 33.9 (Cd), 55.0 (Ce), 124.2 (C2"), 113.0 (C3", d*, ^{4}J = 9.7), 127.9 (C3"a, d*), 104.0 (C4", d*, ^{2}J = 23.4), 158.0 (C5", d*, ^{1}J = 230.2), 110.0 (C6", d*, ^{2}J = 26.1), 113.1 (C7", d*, ^{3}J = 6.2), 134.3 (C7"a, d*, ^{4}J = 18.5), 56.0 (C5′-OCH_3).

*4.1.5.9. 1-{2-[4-(5-methoxy-1H-indol-3-yl)piperidin-1-yl]ethyl}-3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**4i**).* The title compound was isolated as a light brown solid. Yield: 84.0% (0.21 g); m.p. 178–182 °C; ESI-HRMS *m/z* calcd for $C_{29}H_{32}N_4O_4Na$ (M + Na)$^+$ 523.2310, found: 523.2321; ^{1}H NMR (500 MHz, acetone–D_6): δ 4.38 (C3H, dd, $^{3}J_1$ = 9.0, $^{3}J_2$ = 4.0), 2.79 (C4H(1), dd, ^{2}J = 18.0, ^{3}J = 4.5), 3.32 (C4H(2), dd, ^{2}J = 18.0, ^{4}J = 9.0), 7.33 (C2′H, d, ^{3}J = 2.5), 6,81 (C4′H, d, ^{4}J = 2.5), 6.80 (C6′H, dd, ^{3}J = 8.5, ^{4}J = 2.5), 7.31 (C7′H, dd, ^{3}J = 8.5, ^{5}J = 0.5), 3.71 (C1^{x}H$_2$, t, ^{3}J = 7.0), 2.60 (C2^{x}H$_2$, t, ^{3}J = 7.0), 3.10 (CaH(E), CeH(E), pd), 2.17 (CaH(A), CeH(A), m), 1.98 (CbH(E), CdH(E), m), 1.71 (CbH(A), CdH(A), pk), 2.77 (CcH, tt, $^{3}J_{A-A}$ = 12.0, $^{3}J_{A-E}$ = 4.0), 7.02 (C2"H, d, ^{3}J = 2.0), 7.04 (C4"H, d, ^{3}J = 2.5), 6.74 (C6"H, dd, ^{3}J = 8.5, ^{4}J = 2.5), 7.25 (C7"H, dd, ^{3}J = 8.5, ^{5}J = 0.5), 3.78 (OCH_3', s), 3.79 (OCH_3", s), 9.77 (N1′H, bs), 10.08 (N1"H, bs).

*4.1.5.10. 1-{4-[4-(1H-indol-3-yl)piperidin-1-yl]butyl}-3-(5-fluoro-1H-indol-3-yl)pyrrolidine-2,5-dione (**4j**).* The title compound was isolated as a light yellow solid. Yield: 42.0% (0.1 g); m.p. 122–130 °C; ESI-HRMS *m/z* calcd for $C_{29}H_{31}FN_4O_2H$ (M + H)$^+$ 487.2509, found: 487.2499; ^{1}H NMR (500 MHz, acetone–D_6): δ 4.55 (C3H, dd, $^{3}J_1$ = 9.5, $^{3}J_2$ = 5.0), 2.90 (C4H(1), dd, ^{2}J = 18.0, ^{3}J = 5.0), 3.38 (C4H(2), dd, ^{2}J = 18.00, ^{3}J = 9.5), 7.50 (C2′H, d, ^{3}J = 2.5), 7.30 (C4′H, dd, $^{3}J_{H-F}$ = 10.0, ^{4}J = 2.5), 6.93 (C6′H, td, ^{3}J = 9.0, ^{4}J = 2.5), 7.41 (C7′H, dd, ^{3}J = 9.0, $^{4}J_{H-F}$ = 4.5), 3.60 (C1^{x}H$_2$, t, ^{3}J = 7.0), 1.95 (C2^{x}H$_2$, m), 1.71 (C3^{x}H$_2$, q, ^{3}J = 7.0), 3.12 (C4^{x}H$_2$, t, ^{3}J = 8.0), 3.55 (CaH(E), CeH(E), m), 3.07 (CaH (A), Ce(A), m), 2.47 (CbH(E), CdH(E), m) 2.16 (CbH(A), Cd(A), pd) 3.22 (CcH, tt, $^{3}J_{A-A}$ = 12.0, $^{3}J_{A-E}$ = 3.5), 7.14 (C2"H, d, ^{3}J = 2.0), 7.74 (C4"H, d, ^{3}J = 8.0), 7.00 (C5"H, m, $^{3}J_1$ = 8.0, $^{3}J_2$ = 7.0, ^{4}J = 1.0), 7.10 (C6"H, m, $^{3}J_1$ = 8.0, $^{3}J_2$ = 7.0, ^{4}J = 1.5), 7.41 (C7"H, dt, ^{3}J = 8.0, ^{4}J = ^{5}J = 1.0), 10.08 (N1′H, bs), 10.41 (N1"H, bs); ^{13}C NMR (50 MHz, acetone–D_6): δ 179.1 (C2), 38.2 (C3), 36.9 (C4), 177.1 (C5), 125.8 (C2′), 112.7 (C3′, d*), 127.9 (C3′a, d*), 104.5 (C4′, d*, ^{2}J = 23.8), 158.4 (C5′, d*, ^{1}J = 232.4), 110.7 (C6′, d*, ^{2}J = 26.5), 113.5 (C7′, d*, ^{3}J = 9.9), 134.4 (C7′a), 38.8 (C1^x), 25.6 (C2^x), 21.8 (C3^x), 57.0 (C4^x), 53.5 (Ca, Ce), 30.4 (Cb, Cd), 32.3 (Cc), 119.8 (C2"), 119.1 (C3"), 127.3 (C3"a), 119.5 (C4"), 121.6 (C5"), 122.2 (C6"), 112.4 (C7"), 137.8 (C7"a).

*4.1.5.11. 1-{4-[4-(5-fluoro-1H-indol-3-yl)piperidin-1-yl]butyl}-3-(5-fluoro-1H-indol-3-yl)pyrrolidine-2,5-dione (**4k**).* The title compound was isolated as a light yellow solid. Yield: 68.0% (0.17 g); m.p. 120–134 °C (melts with decomposition); ESI-HRMS *m/z* calcd for $C_{29}H_{30}N_4O_2F_2H$ (M + H)$^+$ 505.2415, found: 505.2392; ^{1}H NMR (500 MHz, acetone–D_6): δ 4.44 (C3H, 4d, $^{3}J_1$ = 10.0, $^{3}J_2$ = 4.5, ^{4}J = 0.5), 2.87 (C4H(1), dd, ^{2}J = 18.0, ^{3}J = 5.0), 3.33 (C4H(2), dd, ^{2}J = 18.00, ^{3}J = 9.5), 7.44 (C2′H, d, ^{3}J = 2.5), 7.26 (C4′H, dd, $^{3}J_{H-F}$ = 10.0, ^{4}J = 2.5), 6.93 (C6′H, td, ^{3}J = 9.5, ^{4}J = 2.5), 7.42 (C7′H, 4d, ^{3}J = 9.0, $^{4}J_{H-F}$ = 4.5, ^{5}J = 0.5), 3.58 (C1^{x}H$_2$, t, ^{3}J = 7.0), 1.68 (C2^{x}H$_2$, m), 1.61 (C3^{x}H$_2$, m) 2.54 (C4^{x}H$_2$, t, ^{3}J = 7.0), 3.10 (CaH(E), CeH(E), pd), 2.29 (CaH (A), Ce(A), pt), 2.00 (CbH(E), CdH(E), pd), 1.88 (CbH(A), Cd(A), m), 2.83 (CcH, tt, $^{3}J_{A-A}$ = 12.0, $^{3}J_{A-E}$ = 3.5), 7.18 (C2"H, d, ^{3}J = 2.5), 7.32 (C4"H, dd, $^{3}J_{H-F}$ = 10.5, ^{4}J = 2.5), 6.87 (C6"H, td, $^{3}J_1$ = 9.0, ^{4}J = 2.5), 7.36 (C7"H, 4d, ^{3}J = 9.0, $^{4}J_{H-F}$ = 5.0, ^{5}J = 0.5), 10.14 (N1′H, bs), 10.43 (N1"H, bs); ^{13}C NMR (125 MHz, acetone–D_6): δ 178.8 (C2), 38.7 (C3), 36.8 (C4), 177.0 (C5), 125.7 (C2′), 112.9 (C3′, d*, ^{4}J = 4.4), 127.7 (C3′a, d*, ^{3}J = 9.8), 104.4 (C4′, d*, ^{2}J = 23.4), 158.4 (C5′, d*, ^{1}J = 232.5), 110.7 (C6′, d*, ^{2}J = 26.3), 113.5 (C7′, d*, ^{3}J = 9.7), 134.5 (C7′a), 39.0 (C1^x), 26.3 (C2^x), 24.3 (C3^x), 58.5 (C4^x), 54.7 (Ca, Ce), 33.1, 33.1 (Cb, Cd), 34.0 (Cc), 123.3 (C2"), 121.2 (C3", d*, ^{4}J = 4.9), 127.9 (C3"a, d*, ^{3}J = 9.8), 104.2 (C4" d*, ^{2}J = 23.4), 158.1 (C5", d*, ^{1}J = 231.4), 110.0 (C6", d*, ^{2}J = 26.3), 113.0 (C7", d*, ^{3}J = 9.8), 134.4 (C7"a).

*4.1.5.12. 1-{4-[4-(5-methoxy-1H-indol-3-yl)piperidin-1-yl]butyl}-3-(5-fluoro-1H-indol-3-yl)pyrrolidine-2,5-dione (**4l**).* The title compound was isolated as a light yellow solid. Yield: 64.0% (0. 16 g); m.p. 80–85 °C; ESI-HRMS *m/z* calcd for $C_{30}H_{33}N_4O_3FH$ (M + H)$^+$ 517.2615, found: 517.2630; ^{1}H NMR (500 MHz, acetone–D_6): δ 4.46

(C3H, dd, $^{3}J_1 = 9.0$, $^{3}J_2 = 5.0$), 2.88 (C4H(1), dd, $^{2}J = 17.5$, $^{3}J = 5.0$), 3.34 (C4H(2), dd, $^{2}J = 17.5$, $^{3}J = 9.0$), 7.46 (C2′H, d, $^{3}J = 2.5$), 7.27 (C4′H, dd, $^{3}J_{H-F} = 10.0$, $^{4}J = 3.0$), 6.93 (C6′H, td, $^{3}J = 9.0$, $^{4}J = 2.5$), 7.42 (C7′H, dd, $^{3}J = 9.0$, $^{4}J_{H-F} = 4.0$, $^{5}J = 0.5$), 3.58 (C1^{x}H$_2$, t, $^{3}J = 6.0$), 1.69 (C2^{x}H$_2$, C3^{x}H$_2$, m), 2.65 (C4^{x}H$_2$, bs) 3.19 (CaH(E), CeH(E), pd), 2.44 (CaH(A), Ce(A), bs), ~2.0** (CbH(E), CdH(E), CbH(A), CdH(A)), 2.98 (CcH, m), 7.07 (C2"H, d, $^{3}J = 2.0$), 7.16 (C4"H, d, $^{4}J = 2.5$), 6.75 (C6"H, dd, $^{3}J = 8.5$, $^{4}J = 2.5$), 7.27 (C7"H, d, $^{3}J = 8.0$), 3.81 (OCH$_3$, s), 9.84 (N1′H, bs), 10.38 (N1"H, bs); ^{13}C NMR (125 MHz, acetone−D$_6$): δ 178.8 (C2), 38.7 (C3), 36.8 (C4), 177.0 (C5), 125.7 (C2′), 112.9 (C3′, d*, $^{4}J = 4.8$), 127.8 (C3′a, d*, $^{3}J = 9.7$), 104.4 (C4′, d*, $^{2}J = 24.0$), 158.4 (C5′, d*, $^{1}J = 232.4$), 110.7 (C6′, d*, $^{2}J = 26.4$), 113.5 (C7′, d*, $^{3}J = 9.7$), 134.5 (C7′a), 38.8 (C1^{x}), 26.2 (C2^{x}), 23.8 (C3^{x}),58.25 (C4^{x}), 54.6 (Ca, Ce), 23.6 (Cb, Cd), 33.9 (Cc), 127.9 (C2"), 120.4 (C3"), 122.0 (C3"a), 101.6 (C4"),154.6 (C5"), 112.8 (C6"), 112.4 (C7"), 133.0 (C7"a), 56.2 (OCH$_3$).

*4.1.5.13. 1-{3-[4-(1H-indol-3-yl)piperidin-1-yl]propyl}-3-(5-fluoro-1H-indol-3-yl)pyrrolidine-2,5-dione (**4m**).* The title compound was isolated as a light yellow solid. Yield: 51.9% (0.13 g); m.p. 124−132 °C; ESI-HRMS *m/z* calcd for $C_{28}H_{29}N_4O_2FH$ (M + H)$^{+}$ 473.2353, found: 473.2358; ^{1}H NMR (500 MHz, acetone−D$_6$): δ 4.42 (C3H, dd, $^{3}J_1 = 9.5$, $^{3}J_2 = 5.0$), 2.87 (C4H(1), dd, $^{2}J = 18.0$, $^{3}J = 5.0$), 3.33 (C4H(2), dd, $^{2}J = 18.0$, $^{3}J = 9.5$), 7.44 (C2′H, d, $^{3}J = 2.5$), 7.26 (C4′H, dd, $^{3}J_{H-F} = 10.0$, $^{4}J = 2.5$), 6.94 (C6′H, td, $^{3}J = 9.0$, $^{4}J = 2.5$),7.42 (C7′H, dd, $^{3}J = 9.0$, $^{4}J_{H-F} = 5.0$), 3.64 (C1^{x}H$_2$, t, $^{3}J = 7.0$), 1.87 (C2^{x}H$_2$, CbH(A), CdH(A), m), 2.50 (C3^{x}H$_2$, bs), 3.08 (CaH(E), CeH(E), pd), 2.19 (CaH(A), Ce(A), bs), 2.02 (CbH(E), CdH(E), pd), 2.84 (CcH, m), 7.07 (C2"H, d, $^{3}J = 1.5$), 7.61 (C4"H, d, $^{3}J = 8.0$), 6.98 (C5"H, m, $^{3}J_1 = 8.0$, $^{3}J_2 = 6.0$, $^{4}J = 1.0$), 7.07 (C6"H, m, $^{3}J_1 = 8.5$, $^{3}J_2 = 6.0$, $^{4}J = 1.0$), 7.37 (C7"H, d, $^{3}J = 8.0$), 9.95 (N1′H, bs), 10.36 (N1"H, bs); ^{13}C NMR (125 MHz, acetone−D$_6$): δ 178.7 (C2), 38.7 (C3), 36.8 (C4), 176.9 (C5), 125.7 (C2′), 112.9 (C3′, d*, $^{4}J = 4.9$), 127.7 (C3′a, d*, $^{3}J = 10.3$), 104.4 (C4′, d*, $^{2}J = 23.4$), 158.4 (C5′, d*, $^{1}J = 232.4$), 110.7 (C6′, sd*, $^{2}J = 26.4$), 113.5 (C7′, d*, $^{3}J = 9.7$), 134.5 (C7′a), 37.8 (C1^{x}), 25.6 (C2^{x}), 56.8 (C3^{x}), 54.9 (Ca, Ce), 33.7 (Cb, Cd), 34.4 (Cc), 119.6 (C2", C5"), 121.0 (C3"), 127.7 (C3"a), 119.2 (C4"), 122.0 (C6"), 112.2 (C7"), 137.9 (C7"a).

*4.1.5.14. 1-{3-[4-(5-fluoro-1H-indol-3-yl)piperidin-1-yl]propyl}-3-(5-fluoro-1H-indol-3-yl)pyrrolidine-2,5-dione (**4n**).* The title compound was isolated as a yellow solid. Yield: 47.3% (0.12 g); m.p. 112−140 °C (melts with decomposition); ESI-HRMS *m/z* calcd for $C_{28}H_{28}N_4O_2F_2H$ (M + H)$^{+}$ 491.2259, found: 491.2260; ^{1}H NMR (500 MHz, acetone−D$_6$): δ 4.41 (C3H, 4d, $^{3}J_1 = 9.5$, $^{3}J_2 = 5.0$, $^{4}J = 0.5$), 2.86 (C4H(1), dd, $^{2}J = 18.0$, $^{3}J = 5.0$), 3.33 (C4H(2), dd, $^{2}J = 18.00$, $^{3}J = 9.5$), 7.43 (C2′H, d, $^{3}J = 2.0$), 7.25 (C4′H, dd, $^{3}J_{H-F} = 10.0$, $^{4}J = 2.5$), 6.94 (C6′H, td, $^{3}J = 9.5$, $^{4}J = 2.5$), 7.42 (C7′H, dd, $^{3}J = 9.0$, $^{4}J_{H-F} = 4.5$), 3.63 (C1^{x}H$_2$, t, $^{3}J = 7.0$), 1.84 (C2^{x}H$_2$, m), 2.45 (C3^{x}H$_2$, t, $^{3}J = 7.0$), 3.04 (CaH(E), CeH(E), m), 2.12 (CaH(A), Ce(A), m), 1.79 (CbH(E), CdH(E), m), 1.76 (CbH(A), Cd(A), m), 2.78 (CcH, tt, $^{3}J_{A-A} = 12.0$, $^{3}J_{A-E} = 4.0$), 7.15 (C2"H, d, $^{3}J = 2.0$), 7.29 (C4"H, dd, $^{3}J_{H-F} = 10.0$, $^{4}J = 2.5$), 6.87 (C6"H, td, $^{3}J_1 = 9.5$, $^{4}J = 2.5$), 7.35 (C7"H, dd, $^{3}J = 9.0$, $^{4}J_{H-F} = 4.5$), 10.08 (N1′H, bs), 10.39 (N1"H, bs); ^{13}C NMR (125 MHz, acetone−D$_6$): δ 178.8 (C2), 37.9 (C3), 36.8 (C4), 177.0 (C5), 125.7 (C2′), 112.9 (C3′, d*, $^{4}J = 4.9$), 127.7 (C3′a, d*, $^{3}J = 9.7$), 104.4 (C4′, d*, $^{2}J = 27.3$), 158.4 (C5′, d*, $^{1}J = 232.5$), 110.7 (C6′, d*, $^{2}J = 26.3$), 113.5 (C7′, d*, $^{3}J = 9.7$), 134.5 (C7′a), 38.7 (C1^{x}), 25.8 (C2^{x}), 56.9 (C3^{x}), 55.0 (Ca, Ce), 33.8, 33.7 (Cb, Cd), 34.3 (Cc), 123.2 (C2"), 121.5 (C3", d*, $^{4}J = 4.9$), 128.0 (C3"a, d*, $^{3}J = 9.8$), 104.2 (C4", d*, $^{2}J = 26.8$),158.1 (C5", d*, $^{1}J = 231.5$), 110.0 (C6", d*, $^{2}J = 26.4$), 113.0 (C7", d*, $^{3}J = 9.2$), 134.4 (C7"a).

*4.1.5.15. 1-{3-[4-(5-methoxy-1H-indol-3-yl)piperidin-1-yl]propyl}-3-(5-fluoro-1H-indol-3-yl)pyrrolidine-2,5-dione (**4o**).* The title compound was isolated as a yellow solid. Yield: 55.7% (0.13 g); m.p. 123−126 °C; ESI-HRMS *m/z* calcd for $C_{29}H_{31}N_4O_3FH$ (M + H)$^{+}$ 503.2458, found: 503.2440; ^{1}H NMR (500 MHz, acetone−D$_6$): δ 4.42 (C3H, dd, $^{3}J_1 = 9.5$, $^{3}J_2 = 5.0$), 2.86 (C4H(1), dd, $^{2}J = 18.0$, $^{3}J = 5.0$), 3.33 (C4H(2), dd, $^{2}J = 18.0$, $^{3}J = 9.5$), 7.45 (C2′H, d, $^{3}J = 2.0$), 7.26 (C4′H, dd, $^{3}J_{H-F} = 10.0$, $^{4}J = 2.5$), 6.94 (C6′H, td, $^{3}J = 9.5$, $^{4}J = 2.5$), 7.42 (C7′H, dd, $^{3}J = 9.0$, $^{4}J_{H-F} = 4.5$), 3.64 (C1^{x}H$_2$, t, $^{3}J = 7.0$), 1.88 (C2^{x}H$_2$, CbH(A), CdH(A), m), 2.52 (C3^{x}H$_2$, bs), 3.09 (CaH(E), CeH(E), pd), 2.21 (CaH(A), Ce(A), bs), 2.01 (CbH(E), CdH(E), pd), 2.82 (CcH, m), 7.04 (C2"H, d, $^{3}J = 2.0$), 7.12 (C4"H, d, $^{4}J = 2.0$), 6.74 (C6"H, dd, $^{3}J = 9.0$, $^{4}J = 2.0$), 7.25 (C7"H, dd, $^{3}J = 8.5$, $^{5}J = 0.5$), 3.80 (OCH$_3$, s), 9.79 (N1′H, bs), 10.35 (N1"H, bs); ^{13}C NMR (125 MHz, acetone−D$_6$): δ 178.7 (C2), 38.7 (C3), 36.8 (C4), 176.9 (C5), 125.7 (C2′), 112.9 (C3′, d*, $^{4}J = 4.4$), 127.7 (C3′a, d*, $^{3}J = 9.7$), 104.4 (C4′, d*, $^{2}J = 23.4$), 158.4 (C5′, d*, $^{1}J = 232.5$), 110.7 (C6′, d*, $^{2}J = 26.4$), 113.5 (C7′, d*, $^{3}J = 9.7$), 134.5 (C7′a), 37.8 (C1^{x}), 25.6 (C2^{x}), 56.7 (C3^{x}), 54.9 (Ca, Ce), 33.5 (Cb, Cd), 34.3 (Cc), 128.0 (C2"), 120.9 (C3"), 121.8 (C3"a), 101.6 (C4"), 154.5 (C5"), 112.8 (C6"), 112.3 (C7"), 133.0 (C7"a), 56.0 (OCH$_3$).

*4.1.5.16. 1-{2-[4-(1H-indol-3-yl)piperidin-1-yl]ethyl}-3-(5-fluoro-1H-indol-3-yl)pyrrolidine-2,5-dione (**4p**).* The title compound was isolated as a light yellow solid. Yield: 38.2% (0.13 g); m.p. 123−124 °C; ESI-HRMS *m/z* calcd for $C_{27}H_{27}N_4O_2FNa$ (M + Na)$^{+}$ 481.2029, found: 481.2016; ^{1}H NMR (500 MHz, acetone−D$_6$): δ 4.39 (C3H, dd, $^{3}J_1 = 9.5$, $^{3}J_2 = 5.0$), 2.85-2.78 (C4H(1), CcH, m, $^{2}J = 18.0$, $^{3}J = 5.5$), 3.30 (C4H(2), dd, $^{2}J = 17.5$, $^{4}J = 9.5$), 7.46 (C2′H, d, $^{3}J = 2.0$), 7.29 (C4′H, dd, $^{3}J_{H-F} = 9.5$, $^{4}J = 2.5$), 6.93 (C6′H, td, $^{3}J = 9.0$, $^{4}J = 2.5$), 7.41 (C7′H, dd, $^{3}J = 9.0$, $^{4}J_{H-F} = 4.5$), 4.39 (C1^{x}H$_2$, m), 2.18 (C2^{x}H$_2$, m), 3.12 (CaH(E), CeH(E), m), 2.18 (CaH(A), CeH(A), m), 1.98 (CbH(E), CdH(E), m), 1.73 (CbH(A), CdH(A), m), 7.04 (C2"H, d, $^{2}J = 2.0$), 7.60 (C4"H, d, $^{3}J = 8.0$), 6.97 (C5"H, m), 7.07 (C6"H, m), 7.36 (C7"H, d, $^{3}J_{H-F} = 9.5$, $^{4}J = 2.5$), 9.91 (N1′H, bs), 10.34 (N1"H, bs); ^{13}C NMR (125 MHz, acetone−D$_6$): δ 178.7 (C2), 37.1 (C3), 36.9 (C4), 176.8 (C5), 125.9 (C2'), 113.3 (C3', d, $^{3}J = 4.8$), 127.5 (C3′a, d, $^{3}J = 9.6$), 104.4 (C4'), 158.5 (C5'), 110.7 (C6', d, $^{3}J = 26.5$) 113.5 (C7', $^{3}J = 9.6$), 134.5 (C7′a), 38.7 (C1^{x}), 56.2 (C2^{x}), 55.1 (Ca, Ce), 34.0 (Cb Cd), 34.5 (Cc), 121.0 (C2"), 121.4 (C3"), 127.8 (C3"a), 104.4 (C4", d, $^{3}J = 23.4$), 119.6 (C5"), 122.0 (C6"), 112.7 (C7"), 137.9 (C7"a), 56.2 (C5"-OCH$_3$).

*4.1.5.17. 1-{2-[4-(5-fluoro-1H-indol-3-yl)piperidin-1-yl]ethyl}-3-(5-fluoro-1H-indol-3-yl)pyrrolidine-2,5-dione (**4q**).* The title compound was isolated as a light yellow solid. Yield: 56.5% (0.20 g). Obtained from double amount of starting compounds.; m.p. 108−115 °C; ESI-HRMS *m/z* calcd for $C_{27}H_{26}N_4O_2F_2H$ (M + H)$^{+}$ 477.2102, found: 477.2091; ^{1}H NMR (500 MHz, acetone−D$_6$): δ 4.39 (C3H, dd, $^{3}J_1 = 9.5$, $^{3}J_2 = 5.5$), 2.83 (C4H(1), dd, $^{2}J = 18.0$, $^{3}J = 5.5$), 3.30 (C4H(2), dd, $^{2}J = 18.0$, $^{4}J = 9.5$), 7.45 (C2′H, d, $^{3}J = 2.0$), 7.29−7.26 (C4′H, C4", m), 6.92 (C6′H, td, $^{3}J = 9.0$, $^{4}J = 2.5$), 7.41 (C7′H, dd, $^{3}J = 8.5$, $^{4}J_{H-F} = 4.0$), 3.72 (C1^{x}H$_2$, m), 2.62 (C2^{x}H$_2$, m), 3.13 (CaH(E), CeH(E), m), 2.18 (CaH(A), CeH(A), m), 1.97 (CbH(E), CdH(E), m), 1.70 (CbH(A), CdH(A), pk), 2.77 (CcH, tt, $^{3}J_{A-A} = 12.0$, $^{3}J_{A-E} = 3.5$), 7.12 (C2"H, d, $^{3}J = 2.0$), 6.87 (C6"H, td, $^{3}J = 9.0$, $^{4}J = 2.5$), 7.35 (C7"H, dd, $^{3}J_{H-F} = 8.5$, $^{4}J = 4.5$), 3.78 (OCH$_3$', s), 8.74 (N1′H, bs), 10.34 (N1"H, bs); ^{13}C NMR (125 MHz, acetone−D$_6$): δ 178.7 (C2), 37.0 (C3), 36.9 (C4), 176.8 (C5), 125.9 (C2'), 113.2 (C3'), 127.4 (C3′a), 104.4 (C4'), 158.4 (C5'), 110.7 (C6') 113.5 (C7'), 134.5 (C7′a), 38.7 (C1^{x}), 56.2 (C2^{x}), 54.9 (Ca, Ce), 34.0 (Cb), 33.8 (Cc, Cd), 123.2 (C2"), 121.6 (C3"), 128.1 (C3"a), 104.2 (C4"), 158.1 (C5"), 110.0 (C6"), 112.9 (C7"), 134.4 (C7"a).

*4.1.5.18. 1-{2-[4-(5-methoxy-1H-indol-3-yl)piperidin-1-yl]ethyl}-3-(5-fluoro-1H-indol-3-yl)pyrrolidine-2,5-dione (**4r**).* The title compound was isolated as a yellow solid. Yield: 63.4% (0.23 g). Obtained from double amount of starting compounds.; m.p. 132−140 °C; ESI-HRMS *m/z* calcd for $C_{28}H_{29}N_4O_3FH$ (M + H)$^{+}$ 489.2302, found:

489.2318; ^{1}H NMR (500 MHz, acetone−D_6): δ 4.40 (C3H, dd, $^3J_1 = 9.5$, $^3J_2 = 5.0$), 2.82 (C4H(1), dd, $^2J = 18.0$, $^3J = 4.5$), 3.30 (C4H(2), dd, $^2J = 18.0$, $^4J = 9.5$), 7.46 (C2′H, d, $^3J = 2.0$), 6,93 (C6′H, td, $^3J = 9.0$, $^4J = 2.5$), 7.41 (C7′H, dd, $^3J = 9.0$, $^4J_{H\text{-}F} = 4.5$), 3.71 (C1^{x}H$_2$, m), 2.61 (C2^{x}H$_2$, m), 3.11 (CaH(E), CeH(E), m), 2.17 (CaH(A), CeH(A), m), 2.00 - 1.96 (CbH(E), CdH(E), m), 1.71 (CbH(A), CdH(A), m), 2.77 (CcH, tt, $^3J_{A\text{-}A} = 12.0$, $^3J_{A\text{-}E} = 3.5$), 7.01 (C2''H, d, $^3J = 2.5$), 7.08 (C4''H, d, $^4J = 2.0$), 6.74 (C6''H, dd, $^3J = 8.5$, $^4J = 2.0$), 7.25 (C7''H, d, $^3J = 9.0$), 9.77 (OCH$_3$, s), 9.76 (N1′H, bs), 10.34 (N1''H, bs); ^{13}C NMR (125 MHz, acetone−D_6): δ 178.7 (C2), 37.1 (C3), 36.9 (C4), 158.5 (C5), 125.9 (C2'), 113.4 (C3'), 127.5 (C3′a), 104.4 (C4'), 158.5 (C5'), 110.7 (C6') 113.5 (C7', $^3J = 9.7$), 134.5 (C7′a), 38.7 (C1^x), 55.9 (C2^x), 55.3 (Ca), 34.0 (Cb), 34.4 (Cc), 33.9 (Cd), 55.0 (Ce), 121.7 (C2''), 121.2 (C3''), 128.1 (C3''a), 101.6 (C4''), 154.5 (C5'', d*, $^1J = 230.2$), 112.2 (C6''), 112.7 (C7''), 133.0 (C7''a), 56.2 (C5''-OCH$_3$).

*4.1.6. General procedure for the synthesis of derivatives 3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**6a**–**d**)*

A mixture of appropriate derivatives of 1-(4-bromobutyl)-3-(5-methoxy-1*H*-indol-3-yl)pyrrolidine-2,5-dione (**2a**) or 1-(4-bromobutyl)-3-(1*H*-indol-3-yl)pyrrolidine-2,5-dione (**2g**) (0.002 mol), the appropriate derivatives of 5-substitued-3-(piperidin-3-yl)-1H-indoles hydrochloride (**5a**–**c**) (0.002 mol) and K_2CO_3 (0.010 mol) and 50 mL acetonitrile were stirred and refluxed for 5−7 h. Reaction 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 *v/v*) as an eluent. Proper fractions were identified by TLC and evaporated to dryness giving analytically pure compounds **6a**–**d**.

*4.1.6.1. 1-{4-[3-(1H-indol-3-yl)piperidin-1-yl]butyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**6a**).* The title compound was isolated as a yellow solid. Yield: 44.9% (0.42 g); m.p. 98−105 °C; ESI-HRMS *m/z* calcd for $C_{29}H_{32}N_4O_2H$ (M + H)$^+$ 469.2604, found: 469.2593; ^{1}H NMR (500 MHz, CD_3OD): δ 7.55 (C7"H, m$^\#$), 7.31−7.36 (C4′H, C7′H, C4"H, m$^\#$), 7.13 (C2"H, d, $^3J = 4.5$), 7.05−7.10 (C6′H, C6"H, m$^\#$), 6.93−7.01 (C2′H, C5′H, C5"H, m$^\#$), 4.23 (C3H, dd, $^3J_1 = 9.5$, $^3J_2 = 4.5$), 3.55 (C1^{x}H$_2$, m), 3.07−3.22 (CaH(E), CbH, C4H(2), m$^\#$), 2.96 (CeH(E), pd), 2.80 (C4H(1), 4d, $^2J = 13.5$, $^3J = 4.5$), 2.40−2.46 (C4^{x}H$_2$, m$^\#$), 1.99−2.12 (CaH(A), CeH(A), CcH(E), m), 1.68−1.80 (CdH$_2$, m$^\#$), 1.60 (C2^{x}H$_2$, m$^\#$), 1.45−1.56 (C3^{x}H$_2$, CcH(A), m); ^{13}C NMR (125 MHz, CD_3OD): δ 180.9 (C2, s), 178.8 (C5, s), 138.4 (C7′a, s), 138.1 (C7"a, 2s#), 127.8 (C3"a, s), 127.0 (C3′a, s), 124.0 (C2", 2s$^\#$), 123.0 and 123.0 (C2′, 2s$^\#$), 122.4 (C6", s), 121.7 and 121.7 (C3", 2s$^\#$), 120.3 (C6′, s), 119.6 and 119.6 (C5", 2s$^\#$), 119.4 (C4", s), 119.2 (C5′, s), 118.6 and 118.6 (C4′, 2s$^\#$), 112.8 (C7", s), 112.4 (C7′, s), 112.1 and 112.0 (C3′, 2s$^\#$), 61.5 and 61.4 (Ca, 2s$^\#$), 59.2 (C4^x, s), 54.7 (Ce, s), 39.4 (C1^x, s), 39.4 (C3, s), 37.3 (C4, s), 34.7 (Cb, s), 32.0 and 32.0 (Cc, 2s$^\#$), 26.7 (Cd, 2s$^\#$), 26.1 (C2^x, s), 24.3 and 24.2 (C3^x, 2s$^\#$).

$^\#$ - NMR spectra indicate that the sample contains two different stereoisomers, hence the doubling of signals and appearance of complex multiplets.

*4.1.6.2. 1-{4-[3-(5-fluoro-1H-indol-3-yl)piperidin-1-yl]butyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**6b**).* The title compound was isolated as a yellow solid. Yield: 49.2% (0.48 g); m.p. 94−120 °C (melts with decomposition); ESI-HRMS *m/z* calcd for $C_{29}H_{31}N_4O_2FH$ (M + H)$^+$ 487.2484, found: 487.2498; ^{1}H NMR (500 MHz, CD_3OD): δ 7.32−7.37 (C4′H, C7′H, m), 7.29 (C7"H, dd, $^3J = 9.0$, $^4J_{H\text{-}F} = 2.0$), 7.21−7.26 (C4"H, m, $^3J_{H\text{-}F} = 10.0$, $^4J = 3.5$, $^5J = 2.0$), 7.17 (C2"H, d, $^3J = 2.5$), 7.07 (C2′H, C6′H, m$^\#$), 6.96 (C5′H, m$^\#$), 6.86 (C6"H, m$^\#$), 4.25−4.31 (C3H, m$^\#$), 3.55−3.60 (C1^{x}H$_2$, m$^\#$), 3.14−3.27 (C4H(2), CaH(E), m$^\#$), 3.04−3.13 (CbH, CeH(E), m$^\#$), 2.83 (C4H(1), m$^\#$), 2.55−2.65 (C4^{x}H$_2$, m$^\#$), 2.29 (CaH(A), t, $^2J = {}^3J_{A\text{-}A} = 11.5$), 2.22 (CeH(A), td, $^2J = {}^3J_{A\text{-}A} = 11.5$, $^3J_{A\text{-}E} = 4.0$), 2.02 (CcH(E), pd), 1.72−1.85 (CdH$_2$, m), 1.47−1.66 (CeH(A), C2^{x}H$_2$, C3^{x}H$_2$, m); ^{13}C NMR (125 MHz, CD_3OD): δ 180.9 (C2, s), 178.8 (C5, s), 158.8 (C5", d, $^1J = 232.3$), 138.4 (C7′, s), 134.6 (C7"a, s), 127.9 (C3"a, d, $^3J = 9.7$), 127.0 and 127.0 (C3′a, 2s$^\#$), 124.1 and 124.0 (C2", 2d, $^5J = 1.8$ and $^5J = 1.9^\#$), 122.9 and 122.9 (C2′, 2s$^\#$), 120.3 (C6′, s), 119.2 (C4′, s), 119.2 (C5′, s), 118.0 (C3", 2d$^\#$), 113.2 (C7", d, $^4J = 9.7$), 112.8 (C7′, s), 112.0 and 112.0 (C3′, 2s$^\#$), 110.6 (C6", d, $^2J = 26.4$), 104.0 (C4", d, $^2J = 23.5$), 60.7 and 60.7 (Ca, 2s$^\#$), 58.8 (C4^x, s), 54.3 (Ce, s), 39.5 and 39.5 (C1^x, 2s$^\#$), 39.3 and 39.2 (C3^x, 2s$^\#$), 37.3 (C4, s), 34.3 (Cb, s), 31.4 and 31.4 (Cc, 2s$^\#$), 26.5 and 26.5 (Cd, 2s$^\#$), 25.6 (C2^x, s), 23.8 and 23.7 (C3^x, 2s$^\#$).

$^\#$ - NMR spectra indicate that the sample contains two different stereoisomers, hence the doubling of signals and the complex appearance of multiplets.

*4.1.6.3. 1-{4-[3-(5-methoxy-1H-indol-3-yl)piperidin-1-yl]butyl}-3-(1H-indol-3-yl)pyrrolidine-2,5-dione (**6c**).* The title compound was isolated as a yellow solid. Yield: 51.8% (0.52 g). Obtained from double amount of substrates; m.p. 88−114 °C (melts with decomposition); ESI-HRMS *m/z* calcd for $C_{30}H_{34}N_4O_3H$ (M + H)$^+$ 499.2709, found: 499.2713; ^{1}H NMR (500 MHz, CD_3OD): δ 7.30−7.36 (C4′H, C7′H, m$^\#$), 7.23 (C7"H, m$^\#$), 7.13 (C2"H, d, $^3J = 2.5$), 7.04−7.09 (C6′H, m$^\#$), 7.03 (C4"H, m$^\#$), 6.93−6.98 (C2′H, C5′H, m$^\#$), 6.76 (C6"H, m$^\#$), 4.20−4.26 (C3H, m$^\#$), 3.77 (OCH$_3$, s), 3.55 (C1^{x}H$_2$, m$^\#$), 3.19−3.25 (C4H(2), m$^\#$), 3.12−3.18 (CaH(E), m$^\#$), 3.05−3.12 (CbH, m$^\#$), 3.01 (CeH(E), pd), 2.81 (C4H(1), m$^\#$), 2.50 (C4^{x}H$_2$, m$^\#$), 2.07−2.17 (CaH(A), CeH(A), m$^\#$), 2.02 (CcH(E), pd), 1.70−1.82 (CdH$_2$, m$^\#$), 1.45−1.65 (CcH(A), C2^{x}H$_2$, C3^{x}H$_2$, m$^\#$); ^{13}C NMR (125 MHz, CD_3OD): δ 180.8 (C2, s), 178.8 (C5, s), 154.9 (C5", s), 138.4 (C7′a, s), 133.3 and 133.3 (C7"a, 2s$^\#$), 128.1 (C3"a, s), 127.0 (C3′a, 2s$^\#$), 124.0 (C2", s), 122.9 and 122.9 (C2′, 2s$^\#$), 122.6 and 122.6 (C4", 2s$^\#$), 120.3 (C6′, s), 119.2 (C4′, C5′, s), 117.9 and 117.9 (C3", 2s$^\#$), 113.1 (C6", s), 112.8 (C7", s), 112.6 (C7′, s), 112.0 and 112.0 (C3′, 2s$^\#$), 101.4 (C4", s), 61.1 and 61.1 (Ca, 2s$^\#$), 58.9 and 58.9 (C4^x, 2s$^\#$), 56.3 (OCH$_3$, s), 54.6 (Ce, s), 39.4 (C1^x, s), 39.3 and 39.3 (C3, 2s$^\#$), 37.3 (C4, s), 34.4 (Cb, s), 31.6 and 31.5 (Cc, 2s$^\#$), 26.6 and 26.6 (Cd, 2s$^\#$), 25.9 (C2^x, s), 24.0 and 24.0 (C3^x, 2s$^\#$).

$^\#$ - NMR spectra indicate that the sample contains two different stereoisomers, hence the doubling of signals and the appearance of complex multiplets.

*4.1.6.4. 1-{4-[3-(5-methoxy-1H-indol-3-yl)piperidin-1-yl]butyl}-3-(5-methoxy-1H-indol-3-yl)pyrrolidine-2,5-dione (**6d**).* The title compound was isolated as a yellow solid. Yield: 48.3% (0.55 g); m.p. 102−110 °C; ESI-HRMS *m/z* calcd for $C_{31}H_{36}N_4O_4H$ (M + H)$^+$ 529.2815, found: 529.2811; ^{1}H NMR (500 MHz, CD_3OD): δ 7.19−7.24 (C7′H, C7"H, m$^\#$), 7.10 (C2"H, d, $^3J = 3.5$), 7.02 (C4"H, m$^\#$), 6.97 (C4′H, d, $^4J = 4.5$), 6.85 (C2′H, d, $^3J = 2.0$), 6.74−6.78 (C6′H, C6"H, m$^\#$), 4.18−4.23 (C3H, m$^\#$), 3.77 and 3.77 (C5"-OCH$_3$, 2s$^\#$), 3.74 and 3.74 (C5′-OCH$_3$, 2s$^\#$), 3.54 (C1^{x}H$_2$, m$^\#$), 3.24 (C4H(2), m$^\#$), 3.16 (CaH(E), m$^\#$), 3.10 (CbH, m$^\#$), 3.05 (CeH(E), pd), 2.80 (C4H(1), m$^\#$), 2.50−2.61 (C4^{x}H$_2$, m$^\#$), 2.14−2.25 (CaH(A), CeH(A), m$^\#$), 1.71−1.83 (CcH(E), CdH(E), m$^\#$), 1.44−1.63 (CcH(A), CdH(A), C2^{x}H$_2$, C3^{x}H$_2$, m$^\#$); ^{13}C NMR (125 MHz, CD_3OD): δ 180.9 (C2, s), 178.8 (C5, s), 155.2 (C5′, s), 154.9 (C5", s), 133.6 (C7′a, s), 133.2 (C7"a, s), 128.0 (C3"a, s), 127.5 and 127.5 (C3′a, 2s$^\#$), 124.5 (C2", s), 122.6 and 122.6 (C2′, 2s$^\#$), 117.5 and 117.5 (C3", 2s$^\#$), 113.5 (C6", s), 113.1 (C7", s), 112.9 and 112.9 (C7′, 2s$^\#$), 112.7 and 112.6 (C3′, 2s$^\#$), 111.8 (C6′, s), 101.6 and

101.6 (C4", 2s[#]), 101.4 and 101.4 (C4′, 2s[#]), 60.8 and 60.8 (Ca, 2s[#]), 58.7 (C4[x], s), 56.3 (C5"-OCH_3, s), 56.3 (C5′-OCH_3, s), 54.4 and 54.3 (Ce, 2s[#]), 39.4 (C1[x], s), 39.2 and 39.1 (C3, 2s[#]), 37.1 (C4, s), 34.2 (Cb, s), 31.3 (Cc, s), 26.6 and 26.6 (Cd, 2s[#]), 25.6 (C2[x], s), 23.7 and 23.7 (C3[x], 2s[#]).

[#] - NMR spectra indicate that the sample contains two different stereoisomers, hence the doubling of signals and appearance of complex multiplets.

4.2. Biology evaluation

4.2.1. In vitro experiment

Preparation of solutions of test and reference compounds:

1 mM stock solutions of tested compounds were prepared in DMSO. Serial dilutions of compounds were prepared in 96-well microplate in assay buffers using automated pipetting system epMotion 5070 (Eppendorf). Each compound was tested in 6 concentrations from 10 to 5 to 10−10 M (final concentration).

4.2.1.1. 5-HT_{1A} binding assay. Radioligand binding was performed using membranes from CHO-K1 cells stably transfected with the human 5-HT_{1A} receptor (PerkinElmer). All assays were carried out in duplicates. 50 μl working solution of the tested compounds, 50 μl [^{3}H]-8-OH-DPAT (final concentration 1 nM) and 150 μl diluted membranes (10 μg protein per well) prepared in assay buffer (50 mM Tris, pH 7.4, 10 mM $MgSO_4$, 0,5 mM EDTA, 0.1% ascorbic acid) were transferred to polypropylene 96-well microplate using 96-wells pipetting station Rainin Liquidator (MettlerToledo). Serotonin (10 μM) was used to define nonspecific binding. Microplate was covered with a sealing tape, mixed and incubated for 60 min at 27 °C. The reaction was terminated by rapid filtration through GF/C filter mate presoaked with 0.3% polyethyleneimine for 30 min. Ten rapid washes with 200 μl 50 mM Tris buffer (4 °C, pH 7.4) were performed using automated harvester system Harvester-96 MACH III FM (Tomtec). The filter mates were dried at 37 °C in forced air fan incubator and then solid scintillator MeltiLex was melted on filter mates at 90 °C for 4 min. Radioactivity was counted in MicroBeta2 scintillation counter (PerkinElmer). Data were fitted to a one-site curve-fitting equation with Prism 6 (GraphPad Software) and K_i values were estimated from the Cheng−Prusoff equation.

4.2.1.2. SERT binding assay. Radioligand binding was performed using membranes from HEK-293 cells stably transfected with the human serotonin transporter (PerkinElmer). All assays were carried out in duplicates. 50 μl working solution of the tested compounds, 50 μl [^{3}H]-imipramine (final concentration 2 nM) and 150 μl diluted membranes (9 μg protein per well) prepared in assay buffer (50 mM Tris, pH 7.4, 5 mM KCl, 120 mM NaCl) were transferred to polypropylene 96-well microplate using 96-wells pipetting station Rainin Liquidator (MettlerToledo). Imipramine (10 μM) was used to define nonspecific binding. Microplate was covered with a sealing tape, mixed and incubated for 30 min at 27 °C. The reaction was terminated by rapid filtration through GF/C filter mate presoaked with 0.5% polyethyleneimine for 30 min. Ten rapid washes with 200 μl 50 mM Tris buffer, 154 mM NaCl (4 °C, pH 7.4) were performed using automated harvester system Harvester-96 MACH III FM (Tomtec). The filter mates were dried at 37 °C in forced air fan incubator and then solid scintillator MeltiLex was melted on filter mates at 90 °C for 4 min. Radioactivity was counted in MicroBeta2 scintillation counter (PerkinElmer). Data were fitted to a one-site curve-fitting equation with Prism 6 (GraphPad Software) and K_i values were estimated from the Cheng−Prusoff equation.

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. 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 vol of assay buffer using an Ultra Turrax tissue homogenizer and centrifuged twice at 35.00 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_6R, 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_6R) or 0.6 nM [^{3}H]-5-CT (39.2 Ci/mmol) for 5-HT_7R.

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_6R or 5-HT_7R 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 [52]. Membrane preparation and general assay procedures for cloned receptors were adjusted to a 96-microwell format based on protocols previously described by us [53,54].

4.2.1.5. ADRA1 receptor binding assay. Preparation of solutions of test and reference compounds: 10 mM stock solutions of tested compounds were prepared in DMSO. Serial dilutions of compounds were prepared in 96-well microplate in assay buffers using automated pipetting system epMotion 5070 (Eppendorf). Each compound was tested in 6 concentrations from 10 to 5 to 10−10 M (final concentration).

Radioligand binding was performed using tissue rat cortex. All assays were carried out in duplicates. 50 μl working solution of the tested compounds, 50 μl [^{3}H]-prazosin (final concentration 0.2 nM) and 150 μl tissue suspension prepared in assay buffer (50 mM Tris-

HCl, pH 7.6) were transferred to polypropylene 96-well microplate using 96-wells pipetting station Rainin Liquidator (MettlerToledo). Phentolamine (10 μM) was used to define nonspecific binding. Microplate was covered with a sealing tape, mixed and incubated for 30 min at 30 °C. The reaction was terminated by rapid filtration through GF/B filter mate. Ten rapid washes with 200 μl 50 mM Tris buffer (4 °C, pH 7.6) were performed using 96-well FilterMate harvester (PerkinElmer, USA). The filter mates were dried at 37 °C in forced air fan incubator and then solid scintillator MeltiLex was melted on filter mates at 90 °C for 4 min. The radioactivity on the filter was measured in MicroBeta TriLux 1450 scintillation counter (PerkinElmer, USA). Data were fitted to a one-site curve-fitting equation with Prism 6 (GraphPad Software) and K_i values were estimated from the Cheng−Prusoff equation.

4.2.2. Metabolic stability

Stock solutions of studied compounds were prepared at concentration of 10 mM in DMSO. Working solutions were prepared daily by dilution of stock with reaction buffer or acetonitrile, final concentration of organic solvent did not exceed 1%. Incubation mixture contained 1 μM of a studied derivative, 1 mM of NADPH (Sigma-Aldrich) and 0.5 mg/mL of pooled human liver microsomes (HLM, Sigma-Aldrich) in potassium phosphate buffer (0.1 M, pH 7.4). Incubation was carried out in thermostat at 37 °C and started by addition of studied compound. 50 μL samples were taken at starting point and after 5, 10, 15, 20, 30 min. Enzymatic reaction was terminated by the addition of the equal volume of ice-cold acetonitrile, containing 1 μM of buspirone hydrochloride as internal standard. After collection, samples were immediately centrifuged (10 min, 10000 rpm) and resulted supernatant was directly analysed or kept in −80 °C until LC-MS analysis. Natural logarithm of a compound over IS peak area ratio was plotted versus incubation time. Metabolic half-time ($t_{1/2}$) was calculated from the slope of the linear regression.

LC-MS analysis was performed on an Agilent 1260 system coupled to SingleQuad 6120 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Poroshell EC-C18 (2.1 mm × 150 mm, 2.7 μm, Agilent Technologies, Santa Clara, CA, USA) was used in reversed-phase mode with gradient elution starting with 90% of phase A (0.1% formic acid in water) and 10% of phase B (0.1% dormic acid in acetonitrile). The amount of phase B was linearly increased to 100% in 15 min and equilibrated. Total analysis time was 21 min at 25 °C, flow rate was 0.5 mL/min and the injection volume was 20 μL. The mass spectrometer was equipped with electrospray ion source and operated in positive ionization. Mass analyser was set individually to each compound to detect $[M+H]^+$ protonated molecule. MSD parameters of the ESI source were as follows: nebulizer pressure 40 psig (N_2), drying gas 10 mL/min (N_2), drying gas temperature 300 °C, capillary voltage 3.0 kV, fragmentor voltage 150 V.

4.2.3. In vivo experiments

The experiments were performed on male CD-1 mice (29−42 g). The animals were kept at a room temperature (21 ± 2 °C) on a natural day-night cycle (May−November) and housed under standard laboratory conditions. They had free access to food and tap water before the experiment. Each experimental group consisted of 6−8 animals/dose. All the animals were used only once. 8-Hydroxy-2-(di-*n*-propylamino)tetralin hydro-bromide (8-OH-DPAT, Research Biochemical Inc.) was used as aqueous solutions. Compounds: **4a, 4b, 4c, 4j, 4k** and **4l** were suspended in a 10% aqueous solution of dimethyl sulfoxide (DMSO). Vehicle group was administered as 10% aqueous solution of DMSO. 8-OH-DPAT was injected subcutaneously (*sc*), **4a, 4b, 4c, 4j, 4k** and **4l** were given intraperitoneally (*ip*) in a volume of 10 mL/kg/mice. The obtained data were analysed by Dunnett's test (when one drug was administrated) or by the Newman-Keuls test (when two drugs were administrated).

4.2.3.1. Body temperature in mice. The effects of the tested compounds: **4a, 4b, 4c, 4j, 4k** and **4l** given alone on the rectal body temperature in mice (measured with an Ellab thermometer) were recorded 30, 60, 90 and 120 min after their administration. In the another experiment the effect of WAY100635 (0.1 mg/kg *s.c.* as aqueous solution) on the hypothermia induced by tested compounds and/or 8-OH-DPAT was measured. WAY100635 was administered 15 min before the tested compounds and the rectal body temperature was recorded 30 min and 60 min after injection.

4.2.3.2. Forced swim test in mice. The experiments were performed according to the method of Porsolt et al. (1977). Mice were placed individually into glass cylinders (height 25 cm, diameter 10 cm) containing about 20 cm of water (23 °C). The animals were left in the cylinder for 6min. First 2min of test was count as adaptation period, the total duration of immobility was measured during a 4-min test. The mouse was estimated to be immobile when it remained floating passively, performing slow motion to keep head above the water. Tested compounds were administered 30 min before the test.

4.2.3.3. Locomotor activity of mice (LA). The locomotor activity of mice was measured in a 20-station photo beam activity system (Opto-M3 Activity Meter; Columbus Instruments) composed of Plexiglas locomotor activity chambers (40 x 20 × 15 cm). Mice were placed individually in the chamber, and the total number of ambulation was recorded for the time adequate to the forced swimming test.

4.2.4. The bioavailability assays

The *in vitro* bioavailability prediction assays and the hERG automated patch-clamp experiments were performed by Eurofins Pharma Discovery Services according to the well-known methods. Further methodological details are available on the company website (www.eurofinsdiscoveryservices.com) and the appropriate publications [[55−58]]. For more details see also Supplementary data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.111736.

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