Design, Sustainable Synthesis and Biological Evaluation of a Novel Dual α2A/5-HT7 Receptor Antagonist with Antidepressant-Like Properties

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Abstract: The complex pathophysiology of depression, together with the limits of currently available antidepressants, has resulted in the continuous quest for alternative therapeutic strategies. Numerous findings suggest that pharmacological blockade of α2-adrenoceptors might be beneficial for the treatment of depressive symptoms by increasing both norepinephrine and serotonin levels in certain brain areas. Moreover, the antidepressant properties of 5-HT7 receptor antagonists have been widely demonstrated in a large set of animal models. Considering the potential therapeutic advantages in targeting both α2-adrenoceptors and 5-HT7 receptors, we designed a small series of arylsulfonamide derivatives of (dihydrobenzofuranoxy)ethyl piperidines as dually active ligands. Following green chemistry principles, the designed compounds were synthesized entirely using a sustainable mechanochemical approach. The identified compound 8 behaved as a potent α2A/5-HT7 receptor antagonist and displayed moderate-to-high selectivity over α1-adrenoceptor subtypes and selected serotonin and dopaminergic receptors. Finally, compound 8 improved performance of mice in the forced swim test, displaying similar potency to the reference drug mirtazapine.

Keywords: α2 adrenoceptor antagonist; 5-HT7 receptor antagonist; medicinal mechanochemistry; depression; forced swim test

1. Introduction

Depressive disorder is a common and disabling illness characterized by the presence of behavioral and emotional symptoms (i.e., sleep disturbances, low self-esteem, sadness as well as suicidal ideation) [1]. Although different pharmacotherapeutic options are available (e.g., selective serotonin reuptake inhibitors SSRIs; serotonin/noradrenaline reuptake inhibitors SNRIs; monoamine receptors modulators), the treatment of depressive disorders is still limited. Currently available antidepressants display a delay of therapeutic action, which lasts up to a few weeks in some patients after numerous antidepressant drugs, and numerous unacceptable side effects [2].
The α2-adrenoceptor (α2-AR) is a member of class A of G-protein coupled receptors (GPCRs) canonically associated with heterotrimeric Gi/o subtypes. Its activation leads to inhibition of adenylyl cyclase and voltage-gated calcium channels [3]. Among the identified α2-ARs, α2A-AR subtype is the predominant isoform (90% of all α2-AR) and represents the primary modulators of monoaminergic neurotransmission in CNS [4]. In particular, the high expression of α2A-AR in the hippocampus and cortico-limbic structures together with its involvement in serotonin release [5,6], asserts its pivotal role in cognition, memory, and mood disorders [7].

The 5-HT7 receptor (5-HT7R) represents the latest addition to a subfamily of serotonin GPCRs [8]. All isolated 5-HT7R isoforms are positively coupled to adenylyl cyclase via activation of the Gαs subunit with consequent increasing of intracellular cAMP levels [9]. The distribution of 5-HT7R in specific CNS regions (i.e., the hippocampus and prefrontal cortex) suggests its implications in the control of rapid eye movement sleep, learning, and memory as well as in pathological processes such as affective disorders, neurodegenerative diseases, and cognitive decline [10,11].

A back-drop of evidence has demonstrated the potential of genetic and pharmacological blockade of α2-AR and 5-HT7R in several preclinical models of depression [12–15]. These findings are further supported by the fact that augmentation of the SSRIs and NRSIs with α2-AR or 5-HT7-R antagonists increases their efficiency of monoamine reuptake inhibitors [16–18]. Of note, the antidepressant effects of mianserin and mirtazapine, which display high-to-moderate affinity for several monoaminergic receptors and transporters, is mainly attributed to their antagonism at presynaptic α2A-AR [19,20].

Recent studies in a group of arylsulfonamide derivatives of aryloxyalkylpiperidine [21] identified compound I as a potent 5-HT7-R ligand with affinity for α2-AR in a submicromolar range (Figure 1). Further replacement of the flexible isoproxy moiety with the rigid 2,2-dimethyl-2,3-dihydrofurane moiety increased the affinity for α2-AR, providing the moderate α2-A R ligand 6 (Figure 1). At the same time, this modification maintained high affinity for 5-HT7-R. These findings prompted us to design novel dual acting compounds which behave as α2-AR and 5-HT7-R antagonists.

![Figure 1](image_url). Design strategy for the arylsulfonamide derivatives of (aryloxy)ethyl piperidines 6–19.

Here, we present a medicinal mechanochemistry approach to the generation of a focused library of arylsulfonamides of (aryloxyethyl)piperidines (Figure 1). The impact of the applied modifications on the affinity for α2-AR and 5-HT7-R was first assessed in vitro in radioligand binding studies. The selectivity of the most promising derivatives over structurally related off-target GPCRs (α1-AR, 5-HT1A-R, 5-HT2A-R, 5-HT3R and D2-R) was then investigated, followed by a determination of their antagonistic properties at α2A-AR and 5-HT7-R in cellular assays. Finally, the selected derivatives improved performance of mice in the forced swim test.
2. Results and Discussion

2.1. Chemistry

In the last decade, mechanochemistry, and in particular medicinal mechanochemistry [22,23], has been recognized as an innovative technique for the generation of various organic compounds as well as producing pharmaceutically relevant fragments and functionalities [24–26]. Taking into account the benefits of employing a solid-state approach over classic in-batch procedures (i.e., increased reaction yield, reduction of time, decreased use of organic solvents) [27,28], we developed a mechanochemical approach for the synthesis of the designed derivatives 6–19 (Scheme 1). Initially, alkylation of Boc-protected 4-aminomethylpiperidine with the commercially available 7-(2-bromoethoxy)-2,2-dimethyl-2,3-dihydrobenzofuran 1 was carried out in a 10 mL PTFE jar with a 1.5 cm diameter stainless steel ball using a Retsch vibratory ball mill (vbm) operating at 30 Hz. The use of a slight excess of amine, K$_2$CO$_3$ as base, in the presence of KI, allowed us to achieve high conversion rates for intermediate 2 after a milling time of 140 min (for more details see Table S1). Further scale-up optimization was required to translate reaction conditions to a 35 mL PTFE jar (see Table S1). Increasing the loading of base (from 2 to 3 eq) together with elongation of reaction time (from 140 to 210 min) enabled us to reach a 97% conversion of substrates. After an acidic extraction at pH 3.5 to remove unreacted Boc-protected alicyclic amine, intermediate 2 was isolated with a high 95% purity and 84% yield. Having identified the optimal reaction conditions, the same mechanochemical protocol was applied for the alkylation of Boc protected 4-aminomethylpiperidine to obtain intermediate 3 (96% purity) in a satisfactory yield (81%) (see Table S2). In the next step, primary amine derivatives 4 and 5, obtained upon treatment of Boc-derivative 2 and 3 with gaseous HCl [29,30], reacted in a ball mill with different substituted arylsulfonyl chlorides to generate the designed sulfonamide derivatives.

![Scheme 1](image_url)

**Scheme 1.** Mechanochemical synthesis of final compounds 6–19. Reagents and conditions: (i) vbm 30 Hz, $\phi_{\text{ball}} = 1.5$ cm, total mass of reagents = 500 mg, 35 mL PTFE jar, alkylation agent (1 eq) Boc-protected alicyclic diamine (1.2 eq), K$_2$CO$_3$ (3 eq), KI (0.5 eq), 210 min; (ii) HCl$_{\text{gas}}$, 2 h; (iii) vbm 30 Hz, $\phi_{\text{ball}} = 1.5$ cm, total mass of reagents = 125 mg, 10 mL PTFE jar, primary amine (1 eq), arylsulfonyl chloride (1 eq), K$_2$CO$_3$ (2 eq), 1–10 min.

Hence, the final compounds 6–19 were obtained by milling equimolar amounts of starting materials in the presence of K$_2$CO$_3$ in moderate-to-high yields (65–94%). According to our previously reported findings on sulfonamide bond formation in the solid-state [29], sulfonfylation of the primary amine function was significantly influenced by the nature of the substituent on the phenyl ring of the sulfonyl chloride. Regardless of the type of central amine core, the presence of 4-F and 3-Cl substituents enabled the formation of compounds 6, 7, 14 and 15 with high conversion rates in a relatively shorter time (1 min) than all other tested analogs (see Table S3). The introduction of a second substituent at the 3-chlorophenyl moiety in both subsets (5-Cl, 2-F and 5-Cl, 2-MeO) required longer milling times to guarantee similar conversion rates for the generation of derivatives 8–10.
and 16–17. Notably, compounds 11, 12 and 18 bearing 1-naphthyl and 2-naphthyl moieties displayed the lowest conversion rates amongst the series (<70%) after 10 min of reaction. Prolongation of the milling time for these derivatives did not increase the formation of desired products while causing degradation of substrates, which was not detected in the solution. In contrast, milling isoquinolinyl-4-sulfonyl chloride and primary amines 4 and 5 for 5 min furnished final compounds 13 and 19 with the highest conversion rate amongst the series (90 and 99%, respectively).

2.2. In Vitro Pharmacology

All synthesized compounds were evaluated in $^3$H]clonidine and $^3$H]5-CT binding experiments for their affinity toward $\alpha_2$-AR and 5-HT$_7$-R, respectively (Table 1). The tested compounds showed high-to-low affinity for $\alpha_2$-AR ($K_i = 80–1194$ nM) and for 5-HT$_7$-R ($K_i = 30–727$ nM). Structure–activity relationship (SAR) studies were firstly focused on the impact of the central amine core on the affinity for both biological targets. Compounds with a 4-aminopiperidine scaffold displayed a higher affinity for both $\alpha_2$-AR and 5-HT$_7$-R, than their 4-aminomethylpiperidine analogs (6 vs. 14, 9 vs. 17 and 11 vs. 18).

Next, the impact of the kind of substituents at the arylsulfonamide moiety was analyzed. Based on our data, reporting on the impact of monosubstituted benzenesulfonyl moiety on the affinity for $\alpha$-AR and 5-HT$_7$-R, the analysis was limited to the 3-Cl substitution pattern [31,32]. Although replacement of the 4-F substituent present in the pilot compound 6 with the 3-Cl one (compound 7) decreased the affinity for $\alpha_2$-AR 5-fold ($K_i = 138$ and 649, respectively), this modification was tolerated for interaction with the 5-HT$_7$-R. Regardless of the kind of central amine core, introducing a fluorine atom in the meta position to the 3-Cl-phenylsulfonyl moiety (i.e., 5-Cl, 2-F substitution pattern) improved the affinity of compounds 7 and 15 for both biological targets. In contrast to our previous findings [33], the replacement of one or both halogens with the electron-donating methoxy substituent decreased the affinity for $\alpha_2$-AR and 5-HT$_7$-R ligands (8 vs. 9 and 10 and 16 vs. 17).

Table 1. The binding data of the synthesized compounds 6–19 for $\alpha_2$-AR and 5-HT$_7$-R.

| Compound | Ar | m | $K_i$ [nM] ± SEM |
|----------|----|---|----------------|
|          |    |   | $\alpha_2$      | 5-HT$_7$ |
| 6        | 4-F-phenyl | 0 | 138 ± 4 | 35 ± 22 |
| 7        | 3-Cl-phenyl | 0 | 649 ± 62 | 64 ± 15 |
| 8        | 5-Cl2-3-F-phenyl | 0 | 148 ± 23 | 30 ± 11 |
| 9        | 5-Cl2-2-MeO-phenyl | 0 | 573 ± 56 | 86 ± 26 |
| 10       | 2,5-diMeO-phenyl | 0 | 244 ± 52 | 95 ± 18 |
| 11       | 1-naphthyl | 0 | 366 ± 46 | 50 ± 16 |
| 12       | 2-naphthyl | 0 | 200 ± 51 | 67 ± 21 |
| 13       | 4-isooquinolyl | 0 | 80 ± 42 | 91 ± 25 |
| 14       | 4-F-phenyl | 1 | 1194 ± 44 | 727 ± 65 |
| 15       | 3-Cl-phenyl | 1 | 743 ± 32 | 664 ± 82 |
| 16       | 5-Cl2-2-F-phenyl | 1 | 154 ± 28 | 316 ± 73 |
| 17       | 5-Cl2-2-MeO-phenyl | 1 | 1097 ± 78 | 488 ± 70 |
| 18       | 1-naphthyl | 1 | 907 ± 64 | 366 ± 69 |
| 19       | 4-isooquinolyl | 1 | 298 ± 24 | 387 ± 58 |
|          | Clonidine |    | 2.7 ± 0.3 | NT$^c$ |
|          | SB-267790 |    | NT$^c$ | 3 ± 0.5 |
|          | Mirtazapine | 112 | 265$^e$ |

$^a$ $K_i$ ± SEMs values based on three independent binding experiments in rat cerebral cortex; $^b$ $K_i$ ± SEMs values based on three independent binding experiments in HEK-293 cells; $^c$ Not Tested; $^d$ Data taken from [34] with binding experiments performed in rat cerebral cortex; $^e$ Data taken from [35] with binding experiments performed in cloned human receptors.

The bicyclic 1-naphthyl and 2-naphthyl moiety displayed no significant improvement over the substituted phenyl ring. An exception was observed when the naphthyl
Although the observed potencies were lower than those of reference AR, serotonin 5-HT\textsubscript{\textalpha} was confirmed in HEK-293 cells; \(K_b \pm \text{SEMs values based on three independent binding experiments in HEK293 cells.}\)

The evaluated compounds were classified as potent \(K_i \pm \text{SEMs values based on three independent binding experiments in HEK-293 cells.}\)

| Compd | \(K_i \text{[nM]}\) | \(\alpha_1\) a | 5-HT\textsubscript{1A} b | 5-HT\textsubscript{2A} b | 5-HT\textsubscript{6} b | D\textsubscript{2} b |
|-------|-----------------|----------------|----------------|----------------|----------------|----------------|
| 6     | 761 ± 86        | 221 ± 25       | 538 ± 38       | 839 ± 70       | 327 ± 29       |
| 8     | 1256 ± 101      | 260 ± 29       | 1420 ± 98      | 873 ± 91       | 388 ± 37       |
| 13    | 429 ± 66        | 260 ± 18       | 1422 ± 105     | 1123 ± 114     | 326 ± 25       |

\(\text{Table 2.} \) The binding data of selected compounds 6, 8 and 13 for \(\alpha_1\)-AR, 5-HT\textsubscript{1A}, 5-HT\textsubscript{2A}, 5-HT\textsubscript{6} and D\textsubscript{2}R.

Considering the high and specific distribution of \(\alpha_2\text{A}-\text{AR}\) in CNS [5], and its engagement in controlling noradrenaline-serotonin release in the hippocampus and the corticolimbic structures involved in affective, cognitive and memory processes [39], targeting \(\alpha_2\text{A}-\text{AR}\) subtype might provide more beneficial therapeutic effects than other isolated \(\alpha_2\)-AR isoforms. Thus, the functional activity of 6, 8 and 13 at \(\alpha_2\text{A}-\text{AR}\) and selectivity over \(\alpha_2\text{B}-\text{AR}\) subtypes were assessed in fluorescence-based cellular assays (Table 3) [40]. The evaluated compounds were classified as potent \(\alpha_2\text{A}-\text{AR}\) antagonists (\(K_b = 12–40\ \text{nM}\)). Although the observed potencies were lower than those of reference \(\alpha_2\)-AR antagonist yohimbine, compounds 6 and 8 displayed higher functional selectivity over \(\alpha_2\text{B}-\text{AR}\) subtype (up to seven- and four-fold, respectively). In contrast, 4-isoquinolyl derivatives 13 did not show any preference for the tested \(\alpha_2\)-AR subtype.

| Compd | \(\alpha_2\) | \(\alpha_2\text{A}\) | \(\alpha_2\text{B}\) | 5-HT\textsubscript{7} |
|-------|-------------|----------------|----------------|----------------|
| 6     | 138 ± 44    | 12 ± 4         | 103 ± 25       | 35 ± 22        | 186 ± 37       |
| 8     | 148 ± 23    | 40 ± 11        | 142 ± 31       | 30 ± 11        | 141 ± 43       |
| 13    | 80 ± 42     | 31 ± 8         | 27 ± 13        | 91 ± 25        | 155 ± 30       |
| Yohimbine | 164 ± 55 | 0.81 ± 0.3 | 2.99 ± 0.7 | NT f |

\(\text{Table 3.} \) The antagonist activity of selected compounds 6, 8, 13 and reference yohimbine at \(\alpha_2\text{A}-\text{AR}, \alpha_2\text{B}-\text{AR}\) and 5-HT\textsubscript{7}R.

Next, the antagonistic properties of 6, 8 and 13 at 5-HT\textsubscript{7}R were confirmed in HEK-293 cells, which stably over-express the 5-HT\textsubscript{7}R (Table 3). All tested derivatives inhibited the cAMP production promoted by the administration of the agonist 5-CT, thus behaving as potent antagonists in this cellular setting. To exclude pharmacological effects associated with interaction with 5-HT\textsubscript{1A}R, further functional profiling of 6, 8 and 13 was performed at...
Eurofins (Eurofins Scientific, France), revealing low agonist activity (EC$_{50} > 4 \mu$M) and no antagonist property at 1 µM in cAMP-based assays.

2.3. In Vivo Pharmacology

In view of the findings that the modulation of noradrenergic/serotonin transmissions by targeting α$_2$-AR and the blockade of 5-HT$_7$R are involved in behavioral changes responsible for antidepressant-like effects observed in preclinical models [7,10,41], selected compounds 6, 8 and 13 were assessed for their potential antidepressant properties in the forced swim test using Albino Swiss mice. The clinically used antidepressant mirtazapine was tested as reference. Although mirtazapine displays high-to-moderate affinity for 5-HT$_2A$/2C, 5-HT$_3$ and 5-HT$_7$Rs, its antagonism at presynaptic α$_2A$-AR, which enhances noradrenaline and serotonin release, is mainly related to the observed in vivo antidepressant effect [42].

All tested compounds (administered at dose range of 1–8 mg/kg, ip) shortened the immobility of the mice by about 25% in comparison to control, thus exerting antidepressant-like effects in the FST (F(5,36) = 4.259, $p = 0.0038$; F(5,33) = 4.521, $p = 0.003$; F(7,49) = 3.209, $p = 0.007$; respectively for compounds: 8, 6, 13). In FST, the data sets for the experiments showed normal distribution ($\alpha = 0.05$). Results are shown at Figure 2A,C,E. The effects were similar to those of mirtazapine displayed at the active dose of 16 mg/kg, however the antidepressant effects of 6, 8 and 13 occurred at lower doses (8, 4 and 1 mg/kg, respectively).

![Figure 2. The effect of the tested compounds 6, 8 and 13 and mirtazapine on the immobility of mice in FST (A, C, E) and on locomotor activity (B, D). All compounds or vehicle were administered 30 min (ip) before the test. MIRT—mirtazapine. The results are presented as box plots showing the following data: mean (+), median (horizontal line), upper and lower quartile (the width of the box shows interquartile range), upper and lower extreme (whiskers). Statistical analysis: Shapiro–Wilk test for normality and one-way ANOVA (Dunnett post hoc) *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control group (vehicle); $n = 6$–8 mice per group. Although sedation may be perceived as being therapeutically beneficial in certain stress-related mood disorders [43], it represents an unacceptable side effect, mainly related to some antidepressants. Indeed, about 20% of patients treated with mirtazapine for their depression or anxiety report sedation as a side effect [20].

To evaluate potential sedative activity at the doses used in the behavioral experiments, the influence of tested compounds on the spontaneous locomotor activity of mice was assessed. The data sets for the activity showed normal distribution ($\alpha = 0.05$). Among the studied compounds, only 8 produced an antidepressant-like effect with no influence...
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3. Materials and Methods
3.1. Chemistry
3.1.1. General Chemical Methods

All commercially available reagents were of the highest purity (from Sigma-Aldrich, Fluorochem, AlfaAesar). The milling treatments were carried out in a vibratory ball-mill Retsch MM400 operated at 30 Hz. The milling load was defined as the sum of the mass of the reactants per free volume in the jar and was equal to 15 mg/mL. All reactions using the vibratory ball mill were performed under air.

$^1$H and $^{13}$C NMR spectra were recorded on a JEOL JNM-ECZR500 RS1 (ECZR version) at 500 and 126 MHz, respectively, and were reported in ppm using deuterated solvent for calibration (CDCl$_3$). The $I$ values were reported in hertz (Hz), and the splitting patterns were designated as follows: br s. (broad singlet), br d. (broad doublet), s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), td (triplet of doublets), tt (triplet of triplets), ddd (doublet of doublet of doublets), dq (doublet of quartets), dddd (doublet of doublet of doublet of doublets), m (multiplet).

Mass spectra were recorded on a UPLCMS/MS system consisting of a Waters ACQUITY UPLC (Waters Corporation, Milford, MA, USA) coupled to a Waters TQD mass
spectrometer (electrospray ionization mode ESI-tandem quadrupole). Chromatographic separations were carried out using the Acquity UPLC BEH (bridged ethyl hybrid) C18 column; 2.1 mm × 100 mm, and 1.7 µm particle size, equipped with Acquity UPLC BEH C18 Van Guard precolumn; 2.1 mm × 5 mm, and 1.7 µm particle size. The column was maintained at 40 °C and eluted under gradient conditions from 95% to 0% of eluent A over 10 min, at a flow rate of 0.3 mL min⁻¹. Eluent A: water/formic acid (0.1%, v/v), Eluent B: acetonitrile/formic acid (0.1%, v/v).

Melting points (mp) were determined with a Büchi apparatus and are uncorrected.

Elemental analyses for C, H, N and S were carried out using the elemental Vario EL III Elemental Analyser (Hanau, Germany). All values are given as percentages and were within ±0.4% of the calculated values.

3.1.2. Alkylation of Boc-Protected 4-Aminopiperidine in Ball Mill (Procedure A)

Commercially available bromine derivative 1 (38.9 mg, 0.144 mmol, 1 eq) and Boc-protected alicyclic amine (34.5 mg, 0.172 mmol, 1.2 eq) were introduced in a 10 mL PTFE jar (milling load 15 mg/mL) with one stainless steel ball (φball = 1.5 cm), followed by the addition of previously ground K₂CO₃ (39.7 mg, 0.287 mmol, 2 eq) and KI (14.3 mg, 0.086 mmol, 0.5 eq). The reaction was carried out for 140 min. Then, the product was solubilized in CH₂Cl₂ (10 mL), and the organic phase was washed with KHSO₄ aqueous solution at pH = 3.5 (3 × 5 mL) and saturated NaCl solution (1 × 5 mL), dried over Na₂SO₄, and finally filtered and concentrated under reduced pressure.

3.1.3. Alkylation of Boc-Protected 4-Aminopiperidine and 4-Aminomethylpiperidine in Ball Mill (Procedure B)

Commercially available bromine derivative 1 (1 eq) and Boc-protected alicyclic di-amine (1.2 eq) were introduced in two 35 mL PTFE jars (milling load 15 mg/mL) with one stainless steel ball (φball = 1.5 cm), followed by the addition of previously ground K₂CO₃ (3 eq) and KI (0.5 eq). The reaction was carried out for 210 min at rt. Then, the product was solubilized in CH₂Cl₂ (25 mL), and the organic phase was washed with KHSO₄ aqueous solution at pH = 3.5 (3 × 10 mL) and saturated NaCl solution (1 × 10 mL), dried over Na₂SO₄, and finally filtered and concentrated under reduced pressure. To obtain the desired amount of product, the reaction was carried out twice (4 × 35 mL).

Tert-butyl {1-[2-(2,2-Dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl}carbamate (2)

General procedure B was followed with bromine derivative 1 (134.4 mg, 0.495 mmol, 1 eq), Boc-protected 4-aminopiperidine (119.1 mg, 0.595 mmol, 1.2 eq), previously ground K₂CO₃ (205.4 mg, 1.486 mmol, 3 eq), and KI (49.4 mg, 0.297 mmol, 0.5 eq) to afford intermediate 3 as a white powder (164 mg and 84% yield).

C₂₂H₃₄N₂O₄, MW: 390.52, Monoisotopic Mass: 390.25. UPLC/MS purity 96%, tᵣ = 4.99 min; [M+H]+ 391.4. ¹H NMR (500 MHz, CDCl₃) δ 6.78–6.73 (m, 3H), 4.45 (s, 1H), 4.24–4.17 (m, 2H), 3.48 (d, ²J = 7.1 Hz, 1H), 3.01 (t, ²J = 1.6 Hz, 3H), 3.00–2.95 (m, 2H), 2.88–2.82 (m, 2H), 2.28 (d, ²J = 10.1 Hz, 2H), 1.95 (d, ²J = 11.5 Hz, 2H), 1.50 (s, 1H), 1.48 (s, 6H), 1.43 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 155.3, 147.9, 143.5, 128.7, 120.5, 118.1, 113.6, 87.5, 79.4, 66.7, 57.1, 52.9, 43.4, 32.3, 28.5, 28.4. Mp for C₂₂H₃₄N₂O₄ 118.1–119.8 °C.

Tert-butyl (1-[2-(2,2-Dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl)methyl)carbamate (3)

General procedure B was followed with bromine derivative 1 (132.1 mg, 0.487 mmol, 1 eq), Boc-protected 4-aminomethylpiperidine (125.4 mg, 0.585 mmol, 1.2 eq), previously ground K₂CO₃ (202.1 mg, 1.462 mmol, 3 eq), and KI (48.5 mg, 0.292 mmol, 0.5 eq) to afford intermediate 4 as a white powder (154 mg and 81% yield).

C₂₃H₃₆N₂O₄, MW: 404.55, Monoisotopic Mass: 404.27. UPLC/MS: purity 96%, tᵣ = 5.17 min; [M+H]+ 405.4. ¹H NMR (500 MHz, CDCl₃) δ 6.91–6.60 (m, 3H), 4.65 (dd, ²J = 7.2, 6.5 Hz, 1H),
4.30–4.22 (m, 2H), 3.16–3.10 (m, 2H), 3.04–2.98 (m, 4H), 2.95–2.88 (m, 2H), 2.29–2.22 (m, 2H),
1.75–1.71 (m, 2H), 1.56–1.54 (m, 1H), 1.48 (s, 6H), 1.45 (d, J = 3.8 Hz, 1H), 1.43 (s, 9H). 13C NMR
(126 MHz, CDCl3) δ 156.2, 148.0, 128.7, 120.5, 118.3, 113.8, 87.5, 79.4, 66.4, 57.2, 53.8, 46.0, 43.4, 31.1,
28.5, 28.4. Mp for C17H26N2O2·HCl: 205.0–208.0 °C.

3.1.4. General Procedure for the Deprotection of Boc Function in Solid State (Procedure C)

Intermediate 2 or 3 was submitted to HCl gas for 2 h at rt to afford the primary amine 4
or 5 as white hydrochloride salts, according to previously reported procedures [29].

1-{2-[2,2-Dimethyl-2,3-dihydrobenzofuran-7-yl]oxy}ethylpiperidin-4-amine hydrochloride (4)

General procedure C was followed with derivative 2 (600 mg, 1.537 mmol, 1 eq) to
afford intermediate 4 as a yellow powder (492 mg and 98% yield).

C17H26N2O2·HCl, MW: 326.86, Monoisotopic Mass: 290.20. UPLC/MS: purity 100%,
tr = 2.61 min, [M+H]+ 291.3. 1H NMR for free base (500 MHz, CDCl3) δ 6.76 (dt, J = 8.1,
4.6 Hz, 3H), 4.18 (t, J = 6.4 Hz, 2H), 3.01 (s, J = 3.4 Hz, 2H), 2.99–2.92 (m, 2H), 2.81 (t, J = 6.4 Hz, 2H), 2.20–2.13 (m, 2H), 1.86 (d, J = 15.5 Hz, 1H), 1.51 (s, 1H), 1.49 (s, 6H), 1.46 (d, J = 10.5 Hz, 1H). 13C NMR for free base (126 MHz, CDCl3) δ 147.7, 143.6, 128.5, 120.5, 117.9,
113.0, 87.5, 66.5, 57.0, 52.9, 48.7, 43.4, 35.1, 28.4. Mp for C17H26N2O2·HCl: 139.5–140.3 °C.

1-[2-[2,2-Dimethyl-2,3-dihydrobenzofuran-7-yl]oxy]ethylpiperidin-4-yl)methanamine hydrochloride (5)

General procedure C was followed with derivative 3 (600 mg, 1.483 mmol, 1 eq) to
afford intermediate 5 as a yellow powder (505 mg and 98% yield).

C18H28N2O2·HCl, MW: 340.89 Monoisotopic Mass: 304.22. UPLC/MS: purity 100%,
tr = 2.72 min, [M+H]+ 305.3. 1H NMR for free base (500 MHz, CDCl3) δ 6.75 (dt, J = 9.6, 4.3 Hz,
3H), 4.18 (t, J = 6.5 Hz, 3H), 3.01 (d, J = 8.9 Hz, 2H), 2.80 (t, J = 6.4 Hz, 2H), 2.60 (d, J = 6.0 Hz,
2H), 2.52 (s, 1H), 2.08 (t, J = 11.5 Hz, 2H) 1.73 (d, J = 12.3 Hz, 2H), 1.49 (s, 6H), 1.30–1.23 (m, 4H).
13C NMR for free base (126 MHz, CDCl3) δ 147.7, 143.7, 128.5, 120.5, 117.9, 113.2, 87.5, 66.7, 57.4, 54.2, 47.9, 43.4, 38.7, 29.9, 28.4. Mp for C18H28N2O2·HCl: 110.9–112.4 °C.

3.1.5. Sulfonylation of Primary Amine (Procedure D) for the Preparation of Final
Compounds (6–19)

Intermediate 4 or 5 (1 eq), selected arylsulfonyl chloride (1 eq), and previously ground
K2CO3 (2 eq) were introduced in a 10 mL PTFE jar (milling load 15 mg/mL) with one
stainless steel ball (R = 1.5 cm). The reaction was carried out for 1–10 min at rt. Then,
the crude mixture was solubilized in AcOEt (10 mL), and the organic phase was washed
with KHSO4 aqueous solution at pH = 3.5 (3 × 5 mL), saturated NaCl solution (1 × 5 mL),
dried over Na2SO4, and finally filtered and concentrated under a vacuum. To obtain the
desired amount of product, the reaction was carried out twice (2 × 10 mL).

3.1.6. Sulfonylation of Primary Amine (Procedure E) for the High-Scale Preparation of
Selected Final Compounds (6, 8 and 13)

Intermediate 4 or 5 (1 eq), selected arylsulfonyl chloride (1 eq), and previously ground
K2CO3 (2 eq) were introduced in a 35 mL PTFE jar (milling load 15 mg/mL) with one
stainless steel ball (R = 1.5 cm). The reaction was carried out for 1–5 min at rt. Then, the
crude mixture was solubilized in AcOEt (15 mL), and the organic phase was washed with
KHSO4 aqueous solution at pH = 3.5 (3 × 10 mL), saturated NaCl solution (1 × 10 mL),
dried over Na2SO4, and finally filtered and concentrated under a vacuum. To obtain the
desired amount of product, the reaction was carried out twice (2 × 15 mL).
3.1.7. Characterization of Final Compounds

4-Fluoro-N-[1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl]benzenesulfonamide (6)

General Procedure D was followed with primary amine 4 (51.27 mg, 0.157 mmol, 1 eq), 4-fluorobenzenesulfonyl chloride (30.4 mg, 0.157 mmol, 1 eq), and previously ground K₂CO₃ (43.3 mg, 0.314 mmol, 2 eq) to afford final compound 6 as a white solid, 62.6 mg (89% isolated yield). For the in vivo pharmacological studies, general procedure E was followed with primary amine 4 (205.1 mg, 0.627 mmol, 1 eq), 4-fluorobenzenesulfonyl chloride (121.7 mg, 0.627 mmol, 1 eq), and previously ground K₂CO₃ (173.2 mg, 1.255 mmol, 2 eq) to afford final compound 6 as a white solid, 250.5 mg (85% isolated yield). Compound 6 was converted to the hydrochloride salt according to procedure C.

C₂₂H₂₆FN₂O₄S·HCl: 448.18, Monoisotopic Mass: 448.18. UPLC/MS purity 99%, tᵣ = 4.99 min, [M+H]⁺ 449.3. ¹H NMR (500 MHz, CDCl₃): δ 7.98 (s, 2H), 7.15 (d, J = 6.5 Hz, 2H), 6.86–6.58 (m, 3H), 4.49 (d, J = 30.1 Hz, 2H), 3.65 (d, J = 57.7 Hz, 2H), 3.40 (d, J = 70.9 Hz, 3H), 3.00 (t, J = 9.1 Hz, 3H), 2.83–2.60 (m, 2H), 2.34 (s, 2H), 2.08–1.87 (m, 2H), 1.45 (s, 2H), 1.44 (s, 4H). ¹³C NMR (126 MHz, CDCl₃) δ 166.1 (J₁C-F = 254.5 Hz), 164.0, 140.0, 141.8, 137.4 (J₁C-F = 126.8 Hz), 129.9, 129.8 (J₁C-F = 9.0 Hz), 129.3 (J₁C-F = 8.8 Hz), 120.9, 119.7, 116.6, 116.4, 115.2, 88.1 (J₁C-F = 22.4 Hz), 64.4, 55.8, 52.4, 48.6, 48.4, 45.1, 43.1, 29.9, 28.4, 27.8. Mp for C₂₂H₂₆FN₂O₄S·HCl: 165.0–166.6 °C. Anal. calcd for C₂₂H₂₆FN₂O₄S·HCl: C 56.96, H: 6.23, N: 5.78, S: 6.71; Found C: 57.17, H: 6.32, N: 5.74, S: 6.39.

3-Chloro-N-[1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl]benzenesulfonamide (7)

General Procedure D was followed with primary amine 4 (50.2 mg, 0.154 mmol, 1 eq), 3-chlorobenzenesulfonyl chloride (21.6 µL, 0.154 mmol, 1 eq), and previously ground K₂CO₃ (42.4 mg, 0.307 mmol, 2 eq) to afford final compound 7 as yellow solid, 61.3 mg (86% isolated yield).

C₂₃H₂₇ClN₂O₄S·HCl: 464.15. UPLC/MS purity 95%, tᵣ = 5.15 min, [M+H]⁺ 465.2. ¹H NMR (500 MHz, CDCl₃): δ 7.88 (t, J = 1.9 Hz, 1H), 7.77 (dd, J = 7.8, 1.7, 1.0 Hz, 1H), 7.53 (ddd, J = 8.0, 2.1, 1.1 Hz, 1H), 7.45 (t, J = 7.9 Hz, 1H), 6.80–6.68 (m, 3H), 5.08–4.91 (m, 1H), 4.16 (t, J = 5.9 Hz, 2H), 3.22 (t, J = 10.2 Hz, 1H), 3.00 (s, 2H), 2.92 (d, J = 11.5 Hz, 2H), 2.82 (t, J = 6.0 Hz, 2H), 2.26 (s, 2H), 1.81 (d, J = 12.8 Hz, 2H), 1.65–1.52 (m, 2H), 1.47 (s, 6H). ¹³C NMR (126 MHz, CDCl₃): δ 147.9, 143.4, 143.2, 135.4, 135.3, 132.8, 130.6, 128.7, 127.2, 125.1, 120.5, 118.3, 113.6, 87.6, 66.6, 56.9, 52.3, 50.7, 43.4, 32.8, 29.8, 28.4. Mp for C₂₃H₂₇ClN₂O₄S·HCl: 118.9–120.7 °C.

5-Chloro-2-fluoro-N-[1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl]benzenesulfonamide (8)

General Procedure D was followed with primary amine 4 (49.2 mg, 0.150 mmol, 1 eq), 5-chloro-2-fluorobenzenesulfonyl chloride (34.3 mg, 0.150 mmol, 1 eq), and previously ground K₂CO₃ (41.5 mg, 0.300 mmol, 2 eq) to afford final compound 8 as a colorless oil, 48.7 g (68% isolated yield). For in vivo pharmacological studies, general procedure E was followed with primary amine 4 (196.7 mg, 0.602 mmol, 1 eq), 5-chloro-2-fluorobenzenesulfonyl chloride (137.2 mg, 0.602 mmol, 1 eq), and previously ground K₂CO₃ (166.1 mg, 1.204 mmol, 2 eq) to afford final compound 8 as a colorless oil, 194.7 mg (67% isolated yield). Compound 8 was converted to the hydrochloride salt according to procedure C.

C₂₃H₂₆ClFN₂O₄S·HCl: 483.0. Monoisotopic Mass: 482.14. UPLC/MS purity 100%, tᵣ = 5.37 min, [M+H]⁺ 483.2. ¹H NMR (500 MHz, CDCl₃): δ 7.85 (td, J = 6.4, 2.6 Hz, 1H), 7.56–7.46 (m, 1H), 7.14 (t, J = 9.0 Hz, 1H), 6.85–6.69 (m, 3H), 4.51 (dt, J = 41.8, 4.3 Hz, 1H), 3.71–3.40 (m, 5H), 3.08 (d, J = 11.6 Hz, 1H), 3.03–2.95 (m, 2H), 2.44 (d, J = 13.5 Hz, 2H), 2.30 (s, 2H), 2.07–1.85 (m, 2H), 1.46 (s, 3H), 1.46 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 158.3, 156.3 (J₁C-F = 253.9 Hz), 147.9, 143.4, 134.7, 134.6 (J₁C-F = 8.5 Hz), 131.0, 130.9 (J₁C-F = 15.2 Hz), 129.2, 128.8, 125.1, 120.5.
130.0, 130.0 (1\textsubscript{1}C-F = 3.4 Hz), 129.8, 128.7, 120.5, 118.6, 118.4 (1\textsubscript{2}C-F = 23.1 Hz), 118.2, 113.5, 87.5, 66.6, 56.9, 53.6, 52.3, 51.0, 43.4, 32.7, 28.4. Mp for C\textsubscript{23}H\textsubscript{28}ClFN\textsubscript{2}O\textsubscript{5}: 170.9–173.3 °C. Mp for C\textsubscript{23}H\textsubscript{28}ClFN\textsubscript{2}O\textsubscript{5} HCl: 189.2–191.6 °C. Anal. calculated for C\textsubscript{23}H\textsubscript{28}ClFN\textsubscript{2}O\textsubscript{5} HCl: C: 53.18, H: 5.63, N: 5.39, S: 6.17; Found C: 53.37, H: 5.43, N: 5.54, S: 6.39.

5-Chloro-2-methoxy-N-[[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)oxy]ethyl]piperidin-4-yl]benzenesulfonamide (9)

General Procedure D was followed with primary amine 4 (48.5 mg, 0.148 mmol, 1 eq), 5-chloro-2-methoxybenzenesulfonyl chloride (35.6 mg, 0.148 mmol, 1 eq), and previously ground K\textsubscript{2}CO\textsubscript{3} (41.0 mg, 0.297 mmol, 2 eq) to afford final compound 9 as a white solid, 61.7 mg (84% isolated yield).

C\textsubscript{24}H\textsubscript{33}ClN\textsubscript{2}O\textsubscript{5}S, MW: 495.03, Monoisotopic Mass: 494.16. UPLC/MS purity 95%, t\textsubscript{R} = 5.37 min, [M+H]\textsuperscript{+} 495.3. \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}): 7.89 (d, J = 2.7 Hz, 1H), 7.48 (dd, J = 8.8, 2.7 Hz, 1H), 6.96 (d, J = 8.8 Hz, 1H), 6.79–6.67 (m, 3H), 4.97 (d, J = 8.0 Hz, 1H), 4.16 (t, J = 6.0 Hz, 2H), 3.97 (s, 3H), 3.24 (dd, J = 7.9, 4.0 Hz, 1H), 3.02–2.97 (m, 2H), 2.90 (d, J = 10.7 Hz, 2H), 2.82 (s, 2H), 2.26 (s, 2H), 1.75 (s, 2H), 1.60–1.53 (m, 2H), 1.47 (s, 6H). \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) \textsuperscript{δ} 154.7, 148.0, 143.3, 134.2, 130.5, 129.6, 128.8, 126.1, 120.5, 118.3, 113.8, 113.7, 87.5, 66.7, 56.9, 56.8, 52.3, 43.3, 32.4, 29.8, 28.4. Mp for C\textsubscript{24}H\textsubscript{33}ClN\textsubscript{2}O\textsubscript{5}S: 147.4–149.7 °C.

2,5-Dimethoxy-N-[[1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)oxy]ethyl]piperidin-4-yl]benzenesulfonamide (10)

General Procedure D was followed with primary amine 4 (48.7 mg, 0.149 mmol, 1 eq), 2,5-dimethoxybenzenesulfonyl chloride (35.2 mg, 0.149 mmol, 1 eq), and previously ground K\textsubscript{2}CO\textsubscript{3} (41.1 mg, 0.298 mmol, 2 eq) to afford final compound 6 as a light-yellow solid, 51.1 mg (70% isolated yield).

C\textsubscript{25}H\textsubscript{34}N\textsubscript{2}O\textsubscript{5}S, MW: 490.62, Monoisotopic Mass: 490.21. UPLC/MS purity 100%, t\textsubscript{R} = 4.96 min, [M+H]\textsuperscript{+} 491.3. \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): \textsuperscript{δ} 7.44 (d, J = 3.2 Hz, 1H), 7.05 (dd, J = 9.0, 3.1 Hz, 1H), 6.95 (d, J = 9.0 Hz, 1H), 6.79–6.68 (m, 3H), 4.97 (d, J = 7.7 Hz, 1H), 4.15 (t, J = 6.1 Hz, 2H), 3.92 (s, 3H), 3.81 (s, 3H), 3.24–3.15 (m, 1H), 3.00 (d, J = 1.0 Hz, 2H), 2.79 (d, J = 6.9 Hz, 2H), 2.22 (s, 2H), 1.76 (d, J = 14.5 Hz, 4H), 1.52 (d, J = 11.1 Hz, 2H), 1.46 (s, 6H). \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) \textsuperscript{δ} 153.4, 150.1, 147.95, 143.4, 129.6, 128.7, 120.4, 120.3, 118.2, 114.2, 113.8, 113.7, 87.5, 66.8, 56.9, 56.1, 52.3, 43.3, 32.5, 28.4. Mp for C\textsubscript{25}H\textsubscript{34}N\textsubscript{2}O\textsubscript{5}S: 124.9–125.8 °C.

1-Naphthalene-N-[[1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)oxy]ethyl]piperidin-4-yl]sulfonamide (11)

General Procedure D was followed with primary amine 4 (49.2 mg, 0.151 mmol, 1 eq), 1-naphthalenesulfonyl chloride (34.2 mg, 0.151 mmol, 1 eq), and previously ground K\textsubscript{2}CO\textsubscript{3} (41.6 mg, 0.301 mmol, 2 eq) to afford final compound 11 as a light-brown solid, 48.5 mg (67% isolated yield).

C\textsubscript{27}H\textsubscript{32}N\textsubscript{2}O\textsubscript{5}S, MW: 480.62, Monoisotopic Mass: 480.21. UPLC/MS purity 95%, t\textsubscript{R} = 5.54 min, [M+H]\textsuperscript{+} 481.3. \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): \textsuperscript{δ} 8.61 (dq, J = 8.7, 1.0 Hz, 1H), 8.28 (dd, J = 7.3, 1.3 Hz, 1H), 8.06 (dt, J = 8.4, 1.2 Hz, 1H), 8.00–7.91 (m, 1H), 7.66 (ddd, J = 8.5, 6.9, 1.4 Hz, 1H), 7.59 (ddd, J = 8.0, 6.9, 1.1 Hz, 1H), 7.53 (ddd, J = 8.2, 7.3 Hz, 1H), 6.80–6.62 (m, 3H), 4.97 (s, 1H), 4.09 (t, J = 5.9 Hz, 2H), 3.22–3.15 (m, 1H), 2.99 (s, 2H), 2.82 (d, J = 11.7 Hz, 2H), 2.75 (t, J = 5.9 Hz, 2H), 2.15 (s, 2H), 1.64 (d, J = 11.8 Hz, 2H), 1.49 (d, J = 2.8 Hz, 2H), 1.45 (s, 6H). \textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}): \textsuperscript{δ} 147.8, 143.4, 135.8, 134.4, 134.4, 129.5, 129.3, 128.7, 128.5, 128.2, 127.1, 124.3, 124.3, 120.5, 118.2, 113.3, 87.6, 66.2, 56.8, 52.3, 43.3, 32.5, 28.4. Mp for C\textsubscript{27}H\textsubscript{32}N\textsubscript{2}O\textsubscript{5}S: 170.0–173.7 °C.
2-Naphthalene-N-[1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl]sulfonamide (12)

General Procedure D was followed with primary amine 4 (49.2 mg, 0.151 mmol, 1 eq), 1-naphthalenesulfonyl chloride (34.2 mg, 0.151 mmol, 1 eq), and previously ground K$_2$CO$_3$ (41.6 mg, 0.301 mmol, 2 eq) to afford final compound 12 as a white solid, 47.1 mg (65% isolated yield).

C$_{27}$H$_{32}$N$_2$O$_5$S, MW: 480.62, Monoisotopic Mass: 480.21. UPLC/MS purity 95%, t$_R$ = 5.53 min, [M+H]$^+$ 481.3. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.40 (d, $J = 1.9$ Hz, 1H), 7.95–7.76 (m, 4H), 7.57 (dddd, $J = 17.6$, 8.2, 6.9, 1.4 Hz, 2H), 6.72–6.58 (m, 3H), 4.86 (ddt, $J = 10.2$, 2.3, 1.2 Hz, 1H), 4.07 (t, $J = 5.9$ Hz, 2H), 3.21–3.17 (m, 1H), 2.97–2.90 (m, 2H), 2.88–2.78 (m, 2H), 2.75–2.70 (m, 2H), 2.13 (d, $J = 22.5$ Hz, 2H), 1.73 (d, $J = 12.0$ Hz, 2H), 1.57–1.47 (m, 2H), 1.38 (s, 6H). 

4-Isoquinoline-N-[1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl]sulfonamide (13)

General Procedure D was followed with primary amine 4 (49.2 mg, 0.150 mmol, 1 eq), 4-isooquinolinsulfonyl chloride (34.3 mg, 0.150 mmol, 1 eq), and previously ground K$_2$CO$_3$ (41.6 mg, 0.301 mmol, 2 eq) to afford final compound 13 as white solid, 59.4 mg (82% isolated yield). For in vivo pharmacological studies, general procedure E was followed with primary amine 4 (196.7 mg, 0.602 mmol, 1 eq), 4-isooquinolinsulfonyl chloride (137.0 mg, 0.602 mmol, 1 eq), and previously ground K$_2$CO$_3$ (166.3 mg, 1.204 mmol, 2 eq) to afford final compound 13 as a white solid, 220.3 mg (76% isolated yield). Compound 13 was converted to the hydrochloride salt according to procedure C.

C$_{26}$H$_{31}$N$_3$O$_5$S, MW: 481.61, Monoisotopic Mass: 481.21. UPLC/MS purity 99%, t$_R$ = 4.59 min, [M+H]$^+$ 482.3. $^1$H NMR (500 MHz, CD$_3$OD): $\delta$ 9.95 (s, 1H), 9.10 (s, 1H), 8.84 (d, $J = 8.6$ Hz, 1H), 8.61 (d, $J = 8.1$ Hz, 1H), 8.35 (ddd, $J = 8.4$, 7.1, 1.1 Hz, 1H), 8.20–8.04 (m, 1H), 6.89–6.66 (m, 3H), 4.28 (t, $J = 4.6$ Hz, 2H), 3.65–3.53 (m, 3H), 3.45 (t, $J = 4.6$ Hz, 2H), 3.27 (dt, $J = 1.6$ Hz, 2H), 3.10 (s, 1H), 3.02–2.97 (m, 2H), 1.87 (dd, $J = 69.4$, 13.4 Hz, 4H), 1.42 (s, 1H), 1.41 (s, 4H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 151.2, 147.9, 143.4, 143.3, 137.2, 133.3, 130.3, 129.0, 128.7, 125.9, 122.4, 120.4, 118.1, 113.6, 87.4, 66.7, 56.9, 53.6, 52.3, 43.3, 32.4, 28.3. Mp for C$_{26}$H$_{31}$N$_3$O$_5$S: 175.0–178.9 °C.

4-Fluoro-N-(1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl)benzenesulfonamide (14)

General Procedure D was followed with primary amine 5 (52.5 mg, 0.154 mmol, 1 eq), 4-fluorobenzensulfonyl chloride (30.0 mg, 0.154 mmol, 1 eq), and previously ground K$_2$CO$_3$ (42.5 mg, 0.308 mmol, 2 eq) to afford final compound 14 as yellow solid, 64.1 mg (90% isolated yield).

C$_{24}$H$_{32}$FN$_2$O$_4$S, MW: 462.58, Monoisotopic Mass: 462.2. UPLC/MS purity 100%, t$_R$ = 5.13 min, [M+H]$^+$ 463.3. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.87 (dd, $J = 8.9$, 5.0 Hz, 2H), 7.17 (t, $J = 8.6$ Hz, 2H), 6.82–6.59 (m, 3H), 4.16 (s, 2H), 3.09–3.03 (m, 2H), 3.01 (d, $J = 0.8$ Hz, 2H), 2.83 (t, $J = 5.9$ Hz, 4H), 2.10 (td, $J = 11.8$, 2.5 Hz, 2H), 1.70–1.63 (m, 2H), 1.48 (s, 6H), 1.40–1.20 (m, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 167.1, 165.1 ($^{13}$C-F = 254.6 Hz), 148.6, 144.4, 137.1, 137.1, 130.9, 130.8 ($^{13}$C-F = 9.3 Hz), 129.5, 121.5, 119.0, 117.5, 117.3 ($^{13}$C-F = 22.4 Hz), 113.9, 88.6, 66.7, 58.2, 54.5, 49.5, 44.3, 36.8, 30.1, 29.3. Mp for C$_{24}$H$_{32}$FN$_2$O$_4$S: 189.1–192.4 °C.
3-Chloro-N-{[1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl]methyl}benzensulfonamide (15)

General Procedure D was followed with primary amine 5 (51.5 mg, 0.151 mmol, 1 eq), 3-chlorobenzenesulfonyl chloride (21.3 µL, 0.151 mmol, 1 eq), and previously ground K$_2$CO$_3$ (41.7 mg, 0.302 mmol, 2 eq) to afford final compound 15 as a colorless oil, 60.0 mg (83% isolated yield).

C$_{24}$H$_{31}$ClN$_2$O$_4$S, MW: 479.03, Monoisotopic Mass: 478.17. UPLC/MS purity 100%, t$_R$ = 5.45 min, [M+H]$^+$: 479.3. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.85 (t, $J$ = 1.9 Hz, 1H), 7.74 (dt, $J$ = 7.9, 1.4 Hz, 1H), 7.53 (dd, $J$ = 8.1, 2.1, 1.1 Hz, 1H), 7.45 (t, $J$ = 7.9 Hz, 1H), 6.79–6.69 (m, 3H), 4.18 (t, $J$ = 6.0 Hz, 2H), 3.05 (d, $J$ = 11.4 Hz, 2H), 3.01 (s, 2H), 2.85 (dq, $J$ = 6.1, 2.8 Hz, 4H), 2.16–2.09 (m, 2H), 1.72–1.66 (m, 2H), 1.48 (s, 6H), 1.37–1.23 (m, 3H).

5-Chloro-2-fluoro-N-{[1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl]methyl}benzensulfonamide (16)

General Procedure D was followed with primary amine 5 (50.4 mg, 0.148 mmol, 1 eq), 5-chloro-2-fluorobenzenesulfonyl chloride (33.7 mg, 0.148 mmol, 1 eq), and previously ground K$_2$CO$_3$ (40.9 mg, 0.296 mmol, 2 eq) to afford final compound 16 as a colorless oil, 49.3 mg (67% isolated yield).

C$_{24}$H$_{30}$ClFN$_2$O$_4$S, MW: 497.02, Monoisotopic Mass: 496.16. UPLC/MS purity 100%, t$_R$ = 5.51 min, [M+H]$^+$: 497.3. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 7.86 (dd, $J$ = 6.1, 2.7 Hz, 1H), 7.51 (ddd, $J$ = 8.8, 4.2, 2.7 Hz, 1H), 7.16 (t, $J$ = 9.1 Hz, 1H), 6.82–6.69 (m, 3H), 5.12 (s, 1H), 4.20 (t, $J$ = 6.0 Hz, 2H), 3.08 (d, $J$ = 11.5 Hz, 2H), 3.01 (d, $J$ = 1.0 Hz, 2H), 2.94–2.81 (m, 4H), 2.24–2.11 (m, 2H), 1.79–1.70 (m, 2H), 1.48 (s, 6H), 1.43–1.24 (m, 3H), $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 158.3, 156.3 ($^{1}$J$_{C-F}$ = 253.95 Hz), 147.8, 143.4, 134.8, 134.7 ($^{3}$J$_{C-F}$ = 8.57 Hz), 130.2, 130.1, 130.1 ($^{5}$J$_{C-F}$ = 3.75 Hz), 129.6, 129.4 ($^{7}$J$_{C-F}$ = 15.4 Hz), 128.7, 120.5, 118.6, 118.4, 118.2 ($^{9}$J$_{C-F}$ = 23.3 Hz), 113.4, 87.6, 66.2, 57.2, 53.5, 48.68, 43.4, 35.9, 29.2, 28.4.

5-Chloro-2-methoxy-N-{[1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl]methyl}benzensulfonamide (17)

General Procedure D was followed with primary amine 5 (49.7 mg, 0.146 mmol, 1 eq), 5-chloro-2-methoxybenzenesulfonyl chloride (35.0 mg, 0.146 mmol, 1 eq), and previously ground K$_2$CO$_3$ (40.3 mg, 0.292 mmol, 2 eq) to afford final compound 17 as a yellow solid, 56.4 mg (76% isolated yield).

C$_{25}$H$_{33}$ClN$_2$O$_5$S, MW: 509.06, Monoisotopic Mass: 508.18. UPLC/MS purity 96%, t$_R$ = 5.57 min, [M+H]$^+$: 509.3. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.88–7.84 (m, 1H), 7.54–7.47 (m, 1H), 7.17–6.94 (m, 1H), 6.79–6.71 (m, 3H), 5.11–5.00 (m, 1H), 4.22 (dd, $J$ = 13.8, 6.1 Hz, 2H), 3.96 (s, 2H), 3.16–3.08 (m, 2H), 3.01 (s, 2H), 2.95–2.84 (m, 3H), 2.75 (t, $J$ = 6.7 Hz, 1H), 2.26–2.15 (m, 2H), 1.75 (t, $J$ = 17.9 Hz, 2H), 1.58–1.52 (m, 1H), 1.48 (s, 6H), 1.38–1.33 (m, 2H), $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 154.7, 147.9, 134.3, 130.3, 130.2, 128.7, 128.7, 128.6, 126.1, 120.5, 118.3, 113.6, 87.5, 87.5, 66.5, 66.3, 57.1, 56.9, 53.6, 53.5, 48.8, 48.7, 43.3, 28.4. Mp for C$_{25}$H$_{33}$ClN$_2$O$_5$S: 189.0–191.5°C.

1-Naphthalene-N-{[1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl]methyl}sulfonamide (18)

General Procedure D was followed with primary amine 5 (50.5 mg, 0.148 mmol, 1 eq), 1-naphthalenesulfonyl chloride (33.6 mg, 0.148 mmol, 1 eq), and previously ground K$_2$CO$_3$ (40.9 mg, 0.296 mmol, 2 eq) to afford final compound 18 as a colorless oil, 53.5 mg (73% isolated yield).
C_{28}H_{34}N_{2}O_{3}S, MW: 494.65, Monoisotopic Mass: 494.2. UPLC/MS purity 100%, t<sub>R</sub> = 5.61 min, [M+H]<sup>+</sup> 495.3. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 8.63 (dd, J = 8.6, 1.1 Hz, 1H), 8.23 (dd, J = 7.3, 1.3 Hz, 1H), 8.06 (d, J = 8.2 Hz, 1H), 7.94 (dd, J = 8.2, 1.4 Hz, 1H), 7.68–7.51 (m, 3H), 6.82–6.60 (m, 3H), 4.99–4.94 (m, 1H), 4.17 (t, J = 5.9 Hz, 2H), 2.99 (s, 4H), 2.83 (d, J = 6.2 Hz, 2H), 2.76 (t, J = 6.6 Hz, 2H), 2.11–2.02 (m, 2H), 1.62–1.55 (m, 3H), 1.47 (s, 1H), 1.46 (s, 6H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 147.9, 143.3, 134.6, 134.4, 134.4, 129.8, 129.3, 128.7, 128.6, 128.2, 127.1, 124.4, 124.3, 120.5, 118.2, 113.6, 87.5, 66.3, 57.0, 53.5, 48.6, 43.4, 29.8, 28.4.

4-Isoquinoline-N-[(1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yloxy)ethyl]piperidin-4-yl]methyl)sulfonamide (19)

General Procedure D was followed with primary amine 5 (50.4 mg, 0.148 mmol, 1 eq), 4-isoquinolinolsulfonyl chloride (33.7 mg, 0.148 mmol, 1 eq), and previously ground K<sub>2</sub>CO<sub>3</sub> (40.9 mg, 0.296 mmol, 2 eq) to afford final compound (68.9 mg, 94% isolated yield).

C_{27}H_{33}N_{3}O_{4}S, MW: 495.63, Monoisotopic Mass: 495.22. UPLC/MS purity 100%, t<sub>R</sub> = 4.85 min, [M+H]<sup>+</sup> 496.3. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 9.41 (d, J = 0.9 Hz, 1H), 9.11 (s, 1H), 8.62 (dq, J = 8.6, 0.9 Hz, 1H), 8.10 (dt, J = 8.2, 1.0 Hz, 1H), 7.88 (dd, J = 8.4, 6.9, 1.3 Hz, 1H), 7.74 (dd, J = 8.1, 7.0, 1.0 Hz, 1H), 6.79–6.67 (m, 3H), 5.51 (s, 1H), 4.17 (t, J = 6.0 Hz, 2H), 2.99 (s, 5H), 2.86 (t, J = 5.6 Hz, 2H), 2.82 (t, J = 6.0 Hz, 2H), 2.10 (t, J = 11.8 Hz, 2H), 1.67–1.60 (m, 2H), 1.46 (s, 6H), 1.29–1.24 (m, 2H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 158.0, 147.8, 144.8, 143.3, 133.0, 130.9, 129.7, 129.0, 128.9, 128.7, 128.6, 123.9, 120.5, 118.2, 113.5, 87.5, 66.3, 57.1, 53.5, 48.6, 43.3, 35.7, 29.2, 28.3. Mp for C_{27}H_{33}N_{3}O_{4}S: 114.9–116.0 °C.

3.2. In Vitro Pharmacology

3.2.1. Determination of the Affinity of the Test Compounds at the α<sub>1</sub>- and α<sub>2</sub>–ARs

The affinity of the obtained compounds were evaluated by radioligand binding assays (the ability to displace [<sup>3</sup>H]prazosin and [<sup>3</sup>H]clonidine from α<sub>1</sub>- and α<sub>2</sub>-adrenoceptors, respectively) on rat cerebral cortex [33,44]. The brains were homogenized in 20 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.6) and centrifuged at 20,000 × g for 20 min (0–4 °C). The cell pellet was resuspended in the Tris–HCl buffer and centrifuged again. Radioligand binding assays were performed in plates (MultiScreen/Millipore). The final incubation mixture (final volume 300 µL) consisted of 240 µL of the membrane suspension, 30 µL of [<sup>3</sup>H]prazosin (0.2 nM) or [<sup>3</sup>H]clonidine (2 nM) solution, and 30 µL of the buffer containing seven to eight concentrations (10<sup>−11</sup>–10<sup>−4</sup> M) of the tested compounds. For measuring the unspecific binding, phentolamine, 10 µM (in the case of [<sup>3</sup>H]prazosin) and clonidine, 10 µM (in the case of [<sup>3</sup>H]clonidine) were applied. The incubation was terminated by rapid filtration over glass fibre filters (Whatman GF/C) using a vacuum manifold (Millipore). The filters were then washed twice with the assay buffer and placed in scintillation vials with a liquid scintillation cocktail. Radioactivity was measured in a WALLAC 1409 DSA liquid scintillation counter. All assays were made in duplicate.

3.2.2. Determination of the Affinity of the Test Compounds at the Serotonin 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7</sub> and Dopaminergic D<sub>2</sub> Receptors

All experiments were performed using HEK293 cells stably expressing human 5-HT<sub>1A</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7b</sub>, and D<sub>2l</sub> receptors or CHO-K1 cells with a plasmid containing the human 5-HT<sub>2A</sub> receptor coding sequence (PerkinElmer, Waltham, MA, USA), according to previously reported procedures [38,45,46]. Cells were cultured in 150 cm<sup>2</sup> flasks and, after reaching a 90% confluence, were washed with PBS and the centrifugated (200 g) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol. Cell pellets were subsequently homogenized (in Ultra Turrax tissue homogenizer) and centrifugated twice (35,000 × g for 15 min at 4 °C), with 15 min incubation at 37 °C between the centrifugations. All assays were incubated in dedicated buffers and in a total volume of 200 µL in 96-well microtitre plates for 1 h at 37 °C, except for 5-HT<sub>1AR</sub> and 5-HT<sub>2AR</sub> that was incubated
for 1 h at room temperature. The process of equilibration is terminated by rapid filtration through Unifilter-96 (PerkinElmer) 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 following assay samples containing the proper radioligand were used: 2.5 nM [3H]-8-OH-DPAT (PerkinElmer, #NET929001MC) for 5-HT1A R; 1 nM [3H]-ketanserin (PerkinElmer, #NET791250UC) for 5-HT2A R; 2 nM [3H]-LSD (PerkinElmer, #NET638250UC) for 5-HT6 R; and 0.8 nM [3H]-5-CT (PerkinElmer, #NET975001MC) for 5-HT7 R or 2.5 nM [3H]-raclopride (PerkinElmer, #NET975001MC) for D2L R. Non-specific binding was determined using 10 µM 5-HT for 5-HT1A R and 5-HT7 R, 20 µM mianserin for 5-HT2A R, 10 µM methiothepin for 5-HT6 R, and 10 µM haloperidol for D2L R. Each compound was tested in triplicate at seven concentrations ranging from 10−10 to 10−4 M. The inhibition constants (Ki) were calculated from the Cheng–Prusoff equation [47]. Results were expressed as means of at least three separate experiments.

3.2.3. Determination of the Intrinsic Activity of the Test Compounds at the α2-AR Subtypes

Intrinsic activity assays for α2A-adrenergic receptor were performed according to the instructions of the manufacturer of the assay kit containing the ready to use cells with stable expression of the α2A-adrenoceptor (Invitrogen, Life Technologies, Waltham, MA, USA). Tango™ ADRA2A-bla U2OS DA cells (10,000 cells/well) were plated in a 384-well format and incubated for 20 h. Cells were exposed to Yohimbine (Sigma-Aldrich, Merck, Darmstadt, Germany) for 30 min, then stimulated with an EC80 concentration of UK14,304 (Sigma-Aldrich) in the presence of 0.1% DMSO for 5 h. Cells were then loaded with LiveBLAzer™-FRET B/G substrate for 2 h. Fluorescence emission values at 460 nm and 530 nm were obtained using a standard fluorescence plate reader and the % inhibition plotted against the indicated concentrations of Yohimbine.

The intrinsic activity assays for the α2B-adrenergic receptor were assessed by luminescence detection of calcium mobilization using the recombinant expressed jellyfish photoprotein, aequorin. Measurements were performed with adrenergic α2B AequoScreen cell line (PekinElmer). The cell density in 96-well format measurements was 5000 cells per well. Cell harvesting, coelenterazine h (Invitrogen, cat. no. C 6780) loading and preparation were done according to instructions presented in the AequoScreen Starter Kit Manual (PerkinElmer). Compound concentration series (50 µL/well) were diluted in 0.1% BSA (Intergen) containing assay buffer (D-MEM/F-12, Invitrogen cat. no. 11039) and prepared in white 1/2 Area Plate–96 well microplates (PerkinElmer). The cell suspension was dispensed on the ligands using the POlarStar optima reader injectors. For the antagonist assay, cells were injected (50 µL) into the assay plate with antagonists (50 µL) using the POlarStar optima reader. The antagonist dilution series with four replicates were prepared as instructed in the AequoScreen Starter Kit Manual at the concentrations from 10−11 to 10−6 M/L. The agonist used for α2A-AR cells was Oxymetazoline (Sigma, cat. no. O2378), which at a single concentration was injected (50 µL, final concentration EC80) on the preincubated (15–20 min) mixture of cells and antagonist, and the emitted light was recorded for 20 s.

3.2.4. Determination of the Intrinsic Activity of the Test Compounds at the 5-HT7R

Cells (prepared with the use of Lipofectamine 2000) were maintained at 37 °C in a humidified atmosphere with 5% CO2 and grown in Dulbecco’s Modifier Eagle Medium containing 10% dialyzed fetal bovine serum and 500 mg/mL G418 sulphate. For functional experiments, cells were subcultured in 25 cm diameter dishes, grown to 90% confluence, washed twice with pre-warmed to 37 °C phosphate buffered saline (PBS) and were centrifuged for 5 min (160 × g). The supernatant was aspirated, then the cell pellet was resuspended in stimulation buffer (1 × HBSS, 5 mM HEPES, 0.5 mM IBMX, 0.1% BSA). The cAMP level was measured using the LANCE cAMP detection kit (PerkinElmer), according to the manufacturer’s directions. For the investigation of the antagonist effect on 5-HT7R, the agonist 5-carboxyamidotryptamine (5-CT; EC50 = 1 nM) was used in submaximal
concentration (10nM) to stimulate cAMP production. Cells (5 µL) were incubated with compounds (5 µL) for 30 min at room temperature in 384-well white opaque microtiter plate. After incubation, the reaction was stopped, and cells were lysed by the addition of 10 µL working solution (5 µL Eu-cAMP and 5 µL ULight-anti-cAMP). The assay plate was incubated for 1 h at room temperature. Time-resolved fluorescence resonance energy transfer (TR-FRET) was detected by an Infinite M1000 Pro (Tecan) using instrument settings from LANCE cAMP detection kit manual. 

Kb values were calculated from the Cheng–Prusoff equation specific for the analysis of functional inhibition curves: 

$$K_b = \frac{IC_{50}}{1 + A/EC_{50}}$$

where A is the agonist concentration, IC_{50} is the concentration of the antagonist producing a 50% reduction in the response to the agonist, and EC_{50} is the agonist concentration which causes half of the maximal response [47].

3.3. In Vivo Pharmacology
3.3.1. Animals

Adult male Albino Swiss mice (CD-1, 8 weeks old, 25–30 g: Jagiellonian University Medical College, Krakow, Poland) were used in the study. Animals were housed in groups of 8 in a transparent plastic cage (382 × 220 × 150 mm) at room temperature (22 ± 2 °C), on a 12 h light/dark cycle with ad libitum access to food and water. Mice were handled for one week before starting the experimental procedures. The separate groups of animals were used in the forced swim test and in the locomotor studies. All studies were approved by the Institutional Animal Care and Ethics Committee of the Jagiellonian University (approval no.: 80/2015).

3.3.2. Drug Administration

Mirtazapine (16 mg/kg or 4 mg/kg, Sigma-Aldrich) was dissolved in DMSO and diluted to the appropriate dose with 1% Tween 80, immediately before use (the maximal final DMSO concentration was 2%). Tested compounds were dissolved with 1% Tween 80. Solutions of mirtazapine and the tested compounds were administered intraperitoneally (ip) 30 min prior to the experiment. The control animals were given ip injections of the 2% DMSO in 1% Tween 80 (vehicle). The volume of vehicle or drug solutions was 10 mL/kg.

3.3.3. Forced Swim Test

The experiment was performed on mice according to the method previously described [48,49]. Mice were forced to swim individually in the glass cylinders (height 25 cm, diameter 10 cm) filled with water at 24 ± 1 °C to a depth of 10 cm and left there for 6 min. Following a 2 min habituation period, total time spent immobile was recorded over the next 4 min. The animal was regarded as immobile when it remained floating passively in the water, making only small movements to keep its head above the water.

3.3.4. Spontaneous Locomotor Activity

Locomotor activity was recorded with an Opto M3 multichannel activity monitor, photoresistor actometers connected to a counter for the recording of light-beam interruptions (MultiDevice Software v1.3, Columbus Instruments, Columbus, OH, USA). The mice, after being placed into the cages individually, had their activity evaluated between the 2nd and the 6th minute. The chosen time period corresponded with the time interval considered in the FST. Spontaneous locomotor activity was evaluated as the distance travelled plus the movements of climbing by animals.

3.3.5. Statistical Analysis

Statistical calculations were performed using GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA). The normality of data sets was determined using Shapiro–Wilk test. Comparisons between experimental and control groups were performed by one-way ANOVA, followed by Dunnett post hoc.
4. Conclusions

Based on the finding that concurrent blockade of $\alpha_2$-AR and 5-HT$_7$R might be beneficial in the treatment of depressive disorders, we elaborated a medicinal mechanochemical approach to provide a limited series of arylsulfonamides of (dihydrobenzofuranoxy)ethyl piperidines as dual acting $\alpha_2$/5-HT$_7$R antagonists. Sustainable solid-state protocol furnished designed compounds 6–19 in high yields and purities, limiting the amount of organic solvents as well as the formation of by-products. Further focused SAR studies revealed that the presence of a 4-aminopiperidine central core, together with dihalogenated substituents or a 4-isoquinolyl moiety at the sulfonamide fragment, were responsible for the high affinity of tested compounds for both biological targets. Finally, the study identified 5-chloro-2-fluoro-N-[1-[2-(2,2-dimethyl-2,3-dihydrobenzofuran-7-yl)oxy]ethyl[piperidin-4-yl]benzenesulfonamide (compound 8) as a potent $\alpha_{2A}$/5-HT$_7$R antagonist, which produced an antidepressant-like effect in FST in mice. The effect was similar to that produced by mirtazapine used in a two-fold higher dose, without inducing sedation. Preliminary data for compound 8 are promising enough to warrant further efficacy and safety studies on the potential of dual-acting $\alpha_{2A}$/5-HT$_7$R antagonists in the treatment of affective disorders.

Supplementary Materials: The following are available online: optimization of mechanochemical reactions (Tables S1–S3); MS, $^1$H-NMR and $^{13}$C-NMR spectra of all intermediates and final compounds (Figures S1–S54).

Author Contributions: Conceptualization: V.C., M.K., P.Z.; synthesis: V.C., M.S., K.M.; characterization: M.S., P.K.; in vitro pharmacological studies: G.S. (Grzegorz Satała), A.S., M.B., G.S. (Gabriela Starowicz); in vivo pharmacological studies: M.K., A.D.; validation and data analysis: V.C., X.B., F.L., B.D., A.S., M.K., J.S.; manuscript writing, review and editing: V.C., M.K., M.S., P.Z. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and were approved by the Institutional Animal Care and Ethics Committee of the Jagiellonian University (approval no.: 80/2015).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds are available from the authors.

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