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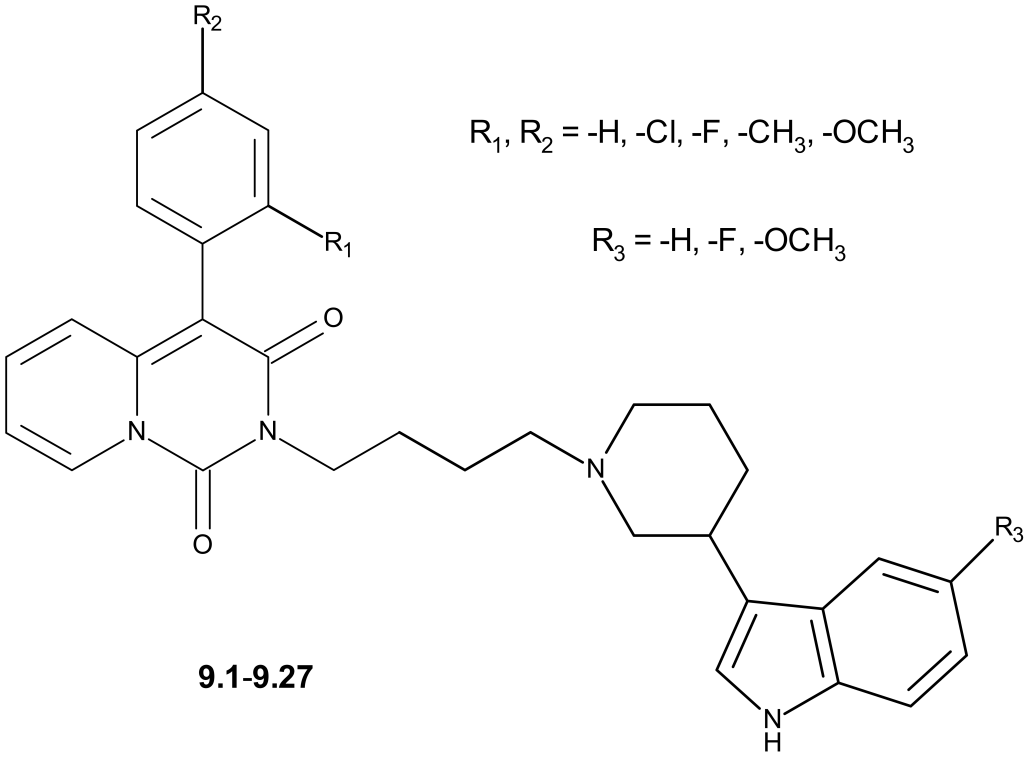

R_1, R_2 = -H, -Cl, -F, $-CH_3$, $-OCH_3$

R_3 = -H, -F, $-OCH_3$

9.1-**9.27**

9.1 (R_1, R_2, R_3 = -H)
K_i 5-HT_{1A} = 22.0 nM
K_i SERT = 68.0 nM

9.7 (R_1, R_3 = -H; R_2 = -F)
K_i 5-HT_{1A} = 46.0 nM
K_i SERT = 46.0 nM

Synthesis of novel pyrido[1,2-c]pyrimidine derivatives with rigidized tryptamine moiety as potential SSRI and 5-HT$_{1A}$ receptor ligands

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Keywords: Antidepressants, Pyrido[1,2-c]pyrimidines, Dual 5-HT$_{1A}$/SERT activity

List of abbreviations: WHO, World Health Organization; 5-HT$_{1A}$R, serotonin 5-HT$_{1A}$ receptors; SERT, serotonin transporter; SAR, structure-activity relationship; K_i, inhibitory constant; NMR, nuclear magnetic resonance; HRMS, high resolution mass spectroscopy; RBA, radioligand binding assay; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; TEA, triethylamine; DMSO, dimethyl sulfoxide; XRD, X-ray diffraction; clogP, calculated logarithm of octanol/water partition coefficient; RBA, radioligand binding assay; FST, forced swimming test

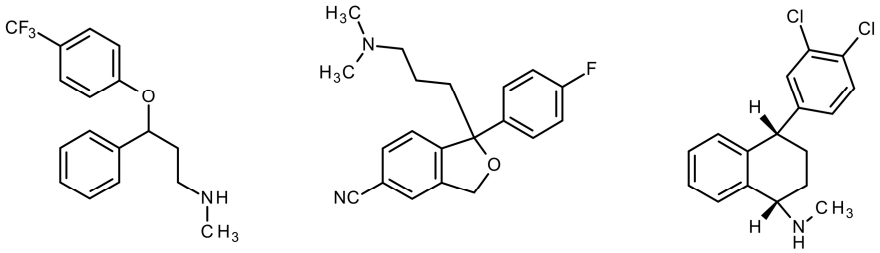
Abstract

The study enabled obtaining a number of new derivatives of 4-aryl-pyrido[1,2-c]pyrimidine **9.1**-**9.27** having conformationally restricted tryptamine moiety. In vitro studies (RBA) have shown that derivatives **9.1**, **9.2**, **9.4**, **9.7**, **9.9**, **9.14** and **9.27** exhibit high affinity to molecular targets 5-HT$_{1A}$ receptor and SERT protein. In general, compounds with an unsubstituted or a para-substituted benzene ring of the pyrido[1,2-c]pyrimidine residue in the terminal part were characterized by higher binding ability, which can be justified by the greater flexibility of the structure. For the selected compounds **9.1**, **9.7**, **9.9** and **9.27**, further *in vitro, in vivo* and metabolic stability tests were performed. The *in vitro* studies in the extended receptor profile (D_2, 5-HT$_{2A}$, 5-HT$_6$ and 5-HT$_7$) indicated their selectivity toward the 5-HT$_{1A}$ receptor and SERT protein. The *in vivo* studies (8-OH-DPAT-induced hypothermia in mice, FST) revealed that the compound **9.1** has the properties of presynaptic agonist of the 5-HT$_{1A}$ receptor, and compound **9.7** demonstrated the properties of a presynaptic antagonist of the 5-HT$_{1A}$ receptor. Metabolic stability studies, in turn, showed that compounds **9.1, 9.7** and **9.9**, having an unsubstituted indole residue, were more resistant to

biotransformation reactions of the first pass phase than was compound **9.27** containing a 5-methoxy-substituted indole residue. The obtained results allowed further optimization of the structure.

1. Introduction

Depression is a mental illness affecting over 350 million people around the world [1]. As many as 80% of patients suffer recurrence after they have recovered [2]. Emotional, somatic and functional disorders accompanying depression make this disease the primary cause of disability in relation to the entire population. According to the WHO, by 2030 serious depressive illness will have become the main cause of disability [3,4].



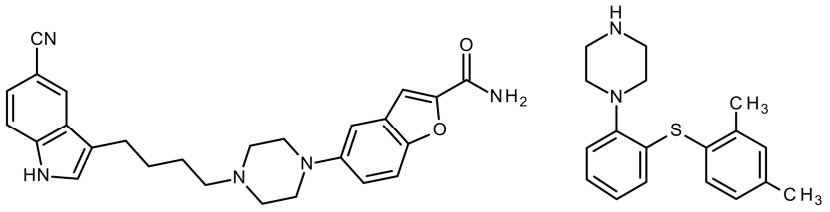
Fluoxetine Citalopram Sertraline

***Fig. 1**. SSRI drug structures.*

A breakthrough in the treatment of depressive disorders was the introduction of SSRIs, which are currently the first-line antidepressants (*Fig. 1*). Their mechanism is based on the serotoninergic system and their molecular target is serotonin transporter protein (SERT). The effectiveness of these therapeutics still leaves much to be desired, as up to 40% patients do not respond to treatment at all [5]. Serotonin reuptake inhibitors are also seriously affected by the need to administer them for a longer period of time (2–6 weeks) before a clear improvement of the patient's mood can be observed, which is due to latency.

Rodent models have shown that 5-HT_{1A} receptor agonists (e.g. 8-OH-DPAT) may have potential high antidepressant activity [6]. These effects are blocked by antagonists of 5-HT_{1A}-R, indicating that said activity is specific for transduction of the 5-HT_{1A} receptor [7,8]. This is probably connected to expression of 5-HT_{1A} heteroreceptors in the limbic system [9]. The 5-HT_{1A} autoreceptors, on the other hand, work in opposition, causing prodepressive effects. Their stimulation leads to hyperpolarization and reduced transmission of seam neurons, reducing the release of serotonin in the projection regions [10]. Therefore, increased stimulation of 5-HT_{1A} autoreceptors during SSRI administration simultaneously inhibits activity of serotonin neurons [11]. Occurrence of the latency period is explained by the desensitization model of 5-HT_{1A} autoreceptors [12,13]. This indicates that long-term antidepressant therapy reduces negative feedback in the serotoninergic system caused by the stimulation of 5-HT_{1A} autoreceptors. This process occurs through desensitization/negative regulation of 5-HT_{1A} autoreceptors, which results in increased serotonin transmission [13]. Accordingly, the use of the 5-HT_{1A} autoreceptor activation mechanism has been introduced as a strategy to supplement the inhibition of serotonin reuptake. The use of agonists or partial agonists of the 5-HT_{1A} receptor for this purpose appears to be a more favorable choice, as the sensitivity of postsynaptic receptors is not reduced even after repeated exposure to the 5-HT_{1A}/SSRI agonist, as opposed to the response to the 5-HT_{1A}/SSRI antagonist [11,14]. The

legitimacy of this approach has been confirmed by the FDA registration of vilazodone and vortioxetine (*Fig. 2*) [15–20].



Vilazodone Vortioxetine

Fig. 2. *SSRI+ drug structures.*

Our laboratory has been conducting research on double-binding ligands to the 5-HT_{1A} receptor and SERT protein among new pyrido[1,2-c]pyrimidine derivatives for a decade. Earlier works described pyrido[1,2-c]pyrimidine derivatives with the fragment of 3-(piperidin-4-yl)-1H-indole in the pharmacophore part; an array of high-binding compounds were obtained for both molecular targets, as well as with an appropriate functional profile for the 5-HT_{1A} receptor – pre- and postsynaptic agonism [21–25].

Our current research has focused on pyrido [1,2-c] pyrimidine derivatives containing a 3-(piperidin-3-yl)-1H-indole residue, where the aminoethyl chain on the indole is conformationally restricted. The research objective was the synthesis of newly designed derivatives of the series 2H-pyrido[1,2-c]pyrimidine based on previously obtained structures in which the pharmacophore part had been redesigned. The introduced 3-(piperidin-3-yl)-1H-indole residue is more similar in its structure to serotonin, and its conformationally constrained limitation (*Fig. 3*) aimed to increase the activity of compounds by increasing the affinity for 5-HT_{1A} and SERT, confirmed in numerous references [26–31]. Moreover, residues with such a structure also have an effect on higher metabolic stability [32].

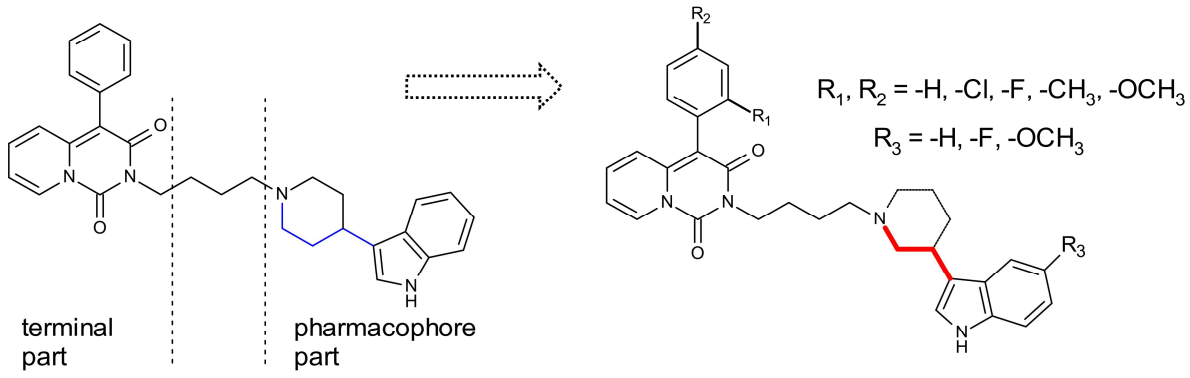


Fig. 3*. Comparison of the leading structure and structures examined in the project.*

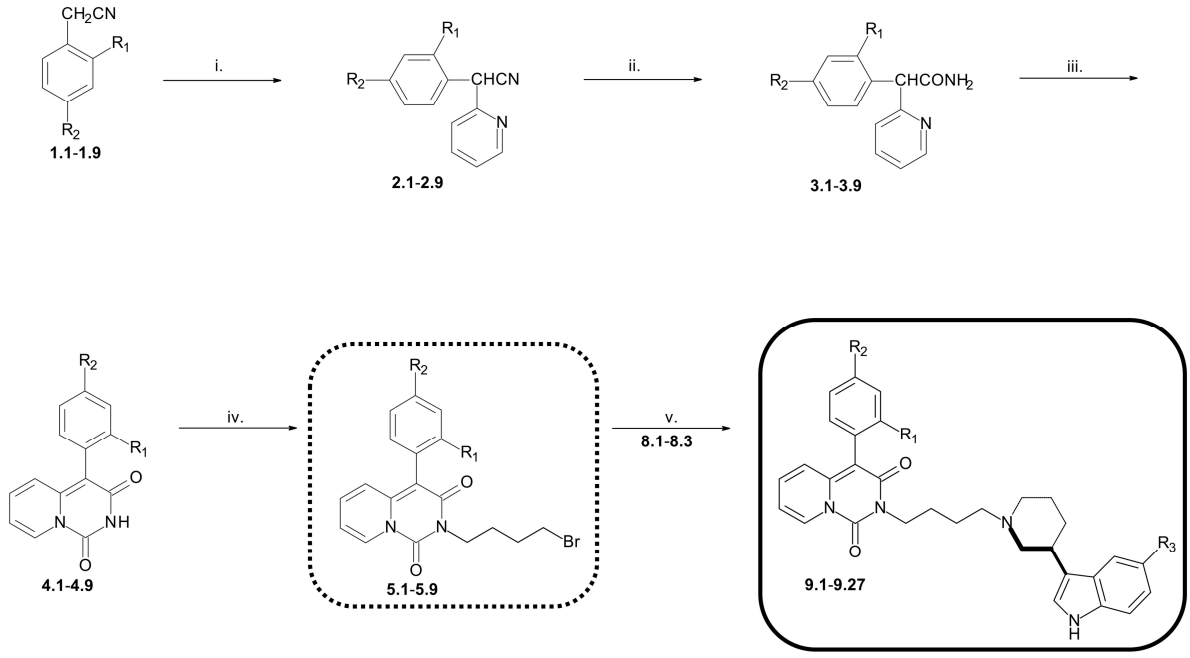
The SAR analysis investigated the effect of R_3 (-H, -F, -OCH_3) substituents present in the pharmacophore part of the indole ring and in the terminal part of the benzene ring of the pyrido[1,2-c]pyrimidine (-H, -Cl, -F, -CH_3, -OCH_3) residue on the affinity of the derivatives obtained for both molecular targets (5-HT_{1A}, SERT). For selected compounds, *in vitro* studies were also performed in the extended receptor profile – D_2, 5-HT_{2A}, 5-HT_6 and 5-HT_7. Their functional profile to the 5-HT_{1A}

receptor (agonism/antagonism) and behavioral profile as well as their metabolic stability were also tested.

2. Results and discussion

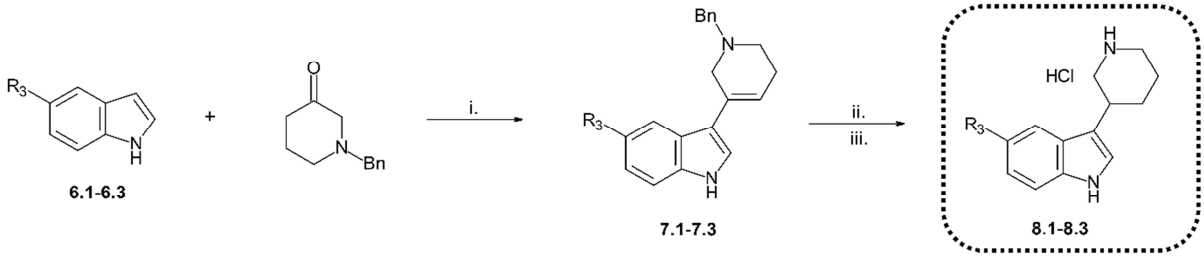
2.1. Chemistry

The designed compounds **9.1–9.27** were obtained by synthesis in accordance with *Scheme 1.* Two synthon series were necessary for the syntheses thereof. The first series of syntons were *N*-bromobutyl-4-aryl-pirydo[1,2-c]pyrimidine derivatives **5.1–5.9**, followed by 3-(piperidin-3-yl)-1H-indole derivatives **8.1–8.3** (*Scheme 2*).



***Scheme 1**. The synthesis pathway of compounds **9.1–9.27**. Reagents and conditions: (i) 2-bromopyridine, KOH, DMSO, Δ; (ii) H_2SO_4, CH_3COOH, Δ; (iii) diethyl carbonate, EtONa, EtOH abs., Δ; (iv) 1,4-dibromobutane, K_2CO_3, acetone, Δ; (v) **8.1–8.3**, acetonitrile, K_2CO_3, Δ.*

Synthons **5.1–5.9** were obtained in accordance with pathways that we had developed earlier [21,23–25]. Another group of synthons **8.1–8.3** was obtained in a two-step synthesis. **7.1–7.3** derivatives of 3-(1-phenyl-1,2,5,6-tetrahydropyridin-3-yl)-1H-indole were obtained based on the method of [33] in condensation of indole derivatives **8.1–8.3** with 1-benzyl-3-piperidone hydrochloride. This formula was modified by replacement of Na/MeOH with KOH and isopropanol, obtaining derivatives **7.1–7.3.** Hydrogenolysis with hydrogenation of compounds **7.1–7.3** was carried out with Pd/C 10% in methanol at a pressure of 5 atm. The obtained bases **8.1–8.3** were converted into hydrochlorides, for which melting points and composition were determined, and ^{1}H, ^{13}C NMR and XRD tests were conducted. Physicochemical data for these compounds have not been reported in the literature so far. There was only the application of base **8.1** indicated [34–36] (*Scheme 2*).



Scheme 2*. The synthesis pathway of substrates: 3-piperidyn-3-yl-1H-indole derivatives* ***8.1*** *(R_3 = -H);* ***8.2*** *(R_3 = -F);* ***8.3*** *(R_3 = -OCH_3). Reagents and conditions: (i) IPA, KOH, Δ, Ar; (ii.) 10% Pd/C, CH_3OH, p, Δ; (iii) CH_3OH/HCl (g).*

The structure and composition of all of the newly obtained derivatives **9.1–9.27** were proven by HRMS and by ^{1}H and ^{13}C NMR spectroscopy. X-ray crystallography tests were performed for the compounds **8.1–8.2.**

Table 1

5-HT_{1A} receptor and SERT binding affinities as well as clogP ("ACD/ChemSketch," 2017) *of 2H-pyrido[1,2-c]pyrimidine derivatives.*

				K_i [nM]		
Compound	**R_1**	**R_2**	**R_3**	**5-HT_{1A}**	**SERT**	**clog P**
9.1	-H	-H	-H	22.0 ± 2.7	68.0 ± 7.5	4.78
9.2	-Cl	-H	-H	50.0 ± 2.0	103.0 ± 10.2	5.16
9.3	-F	-H	-H	53.0 ± 5.0	240.0 ± 6.5	4.83
9.4	-CH_3	-H	-H	102.0 ± 6.5	76.0 ± 7.0	5.24
9.5	-OCH_3	-H	-H	132.0 ± 10.7	411.0 ± 22.9	4.78
9.6	-H	-Cl	-H	294.0 ± 24.5	82.0 ± 6.0	5.50
9.7	-H	-F	-H	46.0 ± 1.5	46.0 ± 2.8	4.79
9.8	-H	-CH_3	-H	63.0 ± 1.5	207.0 ± 15.0	5.24
9.9	-H	-OCH_3	-H	42.0 ± 1.5	41.0 ± 1.8	4.72
9.10	-H	-H	-F	209.0 ± 10.5	8.0 ± 0.5	4.92
9.11	-Cl	-H	-F	378.0 ± 17.4	255.0 ± 17.9	5.29
9.12	-F	-H	-F	218.0 ± 13.5	115.0 ± 22.0	4.97
9.13	-CH_3	-H	-F	376.0 ± 22.0	338.0 ± 45.0	5.38
9.14	-OCH_3	-H	-F	80.0 ± 9.5	118.0 ± 9.4	4.92
9.15	-H	-Cl	-F	155.0 ± 8.5	86.0 ± 11.0	5.63
9.16	-H	-F	-F	147.0 ± 10.5	30.0 ± 1.4	4.93
9.17	-H	-CH_3	-F	136.0 ± 14.0	135.0 ± 14.5	5.38
9.18	-H	-OCH_3	-F	140.0 ± 17.0	27.0 ± 1.5	4.86
9.19	-H	-H	-OCH_3	261.0 ± 20.0	248.0 ± 20.8	4.69
9.20	-Cl	-H	-OCH_3	176.0 ± 17.0	459.0 ± 32.5	5.07
9.21	-F	-H	-OCH_3	130.0 ± 14.0	1670.0 ± 179.0	4.75
9.22	-CH_3	-H	-OCH_3	476.0 ± 4.0	87.0 ± 6.5	5.15
9.23	-OCH_3	-H	-OCH_3	69.0 ± 3.5	386.0 ± 15.0	4.70
9.24	-H	-Cl	-OCH_3	257.0 ± 14.0	490.0 ± 29.5	5.41
9.25	-H	-F	-OCH_3	281.0 ± 16.5	192.0 ± 7.5	4.70
9.26	-H	-CH_3	-OCH_3	333.0 ± 17.5	173.0 ± 16.8	5.15
9.27	-H	-OCH_3	-OCH_3	62.0 ± 3.5	86.0 ± 10.0	4.64
Imipramine					29.0 ± 2.0	

Serotonin	3.0 ± 0.3

Table 2

Binding affinity data on serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7, and dopamine D_2 receptors of the investigated 2H-pyrido[1,2-c]pyrimidine derivatives.

				K_i [nM]			
Compound	**R_1**	**R_2**	**R_3**	**5-HT_{2A}**	**5-HT_6**	**5-HT_7**	**D_2**
9.1	-H	-H	-H	433	435	1115	17670
9.7	-H	-F	-H	435	697	1444	963
9.9	-H	-OCH_3	-H	21	1709	1922	1129
9.27	-H	-OCH_3	-OCH_3	314	690	958	11160
Olanzapine				4.6	7	n.d.	n.d.
Mianserine				2.8	n.d.	n.d.	n.d.
Clozapine				n.d.	n.d.	18	n.d.
Haloperidol				n.d.	n.d.	n.d.	4.5
Apomorphine				n.d.	n.d.	n.d.	42
Chloropromazine				n.d.	n.d.	n.d.	1.8

2.2 Biological evaluation

2.2.1 Radioligand binding assay for 5-HT_{1A} receptor and SERT

Compounds **9.1–9.27** were tested for *in vitro* affinity for 5-HT_{1A} receptor and SERT protein by a radioligand binding assay (*Table 1*).

Binding affinity data for 5-HT_{1A} and SERT for compounds **9.1–9.27** were used in SAR analysis.

The influence of substituents in the ortho (R_1) and para (R_2) positions in the terminal part of the benzene ring of the pyrido [1,2-c] pyrimidine residue and the presence of R_3 (-H, -F, -OCH_3) substituents in the indole residue of the pharmacophore part of the compounds were analyzed.

Analysis of the obtained results showed high affinity of compounds **9.1–9.4**, **9.7–9.9**, **9.14**, **9.23** and **9.27** to the 5-HT_{1A} receptor, with the K_i values of those compounds ranging from 22.0 to 102.0 nM. It can be clearly seen that compounds with an unsubstituted indole ring had higher affinity to the 5-HT_{1A} receptor than did their substituted analogs (R_3 = -F, -OCH_3). In turn, the analysis of the effect of substituents on the terminal part present in the benzene ring showed that derivatives containing a para-substituted ring had greater 5-HT_{1A} binding ability than did ortho-substituted derivatives. Compounds having a methoxy substituent in the terminal portion (**9.5**, **9.9**, **9.14**, **9.18**, **9.23** and **9.27**), regardless of its position in the benzene ring, generally had similar high to moderate binding affinity values toward the 5-HT_{1A} receptor.

While studying the effect of R_1, R_2 and R_3 in both parts of the molecule for their affinity to the SERT protein, it can be concluded that compound **9.10** showed the highest affinity (K_i = 8 nM) to SERT, while the affinity of compounds **9.16** and **9.18** was high (K_i = 27.0–30.0 nM), and the affinity of a series of compounds **9.1**, **9.2**, **9.4**, **9.6**, **9.7**, **9.9**, **9.12**, **9.14–9.16**, **9.18**, **9.22** and **9.27** was high to moderate. This molecular target shows that the highest binding ability was achieved by derivatives in which R_3 = -H or -F in the 3-(piperidin-3-yl)-1H-indole residue of the pharmacophore part. In turn, methoxy-substituted analogs (R_3 = -OCH_3) showed significantly lower activity. As with the 5-HT_{1A} receptor, higher activity against this molecular target was demonstrated by compounds with a para-

substituted benzene ring of the pyrido[1,2-c]pyrimidine residue in the terminal part, and this tendency is even more visible in SERT. NMR studies show that the benzene ring in the ortho-substituted compounds cannot rotate freely due to steric barriers (the NMR spectra show temporary "magnetic stereoisomers" manifested by double instead of single signals of R_1 hydrogens). This is possible in para-substitute compounds, which may imply the hypothesis that a more flexible terminal part can affect higher binding affinity to 5-HT_{1A} and SERT.

The obtained clog P values for the compounds **9.1**–**9.27** are in the range from 4.64 to 5.63. Value presented by the compounds **9.1**, **9.3**, **9.5**, **9.7**, **9.9**, **9.10**, **9.12**, **9.14**, **9.16**, **9.18**, **9.19**, **9.21**, **9.23**, **9.25** and **9.27** were below 5, which meets the condition of proper penetration through the membrane and complies with the Lipinski 'rule of five' [38]. In this respect, they are similar to vortioxetine (clog P = 4.26), an SSRI+ antidepressant available on the market.

During the studies it was possible to obtain compounds **9.1**, **9.2**, **9.4**, **9.7**, **9.9**, **9.14** and **9.27** with high binding affinity to both molecular targets (5-HT_{1A} and SERT) and clog P values lower than 5 (**9.1**, **9.7**, **9.9**, **9.14** and **9.27**).

Among the above-mentioned derivatives, compounds **9.1**, **9.7**, **9.9** and **9.27** were selected to be subject to *in vitro* studies in the extended receptor profile to D_2, 5-HT_{2A}, 5-HT_6 and 5-HT_7 (*Table 2*). The results of the tests showed that these compounds are selective ligands of molecular targets – receptor 5-HT_{1A} and SERT protein – due to the low affinity of the compounds to other receptors tested in the profile.

2.2.2 In vivo studies

To determine the profile of functional activity of the selected ligands, behavioral tests were performed.

Table 3

*The effect of compounds: **9.1**, **9.7**, **9.9** and **9.27** 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 ± 0.2	0.1 ± 0.3	0.1 ± 0.1	0.1 ± 0.3
9.1	5	–0.1 ± 0.2	–0.4 ± 0.1	–0.5 ± 0.1[b]	–0.8 ± 0.1[c]
	20	–0.9 ± 0.3[c]	–0.3 ± 0.3	0.1 ± 0.2	0.2 ± 0.1
		P<0.0001	ns	*P* = 0.0083	*P* = 0.0003
Vehicle	–	0.4 ± 0.2	0.1 ± 0.3	0.1 ± 0.1	0.1 ± 0.3
9.7	5	0.4 ± 0.1	0.4 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
	20	–0.2 ± 0.3	0.5 ± 0.2	0.2 ± 0.2	0.1 ± 0.2
		ns	ns	ns	ns
Vehicle	–	0.4 ± 0.2	0.1 ± 0.3	0.1 ± 0.1	0.1 ± 0.3
9.9	5	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
	20	–1.6 ± 0.3[c]	0.3 ± 0.2	0.0 ± 0.1	0.3 ± 0.2
		P < 0.0001	*P* = 0.0099	ns	ns
Vehicle	–	0.0 ± 0.1	0.1 ± 0.1	–0.1 ± 0.1	–0.1 ± 0.1

9.27	5	–0.2 ± 0.2	0.2 ± 0.3	0.2 ± 0.2	–0.2 ± 0.3
	15	–0.5 ± 0.3	0.2 ± 0.3	0.3 ± 0.4	0.2 ± 0.3
	17.5	–0.6 ± 0.2 [a]	–0.2 ± 0.2	–0.1 ± 0.1	0.1 ± 0.1
	20	–2.4 ± 0.3 [c]	–1.0 ± 0.4 [b]	0.1 ± 0.2	0.0 ± 0.2
		P < 0.0001	*P* = 0.0176	ns	ns
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 30 min before the test, [a]p < 0.05 vs vehicle, [b]p < 0.01 vs vehicle, [c]p < 0.001 vs vehicle, ns = non-significant.

It is known that 8-OH-DPAT as a 5-HT_{1A} receptor agonist can induce hypothermia in mice, through 5-HT_{1A} somatodendritic receptors [39,40]. Moreover, this effect can be abolished by WAY100635 [41], 5-HT_{1A} receptor antagonist. Based on this knowledge, we tested compounds **9.1**, **9.7, 9.9** and **9.27** in a commonly used *in vivo* panel of tests, to assess their functional 5-HT_{1A} receptor activity. Compounds **9.1, 9.9** and **9.27**, as 8-OH-DPAT, induced hypothermia in mice (*Table 3*). Hypothermia induced by compound **9.1** (20 mg/kg) was attenuated by WAY100635 (0.1 mg/kg) (*Table 4*). Concluding, the decrease in mouse body temperature produced by compound **9.1** can be accounted as a measure of its presynaptic 5-HT_{1A} agonistic activity. Tested derivative **9.1** was ineffective in the forced swimming test in mice, so we can conclude lack of postsynaptic 5-HT_{1A} receptor activity [39]. Compound **9.7** (20 mg/kg) decreased the hypothermia induced by 8-OH-DPAT (5 mg/kg) in mice (*Table 5*) demonstrating its presynaptic 5-HT_{1A} receptor antagonist activity.

Table 4

*The effect of WAY100635 (0.1 mg/kg sc) on the hypothermia induced by compounds **9.1** and **9.9**.*

Treatment and dose (mg/kg)	Δt ± SEM (^{0}C)	
	30 min	**60 min**
Vehicle	0.3 ± 0.1	0.3 ± 0.1
Vehicle + **9.1** (20)	–0.9 ± 0.3 [c]	–0.3 ± 0.3
WAY100635 + **9.1** (20)	0.0 ± 0.2 [e]	0.1 ± 0.2
	P = 0.0002	ns
Vehicle	0.2 ± 0.4	0.1 ± 0.2
Vehicle + **9.9** (20)	–1.6 ± 0.3 [c]	0.3 ± 0.2
WAY100635 + **9.9**	–1.4 ± 0.2 [c]	–0.7 ± 0.2 [a]
	P<0.0001	*P* = 0.0216
Vehicle	0.1 ± 0.1	–0.0 ± 0.1
WAY100635	0.3 ± 0.3	0.2 ± 0.3
	ns	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, ns = non-significant.

Table 5

*The effect of compound **9.7** on the hypothermia induced by 8-OH-DPAT (5 mg/kg).*

Treatment and dose (mg/kg)	Δt ± SEM (^{0}C)			
	15 min	30 min	45 min	60 min
Vehicle + vehicle	0.2 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.2
Vehicle + 8-OH-DPAT	–1.9 ± 0.2 [c]	–2.4 ± 0.2 [c]	–2.6 ± 0.2 [c]	–2.4 ± 0.3 [c]
9.7 (20) + 8-OH-DPAT	–1.2 ± 0.2 [c, e]	–1.2 ± 0.3 [c, e]	–1.3 ± 0.3 [c, f]	–1.3 ± 0.3 [c, e]
	P < 0.0001	*P* < 0.0001	*P* < 0.0001	*P* < 0.0001

*Compound **9.7** was administered 45 min before to 8-OH-DPAT, n = 14–15, [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.*

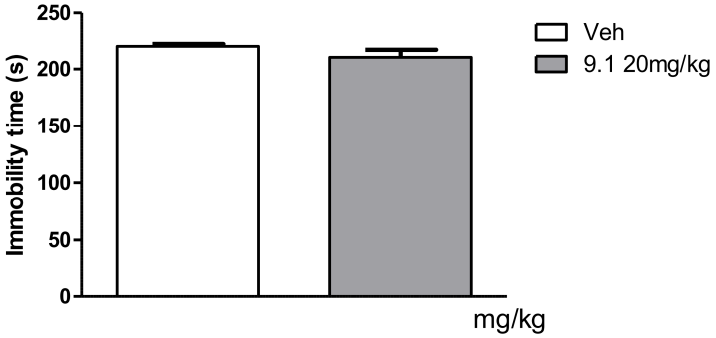


***Fig. 4**. Effect of compound **9.1** on forced swimming test in CD-1 mice*

2.2.3 Metabolic stability evaluation

The results of compound incubations in the presence of pooled HLMs and NADPH are presented in *Table 6*. Metabolic stability is presented in form of a biological half-life value, which allows for easy comparison of compound structures and their susceptibility to phase 1 biotransformation reactions (the result of incubation on the presence of HLMs).

Table 6

Experimental $t_{1/2}$ values along with corresponding SD and RSD%.

Compound	Average $t_{1/2}$ [min] (n = 2)	SD [min]	RSD%
9.1	6.12	0.18	3.07
9.7	5.53	0.14	2.55
9.9	7.40	0.13	1.72
9.27	3.31	0.47	15.92

*Table legend: SD – standard deviation, RSD% – relative standard deviation, expressed as SD/average*100%.*

Results presented in *Table 6* allow a quick assessment of metabolic stability. Even though the biological half-life values for studied compounds were far from high, it is worth noticing that biological half-life value depends on the compound's initial concentration in the incubation mix. As the initial studied compound concentration was 1 µM, such values were to be expected. Of the studied pyrido[1,2-c]pyrimidine derivatives, compounds **9.1** (R_1 = -H, R_2 = -H, R_3 = -H), **9.7** (R_1 = -H, R_2 = -F, R_3 = -H) and **9.9** (R_1 = -H, R_2 = -OCH_3, R_3 = -H) proved to be more resistant to biotransformation reactions. Compound **9.27** (R_1 = -H, R_2 = -OCH_3, R_3 = -OCH_3) was most susceptible to phase 1 biotransformation reactions. Visual comparison of a derivative's chemical structure and its biological half-life allows for an assessment of basic structure–metabolic stability relationships. In the present study, compounds with the highest resistance to phase 1 enzymes possessed a -OCH_3 group in the R_2 position of the terminal portion. Compounds **9.1** and **9.7** possessed a hydrogen and fluoride moiety in that place, respectively. Addition of another -OCH_3 moiety (in derivative **9.27**) in position 5 of the indole ring (R_3) resulted in lowered metabolic stability (biological half-life value of 3.31 min). Therefore, such direction of synthesis should rather be avoided to prevent development of further metabolically unstable derivatives.

3. Materials and methods

3.1. General remarks

Melting points were determined on an Electrothermal IA9200 apparatus with open capillary tubes and are uncorrected. Elemental analyses were performed on a Elementar Vario EL III analyzer and were within 0.4% of the theoretical values. ^{1}H and ^{13}C NMR spectra were obtained on Varian Unity Plus 500 MHz instrument (chemical shifts are reported in δ units). Coupling constants (J) are in hertz (Hz); the internal reference was TMS. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), bs (broad singlet), d (doublet), dd (double doublet), t (triplet), td (triple doublet), ps (pseudotriplet), 4d (quartet of doublets), m (multiplet), E (equatorial), A (axial). For the two-dimensional experiments, the pulse sequences, acquisition and processing parameters were taken from the standard Varian software library. ESI-HRMS spectra were obtained on a Thermo Q-Exactive instrument. Flash column chromatography was carried out on Merck silica gel 60 (230–400 mesh ASTM) using the solvent methylene chloride/methanol/triethylamine (9:1:0.2, 8:2:0.1, v/v) and ethyl acetate/hexane (9:1 v/v). Thin layer chromatography was run on Merck silica gel 60 F254 plates using a mobile phase of methylene chloride/methanol/triethylamine (9:1:0.2, 8:2:0.1, v/v). Compound purity was determined by high performance liquid chromatography (HPLC), and all final test compounds were >95% purity. The HPLC methods used a Phenomenex column C18 (3 µm, 150 mm x 2.00 mm); with a mobile phase of methanol/water/diisopropylamine (80:20:0.008, v/v; detection at 280 nm; flow: 0.2 mL/min; temp 30 ^{o}C.

3.2. Synthesis of compounds

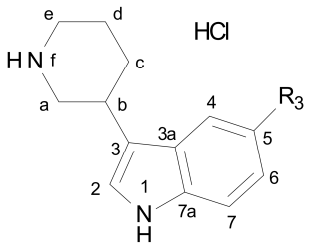
*3.2.1. Preparation of 2-(4-bromobutyl)-4-aryl-pyrido[1,2- c]pyrimidine-1,3-diones (**5.1–5.9**)*

The starting compounds **2.1–2.9**, **3.1–3.9, 4.1–4.9** and **5.1–5.9** were obtained according to procedures described in the literature[21–25].

*3.2.2. General procedure for the synthesis of 3-piperidin-3-yl-1H-indole hydrochlorides (**8.1–8.3**)*

A mixture of 0.01 mol of appropriate indole derivative (**6.1–6.3**), 0.03 mol of *N*-benzyl-3-piperidone and 60 mL of 2 M KOH/isopropanole was stirred in 80 ^{o}C under an argon atmosphere for 8 h. The mixture was further stirred for 8 h at room temperature and poured onto ice/water. The

crude product was extracted with $CHCl_3$ and the organic layers combined and dried with $MgSO_4$. The mixture was then filtered and the filtrate was evaporated to dryness. The crude residue was purified by flash chromatography, using CH_2Cl_2/MeOH/TEA (9:1:0.2 v/v) and ethyl acetate/hexane (9:1 v/v) mixtures. 0.01 mol of the obtained yellow solid (**7.1**–**7.3**)[33] was dissolved in 150 mL of methanol and hydrogenated for 8 h at 30 °C and 1 atm with 0.25 g of 10% Pd/C as catalyst. The catalyst was then filtered off and the filtrate evaporated to one-third and then, after cooling, acidified with HCl/MeOH. The evaporated-to-dryness crude product (**8.1**–**8.3**) was purified by crystallization from methanol.



8.1-8.3

Fig. 5*. Numerical system for NMR spectra interpretation of 3-piperidyn-3-yl-1H-indole derivatives* ***8.1*** *(R_3 = -H);* ***8.2*** *(R_3 = -F);* ***8.3*** *(R_3 = -OCH_3).*

*3.2.2.1. 3-(3-piperidyl)-1H-indole hydrochloride (**8.1**).*

The title compound was isolated as white crystals. Yield: 68.0 %, m.p. 295.2-297.9 °C

^{1}H NMR (500 MHz, D_2O): δ 7.70 (C4"H, 1H, dd, ^3J=8.0), 7.53 (C7"H,1H, m, ^3J=7.0 , ^4J=0.5), 7.27 (C6"H, 1H, m), 7.21 (C2"H, 1H, s), 7.19 (C5"H, 1H, m), 3.57 (CaH(E), 1H, m), 3.48 (CeH(E), 1H, m), 3.30 (CbH(A), 1H, tt, $^3J_{A\text{-}A}$=12.0 , $^3J_{A\text{-}E}$=4.0), 2.99 (CaH(A),CeH(A), 2H, m), 2.14 (CcH(E), 1H, m), 2.05 (CdH(E),1H, m), 1.89 (CdH(A), 1H, m), 1.76 (CcH(A), 1H, m).

^{13}C NMR (125 MHz, D_2O): δ 135.7 (C7"a, s), 124.9 (C3"a, s), 121.8 (C6", s), 121.6 (C2", s), 118.9 (C5", s), 118.0 (C4", s), 114.4 (C3", s), 111.6 (C7", s), 48.3 (Ca,s), 43.7 (Ce, s), 30.6 (Cb,s), 28.3 (Cc, s), 21.9 (Cd, s).

ESI-HRMS m/z: Calcd for $C_{13}H_{17}N_2$ $[M+H]^+$ 201.13917. Found: 201.13874.

$C_{13}H_{17}N_2Cl$: Mol. Wt.: 236.74 g mol^{-1}; Anal. Calc.: Found% (Calc%): C, 65.87 (65.95); H, 7.24 (7.20); N, 11.83 (11.56).

*3.2.2.2. 5-fluoro-3-(3-piperidyl)-1H-indole hydrochloride (**8.2**).*

The title compound was isolated as white crystals. Yield: 70.2 %, m.p. 270.3-273.1 °C

^{1}H NMR (500 MHz, D_2O): δ 7.44 (C7"H, [1H], 4d, ^3J=9.0, $^4J_{H\text{-}F}$=5.0, ^5J=0.5), 7.35 (C4"H, [1H], 4d, $^3J_{H\text{-}F}$=10.0, ^4J=2.5, ^5J=0.5), 7.24 (C2"H, [1H], s), 7.03 (C6"H, [1H], 8d, ^3J=9.5, $^3J_{H\text{-}F}$=8.0, ^4J=2.5, ^pJ=0.5), 3.56 (CaH(E). [1H], m), 3.49 (CeH(E), [1H], m), 3.22 (CbH, [1H], tt, $^3J_{A\text{-}A}$=12.0, $^3J_{A\text{-}E}$=3.5), 3.01 (CeH(A), [1H], td, ^2J=$^3J_{A\text{-}A}$=13.0, $^3J_{A\text{-}E}$=3.5), 2.98 (CaH(A), [1H], t, ^2J=$^3J_{A\text{-}A}$=12.5), 2.01 – 2.14 (CcH(E),CdH(E), [2H], m), 1.83 – 1.95 (CdH(A), [1H], m), 1.72 (CcH(A), [1H], kd, ^2J=$^3J_{A\text{-}A}$=12.0, $^3J_{A\text{-}E}$=4.0).

^{13}C NMR (125 MHz, D_2O): δ 156.9 (C5", d, ^{1}J=231.9), 132.2 (C3", s), 125.1 (C3"a, d, ^{3}J=9.9), 123.3 (C2", s), 114.6 (C7"a, d, ^{4}J=4.8), 112.6 (C7", d, ^{3}J=9.9), 109.8 (C6", d, ^{2}J=26.4), 102.5 (C4", d, ^{2}J=23.8), 48.2 (Ca, s), 43.7 (Ce, s), 30.5 (Cb, s), 28.2 (Cc, s), 21.9 (Cd, s).

ESI-HRMS m/z: Calcd for $C_{13}H_{16}FN_2$ $[M+H]^+$ 219.12975. Found: 219.12935.

$C_{13}H_{16}ClFN_2$: Mol. Wt.: 254.73 g mol^{-1}; Anal. Calc.: Found% (Calc%): C, 61.26 (61.30); H, 6.30 (6.24); N, 11.10 (10.99).

*3.2.2.3. 5-methoxy-3-(3-piperidyl)-1H-indole hydrochloride (**8.3**).*

The title compound was isolated as white crystals. Yield: 68.0 %, m.p. 276.9-278.4 °C

^{1}H NMR (500 MHz, D_2O): δ 7.46 (C7"H, [1H], d, ^{3}J=9.0), 7.27 (C2"H, [1H], s), 7.22 (C4"H, [1H], d, ^{4}J=2.5), 6.96 (C6"H, [1H], dd, ^{3}J=9.0, ^{4}J=2.5), 3.90 (OCH_3, [3H], s), 3.63 (CaH(E), [1H], m), 3.51 (CeH(E), [1H], m), 3.32 (CbH, [1H], tt, $^{3}J_{A\text{-}A}$=12.0, $^{3}J_{A\text{-}E}$=4.0), 3.08 (CaH(A), [1H], t, ^{2}J=$^{3}J_{A\text{-}A}$=12.5), 3.06 (CeH(A), [1H], td, ^{2}J=$^{3}J_{A\text{-}A}$=13.0, $^{3}J_{A\text{-}E}$=3.0), 2.22 (CcH(E), [1H], m), 2.09 (CdH(E), [1H], m), 1.93 (CdH(A), [1H], m), 1.81 (CcH(A), [1H], kd, ^{2}J=$^{3}J_{A\text{-}A}$=13.0, $^{3}J_{A\text{-}E}$=3.5).

^{13}C NMR (125 MHz, D_2O): δ 153.0 (C5", s), 131.6 (C7"a, s), 125.7 (C3"a, s), 123.0 (C2", s), 114.8 (C3", s), 113.1 (C6", s), 112.0 (C7", s), 100.9 (C4", s), 56.3 and 56.3 (OCH_3, 2s), 48.7 (Ca, s), 44.2 (Ce, s), 31.0 (Cb, s), 28.9 (Cc, s), 22.4 (Cd, s).*

ESI-HRMS m/z: Calcd for $C_{14}H_{19}N_2O$ $[M+H]^+$ 231.14974. Found: 231.14941.

$C_{14}H_{19}ClN_2O$: Mol. Wt.: 266.77 g mol^{-1}; Anal. Calc.: Found% (Calc%): C, 62.77 (63.03); H, 7.13 (7.18); N, 10.28 (10.50).

*3.2.3. General procedure for the synthesis of 2-[4-[3-(1H-indol-3-yl)-1-piperidyl]butyl]-4-phenyl-pyrido[1,2-c]pyrimidine-1,3-diones (**9.1–9.27**)*

Compounds **5.1–5.9** (0.75 mmol), **8.1–8.3** (0.75 mmol), K_2CO_3 (1.65 mol) and 25 mL of acetonitrile were stirred at 45 °C for 4–5 h. Reaction time was monitored using TLC. After cooling, the mixture was filtered, and the filtrate evaporated to dryness. The crude residue was purified by flash chromatography, using CH_2Cl_2/MeOH/TEA (9:1:0.2, 8:2:0.1 v/v) mixture. Proper fractions were identified by TLC and evaporated to dryness giving analytically pure compounds **9.1–9.27.**

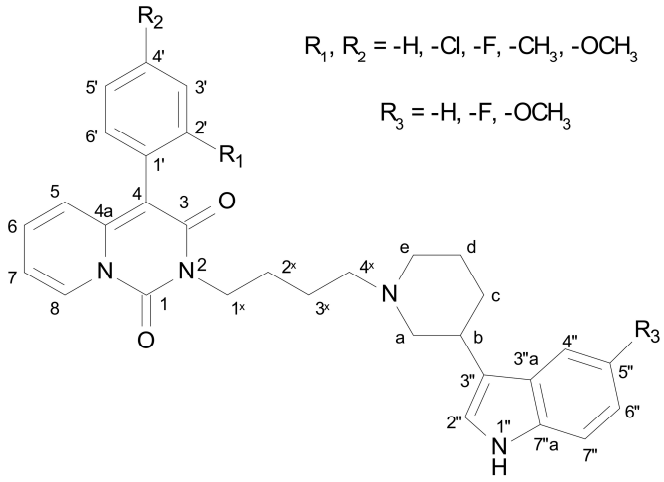


Fig. 6**. Numbering system for NMR spectra interpretation of compounds **9.1–9.27

*3.2.3.1. 2-[4-[3-(1H-indol-3-yl)-1-piperidyl]butyl]-4-phenyl-pyrido[1,2-c]pyrimidine-1,3-dione (**9.1**).*

The title compound was isolated as a yellow powder. Yield: 66.0 %, m.p. 123.6-125.4 °C. HPLC t_R = 5.52 min, 99.5% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.31 (C8H, [1H], d, ^{3}J=8.0), 8.26 (N1"H, [1H], bs), 7.65 (C4"H, [1H], d, ^{3}J=7.5), 7.43 (C3'H,C5'H, [2H], t, ^{3}J=7.0), 7.38 – 7.32 (C4'H,C7"H, [2H], m), 7.30 (C2'H,C6'H, [2H], d, ^{3}J=7.0), 7.16 (C6"H, [1H], m), 7.08 (C5"H, [1H], m), 6.96 (C2"H, [1H], bs), 6.88 (C5H,C6H, [2H], m), 6.36 (C7H, [1H], m), 4.17 (C1^{x}H$_2$, [2H], t, ^{3}J=7.0), 3.25 (CbH,CaH(E), [2H], bs), 3.05 (CeH(E), [1H], bs), 2.54 (C4^{x}H$_2$, [2H], bs), 2.16 (CaH(A),CeH(A), [2H], bs), 2.06 (CcH(E), [1H], pd), 2.00 – 1.60 (CdH$_2$,C2^{x}H$_2$,C3^{x}H$_2$, [6H], m), 1.51 (CcH(A), [1H], m).

^{13}C NMR (125 MHz, $CDCl_3$): δ 160.2 (C3, s), 148.9 (C1, s), 143.6 (C4a, s), 136.2 (C7"a, s), 132.8 (C6, s), 132.4 (C1', s), 131.2 (C2',C6', s), 128.8 (C3',C5', s), 128.0 (C4', s), 127.8 (C8, s), 126.6 (C3"a, s), 121.9 (C6", s), 121.4 (C5, s), 120.4 (C2", s), 119.2 (C5', s), 119,1 (C4", s), 111.2 (C7", s), 110.7 (C7, s), 104.8 (C4, s), 60.2 (Ca, s), 58.4 (C4^x, s), 53.9 (Ce, s), 42.1 (C1^x, s), 33.5 (Cb, s), 30.9 (Cc, s), 25.4 (Cd, s), 25.0 (C2^x, s), 24.0 (C3^x, s).

ESI-HRMS m/z: Calcd for $C_{31}H_{33}N_4O_2$ $[M+H]^+$ 493.26035*. Found: 493.26028.*

*3.2.3.2. 4-(2-chlorophenyl)-2-[4-[3-(1H-indol-3-yl)-1-piperidyl]butyl]pyrido[1,2-c]pyrimidine-1,3-dione (**9.2**).*

The title compound was isolated as a yellow powder. Yield: 90.3 %, m.p. 175.2-177.7 °C. HPLC t_R = 5.63 min, 99.9% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.34 (C8H, [1H], 4t: (1): 8.347, ^{3}J=7.5, ^{4}J=^{5}J=1.0, (2): 8.34, ^{3}J=7.5, ^{4}J=^{5}J=1.0), 8.23 (N1"H, [1H], bs), 7.65 (C4"H, [1H], d, ^{3}J=8.0), 7.49 (C3'H, [1H], m (14 lines)), 7.34 – 7.26 (C4'-6'H,C7"H, [4H], m), 7.15 (C6"H, [1H], m, $^3J_1$=8.0, $^3J_2$=7.0, ^{4}J=1.0), 7.08 (C5"H, [1H], m), 6.96 (C2"H, [1H], bs), 6.93 (C6H, [1H}, m (11 lines)), 6.53 (C5H, [1H], dt, ^{3}J=9.5, ^{4}J=^{5}J=1.0), 6.39 (C7H, [1H], m(14 lines)), 4.18 (C1^{x}H$_2$, [2H], t, ^{3}J=7.5), 3.20 (CaH(E),CbH, [2H], pd), 2.99 (CeH(E), [1H], bs), 2.47 (C4^{x}H$_2$, [2H], bs), 2.07 (CaH(A),CeH(A),CcH(E), [3H], m), 1.78 (CdH$_2$,C2^{x}H$_2$, [4H], m), 1.66 (C3^{x}H$_2$, [2H], m), 1.89 (CcH(A), [1H], m).

^{13}C NMR (125 MHz, $CDCl_3$): δ 159.4 and 159.5 (C3, 2s), 148.9 (C1, s), 143.8 (C4a, s), 136.2 (C7"a, s), 135.2 and 135.7 (C2', 2s), 133.4 (C6, 2s), 133.0 (C6', s), 131.8 (C1', s), 130.0 (C3', s), 129.6 (C4', s), 128.1 (C8, s), 127.3 (C5', 2s), 126.7 (C3"a, s), 121.8 (C6", s), 121.0 (C5, s), 120.3 (C2", s), 119.1 (C5', s), 119.1 (C4", s), 111.2 (C7", s), 110.7 (C7, s), 102.2 (C4, s), 60.7 (Ca, s), 58.5 ($C4^x$, s), 54.0 (Ce, s), 42.3 ($C1^x$, s), 33.7 (Cb, s), 31.2 (Cc, s), 25.5 (Cd, s), 25.0 ($C2^x$,s), 24.5 ($C3^x$, s).

ESI-HRMS m/z: Calcd for $C_{31}H_{32}ClN_4O_2$ $[M+H]^+$ 527.22138. Found: 527.22162.

*3.2.3.3. 4-(2-fluorophenyl)-2-[4-[3-(1H-indol-3-yl)-1-piperidyl]butyl]pyrido[1,2-c]pyrimidine-1,3-dione (**9.3**).*

The title compound was isolated as a yellow powder. Yield: 93.5 %, m.p. 137.8-139.7 °C. HPLC t_R = 5.21 min, 99.9% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 – 8.31 (C8H, [1H], m (12 lines)), 8.15 (N1"H, [1H], bs), 7.7 (C4"H, [1H], d, ^{3}J=8.0), 7.40 – 7.28 (C4'H,C6'H, [2H], m), 7.34 (C7"H, [1H], d, ^{3}J=8.0), 7.24 – 7.19 (C5'H, [1H], m), 7.19 – 7.11 (C3'H,C6"H, [2H], m), 7.09 (C5"H, [1H], m), 7.00 – 6.93 (C2"H,C6H, [2H], m), 6.72 (C5H, [1H], m), 6.43 – 6.38 (C7H, [1H], m (14 lines)), 4.18 ($C1^xH_2$, [2H], t, ^{3}J=7.5), 3.23 (CaH(E),CbH, [2H], bs), 3.02 (CeH(E), [1H], bs), 2.50 ($C4^xH_2$, [2H], bs), 2.20 – 2.00 (CaH(A),CeH(A),CcH(E), [3H], m), 1.80 – 1.60 (CdH_2,$C2^xH_2$,$C3^xH_2$, [6H], m), 1.52 (CcH(A), [1H], m).

^{13}C NMR (125 MHz, $CDCl_3$): δ 160.9 (C2', d, ^{1}J=247.0), 159.6 (C3, s), 148.9 (C1, s), 144.0 (C4a, s), 136.2 (C7"a, s), 133.5 (C6', d), 133.1 (C6, s), 130.1 (C4', d, ^{3}J=8.2), 128.2 (C8, s), 127.3 (C3", s), 126.7 (C3"a, s), 124.4 (C5', d), 121.9 (C6", s), 121.2 (C5, s), 120.3 (C1', d, ^{2}J=16.0), 120.3 (C2", s), 119.2 (C4',C5', s), 116.1 (C3', d, ^{2}J=22.4), 111.2 (C7", s), 110.8 (C7, s), 98.3 (C4, s), 61.0 (Ca, s), 58.5 ($C4^x$, s), 54.0 (Ce, s), 42.3 ($C1^x$, s), 33.0 (Cb, s), 31.0 (Cc, s), 25.5 (Cd, s), 25.0 ($C2^x$, s), 24.0 ($C3^x$, s).

ESI-HRMS m/z: Calcd for $C_{31}H_{32}FN_4O_2$ $[M+H]^+$ 511.25092. Found: 511.25108.

*3.2.3.4. 2-[4-[3-(1H-indol-3-yl)-1-piperidyl]butyl]-4-(o-tolyl)pyrido[1,2-c]pyrimidine-1,3-dione (**9.4**).*

The title compound was isolated as a yellow powder. Yield: 93.7 %, m.p. 142.1-144.8 °C. HPLC t_R = 6.08 min, 99.3% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.43 (N1"H, [1H], bs), 8.31 (C8H, [1H], dt, ^{3}J=7.5, ^{4}J=^{5}J=1.0), 7.64 (C4"H, [1H], d, ^{3}J=8.0), 7.31 (C7"H, [1H], d, ^{3}J=8.0), 7.30 – 7.20 (C4'-6'H, [3H], m), 7.16 – 7.10 (C3'H,C6"H, [2H], m), 7.06 (C5"H, [1H], m, $^3J_1$=8.0, $^3J_2$=7.5, ^{4}J=1.0), 6,923 (C2"H, [1H], bs), 6.86 (C6H, [1H], 4d, $^3J_1$=9.5, $^3J_2$=6.0, ^{4}J=1.0), 6.53 (C5H, [1H], dt, ^{3}J=9.0, ^{4}J=^{5}J=1.0), 6.35 (C7H, [1H], m, $^3J_1$=7.5, $^3J_2$=9.0, ^{4}J=1.0), 4.18 ($C1^xH_2$, [2H], t, ^{3}J=7.5), 3.23 (CaH(E),CbH, [2H], m), 3.02 (CeH(E), [1H], pd), 2.51 ($C4^xH_2$, [2H], bs), 2.13 and 2.10 ([6H], 2s), 2.07 – 2.00 (CaH(A),CeH(A),CcH(E), [3H], m), 1.85 – 1.73 (CdH_2,$C2^xH_2$, [4H], m), 1.67 ($C3^xH_2$, [2H], m), 1.48 (CcH(A), [1H], kd, ^{2}J=$^3J_{A\text{-}A}$=12.5, $^3J_{A\text{-}E}$=3.5).

^{13}C NMR (125 MHz, $CDCl_3$): δ 159.7 (C3, s), 149.0 (C1, s), 143.5 (C4a, s), 138.4 and 138.5 (C2', 2s), 136.2 (C7"a, s), 132.5 (C6, s), 131.5 and 131.6 (C3', 2s), 130.5 (C6', s), 128.3 (C4', s), 128.0 (C8, s), 126.6 (C3"a, s), 126.3 (C5', 2s), 121.8 (C6", s), 121.3 (C5, s), 120.4 (C2", s), 119.1 (C4",C5", s), 111.2 (C7", s), 110.6 (C7, s), 104.0 (C4, s), 60.3 (Ca, s), 58.3 ($C4^x$, s), 53.8 (Ce, s), 42.0 ($C1^x$,s), 33.5 (Cb, s), 31.0 (Cc, s), 25.5 (Cd, s), 25.2 ($C2^x$, s), 23.6 ($C3^x$, s), 19.6 (CH_3, 2s).

ESI-HRMS m/z: Calcd for $C_{32}H_{35}N_4O_2$ $[M+H]^+$ 507.27600*. Found: 507.27636.*

*3.2.3.5. 2-[4-[3-(1H-indol-3-yl)-1-piperidyl]butyl]-4-(2-methoxyphenyl)pyrido[1,2-c]pyrimidine-1,3-dione (**9.5**).*

The title compound was isolated as a yellow powder. Yield: 66.6 %, m.p. 102.5-104.1 °C. HPLC t_R = 5.28 min, 99.9% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.38 (N1"H, [1H], bs), 8.29 (C8H, [1H], m(8 lines)), 7.64 (C4"H, [1H], d, ^{3}J=8.0), 7.36 (C4'H, [1H], m), 7.31 (C7"H, [1H], d, ^{3}J=8.0), 7.19 (C6"H, [1H], m , $^3J_1$=7.5, $^3J_2$=6.0, ^{4}J=1.5), 7.14 (C6'H, [1H], m), 7.07 (C5"H, [1H], m), 7.02 (C5'H, [1H], m), 6.98 – 6.91 (C2"H,C3'H, [2H], m), 6.85 (C6H, [1H], m), 6.61 (C5H, [1H], m), 6.34 (C7H, [1H], m), 7.17 ($C1^xH_2$, [2H], m), 3.72 and 3.69 (OCH_3, [3H], 2s), 3.20 (CbH,CaH(E), [2H], m), 3.00 (CeH(E), [1H], bs), 2.18 – 2.00 (CaH(A),CeH(A),CcH(E), [3H], m), 1.78 (CdH_2,$C2^xH_2$, [4H], m), 1.67 ($C3^xH_2$, [2H], m), 1.48 (CcH(A), kd, $^2J=^3J_{A-A}$=12.5, $^3J_{A-E}$=4.5).

^{13}C NMR (125 MHz, $CDCl_3$): δ 159.9 (C3, s), 157.9 (C2', 2s), 149.1 (C1, s), 143.6 (C4a, s), 136.2 (C7"a, s), 133.0 (C6', 2s), 132.0 (C6, s), 129.6 (C4', s), 127.8 (C8, s), 126.6 (C3"a, s), 123.0 (C3", s), 121.9 (C5, s), 121.7 (C6", s), 121.4 (C1', s), 120.9 (C5', 2s), 120.4 (C2", s), 119.1 (C5", s), 119.1 (C4", s), 111.4 and 111.3 (C3', 2s), 111.2 (C7", s), 110.5 (C7, s), 101.2 (C4, s), 60.4 (Ca, s), 58.5 ($C4^x$, s), 55.6 (OCH_3, 2s), 53.9 (Ce, s), 42.1 ($C1^x$, s), 33.5 (Cb, s), 31.1 (Cc, s), 25.5 (Cd, s), 25.3 ($C2^x$, s), 23.8 ($C3^x$, s).

ESI-HRMS m/z: Calcd for $C_{32}H_{35}N_4O_3$ $[M+H]^+$ 523.27091. Found: 523.27104.

*3.2.3.6. 4-(4-chlorophenyl)-2-[4-[3-(1H-indol-3-yl)-1-piperidyl]butyl]pyrido[1,2-c]pyrimidine-1,3-dione (**9.6**).*

The title compound was isolated as a yellow powder. Yield: 87.8 %, m.p. 229.6-232.3 °C. HPLC t_R = 6.87 min, 97.2% purity.

^{1}H NMR (500 MHz, DMSO – d_6): δ 10.82 (N1"H, [1H], bs), 8.29 (C8H, [1H], dt, ^{3}J=7.5, $^4J=^5$J=1.0), 7.53 (C4"H, [1H], d, ^{3}J=7.5), 7.46 (C2'H,C6'H, [2H], dt, ^{3}J=8.5, ^{4}J=2.5), 7.33 (C7"H, [1H], d, ^{3}J=8.0), 7.28 (C3'H,C5'H, [2H], dt, ^{3}J=8.5, ^{4}J=2.5), 7,139 (C6"H, [1H], 4d, $^3J_1$=9.5, $^3J_2$=6.0, ^{4}J=1.0), 7.12 (C2"H, [1H], d, ^{3}J=2.0), 7.05 (C5"H, [1H], m), 6.95 (C6H, [1H], m), 6.78 (C5H, [1H], dt, ^{3}J=9.5, $^4J=^5$J=1.0), 6.57 (C7H, [1H], m, $^3J_1$=7.5, $^3J_2$=6.0, ^{4}J=1.5), 4.00 ($C1^xH_2$, [2H], t, ^{3}J=7.0), 3.34 (CaH(E),CbH, [2H], bs), 3.03 (CeH(E), [1H], pt), 2.43 ($C4^xH_2$, [2H], bs), 2.07 (CaH(A),CeH(A), [2H], bs), 1.95 (CcH(E), [1H], pd), 1.72 (CdH(E), [1H], bs), 1.65 ($C2^xH_2$, [2H], q, ^{3}J=6.5), 1.53 ($C3^xH_2$,CcH(A),CdH(A), [4H], bs).

^{13}C NMR (125 MHz, $CDCl_3$): δ 160.0 (C3, s), 148.8 (C1, s), 143.7 (C4a, s), 136.2 (C7"a, s), 133.7 (C4', s), 133.0 (C6, s), 132.7 (C3',C5', s), 131.3 (C1', s), 129.0 (C2',C6', s), 128.1 (C8, s), 126.5 (C3"a, s), 122.0 (C6", s), 121.0 (C5, s), 120.4 (C2", s), 119.3 (C5", s), 119.2 (C4", s), 111.2 (C7", s), 110.9 (C7, s), 103.4 (C4, s), 60.1 (Ca, s), 58.3 ($C4^x$, s), 53.8 (Ce, s), 42.1 ($C1^x$, s), 33.2 (Cb, s), 30.7 (Cc, s), 25.3 (Cd, s), 25.0 ($C2^x$, s), 23.3 ($C3^x$, s).

ESI-HRMS m/z: Calcd for $C_{31}H_{32}ClN_4O_2$ $[M+H]^+$ 527.22138*. Found:* 527.22177*.*

*3.2.3.7. 4-(4-fluorophenyl)-2-[4-[3-(1H-indol-3-yl)-1-piperidyl]butyl]pyrido[1,2-c]pyrimidine-1,3-dione (**9.7**).*

The title compound was isolated as a yellow powder. Yield: 69.7 %, m.p. 209.6-212.2 °C. HPLC t_R = 5.56 min, 99.7% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.32 (N1"H, [1H], bs), 8.31 (C8H, [1H], dt, ^{3}J=7.5), 7.65 (C7"H,[1H], d, ^{3}J=8.0), 7.26 (C2'H,C6'H, [2H], pt), 7.16 (C6"H, [1H], m), 7.13 – 7.05 (C3'H,C5'H,C5"H, [3H], m), 6.96 (C2"H, [1H], d, ^{3}J=1.5), 6.91 (C6H, [1H], 4d, $^3J_1$=9.5, $^3J_2$=6.5, ^{4}J=1.0), 6.83 (C5H, [1H], dt, ^{3}J=9.5), 6.37 (C7H, [1H], m, $^3J_1$=7.5, $^3J_2$=6.0, ^{4}J=1.0), 4.16 (C1^{x}H$_2$, [2H], t, ^{3}J=7.0), 3.26 (CaH(E),CbH, [2H], m), 3.07 (CeH(E), [1H], bs), 2.54 (C4^{x}H$_2$, [2H], bs), 2.16 (CaH(A),CeH(A), [2H], bs), 2.06 (CcH(E), [1H], pd), 2.00 – 1.60 (C2^{x}H$_2$,C3^{x}H$_2$,CdH$_2$, [6H], m), 1.51 (CcH(A), [1H], kd, $^2J=^3J_{A\text{-}A}$=12.5, $^3J_{A\text{-}E}$=3.5).

^{13}C NMR (125 MHz, $CDCl_3$): δ 162.3 (C4', d, ^{1}J=247.0), 160.2 (C3, s), 148.8 (C1, s), 143.7 (C4a, s), 136.2 (C7"a, s), 133.0 (C2',C6', d, ^{3}J=8.0), 132.8 (C6, s), 128.6 (C1', d, ^{4}J=3.3), 128.1 (C8, s), 126.5 (C3"a, s), 121.9 (C6", s), 121.1 (C5, s), 120.4 (C2", s), 119.2 (C5", s), 119.1 (C4", s), 115.8 (C3',C5', d, ^{2}J=21.5), 115.0 (C3", s), 111.2 (C7", s), 110.8 (C7, s), 103.6 (C4, s), 60.2 (Ca, s), 58.3 (C4^x, s), 53.9 (Ce, s), 42.1 (C1^x, s), 33.4 (Cb, s), 30.8 (Cc, s), 25.4 (Cd, s), 25.1 (C2^x, s), 23.5 (C3^x, s).

ESI-HRMS m/z: Calcd for $C_{31}H_{32}FN_4O_2$ $[M+H]^+$ 511.25092. *Found:* 511.25112.

*3.2.3.8. 2-[4-[3-(1H-indol-3-yl)-1-piperidyl]butyl]-4-(p-tolyl)pyrido[1,2-c]pyrimidine-1,3-dione (**9.8**).*

The title compound was isolated as a yellow powder. Yield: 74.0 %, m.p. 217.8-219.9 °C. HPLC t_R = 6.97 min, 96.6% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.55 (N1"H, [1H],bs), 8.28 (C8H, [1H], d, ^{3}J=7.5), 7.64 (C4"H, [1H], d, ^{3}J=8.0), 7.33 (C7"H, [1H], d, ^{3}J=8.0), 7.23 (C2'H,C6'H, [2H], pd), 7.17 (C3'H,C5'H, [2H], pd), 7.14 (C6"H, [1H], m), 7.06 (C5"H, [1H], m), 6.92 (C2"H, [1H], bs), 6.88 (C5H,C6H, [2H], m), 6.34 (C7H, [1H], m, $^3J_1$=7.5, $^3J_2$=5.5, ^{4}J=2.0), 4.16 (C1^{x}H$_2$, [2H], t, ^{3}J=7.0), 3.35 (CbH, [1H], bs), 3.26 (CaH(E), [1H], pd), 3.12 (CeH(E), [1H], bs), 2.60 (C4^{x}H$_2$, [2H], bs), 2.38 (CH$_3$, [3H], s), 2.19 (CaH(A),CeH(A), [2H], m), 2.04 (CcH(E), [1H], pd), 1.95 (CdH(E), [1H], bs), 1.83 – 1.65 (CdH(A),C2^{x}H$_2$,C3^{x}H$_2$, [5H],m), 1.48 (CcH(A), [1H], kd, $^2J=^3J_{A\text{-}A}$=12.5, $^3J_{A\text{-}E}$=3.5).

^{13}C NMR (125 MHz, $CDCl_3$): δ 160.3 (C3, s), 148.9 (C1, s), 143.5 (C4a, s), 137.5 (C4', s), 136.2 (C7"a, s), 132.3 (C6, s), 131.0 (C3',C5', s), 129.7 (C1', s), 129.5 (C2',C6', s), 127.9 (C8, s), 126.4 (C3"a, s), 121.9 C6", s), 121.5 (C5, s), 120.5 (C2", s), 119.2 (C5", s), 119.1 (C4", s), 118.0 (C3", s), 111.3 (C7", s), 110.7 (C7, s), 104.7 (C4, s), 59.8 (Ca, s), 58.0 (C4^x, s), 53.5 (Ce, s), 41.8 (C1^x, s), 32.9 (Cb, s), 30.5 (Cc, s), 25.2 {Cd, s), 24.7 (C2^x, s), 23.1 (C3^x, s), 21.3 (CH$_3$, s).

ESI-HRMS m/z: Calcd for $C_{32}H_{35}N_4O_2$ $[M+H]^+$ 507.27600. *Found:* 507.27579.

*3.2.3.9. 2-[4-[3-(1H-indol-3-yl)-1-piperidyl]butyl]-4-(4-methoxyphenyl)pyrido[1,2-c]pyrimidine-1,3-dione (**9.9**).*

The title compound was isolated as a yellow powder. Yield: 92.6 %, m.p. 117.2-119.8 °C. HPLC t_R = 5.70 min, 99.7% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.29 (C8H, [1H], dt, ^{3}J=7.5, $^4J=^5$J=1.0), 8.23 (N1"H, [1H], bs), 7.65 (C4"H, [1H], dd, ^{3}J=8.0, ^{4}J=0.5), 7.32 (C7"H, [1H], dt, ^{3}J=8.0, $^4J=^5$J=1.0), 7.22 (C2'H,C6'H, [2H], dt, ^{3}J=9.0, ^{4}J=3.0), 7.15 (C6"H, [1H], m, $^3J_1$=8.0, $^3J_2$=7.0, ^{4}J=1.0), 7.08 (C5"H, [1H], m, $^3J_1$=8.0, $^3J_2$=7.0, ^{4}J=1.0), 6.96 (C3'H,C5'H,C2"H, [3H], m), 6.89 (C5H, [1H], m, ^{3}J=9.5, ^{4}J=1.5, ^{5}J=1.0), 6.86 (C6H, [1H], 4d, $^3J_1$=9.5,

$^{3}J_{2}$=6.0, ^{4}J=1.5), 6.33 (C7H, [1H], m, $^{3}J_{1}$=7.5, $^{3}J_{2}$=6.0, ^{4}J=1.5), 4.17 (C1$^{x}H_{2}$, [2H], m), 3.83 (OCH_{3}, [3H], s), 3,178 (CbH,CaH(E), [2H], m), 2.97 (CeH(E), [1H], pd), 2.44 (C4$^{x}H_{2}$, [2H], t, ^{3}J=7.5), 2.13 – 1.98 (CaH(A),CeH(A),CcH(E), [3H], m), 1.82 – 1.73 (C2$^{x}H_{2}$,CdH_{2}, [4H], m), 1.64 (C3$^{x}H_{2}$, [2H], q, ^{3}J=7.5), 1.50 (CcH(A), [1H], m).

^{13}C NMR (125 MHz, $CDCl_{3}$): δ 160.4 (C3, s), 159.1 (C4', s), 148.9 (C1, s), 143.5 (C4a, s), 136.2 (C7"a, s), 132.3 (C2',C6', s), 132.1 (C6, s), 127.9 (C8, s), 126.7 (C3"a, s), 124.9 (C1', s), 121.8 (C6", s), 121.6 (C5, s), 120.3 (C2", s), 119.6 (C3", s), 119.1 (C5", s), 119.1 (C4", s), 114.3 (C3',C5', s), 111.1 (C7", s), 110.5 (C7, s), 104.6 (C4, s), 60.8 (Ca, s), 58.7 (C4^{x}, s), 55.3 (OCH_{3}, s), 54.2 (Ce, s), 42.4 (C1^{x}, s), 33.9 (Cb, s), 31.4 (Cc, s), 25.6 (Cd, s), 25.0 (C2^{x}, s), 24.0 (C3^{x},s).

ESI-HRMS m/z: Calcd for $C_{32}H_{35}N_{4}O_{3}$ $[M+H]^{+}$ 523.27092*. Found:* 523.27130*.*

*3.2.3.10. 2-[4-[3-(5-fluoro-1H-indol-3-yl)-1-piperidyl]butyl]-4-phenyl-pyrido[1,2-c]pyrimidine-1,3-dione (**9.10**).*

The title compound was isolated as a yellow powder. Yield: 94.5 %, m.p. 103.8-105.4 °C. HPLC t_{R} = 5.96 min, 99.9% purity.

^{1}H NMR (500 MHz, $CDCl_{3}$): δ 8.39 (N1"H, [1H], bs), 8.32 (C8H, [1H], d, ^{3}J=7.5), 7.43 (C3'H,C5'H, [2H], t, ^{3}J=7.5), 7.35 (C4'H, [1H], tt, ^{3}J=7.5), 7.30 (C2'H,C6'H, [2H], dd, ^{3}J=7.5), 7.26 (C4"H, [1H], dd, $^{3}J_{H\text{-}F}$=10.0, ^{4}J=2.5), 7.20 (C7"H, [1H], dd, ^{3}J=9.0, $^{4}J_{H\text{-}F}$=4.0), 7.00 (C2"H, [1H], bs), 6.88(C5H,C6H,C6"H, [3H], m), 6.36 (C7H, [1H], m), 4.18 (C1$^{x}H_{2}$, [2H], m), 3.13 (CbH,CaH(E), [2H], pd), 2.96 (CeH(E), [1H], pd), 2.46 (C4$^{x}H_{2}$, [2H], t, ^{3}J=7.0), 2.11 (CaH(A),CeH(A), [2H], m), 2.02 (CcH(E), [1H], m), 1.78 (C2$^{x}H_{2}$,CdH_{2}, [4H], m), 1.65 (C3$^{x}H_{2}$, q, ^{3}J=7.5), 1.47 (CcH(A), [1H], m).

^{13}C NMR (125 MHz, $CDCl_{3}$): δ 160.2 (C3, s), 157.5 (C5", d, ^{1}J=234.0), 148.9 (C1, s), 143.6 (C4a, s), 132.8 (C7"a, s), 132.7 (C1', s), 132.4 (C6, s), 131.2 (C2',C6', s), 128.7 (C3',C5', s), 127.9 (C4', s), 127.8 (C8, s), 127.0 (C3"a, d, ^{3}J=9.4), 122.3 (C2", s), 121.4 (C5, s), 119.4 (C3", d), 111.7 (C7", d, ^{3}J=9.6), 110.7 (C7, s), 110.1 (C6",d, ^{2}J=26.3), 104.8 (C4, s), 103.9 (C4", d, ^{2}J=23.3), 60.4 (Ca, s), 58.5 (C4^{x}, s), 54.0 (Ce, s), 42.3 (C1^{x}, s), 33.6 (Cb, s), 31.1 (Cc, s), 25.5 (C2^{x},s), 25.5 (Cd,s), 24.0 (C3^{x}, s).

ESI-HRMS m/z: Calcd for $C_{31}H_{32}FN_{4}O_{2}$ $[M+H]^{+}$ 511.25093*. Found:* 511.25105*.*

*3.2.3.11. 4-(2-chlorophenyl)-2-[4-[3-(5-fluoro-1H-indol-3-yl)-1-piperidyl]butyl]pyrido[1,2-c]pyrimidine-1,3-dione (**9.11**).*

The title compound was isolated as a yellow powder. Yield: 93.0 %, m.p. 114.6-116.9 °C. HPLC t_{R} = 5.90 min, 99.9% purity.

^{1}H NMR (500 MHz, $CDCl_{3}$): δ 8.36 (N1"H, [1H], bs), 8.36 and 8.35 (C8H, [1H], m (10 lines)), 7.51 – 7.46 (C3'H, [1H], m (12 lines)), 7.35 – 7.26 (C4'-6'H,C4"H, [4H], m), 7.22 (C7"H, [1H], dd, ^{3}J=8.5, $^{4}J_{H\text{-}F}$=4.5), 7.01 (C2"H, [1H], pt), 6.95 (C6H, [1H], m (11 lines)), 6.89 (C6"H, [1H], td, ^{3}J=9.0, ^{4}J=2.5), 6.54 (C5H, [1H], dt), 6.41 (C7H, [1H], 2m (14 lines), $^{3}J_{1}$=8.0, $^{3}J_{2}$=6.0, ^{4}J=1.0), 4.19 (C1$^{x}H_{2}$, [2H], m), 3.14 (CbH,CaH(E), [2H], pd), 2.99 (CeH(E), [1H], bs), 2.48 (C4$^{x}H_{2}$, [2H], bs), 2.08 (CaH(A),CeH(A), [2H], m), 2.02 (CcH(E), [1H], pd), 1.78 (C2$^{x}H_{2}$,CdH_{2}, [4H], m), 1.66 (C3$^{x}H_{2}$, [2H], q, ^{3}J=7.0), 1.47 (CcH(A), [1H], m).

^{13}C NMR (125 MHz, $CDCl_{3}$): δ 159.5 (C3, 2s), 157.5 (C5', d, ^{1}J=234.1), 148.9 (C1, s), 143.9 (C4a, 2s), 135.7 (C2', 2s),133.4 (C6', 2s), 133.1 (C1', s), 131.8 (C7"a, s), 130.0 (C3', 2s), 129.6 (C4', s), 128.1 (C8,

s), 127.3 (C5', 2s), 127.0 (C3"a, d, ^{3}J=9.2), 122.3 (C2', s), 121.0 (C5, s), 119.3 (C3", s), 111.8 (C7", d, ^{3}J=9.7), 110.8 (C7, s), 110.1 (C6", d, ^{2}J=26.3), 104.0 (C4", d, ^{2}J=23.1), 102.2 and 102.1 (C4, 2s), 60.3 (Ca, s), 58.4 (C4^{x}, s), 53.9 (Ce, s), 42.2 (C1^{x}, s), 33.6 (Cb, s), 31.1 (Cc, s), 25.5 (Cd, s), 25.2 (C2^{x}, s), 23.7 (C3^{x}, s).

ESI-HRMS m/z: Calcd for $C_{31}H_{31}ClFN_4O_2$ [M+H]$^{+}$ 545.21196*. Found:* 545.21194.

*3.2.3.12. 4-(2-fluorophenyl)-2-[4-[3-(5-fluoro-1H-indol-3-yl)-1-piperidyl]butyl]pyrido[1,2-c]pyrimidine-1,3-dione (**9.12**).*

The title compound was isolated as a yellow powder. Yield: 92.8 %, m.p. 109.3-111.1 °C. HPLC t_R = 5.42 min, 99.9% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.54 (N1"H, [1H],bs), 8.35 (C8H, [1H], m (10 lines)), 7.40 – 7.28 (C4'H,C6'H, [2H], m), 7.26 (C4"H, [1H], dd, $^{3}J_{H\text{-}F}$-9.5, ^{4}J=2.5), 7.24 – 7.17 (C5'H,C7"H, [2H], m), 7.17 – 7.10 (C3'H, [1H], m), 6.99 (C2"H, [1H], bs), 6.96 (C6H, [1H], m), 6.87 (C6"H, [1H], td, ^{3}J=9.0, ^{4}J=2.4), 6.72 (C5H, [1H], d, ^{3}J=9.5), 6.41 (C7H, [1H], m(13 lines)), 4.17 (C1$^{x}H_2$, [2H], m), 3.14 (CbH,CaH(E), [2H], pd), 2.98 (CeH(E), [1H], pd), 2.48 (C4$^{x}H_2$, [2H], pt), 2.17 – 1.96 (CaH(A),CeH(A),CcH(E), [3H], m), 1.78 (CdH$_2$,C2$^{x}H_2$, [4H], m), 1.66 (C3$^{x}H_2$, [2H], q, ^{3}J=7.5), 1.45 (CcH(A), [1H], m).

^{13}C NMR (125 MHz, $CDCl_3$): δ 160.8 (C2', d, ^{1}J=248.0), 159.6 (C3, 2s), 157.4 (C5', d, ^{1}J=234.0), 148.8 (C1, s), 144.0 (C4a, s), 133.4 (C6', 2d, ^{3}J=2.5 i ^{3}J=2.8), 133.2 (C6, s), 132.7 (C7", s), 130.0 (C4', d, ^{3}J=8.3), 128.1 (C8, s), 126.9 (C3"a, d, ^{3}J=9.6), 124.4 (C5', d, ^{4}J=2.4), 122.3 (C2", s), 121.1 (C5, s), 120.3 (C1', d, ^{2}J=16.1), 119.1 (C3", s), 116.0 (C3', 2d, ^{2}J=22,1 i ^{2}J=22.3), 111.8 (C7", d, ^{3}J=9.6), 110.8 (C7, s), 110.0 (C6", d, ^{2}J=26.4), 103.9 (C4", d, ^{2}J=23.3), 98.2 (C4, 2s), 60.3 (Ca, s), 58.4 (C4^{x}, s), 53.8 (Ce, s), 42.3 (C1^{x}, s), 33.5 (Cb, s), 31.0 (Cc, s), 25.4 (Cd, s), 25.2 (C2^{x}, s), 23.8 (C3^{x}, s).

ESI-HRMS m/z: Calcd for $C_{31}H_{31}F_2N_4O_2$ [M+H]$^{+}$ 529.24151*. Found:* 529.24169.

*3.2.3.13. 2-[4-[3-(5-fluoro-1H-indol-3-yl)-1-piperidyl]butyl]-4-(o-tolyl)pyrido[1,2-c]pyrimidine-1,3-dione (**9.13**).*

The title compound was isolated as a yellow powder. Yield: 97.6 %, m.p. 127.5-130.2 °C. HPLC t_R = 6.35 min, 98.7% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.57 (N1"H, [1H], bs), 8.35 – 8.30 (C8H, [1H], m), 7.32 – 7.20 (C4'-6'H,C4"H, [4H], m), 7.18 (C7"H, [1H], dd, ^{3}J=9.0, $^{4}J_{H\text{-}F}$=4.5), 7.12 (C3'H, [1H], m), 6.95 (C2"H, [1H], bs), 6.90 – 6.82 (C6H,C6"H , [2H], m), 6.53 (C5H, [1H], d, ^{3}J=9.5), 6.36 (C7H, [1H], m, $^{3}J_1$=7.5, $^{3}J_2$=6.0, ^{4}J=1.0), 4.19 (C1$^{x}H_2$, [2H], m), 3.11 (CbH,CaH(E), [2H], m), 2.96 (CeH(E), [1H], pd), 2.46 (C4$^{x}H_2$, [2H], t, ^{3}J=6.0), 2.13 and 2.10 (CH_3, [3H], 2s), 2.02 (CaH(A),CeH(A),CcH(E), [3H], m), 1.77 (C2$^{x}H_2$,CdH$_2$, [4H], m), 1.65 (C3$^{x}H_2$, [2H], m), 1.45 (CcH(A), [1H], m).

^{13}C NMR (125 MHz, $CDCl_3$): δ 159.7 (C3, s), 157.4 (C5", d, ^{1}J=234.0), 149.0 (C1, s), 143.5 (C4a, s), 138.5 (C2', 2s), 132.7 (C1', s), 132.5 (C6, s), 132.1 (C7"a, s), 131.6 (C3' 2s), 130.5 (C6', s), 128.3 (C4', s), 128.0 (C8, s), 126.9 (C3"a, d, ^{3}J=9.6), 126.3 (C5', 2s), 122.4 (C2", s), 121.3 (C5, s), 119.1 (C3", s), 111.8 (C7", d, ^{3}J=9.6), 110.6 (C7, s), 109.9 (C6", d, ^{2}J=26.3), 104.0 (C4, s), 103.8 (C4", d, ^{2}J=23.3), 60.4 (Ca, s), 58.5 (C4^{x}, s), 53.9 (Ce, s), 42.1 (C1^{x}, s), 33.6 (Cb, s), 31.1 (Cc, s), 25.5 (Cd, s), 25.3 (C2^{x}, s), 23.9 (C3^{x}, s), 19.6 (CH_3, 2s).

ESI-HRMS m/z: Calcd for $C_{32}H_{34}FN_4O_2$ $[M+H]^+$ 525.26658*. Found:* 525.26687*.*

*3.2.3.14. 2-[4-[3-(5-fluoro-1H-indol-3-yl)-1-piperidyl]butyl]-4-(2-methoxyphenyl)pyrido[1,2-c]pyrimidine-1,3-dione (**9.14**).*

The title compound was isolated as a yellow powder. Yield: 92.0 %, m.p. 110.0-112.3 °C. HPLC t_R = 5.48 min, 99.9% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.31 (C8H, [1H], m (8 lines)), 8.27 (N1"H, [1H], bs), 7.36 (C4'H, [1H], m (8 lines)), 7.27 (C4"H, [1H], m), 7.22 (C7"H, [1H], dd, ^3J=9.0, $^4J_{H-F}$=4.5), 7.21 (C6'H, [1H], m), 7.03 (C3'H,C2"H, [2H], m), 6.96 (C5'H, [1H], m,(6 lines)), 6.92 – 6.82 (C6H,C6"H, [2H], m), 6.61 (C5H, [1H], dt, ^3J=9.5, ^4J=^5J=1.0), 6.35 (C7H, [1H], m (10 lines)), 4.17 ($C1^xH_2$, [2H], m (11 lines)), 3.73 and 3.70 (OCH_3, [3H], 2s), 3.15 (CbH,CaH(E), [2H], bs), 2.99 (CeH(E), [1H], bs), 2.50 ($C4^xH_2$, [2H], bs), 2.13 (CaH(A),CeH(A), [2H], pd), 2.02 (CcH(E), [1H], pd), 1.78 ($C2^xH_2$,CdH_2, [4H], m), 1.67 ($C3^xH_2$, [2H], m), 1.48 (CcH(A), [1H], m).

^{13}C NMR (125 MHz, $CDCl_3$): δ 159.5 (C3, s), 157.4 (C2', 2s), 157.04 (C5", d, ^1J=231.1), 148.6 (C1, s), 143.2 (C4a, s), 132.5 (C6, 2s), 132.2 (C7"a, s), 131.5 (C6', s), 129.2 (C4', s), 127.4 (C8, s), 126.5 (C3"a, s), 121.9 (C2', s), 121.4 (C5, s), 121.0 (C1', s), 120.5 (C5', 2s), 118.0 (C3", s), 111.3 (C7", d, ^3J=9.7), 110.9 (C3', 2s), 110.0 (C7, s), 109.7 (C6", d, ^2J=26.4), 103.5 (C4", d, ^2J=23.3), 100.7 (C4, s), 60.0 (Ca, s), 58.0 ($C4^x$, s), 55.1 (OCH_3, 2s), 53.4 (Ce, s), 41.5 ($C1^x$, s), 33.0 (Cb, s), 30.5 (Cc, s), 25.0 (Cd, s), 25.0 ($C2^x$, s), 24.5 ($C3^x$, s).

ESI-HRMS m/z: Calcd for $C_{32}H_{34}FN_4O_3$ $[M+H]^+$ 541.26149*. Found:* 541.26142*.*

*3.2.3.15. 4-(4-chlorophenyl)-2-[4-[3-(5-fluoro-1H-indol-3-yl)-1-piperidyl]butyl]pyrido[1,2-c]pyrimidine-1,3-dione (**9.15**).*

The title compound was isolated as a yellow powder. Yield: 59.0 %, m.p. 233.3-234.9 °C. HPLC t_R = 7.18 min, 98.5% purity.

^{1}H NMR (500 MHz, DMSO d_6): δ 10.96 (N1"H, [1H], bs), 8.29 (C8H, [1H], dt, ^3J=7.0, ^4J=^5J=1.5), 7.46 (C2'H,C6'H, [2H], dt, ^3J=8.5, ^4J=2.5), 7.32 (C7"H, [1H], dd, ^3J=8.5, $^4J_{H-F}$=4.5), 7.28 (C4"H, [1H], dd), 7.27 {C3'H,C5'H, [2H], dt, ^3J=8.0, ^4J=2.5), 7.21 (C2"H, [1H], d, ^3J=2.5), 7.13 (C6H, [1H], 4d, $^3J_1$=9.5, $^3J_2$=6.5, ^4J=1.0), 6.89 (C6"H, [1H], td, ^3J=9.0, ^4J=2.5), 6.77 (C5H, [1H], dt, ^3J=9.5, ^4J=^5J=1.0), 6.57 (C7H, [1H], m, $^3J_1$=7.5, $^3J_2$=6.5, ^4J=1.0), 4,000 ($C1^xH_2$, [2H], t, ^3J=7.5), 3.15 – 2.90 (CbH,CaH(E),CeH(E), [3H], m), 2.49 ($C4^xH_2$, [2H], bs), 2.16 (CaH(A),CeH(A), [2H], bs), 1.93 (CcH(E), [1H], pd), 1.80 – 1.60 ($C2^xH_2$,CdH_2, [4H], m), 1.55 ($C3^xH_2$, [2H], bs), 1.49 (CcH(A), [1H], m).

^{13}C NMR (125 MHz, DMSO d_6): δ 159.1 (C3, s), 156.5 (C5", d, ^1J=230.8), 148.4 (C1, s), 143.3 (C4a, s), 134.1 (C7"a, s), 133.2 (C3',C6', s), 132.8 (C6, s), 132.3 (C4', s), 131.9 (C1', s), 128.4 (C2',C6', s), 128.1 (C8, s), 126.4 (C3"a, d, ^3J=9.6), 123.4 (C2", s), 120.3 (C5, s), 118.0 (C3", s), 112.4 (C7", d, ^3J=9.8), 111.3 (C7, s), 109.0 (C6", d, ^2J=26.2), 103.1 (C4", d, ^2J=23.0), 101.9 (C4, s), 60.0 (Ca, s), 57.4 ($C4^x$, s), 53.1 (Ce, s), 41.5 ($C1^x$, s), 33.0 (Cb, s), 30.7 (Cc, s), 25.0 ($C2^x$, s), 24.8 (Cd, s), 24.0 ($C3^x$, s).

ESI-HRMS m/z: Calcd for $C_{31}H_{31}ClFN_4O_2$ $[M+H]^+$ 545.21196*. Found:* 545.21225*.*

*3.2.3.16. 4-(4-fluorophenyl)-2-[4-[3-(5-fluoro-1H-indol-3-yl)-1-piperidyl]butyl]pyrido[1,2-c]pyrimidine-1,3-dione (**9.16**).*

The title compound was isolated as a yellow powder. Yield: 72.1 %, m.p. 102.3-104.7 °C. HPLC t_R = 5.79 min, 99.9% purity.

^{1}H NMR (500 MHz, DMSO d_6): *δ 10.92 (N1"H, [1H], bs), 8.28 (C8H, [1H], dt, ^{3}J=7.5, ^{4}J=^{5}J=1.0), 7.31 (C7"H, [1H], dd, ^{3}J=9.0, $^4J_{H-F}$=5.0), 7.28 (C2'H,C6'H,C4"H, [3H], m), 7.23 (C3'H,C5'H, [2H], tt, ^{3}J=9.0, ^{4}J=2.5), 7.20 (C2"H, [1H], ^{3}J=2.5), 7.12 (C6H, [1H], 4d, $^3J_1$=8.5, $^3J_2$=6.0, ^{4}J=1.0), 6.88 (C6"H, [1H], td, ^{3}J=9.5, ^{4}J=2.5), 6.74 (C5H, [1H], dt, ^{3}J=9.5, ^{4}J=^{5}J=1.0), 6.55 (C7H, [1H], m, $^3J_1$=7.5, $^3J_2$=6.0, ^{4}J=1.0), 4.00 (C1^{x}H$_2$, [2H], t, ^{3}J=7.5), 2.96 (CbH,CaH(E),CeH(E), [3H], m), 2.36 (C4^{x}H$_2$, [2H], bs), 2.01 (CaH(A),CeH(A), [2H], bs), 1.92 (CcH(E), [1H], pd), 1.65 (C2^{x}H$_2$,CdH$_2$, [4H], m), 1.51 (C3^{x}H$_2$, [2H], m), 1.43 (CcH(A), [1H], m).*

^{13}C NMR (125 MHz, DMSO d_6): *δ 161.4 (C4', d, ^{1}J=244.0), 159.3 (C3, s), 156.5 (C5", d, ^{1}J=230.9), 148.4 (C1, s), 143.3 (C4a, s), 133.8 (C7"a, s), 133.3 (C2',C6', d, ^{3}J=8.0), 132.8 (C6, s), 129.6 (C1', d, ^{4}J=3.0), 128.0 (C8, s), 126.5 (C3"a, d, ^{3}J=9.7), 123.3 (C2", s), 120.3 (C5, s), 118.0 (C3", s), 115.3 (C3',C5', d, ^{2}J=21.2), 112.3 (C7", d, ^{3}J=9.7), 111.1 (C7, s), 108.9 (C6", d, ^{2}J=26.0), 103.1 (C4", d, ^{2}J=23.0), 102.2 (C4, s), 60.2 (Ca, s), 57.8 (C4^x, s), 53.5 (Ce, s), 41.6 (C1^x, s), 33.3 (Cb, s), 31.1 (Cc, s), 25.2 (C2^x, s), 25.0 (Cd, s), 23.8 (C3^x, s).*

ESI-HRMS m/z: Calcd for $C_{31}H_{31}F_2N_4O_2$ [M+H]$^+$ 529.24151*. Found:* 529.24167.

*3.2.3.17. 2-[4-[3-(5-fluoro-1H-indol-3-yl)-1-piperidyl]butyl]-4-(p-tolyl)pyrido[1,2-c]pyrimidine-1,3-dione (**9.17**).*

The title compound was isolated as a yellow powder. Yield: 68.6 %, m.p. 129.1-132.7 °C. HPLC t_R = 7.27 min, 99.3% purity.

^{1}H NMR (500 MHz, DMSO d_6): *δ 10.93 (N1"H, [1H], bs), 8.26 (C8H, [1H], dt, ^{3}J=7.5, ^{4}J=^{5}J=1.0), 7.32 (C7"H, [1H], dd, ^{3}J=8.5, $^4J_{H-F}$=5.0), 7.27 (C4"H, [1H], dd), 7.21 (C2'H,C6'H,C2"H, [3H], m), 7.13 (C3'H,C5'H, [2H], dt, ^{3}J=8.0, ^{4}J=1.5), 7.08 (C6H, [1H], 4d, $^3J_1$=9.5, $^3J_2$=6.5, ^{4}J=1.0), 6.89 (C6"H, [1H], td, ^{3}J=9.5, ^{4}J=2.5), 6.75 (C5H, [1H], dt, ^{3}J=9.5, ^{4}J=^{5}J=1.0), 6.52 (C7H, [1H], m, $^3J_1$=7.5, $^3J_2$=6.5, ^{4}J=1.5), 3.99 (C1^{x}H$_2$, [2H], t, ^{3}J=7.5), 2.98 (CbH,CaH(E),CeH(E), [3H], m), 2.36 (C4^{x}H$_2$, [2H], bs), 2.34 (CH$_3$, [3H], s), 2.03 (CaH(A),CeH(A), [2H], bs), 1.92 (CcH(E), [1H], pd), 1.64 (C2^{x}H$_2$,CdH$_2$, [4H], m), 1.52 (C3^{x}H$_2$, [2H], bs), 1.45 (CcH(A), [1H], bs).*

^{13}C NMR (125 MHz, DMSO d_6): *δ 159.3 (Cs, s), 156.5 (C5", d, ^{1}J=230.9), 148.4 (C1, s), 143.1 (C4a, s), 136.5 (C4', s), 133.4 (C7"a, s), 132.8 (C6, s), 131.1 (C3',C5', s), 130.3 (C1', s), 129.0 (C2',C6', s), 127.8 (C8, s), 126.5 (C3"a, d, ^{3}J=9.3), 123.3 (C2", s), 120.6 (C5, s), 117.0 (C3", s), 112.3 (C7', d, ^{3}J=9.9), 111.0 (C7, s), 108.9 (C6", d, ^{2}J=26.2), 107.2 (C4, s), 103.1 (C4", d, ^{2}J=23.0), 60.0 (Ca, s), 57.7 (C4^x, s), 53.4 (Ce, s), 41.5 (C1^x, s), 33.2 (Cb, s), 31.1 (Cc, s), 25.0 (C2^x, s), 24.9 (Cd, s), 24.0 (C3^x, s), 20.8 (CH$_3$, s).*

ESI-HRMS m/z: Calcd for $C_{32}H_{34}FN_4O_2$ [M+H]$^+$ 525.26658*. Found:* 525.26668*.*

*3.2.3.18. 2-[4-[3-(5-fluoro-1H-indol-3-yl)-1-piperidyl]butyl]-4-(4-methoxyphenyl)pyrido[1,2-c]pyrimidine-1,3-dione (**9.18**).*

The title compound was isolated as a yellow powder. Yield: 88.3 %, m.p. 118.7-122.1 °C. HPLC t_R = 5.90 min, 99.9% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.48 (N1"H, [1H], bs), 8.30 (C8H, [1H], dt, ^{3}J=7.5, ^{4}J=^{5}J=1.0), 7.26 (C4"H, [1H], dd, $^3J_{H-F}$=10.0, ^{4}J=2.5), 7.23 (C7"H, [1H], dd, ^{3}J=8.5, $^4J_{H-F}$=4.5), 7.21 (C2'H,C6'H, [2H], d, ^{3}J=8.5), 6.99 (C2"H, [1H], bs), 6.95 (C3'H,C5'H, [2H], d, ^{3}J=8.5), 6.91 – 6.84 (C5H,C6H,C6"H, [3H], m), 6.35 (C7H, [1H], m, $^3J_1$=7.5, $^3J_2$=5.5, ^{4}J=2.5), 4.17 (C1^{x}H$_2$, [2H], m), 3.83 (OCH_3, [3H], s), 3.18 CaH(E),CbH, [2H], pd), 3.03 (CeH(E), [1H],bs), 2.53 (C4^{x}H$_2$, [2H], bs), 2.16 (CaH(A),CeH(A), [2H], m), 2.02 (CcH(E), [1H], pd), 1.84 (CdH(E),[1H], bs), 1.78 (CdH(A),C2^{x}H$_2$, [3H],m), 1.69 (C3^{x}H$_2$, [2H], m), 1.47 (CcH(A), [1H], kd, $^3J_{A-A}$=12.5, $^3J_{A-E}$=4.0).

^{13}C NMR (125 MHz, $CDCl_3$): δ 160.5 (C3,s), 159.1 (C4', s), 157.5 (C5", d, ^{1}J=234.2), 148.9 (C1, s), 143.6 (C4a, s), 132.7 (C7"a, s), 132.3 (C2',C6', s), 132.2 (C6, s), 127.9 (C8, s), 126.8 (C3"a, d, ^{3}J=9.9), 124.8 (C1', s), 122.4 (C2", s), 121.5 (C5, s), 117.0 (C3", d), 114.3 (C3',C5' s), 111.8 (C7", d, ^{3}J=9.7), 110.7 (C7, s), 110.1 (C6", d, ^{2}J=26.5), 104.4 (C4, s), 103.9 (C4", d, ^{2}J=23.4), 60.0 (Ca, s), 58.3 (C4^x, s), 55.3 (OCH_3, s), 53.8 (Ce, s), 42.1 (C1^x, s), 33.4 (Cb, s), 30.9 (Cc, s), 25.4 (Cd, s), 25.0 (C2^x, s), 24.0 (C3^x, s).

ESI-HRMS m/z: Calcd for $C_{32}H_{34}FN_4O_3$ $[M+H]^+$ 541.26149*. Found:* 541.26178*.*

*3.2.3.19. 2-[4-[3-(5-methoxy-1H-indol-3-yl)-1-piperidyl]butyl]-4-phenyl-pyrido[1,2-c]pyrimidine-1,3-dione (**9.19**).*

The title compound was isolated as a yellow powder. Yield: 85.0 %, m.p. 94.9-96.1 °C. HPLC t_R = 5.29 min, 99.9% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.30 (N1"H, [1H], bs), 8.30 (C8H, [1H], dt, ^{3}J=7.5, ^{4}J=^{5}J=1.0), 7.43 (C3'H,C5'H, [2H], t, ^{3}J=7.0), 7.35 (C4'H, [1H], tt, ^{3}J=7.0, ^{4}J=1.5), 7.29 (C2'H,C6'H, [2H], m, ^{3}J=7.0), 7.21 (C7"H, [1H], d, ^{3}J=8.5), 7.10 (C4"H, [1H], d, ^{4}J=2.5), 6.92 (C2"H, [1H], d, ^{3}J=2.0), 6.88 (C5H,C6H, [2H], m), 6.82 (C6"H, [1H], dd, ^{3}J=9.0, ^{4}J=2.5), 6.36 (C7H, [1H], m, $^3J_1$=7.5, $^3J_2$=5.0, ^{4}J=2.5), 4.17 (C1^{x}H$_2$, [2H], m), 3.83 (OCH_3, [3H], s), 3.26 (CbH,CaH(E), [2H], pd), 3.10 (CeH(E), [1H], bs), 2.57 (C4^{x}H$_2$, [2H], bs), 2.13 (CaH(A),CeH(A), [2H], m), 2.05 (CcH(E), [1H], pd), 1.85 – 1.55 (CdH$_2$,C2^{x}H$_2$,C3^{x}H$_2$, [6H], m), 1.47 (CcH(A), [1H], kd, $^3J_{A-A}$=13.0, $^3J_{A-E}$=4.0).

^{13}C NMR (125 MHz, $CDCl_3$): δ 160.2 (C3, s), 153.8 (C5", s), 148.9 (C1, s), 143.6 (C4a, s), 132.8 (C6, s), 132.5 (C1', s), 131.3 (C7"a, s), 131.2 (C2',C6', s), 128.8 (C3',C5', s), 127.9 (C4', s), 127.8 (C8, s), 126.9 (C3"a, s), 121.4 (C5, s), 121.1 (C2", s), 119.0 (C3", s), 112.3 (C6", s), 112.0 (C7", s), 110.8 (C7, s), 104.8 (C4, s), 100.7 (C4", s), 60.2 (Ca, s), 58.2 (C4^x, s), 56.0 (OCH_3, s), 53.6 (Ce, s), 42.0 (C1^x, s), 33.1 (Cb, s), 30.6 (Cc, s), 25.3 (Cd, s), 24.9 (C2^x, s), 23.3 (C2^x, s).

ESI-HRMS m/z: Calcd for $C_{32}H_{35}N_4O_3$ $[M+H]^+$ 523.27092*. Found:* 523.27118*.*

*3.2.3.20. 4-(2-chlorophenyl)-2-[4-[3-(5-methoxy-1H-indol-3-yl)-1-piperidyl]butyl]pyrido[1,2-c]pyrimidine-1,3-dione (**9.20**).*

The title compound was isolated as a yellow powder. Yield: 76.1 %, m.p. 117.3-120.2 °C. HPLC t_R = 5.23 min, 99.9% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.35 and 8.34 (C8H, [1H], m (10 lines)), 8.14 (N1"H< [1H], bs), 7.49 (C3'H, [1H], m), 7.35 – 7.26 (C4'-6'H, [3H], m), 7.21 (C7"H, [1H], d, ^{3}J=8.5), 7.09 (C2"H, [1H], d, ^{3}J=2.5), 6.94

(C6H,C4"H, [2H], m), 6.82 (C6"H, [1H], dd, ^{3}J=8.5, ^{4}J=2.5), 6.53 (C5H, [1H], dt, ^{3}J=9.5, ^{4}J=^{5}J=1.0), 6.40 (C7H, [1H], m (13 lines)), 4.18 (C1$^{x}H_2$, [2H] m), 3.84 and 3.83 (OCH_3, [3H], 2s), 3.19 (CbH,CaH(E), [2H], pd), 3.00 (CeH(E), [1H], bs), 2.48 (C4$^{x}H_2$, [2H], bs), 2,05 (CaH(A),CeH(A),CcH(E), [3H], pd), 1.79 (CdH_2,C2$^{x}H_2$, [4H], m), 1.66 (C3$^{x}H_2$, [2H], m), 1.47 (CcH(A), [1H], m).

^{13}C NMR (125 MHz, $CDCl_3$): δ 159.5 (C3, 2s), 153.8 (C5", s), 148.9 (C1, s), 143.8 (C4a, s), 135.7 (C2', 2s), 133.4 (C6, s), 133.1 (C6', s), 131.7 (C1', s), 131.3 (C7"a, s), 130.0 (C3', s), 129.6 (C4', s), 128.1 (C8, s), 127.3 (C5', 2s), 127.0 (C3"a, s), 121.0 (C5,C2", s), 119.0 (C3", s), 112.2 (C6", 2s), 111.9 (C7", s), 110.8 (C7, s), 102.2 (C4, s), 100.7 (C4", 2s), 60.8 (Ca, s), 58.5 (C4^{x}, s), 55.9 (OCH_3, s), 53.8 (Ce, s), 42.2 (C1^{x}, s), 33.6 (Cb, s), 31.1 (Cc, s), 25.5 (Cd, s), 25.0 (C2^{x}, s), 23.9 (C3^{x}, s).

ESI-HRMS m/z: Calcd for $C_{32}H_{34}ClN_4O_3$ $[M+H]^+$ 557.23194*. Found:* 557.23212*.*

*3.2.3.21. 4-(2-fluorophenyl)-2-[4-[3-(5-methoxy-1H-indol-3-yl)-1-piperidyl]butyl]pyrido[1,2-c]pyrimidine-1,3-dione (**9.21**).*

The title compound was isolated as a yellow powder. Yield: 77.9 %, m.p. 126.5-128.7 °C. HPLC t_R = 4.85 min, 99.9% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.35 and 8.34 (C8H, [1H], m(12 lines)), 8.33 (N1"H, [1H], bs), 7.37 (C4'H, [1H], m(22 lines)), 7.30 (C6'H, [1H], m(10 lines)), 7.23 (C7"H, [1H], d, ^{3}J=9.0), 7.19 (C5'H, [1H], m(8 lines)), 7.16 – 7.10 (C3'H,C4"H, [2H], m), 6.97 (C6H, [1H], m(14 lines)), 6.93 (C2"H, [1H], m), 6.82 (C6"H, [1H], dd, ^{3}J=8.5, ^{4}J=2.0), 6.72 (C5H, [1H], m), 6.42 (C7H, [1H], m(12 lines)), 4.17 (C1$^{x}H_2$, [2H], pt), 3.84 (OCH_3, [3H], s), 3.30 (CbH,CaH(E), [2H], pd), 3.15 (CeH(E), [1H], bs), 2.62 (C4$^{x}H_2$, [2H], bs), 2.18 (CaH(A),CeH(A), [2H], bs), 2.06 (CcH(E), [1H], pd), 1.96 (CdH(E), [1H], bs), 1.85 – 1.65 (C2$^{x}H_2$,C3$^{x}H_2$,CdH(A), [5H], m), 1.48 (CcH(A), [1H], m).

^{13}C NMR (125 MHz, $CDCl_3$): δ 160.8 (C2', d, ^{1}J=246,9), 159.7 and 159.6 (C3, 2s), 153.9 (C5", s), 148.8 (C1, s), 144.1 (C4a, 2s), 133.4 (C6', 2d, ^{3}J=2.6, ^{3}J=2.8), 133.3 and 133.2 (C6, 2s), 131.0 (C4', d, ^{3}J=8.2), 128.2 (C8, 2s), 126.8 (C3"a, s), 124.5 and 124.4 (C5', 2d, ^{4}J=3.1, ^{4}J=3.4), 121.1 (C5,C2", 2s), 120.3 (C1', d, ^{2}J=16.0), 116.0 (C3', 2d, ^{2}J=22.3, ^{2}J=22.1), 112.3 (C6", 2s), 112.0 (C7", s), 110.9 (C7, 2s), 100.7 and 100.6 (C4", 2s), 98.2 (C4, 2s), 60.0 (Ca, s), 58.0 (C4^{x}, s), 56.0 (OCH_3, s), 53.5 (Ce, s), 41.9 (C1^{x}, s), 32.9 (Cb, s), 25.2 (Cc, s), 24.7 (Cd, s), 25.0 (C2^{x}, s), 22.8 (C3^{x}, s).

ESI-HRMS m/z: Calcd for $C_{32}H_{34}FN_4O_3$ $[M+H]^+$ 541.26149. Found: 541.26166*.*

*3.2.3.22. 2-[4-[3-(5-methoxy-1H-indol-3-yl)-1-piperidyl]butyl]-4-(o-tolyl)pyrido[1,2-c]pyrimidine-1,3-dione (**9.22**).*

The title compound was isolated as a yellow powder. Yield: 73.0 %, m.p. 104.5-105.6 °C. HPLC t_R = 5.59 min, 99.6% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.31 (C8H, [1H], dt, ^{3}J=7.5, ^{4}J=^{5}J=1.0), 8.12 (N1"H, [1H], bs), 7.30 – 7.22 (C4'-6'H, [3H], m(20 lines)), 7.21 (C7"H, [1H], dd, ^{3}J=9.0, ^{5}J=0.5), 7.12 (C3'H, [1H], m(9 lines)), 7.09 (C4"H, [1H], d, ^{4}J=2.5), 6.94 (C2"H, [1H], bs), 6.87 (C6H, [1H], 4d, $^{3}J_1$=9.0, $^{3}J_2$=6.5, ^{4}J=1.5), 6.82 (C6"H, [1H], dd, ^{3}J=8.5, ^{4}J=2.5), 6.54 (C5H, [1H], dt, ^{3}J=9.5, ^{4}J=^{5}J=1.0), 6.36 (C7H, [1H], m, $^{3}J_1$=7.5, $^{3}J_2$=6.5, ^{4}J=1.5), 4.18 (C1$^{x}H_2$, [2H], m), 3.84 and 3.83 (OCH_3, [3H], 2s), 3.20 (CbH,CaH(E), [2H] pd), 2.49 (C4$^{x}H_2$, [2H], bs), 2.13 and 2.11 (CH_3, [3H], 2s), 2.14 – 2.00 (CaH(A),CeH(A),CcH(E), [3H], m), 1.79 (C2$^{x}H_2$,CdH_2, [4H], m), 1.67 (C3$^{x}H_2$,]2H], m), 1.49 (CcH(A), [1H], m).

^{13}C NMR (125 MHz, $CDCl_3$): *δ 159.7 (C3, s), 153.8 (C5", s), 149.1 C1, s), 143.5 (C4a, s), 138.5 (C2', 2s), 132.5 (C1', s), 132.1 (C6, s), 131.6 (C3', 2s), 131.3 (C7"a, s), 130.5 (C6', s), 128.3 (C4', s), 128.0 (C8, s), 127.0 (C3"a, s), 126.4 (C5', s), 121.3 (C5, s), 121.1 (C3", s), 112.2 (C6", s), 111.9 (C7", s), 110.5 (C7, s), 104.0 (C4, s), 100.7 (C4", 2s), 60.7 (Ca, s), 58.5 (C4^x, s), 56.0 (OCH_3, 2s), 53.9 (Ce, s), 42.1 (C1^x, s), 33.5 (Cb, s), 31.0 (Cc, s), 25.5 (Cd, s), 25.0 (C2^x, s), 23.8 (C3^x, s), 19.6 (CH_3, 2s).*

ESI-HRMS m/z: Calcd for $C_{33}H_{37}N_4O_3$ $[M+H]^+$ 537.28657. Found: 537.28658.

*3.2.3.23. 2-[4-[3-(5-methoxy-1H-indol-3-yl)-1-piperidyl]butyl]-4-(2-methoxyphenyl)pyrido[1,2-c]pyrimidine-1,3-dione (**9.23**).*

The title compound was isolated as a yellow powder. Yield: 62.5 %, m.p. 106.6-108.3 °C. HPLC t_R = 4.90 min, 99.9% purity.

^{1}H NMR (500 MHz, $CDCl_3$): *δ 8.42 (N1"H, [1H], bs), 8.29 (C8H, [1H], m (14 lines)), 7.36 (C4'H, [1H], m (12 lines)), 7.20 (C7"H, [1H], dd, ^{3}J=8.5, ^{5}J=0.5), 7.18 (C6'H, [1H], m (6 lines)), 7.10 (C4"H, [1H], pt), 7.02 (C5'H, [1H], m (8 lines)), 6.96 (C3'H, [1H], m (8 lines)), 6.89 (C2"H, [1H], bs), 6.87 (C6H, [1H], m (12 lines)), 6.81 (C6"H, [1H], dd, ^{3}J=8.5, ^{4}J=2.0), 6.61 (C5H, [1H], dt), 6.35 (C7H, [1H], m (10 lines)), 4.16 (C1^{x}H$_2$, [2H], m), 3.84 (C5"-OCH_3, [3H], s), 3.72 and 3.67 (C2'-OCH_3, [3H], 2s), 3.27 (CbH,CaH(E), [2H], m), 3.12 (CeH(E), [1H], bs), 2.60 (C4^{x}H$_2$, [2H], bs), 2.15 (CeH(A), [1H], bs), 2.04 (CcH(E), [1H], pd), 1.85 – 1.65 (CdH$_2$,C2^{x}H$_2$,C3^{x}H$_2$, [6H], m), 1.45 (CcH(A), [1H], kd, $^3J_{A\text{-}A}$=12.5, $^3J_{A\text{-}E}$=3.5).*

^{13}C NMR (125 MHz, $CDCl_3$): *δ 159.9 (C3, s), 157.9 (C2', 2s), 153.8 (C5", s), 149.1 (C1, s), 143.6 (C4a, s), 133.0 (C6', 2s), 132.0 (C6, s), 131.3 (C7"a, s), 129.7 (C4', s), 127.8 (C8, s), 127.0 (C3"a, s), 121.9 (C5, s), 121.4 (C1', s), 121.1 (C2", s), 121.0 and 120.9 (C5', 2s), 119.0 (C3", s), 112.3 (C6", s), 111.9 (C7', s), 111.4 (C3', 2s), 110.5 (C7, s), 101.2 (C4, s), 100.7 (C4", 2s), 60.7 (Ca, s), 58.5 (C4^x, s), 56.0 (5"-OCH_3, s), 55.6 (C2'-OCH_3, 2s), 53.8 (Ce, s), 42.1 (C1^x, s), 33.5 (Cb, s), 31.0 (Cc, s), 25.5 (Cd, s), 25.0 (C2^x, s), 23.7 (C3^x, s).*

ESI-HRMS m/z: Calcd for $C_{33}H_{37}N_4O_4$ $[M+H]^+$ 553.28148. Found: 553.28179.

*3.2.3.24. 4-(4-chlorophenyl)-2-[4-[3-(5-methoxy-1H-indol-3-yl)-1-piperidyl]butyl]pyrido[1,2-c]pyrimidine-1,3-dione (**9.24**).*

The title compound was isolated as a yellow powder. Yield: 65.1 %, m.p. 111.8-114.4 °C. HPLC t_R = 6.24 min, 98.4% purity.

^{1}H NMR (500 MHz, $CDCl_3$): *δ 8.32 (C8H, [1H], dt, ^{3}J=7.5, ^{4}J=^{5}J=1.0), 8.13 (N1"H, [1H], bs), 7.9 (C2'H,C6'H, [2H], dt, ^{3}J=8.5), 7.25 – 7.21 (C3'H,C5'H,C7"H, [3H], m), 7.10 (C4"H, [1H], d, ^{4}J=2.0), 6.95 – 6.91 (C6H,C2"H, [2H], m), 6.87 – 6.81 (C5H,C6"H, [2H], m), 6.387 (C7H, [1H], m, $^3J_1$=7.5, $^3J_2$=6.0, ^{4}J=1.0), 4.16 (C1^{x}H$_2$, [2H], t, ^{3}J=7.5), 3.84 (OCH_3, [3H], s), 3.24 (CbH,CaH(E), [2H], bs), 3.07 (CeH(E), [1H], bs), 2.53 (C4^{x}H$_2$, [2H], bs), 2.08 (CaH(A),CeH(A),CcH(E), [3H], m), 1.85 – 1.73 (CdH$_2$,C2^{x}H$_2$, [4H], m), 1.69 (C3^{x}H$_2$, [2H], bs), 1.49 (CcH(A), [1H], kd, $^3J_{A\text{-}A}$=12.5, $^3J_{A\text{-}E}$=4.0).*

^{13}C NMR (125 MHz, $CDCl_3$): *δ 160.0 (C3, s), 153.8 (C5", s), 148.8 (C1, s), 143.7 (C4a, s), 133.7 (C4', s), 133.0 (C6, s), 132.7 (C3',C5', s), 131.3 (C1', s), 131.3 (C7"a, s), 129.0 (C2',C6', s), 128.1 (C8, s), 127.0 (C3"a, s), 121,036 (C2", s), 121.0 (C5, s), 118.5 (C3", s), 112.2 (C6", s), 111.9 (C7", s), 110.9 (C7, s), 103.4 (C4, s), 100.7 (C4", s), 60.5 (Ca, s), 58.4 (C4^x, s), 56.0 (OCH_3, s), 53.8 (Ce, s), 42.2 (C1^x, s), 33.4 (Cb, s), 30.8 (Cc, s), 25.4 (Cd, s), 25.2 (C2^x, s), 23.6 (C3^x, s).*

ESI-HRMS m/z: Calcd for $C_{32}H_{34}ClN_4O_3$ $[M+H]^+$ 557.23194*. Found:* 557.23219*.*

*3.2.3.25. 4-(4-fluorophenyl)-2-[4-[3-(5-methoxy-1H-indol-3-yl)-1-piperidyl]butyl]pyrido[1,2-c]pyrimidine-1,3-dione (**9.25**).*

The title compound was isolated as a yellow powder. Yield: 63.1 %, m.p. 105.5-107.7 °C. HPLC t_R = 5.17 min, 99.9% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.31 (C8H, [1H], dt, ^3J=7.0, ^4J=^5J=1.5), 8.15 (N1"H, [1H], bs), 7.26 (C2'H,C6'H, [2H], m), 7.23 (C7"H, [1H], dd, ^3J=8.5, ^5J=0.5), 7.14 – 7.08 (C3'H,C5'H,C4"H, [3H], m), 6.94 (C2"H, [1H], d, ^3J=2.0), 6.92 (C6H, [1H], 4d, $^3J_1$=9.5, $^3J_2$=6.0,^4J=1.0), 6.85 – 6.81 (C5H,C6"H, [2H], m), 6.38 (C7H, [1H], m, $^3J_1$=7.5, $^3J_2$=6.5, ^4J=1.5), 4.16 (C1^{x}H$_2$, [2H], t, ^3J=7.0), 3.84 (OCH_3, [3H], s), 3.26 (CbH,CaH(E), [2H], pd), 3.09 (CeH(E), [1H], bs), 2.55 (C4^{x}H$_2$, [2H], bs), 2.12 (CaH(A),CeH(A), [2H], bs), 2.07 (CcH(E), [1H], pd), 1.95 – 1.60 (CdH$_2$,C2^{x}H$_2$,C3^{x}H$_2$, [6H], m), 1.50 (CcH(A), [1H], kd, $^3J_{A-A}$=12.5, $^3J_{A-E}$=4.0).

^{13}C NMR (125 MHz, $CDCl_3$): δ 162.3 (C4', d, ^1J=247.0), 160.2 (C3, s), 153.8 (C5", s), 148.8 (C1, s), 143.7 (C4a, s), 133.0 (C2',C6', d, ^3J=8.2), 132.8 (C6, s), 131.3 (C7"a, s), 128.6 (C1', d, ^4J=3.4), 128.0 (C8, s), 126.9 (C3"a, s), 121.1 (C5, s), 121.1 (C2", s), 118.4 (C3", s), 115.8 (C3',C5', d, ^2J=21.5), 112.3 (C6", s), 111.9 (C7", s), 110.8 (C7, s), 103.6 (C4, s), 100.7 (C4", s), 60.3 (Ca, s), 58.3 (C4^x, s), 56.0 (OCH_3, s), 53.8 (Ce, s), 42.1 (C1^x, s), 33.3 (Cb, s), 30.7 (Cc, s), 25.4 (Cd, s), 25.1 (C2^x, s), 23.5 (C3^x, s).

ESI-HRMS m/z: Calcd for $C_{32}H_{34}FN_4O_3$ $[M+H]^+$ 541.26149. Found: 541.26171*.*

*3.2.3.26. 2-[4-[3-(5-methoxy-1H-indol-3-yl)-1-piperidyl]butyl]-4-(p-tolyl)pyrido[1,2-c]pyrimidine-1,3-dione (**9.26**).*

The title compound was isolated as a yellow powder. Yield: 67.8 %, m.p. 112.1-113.9 °C. HPLC t_R = 6.40 min, 97.3% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.29 (C8H, [1H], dt, ^3J=7.5, ^4J=^5J=1.0), 8.28 (N1"H, [1H], bs), 7.23 (C2'H,C6'H, [2H], d, ^3J=8.5), 7.21 (C7"H, [1H], d, ^3J=9.0), 7.18 (C3'H,C5'H, [2H], d, ^3J=8.5), 7.10 (C4"H, [1H], d, ^4J=2.0), 6.92 (C2"H, [1H], d, ^3J=2.0), 6.88 (C5H,C6H, [2H], m), 6.82 (C6"H. [1H], dd, ^3J=8.5, ^4J=2.0), 6.35 (C7H, [1H], m, $^3J_1$=7.5, $^3J_2$=6.0, ^4J=2.5), 4.16 (C1^{x}H$_2$, [2H], m), 3.84 (OCH_3, [3H], s), 3.25 (CbH,CaH(E), [2H], pd), 3.09 (CeH(E), [1H], bs), 2.56 (C4^{x}H$_2$, [2H], bs), 2.38 (CH_3, [3H], s), 2.12 (CaH(A),CeH(A), [2H], m), 2.05 (CcH(E), [1H], pd), 1.85 – 1.65 (CdH$_2$,C2^{x}H$_2$,C3^{x}H$_2$, [6H], m), 1.47 (CcH(A), [1H], kd, $^3J_{A-A}$=12.5, $^3J_{A-E}$=4.0).

^{13}C NMR (125 MHz, $CDCl_3$): δ 160.29 (C3, s), 153.8 (C5", s), 148.9 (C1, s), 143.5 (C4a, s), 137.5 (C4', s), 132.2 (C6, s), 131.3 (C7"a, s), 131.0 (C3',C5', s), 129.7 (C1', s), 129.5 (C2',C6', s), 127.9 (C8, s), 126.9 (C3"a, s), 121.5 (C5, s), 121.1 (C2", s), 118.5 (C3", s), 112.2 (C6", s), 112.0 (C7", s), 110.7 (C7, s), 104.8 (C4, s), 100.7 (C4", s), 60.3 (Ca, s), 58.2 (C4^x, s), 56.0 (OCH_3, s), 53.6 (Ce, s), 42.0 (C1^x, s), 33.2 (Cb, s), 30.6 (Cc, s), 25.3 (Cd, s), 25.0 (C2^x, s), 23.4 (C3^x, s), 21.3 (CH_3, s).

ESI-HRMS m/z: Calcd for $C_{33}H_{37}N_4O_3$ $[M+H]^+$ 537.28657. Found: 537.28677*.*

*3.2.3.27. 2-[4-[3-(5-methoxy-1H-indol-3-yl)-1-piperidyl]butyl]-4-(4-methoxyphenyl)pyrido[1,2-c]pyrimidine-1,3-dione (**9.27**).*

The title compound was isolated as a yellow powder. Yield: 59.8 %, m.p. 90.4-91.6 °C. HPLC t_R = 5.33 min, 99.5% purity.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.29 (N1"H, [1H], bs), 8.29 (C8H, [1H], dt, ^{3}J=7.5, ^{4}J=^{5}J=1.0), 7.21 (C2'H,C6'H,C7"H, [3H], m), 7.11 (C4"H, [1H], d, ^{4}J=2.5), 6.96 (C3'H,C5'H, [2H], d, ^{3}J=8.5), 6.91 (C2"H, [1H], d, ^{3}J=1.5), 6.89 – 6.86 (C5H,C6H, [2H], m), 6.822 (C6"H, [1H], dd, ^{3}J=8.5, ^{4}J=2.5), 6.35 (C7H, [1H], m, $^3J_1$=7.5, $^3J_2$=4.5, ^{4}J=3.0), 4.16 (C1^{x}H$_2$, [2H], t, ^{3}J=7.0), 3.84 (C5"-OCH$_3$, [3H], s), 3.83 (C4'-OCH$_3$, [3H], s), 3.29 (CbH,CaH(E), [2H], m), 3.13 (CeH(E), [1H], bs), 2.61 (C4^{x}H$_2$, [2H], bs), 2.18 (CaH(A),CeH(A), [2H], bs), 2.06 (CcH(E), [1H], pd), 1.85 – 1.70 (CdH$_2$,C2^{x}H$_2$,C3^{x}H$_2$, [6H], m), 1.48 (CcH(A), [1H], kd, $^3J_{A-A}$=12.5, $^3J_{A-E}$=3.5).

^{13}C NMR (125 MHz, $CDCl_3$): δ 160.4 (C3, s), 159.13 (C4', s), 153.8 (C5", s), 148.9 (C1, s), 143.5 (C4a, s), 132.3 (C2',C6', s), 132.2 (C6, s), 131.3 (C7"a, s), 127.9 (C8, s), 127.0 (C3"a, s), 124.9 (C1', s), 121.6 (C5, s), 121.1 (C2", s), 119.0 (C3", s), 114.3 (C3',C5', s), 112.3 (C6", s), 111.9 (C7", s), 110.6 (C7, s), 104.5 (C4, s), 100.7 (C4", s), 60.6 (Ca, s), 58.5 (C4^x, s), 56.0 (C5'-OCH$_3$, s), 55.3 (4'-OCH$_3$, s), 53.9 (Ce, s), 42.2 (C1^x, s), 33.6 (Cb, s), 31.0 (Cc, s), 25.5 (Cd, s), 25.0 (C2^x, s), 23.9 (C3^x, s).

ESI-HRMS m/z: Calcd for $C_{33}H_{37}N_4O_4$ $[M+H]^+$ 553.28148. Found: 553.28186.

2.3. X-ray crystallography

The diffraction data for **8.1** and **8.2** were collected at room temperature with a KM4 (Cu Kα, λ = 1.54173 Å) diffractometer. The data were corrected for Lorentz and polarization effects and a multi-scan absorption correction applied. The structure was solved using direct methods implemented in SHELXS-97, and refined by the full matrix least-squares on F2 with the SHELXL-97 program [42]. All non-H atoms were refined with anisotropic displacement parameters. The H atoms attached to carbon were positioned geometrically and refined using the riding model with U_{ISO}(H) = 1.2 U_{eq}(C). The oxygen-bonded H atoms were found in the difference-Fourier map and included at fixed positions with isotropic displacement parameters. The data were included in supplementary information.

2.4. Biological tests

2.4.1. In vitro tests

2.4.1.1. 5-HT$_{1A}$ binding assay

Radioligand binding was performed using membranes from CHO-K1 cells stably transfected with the human 5-HT$_{1A}$ receptor (Perkin Elmer). All assays were carried out in duplicate. Working solution (50 µL) of the tested compounds, 50 µL [3H]-8-OH-DPAT (spec. act. 139.7 Ci/mmol, 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 a polypropylene 96-well microplate using a Rainin Liquidator 96-well pipetting station (Mettler Toledo). Serotonin (10 µM) was used to define nonspecific binding. The 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 a Harvester-96 MACH III

FM automated harvester system (Tomtec). The filter mates were dried at 37 °C in a forced-air fan incubator and then MeltiLex solid scintillator was melted onto the filter mates at 90 °C for 4 min. Radioactivity was counted in a MicroBeta2 scintillation counter (PerkinElmer). Data were fitted to a one-site curve-fitting equation with Prism 6 (GraphPad Software) and Ki values were estimated from the Cheng–Prusoff equation [43]:

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

L_O – labeled ligand concentration

K_D – dissociation constant of labeled ligand

K_d value was determined in our experimental conditions.

2.4.1.2. SERT binding assay

Radioligand binding was performed using rat cortex tissue. All assays were carried out in duplicate. Working solution (50 µL) of the tested compounds, 50 µL [^{3}H]-citalopram (spec. act. 84.5 Ci/mmol, final concentration 1.0 nM) and 150 µL tissue suspension prepared in assay buffer (50 mM Tris, pH 7.7; 150 mM NaCl; 5 mM KCl) were transferred to a polypropylene 96-well microplate using a Rainin Liquidator 96-well pipetting station (Mettler Toledo). Imipramine (10 µM) was used to define nonspecific binding. The microplate was covered with sealing tape, mixed and incubated for 60 min at 24 °C. The reaction was terminated by rapid filtration through a GF/B filter mate presoaked with 0.3% polyethyleneimine for 30 min. Ten rapid washes with 200 µL of 50 mM Tris buffer (4 °C, pH 7.7) were performed using a Harvester-96 MACH III FM automated harvester system (Tomtec). The filter mates were dried at 37 °C in a forced-air fan incubator and then solid scintillator MeltiLex was melted onto the filter mates at 90 °C for 5 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 [43]. K_d value was determined in our experimental conditions.

2.4.1.3. 5-HT$_{2A}$, 5-HT$_6$, 5-HT$_7$ and D$_2$ binding assays

In vitro radioligand binding assays for 5-HT$_{2A}$, 5-HT$_6$, 5-HT$_7$ and D$_2$ receptors were carried out using methods published by Zajdel et al.[44]. For the assays, HEK293 cell cultures stably expressing the investigated human receptors were used. Cell pellets were thawed and homogenized in 20 volumes of assay buffer using an Ultra Turrax tissue homogenizer and centrifuged twice at 35000 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 5-HT$_{2A}$ receptors: 50 mM Tris-HCl, 0.1 mM EDTA, 4 mM $MgCl_2$ and 0.1% ascorbate; for 5-HT$_6$ receptors: 50 mM Tris-HCl, 0.5 mM EDTA and 4 mM $MgCl_2$; for 5-HT$_7$ receptors: 50 mM Tris-HCl, 4 mM $MgCl_2$, 10 mM pargyline and 0.1% ascorbate; for D$_2$ receptors: 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. All assays were incubated in a total volume of 200 mL in 96-well microtitre plates for 1 h at 37 °C, except for 5-HT$_{2A}$ receptors which were incubated at room temperature for 1.5 h. 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

(PerkinElmer, USA). For displacement studies, the assay samples contained as radioligands (PerkinElmer): 2 nM [3 H]-ketanserin (spec. act. 53.4 Ci/mmol) for 5-HT_{2A} receptors; 2 nM [^{3}H]-LSD (spec. act. 83.6 Ci/mmol) for 5-HT_6 receptors; 0.6 nM [^{3}H]-5-CT (spec. act. 39.2 Ci/mmol) for 5-HT_7 receptors and [^{3}H]-raclopride (spec. act. 76.0 Ci/mmol) for D_2 receptors. Nonspecific binding was defined with 10 mM of 5-HT in 5-HT_7 receptors binding experiments, whereas 10 mM chlorpromazine, 10 mM methiothepine, or 1 mM (+)butaclamol were used in 5-HT_{2A}, 5-HT_6 and D_2 receptors assays, 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–Prushoff equation [43]. Results were expressed as means of at least two separate experiments.

2.4.2. In vivo tests

All studies were performed according to the guidelines of the European Community Council (Directive 86/609/EEC) and were approved by the Ethical Committee of the Institute of Pharmacology. The experiments were performed on male CD-1 mice (23–40 g). The animals were kept at room temperature (21 ± 2 ^{o}C) on a natural day-night cycle (March–October) 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 (except 14–15 animals/dose in the hypothermia induced by 8-OH-DPAT and **9.7**). All the animals were used only once. 8-Hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT, Research Biochemical Inc.) was used as aqueous solution. Compounds **9.1**, **9.7**, **9.9** and **9.27** were suspended in a 10% aqueous solution of dimethyl sulfoxide (DMSO). Vehicle group was administered as 10% aqueous solution of dimethyl sulfoxide (DMSO). 8-OH-DPAT was injected subcutaneously (sc); **9.1**, **9.7**, **9.9** and **9.27** were given intraperitoneally (ip) in a volume of 10 mL/kg/mouse. The obtained data were analyzed by Dunnett's test (one drug administration) or by the Newman-Keuls test (two drugs administration).

2.4.2.1. Body temperature in mice

The effects of the tested compounds **9.1**, **9.7**, **9.9** and **9.27** given alone on the rectal body temperature in mice (measured with an Ellab thermometer) were recorded 30, 60, 90 and 120 min after their administration. In a separate experiment, the effect of WAY100635 (0.1 mg/kg sc) on the hypothermia induced by the 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. In an independent experiment, the effect of compound **9.7** (with no effect on body temperature) on the hypothermia induced by 8-OH-DPAT (5 mg/kg sc) was tested. Compound **9.7** was administered 45 min before 8-OH-DPAT, and the rectal body temperature was recorded 15, 30, 45 and 60 min later. The absolute mean body temperatures were within a range 36.7 ± 0.5 ^{o}C The results were expressed as a change in body temperature (Δt) with respect to the basal body temperature, as measured at the beginning of the experiment.

2.4.2.2. Forced swimming test in mice

The experiments were performed according to the method of [45]. Mice were placed individually into glass cylinders (height 25 cm, diameter 10 cm) containing 20 cm of water and maintained at 23 ^{o}C. The animals were left in the cylinder for 6 min. After the first 2 min adaptation period, the total duration of immobility was measured during a 4-min test. The mouse was judged 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.

2.4.3. Metabolic stability

Stock solutions of studied compounds were prepared at a concentration of 100 µM in 1:1 acetonitrile/water. Incubation mixes consisted of 1 µM of studied compound, 100 µM of NADPH in phosphate buffer and 1 mg/mL of pooled human liver microsomes (HLMs) (Sigma-Aldrich, St. Louis, MO, USA) in potassium phosphate buffer (0.1 M, pH 7.4). Incubations were carried out in 96-well plates at 37 °C. Incubation mixtures (excluding compound solution) were subjected to 5 min pre-incubation and started by addition of 10 µL of compound stock solution. After 0, 5, 10, 15, and 30 min 25 µl samples of incubation reaction were added to the equal volume of ice-cold acetonitrile containing 1 µM of IS (buspirone hydrochloride). Control incubations were performed without NADPH to assess possible chemical instability. All samples were immediately centrifuged (10 min, 10 000 rpm) and the resulting supernatant was directly subjected to LC-MS analysis.

LC-MS analysis was performed on an Agilent 1260 system coupled to SingleQuad 6120 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). A Poroshell C18 EC120 column (3.0 x 100 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 deionized water) and 10% of phase B (0.1% formic acid in acetonitrile). The gradient elution program was 0.00–10.00 min, 10%-95% B; 10.01–10.02 min, 95%-10% B; 10.02–15.00 min, 10% B. Total analysis time was 15 min at 40 °C, flow rate was 0.4 mL/min and the injection volume was 5 µL. The mass spectrometer was equipped with an electrospray ionization source in positive ionization mode. The mass analyzer was set individually to each derivative to detect pseudomolecular ions [$M+H^+$]. MSD parameters of the ESI source were as follows: nebulizer pressure 35 psig (N_2), drying gas 12 L/min (N_2), drying gas temperature 300 °C, capillary voltage 3.0 kV, fragmenter voltage 70 V.

4. Conclusions

The study enabled us to obtain a number of new derivatives of 4-aryl-pyrido[1,2-c]pyrimidine **9.1**–**9.27** having a conformationally restricted residue of 3-(3-piperidinyl)-1H-indole (**9.1**–**9.9**), 5-fluoro-3-(3-piperidinyl)-1H-indole (**9.10**–**9.18**) or 5-methoxy-3-(3-piperidinyl)-1H-indole (**9.19**–**9.27**). In vitro studies have shown that derivatives **9.1**, **9.2**, **9.4**, **9.7**, **9.9**, **9.14** and **9.27** have high affinity to both molecular targets 5-HT_{1A} receptor and SERT protein (5-HT_{1A} K_i = 41–118 nM; SERT K_i = 22–102 nM). The remaining compounds showed moderate to weak affinity to 5-HT_{1A} and SERT. In general, compounds with an unsubstituted or a para-substituted benzene ring of the pyrido[1,2-c]pyrimidine residue in the terminal part were characterized by higher binding affinity, which can be justified by the greater flexibility of the structure. For the selected compounds **9.1**, **9.7**, **9.9** and **9.27**, further *in vitro, in vivo* and metabolic stability tests were performed. The *in vitro* studies in the extended receptor profile (D_2, 5-HT_{2A}, 5-HT_6 and 5-HT_7) indicated their selectivity toward the 5-HT_{1A} receptor and SERT protein. The *in vivo* studies of these compounds (8-OH-DPAT-induced hypothermia in mice, FST) revealed that compound **9.1** had the properties of presynaptic agonist of the 5-HT_{1A} receptor, but did not show any postsynaptic activity [39]. In turn, compound **9.7** demonstrated the properties of a presynaptic antagonist of the 5-HT_{1A} receptor. Metabolic stability studies showed that compounds **9.1, 9.7** and **9.9**, having an unsubstituted indole residue (R_3 = -H), were more resistant to biotransformation reactions of the first pass phase than compound **9.27**, which contains a 5-methoxy substituted indole residue (R_3 = -OCH_3). The presented results allow further optimization of the structure in order to obtain a novel potential antidepressant agent within the group of pyrido[1,2-c]pyrimidine derivatives.

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- A series of novel pyrido[1,2-c]pyrimidine derivatives with rigidized tryptamine moiety have been synthesized.
- All compounds were evaluated for SSRI/5-HT_{1A} activities *in vitro.*
- For the potent compounds further in vitro, in vivo and metabolic stability tests were performed.
- **9.1** has the properties of presynaptic agonist of the 5-HT_{1A} receptor.
- **9.7** demonstrated the properties of a presynaptic antagonist of the 5-HT_{1A} receptor.