Synthesis of Novel Pyrido[1,2-c]pyrimidine Derivatives with 6-Fluoro-3-(4-piperidynyl)-1,2-benzisoxazole Moiety as Potential SSRI and 5-HT$_{1A}$ Receptor Ligands

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Abstract: Two series of novel 4-aryl-2H-pyrido[1,2-c]pyrimidine (6a–i) and 4-aryl-5,6,7,8-tetrahydropyrido[1,2-c]pyrimidine (7a–i) derivatives were synthesized. The chemical structures of the new compounds were conﬁrmed by $^1$H and $^{13}$C NMR spectroscopy and ESI-HRMS spectrometry. The affinities of all compounds for the 5-HT$_{1A}$ receptor and serotonin transporter protein (SERT) were determined by in vitro radioligand binding assays. The test compounds demonstrated very high binding affinities for the 5-HT$_{1A}$ receptor of all derivatives in the series (6a–i and 7a–i) and generally low binding affinities for the SERT protein, with the exception of compounds 6a and 7g. Extended afﬁnity tests for the receptors D$_2$, 5-HT$_{2A}$, 5-HT$_6$ and 5-HT$_7$ were conducted with regard to selected compounds (6a, 7g, 6d and 7i). All four compounds demonstrated very high affinities for the D$_2$ and 5-HT$_{2A}$ receptors. Compounds 6a and 7g also had high affinities for 5-HT$_7$, while 6d and 7i held moderate affinities for this receptor. Compounds 6a and 7g were also tested in vivo to identify their functional activity proﬁles with regard to the 5-HT$_{1A}$ receptor, with 6a demonstrating the activity proﬁle of a presynaptic agonist. Metabolic stability tests were also conducted for 6a and 6d.

Keywords: antidepressants; pyrido[1,2-c]pyrimidines; dual 5-HT$_{1A}$/SERT activity; drug design

1. Introduction

The serotonergic nervous system plays a substantial role in regulating mood, diurnal rhythm, cognitive functions, memory, thermoregulation and anxiety, while also contributing to many other vital functions [1]. Numerous studies in recent years have conﬁrmed that disturbances in serotonergic neurotransmission are closely related to central nervous system (CNS) disorders such as depression, anxiety, schizophrenia or obsessive compulsive disorder (OCD) [2,3]. There is an increase in patients with these depressive disorders, which represent the fourth most common class of medical conditions and affect approximately 20% of the population. Consequently, there is a growing interest in novel modulators of the serotonergic system [4]. The World Health Organization predicts that by 2030, unipolar depression will be the primary reason for inability to work worldwide, with depression...
and anxiety affecting approximately 100 million people in Europe alone [5]. At the same time, drugs currently used to treat depression are far from satisfactory [2].

The introduction of selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine or citalopram in the 1980s marked a turning point in the pharmacotherapy of depression (Figure 1) [6]. SSRIs demonstrate low affinities for adrenergic, histaminic and muscarinic receptors, contributing to their limited adverse effects, better tolerability and higher therapeutic index than tricyclic antidepressants or monoamine oxidase inhibitors [6–8]. Therapeutic efficacy of SSRIs has been documented for unipolar depression, anxiety disorder, posttraumatic stress disorder, OCD and negative symptoms of schizophrenia [9]. However, SSRIs are not free from adverse effects, which include insomnia, nausea, sexual dysfunction and a possible effect on myocardial ion channels [9,10]. One serious drawback of SSRIs is their long therapeutic latency (therapeutic effects become evident two to three weeks after administration) and limited efficacy (visible effects are only seen in approximately 60% of patients) [7]. Given the high probability of suicide in depressive patients, drugs without a latency period are extremely important [1,4].

Figure 1. Chemical structures of fluoxetine and citalopram.

5-HT$_{1A}$ receptors play an important role in the self-regulation of the serotonergic system [2]. They may function both as presynaptic (autoreceptors) or postsynaptic receptors. The 5-HT$_{1A}$ presynaptic receptors are found in the neurons and dendrites of brainstem raphe nuclei. Upon stimulation, they release endogenous serotonin into the synaptic cleft, reducing transmission across serotonergic neurons. On the other hand, postsynaptic 5-HT$_{1A}$ neurons are stimulated in somatodendritic nerve endings in the cortico-limbic area of the CNS, increasing transmission via serotonergic neurons [7,11,12]. In fact, a number of 5-HT$_{1A}$ agonists are currently undergoing various phases of clinical studies or have already been approved for marketing. Their pharmacological activity is not limited to the treatment of depression, but may also be used in the treatment of anxiety (osemozotan, phase II), schizophrenia (bifeprunox, phase III) or pain (befiradol, phase II) (Figure 2) [13].

Figure 2. Chemical structures of osemozotan, bifeprunox and befiradol.

The aforementioned SSRI latency period is associated with changes in adaptive processes within the CNS that result in increased serotonergic neurotransmission via postsynaptic 5-HT$_{1A}$ receptors [8,14]. The therapeutic effects seen during SSRI administration are the combined result of neurochemical changes in the brain, including desensitization of 5-HT$_{1A}$ autoreceptors, downregulation of receptor responsivity to neurotransmitters,
changes in signal transmission, neurotropism and hippocampal neurogenesis [15]. One key consequence of desensitization of somatodendric 5-HT<sub>1A</sub> autoreceptors on brainstem raphe nuclei is increasing serotonin levels in synaptic clefts [7,16].

In 1993, Artigas proposed that coadministration of a 5-HT<sub>1A</sub> receptor antagonist with SSRIs should potentiate the antidepressant effect through accelerating the desensitization of 5-HT<sub>1A</sub> autoreceptors, thus strengthening their function [17]. This hypothesis was confirmed upon coadministration of an SSRI with the partial 5-HT<sub>1A</sub> antagonist pindolol [7]. Unfortunately, 5-HT<sub>1A</sub> antagonists were nonselective and simultaneously blocked pre- and postsynaptic receptors, an undesirable effect when treating depression [9]. A more promising direction in the search for next-generation antidepressants looks at agonists of both the 5-HT<sub>1A</sub> receptor and SSRI. Such compounds have a potential for accelerating desensitization and downregulation of autoreceptors, while directly stimulating postsynaptic serotonergic neurons. As a result of this process, the concentration of endogenous serotonin in the synaptic cleft increases [18]. Most importantly, the sensitivity of postsynaptic receptors does not decrease with prolonged administration [18,19]. This approach was positively verified by the introduction of vilazodone (Viibryd) to treat depression (Figure 3). Vilazodone was the first of several SSRI+ drugs whose mechanism of action involves both agonism towards the 5-HT<sub>1A</sub> receptors and serotonin transporter protein (SERT) inhibition [20–22]. In 2013, the Food and Drug Administration approved vortioxetine as another SSRI+ agent with an extended receptor activity profile for pharmacotherapy of depression (Figure 3). Vortioxetine acts as an SSRI, an agonist of the 5-HT<sub>1A</sub> receptor, a partial agonist of the 5-HT<sub>1B</sub> and an antagonist of the 5-HT<sub>3</sub> and 5-HT<sub>7</sub> receptors [23].

Figure 3. Chemical structures of vilazodone and vortioxetine.

This work describes the synthesis and results of pharmacological testing of a series of novel derivatives of 4-aryl-2H-pyrido[1,2-<i>c</i>]pyrimidine, characterized by double binding for the 5-HT<sub>1A</sub> receptor and SERT protein. Selected compounds were tested further to determine their activity towards other molecular targets, such as the 5-HT<sub>2A</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7</sub>, and D<sub>2</sub> receptors.

The research presented in this paper is a continuation of a long-term research project conducted in our department, where ligands are tested for a double binding affinity for both the SERT protein and 5-HT<sub>1A</sub> receptors [24–28]. Two series of novel derivatives of 4-aryl-2H-pyrido[1,2-<i>c</i>]pyrimidine and 4-aryl-5,6,7,8-tetrahydropyrido[1,2-<i>c</i>]pyrimidine were designed, based on lead compounds (I–IV) that had been synthesized previously by the same research group and had demonstrated a high affinity for both the 5-HT<sub>1A</sub> receptors and SERT protein (Figure 4) [26,27].

Modifications of lead compounds involved a change in the pharmacophore part via the introduction of a 6-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole residue. This addition should increase affinity for the 5-HT<sub>1A</sub> receptors and SERT protein, and thus these new compounds would potentially demonstrate dual binding affinity, appropriate functional activity and affinity for other molecular targets (e.g., 5-HT<sub>2A</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7</sub>, and D<sub>2</sub>).

This study aimed to investigate the effect of (i.) introducing a 6-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole residue to the pharmacophore, (ii.) the degree of saturation of the pyrido[1,2-<i>c</i>]pyrimidine residue in the terminal segment and (iii.) substituents in the 4-aryl-pyrido[1,2-<i>c</i>]pyrimidine moiety on affinity for both the 5-HT<sub>1A</sub> receptor and SERT protein and other receptors (5-HT<sub>2A</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7</sub>, D<sub>2</sub>) in extended receptor profile tests. Further testing probed the effect of these modifications on the functional activity (agonism-antagonism) and metabolic stability of the compounds of interest.
Figure 4. Comparison of the novel derivatives of 4-aryl-2H-pyrido[1,2-c]pyrimidine (6a–i) and 4-aryl-5,6,7,8-tetrahydropyrido[1,2-c]pyrimidine (7a–i), designed in this paper with lead compounds (I–IV).

2. Results and Discussion

2.1. Chemistry

The designed target compounds (6a–i) and (7a–i) were obtained in a multistage synthesis process (Figure 5). The starting materials were phenylacetonitrile derivatives subjected to C-arylation with 2-bromopyridine over KOH. This reaction yielded respective derivatives of α-(2-pyridyl)-α-(aryl)-acetonitriles (1a–i), which were subsequently hydrolyzed in an acidic environment to obtain respective α-(2-pyridyl)-α-(aryl)-acetamides (2a–i). These amides were then reacted with diethyl carbonate via a cyclocondensation process, producing derivatives of 4-aryl-2H-pyrido[1,2-c]pyrimidine-1,3-dione (3a–i). All the compounds (1a–i), (2a–i), (3a–i) were obtained according to an original method. The imides (3a–i) were then N-alkylated with 1,4-dibromobuthane to produce N-bromobuthyl derivatives of 4-aryl-2H-pyrido[1,2-c]pyrimidine-1,3-dione (4a–i). Some of the 2-(4-bromobuthyl)-4-aryl-pyrido[1,2-c]pyrimidine-1,3-dione (4a–i) derivatives were subjected to catalytic reduction over 10% Pd/C, yielding 2-(4-bromobuthyl)-4-aryl-5,6,7,8-tetrahydropyrido[1,2-c]pyrimidine-1,3-dione (5a–i) derivatives. The target compounds (6a–i) and (7a–i) were obtained by reacting the bromobuthyl derivatives of (4a–i) and (5a–i) with 6-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole. The chemical structures and purity of the newly synthesized compounds (6a–i) and (7a–i) were confirmed by 1H and 13C NMR spectroscopy, as well as LC/MS and HRMS spectrometry. The investigated compounds were subsequently tested in vitro and in vivo as free bases.
Figure 5. Schematic of the syntheses of compounds 6a–i and 7a–i. Reagents and conditions: (i) 2-bromopyridine, KOH, DMSO, 50 °C; (ii) H₂SO₄, CH₃COOH, 100 °C; (iii) (C₂H₅)₂CO₃, EtONa, EtOH reflux; (iv) 1,4-dibromobutane, acetone, K₂CO₃, reflux; (v) H₂, 10% Pd/C, EtOH, 60 atm., 50 °C; (vi) 6-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole, K₂CO₃, CH₃CN, 45 °C.

2.2. Biological Evaluation

2.2.1. Radioligand Binding Assay for 5-HT₁A and SERT

The target compounds (6a–i) and (7a–i) were assessed for in vitro affinity for the 5-HT₁AR receptor and SERT protein by radioligand binding assays [26,29,30]. The results, which were subsequently used for structure–activity relationship (SAR) analysis, can be found in Table 1. Various substituents at the ortho or para position of the benzene ring—as well as the degree of saturation of the pyrido[1,2-c]pyrimidine residue—were investigated with regard to their effect on the binding affinity of the compounds (6a–i) and (7a–i). The resulting data on 5-HT₁AR binding affinity indicated very high affinity of the following ligands: 6c (Kᵢ = 7.0 nM), 6g (Kᵢ = 10.0 nM), 6d (Kᵢ = 11.0 nM), 6e (Kᵢ = 15.0 nM), 6i (Kᵢ = 17.0 nM), 6b (Kᵢ = 20.0 nM), 6a (Kᵢ = 23.0 nM) and 6f (Kᵢ = 30 nM), with 6f being the only compound with a binding affinity at the level of Kᵢ = 74 nM (Table 1). The compounds (7a–i) (4-aryl-5,6,7,8-tetrahydropyrido[1,2-c]pyrimidine derivatives) demonstrated slightly lower binding affinity for the 5-HT₁AR compared to (6a–i). Very high binding affinity was noted for 7g (Kᵢ = 5.0 nM), 7i (Kᵢ = 9.5 nM), 7h (Kᵢ = 25.0 nM), 7a (Kᵢ = 27.0 nM), 7b (Kᵢ = 35.0 nM) and 7e (Kᵢ = 36.0 nM); the other three derivatives demonstrated high binding affinity, viz., 7f (Kᵢ = 52.0 nM), 7c (Kᵢ = 62.0 nM) and 7d (Kᵢ = 71.0 nM). A comparison of
binding affinity data for the ligands (6a–i) and (7a–i) indicated that the degree of saturation had little effect, with the 4-aryl-2H-pyrido[1,2-c]pyrimidine derivatives (6a–i) being slightly superior to the 4-aryl-5,6,7,8-tetrahydropyrido[1,2-c]pyrimidine derivatives (7a–i).

Table 1. 5-HT\textsubscript{1A} receptor and SERT binding affinities, as well as cLogP [31] of 4-aryl-2H-pyrido[1,2-c]pyrimidine derivatives 4-aryl-2H-pyrido[1,2-c]pyrimidine (6a–i) and 4-aryl-5,6,7,8-tetrahydropyrido[1,2-c]pyrimidine derivatives (7a–i).

| Compound | R   | R\textsubscript{1} | 5-HT\textsubscript{1A} | SERT  | cLogP |
|----------|-----|-------------------|------------------------|-------|-------|
| 6a       | –H  | –H                | 23.0 ± 1.0             | 32.0 ± 3.6 | 4.32  |
| 6b       | –CH\textsubscript{3} | –H | 30.0 ± 3.5 | >5000 | 4.81  |
| 6c       | –OCH\textsubscript{3} | –H | 7.0 ± 1.0 | >5000 | 4.30  |
| 6d       | –Cl | –H                | 11.0 ± 1.4             | 373.0 ± 32.0 | 4.98  |
| 6e       | –F  | –H                | 15.0 ± 0.6             | >1000 | 4.52  |
| 6f       | –H  | –CH\textsubscript{3} | 74.0 ± 4.0             | 772.0 ± 36.0 | 4.81  |
| 6g       | –H  | –OCH\textsubscript{3} | 10.0 ± 1.1             | 730.0 ± 79.0 | 4.98  |
| 6h       | –H  | –Cl               | 21.0 ± 1.5             | 310.0 ± 1.0  | 4.98  |
| 6i       | –H  | –F                | 17.0 ± 2.0             | 342.0 ± 28.8 | 4.55  |
| 6j       | –H  | –H                | 27.0 ± 2.0             | 520.0 ± 58.8 | 5.02  |
| 6k       | –H  | –CH\textsubscript{3} | 35.0 ± 3.5             | >1000 | 5.51  |
| 6l       | –OCH\textsubscript{3} | –H | 62.0 ± 6.0 | 878.0 ± 88.0 | 5.00  |
| 6m       | –Cl | –H                | 71.0 ± 6.9             | 310.0 ± 34.5 | 5.68  |
| 6n       | –F  | –H                | 36.0 ± 4.1             | >1000 | 5.22  |
| 6o       | –H  | –CH\textsubscript{3} | 52.0 ± 2.5             | 1773.0 ± 180.0 | 5.90  |
| 6p       | –H  | –OCH\textsubscript{3} | 5.0 ± 0.5              | 48.0 ± 2.4  | 5.00  |
| 6q       | –H  | –Cl               | 25.0 ± 3.0             | 290.0 ± 22.7 | 5.68  |
| 6r       | –H  | –F                | 9.5 ± 1.1              | 311.0 ± 35.0 | 5.22  |

| Reference compound | 3.6 ± 0.4 | 4.8 ± 0.5 | 17.0 ± 1.3 |

An analysis of the effect of the substituents in the benzene ring of the 4-aryl-2H-pyrido[1,2-c]pyrimidine residue on 5-HT\textsubscript{1A}R affinity of the ligands (6a–i) showed that the presence of a substituent at the ortho position generally increased binding affinity, compared to ligands with a substituent at the para position. The most marked effect on affinity was exerted by substituents in the compounds (6a–i) in the following order: 6c (o-OCH\textsubscript{3}) > 6g (p-OCH\textsubscript{3}) > 6d (o-Cl) > 6e (o-F) > 6i (p-CI) > 6a (-H) > 6b (o-CH\textsubscript{3}) > 6f (p-CH\textsubscript{3}). Analysis of the effect of substituents on the binding affinity of the derivatives of the 4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine (7a–i) series revealed the most marked effect on binding affinity of substituents at the para position in the following order: 7g (p-OCH\textsubscript{3}) > 7i (p-F) > 7h (p-Cl) > 7a (-H) > 7b (o-CH\textsubscript{3}) > 7e (o-F) > 7f (p-CH\textsubscript{3}) > 7c (o-CH\textsubscript{3}) > 7d (o-Cl).

Data on the affinity of the ligands (6a–i) and (7a–i) for SERT protein generally indicated poor binding affinity of most of the compounds. Consequently, it is difficult to determine the effect of the degree of saturation and substituents of the 4-aryl-pyrido[1,2-c]pyrimidine residue in the test compounds on their binding affinity. High binding affinity was only demonstrated for 6a (K\textsubscript{i} = 32.0 nM) and 7g (K\textsubscript{i} = 48.0 nM). The binding affinity values for the other ligands in both series are low (K\textsubscript{i} from 310–>5000 nM for series 6 and K\textsubscript{i} = 290–1773 nM for series 7).

The compounds 6a, 6d, 7g and 7i were selected for in vitro studies. The compounds 6a and 7g showed very high affinity for the 5-HT\textsubscript{1A} receptor and SERT protein, while the compounds 6d and 7i demonstrated very high affinity for the 5-HT\textsubscript{1A} receptor and poor affinity for SERT. The compounds were tested for their multiple receptor binding affinity,

An analysis of the effect of the substituents in the benzene ring of the 4-aryl-2H-pyrido[1,2-c]pyrimidine residue on 5-HT\textsubscript{1A} receptor and SERT binding affinities, as well as cLogP [31] of 4-aryl-2H-pyrido[1,2-c]pyrimidine derivatives 4-aryl-2H-pyrido[1,2-c]pyrimidine (6a–i) and 4-aryl-5,6,7,8-tetrahydropyrido[1,2-c]pyrimidine derivatives (7a–i).
with special regard to their affinity for the receptors 5-HT2A, 5-HT6, 5-HT7 and D2, whose role in the pathomechanism of depression has been well documented [15,32] (Table 2). It has been confirmed that disturbed dopaminergic neurotransmission in the mesolimbic and nigrostriatal regions also contributes significantly to the development of depression [32]. Consequently, intensive research is under way on a new class of potential drugs which exert their effects through interaction with D2/5-HT1A [33]; where the ligand would be a partial agonist of the 5-HT1A receptor and would induce postsynaptic serotonergic neurotransmission, which would increase dopamine levels in the mPFC (medial prefrontal cortex) [34]. The role of 5-HT2A receptors in the treatment of schizophrenia has also been well documented in numerous publications. Accordingly, multireceptor studies of the selected ligands 6a, 6d, 7g and 7i—including this molecular target—appear well justified. The role of the serotonergic receptors 5-HT6 and 5-HT7 in the pathomechanism of CNS disorders, including depression, has been well documented and presented in numerous papers [15], and so have the satisfactory results of treatment with multireceptor drugs, such as Aripiprazole, Clozapine [35] or Vortioxetine [23]. This encouraged us to investigate the extended affinity profile of selected compounds [36], targeting 5-HT2A, 5-HT6, 5-HT7 and D2 receptors.

Table 2. Binding affinity data on serotonin 5-HT1A, 5-HT2A, 5-HT6, 5-HT7 receptors, SERT protein, and dopamine D2 receptor of the investigated 4-aryl-2H-pyrido[1,2-c]pyrimidine (6a, 6d) and 4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine (7g, 7i) derivatives.

| Compound | R1 | R2 | 5-HT1A | SERT | 5-HT2A | 5-HT6 | 5-HT7 | D2 |
|----------|----|----|--------|------|--------|-------|-------|----|
| 6a       | -H | -H | 23.0 ± 1.0 | 32.0 ± 3.6 | 17 ± 3 | 376 ± 58 | 62 ± 5 | 7 ± 2 |
| 6d       | -Cl | -H | 11.0 ± 1.4 | 373.0 ± 32.0 | 20 ± 4 | 709 ± 135 | 109 ± 14 | 9 ± 2 |
| 7g       | -H | -OCH3 | 5.0 ± 0.5 | 48.0 ± 2.4 | 16 ± 2 | 400 ± 32 | 94 ± 11 | 10 ± 1 |
| 7i       | -H | -F | 9.5 ± 1.1 | 311.0 ± 35.0 | 44 ± 6 | 740 ± 183 | 161 ± 27 | 17 ± 2 |

| Reference Compound |
|---------------------|
| Olanzapine [37]     | 4.6 ± 0.9 | 7 ± 1 | n.d. | n.d. |
| Mianserin [37]      | 2.8 ± 0.5 | n.d.  | n.d. | n.d. |
| Clozapine [37]      | n.d.     | n.d.  | 18 ± 2 | n.d. |
| Haloperidol [37]    | n.d.     | n.d.  | n.d.  | 4.5 ± 0.7 |
| Apomorphine [37]    | n.d.     | n.d.  | n.d.  | 42 ± 6 |
| Chlorpromazine [37] | n.d.     | n.d.  | n.d.  | 1.8 ± 0.3 |

n.d. = not determined.

Table 2 presents binding affinities of the compounds 6a and 7g, which exhibited very high binding affinity for the receptors 5-HT1A, 5-HT2A, SERT and D2, and high binding affinity for 5-HT7. The compounds 6d and 7i, in turn, demonstrated very high affinity for the receptors 5-HT1A, 5-HT2A and D2 and moderate affinity for the receptor 5-HT7, with low affinity for the receptors 5-HT6 and SERT. The in vitro data for both groups of compounds can be seen as a good starting point for further research on multireceptor ligands in the treatment of depressive disorder or schizophrenia. Additionally, cLogP values for the compounds in the 4-aryl-2H-pyrido[1,2-c]pyrimidine (6a–i) series ranged from 4.30 to 4.98, while cLogP values for the series of 4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine (7a–i) derivatives ranged from 5.00-5.90. These values are given in Table 1. A comparison of cLogP values for both ligand series shows that all cLogP values for the (6a–i) series do not exceed 5.00—which, according to Lipiński [38,39] is a cut-off value for transmembrane penetration required of candidate drugs. The derivatives 6a (R1 = -H, R2 = -H) and 6c (R = -OCH3, R1 = H) had a cLogP value of 4.32 and 4.30, respectively, which can be compared to the cLogP value of 4.26 [30] of Vortioxetine, a well-known antidepressant of the SSRI/5-HT1A class. At the same time, ligands of the (7a–i) series
demonstrated cLogP values >5, which is not pharmacologically desirable, according to Lipinski’s rule [38].

2.2.2. In Vivo Studies

To determine the profile of functional activity of the selected ligands, behavioral tests were performed. It is known that 8-OH-DPAT, a 5-HT₁A receptor agonist, can induce hypothermia in mice, through 5-HT₁A somatodendritic receptors [30,40]. Moreover, this effect can be abolished by WAY-100635 [41], a 5-HT₁A receptor antagonist. Based on this knowledge, we tested compounds 6a (R = -H, R₁ = -H) and 7g (R = -H, R₁ = -OCH₃) in a commonly used in vivo panel of tests, to assess their functional 5-HT₁A receptor activity. Test compounds 6a and 7g, like 8-OH-DPAT, induced hypothermia in mice (Table 3).

Table 3. The effect of compounds 6a and 7g on body temperature in mice.

| Treatment          | Dose (mg/kg) | Δt ± SEM (°C) |
|--------------------|-------------|---------------|
|                    | 30 min      | 60 min        | 90 min        | 120 min       |
| Vehicle            | -           | -             | -             | -             |
| 6a                 | -0.2 ± 0.2  | -0.0 ± 0.2    | -0.1 ± 0.1    | -0.0 ± 0.1    |
| 5                  | -2.0 ± 0.2  | -3.2 ± 0.5    | -3.1 ± 0.5    | -2.8 ± 0.5    |
| 2.5                | -1.6 ± 0.2  | -2.5 ± 0.3    | -2.4 ± 0.3    | -2.0 ± 0.2    |
| 1.25               | -1.7 ± 0.3  | -1.7 ± 0.3    | -1.6 ± 0.2    | -1.6 ± 0.3    |
| 0.6                | -1.0 ± 0.2  | -1.2 ± 0.1    | -0.9 ± 0.1    | -0.8 ± 0.2    |
| Vehicle            | -           | 0.0 ± 0.1     | -0.1 ± 0.1    | -0.1 ± 0.1    |
| 7g                 | -0.0 ± 0.2  | 0.1 ± 0.2     | 0.1 ± 0.2     | 0.1 ± 0.2     |
| 5                  | -2.1 ± 0.1  | -3.4 ± 0.3    | -3.9 ± 0.5    | -4.2 ± 0.8    |
| 2.5                | -2.2 ± 0.2  | -2.8 ± 0.3    | -2.9 ± 0.3    | -3.0 ± 0.4    |
| 1.25               | -1.5 ± 0.3  | -1.7 ± 0.3    | -1.3 ± 0.4    | -1.0 ± 0.3    |
| Vehicle            | -           | 0.2 ± 0.1     | 0.1 ± 0.2     | 0.1 ± 0.2     |
| WAY-100635         | 0.1         | 0.1 ± 0.2     | -0.1 ± 0.2    | -0.2 ± 0.1    |
| 8-OH-DPAT          | 5           | -1.7 ± 0.2    | -1.1 ± 0.2    | 0.3 ± 0.3     |

The investigated compounds were administered 30 min before the test, a p < 0.05 vs vehicle, b p < 0.001 vs vehicle, ns = non-significant.

WAY-100635 (0.1 mg/kg) diminished hypothermia induced by compound 6a (0.6 mg/kg) by 50% (Table 4).

Table 4. The effect of WAY-100635 (0.1 mg/kg sc) on the hypothermia induced by compounds 6a and 7g.

| Treatment and Dose (mg/kg) | Δt ± SEM (°C) |
|---------------------------|---------------|
|                           | 30 min        | 60 min        |
| Vehicle                   | 0.0 ± 0.1     | 0.0 ± 0.0     |
| Vehicle + 6a (0.6)        | -1.0 ± 0.2    | -1.2 ± 0.1    |
| WAY-100635 + 6a           | -0.5 ± 0.2    | -0.4 ± 0.2    |
|                           | p < 0.0001    | p < 0.0001    |
| Vehicle                   | -0.3 ± 0.1    | -0.2 ± 0.1    |
| Vehicle + 7g (1.25)       | -1.5 ± 0.3    | -1.7 ± 0.3    |
| WAY-100635 + 7g           | -1.2 ± 0.2    | -0.7 ± 0.3    |
|                           | p < 0.0001    | p < 0.0005    |
| Vehicle                   | 0.1 ± 0.1     | -0.0 ± 0.1    |
| WAY-100635                | 0.5 ± 0.3     | 0.2 ± 0.3     |

WAY-100635 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 < 0.001 vs compound group, ns = non-significant.
In conclusion, the decrease in mouse body temperature produced by compound 6a can be accounted as a measure of its presynaptic 5-HT\textsubscript{1A} agonistic activity. Tested compound 6a was ineffective in the forced swimming test in mice (Figure 6), so we can conclude lack of postsynaptic 5-HT\textsubscript{1A} receptor activity [40].

Figure 6. Effect of compound 6a on forced swimming test in CD-1 mice.

2.2.3. Metabolic Stability Evaluation

We decided to carry out preliminary tests of metabolic stability for selected compounds, as metabolic stability is an important index of a compound’s pharmacokinetics. Such studies are routinely performed at earlier stages of studies of potential new drugs. They are extremely important, as many known valuable compounds possessing high desirable pharmacological activity are disqualified at later clinical stages on account of an undesirable pharmacokinetic profile with regard to metabolic stability. Thus, the preliminary studies in this regard performed by us appeared advisable.

A compound with poor metabolic stability will not reach appropriate therapeutic levels for a given molecular target. High metabolic stability of a candidate drug or its metabolite can, in turn, potentially cause higher toxicity or adverse effects. On the other hand, the identification of inhibition or induction of cytochrome P-450 isoenzymes, which mediate the metabolism of most drugs, allows for predicting potential drug-drug interactions [42,43]. The results of a metabolic stability study in the presence of pooled human liver microsomes (HLMs) and nicotinamide adenine dinucleotide phosphate (NADPH) are shown in Table 5. Metabolic stability is presented in the form of biological half-life value, which allows for easy comparison of compounds’ structure and their susceptibility to phase 1 biotransformation reactions (the result of incubation on the presence of human liver microsomes). Results presented in Table 5 allow for the quick assessment of metabolic stability.

Table 5. Experimental t\textsubscript{1/2} values along with corresponding SD and RSD%.

| Compound | Average t\textsubscript{1/2} [min] (n = 2) | SD [min] | RSD% |
|----------|----------------------------------------|----------|------|
| 6a       | 3.61                                   | 0.43     | 11.18|
| 6d       | 3.20                                   | 0.09     | 2.81 |

Table legend: SD—standard deviation, RSD%—relative standard deviation, expressed as SD/average \times 100%.

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 \mu M, such values were to be expected. Of the studied pyrido[1,2-c]pyrimidine derivatives, compounds 6a (R = -H, R\textsubscript{1} = -H) and 6d (R = -Cl, R\textsubscript{1} = -H) were most susceptible to phase 1 biotransformation reactions. The metabolic stability investigations for the compounds 6a and 6d showed their sensitivity to the activity of human liver microsomes, which is most likely associated with the presence of a 6-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole residue in the pharmacophore part. These results will prompt further studies in search of compounds with greater metabolic stability in this group of derivatives. The justification
for further research stems from the fact that the novel compounds revealed very high affinity for a number of receptors and were also characterized by appropriate cLogP values.

3. Materials and Methods

3.1. General Remarks

Reagents and solvents were purchased from commercial suppliers: Sigma-Aldrich, TCI, Alfa Aesar and Chempur. The purity of the obtained samples was routinely confirmed by TLC using Merck plates (Kieselgel 60 F\textsubscript{254}). Melting points (m.p.) were determined using the Electrothermal IA 9200 apparatus with open capillary tubes and were not corrected. \textsuperscript{1}H and \textsuperscript{13}C NMR spectra were recorded on a Bruker AVANCE III HD (500 MHz) instrument in CDCl\textsubscript{3} (chemical shifts are reported in δ units), with the use of TMS as the internal reference. The following abbreviations were used to describe peak patterns when appropriate: s (singlet), 2s (double singlet), d (doublet), dd (double doublet), dt (double triplet), t (triplet), td (triple doublet), 4d (quartet of doublets), m (multiplet), q (quartet), qu (quintet). Coupling constants (J) are in hertz (Hz). Numbering system, which was used in NMR spectra interpretation is shown in Figure 7. ESI-HRMS spectra were obtained on a Thermo Q-Exactive instrument. Flash column chromatography was carried out using Merck Silicagel (40–63 µm) Geduran\textsuperscript{®} Si 60 and a mixture of toluene:ethylacetate:methanol (10:4.3 v/v), methylene chloride:methanol:triethylamine (64:2:0.4 v/v), chloroform:methanol (9:1 v/v) as an eluent. Thin layer chromatography was performed on Merck Silicagel (Kieselgel 60 F\textsubscript{254}) plates, where the mobile phase was composed of toluene, dioxane, ethanol, and 25% ammonia (9.0:5.0:1.0:0.3 v/v) or methylene chloride, methanol, triethylamine (16.0:1.0:0.2 v/v). Plates were visualized by UV light (254 nm).

3.2. Synthesis of Compounds

3.2.1. Procedure for the Synthesis of 2-(4-Bromobutyl)-4-aryl-pyrido[1,2-c]pyrimidine-1,3-diones (4a–i) and 2-(4-bromobutyl)-4-aryl-5,6,7,8-tetrahydropyrido[1,2-c]pyrimidine-1,3-diones (5a–i)

Compounds (4a–i) and (5a–i) were obtained according to the previously described procedures [26,37,44].

3.2.2. General Procedure for the Synthesis of Derivatives of 4-Aryl-2H-pyrido[1,2-c]pyrimidine-1,3-dione (6a–i) and 4-aryl-5,6,7,8-tetrahydropyrido[1,2-c]pyrimidine-1,3-dione (7a–i)

The appropriate bromobutyl derivatives (4a–i) or (5a–i) (0.75 mmol), 6-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole (0.75 mmol) and K\textsubscript{2}CO\textsubscript{3} (2 mmol) were suspended in acetonitrile (25 mL). The reaction mixture was carried out at 45 °C and stirred for 8–12 h. The reaction time was determined using TLC. The mixture was filtered to remove inorganic salts, and the solvent was removed from the filtrate under vacuum. The residue was purified by column chromatography (flash or gravity technique) using toluene:ethylacetate:methanol (10:4.3 v/v), methylene chloride:methanol:triethylamine (64:2:0.4 v/v), chloroform:methanol (9:1 v/v) as an eluent. Appropriate fractions were identified by TLC and evaporated to give compounds (6a–i) or (7a–i).

![Figure 7. Numbering system for NMR spectra interpretation of compounds (6a-i) and (7a-i).](image-url)
The title compound was isolated as a yellow powder. Yield: 20.5%; m.p. 132–133 °C.

\[ \text{H NMR (500 MHz, CDCl}_3\text{): } \delta \text{ 8.33 (C8H, dt, } J = 7.5, \ J = 3) = 5\), 7.75 (C4'H, dd, } J = 8.5, \ J = 5\), 7.42–7.47 (C2'H, C6'H, m), 7.35 (C4'H, tt, } J = 7.5, \ J = 1.5), 7.30–7.33 (C3'H, C5'H, m), 7.22 (C7'H, 4d, } J = 8.5, \ J = 2.5, \ J = 0.5), 7.04 (C5'H, td, } J = 9.0, \ J = 4), 6.89–6.91 (C5H, C6H, m), 6.38 (C7H, m, } J = 8.0, \ J = 4.5, \ J = 3.5), 4.20 (C1'H2, t, } J = 7.5), 3.11 (CaH(E), CbH(E), CcH, c), 2.52 (C4'H2, t, } J = 7.0), 2.02–2.30 (CaH(A), CbH(A), CdH(A), CbH(E), CdH(E), m), 1.81 (C2'H2, q, } J = 7.5), 1.68 (C3'H2, q, } J = 7.5).

13C NMR (125 MHz, CDCl3): } \delta 164.1 (C6", d, } J = 250.6*), 163.9 (C7"a, d, } J = 13.6*), 160.9 (C3, s), 160.1 (C3", s), 148.3 (C1, s), 143.5 (C4a, s), 132.8 (C1", s), 132.4 (C6, s), 131.2 (C2", C6", s), 128.8 (C3", C5", s), 127.9 (C4', s), 127.8 (C8, s), 122.8 (C4", d, } J = 10.2*), 121.4 (C5, s), 117.2 (C3"a, s), 112.3 (C5", d, } J = 25.3*), 110.7 (C7, s), 104.9 (C4, s), 97.3 (C7", d, } J = 26.7*), 58.3 (C4", s), 53.4 i, m (4.3 (Ca i lub Ce, 2s), 42.3 (C1", s), 34.4 (Cc, s), 30.1 (Cb, Cd, s), 25.4 (C2", s), 24.1 (C3", s).

ESI-HRMS m/z: Calcd for C30H30FN4O3 [M + H]+ 513.2296. Found: 513.2304

The title compound was isolated as a yellow powder. Yield: 79.7%; m.p. 120–122 °C.

\[ \text{H NMR (500 MHz, CDCl}_3\text{): } \delta \text{ 8.33 (C8H, dt, } J = 7.5, \ J = 3) = 5\), 7.78 (C4'H, dd, } J = 8.5, \ J = 5\), 7.21–7.33 (C4'-6'H, C7"H, [4H], m), 7.14 (C3"H, dd, } J = 8.0, \ J = 1.5), 7.05 (C5"H, td, } J = 9.0, \ J = 2.0), 6.88 (C6'H, 4d, } J = 9.5, \ J = 6.0, \ J = 1.5), 6.56 (C5H, dt, } J = 9.5, \ J = 4), 6.38 (C7H, m, } J = 7.5, \ J = 6.0, \ J = 1.0), 6.40 (C1'H2, t, } J = 7.5), 3.08 (CaH(E), CbH(E), CcH, m), 2.48 (C4'H2, t, } J = 7.5), 2.15 (C3H3, s), 2.0–2.2 (C4a, Ca, Cb, CbH2, Cdd, m), 1.80 (C2'H2, q, } J = 7.5), 1.65 (C3'H2, q, } J = 7.5).

13C NMR (125 MHz, CDCl3): } \delta 164.0 (C6", d, } J = 250.6*), 163.8 (C7"a, d, } J = 13.6*), 161.0 (C3", s), 159.6 (C3, s), 149.1 (C1, s), 143.4 (C4a, s), 138.4 (C2", s), 132.4 (C1", s), 132.1 (C6, s), 131.5 (C6", s), 130.5 (C3', s), 128.4 (C4', s), 128.0 (C8, s), 126.4 (C5', s), 122.7 (C4", d, } J = 11.1*), 121.4 (C5, s), 117.2 (C3"a, s), 112.3 (C5", d, } J = 25.3*), 110.5 (C7, s), 104.1 (C4, s), 97.4 (C7", d, } J = 26.7*), 58.4 (C4", s), 53.4 (Ca, Ce, s), 42.2 (C1", s), 34.5 (Cc, s), 30.3 (Cb, Cd, s), 25.5 (C2", s), 24.2 (C3", s), 19.6 (CH2, s).

ESI-HRMS m/z: Calcd for C31H32FN4O4 [M + H]+ 527.2453. Found: 527.2461

The title compound was isolated as a yellow powder. Yield: 71.1%; m.p. 65–70 °C.

\[ \text{H NMR (500 MHz, CDCl}_3\text{): } \delta \text{ 8.32 (C8H, dt, } J = 7.5, \ J = 3) = 5\), 7.73 (C4'H, dd, } J = 8.5, \ J = 5\), 7.37 (C4'H, 4d, } J = 8.0, \ J = 3.2), 7.22 (C6'H, C7"H, m), 7.01–7.07 (C5'H, C5"H, m), 6.88 (C6'H, 4d, } J = 9.5, \ J = 6.5, \ J = 1.5), 6.63 (C5H, dt, } J = 9.0, \ J = 4), 5.37 (C7H, m, } J = 7.5, \ J = 6.5, \ J = 1.5), 4.19 (C1'H2, t, } J = 7.5), 3.76 (CH3C, s), 3.08 (CaH(E), CbH(E), CcH, m), 2.48 (C4'H2, t, } J = 7.5), 2.0–2.2 (CaA, CeA, CbH2, CddH2, m), 1.80 (C2'H2, q, } J = 7.0), 1.66 (C3'H2, q, } J = 7.0).

13C NMR (125 MHz, CDCl3): } \delta 164.0 (C6", d, } J = 250.4*), 163.8 (C7"a, d, } J = 13.6*), 161.1 (C3", s), 159.9 (C3, s), 157.9 (C1", s), 149.1 (C1, s), 143.6 (C4a, s), 133.0 (C6", s), 131.9 (C6, s), 129.6 (C4', s), 127.8 (C8, s), 122.8 (C4", d, } J = 11.1*), 121.9 (C1', s), 121.4 (C5, s), 120.9 (C5", s), 117.2 (C3"a, s), 112.3 (C5", d, } J = 25.1*), 111.4 (C3", s), 110.5 (C7, s), 101.2 (C4, s), 97.3 (C7", d, } J = 26.7*), 58.4 (C4", s), 55.6 (CH3C, s), 53.5 (Ca, Ce, s), 42.3 (C1", s), 34.5 (Cc, s), 30.3 (Cb, Cd, s), 25.5 (C2", s), 24.2 (C3", s).

ESI-HRMS m/z: Calcd for C31H32FN4O4 [M + H]+ 543.2420. Found: 543.2410
4-(2-Chlorophenyl)-2-[[4-(6-fluoro-1,2-benzoxazol)-1-piperidyl]butyl]-pyrido[1,2-c]pyrimidine-1,3-dione 6d

The title compound was isolated as a yellow powder. Yield: 71.7%, m.p. 69–74 °C.

1H NMR (500 MHz, CDCl3): δ 8.37 (C8H, dt, 3J = 7.5, 4J = 1.0), 7.75 (C4'H, bs), 7.52 (C3'H, m), 7.29–7.37 (C4'H, C5'H, C6'H, m), 7.23 (C7'H, 4d, 3J = 8.5, 4J = 2.5, 5J = 0.5), 7.05 (C5'H, td, 3J = 8.5, 4J = 2.0), 6.97 (C6'H, 4d, 3J = 9.5, 4J = 6.0, 5J = 1.0), 6.56 (C5H, dt, 3J = 9.0, 4J = 1.0), 6.43 (C7H, m, 3J = 7.5, 4J = 6.5, 5J = 1.5), 4.21 (C1'H2, m), 3.10 (CaH(E), CeH(E), CcH, bs), 2.51 (C4'H2, bs), 2.0–2.3 (CaH(A), CeH(A), CbH2, CdH2), 1.81 (C2'H2, m), 1.67 (C3'H2, m).

13C NMR (125 MHz, CDCl3): δ 164.1 (C6′, d, 1J = 250.6*), 163.9 (C7′a, d, 3J = 13.6*), 161.0 (C3′′, s), 159.5 (C3, s), 149.0 (C1, s), 143.8 (C4a, s), 135.7 (C6′, s), 133.4 (C6′, s), 133.1 (C1′, s), 131.7 (C6, s), 130.0 (C3′, s), 129.7 (C4′, s), 128.1 (C8, s), 127.3 (C5′, s), 122.8 (C4′, d, 3J = 10.7*), 121.1 (C5′, s), 117.2 (C3′a, s), 122.3 (C5′, d, 2J = 25.1*), 110.8 (C7, s), 102.2 (C4, s), 97.4 (C7′, d, 2J = 26.7*), 58.3 (C4′, s), 53.4 (Ca, Ce, s), 42.2 (C1, s), 34.4 (Cc, s), 30.2 (Cb, Cd, s), 25.4 (C2′, s), 24.0 (C2′, s).

ESI-HRMS m/z: Calcd for C36H29ClF4N3O3 [M + H]+ 547.1907. Found: 547.1915

4-(2-Fluorophenyl)-2-[[4-(6-fluoro-1,2-benzoxazol)-1-piperidyl]butyl]-pyrido[1,2-c]pyrimidine-1,3-dione 6e

The title compound was isolated as a yellow powder. Yield: 63.2%, m.p. 58–62 °C.

1H NMR (500 MHz, CDCl3): δ 8.37 (C8H, dt, 3J = 7.5, 4J = 1.0), 7.74 (C4'H, m), 7.35–7.43 (C4'H, m), 7.33 (C5'H, td, 3J = 7.5, 4J = 2.0), 7.23 (C6'H, C7'H, m), 7.17 (C3'H, m, 3J = 1.0, 4J = 1.0), 7.04 (C5'H, td, 3J = 8.5, 4J = 2.5), 6.98 (C6'H, 4d, 3J = 9.5, 4J = 1.5), 6.74 (dt) and 6.74 (dt) (C5H, 3J = 9.0, 4J = 5J = 1.5*), 6.43 (C7H, m, 3J = 7.5, 4J = 6.5, 5J = 1.5), 4.20 (C1'H2, C′, 3J = 7.5), 3.09 (CaH(E), CbH(E), CcH, pd), 2.49 (C4'H2, bs), 2.00–2.30 (CaH(A), CeH(A), CbH2, CdH2, m), 1.81 (C2'H2, q, 3J = 7.5), 1.66 (C3'H2, q, 3J = 7.5).

13C NMR (125 MHz, CDCl3): δ 164.1 (C6′, d, 1J = 250.4*), 163.9 (C7′a, d, 3J = 13.5*), 161.1 (C3′′, s), 160.9 (C2′, d, 3J = 246.9*), 159.6 (C3, s), 148.9 (C1, s), 144.0 (C4a, s), 133.4 (C6′, d, 3J = 3.0*), 133.1 (C6, s), 130.1 (C4′, d, 3J = 8.2*), 128.2 (C8, s), 124.4 (C5′, d, 4J = 3.5*), 122.8 (C4′, d, 3J = 11.1*), 121.2 (C5, s), 120.3 (C1′, d, 2J = 16.0*), 117.2 (C7′a, s), 116.1 (C3′, d, 2J = 22.3*), 112.3 (C5′, d, 2J = 25.4*), 110.8 (C7, s), 98.4 (C4, s), 97.4 (C7′, d, 2J = 26.6*), 58.4 (C4′, s), 53.5 (Ca, Ce, s), 42.4 (C1′, s), 34.5 (Cc, s), 30.3 (Cb, Cd, s), 25.5 (C2′, s), 24.2 (C3′, s).

ESI-HRMS m/z: Calcd for C36H29ClF4N3O3 [M + H]+ 531.2202. Found: 531.2212

4-(4-Methylphenyl)-2-[[4-(6-fluoro-1,2-benzoxazol)-1-piperidyl]butyl]-pyrido[1,2-c]pyrimidine-1,3-dione 6f

The title compound was isolated as a yellow powder. Yield: 71.1%, m.p. 158–159 °C.

1H NMR (500 MHz, CDCl3): δ 8.32 (C8H, dt, 3J = 7.5, 4J = 1.0), 7.78 (C4'H, bs), 7.18–7.26 (C2'H, C3'H, C5'H, C6'H, C7'H, m), 7.05 (C5'H, td, 3J = 9.0, 4J = 2.0), 6.86–6.93 (C5H, CeH, m), 6.37 (C7H, m, 3J = 7.5, 4J = 5.5, 5J = 1.5), 4.19 (C1'H2, t, 3J = 7.5), 3.14 (CaH(E), CeH(E), CcH, bs), 2.57 (C4'H2, bs), 2.39 (Ch3, s), 2.03–2.35 (CaH(A), CcH(A), CbH2, CdH2, m), 1.81 (C2'H2, q, 3J = 7.5), 1.70 (C3'H2, bs).

13C NMR (125 MHz, CDCl3): δ 164.1 (C6′, d, 1J = 250.7*), 163.9 (C7′a, d, 3J = 13.6*), 160.8 (C3′′, s), 165.0 (C3, s), 149.0 (C1, s), 143.5 (C4a, s), 137.6 (C6, s), 132.2 (C4′, s), 131.0 (C2′, C6′, s), 129.7 (C1′, s), 129.5 (C3′, C5′, s), 127.9 (C8, s), 122.8 (C4′, d), 121.6 (C5, s), 117.1 (C3′a, bs), 112.4 (C5′, d, 2J = 25.1*), 110.6 (C7H, s), 104.9 (C4, s), 97.4 (C7′, d, 2J = 26.8*), 58.2 (C4′, s), 53.2 (Ca, Ce, s), 42.1 (C1′, s), 34.2 (Cc, s), 30.0 (Cb, Cd, s), 25.4 (C2′, s), 23.7 (C3′, s), 21.3 (Ch3, s).

ESI-HRMS m/z: Calcd for C31H32F4N3O3 [M + H]+ 527.2453. Found: 527.2459
4-(4-Methoxyphenyl)-2-[4-[4-(6-fluoro-1,2-benzoxazol)-1-piperidyl]butyl]-pyrido[1,2-c]pyrimidine-1,3-dione 6g

The title compound was isolated as a yellow powder. Yield: 78.9%; m.p. 163–166 °C. 1H NMR (500 MHz, CDCl3): δ 8.31 (C8H, dt, 3J = 7.5, 4J = 5J = 1.0), 7.73 (C4"H, dd, 3J = 8.5, 4J = 5J = 5.0), 7.24 (C2"H, C6"H, dt, 3J = 8.5, 4J = 3.0), 7.21–7.24 (C7"H, m*), 7.04 (C5"H, td, 3J = 9.0, 4J = 2.0), 6.98 (C3"H, C5"H, dt, 3J = 8.5, 4J = 3.0), 6.88–6.94 (C5H, C6H, m), 6.37 (C7H, m, 3J1 = 8.0, 3J2 = 6.0, 4J = 1.5), 4.19 (C1H, t, 3J = 7.5), 3.84 (OHCH2, s), 3.09 (CaE, Ce(E), Cc, 3.09, m), 2.47 (C4H2, t, 3J = 7.5), 2.00–2.20 (Ca(A), Ce(A), CbH2, CdH2, m), 1.80 (C2H2, q, 3J = 7.5), 1.65 (C3H2, q, 3J = 7.7).

13C NMR (125 MHz, CDCl3): δ 164.1 (C6", d, 1J = 250.4*), 163.8 (C7"a, d, 3J = 13.6*), 161.1 (C3", s), 160.4 (C3, s), 159.1 (C4", s), 149.0 (C1, s), 143.5 (C4a, s), 132.3 (C2", C6", s), 132.1 (C5, s), 127.9 (C8, s), 124.8 (C1', s), 122.7 (C4", d, 3J = 11.1*), 121.6 (C5, s), 117.2 (C3"a, s), 114.3 (C3", C5", s), 112.3 (C5", s, 2J = 25.3*), 110.6 (C7, s), 104.6 (C4, s), 97.4, d, 2J = 26.8*), 58.4 (C4", s), 55.3 (OHCH3, s), 53.5 (C, Ce, s), 42.4 (C1", s), 34.5 (Cc, s), 30.4 (Cb, Cd, s), 25.5 (C2", s), 24.3 (C3", s).

ESI-HRMS m/z: Calcd for C31H32FN4O4 [M + H]+ 543.2402. Found: 543.2411

4-(4-Chlorophenyl)-2-[4-[4-(6-fluoro-1,2-benzoxazol)-1-piperidyl]butyl]-pyrido[1,2-c]pyrimidine-1,3-dione 6h

The title compound was isolated as a yellow powder. Yield: 63.2%; m.p. 152–155 °C. 1H NMR (500 MHz, CDCl3): δ 8.35 (C8H, dt, 3J = 7.5, 4J = 5J = 1.0), 7.76 (C4"H, bs), 7.25–7.29 (C3"H, C5"H, dt, 3J = 8.5, 4J = 2.5), 7.23 (C7"H, 4d, 3JHF = 8.5, 4J = 2.0, 5J = 0.5), 7.40–7.44 (C2"H, C6"H, dt, 3J = 8.5, 4J = 2.5), 7.00 (C5"H, td, 3J = 8.5, 4J = 2.0), 6.89 (C6H, m, 3J = 9.0, 4J = 1.5), 6.61 (C7H, m, 3J1 = 7.5, 3J2 = 6.0, 4J = 1.0), 4.19 (C1H, t, 3J = 7.5), 3.13 (CaH(E), CeH(E), CcH, bs), 2.55 (C4H2, bs), 2.0–2.4 (Ca(H)A, Ce(H)A, CbH2, CdH2, m), 1.80 (C2H2, q, 3J = 7.0), 1.70 (C3"H, bs).

13C NMR (125 MHz, CDCl3): δ 164.1 (C6", d, 1J = 250.7*), 163.9 (C7"a, d, 3J = 13.7*), 160.8 (C3", s), 160.0 (C3, s), 148.8 (C1, s), 143.7 (C4", s), 133.7 (C4", s), 132.6 (C3", C5", s), 131.2 (C1", s), 129.0 (C2", C6", s), 128.1 (C8, s), 122.8 (C4", d, 3J = 9.4*), 121.1 (C5, s), 117.1 (C3", s), 112.4 (C5", d, 2J = 24.9*), 110.9 (C7, s), 103.5 (C4, s), 97.4 (C7", d, 2J = 26.8*), 58.2 (C4", s), 53.3 (Ca, Ce, s), 42.3 (C1", s), 34.3 (Cc, s), 29.9 (Cb, Cd, s), 25.4 (C2", s), 23.8 (C3", s).

ESI-HRMS m/z: Calcd for C30H29ClF4N4O3 [M + H]+ 547.1907. Found: 547.1916

4-(4-Fluorophenyl)-2-[4-[4-(6-fluoro-1,2-benzoxazol)-1-piperidyl]butyl]-pyrido[1,2-c]pyrimidine-1,3-dione 6i

The title compound was isolated as a yellow powder. Yield: 17.6%; m.p. 102–107 °C. 1H NMR (500 MHz, CDCl3): δ 8.34 (C8H, dt, 3J = 7.0, 4J = 5J = 1.5), 7.75 (C4"H, bs), 7.29 (C2"H, C6"H, m), 7.23 (C7"H, 4d, 3JHF = 8.5, 4J = 2.0, 5J = 0.5), 7.14 (C3"H, C5"H, tt, 3J = 8.5), 7.052 (C5"H, td, 3J = 8.5, 4J = 2.0), 6.94 (C6H, 4d, 3J1 = 9.0, 3J2 = 6.0, 4J = 1.5), 6.87 (C5H, dt, 3J = 9.0, 4J = 5J = 1.5), 6.41 (C7H, m, 3J1 = 7.5, 3J2 = 6.0, 4J = 1.5), 4.19 (C1H, t, 3J = 7.5), 3.12 (CaH(E), CeH(E), CcH, bs), 2.53 (C4H2, bs), 2.0–2.3 (Ca(H)A, Ce(H)A, CbH2, CdH2, m), 1.81 (C2H2, q, 3J = 7.5), 1.65 (C3"H, bs).

13C NMR (125 MHz, CDCl3): δ 164.1 (C6", d, 1J = 250.8*), 163.9 (C7"a, d, 3J = 13.5*), 162.3 (C4"H, d, 1J = 247.2*), 161.0 (C3", s), 160.2 (C3, s), 148.9 (C1, s), 143.7 (C4a, s), 133.0 (C2", C6", d, 3J = 8.2*), 132.8 (C6, s), 128.6 (C1", d, 3J = 3.5*), 128.1 (C8, s), 122.8 (C4", d, 3J = 10.8*), 121.2 (C5, s), 117.2 (C3", s), 115.8 (C3", C9", d, 2J = 21.5*), 112.4 (C5", d, 2J = 24.9*), 110.8 (C7, s), 103.8 (C4, s), 97.4 (C7", d, 2J = 26.8*), 58.4 (C4", s), 53.4 (Ca, Ce, s), 42.3 (C1", s), 34.4 (Cc, s), 30.1 (Cb, Cd, s), 25.4 (C2", s), 24.1 (C3", s).

ESI-HRMS m/z: Calcd for C32H27F2N4O3 [M + H]+ 531.2202. Found: 531.2211
4-Phenyl-2-[4-(6-fluoro-1,2-benzoazol-3-yl)-1-piperidyl]butyl]-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione 7a

The title compound was isolated as a white powder. Yield: 81.6%; m. p. 110–113 °C.

\[ \begin{align*}
\text{H NMR} & : \delta 7.78 (C4'H, bs), 7.40 (C3'H, C5'H, t, J = 7.5), 7.33 (C4'H, tt, J = 7.5), 7.23 (C7'H, dd, J_{HF} = 8.0, J = 2.0), 7.19 (C2'H, C6'H, dt, J = 7.5), 7.05 (C5'H, td, J = 9.0, J = 2.0), 4.95 (C1'H, t, J = 7.5), 3.95 (C8'H, t, J = 6.5), 3.14 (Cah(E), Ceh(E), Cch, bs), 2.54 (C4'H2, C5'H2, m), 2.05–2.35 (Cah(A), Ceh(A), Cdh2, m), 1.93 (C7'H2, q, J = 6.5), 1.71 (C2'H2, C3'H2, C6'H2, m).
\end{align*}\]

\[ \begin{align*}
\text{C NMR} & : \delta 164.1 (C6'', d, J = 250.6), 163.9 (C7''a, d, J = 13.4), 162.0 (C5, s), 160.8 (C3'', s), 151.7 (C1, s), 149.8 (C4a, s), 133.3 (C1', s), 130.7 (C2', C6', s), 128.5 (C3', C5', s), 127.7 (C4', s), 122.9 (C4', d*), 117.1 (C3'a, s), 112.4 (C5'', d, J = 25.2), 112.4 (C4, s), 97.4 (C7'', d, J = 26.7), 58.3 (C4', s), 53.3 (Ca, Ce, s), 42.7 (C8, s), 41.3 (C1', s), 34.1 (Cc, s), 29.8 (Cb, Cd, s), 26.7 (C5, s), 25.6 (C2', s), 23.9 (C3', s), 21.8 (C7, s), 18.6 (C6, s).
\end{align*}\]

ESI-HRMS m/z: Calcd for C_{30}H_{34}F_{10}N_{3}O_{3} [M + H]^+ 517.2609. Found: 517.2617

4-(2-Methylphenyl)-2-[4-(6-fluoro-1,2-benzoazol-1-yl)-1-piperidyl]butyl]-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione 7b

The title compound was isolated as an oil. Yield: 97.4%.

\[ \begin{align*}
\text{H NMR} & : \delta 7.75 (C4'H, bs), 7.18–7.26 (C4'-6'H, C7''H, m), 7.05 (C3'H, C5'H, m), 4.05 (C1'H2, t, J = 7.0), 3.93 (C8'H2, m), 3.09 (Cah(E), Ceh(E), Cch, pd), 2.49 (C4'H2, bs), 2.45 (C5'H1, m), 2.27 (C5', m), 2.00–2.23 (Cah(A), Ceh(A), Cbh2, CdH2, m), 2.14 (OCH3, s), 1.93 (C7'H2, m), 1.60–1.78 (C2'H2, C3'H2, C6'H2, m).
\end{align*}\]

\[ \begin{align*}
\text{C NMR} & : \delta 164.1 (C6'', d, J = 250.4), 163.9 (C7''a, d, J = 13.6), 161.4 (C3, s), 160.1 (C3'', s), 151.9 (C1, s), 149.6 (C4a, s), 137.6 (C2', s), 132.9 (C1', s), 130.7 (C6', s), 130.3 (C3', s), 128.2 (C4', s), 126.2 (C5', s), 122.8 (C4', d, J = 10.8), 117.2 (C3'a, s), 112.4 (C5'', d, J = 25.3), 111.8 (C4, s), 97.4 (C7'', d, J = 26.7), 58.4 (C4', s), 53.4 (Ca, Ce, s), 42.9 (C8, s), 41.3 (C1', s), 34.5 (Cc, s), 30.2 (cb, Cc, s), 26.5 (C5, s), 25.7 (C2', s), 24.1 (C3', s), 21.9 (C7, s), 19.7 (CH3, s), 18.6 (C6, s).
\end{align*}\]

ESI-HRMS m/z: Calcd for C_{36}H_{36}F_{10}N_{3}O_{3} [M + H]^+ 531.2766. Found: 531.2774

4-(2-Methoxyphenyl)-2-[4-(6-fluoro-1,2-benzoazol-1-yl)-1-piperidyl]butyl]-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione 7c

The title compound was isolated as an oil. Yield: 71.1%.

\[ \begin{align*}
\text{H NMR} & : \delta 7.79 (C4'H, bs), 7.33 (C4'H, 4d, J_{1} = 8.5, J_{2} = 7.5, J = 1.5), 7.23 (C7''H, 4d, J_{HF} = 8.5, J = 2.0, J = 0.5), 7.11 (C6'H, dd, J = 7.5, J = 1.5), 7.06 (C5''H, td, J = 9.0, J = 2.0), 6.99 (C5'H, td, J = 7.5, J = 1.0), 6.94 (C3'H, dd, J = 8.5, J = 1.0), 4.03 (C1'H, t, J = 7.0), 3.95 (C8'H, t, J = 13.5, J = 7.0), 3.90 (C8'H2, dt, J = 13.5, J = 7.0), 3.78 (OCH3, s), 3.14 (Cah(E), Ceh(E), Cch, bs), 2.56 (C4'H2, bs), 2.43 (C5H2, m), 2.00–2.35 (Cah(A), Ceh(A), Cbh2, CdH2, m), 1.92 (C7'H2, q, J = 6.5), 1.61–1.80 (C2'H2, C3'H2, C6'H2, m).
\end{align*}\]

\[ \begin{align*}
\text{C NMR} & : \delta 164.1 (C6'', d, J = 250.8), 163.9 (C7''a, d, J = 13.3), 161.8 (C5, s), 160.8 (C3'', s), 157.3 (C2', s), 151.9 (C1, s), 150.2 (C4a, s), 132.3 (C6', s), 129.5 (C4', s), 122.9 (C4', s), 122.0 (C1', s), 120.8 (C5', s), 117.1 (C3'a, s), 112.5 (C5'', d, J = 25.5), 111.1 (C3', s), 108.5 (C4, s), 97.4 (C7'', d, J = 26.8), 58.3 (C4', s), 55.5 (OCH3, s), 53.3 (Ca, Ce, s), 42.9 (C8, s), 41.1 (C1', s), 34.2 (Cc, s), 29.9 (Cb, Cc, s), 26.3 (C5, s), 25.5 (C2', s), 23.8 (C3', s), 21.8 (C7, s), 18.5 (C6, s).
\end{align*}\]

ESI-HRMS m/z: Calcd for C_{36}H_{36}F_{10}N_{3}O_{3} [M + H]^+ 547.2715. Found: 547.2726

4-(2-Chlorophenyl)-2-[4-(6-fluoro-1,2-benzoazol-1-yl)-1-piperidyl]butyl]-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione 7d

The title compound was isolated as an oil. Yield: 51.4%.
The title compound was isolated as a white powder. Yield: 55.3%; m.p. 107–110 °C.

\[ \text{H NMR (500 MHz, CDCl}_3): \delta 7.71 (C4'H, pt), 7.25 (C7'H, dd, \int J_H = 8.5, \int J_F = 2.0, \int J_H = 0.5), 7.18–7.21 (C4'H, m), 7.04 (C5'H, td, \int J = 9.0, \int J = 2.0), 6.05 (C1'H, t, \int J = 7.0), 3.98 (C8H1), dt, \int J = 13.5, \int J = 6.5), 3.90 (C8H2, dt, \int J = 13.5, \int J = 6.5), 3.06 (C8H(E), C8H(F), C8H2), 2.44 (C4'H, pt), 2.11 (C5'H, m), 2.00–2.17 (C6H(A), C6H(B), C6H2, C6H3, m), 1.94 (C7'H, m), 1.65–1.81 (C2'H, C2'H, m), 1.61 (C3'H, q, \int J = 5.5). \]

\[ \text{ESI-HRMS m/z: Calcd for C33H35ClF4N5O3 [M + H]^+ 551.2220. Found: 551.2230} \]

4-(2-Fluorophenyl)-2-[4-(6-fluoro-1,2-benzoxazol)-1-piperidyl]butyl]-5,6,7,8-tetrahydro-3,5-dione 7e

The title compound was isolated as a white oil. Yield: 76.3%.

\[ \text{H NMR (500 MHz, CDCl}_3): \delta 7.72 (C4'H, dd, \int J = 3.6, \int J_H = 5.5), 7.31–7.37 (C4'H, m), 7.20–7.25 (C7'H, C7'H, m), 7.19 (C5'H, td, \int J = 7.5, \int J = 1.0), 7.12 (C3'H, td, \int J = 9.0), 7.04 (C5'H, td, \int J = 7.5), 3.97 (C8H1), dt, \int J = 14.0, \int J = 7.0 **), 3.92 (C8H2), dt, \int J = 14.0, \int J = 7.0 **), 3.07 (C8H(E), C8H(F), C8H2), 2.47–2.57 (C5'H, m**), 2.45 (C4'H, t, \int J = 7.0), 2.00–2.19 (C6H(A), C6H(B), C6H2, C6H3, m), 1.94 (C7'H, q, \int J = 7.0), 1.65–1.81 (C2'H, C2'H, m), 1.61 (C3'H, q, \int J = 7.5). \]

\[ \text{ESI-HRMS m/z: Calcd for C33H33ClF4N5O3 [M + H]^+ 551.2220. Found: 551.2230} \]

4-(4-Methylphenyl)-2-[4-(6-fluoro-1,2-benzoxazol)-1-piperidyl]butyl]-5,6,7,8-tetrahydro-3,5-dione 7f

The title compound was isolated as a white powder. Yield: 55.3%; m.p. 107–110 °C.

\[ \text{H NMR (500 MHz, CDCl}_3): \delta 7.71 (C4'H, pt), 7.25 (C7'H, dd, \int J_H = 8.5, \int J_F = 2.0, \int J_H = 2.0), 7.20 (C2'H, C6'H, d, \int J = 8.0), 7.08 (C5'H, C5'H, d, \int J = 8.0), 7.04 (C5'H, td, \int J = 9.0, \int J = 2.0), 4.04 (C1'H, t, \int J = 7.0), 3.94 (C8H2, C8H3, d, \int J = 6.0), 3.06 (C8H(E), C8H(F), C8H2), 2.54 (C5'H, t, \int J = 6.5), 2.44 (C4'H, pt), 2.36 (C8H3), 2.00–2.17 (C6H(A), C6H(E), C6H2, C6H3, m), 1.92 (C7'H2, q, \int J = 6.5), 1.65–1.77 (C2'H2, C6'H2, m), 1.605 (C3'H, q, \int J = 6.5). \]

\[ \text{ESI-HRMS m/z: Calcd for C33H33F2N4O3 [M + H]^+ 535.2515. Found: 535.2522} \]

4-(4-Methoxyphenyl)-2-[4-(6-fluoro-1,2-benzoxazol)-1-piperidyl]butyl]-5,6,7,8-tetrahydro-3,5-dione 7g

The title compound was isolated as a white powder. Yield: 78.9%; m.p. 108–110 °C.

\[ \text{H NMR (500 MHz, CDCl}_3): \delta 7.75 (C4'H, bs), 7.23 (C7'H, dd, \int J_H = 8.5, \int J_F = 2.0, \int J_H = 2.0), 7.12 (C2'H, C6'H, d, \int J = 8.5, \int J = 3.0), 7.05 (C5'O, td, \int J = 9.0, \int J = 2.0), 6.93 (C3'H, C5'H, m). \]
162.3 (C4, s), 18.5 (C6, s). 131.776 (C1, s), 160.8 (C3", s), 151.6 (C1, s), 150.0 (C4a, s), 133.715 (C4, s), 18.6 (C6, s).

ESI-HRMS m/z: Calcd for C31H38FN4O4 [M + H]+ 547.2715. Found: 547.2724

4-(4-Chlorophenyl)-2-[4-[4-(6-fluoro-1,2-benzoxazol)-1-piperidyl]butyl]-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione 7h

The title compound was isolated as a white powder. Yield: 71.1%; m.p. 116–120 °C.

1H NMR (500 MHz, CDCl3): δ 7.77 (C4"H, bs), 7.37 (C2"H, C6"H, dt, J = 9.0, 4J = 2.5), 7.23 (C7"H, 4d, 3J = 8.5, 4J = 2.0, 3J = 0.5), 7.15 (C3"H, C5"H, dt, J = 8.5, 4J = 2.5), 7.06 (C5"H, td, J = 9.0, 4J = 2.0), 4.04 (C1"H2, t, 3J = 7.5), 3.94 (C8H2, t, 3J = 6.5), 3.14 (CaH(E), CcH, ss), 2.52 (C4"H2, C5"H2, m), 2.02–2.40 (CaH(A), CcH(E), CcH, CcH2, m), 1.94 (C4H2, q, 3J = 6.5), 1.72 (C2"H2, C3"H2, C6H2, m).

13C NMR (125 MHz, CDCl3): δ 71.3 (CaH(E), C5H(E), CcH(E), CcH, bs), 2.52 (C5H2, m), 2.02–2.22 (CaH(A), CcH(E), CcH, CcH2, m), 1.93 (C7H2, q, 3J = 7.0), 1.72 (C2"H2, C6H2, m), 1.61 (C2"H2, C3"H2, q, 3J = 7.0).

13C NMR (125 MHz, CDCl3): δ 160.2 (C6", s), 159.7 (C7", s), 137.4 (C8", s), 129.2 (C'1", s), 123.4 (C4", d, 3J = 7.5), 113.7 (C3"a, s), 115.5 (C3", C5", d, 2J = 25.5), 111.4 (C4, s), 97.4 (C7"H, m), 2.20–2.22 (CaH(A), CcH(E), CcH, CcH2, pd), 1.93 (C7H2, q, 3J = 7.0), 1.72 (C2"H2, C6H2, m), 1.61 (C2"H2, C3"H2, q, 3J = 7.0).

3.3. Biological Tests

3.3.1. In Vitro Tests

5-HT1A Binding Assay

Radioligand binding was performed using membranes from CHO-K1 cells stably transfected with the human 5-HT1A receptor (PerkinElmer, Fremont, CA, USA). All assays were carried out in duplicate. Then, 50 µL working solution of the tested compounds, 50 µL [3H]-8-OH-DPAT (final concentration 1 nM) and 150 µL diluted membranes (20 µg protein per well) prepared in assay buffer (50 mM Tris, pH 7.4, 10 mM MgSO4, 0.5 mM EDTA, 0.1% ascorbic acid) were transferred to a polypropylene 96-well microplate
using 96-wells pipetting station Rainin Liquidator (Mettler Toledo, Columbus, OH, USA). 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/B filter mate, pre-soaked with 0.5% polyethyleneimine for 30 min. Ten rapid washes with 200 µL 50 mM Tris buffer (4 °C, pH 7.4) were performed using an automated harvester system—Harvester-96 MACH III FM (Tomtec, Hamden, CT, USA). The filter mates were dried at 37 °C in a forced-air fan incubator, and then solid scintillator MeltiLex was melted on filter mates at 90 °C for 6 min. The radioactivity on the filter was measured in a MicroBeta TriLux 1450 scintillation counter (PerkinElmer, Waltham, MA, USA). Data were fitted to a one-site curve-fitting equation with Prism 6 (GraphPad Software, San Diego, CA, USA) and Ki values were estimated from the Cheng–Prusoff equation.

**SERT Binding Assay**

Radioligand binding was performed using rat cortex tissue. All assays were carried out in duplicate. First, 50 µL working solution of the tested compounds, 50 µL [³H]-citalopram (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 polystyrene 96-well microplate using 96-wells pipetting station Rainin Liquidator (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 GF/B filter mate presoaked with 0.3% polyethyleneimine for 30 min. Ten rapid washes with 200 µL 50 mM Tris buffer (4 °C, pH 7.7) were performed using an automated harvester system—Harvester-96 MACH III FM (Tomtec). The filter mates were dried at 37 °C in a forced-air fan incubator and then solid scintillator MeltiLex was melted on filter mates at 90 °C for 6 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 Ki values were estimated from the Cheng–Prusoff equation.

**5-HT₂A, 5-HT₆, 5-HT₇ and D₂ Binding Assay**

In vitro radioligand binding assays for 5-HT₂A, 5-HT₆, 5-HT₇ and D₂ receptors were carried out using methods published by Zajdel et al. [45]. For the assays, HEK293 cell cultures stably expressing the investigated human receptors were used. Cell pellets were thawed and homogenized in 20 vol of assay buffer using an Ultra Turrax tissue homogenizer, then 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-HT₂A receptors—50 mM Tris-HCl, 0.1 mM EDTA, 4 mM MgCl₂ and 0.1% ascorbate; for 5-HT₆ receptors—50 mM Tris-HCl, 0.5 mM EDTA, 4 mM MgCl₂; for 5-HT₇ receptors—50 mM Tris-HCl, 4 mM MgCl₂, 10 µM pargyline and 0.1% ascorbate; for D₂ receptors—50 mM Tris-HCl, 1 mM EDTA, 4 mM MgCl₂, 120 mM NaCl, 5 mM KCl, 1.5 mM CaCl₂ and 0.1% ascorbate. All assays were incubated in total volume of 200 mL in 96-well microtitre plates for 1 h at 37 °C, except for 5-HT₂A 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 [³H]-ketanserin (spec. act. 53.4 Ci/mmol) for 5-HT₂A receptors; 2 nM [³H]-LSD (spec. act. 83.6 Ci/mmol) for 5-HT₆ receptors; 0.6 nM [³H]-5-CT (spec. act. 39.2 Ci/mmol) for 5-HT₇ receptors and [³H]-raclopride (spec. act. 76.0 Ci/mmol) for D₂ receptors. Nonspecific binding was defined with 10 µM chlorpromazine, 10 µM methiotepine, or 1 µM (+) butaclamol used in 5-HT₂A, 5-HT₆ and D₂ receptor assays, respectively. Each compound was tested in triplicate at 7–8 concentrations (10⁻¹¹–10⁻⁴ M). The inhibition constants (Ki) were calculated from the Cheng–Prusoff equation [46]. Results were expressed as means of at least two separate experiments.
3.3.2. 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 (88/2016, 05/31/2016). The experiments were performed on male CD-1 mice (23–40 g). The animals were kept at room temperature (21 ± 2 °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. 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 solution. Compounds 6a and 7g were suspended in a 10% aqueous solution of dimethyl sulphoxide (DMSO). Vehicle group was administered as 10% aqueous solution of dimethyl sulphoxide (DMSO). 8-OH-DPAT was injected subcutaneously (sc); 6a and 7g were given intraperitoneally (ip) in a volume of 10 mL/kg/mice. The obtained data were analyzed by Dunnett’s test (one drug administration) or by the Newman–Keuls test (two drugs administrations). Forced swim test: the obtained data was evaluated by one-way analysis of variance (ANOVA) followed by the Dunnett’s multiple comparisons test: p < 0.05 was considered significant.

Body Temperature in Mice

The effects of the tested compounds 6a and 7g given alone, on the rectal body temperature in mice (measured with an Ellab thermometer) were recorded 30, 60, 90 and 120 min after their administration. In a separate experiment the effect of WAY-100635 (0.1 mg/kg s.c.) on the hypothermia induced by tested compounds was measured. WAY-100635 was administered 15 min before the tested compounds and the rectal body temperature was recorded 30 min and 60 min after injection. The absolute mean body temperatures were within a range 36.7 ± 0.5 °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.

Forced Swim Test in Mice

The forced swim test (FST) was carried out according to the method of Porsolt at al. [47]. Mice were placed individually into glass cylinders (height 25 cm, diameter 10 cm) containing 20 cm of water and maintained at 23 ± 1 °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 the last 4 min test. The mouse was judged to be immobile when it remained floating passively, performing slow motions to keep its head above the water. Tested compounds were administered 30 min before test.

Metabolic Stability

Stock solutions of studied compounds were prepared at concentration of 100 µM in 1:1 acetonitrile/water mixture. Incubation mixes consisted 1 µM of a studied compound, 100 µM of NADPH in phosphate buffer and 1 mg/mL of pooled HLMs (Sigma-Aldrich, St. Louis, MO, USA) in potassium phosphate buffer (0.1 M, pH 7.4). Incubation was carried out in 96-well plates at 37 °C. Incubation mixtures (excluding compound solution) were subjected to 5 min preincubations, 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 × 100 mm², 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%
The gradient elution program was: 0.00–10.00 min, 10–95% B; 10.01 min–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 and was in positive ionization mode. The mass analyzer was set individually to each derivative to detect pseudomolecular ions [M + H]+. Mass spectrometry detector (MSD) parameters of the ESI source were as follows: nebulizer pressure—35 psig (N2); drying gas 12 L/min (N2); drying gas temperature—300 °C; capillary voltage—3.0 kV; fragmenter voltage—70 V.

4. Conclusions

The use of antidepressants or neuroleptic drugs acting through an extended receptor profile is now becoming a widely used therapeutic approach. The paper describes the synthesis and biological studies in vitro and in vivo (binding affinity for receptor, functional profile and metabolic stability) of new 4-aryl-2H-pyrido[1,2-c]pyrimidine derivatives (6a–i), as well as 4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidines (7a–i), having a 6-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole residue in the pharmacophore ligand part. Receptor studies showed their sensitivity to the action of human liver microsomes. Low stability may be due to the introduction of the 6-fluoro-3-(4-piperidinyl)-1,2-benzisoxazole residue into the pharmacophore part.

Analyses of the obtained results of affinities of the derivatives (6a–i) and (7a–i) for the 5-HT1A receptor, showed that the derivatives of both series have very high bindings (6a–i 5-HT1A Ki = 7.0–30.0 nM, and for 7a–i Ki = 5.0–52.0 nM), where the series (6a–i) showed higher (more active derivatives) activity against compounds of the series (7a–i).

Thus, when examining the effect of the degree of hydrogenation of the terminal part of ligands on the affinity for the 5-HT1A receptor for both series (6a–i) and (7a–i), it can be concluded that it was low. Derivatives of both series (6a–i) and (7a–i) generally showed low affinity for SERT protein with the exception of 6a (SERT Ki = 32.0 nM) and 7g (SERT Ki = 5.0 nM; SERT Ki = 48.0 nM) showed their desirable very high binding to both molecular targets.

Analyses of the obtained study results encourage further optimization of the obtained ligand structures in the search for new pyrido[1,2-c]pyrimidine derivatives with potential antidepressant activity from the SSRI+ group.
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