Journal Pre-proof

Multifunctional 6-fluoro-3-[3-(pyrrolidin-1-yl)propyl]-1,2-benzoxazoles targeting behavioral and psychological symptoms of dementia (BPSD)

Adam Bucki, Monika Marcinkowska, Joanna Śniecikowska, Agnieszka Zagórska, Marek Jamrozik, Maciej Pawłowski, Monika Głuch-Lutwin, Agata Siwek, Magdalena Jakubczyk, Karolina Pytka, Magdalena Jastrzębska-Więsek, Anna Partyka, Anna Wesołowska, Paweł Mierzejewski, Marcin Kołaczkowski

PII: S0223-5234(20)30116-1

DOI: https://doi.org/10.1016/j.ejmech.2020.112149

Reference: EJMECH 112149

To appear in: *European Journal of Medicinal Chemistry*

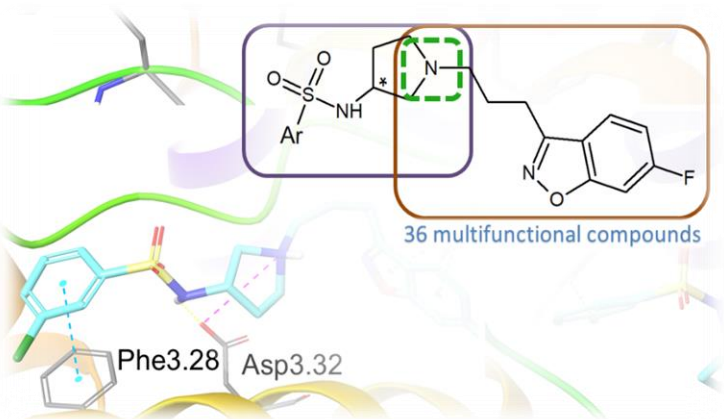
Received Date: 13 November 2019

Revised Date: 12 February 2020

Accepted Date: 13 February 2020

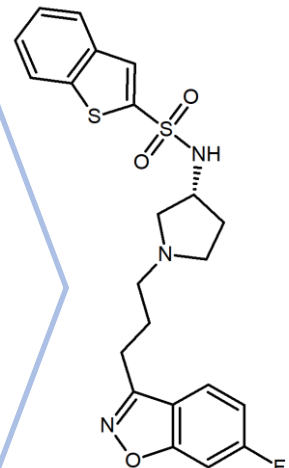
Please cite this article as: A. Bucki, M. Marcinkowska, J. Śniecikowska, A. Zagórska, M. Jamrozik, M. Pawłowski, M. Głuch-Lutwin, A. Siwek, M. Jakubczyk, K. Pytka, M. Jastrzębska-Więsek, A. Partyka, A. Wesołowska, Paweł. Mierzejewski, M. Kołaczkowski, Multifunctional 6-fluoro-3-[3-(pyrrolidin-1-yl)propyl]-1,2-benzoxazoles targeting behavioral and psychological symptoms of dementia (BPSD), *European Journal of Medicinal Chemistry* (2020), doi: https://doi.org/10.1016/j.ejmech.2020.112149.

Computer-aided drug design



Pharmacological profile combining high affinity for the 5-HT2A, 5-HT6, 5-HT7 and D2 receptors

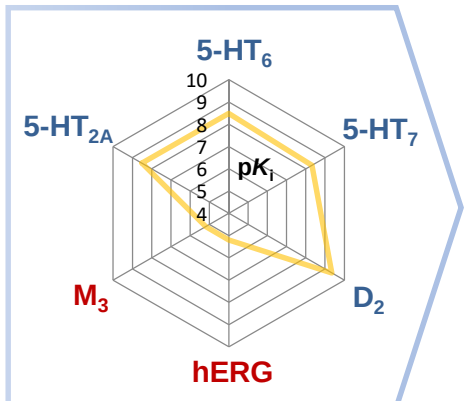
Lead compound of drug-like properties



CNS MPO 4.4

Clint = 88 µL/min/mg HLM

In vitro affinity



Antagonist functional activity

Behavioral studies

In vivo activity:

✓ Antipsychotic-like

✓ Anxiolytic-like

✓ Antidepressant-like

Cause no:

✓ memory deficits

✓ motor impairments

Potential application in BPSD

Multifunctional 6-Fluoro-3-[3-(pyrrolidin-1-yl)propyl]-1,2-benzoxazoles Targeting Behavioral And Psychological Symptoms Of Dementia (BPSD)

Adam Bucki[a,c*], Monika Marcinkowska[a], Joanna Śniecikowska[a,c], Agnieszka Zagórska[a], Marek Jamrozik[a], Maciej Pawłowski[a], Monika Głuch-Lutwin[a] , Agata Siwek[a], Magdalena Jakubczyk[a], Karolina Pytka[a], Magdalena Jastrzębska-Więsek[a] , Anna Partyka[a], Anna Wesołowska[a], Paweł Mierzejewski[b] and Marcin Kołaczkowski[a,c]

[a] Faculty of Pharmacy, Jagiellonian University Medical College,

9 Medyczna Street, 30-688 Kraków, Poland

[b] Institute of Psychiatry and Neurology, 9 Sobieskiego Street, 02-957 Warsaw, Poland

[c] Adamed Pharma S.A., Pienkow, 6A Mariana Adamkiewicza Street, 05-152 Czosnów, Poland

*Corresponding Author Information:

Phone: (+48)126205460; Fax: (+48)126205458

E-mail: adam.bucki@uj.edu.pl

ABSTRACT

Patients suffering from dementia experience cognitive deficits and 90% of them show non-cognitive behavioral and psychological symptoms of dementia (BPSD). The spectrum of BPSD includes agitation, depression, anxiety and psychosis. Antipsychotics, e.g. quetiapine, have been commonly used off-label to control the burdensome symptoms, though they cause serious side effects and further cognitive impairment. Therefore, the development of targeted therapy for BPSD, suitable for elderly patients, remains relevant.

A multitarget-directed ligand, acting on serotonin 5-HT_{2A} and dopamine D_2 receptors (R) and thus exerting anti-aggressive and antipsychotic activity, as well as on 5-HT_6Rs and 5-HT_7Rs (potential pro-cognitive, antidepressant and anxiolytic activity), poses a promising strategy for the treatment of BPSD. Antitargeting muscarinic M_3R and hERG channel is expected to reduce the risk of side effects. We obtained a series of stereoisomeric compounds by combining 6-fluoro-1,2-benzoxazole moiety and arylsulfonamide fragment through pyrrolidin-1-yl-propyl linker.

N-[(3*R*)-1-[3-(6-fluoro-1,2-benzoxazol-3-yl)propyl]pyrrolidin-3-yl]-1-benzothiophene-2-sulfonamide showed a substantial affinity for the targets of interest (pK_i = 8.32 – 9.35) and no significant interaction with the antitargets. Functional studies revealed its antagonist efficacy (pK_B = 7.41 – 9.03). The lead compound showed a promising profile of antipsychotic-like activity in amphetamine- and MK-801-induced hyperlocomotion (MED = 2.5 mg/kg), antidepressant-like, as well as anxiolytic-like activity in mice (MED = 0.312 and 1.25 mg/kg in the forced swim and four-plate tests, respectively). Notably, the novel compound didn't affect spontaneous locomotor activity, nor induced catalepsy or memory deficits (step-through passive avoidance test) in therapeutically relevant doses, which proved its benign safety profile. The overall pharmacological characteristics of the lead compound

outperformed the reference drug quetiapine, making it a promising option for evaluation in the treatment of BPSD.

KEYWORDS: MTDL; BPSD; psychosis in dementia; Alzheimer's disease; serotonin receptors; arylsulfonamide.

ABBREVIATIONS: BPSD, behavioral and psychological symptoms of dementia; AD, Alzheimer's disease; MTDL, multitarget-directed ligand; MED, minimum effective dose; 5-HT_{2A}R, 5-HT_{2A} serotonin receptor; 5-HT_6R, 5-HT_6 serotonin receptor; 5-HT_7R, 5-HT_7 serotonin receptor; D_2R, D_2 dopamine receptor; M_3R, M_3 muscarinic receptor; CNS-MPO, central nervous system multiparameter optimization.

INTRODUCTION

According to the World Health Organization (WHO), dementia affects 47.5 million people worldwide and each year there are approximately 7.7 million new reports [1]. Dementia is typically associated with cognitive impairment and memory loss, however, the most burdensome signs are so-called behavioral and psychological symptoms of dementia (BPSD). The spectrum of BPSD includes agitation, irritability, psychosis, depression and anxiety. These symptoms not only affect the quality of life of patients living with the disease but also poses a heavy burden for their families and caregivers [2].

It is estimated that at least one or more symptoms of psychological or behavioral disturbances will manifest in almost all (about 90%) of dementia patients in the course of their disease. Approximately 50% of patients with Alzheimer's disease (AD) and other dementias develop psychosis manifesting mainly in hallucinations and delusions [3]. The first patient, reported by Alois Alzheimer in the early 20th century, experienced psychotic symptoms in fact [4]. The remaining signs and symptoms occur equally often; depression affects 40-60% of dementia patients, while over 20% suffer from anxiety disorders. These rates were 3 to 4 times higher than those estimated for people aged 65 and elder without dementia [5,6]. Treatment of psychological and behavioral abnormalities in dementia patients is exceptionally intractable because no pharmacotherapy has been designed and approved in this indication to date. Given the lack of dedicated therapies, available atypical antipsychotics have been commonly used off-label for psychosis and severe agitation in dementia. However, these medications show limited clinical effectiveness and cause many serious side effects such as weight gain, type II diabetes, QTc interval prolongation, hyperlipidemia, myocarditis, extrapyramidal syndrome (EPS), and anticholinergic effects. Moreover, long-term treatment with the present antipsychotics causes cognitive impairment

that is particularly undesirable regarding elderly patients who already suffer from memory deficits [7]. Therefore, only short-term use of the lowest doses is acceptable for selected antipsychotics, e.g. risperidone and quetiapine [8–10]. Depressive disorders in dementia patients are treated with known classes of antidepressants, nevertheless, results from different studies are contradictory. Only a few antidepressants (e.g. citalopram and sertraline) appear to be effective and well-tolerated in patients suffering from major depression in AD. On the other hand, antidepressants were ineffective in patients with mild to moderate depression. Moreover, there is no specific data on the treatment of depression in vascular dementia or other non-Alzheimer types of dementia [5]. Therefore, the development of more effective and safe therapeutic option that does not cause further cognitive impairment and therefore is suitable for elderly patients, remains an unmet clinical need.

It's been demonstrated that serotoninergic, dopaminergic and cholinergic systems are particularly involved in the pathogenesis of BPSD, and the role of serotoninergic 5-HT_6, 5-HT_{2A}, 5-HT_7 receptors and dopamine D_2 receptors as therapeutic targets appear to be evident [2]. The relevance of serotoninergic modulation in the treatment of psychosis and cognitive impairment was investigated as a favourable component of the mechanism of action of atypical antipsychotic drugs [11]. The validity of this approach was confirmed in clinical trials, as pimavanserin, the 5-HT_{2A} receptors antagonist, proved its positive efficacy in patients with dementia-related psychosis. It showed antipsychotic activity and acceptable tolerability without negative effect on cognition in phase 2 placebo-controlled study [12]. Treatment with pimavanserin resulted in statistically significant longer time to relapse of psychosis compared to placebo in phase 3 clinical study [13,14].

A growing body of evidence testifies that selective antagonists of serotoninergic 5-HT_6

receptors exhibit procognitive effect due to enhancing cholinergic transmission in the prefrontal cortex. Therapeutic potential of 5-HT_6 receptor antagonists has been studied in clinical trials. The most encouraging results were obtained in phase II clinical trials with $5HT_6$ receptor antagonists: SB 742457 and Lu AE58054, which improved cognitive functions in patients with Alzheimer's disease [15]. These results gave hope for the therapeutic potential of 5-HT_6 receptor antagonists in dementia patients. Besides the procognitive properties, 5-HT_6 receptor antagonists have shown significant antidepressant activity related to an enhancement of dopaminergic and noradrenergic transmission, as well as an anxiolytic effect due to interaction with GABAergic system [16]. Similar pharmacological effects were observed after administration of selective 5-HT_7 receptor antagonist SB-269970. Serotonin 5-HT_7 receptors are located in brain regions associated with the processes of learning and memory. Preclinical studies with selective 5-HT_7 receptor antagonists exposed their ability to elicit antipsychotic and antidepressant-like effects [17]. Moreover, it's been proven that blockade of the 5-HT_{2A} and 5-HT_6 receptors can alleviate EPS induction caused by e.g. dopamine antagonism [18]. The abovementioned data suggest possible efficacy of the selected serotonin receptors antagonists in the management of BPSD.

It has been widely accepted that one of the most advantageous approaches in the modern drug discovery for diseases of complex aetiology, is the development of a multitarget-directed ligand (MTDL). This strategy relies on the design of a single molecule that interacts simultaneously with a range of biological targets of high therapeutic relevance. Such a multifunctional ligand offers several advantages over a pharmacological cocktail, made up from several separate drugs, in terms of pharmacokinetics and safety. Following this strategy, we identified specific molecular targets, modulation of which might contribute to effective pharmacotherapy of BPSD. The interaction with the 5-HT_{2A} and D_2 receptors is

supposed to provide anti-aggressive and antipsychotic activity, while antagonism of the 5-HT_6 and 5-HT_7 receptors is expected to contribute to the pro-cognitive, antidepressant and anxiolytic activity of the designed MTDLs.

Herein we present the computer-aided design, chemical synthesis and biological evaluation of innovative ligands aimed as a potential treatment of behavioral and psychological symptoms in the fragile population of elderly patients.

MATERIALS AND METHODS

Molecular modeling

Computer-aided design was based on molecular property analysis and prediction of ligand-target interactions. To this end, docking to the previously developed homology models of the 5-HT_{2A}, 5-HT_6, 5-HT_7 and D_2 receptors was performed. Procedure for obtaining ligand-optimized models of high predictive value, utilizing Induced-fit docking (IFD), was characterized in detail previously [19–21]. The 5-HT_{2A}R model was based on the 5-HT_{2B}R crystal structure 4IB4 [22]. The alignment of the 5-HT_{1A}R amino acid sequence (UniProt P28223) was predicted by hhsearch model via Genesilico Metaserver (overall amino acid sequence identity 48%) [23]. Homology model was obtained via SwissModel platform [24] and was validated by processing in Protein Preparation Wizard. Ligand-based optimization of the binding site, performed using IFD protocol, as well as cross docking-based (Glide XP) assessment of model predictive value, were carried out using various chemical classes of high affinity 5-HT_{2A}R ligands and resulted in a variety of conformational models that served as molecular targets in docking studies. Glide XP flexible docking procedure was carried out using OPLS3 force field and default parameters. H-bond constraint, as well as centroid of a

grid box for docking to the receptor models were located on Asp3.32. Selection of the best complex was based on scoring function and visual analysis of binding mode. Ligand structures were optimized using LigPrep tool. Glide, induced fit docking (IFD), LigPrep and Protein Preparation Wizard were implemented in Small-Molecule Drug Discovery Suite (Schrödinger, Inc.), which was licensed for Adamed Pharma S.A.

Instant JChem was used for structure database management, search and property prediction, Instant JChem 15.12.14.0, 2015, ChemAxon (http://www.chemaxon.com). Metabolic pathways and clearance prediction was conducted using ADMET Predictor (Simulations Plus Inc.).

The designed structures were tested for compliance with rules evaluating bioavailability of a compound after oral administration – Lipinski's rule of five and Veber filter. The first one assumes that compounds having $LogP_{o/w}$ (octanol/water partition coefficient) lower than 5, molecular weight (MW) below 500, less than 10 H-bond acceptors (HBA), and less than 5 H-bond donors (HBD) are more likely to show favourable bioavailability [25]. The Veber rule extends the range of parameters by rotatable bonds (preferably RB < 10) and topological polar surface area (preferably TPSA < 140 A^2) [26]. Molecules that obey the above restrictions and are characterized by low basicity (the most favourable pK_a values below 8, due to P-gp interactions and ionization issues) are more likely to show preferable membrane permeability [27]. CNS MPO values were calculated using the above data in CNS MPO calculator [28]. Moreover, the designed structures were examined using SwissADME [29] tool for known classes of reactive assay interference compounds (PAINS), that would disturb biological in vitro studies.

Chemical synthesis

General chemistry information

Unless otherwise indicated, all the starting materials were purchased from commercial suppliers and were used for synthesis without further purification. Analytical thin-layer chromatography (TLC) was performed on Merck Kieselgel 60 F_{254} (0.25 mm) pre-coated aluminium sheets (Merck, Darmstadt, Germany). Chromatograms were visualized using a 254 nm UV lamp. Column chromatography was performed using silica gel (particle size 0.063–0.200 mm; 70–230 Mesh ATM) purchased from Merck. The UPLC-MS or UPLC-MS/MS analyses were run on UPLC-MS/MS system comprising Waters ACQUITY UPLC (Waters Corporation, Milford, MA, USA) coupled with Waters TQD mass spectrometer (electrospray ionization mode ESI with tandem quadrupole). Chromatographic separations were carried out using the ACQUITY UPLC BEH (bridged ethyl hybrid) C_{18} column: 2.1 × 100 mm and 1.7 µm particle size. The column was maintained at 40 °C and eluted under gradient conditions using 95% to 0% of eluent A over 10 min, at a flow rate of 0.3 mL/min. Eluent A: 0.1% solution of formic acid in water (v/v); eluent B: 0.1% solution of formic acid in acetonitrile (v/v). A total of 10 µl of each sample were injected, and chromatograms were recorded using Waters eλ PDA detector. The spectra were analyzed in the range of 200–700 nm with 1.2 nm resolution and at a sampling rate of 20 points/s. MS detection settings of Waters TQD mass spectrometer were as follows: source temperature 150 °C, desolvation temperature 350 °C, desolvation gas flow rate 600 l/h, cone gas flow 100 L/h, capillary potential 3.00 kV, and cone potential 20 V. Nitrogen was used for both nebulizing and drying. The data were obtained in a scan mode ranging from 50 to 1000 m/z at 0.5 s intervals; 8 scans were summed up to obtain the final spectrum. Collision activated dissociation (CAD) analyses were carried out with the energy of 20 eV, and all the fragmentations were observed in the source. Consequently, the ion spectra were obtained in

the range from 50 to 500 m/z. MassLynx V 4.1 software (Waters) was used for data acquisition. Standard solutions (1 mg/mL) of each compound were prepared in a mixture comprising analytical grade acetonitrile/water (1/1, v/v). The UPLC/MS purity of all the test compounds and key intermediates was determined to be >95%. ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra were obtained in a Varian Mercury spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$, CD_3OD or DMSO-d_6 operating at 300 MHz (^{1}H NMR), 75 MHz (^{13}C NMR), and 282 MHz (^{19}F NMR). Chemical shifts are reported in terms of δ values (ppm) relative to TMS δ = 0 (^{1}H) as internal standard. The *J* values are expressed in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), dt (doublet of triplets), dtd (doublet of triplet of doublets), t (triplet), td (triplet of doublets), tdd (triplet of doublet of doublets), q (quartet), quin (quintet), m (multiplet).

Synthetic procedures

General procedure for the synthesis of *R* or *S* enantiomer of tert-butyl (1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)carbamate (**I – II**).

A mixture of 3-(3-chloropropyl)-6-fluorobenzo[d]isoxazole (1.0 equiv, 10.0 mmol), appropriate *tert*-butyl pyrrolidin-3-ylcarbamate (R or S enantiomer), (1.1 equiv, 11.0 mmol), potassium carbonate (3.0 equiv, 30.0 mmol) and catalytic amount of potassium iodide in acetonitrile (60 mL) was stirred at 60 °C for 48 h. After that time, reaction mixture was cooled to the room temperature, the solid was filtrated and the solvent was evaporated under the reduced pressure. Next, the crude product was purified by column chromatography over silica gel using dichloromethane/methanol (95/5, v/v) as eluent.

(R)-tert-butyl (1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)carbamate (**I**)**.** The title compound was prepared starting from 3-(3-chloropropyl)-6-fluorobenzo[d]isoxazole (1.0 equiv, 10.0 mmol, 2.13 g), (*R*)-*tert*-butyl pyrrolidin-3-ylcarbamate (1.1 equiv, 11.0 mmol, 2.05 g) and potassium iodide. Yield 36%, yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.61 (dd, *J* = 5.0, 8.8 Hz, 1H), 7.23 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.06 (dt, *J* = 2.2, 8.8 Hz, 1H), 4.90 (br s, 1H), 4.18 (br s, 1H), 3.03 (t, *J* = 7.4 Hz, 2H), 2.92 (br s, 1H), 2.73–2.57 (m, 4H), 2.47–2.35 (m, 1H), 2.26 (dt, *J* = 8.3, 13.1 Hz, 1H), 2.16–2.02 (m, 2H), 1.73–1.57 (m, 1H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 163.6 (d, *J* = 13.8 Hz), 164.3 (d, *J* = 250.2 Hz), 157.9, 155.3, 122.2 (d, *J* = 11.1 Hz), 118.2 (d, *J* = 1.7 Hz), 112.5 (d, *J* = 25.4 Hz), 97.3 (d, *J* = 27.0 Hz), 79.4, 60.7, 55.0, 52.7, 49.6, 32.2, 28.4 (3C), 26.0, 22.9; Formula $C_{19}H_{26}FN_3O_3$; MS (ESI^+): m/z 364 $[M+H]^+$

(S)-tert-butyl (1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)carbamate (**II**). The title compound was prepared starting from 3-(3-chloropropyl)-6-fluorobenzo[d]isoxazole (1.0 equiv, 10.0 mmol, 2.13 g), (*R*)-*tert*-butyl pyrrolidin-3-ylcarbamate (1.1 equiv, 11.0 mmol, 2.05 g) and potassium iodide. Yield 48%, yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.61 (dd, *J* = 5.0, 8.8 Hz, 1H), 7.23 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.06 (dt, *J* = 2.2, 8.8 Hz, 1H), 4.90 (br s, 1H), 4.18 (br s, 1H), 3.03 (t, *J* = 7.4 Hz, 2H), 2.92 (br s, 1H), 2.73–2.57 (m, 4H), 2.47–2.35 (m, 1H), 2.26 (dt, *J* = 8.3, 13.1 Hz, 1H), 2.16–2.02 (m, 2H), 1.73–1.57 (m, 1H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 13.8 Hz), 163.6 (d, *J* = 250.2 Hz), 157.9, 155.3, 122.2 (d, *J* = 11.1 Hz), 118.2 (d, *J* = 1.7 Hz), 112.5 (d, *J* = 25.4 Hz), 97.3 (d, *J* = 27.0 Hz), 79.4, 60.7, 55.0, 52.7, 49.6, 32.2, 28.4, 26.0, 22.9; Formula $C_{19}H_{26}FN_3O_3$; MS (ESI^+): m/z 364 $[M+H]^+$

General procedure for the deprotection of the tert-butyloxycarbonyl group (**III - IV**).

The appropriate enantiomer of 3-(BOC-amino)pyrrolidine (**I** or **II**) (1.0 equiv, 4.26 mmol) was mixed with 1.0 M solution of hydrochloric acid in ethyl acetate (80 ml), and stirred for 18 h (until the disappearance of starting materials - TLC monitoring). Then ethyl acetate was removed under reduced pressure to obtain dark oil, which was washed with ethyl acetate and dried under the vacuum. The intermediates **III** and **IV** were used in the next step without further purification.

(R)-1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-amine hydrochloride (**III**). The title compound was prepared using intermediate **I** (1.0 equiv, 4.26 mmol, 1.55 g) and 80 ml of 1.0 M HCl in EtOAc. Yield 96%, brown oil, ^{1}H NMR (300 MHz, CD_3OD, δ): 7.85 (dd, *J* = 5.3, 8.8 Hz, 1H), 7.36 (dd, *J* = 1.9, 8.8 Hz, 1H), 7.15 (dt, *J* = 2.2, 9.0 Hz, 1H), 4.24–4.04 (m, 1H), 3.99–3.77 (m, 1H), 3.68–3.38 (m, 3H), 3.27 (td, *J* = 1.7, 3.3 Hz, 2H), 3.15 (t, *J* = 7.2 Hz, 2H), 2.76–2.44 (m, 1H), 2.39–2.15 (m, 3H), N*H* protons not detected; Formula $C_{14}H_{19}ClFN_3O$; MS (ESI^+): m/z 264 $[M+H]^+$

(S)-1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-amine hydrochloride (**IV**). The title compound was prepared using intermediate **II** (1.0 equiv, 4.26 mmol, 1.55 g) and 80 ml of 1.0 M HCl in EtOAc. Yield 91%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.87 (dd, *J* = 5.1, 8.7 Hz, 1H), 7.26 (dd, *J* = 2.1, 8.7 Hz, 1H), 7.20 (dt, *J* = 2.2, 9.0 Hz, 1H), 4.30–4.08 (m, 2H), 4.03–3.80 (m, 2H), 3.73–3.70 (m, 2H), 3.48–3.46 (m, 3H), 2.95 (t, *J* = 7.3 Hz, 2H), 2.88 (dd, *J* = 8.6, 16.5 Hz, 2H), 2.17 (td, *J* = 7.6, 15.5 Hz, 1H), 1.70-1.41 (br s,1H); 1.51 (br s, 1H); Formula $C_{14}H_{19}ClFN_3O$; MS (ESI^+): m/z 264 $[M+H]^+$

General procedure for the preparation of sulfonamide derivatives 1 – 36.

Cesium carbonate (2.0 equiv), catalytic amount of DMAP and suitable arylsulfonyl chloride {*1–18*} (1.2 equiv) were added to a suspension of the amine **III** or **IV** (1.0 equiv) in dry dichloromethane at room temperature. The reaction mixture was stirred for 12 hours, then the cesium carbonate was filtrated and the solvent was evaporated under the reduced pressure. Crude sulfonamides were purified by column chromatography over silica gel using *n*-hexane/diethyl ether/dichloromethane/methanol (20/20/55/5, v/v) as eluent. The final compounds were obtained with good overall yield (46 – 80%).

(R)-3-chloro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzenesulfonamide (**1**). The title compound was prepared using intermediate **III** (1.0 equiv, 0.44 mmol, 0.132 g), 3-chlorobenzene-1-sulfonyl chloride (1.2 equiv, 0.53 mmol, 0.112 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.88 mmol, 0.288 g) in DCM (5 mL). Yield 58%, yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.86 (t, *J* = 2.1 Hz, 1H), 7.75 (td, *J* = 1.3, 7.9 Hz, 1H), 7.58 (dd, *J* = 5.0, 8.5 Hz, 1H), 7.55–7.50 (m, 1H), 7.48–7.40 (m, 1H), 7.23 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.07 (dt, *J* = 2.3, 8.8 Hz, 1H), 5.08 (br s, 1H), 3.89–3.79 (m, 1H), 2.98 (t, *J* = 7.3 Hz, 2H), 2.76 (dt, *J* = 3.5, 8.5 Hz, 1H), 2.52–2.43 (m, 3H), 2.43–2.34 (m, 1H), 2.22–2.12 (m, 1H), 2.12–2.02 (m, 1H), 2.01–1.89 (m, 2H), 1.60–1.47 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 163.5 (d, *J* = 13.8 Hz), 164.2 (d, *J* = 250.2 Hz), 158.1, 142.7, 135.3, 132.7, 130.4, 127.1, 125.1, 122.1 (d, *J* = 10.4 Hz), 118.2 (d, *J* = 1.7 Hz), 112.5 (d, *J* = 26.0 Hz), 97.4 (d, *J* = 27.0 Hz), 60.5, 54.5, 52.9, 51.8, 32.5, 26.2, 22.9; Formula $C_{20}H_{21}ClFN_3O_3S$; MS (ESI^+): m/z 438 $[M+H]^+$

(S)-3-chloro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzenesulfonamide (**2**)**.** The title compound was prepared using intermediate **IV** (1.0 equiv, 0.44 mmol, 0.132 g), 3-chlorobenzene-1-sulfonyl chloride (1.2 equiv, 0.53 mmol, 0.112 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.88 mmol, 0.288 g) in

DCM (5 mL). Yield 48%, yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.86 (t, *J* = 2.1 Hz, 1H), 7.74 (td, *J* = 1.3, 7.9 Hz, 1H), 7.61–7.51 (m, 3H), 7.47–7.42 (m, 1H), 7.25 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.07 (dt, *J* = 2.3, 8.8 Hz, 1H), 3.85–3.73 (m, 1H), 2.99 (t, *J* = 7.3 Hz, 2H), 2.76 (dt, *J* = 3.5, 8.5 Hz, 1H), 2.52–2.46 (m, 3H), 2.41–2.39 (m, 1H), 2.14–1.92 (m, 4H), 1.55–1.49 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 13.8 Hz), 163.5 (d, *J* = 250.2 Hz), 158.1, 142.6, 135.3, 132.7, 130.4, 127.1, 125.1, 122.1 (d, *J* = 10.4 Hz), 118.2 (d, *J* = 1.7 Hz), 112.5 (d, *J* = 26.0 Hz), 97.4 (d, *J* = 27.0 Hz), 60.5, 54.5, 52.9, 51.8, 32.4, 26.1, 22.8; Formula $C_{20}H_{21}ClFN_3O_3S$; MS (ESI^+): m/z 438 $[M+H]^+$

(R)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-3-methylbenzenesulfonamide (**3**). The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), 3-methylbenzene-1-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.076 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 58%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.70–7.63 (m, 2H), 7.59 (dd, *J* = 5.0, 8.5 Hz, 1H), 7.39–7.33 (m, 2H), 7.23 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.07 (dt, *J* = 2.3, 8.8 Hz, 1H), 4.89 (br s, 1H), 3.83 (br s, 1H), 2.97 (t, *J* = 7.3 Hz, 2H), 2.74 (dt, *J* = 4.1, 8.8 Hz, 1H), 2.51–2.43 (m, 3H), 2.42–2.35 (m, 4H), 2.24–2.14 (m, 1H), 2.13–1.99 (m, 1H), 1.99–1.89 (m, 2H), 1.59–1.46 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz), 163.6 (d, *J* = 13.8 Hz), 158.1, 140.6, 139.3, 133.4, 129.0, 127.4, 124.1, 122.1 (d, *J* = 10.4 Hz), 118.2, 112.5 (d, *J* = 26.0 Hz), 97.4 (d, *J* = 27.0 Hz), 60.5, 54.6, 52.7, 52.0, 32.5, 26.2, 22.9, 21.3; Formula $C_{21}H_{24}FN_3O_3S$; MS (ESI^+): m/z 418 $[M+H]^+$

(S)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-3-methylbenzenesulfonamide (**4**). The title compound was prepared using intermediate **IV** (1.0 equiv, 0.33 mmol, 0.100 g), 3-methylbenzene-1-sulfonyl chloride (1.2 equiv, 0.40 mmol,

0.076 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 67%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.66–7.60 (m, 2H), 7.57 (dd, *J* = 5.0, 8.5 Hz, 1H), 7.38–7.31 (m, 3H), 7.22 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.07 (dt, *J* = 2.3, 8.8 Hz, 1H), 3.82 (br s, 1H), 2.95 (t, *J* = 7.3 Hz, 2H), 2.70 (dt, *J* = 4.1, 8.8 Hz, 1H), 2.50–2.38 (m, 7H), 2.22–2.19 (m, 1H), 2.06–1.88 (m, 4H), 1.59–1.41 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 13.8 Hz), 163.6 (d, *J* = 250.2 Hz), 158.1, 140.6, 139.3, 133.4, 129.0, 127.4, 124.1, 122.2 (d, *J* = 10.4 Hz), 118.2, 112.5 (d, *J* = 26.0 Hz), 97.1 (d, *J* = 27.0 Hz), 60.4, 54.6, 52.6, 52.1, 32.3, 26.2, 22.8, 21.3; Formula $C_{21}H_{24}FN_3O_3S$; MS (ESI^+): m/z 418 $[M+H]^+$

(R)-4-fluoro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzenesulfonamide (**5**). The title compound was prepared using intermediate **III** (1.0 equiv, 0.42 mmol, 0.126 g), 4-fluorobenzene-1-sulfonyl chloride (1.2 equiv, 0.51 mmol, 0.098 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.84 mmol, 0.275 g) in DCM (5 mL). Yield 54%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.93–7.84 (m, 2H), 7.58 (dd, *J* = 5.0, 8.5 Hz, 1H), 7.23 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.20–7.12 (m, 2H), 7.06 (dt, *J* = 1.8, 8.8 Hz, 1H), 5.02 (br s, 1H), 3.81 (br s, 1H), 2.98 (t, *J* = 7.6 Hz, 2H), 2.74 (dt, *J* = 4.4, 8.6 Hz, 1H), 2.51–2.43 (m, 3H), 2.42–2.33 (m, 1H), 2.23–2.13 (m, 1H), 2.07 (tdd, *J* = 4.2, 8.6, 12.9 Hz, 1H), 2.01–1.89 (m, 2H), 1.59–1.46 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.0 (d, *J* = 254.0 Hz), 164.2 (d, *J* = 250.2 Hz), 163.6 (d, *J* = 15.0 Hz), 158.1, 136.9 (d, *J* = 4.3 Hz), 129.7 (d, *J* = 9.2 Hz, 2C), 122.1 (d, *J* = 11.5 Hz), 118.2, 116.3 (d, *J* = 22.0 Hz, 2C), 112.5 (d, *J* = 26.0 Hz), 97.4 (d, *J* = 27.0 Hz), 60.5, 54.5, 52.7, 51.9, 32.5, 26.2, 22.9; Formula $C_{20}H_{21}F_2N_3O_3S$; MS (ESI^+): m/z 422 $[M+H]^+$

(S)-4-fluoro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzenesulfonamide (**6**). The title compound was prepared using intermediate **IV** (1.0 equiv, 0.42 mmol, 0.126 g), 4-fluorobenzene-1-sulfonyl chloride (1.2 equiv, 0.51 mmol, 0.098 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.84 mmol, 0.275 g) in DCM (5

mL). Yield 57%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.91–7.85 (m, 2H), 7.58 (dd, *J* = 5.0, 8.5 Hz, 1H), 7.25–7.14 (m, 3H), 7.07 (dt, *J* = 1.8, 8.8 Hz, 1H), 5.03 (br s, 1H), 3.83 (br s, 1H), 2.98 (t, *J* = 7.6 Hz, 2H), 2.75 (dt, *J* = 4.4, 8.6 Hz, 1H), 2.51–2.46 (m, 3H), 2.40–2.35 (m, 1H), 2.20–1.92 (m, 5H), 1.59–1.42 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 166.7, 165.9 (d, *J* = 254.0 Hz), 163.6 (d, *J* = 250.2 Hz),163.2 (d, *J* = 15.0 Hz), 162.6, 137.1, 136.9 (d, *J* = 4.3 Hz), 129.7 (d, *J* = 9.2 Hz), 122.2 (d, *J* = 11.5 Hz), 118.2, 116.3 (d, *J* = 22.0 Hz), 112.5 (d, *J* = 26.0 Hz), 97.4 (d, *J* = 27.0 Hz), 60.5, 54.5, 52.7, 51.9, 32.4, 26.1, 22.9; Formula $C_{20}H_{21}F_2N_3O_3S$; MS (ESI^+): m/z 422 $[M+H]^+$

(R)-3-chloro-4-fluoro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzenesulfonamide (**7**)**.** The title compound was prepared using intermediate **III** (1.0 equiv, 0.51 mmol, 0.152 g), 3-chloro-4-fluorobenzene-1-sulfonyl chloride (1.2 equiv, 0.61 mmol, 0.140 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 1.02 mmol, 0.331 g) in DCM (5 mL). Yield 46%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.95 (dd, *J* = 2.3, 6.4 Hz, 1H), 7.81–7.73 (m, 1H), 7.58 (dd, *J* = 4.7, 8.8 Hz, 1H), 7.30–7.20 (m, 2H), 7.11–7.02 (m, 1H), 5.11 (br s, 1H), 3.83 (tdd, *J* = 3.0, 5.7, 8.2 Hz, 1H), 2.98 (t, *J* = 7.6 Hz, 2H), 2.84–2.74 (m, 1H), 2.54–2.45 (m, 3H), 2.43–2.34 (m, 1H), 2.22–2.14 (m, 1H), 2.13–2.03 (m, 1H), 2.02–1.90 (m, 2H), 1.62–1.48 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.3 (d, *J* = 250.2 Hz), 163.6 (d, *J* = 13.8 Hz), 160.5 (d, *J* = 257.0 Hz), 158.1, 138.1 (d, *J* = 4.1 Hz), 130.0 (2C), 127.4 (d, *J* = 8.1 Hz), 122.1 (d, *J* = 10.4 Hz), 118.2, 117.4 (d, *J* = 22.0 Hz), 112.5 (d, *J* = 26.0 Hz), 97.4 (d, *J* = 27.0 Hz), 60.5, 54.4, 52.9, 51.8, 32.5, 26.1, 22.9; Formula $C_{20}H_{20}ClF_2N_3O_3S$; MS (ESI^+): m/z 456 $[M+H]^+$

(S)-3-chloro-4-fluoro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzenesulfonamide (**8**). The title compound was prepared using intermediate **IV** (1.0 equiv, 0.51 mmol, 0.152 g), 3-chloro-4-fluorobenzene-1-sulfonyl chloride (1.2 equiv, 0.61 mmol, 0.140 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 1.02 mmol,

0.331 g) in DCM (5 mL). Yield 41%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.95 (dd, *J* = 2.3, 6.4 Hz, 1H), 7.81–7.73 (m, 1H), 7.58 (dd, *J* = 4.7, 8.8 Hz, 1H), 7.27-7.24 (m, 2H), 7.09–7.08 (m, 1H), 3.73 (tdd, *J* = 3.0, 5.7, 8.2 Hz, 1H), 2.99 (t, *J* = 7.6 Hz, 2H), 2.84–2.35 (m, 5H), 2.23–1.86 (m, 5H), 1.69–1.43 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 250.2 Hz), 163.6 (d, *J* = 13.8 Hz), 162.6 (d, *J* = 257.0 Hz), 158.8, 157.8, 137.8 (d, *J* = 4.1 Hz), 130.0, 127.4 (d, *J* = 8.1 Hz), 122.2 (d, *J* = 10.4 Hz), 117.6, 117.3 (d, *J* = 22.0 Hz), 112.7 (d, *J* = 26.0 Hz), 97.5 (d, *J* = 27.0 Hz), 60.2, 54.5, 52.6, 51.2, 32.1, 25.5, 22.8; Formula $C_{20}H_{20}ClF_2N_3O_3S$; MS (ESI^+): m/z 456 $[M+H]^+$

(R)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)naphthalene-1-sulfonamide (**9**). The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), naphtalene-1-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.090 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 80%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.62 (d, *J* = 8.8 Hz, 1H), 8.27 (dd, *J* = 1.2, 7.0 Hz, 1H), 8.05 (d, *J* = 8.2 Hz, 1H), 7.89 (d, *J* = 7.6 Hz, 1H), 7.69–7.60 (m, 1H), 7.58–7.47 (m, 3H), 7.23 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.05 (dt, *J* = 2.1, 8.9 Hz, 1H), 5.20 (br s, 1H), 3.79 (br s, 1H), 2.87 (dt, *J* = 1.2, 7.6 Hz, 2H), 2.66 (dt, *J* = 4.4, 8.6 Hz, 1H), 2.47–2.25 (m, 3H), 2.24–2.15 (m, 1H), 2.08 (dt, *J* = 7.0, 8.8 Hz, 1H), 1.93 (dtd, *J* = 4.4, 8.8, 13.2 Hz, 1H), 1.79 (quin, *J* = 7.3 Hz, 2H), 1.52–1.37 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 158.1, 135.3, 134.3, 134.2, 129.6, 129.1, 128.4, 128.1, 126.8, 124.3, 124.2, 122.2 (d, *J* = 11.5 Hz), 118.2, 112.5 (d, *J* = 26.0 Hz), 97.3 (d, *J* = 27.0 Hz), 60.1, 54.4, 52.9, 51.9, 32.3, 26.2, 22.8; Formula $C_{24}H_{24}FN_3O_3S$; MS (ESI^+): m/z 454 $[M+H]^+$

(S)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)naphthalene-1-sulfonamide (**10**). The title compound was prepared using intermediate **IV** (1.0 equiv, 0.33

mmol, 0.100 g), naphtalene-1-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.090 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 83%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.62 (d, *J* = 8.8 Hz, 1H), 8.26 (dd, *J* = 1.2, 7.0 Hz, 1H), 8.05 (d, *J* = 8.2 Hz, 1H), 7.88 (d, *J* = 7.6 Hz, 1H), 7.66–7.50 (m, 3H), 7.24 (dd, *J* = 2.1, 8.5 Hz, 2H), 7.06 (dt, *J* = 2.1, 8.9 Hz, 1H), 3.72 (br s, 1H), 2.88 (dt, *J* = 1.2, 7.6 Hz, 2H), 2.67 (dt, *J* = 4.4, 8.6 Hz, 1H), 2.46–2.31 (m, 3H), 2.22–2.01 (m, 3H), 1.92 (dtd, *J* = 4.4, 8.8, 13.2 Hz, 1H), 1.79 (quin, *J* = 7.3 Hz, 2H), 1.52–1.33 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 250.2 Hz), 163.4 (d, *J* = 13.8 Hz), 162.6, 158.1, 135.3, 134.2, 129.6, 129.1, 126.8, 128.1, 124.3, 122.1 (d, *J* = 11.5 Hz), 118.1, 112.6 (d, *J* = 26.0 Hz), 112.3, 97.4 (d, *J* = 27.0 Hz), 97.1, 60.1, 54.4, 52.8, 51.9, 32.1, 26.1, 22.7; Formula $C_{24}H_{24}FN_3O_3S$; MS (ESI^+): m/z 454 $[M+H]^+$

(R)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)naphthalene-2-sulfonamide (**11**)**.** The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), naphthalene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.090 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 68%, yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.43 (d, *J* = 1.8 Hz, 1H), 8.01–7.76 (m, 4H), 7.67–7.58 (m, 2H), 7.55 (dd, *J* = 5.3, 8.8 Hz, 1H), 7.21 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.03 (dt, *J* = 2.3, 8.8 Hz, 1H), 5.18 (br s, 1H), 3.87 (br s, 1H), 2.94 (t, *J* = 7.3 Hz, 2H), 2.72 (dt, *J* = 4.4, 8.6 Hz, 1H), 2.52–2.30 (m, 4H), 2.14–2.08 (m, 1H), 2.08–1.97 (m, 1H), 1.91 (quin, *J* = 7.2 Hz, 2H), 1.60–1.47 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 158.1, 137.6, 134.7, 132.2, 129.5, 129.2, 128.8, 128.3, 127.9, 127.6, 122.3, 122.1 (d, *J* = 11.5 Hz), 118.2, 112.5 (d, *J* = 26.0 Hz), 97.3 (d, *J* = 27.0 Hz), 60.5, 54.5, 52.8, 52.0, 32.5, 26.2, 22.8; Formula $C_{24}H_{24}FN_3O_3S$; MS (ESI^+): m/z 454 $[M+H]^+$

(S)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)naphthalene-2-sulfonamide (**12**). The title compound was prepared using intermediate **IV** (1.0 equiv, 0.33 mmol, 0.100 g), naphthalene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.090 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 68%, yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.43 (d, *J* = 1.8 Hz, 1H), 7.96–7.81 (m, 4H), 7.67–7.53 (m, 2H), 7.23 (dd, *J* = 2.1, 8.5 Hz, 2H), 7.04 (dt, *J* = 2.3, 8.8 Hz, 1H), 3.88 (br dd, 1H), 2.95 (t, *J* = 7.3 Hz, 2H), 2.74 (dt, *J* = 4.4, 8.6 Hz, 1H), 2.52–2.34 (m, 4H), 2.20–1.87 (m, 5H), 1.56–1.52 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 250.2 Hz), 163.6 (d, *J* = 13.8 Hz), 162.5, 158.1, 137.6, 134.7, 132.1, 129.5, 128.8, 128.3, 127.6, 127.4, 122.3, 122.1 (d, *J* = 11.5 Hz), 118.2, 112.6 (d, *J* = 26.0 Hz), 97.3 (d, *J* = 27.0 Hz), 60.4 54.5, 52.7, 52.0, 32.4, 26.2, 22.8; Formula $C_{24}H_{24}FN_3O_3S$; MS (ESI^+): m/z 454 $[M+H]^+$

(R)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-6-methoxynaphthalene-2-sulfonamide (**13**). The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), 6-methoxynaphthalene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.103 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 75%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.34 (s, 1H), 7.88–7.75 (m, 3H), 7.55 (dd, *J* = 4.7, 8.8 Hz, 1H), 7.26–7.18 (m, 2H), 7.16 (d, *J* = 2.3 Hz, 1H), 7.04 (dt, *J* = 2.1, 8.9 Hz, 1H), 5.11 (br s, 1H), 3.95 (s, 3H), 3.86 (br s, 1H), 2.94 (t, *J* = 7.6 Hz, 2H), 2.72 (dt, *J* = 4.1, 8.8 Hz, 1H), 2.52–2.40 (m, 3H), 2.40–2.32 (m, 1H), 2.22–2.11 (m, 1H), 2.10–1.98 (m, 1H), 1.92 (quin, *J* = 7.3 Hz, 2H), 1.60–1.47 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 159.9, 158.1, 136.5, 135.1, 130.7, 128.2, 128.1, 127.5, 123.0, 122.1 (d, *J* = 11.5 Hz), 120.5, 118.2, 112.5 (d, *J* = 26.0 Hz), 105.8, 97.3 (d, *J* = 27.0 Hz), 60.5, 55.5, 54.6, 52.7, 52.0, 32.5, 26.2, 22.8; Formula $C_{25}H_{26}FN_3O_4S$; MS (ESI^+): m/z 484 $[M+H]^+$

(S)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-6-methoxynaphthalene-2-sulfonamide (**14**)**.** The title compound was prepared using intermediate **IV** (1.0 equiv, 0.33 mmol, 0.100 g), 6-methoxynaphthalene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.103 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 78%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.34 (s, 1H), 7.83–7.79 (m, 3H), 7.54 (dd, *J* = 4.7, 8.8 Hz, 1H), 7.26–7.19 (m, 2H), 7.15 (d, *J* = 2.3 Hz, 1H), 7.03 (dt, *J* = 2.1, 8.9 Hz, 1H), 3.94 (s, 3H), 3.87 (d, *J* = 4.7 Hz, 1H), 2.93 (t, *J* = 7.6 Hz, 2H), 2.50–2.48 (m, 1H), 2.46–2.42 (m, 5H), 2.20–1.92 (m, 4H), 1.37–1.25 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 250.2 Hz), 163.4 (d, *J* = 13.8 Hz), 162.6, 159.9, 158.1, 136.5, 135.1, 130.7, 128.1, 127.5, 123.0, 122.5, 120.5 (d, *J* = 11.5 Hz), 118.2, 112.5 (d, *J* = 26.0 Hz), 105.8, 97.3 (d, *J* = 27.0 Hz), 60.5, 55.5, 54.6, 52.7, 52.0, 32.4, 26.1, 22.8; Formula $C_{25}H_{26}FN_3O_4S$; MS (ESI^+): m/z 484 $[M+H]^+$

(R)-6-chloro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)naphthalene-2-sulfonamide **(15).** The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), 6-chloronaphthalene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.104 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 53%, yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.41 (s, 1H), 7.95–7.80 (m, 4H), 7.59–7.50 (m, 2H), 7.22 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.05 (dt, *J* = 2.3, 8.8 Hz, 1H), 5.38 (br s, 1H), 3.88 (br s, 1H), 2.95 (t, *J* = 7.6 Hz, 2H), 2.76 (dt, *J* = 4.1, 8.8 Hz, 1H), 2.54–2.40 (m, 3H), 2.40–2.32 (m, 1H), 2.22–2.09 (m, 1H), 2.08–1.99 (m, 1H), 1.93 (quin, *J* = 7.3 Hz, 2H), 1.62–1.46 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 158.0, 138.1, 135.3, 134.8, 130.7, 130.4, 128.6, 128.6, 128.1, 126.7, 123.5, 122.1 (d, *J* = 11.5 Hz), 118.1, 112.5 (d, *J* = 26.0 Hz), 97.4 (d, *J* = 26.5 Hz), 60.5, 54.5, 52.8, 51.9, 32.4, 26.1, 22.8; Formula $C_{24}H_{23}ClFN_3O_3S$; MS (ESI^+): m/z 489 $[M+H]^+$

(S)-6-chloro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)naphthalene-2-sulfonamide (**16**)**.** The title compound was prepared using intermediate **IV** (1.0 equiv, 0.33 mmol, 0.100 g), 6-chloronaphthalene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.104 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 48%, yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.41 (s, 1H), 7.93–7.84 (m, 4H), 7.56–7.53 (m, 2H), 7.23 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.06 (dt, *J* = 2.3, 8.8 Hz, 1H), 3.95 (br s, 1H), 2.96 (t, *J* = 7.6 Hz, 2H), 2.75 (dt, *J* = 4.1, 8.8 Hz, 1H), 2.59–2.39 (m, 4H), 2.77–1.94 (m, 5H), 1.42–1.18 (m, 1H);

^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 162.6, 158.0, 138.1, 135.3, 134.8, 130.7, 130.4, 128.6, 128.1, 126.7, 123.5, 122.1 (d, *J* = 11.5 Hz), 118.1, 112.5 (d, *J* = 26.0 Hz), 97.4 (d, *J* = 26.5 Hz), 60.5, 54.5, 52.8, 51.9, 32.4, 26.1, 22.8; Formula $C_{24}H_{23}ClFN_3O_3S$; MS (ESI^+): m/z 489 $[M+H]^+$

(R)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-1-methyl-1H-indole-4-sulfonamide (**17**). The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), 1-methyl-1*H*-indole-4-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.092 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 61%, brown oil, ^{1}H (300 MHz, $CDCl_3$, δ): 7.72–7.66 (m, 1H), 7.59–7.49 (m, 2H), 7.29–7.25 (m, 1H), 7.24–7.20 (m, 1H), 7.15 (d, *J* = 3.5 Hz, 1H), 7.09–7.01 (m, 1H), 6.88–6.84 (m, 1H), 5.03 (br d, *J* = 8.8 Hz, 1H), 3.81 (s, 4H), 2.93–2.83 (m, 2H), 2.64 (dt, *J* = 4.1, 8.8 Hz, 1H), 2.47–2.18 (m, 4H), 2.09 (dt, *J* = 6.7, 8.9 Hz, 1H), 1.99–1.87 (m, 1H), 1.86–1.77 (m, 2H), 1.49–1.35 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 158.2, 137.5, 131.3, 130.7, 124.5, 122.2 (d, *J* = 11.5 Hz), 120.6, 120.6, 118.2, 114.1, 112.5 (d, *J* = 26.0 Hz), 100.6, 97.3 (d, *J* = 26.5 Hz), 60.2, 54.6, 52.7, 52.1, 33.2, 32.3, 26.2, 22.8; Formula $C_{23}H_{25}FN_4O_3S$; MS (ESI^+): m/z 457 $[M+H]^+$

(S)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-1-methyl-1H-indole-4-sulfonamide (**18**). The title compound was prepared using intermediate **IV** (1.0 equiv, 0.33 mmol, 0.100 g), 1-methyl-1*H*-indole-4-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.092 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 68%, brown oil, ^{1}H (300 MHz, $CDCl_3$, δ): 7.72–7.66 (m, 1H), 7.59–7.49 (m, 3H), 7.30–7.19 (m, 2H), 7.15 (d, *J* = 3.5 Hz, 1H), 7.09–7.01 (m, 1H), 6.88–6.84 (m, 1H), 3.81 (s, 4H), 2.93–2.83 (m, 2H), 2.64 (dt, *J* = 4.1, 8.8 Hz, 1H), 2.47–2.18 (m, 4H), 2.09 (dt, *J* = 6.7, 8.9 Hz, 1H), 2.00–1.77 (m, 3H), 1.49–1.35 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 158.2, 137.5, 131.3, 130.7, 124.5, 122.2 (d, *J* = 11.5 Hz), 120.6, 120.6, 118.2, 114.1, 112.5 (d, *J* = 26.0 Hz), 100.6, 97.3 (d, *J* = 26.5 Hz), 60.2, 54.6, 52.7, 52.1, 33.2, 32.3, 26.2, 22.8; Formula $C_{23}H_{25}FN_4O_3S$; MS (ESI^+): m/z 457 $[M+H]^+$

(R)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-1-methyl-1H-indole-5-sulfonamide (**19**)**.** The title compound was prepared using intermediate **III** (1.0 equiv, 0.84 mmol, 0.250 g), 1-methyl-1*H*-indole-5-sulfonyl chloride (1.2 equiv, 1.00 mmol, 0.230 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 1.67 mmol, 0.545 g) in DCM (15 mL). Yield 72%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.19 (d, *J* = 1.8 Hz, 1H), 7.67 (dd, *J* = 1.8, 8.8 Hz, 1H), 7.57 (dd, *J* = 5.3, 8.8 Hz, 1H), 7.37 (d, *J* = 8.8 Hz, 1H), 7.22 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.16 (d, *J* = 2.9 Hz, 1H), 7.09–7.00 (m, 1H), 6.60–6.56 (m, 1H), 4.88 (br s, 1H), 3.83 (s, 4H), 2.94 (t, *J* = 7.3 Hz, 2H), 2.69 (dt, *J* = 4.1, 8.8 Hz, 1H), 2.52–2.30 (m, 4H), 2.22–2.11 (m, 1H), 2.09–1.96 (m, 1H), 1.95–1.85 (m, 2H), 1.58–1.43 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz),163.5 (d, *J* = 13.8 Hz), 158.1, 138.3, 131.1 (2C), 127.8, 122.2 (d, *J* = 10.4 Hz), 121.5, 119.9, 118.2, 112.5 (d, *J* = 26.0 Hz), 109.8, 102.7, 97.3 (d, *J* = 27.0 Hz), 60.5, 54.6, 52.6, 52.1, 33.1, 32.4, 26.3, 22.8; Formula $C_{23}H_{25}FN_4O_3S$; MS (ESI^+): m/z 457 $[M+H]^+$

(S)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-1-methyl-1H-indole-5-sulfonamide (**20**)**.** The title compound was prepared using intermediate **IV** (1.0 equiv, 0.84 mmol, 0.250 g), 1-methyl-1*H*-indole-5-sulfonyl chloride (1.2 equiv, 1.00 mmol, 0.230 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 1.67 mmol, 0.545 g) in DCM (15 mL). Yield 70%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.17 (d, *J* = 1.8 Hz, 1H), 7.68 (dd, *J* = 1.8, 8.8 Hz, 1H), 7.56 (dd, *J* = 5.3, 8.8 Hz, 1H), 7.36 (d, *J* = 8.8 Hz, 1H), 7.33 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.15 (d, *J* = 2.9 Hz, 1H), 7.02–6.99 (m, 2H), 6.56–6.55 (m, 1H), 5.21 (br s, 1H), 3.80 (s, 3H), 2.89 (t, *J* = 7.3 Hz, 2H), 2.66 (dt, *J* = 4.1, 8.8 Hz, 1H), 2.43–2.40 (m, 4H), 2.19–2.16 (m, 1H), 1.87–1.83 (m, 3H), 1.26–1.23 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.8 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 162.5, 158.1, 138.2, 131.1, 127.8, 122.4 (d, *J* = 10.4 Hz), 121.4, 119.9, 112.6, 112.3 (d, *J* = 26.0 Hz), 109.8, 102.6, 97.3 (d, *J* = 27.0 Hz), 60.4, 54.6, 52.6, 52.1, 33.1, 32.3, 26.2, 22.8; Formula $C_{23}H_{25}FN_4O_3S$; MS (ESI^+): m/z 457 $[M+H]^+$

(R)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzofuran-2-sulfonamide (**21**)**.** The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), benzofuran-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.087 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 61%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.67 (d, *J* = 7.6 Hz, 1H), 7.57 (dd, *J* = 5.0, 8.5 Hz, 1H), 7.54–7.48 (m, 1H), 7.48–7.41 (m, 1H), 7.39–7.29 (m, 2H), 7.23 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.06 (dt, *J* = 1.8, 8.8 Hz, 1H), 5.36 (br s, 1H), 4.07–3.93 (m, 1H), 2.97 (t, *J* = 7.3 Hz, 2H), 2.86–2.75 (m, 1H), 2.62–2.35 (m, 4H), 2.23–2.04 (m, 2H), 1.95 (quin, *J* = 7.2 Hz, 2H), 1.69–1.52 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 158.1, 155.6, 150.6, 127.6, 126.0, 124.2, 122.9, 122.1 (d, *J* = 11.5 Hz), 118.2, 112.5 (d, *J* = 25.4 Hz), 112.2, 112.1, 97.4 (d, *J* = 26.5 Hz), 60.5, 54.5, 53.2, 51.9, 32.5, 26.2, 22.9; Formula $C_{22}H_{22}FN_3O_4S$; MS (ESI^+): m/z 444 $[M+H]^+$

(S)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzofuran-2-sulfonamide (**22**)**.** The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), benzofuran-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.087 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 66%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.67-7.05 (m, 7H), 7.05-6.98 (m, 2H), 4.04–3.99 (m, 1H), 2.96 (t, *J* = 7.3 Hz, 2H), 2.84–2.77 (m, 1H), 2.61–2.39 (m, 4H), 2.21–2.12 (m, 2H), 1.95 (quin, *J* = 7.2 Hz, 2H), 1.64–1.60 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 250.2 Hz), 163.6 (d, *J* = 13.8 Hz), 162.6, 158.1, 155.6, 150.6, 127.6, 126.0, 124.2, 122.9, 122.2 (d, *J* = 11.5 Hz), 118.2, 112.5 (d, *J* = 25.4 Hz), 112.1, 97.5 (d, *J* = 26.5 Hz), 60.5, 54.6, 53.1, 52.0, 32.5, 26.1, 22.9; Formula $C_{22}H_{22}FN_3O_4S$; MS (ESI^+): m/z 444 $[M+H]^+$

(R)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzo[b]thiophene-3-sulfonamide (**23**). The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), benzo[b]thiophene-3-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.093 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 78%, yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.23 (s, 1H), 8.16 (d, *J* = 8.2 Hz, 1H), 7.86 (d, *J* = 8.8 Hz, 1H), 7.55 (dd, *J* = 5.3, 8.8 Hz, 1H), 7.50–7.36 (m, 2H), 7.23 (dd, *J* = 2.3, 8.8 Hz, 1H), 7.05 (dt, *J* = 2.1, 8.9 Hz, 1H), 5.18 (br s, 1H), 3.86 (br s, 1H), 2.95–2.86 (m, 2H), 2.76–2.65 (m, 1H), 2.52–2.22 (m, 4H), 2.18–1.93 (m, 2H), 1.84 (quin, *J* = 7.2 Hz, 2H), 1.55–1.40 (m, 1H); ^{19}F NMR (282 MHz, $CDCl_3$, δ): -109.6 (s, 1F); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 158.0, 140.4, 134.3, 134.2, 133.5, 125.8, 125.7, 123.0, 122.1 (d, *J* = 11.5 Hz), 118.2, 112.5 (d, *J* = 26.0 Hz), 110.0, 97.3 (d, *J* = 26.5 Hz), 60.1, 54.4, 52.9, 51.9, 32.4, 26.1, 22.7; Formula $C_{22}H_{22}FN_3O_3S_2$; MS (ESI^+): m/z 460 $[M+H]^+$

(S)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzo[b]thiophene-3-sulfonamide (**24**)**.** The title compound was prepared using intermediate **IV** (1.0 equiv, 0.33 mmol, 0.100 g), benzo[b]thiophene-3-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.093 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 79%, yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.23 (s, 1H), 8.15 (d, *J* = 8.2 Hz, 1H), 7.87 (d, *J* = 8.8 Hz, 1H), 7.55 (dd, *J* = 5.3, 8.8 Hz, 1H), 7.47–7.35 (m, 2H), 7.25-7.22 (m, 1H), 7.06 (dt, *J* = 2.1, 8.9 Hz, 1H), 5.20 (br s, 1H), 3.64 (br s, 1H), 2.97–2.90 (m, 3H), 2.89–2.51 (m, 1H), 2.47–2.43 (m, 4H), 2.14–2.14 (m, 2H), 1.89 (quin, *J* = 7.2 Hz, 2H); ^{19}F NMR (282 MHz, $CDCl_3$, δ): -109.6 (s, 1F); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 157.8, 140.2, 134.3, 133.8, 125.7, 122.9, 122.1 (d, *J* = 11.5 Hz), 118.2, 112.5 (d, *J* = 26.0 Hz), 110.0, 97.5 (d, *J* = 26.5 Hz), 97.1, 60.2, 54.5, 52.8, 51.9, 32.3, 29.7, 25.9, 22.8; Formula $C_{22}H_{22}FN_3O_3S_2$; MS (ESI^+): m/z 460 $[M+H]^+$

(R)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzo[b]thiophene-2-sulfonamide (**25**)**.** The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), benzo[b]thiophene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.093 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 69%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.87–7.71 (m, 3H), 7.52 (dd, *J* = 5.3, 8.8 Hz, 1H), 7.47–7.35 (m, 2H), 7.18 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.04–6.96 (m, 1H), 5.45 (br s, 1H), 3.99–3.88 (m, 1H), 2.92 (t, *J* = 7.6 Hz, 2H), 2.82–2.70 (m, 1H), 2.57 (dd, *J* = 2.6, 9.7 Hz, 1H), 2.50–2.34 (m, 3H), 2.20–2.03 (m, 2H), 1.97–1.86 (m, 2H), 1.68–1.54 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 158.0, 141.9, 141.7, 137.6, 129.2, 127.2, 125.6, 125.5, 122.7, 122.1 (d, *J* = 10.4 Hz), 118.2, 112.5 (d, *J* = 26.0 Hz), 97.3 (d, *J* = 26.5 Hz), 60.4, 54.6, 53.1, 52.0, 32.3, 26.1, 22.9; Formula $C_{22}H_{22}FN_3O_3S_2$; MS (ESI^+): m/z 460 $[M+H]^+$

(S)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzo[b]thiophene-2-sulfonamide (**26**). The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), benzo[b]thiophene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.093 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 67%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.89–7.80 (m, 3H), 7.56 (dd, *J* = 5.3, 8.8 Hz, 1H), 7.48–7.42 (m, 2H), 7.24 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.13–6.99 (m, 1H), 4.04–3.96 (m, 1H), 2.97 (t, *J* = 7.6 Hz, 2H), 2.89–2.76 (m, 1H), 2.59 (dd, *J* = 2.6, 9.7 Hz, 1H), 2.52–2.35 (m, 3H), 2.23–2.02 (m, 2H), 2.01–1.91 (m, 3H), 1.67–1.65 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 162.6, 158.0, 141.9, 141.7, 137.6, 129.2, 127.2, 125.6, 122.7, 122.2 (d, *J* = 10.4 Hz), 112.5 (d, *J* = 26.0 Hz), 112.3, 97.3 (d, *J* = 26.5 Hz), 60.4, 54.5, 53.1, 51.9, 32.7, 26.1, 22.9; Formula $C_{22}H_{22}FN_3O_3S_2$; MS (ESI^+): m/z 460 $[M+H]^+$

(R)-6-chloro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzo[b]thiophene-2-sulfonamide (**27**). The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), 6-chlorobenzo[b]thiophene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.107 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 76%, yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.84–7.67 (m, 3H), 7.53 (dd, *J* = 5.3, 8.8 Hz, 1H), 7.36 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.21–7.14 (m, 1H), 7.01 (dt, *J* = 2.3, 8.8 Hz, 1H), 5.30 (br s, 1H), 3.97–3.88 (m, 1H), 2.93 (t, *J* = 7.6 Hz, 2H), 2.82–2.70 (m, 1H), 2.53 (dd, *J* = 2.9, 10.0 Hz, 1H), 2.49–2.31 (m, 3H), 2.18–2.03 (m, 2H), 1.99–1.85 (m, 2H), 1.67–1.51 (m, 1H); ^{19}F NMR (282 MHz, $CDCl_3$, δ): -109.4 (s, 1F); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 158.1, 142.5, 136.0, 133.5, 132.3, 128.6, 126.6, 126.4, 122.3, 122.1 (d, *J* = 11.5 Hz), 118.2, 112.5 (d, *J* = 26.0 Hz), 97.4 (d, *J* = 26.5 Hz), 60.4, 54.5, 53.2, 51.9, 32.4, 26.2, 22.9; Formula $C_{22}H_{21}ClFN_3O_3S_2$; MS (ESI^+): m/z 494 $[M+H]^+$

(S)-6-chloro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzo[b]thiophene-2-sulfonamide (**28**). The title compound was prepared using intermediate **IV** (1.0 equiv, 0.33 mmol, 0.100 g), 6-chlorobenzo[b]thiophene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.107 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 71%, yellow oil, ^{1}H NMR (300 MHz, $CDCl_3$, 7.82–7.80 (m, 2H), 7.78-7.76 (m, 1H), 7.57-7.43 (m, 1H), 7.41 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.2-7.54 (m,1H), 7.06–6.96 (m, 1H), 4.00–3.93 (m, 1H), 3.51-3.44 (m, 1H), 3.03-2.80 (m, 3H), 2.55-2.40 (m, 3H), 2.17-2.15 (m, 2H), 1.33-1.18(m, 3H); ^{19}F NMR (282 MHz, $CDCl_3$, δ): -109.4 (s, 1F); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 158.1, 142.6, 136.0, 133.6, 133.6, 128.6, 126.6, 126.4, 122.3, 122.1 (d, *J* = 11.5 Hz), 118.2, 112.4 (d, *J* = 26.0 Hz), 97.6 (d, *J* = 26.5 Hz), 60.4, 54.5, 53.2, 51.8, 32.4, 26.2, 22.8; Formula $C_{22}H_{21}ClFN_3O_3S_2$; MS (ESI^+): m/z 494 $[M+H]^+$

(*R*)-6-fluoro-*N*-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzo[b]thiophene-2-sulfonamide (**29**)**.** The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), 6-fluorobenzo[b]thiophene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.100 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 67%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.87–7.78 (m, 2H), 7.58 (dd, J = 5.3, 8.8 Hz, 1H), 7.51 (dd, *J* = 2.3, 8.8 Hz, 1H), 7.26–7.15 (m, 2H), 7.06 (dt, *J* = 1.8, 8.8 Hz, 1H), 5.20 (br s, 1H), 4.01–3.92 (m, 1H), 2.98 (t, *J* = 7.6 Hz, 2H), 2.86–2.76 (m, 1H), 2.58 (dd, *J* = 2.9, 10.0 Hz, 1H), 2.53–2.45 (m, 2H), 2.41 (dd, *J* = 5.9, 10.0 Hz, 1H), 2.23–2.07 (m, 2H), 2.03–1.90 (m, 2H), 1.71–1.56 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz), 163.6 (d, *J* = 13.8 Hz), 161.9 (d, *J* = 249.0 Hz), 158.1, 134.1 (d, *J* = 2.2 Hz), 133.1, 128.7, 127.3, 127.0(d, *J* = 9.3 Hz), 122.1 (d, *J* = 11.5 Hz), 118.2, 115.1 (d,

J = 24.3 Hz), 112.5 (d, *J* = 26.0 Hz), 108.8 (d, *J* = 25.2 Hz), 97.4 (d, *J* = 26.5 Hz), 60.4, 54.5, 53.2, 51.8, 32.4, 26.2, 22.9; Formula $C_{22}H_{21}F_2N_3O_3S_2$; MS (ESI^+): m/z 478 $[M+H]^+$

(S)-6-fluoro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)benzo[b]thiophene-2-sulfonamide (**30**)**.** The title compound was prepared using intermediate **IV** (1.0 equiv, 0.33 mmol, 0.100 g), 6-fluorobenzo[b]thiophene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.100 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 69%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.87–7.80 (m, 2H), 7.60–7.55 (m, 2H), 7.28–7.21 (m, 2H), 7.05 (dt, *J* = 1.8, 8.8 Hz, 1H), 3.97 (br s, 1H), 2.96 (t, *J* = 7.6 Hz, 2H), 2.86–2.80 (m, 1H), 2.56 (dd, *J* = 2.9, 10.0 Hz, 1H), 2.51–2.40 (m, 3H), 2.18–1.93 (m, 5H), 1.66–1.42 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 171.8 (d, *J* = 250.2 Hz), 163.6 (d, *J* = 13.8 Hz), 162.6 (d, *J* = 249.0 Hz), 158.1, 154.0 (d, *J* = 2.2 Hz), 137.6, 128.7, 127.1, 126.9 (d, *J* = 9.3 Hz), 122.0 (d, *J* = 11.5 Hz), 118.2, 115.1 (d, *J* = 24.3 Hz), 112.5 (d, *J* = 26.0 Hz), 108.8 (d, *J* = 25.2 Hz), 97.4 (d, *J* = 26.5 Hz), 60.4, 54.5, 53.2, 51.9, 32.4, 26.2, 22.9; Formula $C_{22}H_{21}F_2N_3O_3S_2$; MS (ESI^+): m/z 478 $[M+H]^+$

(R)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-5-methylbenzo[b]thiophene-2-sulfonamide (**31**). The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), 5-methylbenzo[b]thiophene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.099 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 62%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.78 (s, 1H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.64 (s, 1H), 7.57 (dd, *J* = 4.7, 8.8 Hz, 1H), 7.29 (dd, *J* = 1.8, 8.2 Hz, 1H), 7.22 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.10–7.00 (m, 1H), 5.47 (br s, 1H), 4.04–3.92 (m, 1H), 2.97 (t, *J* = 7.6 Hz, 2H), 2.88–2.76 (m, 1H), 2.62 (dd, *J* = 2.6, 9.7 Hz, 1H), 2.55–2.38 (m, 6H), 2.25–2.06 (m, 2H), 2.02–1.91 (m, 2H), 1.73–1.57 (m, 1H); ^{13}C NMR (75 MHz,

$CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 158.0, 141.8, 138.9, 138.0, 135.5, 129.1, 128.9, 125.3, 122.3, 122.1 (d, *J* = 11.5 Hz), 118.2, 112.5 (d, *J* = 25.1 Hz), 97.3 (d, *J* = 26.5 Hz), 60.3, 54.6, 53.1, 52.0, 32.3, 26.0, 22.9, 21.3; Formula $C_{23}H_{24}FN_3O_3S_2$; MS (ESI^+): m/z 474 $[M+H]^+$

(S)-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-5-methylbenzo[b]thiophene-2-sulfonamide (**32**). The title compound was prepared using intermediate **IV** (1.0 equiv, 0.33 mmol, 0.100 g), 5-methylbenzo[b]thiophene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.099 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 65%, brown oil, ^1H NMR (300 MHz, $CDCl_3$, δ): 7.78 (s, 1H), 7.69 (d, *J* = 8.8 Hz, 1H), 7.65–7.57 (m, 2H), 7.25–7.22 (m, 3H), 7.06–7.03 (m, 1H), 4.00–3.92 (m, 1H), 2.98 (t, *J* = 7.6 Hz, 2H), 2.96–2.77 (m, 1H), 2.66–2.48 (m, 6H), 2.21–2.17 (m, 2H), 2.04–1.96 (m, 3H), 1.23–1.38 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 162.6, 157.9, 141.7, 138.9, 137.9, 135.5, 129.0, 125.3, 122.3, 122.1 (d, *J* = 11.5 Hz), 118.1, 112.5 (d, *J* = 25.1 Hz), 97.3 (d, *J* = 26.5 Hz), 60.3, 54.5, 53.0, 51.9, 32.3, 25.8, 22.9, 21.3; Formula $C_{23}H_{24}FN_3O_3S_2$; MS (ESI^+): m/z 474 $[M+H]^+$

(R)-5-chloro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-3-methylbenzo[b]thiophene-2-sulfonamide (**33**)**.** The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), 5-chloro-3-methylbenzo[b]thiophene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.112 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 57%, brown oil, ^1H NMR (300 MHz, $CDCl_3$, δ): 7.77 (d, *J* = 1.8 Hz, 1H), 7.72 (d, *J* = 8.8 Hz, 1H), 7.57 (dd, *J* = 5.3, 8.8 Hz, 1H), 7.43 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.23 (dd, *J* = 1.8, 8.8 Hz, 1H), 7.11–7.01 (m, 1H), 5.28 (br s, 1H), 3.97 (br s, 1H), 2.97 (t, *J* = 7.3 Hz, 2H), 2.86–2.74 (m, 1H), 2.64 (s, 3H), 2.56 (dd, *J* = 2.6, 9.7

Hz, 1H), 2.51–2.44 (m, 2H), 2.39 (dd, *J* = 5.9, 10.0 Hz, 1H), 2.21–2.05 (m, 2H), 2.01–1.88 (m, 2H), 1.68–1.53 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.2 (d, *J* = 250.2 Hz), 163.5 (d, *J* = 13.8 Hz), 158.1, 140.9, 138.0, 137.5, 135.6, 131.5, 127.8, 123.7, 123.3, 122.1 (d, *J* = 11.5 Hz), 118.2, 112.5 (d, *J* = 25.2 Hz), 97.4 (d, *J* = 26.5 Hz), 60.5, 54.5, 53.1, 51.9, 32.4, 26.2, 22.9, 12.3; Formula $C_{23}H_{23}ClFN_3O_3S_2$; MS (ESI^+): m/z 508 $[M+H]^+$

(S)-5-chloro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-3-methylbenzo[b]thiophene-2-sulfonamide (**34**). The title compound was prepared using intermediate **IV** (1.0 equiv, 0.33 mmol, 0.100 g), 5-chloro-3-methylbenzo[b]thiophene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.112 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 61%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.78–7.72 (m, 2H), 7.57 (dd, *J* = 5.3, 8.8 Hz, 1H), 7.43 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.25 (dd, *J* = 1.8, 8.8 Hz, 2H), 7.11–7.07 (m, 1H), 4.06 (br s, 1H), 2.97 (t, *J* = 7.3 Hz, 2H), 2.75–2.33 (m, 8H), 2.21–2.08 (m, 2H), 2.05–2.0 (m, 2H), 1.42–1.20 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 250.2 Hz), 164.5 (d, *J* = 13.8 Hz), 157.6, 140.9, 138.0, 137.5, 135.6, 131.5, 127.8, 123.7, 123.3, 122.1 (d, *J* = 11.5 Hz), 118.2, 112.5 (d, *J* = 25.2 Hz), 97.4 (d, *J* = 26.5 Hz), 60.5, 54.5, 52.8, 52.0, 32.2, 26.9, 22.7, 12.3; Formula $C_{23}H_{23}ClFN_3O_3S_2$; MS (ESI^+): m/z 508 $[M+H]^+$

(R)-5-fluoro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-3-methylbenzo[b]thiophene-2-sulfonamide (**35**)**.** The title compound was prepared using intermediate **III** (1.0 equiv, 0.33 mmol, 0.100 g), 5-fluoro-3-methylbenzo[b]thiophene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.106 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 67%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.75 (dd, *J* = 4.7, 8.8 Hz, 1H), 7.57 (dd, *J* = 5.3, 8.8 Hz, 1H), 7.45 (dd, *J* = 2.3, 9.4 Hz, 1H), 7.28–7.20 (m, Hz, 2H), 7.06 (dt, *J* = 2.3, 8.8 Hz, 1H), 5.18 (br s, 1H), 4.02–3.93 (m,

1H), 3.01–2.94 (m, 2H), 2.86–2.77 (m, 1H), 2.64 (s, 3H), 2.57 (dd, *J* = 2.9, 10.0 Hz, 1H), 2.52–2.44 (m, 2H), 2.38 (dd, *J* = 5.9, 9.4 Hz, 1H), 2.21–2.05 (m, 2H), 1.95 (quin, *J* = 7.2 Hz, 2H), 1.68–1.55 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 164.3 (d, *J* = 250.2 Hz), 163.6 (d, *J* = 16.1 Hz), 161.1 (d, *J* = 245.0 Hz), 158.0, 140.8, 138.4, 135.9 (d, *J* = 4.6 Hz), 134.8, 124.0 (d, *J* = 9.2 Hz), 122.1 (d, *J* = 11.5 Hz), 118.2, 116.4 (d, *J* = 26.0 Hz), 112.5 (d, *J* = 26.0 Hz), 109.2 (d, *J* = 23.5 Hz), 97.4 (d, *J* = 26.5 Hz), 60.5, 54.5, 53.1, 51.9, 32.4, 26.2, 22.9, 12.4; Formula $C_{23}H_{23}F_2N_3O_3S_2$; MS (ESI^+): m/z 492 $[M+H]^+$

(S)-5-fluoro-N-(1-(3-(6-fluorobenzo[d]isoxazol-3-yl)propyl)pyrrolidin-3-yl)-3-methylbenzo[b]thiophene-2-sulfonamide (**36**)**.** The title compound was prepared using intermediate **IV** (1.0 equiv, 0.33 mmol, 0.100 g), 5-fluoro-3-methylbenzo[b]thiophene-2-sulfonyl chloride (1.2 equiv, 0.40 mmol, 0.106 g), DMAP (catalytic amount) and cesium carbonate (2.0 equiv, 0.67 mmol, 0.218 g) in DCM (5 mL). Yield 71%, brown oil, ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.73–7.53 (m, 1H), 7.43–7.40 (m, 1H) 7.40 (dd, *J* = 2.3, 9.4 Hz, 1H), 7.22–7.21 (m, 2H), 7.18–7.03 (m, 2H), 4.00–3.96 (m, 1H), 2.98–2.81 (m, 3H), 2.66–2.62 (m, 3H), 2.57–2.43 (m, 3H), 2.38 (dd, *J* = 5.9, 9.4 Hz, 1H), 2.17–2.01 (m, 2H), 1.95–1.92 (m, 2H), 1.63–1.64 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 165.9 (d, *J* = 250.2 Hz), 163.4 (d, *J* = 16.1 Hz), 162.6 (d, *J* = 245.0 Hz), 159.4, 140.9, 138.4, 135.9 (d, *J* = 4.6 Hz), 134.6, 124.0 (d, *J* = 9.2 Hz), 122.2 (d, *J* = 11.5 Hz), 118.2, 116.4 (d, *J* = 26.0 Hz), 112.5 (d, *J* = 26.0 Hz), 109.2 (d, *J* = 23.5 Hz), 97.4 (d, *J* = 26.5 Hz), 60.4, 54.5, 53.1, 52.9, 32.3, 26.0, 22.8, 12.4; Formula $C_{23}H_{23}F_2N_3O_3S_2$; MS (ESI^+): m/z 492 $[M+H]^+$

In vitro studies

The tested compounds were examined for known classes of assay interference compounds. According to SwissADME tool, none of the compounds contains substructural features recognized as PAINS (Pan Assay Interference Compounds) [29].

Radioligand Binding Assays for 5-HT_{2A}, 5-HT_6, 5-HT_7, D_2 and M_3 receptors

The Radioligand Binding Assays and functional studies for 5-HT_{2A}, 5-HT_6, 5-HT_7 and D_2 receptors were carried out at the Faculty of Pharmacy, Jagiellonian University Medical College. The remaining functional activity assays (α_{2C}, 5-HT_{2C} and H_1 receptors) and M_3 receptor binding were performed by Cerep SA (Le Bois l'Evêque, Poitiers, France). Inhibition of hERG channel was tested by ChanTest (USA).

Preparation of solutions of test and reference compounds. 10 mM stock solutions of test compounds were prepared in DMSO. Serial dilutions of compounds were prepared in 96-well microplate in assay buffers using automated pipetting system epMotion 5070 (Eppendorf). Each compound was tested in 8 concentrations from 1.0E-05 to 1.0E-12 M (final concentration).

Receptor Binding Assay. Radioligand binding was performed using membranes from CHO-K1 cells stably transfected with the human 5-HT_{2A}, 5-HT_6, 5-HT_7 and D_2 receptors (PerkinElmer). Reaction mix included 50 µL solution of the test compound, 50 µL of specific radioligand and 150 µL of diluted membranes. Specific assay conditions for each receptor are shown in table 1. All assays were carried out in duplicates. The reaction was terminated by rapid filtration through GF/B filter mate presoaked with 0.5% polyethyleneimine for 30 minutes. 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). The filter mates were dried at 37 °C in forced air fan incubator and then solid scintillator MeltiLex was melted

on filter mates at 90 °C for 5 minutes. Radioactivity was counted in MicroBeta2 scintillation counter (PerkinElmer). Data were fitted to a one-site curve-fitting equation with Prism 6 (GraphPad Software) and K_i values were estimated from the Cheng−Prusoff equation.

Table 1. Radioligand Binding Assay conditions

Receptor	**Radioligand**	**Blank (nonspecific)**	**Buffer**	**Incubation conditions**
5-HT$_{2A}$	[^{3}H]-ketanserin	10 µM mianserin	50 mM Tris, pH 7.4, 4 mM $CaCl_2$, 0.1% ascorbic acid	60 min, 27 ˚C
5-HT$_6$	[^{3}H]-LSD	10 µM methiothepin	50 mM Tris-HCl pH 7.4 10 mM $MgCl_2$, 0.1 mM EDTA	60 min, 37 ˚C
5-HT$_7$	[^{3}H]-LSD	10 µM methiothepin	50 mM Tris-HCl pH 7.4 10 mM $MgSO_4$, 0.5 mM EDTA	120 min, 27 ˚C
D$_2$	[^{3}H]-methylspiperone	10 µM haloperidol	NHME	60 min, 30 ˚C
M$_3$	[^{3}H]4-DAMP	1 µM atropine	For conditions see [30]	60 min, RT

The radioligand binding assays, results of which are shown in table 3, were performed in 3 or 4 independent experiments (M_3 receptor assay in 1 experiment) in duplicate (n=2).

Functional activity assays for the 5 HT$_{2A}$, 5-HT$_6$ and D$_2$ receptors

Test and reference compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM. Two independent experiments in duplicates were performed and 6

to 10 concentrations were tested. A cellular aequorin-based functional assay was performed with recombinant CHO-K1 cells expressing mitochondrially targeted aequorin, human GPCR, and the promiscuous G protein $G\alpha_{qi/5}$ for D_2, G protein $G\alpha_{16}$ for 5-HT_{2A} and 5-HT_6 receptors. After thawing, cells were transferred to assay buffer (DMEM/HAM's F12 with 0.1% protease-free BSA) and centrifuged. The cell pellet was resuspended in assay buffer and coelenterazine h was added at final concentrations of 5 µM. The cells suspension was incubated at 16 °C, protected from light with constant agitation for 16 h and then diluted with assay buffer to the concentration of 100,000 cells/ml. After 1 h of incubation, 50 µl of the cells suspension was dispensed using automatic injectors built into the radiometric and luminescence plate counter MicroBeta2 LumiJET (PerkinElmer, USA) into white opaque 96-well microplates preloaded with test compounds. Immediate light emission generated following calcium mobilization was recorded for 30- 60 s. In antagonist mode, after 15-30 min of incubation, the reference agonist was added to the above assay mix and light emission was recorded again. The final concentration of the reference agonist was equal to EC_{80}: α-methylserotonin 30 nM for 5-HT_{2A} receptor, serotonin 40 nM for 5-HT_6 receptor and apomorphine 30 nM for the D_2 receptor.

Functional activity assays for the 5-HT_7 receptor

Test and reference compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM. Serial dilutions were prepared in 96-well microplate in assay buffer and 6 to 10 concentrations were tested. For the 5-HT_7, adenylyl cyclase activity was monitored using cryopreserved CHO-K1 cells with expression of the human serotonin 5-HT_7 receptor. A functional assay based on cells with expression of the human 5-hydroxytryptamine (serotonin) receptor type 7 was performed. CHO-K1 cells were

transfected with a beta-lactamase (bla) reporter gene under control of the cyclic AMP response element (CRE) (Life Technologies). Thawed cells were resuspended in stimulation buffer (HBSS, 5 mM HEPES, 0.5 IBMX, and 0.1% BSA at pH 7.4) at 2x105 cells/ml. The same volume (10 µl) of cell suspension was added to the tested compounds. Samples were loaded onto a white opaque half area 96-well microplate. The antagonist response experiment was performed with 10 nM serotonin as the reference agonist for the 5-HT_7 receptor. The agonist and antagonist were added simultaneously. Cell stimulation was performed for 1 h at room temperature. After incubation, cAMP measurements were performed with homogeneous TR-FRET immunoassay using the LANCE Ultra cAMP kit (PerkinElmer, USA). 10 µl of EucAMP Tracer Working Solution and 10 µl of ULight-anti-cAMP Tracer Working Solution were added, mixed, and incubated for 1 h. The TR-FRET signal was read on an EnVision microplate reader (PerkinElmer, USA).

IC_{50} and EC_{50} were determined by non-linear regression analysis using GraphPad Prism 7.0 software. The logIC_{50} was used to obtain the K_b by applying the Cheng-Prusoff approximation.

Inhibition of hERG potassium channel

Blockade of hERG-mediated potassium currents was carried out in duplicate by ChanTest (Cleveland, Ohio) and expressed as mean % of inhibition at 1.0E-06 M (Table 3). Ability to block hERG potassium channels was determined using the electrophysiological method and cloned hERG potassium channels (KCNH2 gene, expressed in CHO cells) as biological material. The effects were evaluated using IonWorks Quattro™ system (Molecular Devices Corporation, Union City CA). hERG current was elicited using a pulse pattern with

fixed amplitudes (conditioning pre-pulse: -80 mV for 25 ms; test pulse: +40 mV for 80 ms) from a holding potential of 0 mV. hERG current was measured as a difference between the peak current at 1 ms after the test step to +40 mV and the steady-state current at the end of the step to +40 mV. Data acquisition and analyses were performed using the IonWorks Quattro™ system operation software (version 2.0.2; Molecular Devices Corporation, Union City, CA). Data were corrected for leak current. The hERG block was calculated as % Block = $(1 - I_{TA} / I_{Control}) \times 100\%$, where $I_{Control}$ and I_{TA} were the currents elicited by the test pulse in control and in the presence of a test compound, respectively. hERG inhibition of reference E-4031 in this assay was determined IC_{50} = 89 nM.

In vivo studies

Subjects. The experiments (except of step-through passive avoidance) were performed on male Swiss albino mice (22–26 g) purchased from a licensed breeder Staniszewska (Ilkowice, Poland) or male CD-1 mice (accredited animal facility Jagiellonian University Medical College, Kraków, Poland) and mice were kept in groups of ten to Makrolon type 3 cages (dimensions 26.5 × 15 × 42 cm). The animals were kept in an environmentally controlled rooms (ambient temperature 22 ± 2 °C; relative humidity 50–60%; 12:12 light:dark cycle, lights on at 8:00). They were allowed to acclimatize to the environment for one week before the commencement of the experiments. Standard laboratory food (Ssniff M-Z) and filtered water were freely available. All experimental procedures were carried out under EU Directive 2010/63/EU and approved by the I Local Ethics Committee for Experiments on Animals of the Jagiellonian University in Krakow, Poland. The animals were used only once in each test and then killed by cervical dislocation.

All the experiments were conducted in the light phase between 9:00 AM and 2:00 PM and each experimental group consisted of 8–10 randomly selected animals. The experiments were performed by an observer unaware of the treatment administered.

d-Amphetamine- or MK-801 induced hyperactivity in CD-1 mice. The locomotor activity was recorded with an Opto M3 multi-channel activity monitor (MultiDevice Software v.1.3, Columbus Instruments). The CD-1 mice were individually placed in plastic cages (22 × 12 × 13 cm) for 30 min habituation period, and then ambulation was counted during 120 min with data recording every 5 min. The cages were cleaned up with 70% ethanol after each mouse.

Spontaneous locomotor activity in CD-1 and Swiss albino mice. The locomotor activity was recorded according to the method described above. CD-1 mice were observed during a 120-min session with data recording every 5 min, and the mobility of Swiss albino mice was counted during the first minute or from 2 to 6 min of the test, i.e. the time equal to the observation periods in FPT and FST, respectively.

Bar test in CD-1 mice. Animal's forelimbs were draped over a thin, cylindrical horizontal rod elevated 4 cm above the tabletop at 30, 60 and 120 min after administration of a test compound. The length of time the animal touched the bar with both front paws was measured up to a pre-set cut-off time of 60 s. A maximum of three trials was used for each animal. In the bar test, a scoring system used by Ögren *et al.* was employed [31]. Results of each trial were scored as follows: 0 for holding the position for <15 s, 1 for holding for 15-29.9 s, 2 for holding for 30-59.9 s, and a maximum score of 3 for staying on the bar for ≥60 s. The minimum cataleptogenic dose (MED) was defined as the lowest dose inducing catalepsy mean score of ≥ 1 at 30, 60 or 120 min post-treatment.

Forced swim test in Swiss albino mice. The experiment was carried out according to the method of Porsolt *et al.* [32]. Briefly, mice were individually placed in a glass cylinder (25 cm high; 10 cm in diameter) containing 10 cm of water maintained at 23–25 °C and were left there for 6 min. A mouse was regarded as immobile when it remained floating on the water, making only small movements to keep its head above it. The total duration of immobility was recorded during the last 4 min of a 6-min test session

Four-plate test in Swiss albino mice. The four-plate apparatus (BIOSEB) consists of a cage (25 x 18 x 16 cm) floored by four identical rectangular metal plates (8 x 11 cm) separated from one another by a gap of 4 mm. The top of the cage is covered by a transparent Perspex lid that prevents escape behavior. The plates are connected to a device that can generate electric shocks. Following a 15-s habituation period, the animal's motivation to explore a novel environment is suppressed by an electric foot shock (0.8 mA, 0.5 s) every time it moves from one plate to another during a 1-min test session. This action is referred to as a 'punished crossing' and is followed by a 3 s shock interval, during which the animal can move across plates without receiving a shock [33].

Drugs. The following drugs were used: d-amphetamine (sulfate, Sigma-Aldrich), MK-801 (hydrogen maleate, Sigma-Aldrich), quetiapine (Adamed Pharma S.A.) and the tested compounds **12**, **25** and **26**. d-Amphetamine and MK-801 were dissolved in distilled water; the remaining compounds were suspended in a 1% aqueous solution of Tween 80 immediately before administration. All agents, except d-amphetamine that was given subcutaneously (s.c.), were administered intraperitoneally (i.p.) in a volume of 10 ml/kg. In locomotor activity tests, d-amphetamine or MK-801 were injected simultaneously with quetiapine or investigated compounds just before the start of the test. In the bar test

quetiapine, compounds **12**, **25** and **26** were given just before the start of the test. In the FST and the FPT quetiapine or investigated compounds were administered 60 minutes before the test.

Step-through passive avoidance task in mice after acute administration. Adult male mice (stock's name: CD-1, 18–21 g), purchased from Animal House at the Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland were used in these experiments. The groups of 15 mice were kept to a plastic cage (60 cm × 38 cm × 20 cm) at room temperature (22 ± 2 °C), on 12 h light/dark cycles (the lights turned on at 7:00 a.m., and off at 7:00 p.m.).

Step-through passive avoidance task was performed according to the method previously described [34,35]. The apparatus for step-through passive avoidance task consisted of two compartments, separated by an automated sliding door (LE872, Bioseb, France). For the acquisition session, mice were placed individually in an illuminated white compartment (20 cm × 21 cm × 20 cm, 1000 lx) with the closed door to a smaller dark compartment (7.3 cm × 7.5 cm × 14 cm, 10 lx) equipped with an electric grid floor (stainless steel rods through which an electric footshock is delivered). After the 30s the door to a smaller compartment was opened. Immediately after the mouse entered the smaller dark compartment, the door closed, and the rodent was punished by an inescapable electric foot shock (0.2 mA for 2 s). The mice, which did not enter the dark compartment within 50 s were excluded from the study. On the following day (24 h later), the trained animals were placed again into the illuminated compartment and observed up to 300 s (retention session). The experimental procedure was similar to the acquisition session, but this time, mice did not receive the electric shock after the entrance to the smaller dark compartment. Mice, which

avoided the dark compartment for 300 s were considered to remember the task. ADN-1459 (0.3125 – 2.5 mg/kg) was injected (i.p.) 30 min before the acquisition trial. Control groups were administered (i.p.) with saline.

Data analysis and statistics. Results are presented as means ± S.E.M. The comparisons between experimental and control groups were performed by one-way ANOVA followed by Bonferroni test post hoc. A value of $p \leq 0.05$ was considered to be significant.

RESULTS

Design

In the course of our research aimed at the development of potential treatment of BPSD, we have explored various possibilities of modulating specific molecular targets to develop compounds demonstrating antipsychotic activity without disturbing cognitive functions [36]. To reach the main goal in proof-of-concept studies we have developed several series of drug-like compounds combining affinity for a defined set of pharmacologically relevant biological targets [20,37,38]. Our previous scientific work, involving molecular docking to homology models and analysis of interactions, revealed that affinity for the 5-HT_7 and the 5-HT_6 receptors might be achieved by incorporation of an accessory arylsulfonamide moiety to the appropriate core structure containing ionizable amine group, capable of forming an ionic bond with Asp3.32 residue [20,37]. The former fragment is responsible for interactions with the binding site residues located between transmembrane helices (TMHs) 2, 3 and 7, rendering interactions of selective ligands of the 5-HT_7 and the 5-HT_6 receptors, e.g. SB-258719 [39] and SB-271046 [40], respectively (Figure 1). Guided by a structure-based molecule design approach, we decided to combine the

arylsulfonamide fragment with the 6-fluoro-1,2-benzoxazole scaffold that primarily secures antagonism of the D_2 and the 5-HT_{2A} receptors. Such a mechanism of action is characteristic to risperidone or neflumozide, and is exerted by stacking the 6-fluoro-1,2-benzoxazole with aromatic residues (mainly Phe6.52) found in the cavity formed by TMHs 3, 5 and 6 (Figure 1). Finally, the key structural feature characteristic for monoamine receptors ligands – basic nitrogen atom – was included in the heterocyclic ring linked by propylene chain to 6-fluoro-1,2-benzoxazole. The geometries of the molecules containing piperidine and pyrrolidine as nitrogen-containing heterocyclic rings were examined in docking studies. The examples of both the chemotypes were then synthesized and evaluated for in vitro binding as promising starting points for the new series. The original propylpiperidine found in neflumozide scored well in docking studies, which was reflected in high affinity for the 5-HT_{2A} and 5-HT_7 receptors, however, showed weak affinity for the 5-HT_6 and the D_2 receptors. The *N*-{1-[3-(6-fluoro-1,2-benzoxazol-3-yl)propyl]piperidin-4-yl}naphthalene-1-sulfonamide in 1.0E-06 M screening concentration showed only 76% of inhibition of control specific binding at the D_2 receptor and 40% at the 5-HT_6 receptor. For comparison, results for its pyrrolidine-containing counterpart (compound **5**) reached 95% and 97% in the respective assays and therefore pyrrolidine became the core structure in the presented series. The basic amine provided charge-reinforced H-bond (salt bridge) anchoring the molecules to Asp3.32 residue, which is present in each of the targeted receptors (Figure 1) [41–44]. The design stage resulted in a series of 36 compounds (18 individual structures and their stereoisomers) containing commercially-available arylsulfonamides that satisfied the aforementioned binding mode requirements studied in silico (table 3) [45].

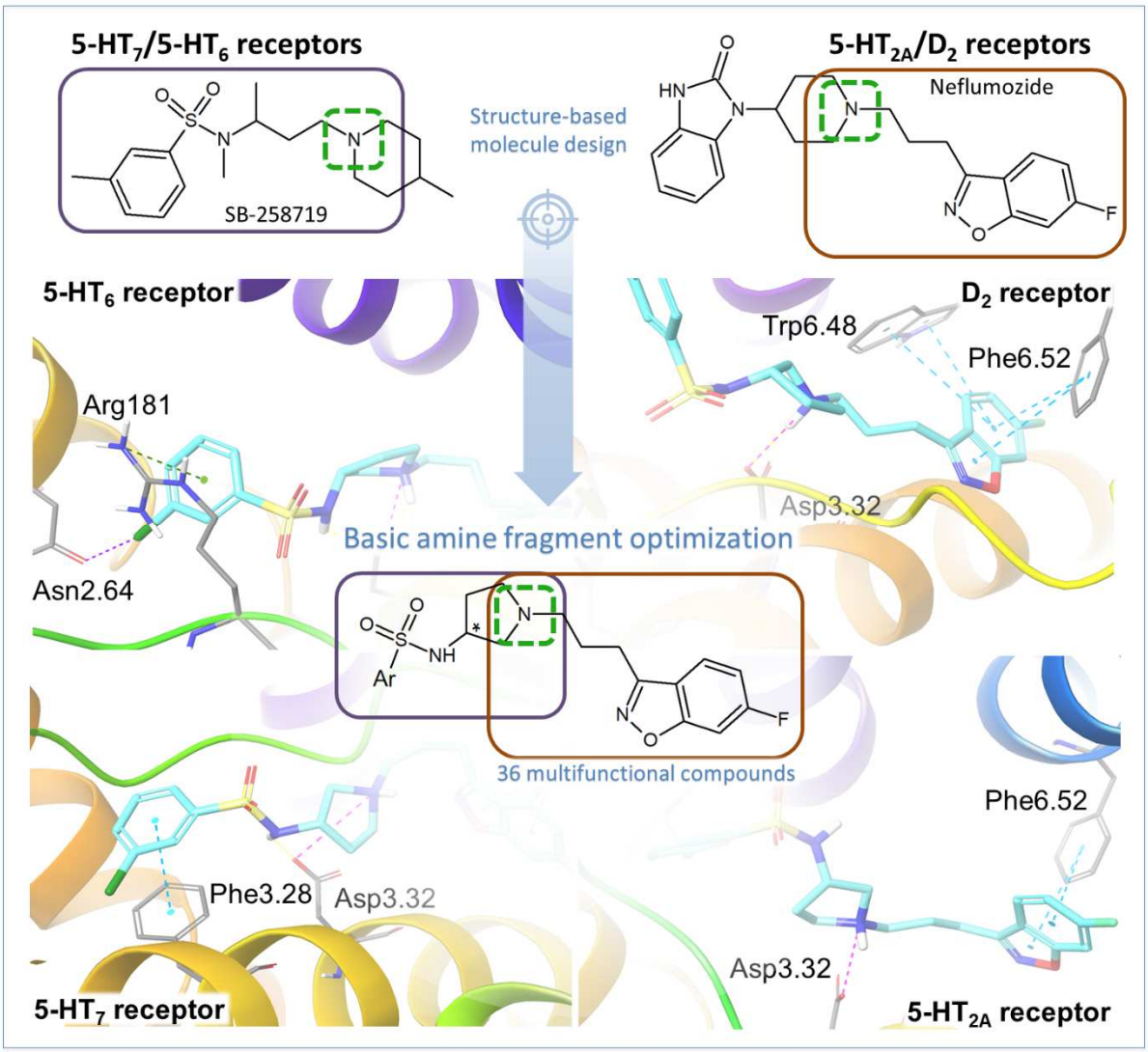


Figure 1. Design of multifunctional ligands and the proposed binding mode of the representative **compound 2** in the targeted receptors**.** The arylsulfonamide fragment together with alkylarylamine moiety satisfy favourable interactions for the 5-HT_7 and 5-HT_6 receptor binding sites, rendering interactions of their reference ligands [39,40,46]. 6-fluoro-1,2-benzoxazole linked to the propylamine moiety constitutes a pharmacophore for the 5-HT_{2A} and D_2 receptor blocking properties [41]. Piperidine ring from neflumozide was hopped to pyrrolidine during structure optimization. The design stage resulted in a series of 36 stereoisomeric ligands potentially characterized by high affinity for the desirable biological targets. Key amino acid residues engaged in ligand binding (within 4 Å from the ligand atoms) are displayed as thick sticks together with their interactions: salt bridges (dotted magenta lines), H-bonds (dotted yellow lines), halogen bonds (dotted violet lines), π−π stacking (dotted cyan lines), or cation−π (dotted green lines). The detailed complexes are shown in Supplementary Material, Figures S1−S4.

The novel compounds complied with Lipinski's rule of five and Veber filter in terms of molecular properties determining favourable bioavailability after oral administration (except compounds **33** and **34** due to exceeded molecular weight) and might be therefore considered drug-like. Moreover, the pKa values of the compounds' most basic nitrogen atoms didn't exceed 8, which limits the risk of poor cell membrane permeability due to ionization issues or P-gp efflux susceptibility. The series was characterized by high values of CNS MPO (Central Nervous System Multiparameter Optimization) tool reaching up to 5.2 (median 4.3). Compounds characterized by CNS MPO values > 4 proved a low risk of attrition caused by ADMETox issues in a group of centrally-acting drugs [28]. All of the determined molecular properties (Table 2) indicated favourable bioavailability, which enabled further in vivo studies. Moreover, none of the compounds contained substructural features recognized as pan assay interference compounds (PAINS, reported by SwissADME).

Prediction of the biotransformation pathways suggested CYP3A4-dependent N-dealkylation to be the main route of metabolism. In the case of compound **25**, the resulting *N*-(pyrrolidin-3-yl)-1-benzothiophene-2-sulfonamide and 3-(6-fluoro-1,2-benzoxazol-3-yl)propanoic acid accounted for 40% of total metabolites (the predicted metabolic pathways are shown in supplementary material figure 5). In human liver microsomes, the compound was characterized by acceptable predicted metabolic stability (Clint = 82.1 µL/min/mg), which was confirmed by in vitro studies (Clint = 88 µL/min/mg HLM).

Table 2. Predicted and calculated molecular parameters of the compounds showing drug-like properties, possibly favourable bioavailability and low risk of attrition.

		Lipinski's rule of 5				Veber filter		CNS MPO
Compound	pK_a	LogP	MW	HBD	HBA	RB	TPSA	Value
1,2	7.6	3.1	437.9	1	4	6	75.4	4.8
3,4	7.6	3.1	417.5	1	4	6	75.4	5
5,6	7.6	2.8	421.5	1	4	6	75.4	5.2
7,8	7.5	3.2	455.9	1	4	6	75.4	4.6
9,10	7.7	3.6	453.5	1	4	6	75.4	4.3
11,12	7.6	3.6	453.5	1	4	6	75.4	4.3
13,14	7.6	3.5	483.6	1	5	7	84.7	4.2
15,16	7.6	4.2	488.0	1	4	6	75.4	3.4
17,18	7.7	2.9	456.5	1	4	6	80.4	4.9
19,20	7.6	2.9	456.5	1	4	6	80.4	4.9
21,22	7.3	2.5	443.5	1	4	6	88.6	5
23,24	7.2	3.5	459.6	1	4	6	75.4	4.1
25,26	7.6	3.3	459.6	1	4	6	75.4	4.4
27,28	7.2	4.1	494.0	1	4	6	75.4	3.3
29,30	7.2	3.7	477.5	1	4	6	75.4	3.9
31,32	7.2	4.0	473.6	1	4	6	75.4	3.5
33,34	7.3	4.7	508.0	1	4	6	75.4	3
35,36	7.3	4.2	491.6	1	4	6	75.4	3.3

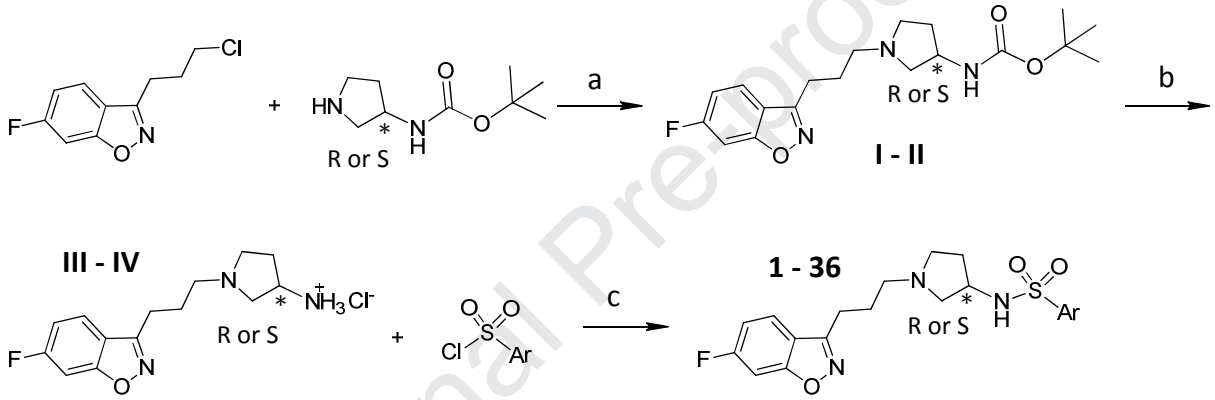
Properties calculated using InstantJChem software (ChemAxon): LogP - Predicted octanol/water partition coefficient; MW – molecular weight; HBD – hydrogen bond donor; HBA – hydrogen bond acceptor; RB –

rotatable bonds; TPSA – total polar surface area. CNS MPO – Multi-Parameter Optimization for Central Nervous System-active drugs (value 0 – 6).

Synthesis

The designed compounds (**1–36**) were prepared in the three-step synthesis as presented in **Scheme 1.**

Scheme 1. General synthesis of the final compounds **1–36**[a]



[a]Reagents and conditions: (a) K_2CO_3, KI, CH_3CN, 60 °C, 48 h; (b) HCl in EtOAc, rt, 18 h; (c) Cs_2CO_3, DMAP, DCM, r.t., 12 h. "Ar" structures shown in table 2.

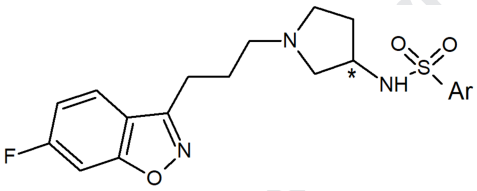
In the first step, the alkylation of appropriate 3-(BOC-amino)pyrrolidine (R or S) with the 3-(3-chloropropyl)-6-fluorobenzo[d]isoxazole in the presence of potassium carbonate, a catalytic amount of potassium iodide in acetonitryle at 60 °C, afforded corresponding enantiomers **I–II**. In the next step, the *tert*-butyloxycarbonyl group of compounds **I–II** was removed using 1.0 M solution of hydrochloric acid in ethyl acetate to afford intermediates **III–IV**, which were next reacted with various commercially available sulfonyl chlorides {*1–18*} to provide the sulfonamides **1–36**. The final reaction was carried out in dichloromethane, in the presence of caesium carbonate and a catalytic amount of 4-dimethylaminopyridine

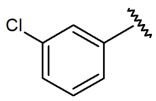
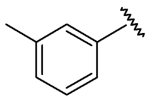
(DMAP). The structures of the final molecules are presented in **Table 3.** The chemical structures of the test compounds and key intermediates were confirmed by ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectroscopy, and the purity of over 95% was determined using UPLC/MS.

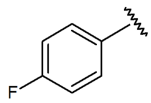
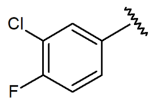
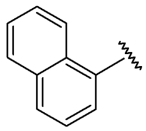
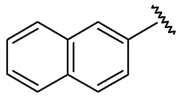
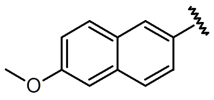
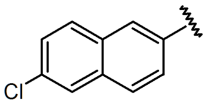
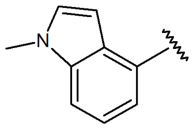
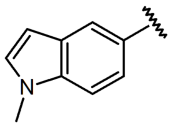
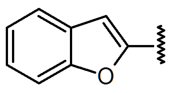
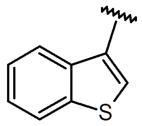
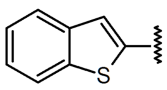
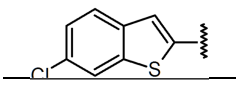
In vitro pharmacology

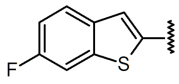
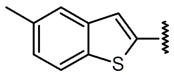
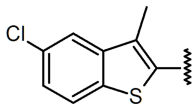
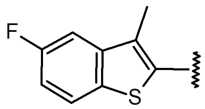
The synthesized series of compounds was submitted to radioligand binding assays, measuring their affinity for the serotonin 5-HT$_{2A}$, 5-HT$_6$, 5-HT$_7$, and dopamine D$_2$ receptors (Table 3). For the most active analogues, we have evaluated affinity for muscarine M$_3$ receptors and interaction with hERG channel in automated patch clamp assay.

Table 3. Structure and receptor binding profile of compounds **1–36**.



Cmpd	Ar	R-S	pK_i ± SEM [a]				% activity at 1.0E-06 M	
			5-HT$_{2A}$R	5-HT$_6$R	5-HT$_7$R	D$_2$R	M$_3$R[b]	hERG[c]
1		R	8.38 ± 0.05	7.56 ± 0.02	8.56 ± 0.03	7.25 ± 0.02		
2		S	8.81 ± 0.01	8.05 ± 0.02	7.55 ± 0.03	7.00 ± 0.00		
3		R	8.37 ± 0.03	7.58 ± 0.03	8.77 ± 0.02	7.50 ± 0.04		
4		S	8.99 ± 0.07	7.72 ± 0.03	7.89 ± 0.03	6.98 ± 0.00		

5		R	7.94 ± 0.02	7.46 ± 0.03	8.40 ± 0.02	7.02 ± 0.01		
6		S	7.81 ± 0.04	7.43 ± 0.03	7.50 ± 0.04	6.49 ± 0.02		
7		R	8.28 ± 0.02	8.33 ± 0.03	8.84 ± 0.03	7.25 ± 0.02		
8		S	8.19 ± 0.03	8.12 ± 0.03	7.59 ± 0.02	6.77 ± 0.02		
9		R	9.35 ± 0.05	8.75 ± 0.03	8.77 ± 0.03	7.69 ± 0.01	9	
10		S	8.96 ± 0.02	8.59 ± 0.07	8.14 ± 0.02	7.49 ± 0.01	12	
11		R	9.61 ± 0.09	8.40 ± 0.03	8.26 ± 0.05	9.70 ± 0.03		29
12		**S**	**9.48 ± 0.04**	**8.41 ± 0.04**	**8.30 ± 0.06**	**7.85 ± 0.02**	**12**	**16**
13		R	9.03 ± 0.07	7.41 ± 0.04	6.93 ± 0.03	8.51 ± 0.03	15	
14		S	8.24 ± 0.08	7.2 ± 0.02	6.92 ± 0.06	7.25 ± 0.01	22	
15		R	8.27 ± 0.03	8.05 ± 0.05	6.90 ± 0.03	8.59 ± 0.09	3	23
16		S	7.71 ± 0.03	7.61 ± 0.05	6.61 ± 0.01	6.99 ± 0.01	9	20
17		R	8.04 ± 0.06	8.33 ± 0.05	8.01 ± 0.02	8.04 ± 0.02		
18		S	8.89 ± 0.06	8.07 ± 0.05	7.37 ± 0.04	7.42 ± 0.01		
19		R	9.61 ± 0.09	8.24 ± 0.01	8.19 ± 0.03	9.03 ± 0.03	0	
20		S	9.35 ± 0.05	7.66 ± 0.02	7.42 ± 0.05	7.91 ± 0.05	0	
21		R	9.46 ± 0.06	8.30 ± 0.06	8.16 ± 0.04	8.91 ± 0.04	11	
22		S	9.61 ± 0.09	8.01 ± 0.04	8.14 ± 0.07	8.25 ± 0.02	39	
23		**R**	**9.38 ± 0.09**	**8.93 ± 0.04**	**8.30 ± 0.03**	**7.70 ± 0.04**	**25**	**0**
24		S	8.96 ± 0.04	8.69 ± 0.05	7.80 ± 0.02	7.36 ± 0.01	23	0
25		**R**	**8.50 ± 0.03**	**8.49 ± 0.05**	**8.32 ± 0.02**	**9.35 ± 0.09**	**11**	**2**
26		S	9.13 ± 0.03	7.96 ± 0.02	7.74 ± 0.04	7.79 ± 0.02	26	17
27		R	9.10 ± 0.07	8.04 ± 0.03	6.64 ± 0.02	8.08 ± 0.04	37	

28		S	8.02 ± 0.02	7.21 ± 0.03	6.77 ± 0.02	7.46 ± 0.01	38	
29		R	9.40 ± 0.06	8.05 ± 0.03	7.60 ± 0.04	8.85 ± 0.08	9	29
30		S	8.96 ± 0.13	7.42 ± 0.03	7.03 ± 0.01	7.75 ± 0.02	12	23
31		R	9.23 ± 0.07	7.82 ± 0.04	6.64 ± 0.03	8.76 ± 0.04	12	23
32		S	8.87 ± 0.17	7.42 ± 0.04	7.19 ± 0.02	7.62 ± 0.02	15	20
33		R	8.55 ± 0.02	7.93 ± 0.02	6.88 ± 0.02	8.05 ± 0.02	17	
34		S	8.05 ± 0.05	7.51 ± 0.06	6.66 ± 0.02	7.41 ± 0.01	33	
35		R	9.10 ± 0.06	8.38 ± 0.03	7.53 ± 0.02	9.20 ± 0.02		
36		S	8.41 ± 0.11	7.84 ± 0.04	7.19 ± 0.04	7.82 ± 0.02		
	Mianserin		8.72 ± 0.03					
	Methiothepin			9.01 ± 0.02	9.26 ± 0.02			
	Haloperidol					8.73 ± 0.01		

[a] Data expressed as the mean value of at least three independent experiments ± SEM. [b] % inhibition of control specific binding at the concentration of 1.0E-06 M. [c] % inhibition of hERG-mediated potassium currents at the concentration of 1.0E-06 M. Assays carried out in duplicate (n=2). Affinities of reference compounds for the antitargets are as follows: M_3R pK_i = 9.35 (4-DAMP), hERG pIC_{50} = 7.05 (E-4031).

The vast majority of the synthesized compounds showed high affinity for the desired targets (pK_i = 6.49 – 9.7) and relatively low affinity for antitargets – the muscarinic M_3 receptors and hERG channel (< 50% inhibition of control binding and potassium current inhibition at 1 μM, respectively). It has been found that R enantiomers were slightly more active comparing to S analogues, having a more balanced affinity for the targets of interest. Notably, all the compounds possessed a very high affinity for the serotonin 5-HT_{2A} receptors with pK_i values 7.71 – 9.61 and for the 5-HT_6 receptors (pK_i = 7.2 – 8.93). Binding affinity for

the 5-HT_7 and the dopamine D_2 receptors varied depending on the structure of an aryl substituent. Clearly, derivatives containing phenyl rings bound to the 5-HT_7 receptors with high affinity (pK_i = 7.5 – 8.84), while their affinity for the D_2 receptors was substantially lower (pK_i = 6.49 – 7.5). On the other hand, replacement of the phenyl substituent with bulkier bicyclic moiety gave the opposite results. The affinity of biaryl derivatives for the D_2 receptors was remarkably high (pK_i = 6.99 – 9.7), whereas binding to the 5-HT_7 receptors depended significantly on the aromatic moiety. Incorporation of benzofuran or indole rings resulted in analogues displaying relatively high affinity (pK_i 7.37 – 8.19), although the highest affinity for the 5-HT_7 receptors was observed in the case of naphthalene and benzothiophene derivatives (pK_i 7.74 – 8.77). However, the introduction of additional substituents into naphthalene or benzothiophene rings decreased significantly binding to the 5-HT_7 receptors (pK_i 6.61 – 7.6). For further investigation, we chose compounds characterized by a superior affinity for all the targeted serotonin receptors (pK_i over 8.3, which corresponds to K_i below 5 nM), and dopamine D_2 receptors (pK_i over 7.7; K_i below 20 nM) – compounds **12**, **23** and **25**. Moreover, we selected an enantiomer of the most promising compound **25 -** compound **26 -** for a direct comparison of stereoisomers.

Table 4. Functional data for the selected compounds.

Cmpd	Antagonist effect pK_B ± SD						
	D_2R[a]	5-$HT_{2A}R$[a]	5-HT_6R[a]	5-HT_7R[a]	$\alpha_{2C}R$[b]	5-$HT_{2C}R$[b]	H_1R[b]
12	7.40 ± 0.08	8.13 ± 0.24	7.07 ± 0.21	7.00 ± 0.16			
23	7.61 ± 0.31	8.66 ± 0.15	6.97 ± 0.44	7.89 ± 0.10			
25	9.03 ± 0.62	8.79 ± 0.19	7.41 ± 0.01	8.72 ± 0.82	N.C.	N.C.	N.C.
26	7.30 ± 0.09	8.10 ± 0.31	7.03 ± 0.25	7.17 ± 0.24			
Chlorpromazine	8.84 ± 0.06						

Pimavanserin	9.17 ± 0.11		
SB 742457		9.21 ± 0.16	
SB 269970			9.55 ± 0.21

[a] Data expressed as the mean value of two independent experiments ± SD. [b] N.C.: K_B value not calculable. Concentration-response curve shows less than 25% effect at the highest validated testing concentration. All assays carried out in duplicate (n=2). Blank spaces – compounds not tested in the assays. Antagonist effect of reference compounds for the antitargets are as follows: α_{2C}R pK_B = 8.62 (rauwolscine), 5-HT_{2C} pK_B = 8.39 (SB 206553), H_1R pK_B = 9.57 (pyrilamine).

Functional studies of the selected compounds resulted in advantageous nanomolar K_B values (pK_B 6.97 – 9.03) in the assays involving the 5-HT_{2A}, 5-HT_6, 5-HT_7 and D_2 receptors. The outcomes reflected the ability of the compounds to block the receptor activation and verified the design assumptions to obtain efficient antagonists of the selected receptors. It is worth noting that no activation of any target receptors has been detected. The most promising results were observed for the 2-benzothiophene derivative (compound **25**), which was therefore tested against the antitargets (α_{2C}, 5-HT_{2c} and H_1 receptors), showing no significant functional effects (Table 4).

Behavioral evaluation

The antipsychotic activity of the selected compounds **12**, **25,** and **26** was evaluated in amphetamine-induced and MK-801-induced hyperactivity tests in mice. The cataleptogenic properties of the compounds were investigated using the bar test. Furthermore, antidepressant- and anxiolytic-like effects of the most antipsychotic-active compound **25** were studied in the forced swim (FST) and four-plate (FPT) tests, respectively. The influence of the effective doses recorded in the aforementioned tests on spontaneous locomotor

activity in mice was studied, to exclude the possibility of competing behaviors, such as increased general locomotor activity or sedation, and to determine the specificity of the observed effects. Finally, the effect of **25** on cognitive function was evaluated in the mouse step-through passive avoidance test (a hippocampus-dependent memory task). Quetiapine, the antipsychotic drug used in BPSD treatment [9], was used as a reference in behavioral experiments.

Table 5. Characterization of compounds **12**, **25** and **26** in behavioral *in vivo* pharmacological tests in comparison to reference drug - quetiapine. Tests carried out on CD-1 and Swiss albino mice after intraperitoneal (i.p.) administration.

Test procedures	**12**	**25**	**26**	**Quetiapine**
	MED (dose range tested) [mg/kg]			
Catalepsy (bar test)	> 30 (3-30)	30 (3-30)	3 (0.3-10)	30 (3-30)
Spontaneous locomotor activity	> 10[b] (10)	> 5[b] (1.25-5)	> 10[b] (10)	10[b] (0.312-10)
Amphetamine-induced hyperactivity	10 (1.25-10)	2.5 (1.25-5)	5 (1.25-5)	5 (2.5-5)
MK-801-induced hyperactivity	10 (2.5-10)	2.5 (1.25-5)	10 (2.5-10)	10 (1.25-10)
Forced swim test (FST)		0.312 (0.156-0.625)		10 (5-20)
Four-plate test (FPT)		1.25 (0.625-2.5)		N.A.[c] (0.625–5)
Passive avoidance test (PA)		N.D.[D] (0.3125 – 2.5)		

[a] MED – minimum effective dose, [b] MSD – minimum sedative dose, [c] N.A. – not active, [D] N.D. – no memory deficits. Blank spaces – compounds not tested in the assays. Results for the essential comparators are as follows: FST MED = 2.5 mg/kg (citalopram, tested in dose range of 1.25 - 5 mg/kg); FPT MED = 1.25 mg/kg (diazepam, tested in dose range of 0.625 - 5 mg/kg); PA MED > 2 mg/kg (aripiprazole induced no memory deficits in dose range of 0.25 - 2 mg/kg) [20,47].

Amphetamine- and MK-801-induced hyperactivity tests were intended to reflect the potential efficacy of drugs in the treatment of psychotic symptoms, agitation and aggression. All the tested compounds - **12** (10 mg/kg), **25** (2.5 mg/kg) and **26** (5 mg/kg) significantly inhibited the amphetamine-evoked hyperactivity. Similar effects were also observed in the MK-801-induced hyperactivity test, where **12** was active at a dose of 10 mg/kg, **25** at 2.5 mg/kg and **26** at 10 mg/kg. The antipsychotic activity noticed in those models appeared to be specific since the compounds administered in effective doses had no influence on spontaneous locomotor activity. By comparison, quetiapine significantly attenuated the hyperactivity evoked by amphetamine and MK-801 at doses of 5 and 10 mg/kg, respectively; however, quetiapine administered at the higher dose (10 mg/kg) decreased spontaneous locomotor activity of animals (Table 5).

Since the compounds have been targeted to a particularly sensitive group of elderly patients, considerable efforts have been made to design drugs that would not produce extrapyramidal symptoms (EPS). We examined cataleptogenic potential of the tested compounds in the bar test, which is recognized as an important rodent model for predicting EPS liability in human [48]. The minimum cataleptogenic dose (minimum effective dose, MED) values for **26** (3 mg/kg) were similar to those active in the MK-801-induced hyperactivity test, whereas **25** (30 mg/kg) elicited catalepsy in 12-fold higher dose, and **12** (> 30 mg/kg) didn't induce catalepsy in up to 3-fold higher dose, than their effective antipsychotic doses. The MED of quetiapine in the bar test was 30 mg/kg (Table 5). The discussed results designated compound **25** for further in vivo activity evaluation.

The extended behavioral studies included determination of the antidepressant and anxiolytic potential of **25** in the forced swim test (FST) and the four-plate test (FPT) in mice.

Our results showed that in FST compound **25** in a single dose of 0.312 mg/kg showed antidepressant-like properties, reducing mice immobility time by 21%. It is worth noting that quetiapine displayed the same effect but in a distinctly higher dose (10 mg/kg). The results obtained in FPT demonstrated anxiolytic-like activity of **25** administered at a dose of 1.25 mg/kg, while quetiapine (tested in 0.625–5 mg/kg) was inactive in that test (Table 5). Effective antidepressant and/or anxiolytic doses of the investigated agents did not stimulate the spontaneous locomotor activity in mice during the 2–6-min or 1-min sessions (that is, at the observation time in FST and FPT, respectively) (data not shown). Finally, the effect of compound **25** on cognitive function in the step-through passive avoidance task in mice was evaluated. In the acquisition trial of the passive avoidance task, the substance did not affect latency times (data not shown). Similarly, in the retention trial, the compound did not influence the latency time, which implicated lack of memory deficits caused by compound **25** (Figure 2).

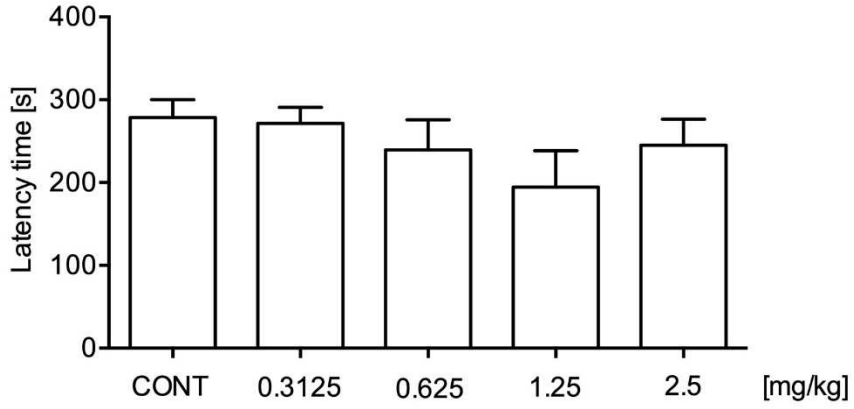


Figure 2. The effect of compound **25** on cognitive function in the step-through passive avoidance task in mice. Compound **25** was administered intraperitoneally (i.p.) 30 min before the acquisition trial of the step-through passive avoidance test. Control (CONT) group received saline (i.p) 30 min before the experiment. Statistical analysis: one-way ANOVA (Bonferroni post hoc; n = 10 mice per group).

Compound **25** proved to pose a competitive and promising alternative to quetiapine in behavioral evaluation in mice.

CONCLUSIONS

The currently described project was aimed at the discovery of a multitarget-directed ligand that would be therapeutically active in managing non-cognitive manifestations of dementia – psychosis, depression and anxiety – not disturbing memory at the same time. The concept was to incorporate selective and balanced antagonism for a particular set of serotoninergic (5-HT_{2A}, 5-HT_6 and 5-HT_7) and dopaminergic (D_2) receptors into a drug-like molecule, not affecting anti-targets that would hamper its efficacy or cause adverse effects (muscarinic receptors, hERG channel, adrenergic α_{2C}, serotoninergic 5-HT_{2C} or histaminergic H_1 receptors). To this end, arylsulfonamide derivatives of 6-fluoro-3-[3-(pyrrolidin-1-yl)propyl]-1,2-benzoxazole were designed with the aid of computational techniques and stereoselectively synthesized. Their chemical structures were confirmed by ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectroscopy, and their purity was determined using UPLC/MS. All the 36 original chemical entities were tested for affinity for the biological targets of interest, and the selected compounds were verified in functional activity assays. Finally, in vivo evaluation of 3 compounds was carried out. The overall pharmacological characteristics of the lead compound **25** (*N*-[(3*R*)-1-[3-(6-fluoro-1,2-benzoxazol-3-yl)propyl]pyrrolidin-3-yl]-1-benzothiophene-2-sulfonamide) satisfied the targeted objectives of proof-of-concept studies – the compound showed antipsychotic-like effect and did not influence cognitive functions negatively in behavioral animal studies. Moreover, it demonstrated robust antidepressant-like, as well as anxiolytic-like activity, thus outperforming the reference drug quetiapine. The

present study provided grounds for considering compounds characterized by the given mechanism of action as potential candidates for further evaluation in the treatment of BPSD.

AUTHOR INFORMATION

Corresponding Author

Phone: (48)6205460 Fax: (48)126205458.

E-mail: adam.bucki@uj.edu.pl

Author Contributions

All authors have contributed and have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): AB and JS have received honoraria from and MK is an employee of Adamed Pharma S.A. The other authors report no conflict of interest and have nothing to disclose.

ACKNOWLEDGMENT

This work was supported by Adamed Pharma S.A., The National Science Centre (NCN) grant No. DEC-2014/15/D/NZ7/01789 and Jagiellonian University Medical College (N42/DBS/000017).

REFERENCES

[1] WHO | Dementia, WHO. (n.d.). http://www.who.int/mediacentre/factsheets/fs362/en/ (accessed March 16, 2018).
[2] D.V. Jeste, S.I. Finkel, Psychosis of Alzheimer's disease and related dementias: Diagnostic criteria for a distinct syndrome, Am. J. Geriatr. Psychiatry. 8 (2000) 29–34.
[3] S.E. Arnold, B.R. Franz, J.Q. Trojanowski, Elderly patients with schizophrenia exhibit infrequent neurodegenerative lesions, Neurobiol. Aging. 15 (1994) 299–303. https://doi.org/10.1016/0197-4580(94)90024-8.
[4] M. Strassnig, M. Ganguli, About a Peculiar Disease of the Cerebral Cortex, Psychiatry Edgmont. 2 (2005) 30–33.

[5] C.G. Lyketsos, J. Olin, Depression in Alzheimer's disease: overview and treatment, Biol. Psychiatry. 52 (2002) 243–252. https://doi.org/10.1016/S0006-3223(02)01348-3.

[6] E.C. Hersch, S. Falzgraf, Management of the behavioral and psychological symptoms of dementia, Clin. Interv. Aging. 2 (2007) 611–621.

[7] D.V. Jeste, D. Blazer, D. Casey, T. Meeks, C. Salzman, L. Schneider, P. Tariot, K. Yaffe, ACNP White Paper: Update on Use of Antipsychotic Drugs in Elderly Persons with Dementia, Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 33 (2008) 957–970. https://doi.org/10.1038/sj.npp.1301492.

[8] C. Ballard, A. Corbett, R. Chitramohan, D. Aarsland, Management of agitation and aggression associated with Alzheimer's disease: controversies and possible solutions, Curr. Opin. Psychiatry. 22 (2009) 532–540. https://doi.org/10.1097/YCO.0b013e32833111f9.

[9] P.N. Tariot, L. Schneider, I.R. Katz, J.E. Mintzer, J. Street, M. Copenhaver, C. Williams-Hughes, Quetiapine Treatment of Psychosis Associated With Dementia: A Double-Blind, Randomized, Placebo-Controlled Clinical Trial, Am. J. Geriatr. Psychiatry. 14 (2006) 767–776. https://doi.org/10.1097/01.JGP.0000196628.12010.35.

[10] J.M. Ringman, L. Schneider, Treatment Options for Agitation in Dementia, Curr. Treat. Options Neurol. 21 (2019) 30. https://doi.org/10.1007/s11940-019-0572-3.

[11] H.Y. Meltzer, B.W. Massey, M. Horiguchi, Serotonin receptors as targets for drugs useful to treat psychosis and cognitive impairment in schizophrenia, Curr. Pharm. Biotechnol. 13 (2012) 1572–1586.

[12] C. Ballard, C. Banister, Z. Khan, J. Cummings, G. Demos, B. Coate, J.M. Youakim, R. Owen, S. Stankovic, ADP Investigators, Evaluation of the safety, tolerability, and efficacy of pimavanserin versus placebo in patients with Alzheimer's disease psychosis: a phase 2, randomised, placebo-controlled, double-blind study, Lancet Neurol. 17 (2018) 213–222. https://doi.org/10.1016/S1474-4422(18)30039-5.

[13] ACADIA Pharmaceuticals Announces Pivotal Phase 3 HARMONY Trial Stopped Early for Positive Efficacy as Pimavanserin Meets the Primary Endpoint in Patients with Dementia-Related Psychosis, ACADIA Pharm. Inc. (n.d.). http://ir.acadia-pharm.com/news-releases/news-release-details/acadia-pharmaceuticals-announces-pivotal-phase-3-harmony-trial (accessed September 11, 2019).

[14] M. Marcinkowska, J. Śniecikowska, N. Fajkis, P. Paśko, W. Franczyk, M. Kołaczkowski, Management of Dementia-Related Psychosis, Agitation and Aggression: A Review of the Pharmacology and Clinical Effects of Potential Drug Candidates, CNS Drugs. (2020). https://doi.org/10.1007/s40263-020-00707-7.

[15] D. Wilkinson, K. Windfeld, E. Colding-Jørgensen, Safety and efficacy of idalopirdine, a 5-HT6 receptor antagonist, in patients with moderate Alzheimer's disease (LADDER): a randomised, double-blind, placebo-controlled phase 2 trial, Lancet Neurol. 13 (2014) 1092–1099. https://doi.org/10.1016/S1474-4422(14)70198-X.

[16] I.E.M. de Jong, A. Mørk, Antagonism of the 5-HT6 receptor - Preclinical rationale for the treatment of Alzheimer's disease, Neuropharmacology. 125 (2017) 50–63. https://doi.org/10.1016/j.neuropharm.2017.07.010.

[17] M.N. Modica, E. Lacivita, S. Intagliata, L. Salerno, G. Romeo, V. Pittalà, M. Leopoldo, Structure–Activity Relationships and Therapeutic Potentials of 5-HT7 Receptor Ligands: An Update, J. Med. Chem. (2018). https://doi.org/10.1021/acs.jmedchem.7b01898.

[18] Y. Ohno, N. Kunisawa, S. Shimizu, Antipsychotic Treatment of Behavioral and Psychological Symptoms of Dementia (BPSD): Management of Extrapyramidal Side Effects, Front. Pharmacol. 10 (2019). https://doi.org/10.3389/fphar.2019.01045.

[19] M. Kołaczkowski, A. Bucki, M. Feder, M. Pawłowski, Ligand-Optimized Homology Models of D1 and D2 Dopamine Receptors: Application for Virtual Screening, J. Chem. Inf. Model. 53 (2013) 638–648. https://doi.org/10.1021/ci300413h.

[20] A. Bucki, M. Marcinkowska, J. Śniecikowska, K. Więckowski, M. Pawłowski, M. Głuch-Lutwin, A. Gryboś, A. Siwek, K. Pytka, M. Jastrzębska-Więsek, A. Partyka, A. Wesołowska, P. Mierzejewski, M. Kołaczkowski, Novel 3-(1,2,3,6-Tetrahydropyridin-4-yl)-1H-indole-Based Multifunctional Ligands with Antipsychotic-Like, Mood-Modulating, and Procognitive Activity, J. Med. Chem. 60 (2017) 7483–7501. https://doi.org/10.1021/acs.jmedchem.7b00839.

[21] A. Więckowska, M. Kołaczkowski, A. Bucki, J. Godyń, M. Marcinkowska, K. Więckowski, P. Zaręba, A. Siwek, G. Kazek, M. Głuch-Lutwin, P. Mierzejewski, P. Bienkowski, H. Sienkiewicz-Jarosz, D. Knez, T. Wichur, S. Gobec, B. Malawska, Novel multi-target-directed ligands for Alzheimer's disease: Combining cholinesterase inhibitors and 5-HT6 receptor antagonists. Design, synthesis and biological evaluation, Eur. J. Med. Chem. 124 (2016) 63–81. https://doi.org/10.1016/j.ejmech.2016.08.016.

[22] D. Wacker, C. Wang, V. Katritch, G.W. Han, X.-P. Huang, E. Vardy, J.D. McCorvy, Y. Jiang, M. Chu, F.Y. Siu, W. Liu, H.E. Xu, V. Cherezov, B.L. Roth, R.C. Stevens, Structural features for functional selectivity at serotonin receptors, Science. 340 (2013) 615–619. https://doi.org/10.1126/science.1232808.

[23] M.A. Kurowski, J.M. Bujnicki, GeneSilico protein structure prediction meta-server, Nucleic Acids Res. 31 (2003) 3305–3307. https://doi.org/10.1093/nar/gkg557.

[24] L. Bordoli, T. Schwede, Automated Protein Structure Modeling with SWISS-MODEL Workspace and the Protein Model Portal, Methods Mol. Biol. Clifton NJ. 857 (2012) 107–136. https://doi.org/10.1007/978-1-61779-588-6_5.

[25] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 46 (2001) 3–26.

[26] D.F. Veber, S.R. Johnson, H.-Y. Cheng, B.R. Smith, K.W. Ward, K.D. Kopple, Molecular properties that influence the oral bioavailability of drug candidates, J. Med. Chem. 45 (2002) 2615–2623.

[27] Z. Rankovic, CNS Drug Design: Balancing Physicochemical Properties for Optimal Brain Exposure, J. Med. Chem. 58 (2015) 2584–2608. https://doi.org/10.1021/jm501535r.

[28] T.T. Wager, X. Hou, P.R. Verhoest, A. Villalobos, Central Nervous System Multiparameter Optimization Desirability: Application in Drug Discovery, ACS Chem. Neurosci. 7 (2016) 767–775. https://doi.org/10.1021/acschemneuro.6b00029.

[29] A. Daina, O. Michielin, V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep. 7 (2017) 42717. https://doi.org/10.1038/srep42717.

[30] E.G. Peralta, A. Ashkenazi, J.W. Winslow, D.H. Smith, J. Ramachandran, D.J. Capon, Distinct primary structures, ligand-binding properties and tissue-specific expression of four human muscarinic acetylcholine receptors., EMBO J. 6 (1987) 3923–3929.

[31] S.O. Ogren, H. Hall, C. Köhler, O. Magnusson, S.E. Sjöstrand, The selective dopamine D2 receptor antagonist raclopride discriminates between dopamine-mediated motor functions, Psychopharmacology (Berl.). 90 (1986) 287–294.

[32] R.D. Porsolt, A. Bertin, M. Jalfre, Behavioral despair in mice: a primary screening test for antidepressants, Arch. Int. Pharmacodyn. Ther. 229 (1977) 327–336.

[33] C. Aron, P. Simon, C. Larousse, J.R. Boissier, Evaluation of a rapid technique for detecting minor tranquilizers, Neuropharmacology. 10 (1971) 459–469.

[34] K. Pytka, K. Gawlik, D. Pawlica-Gosiewska, J. Witalis, A. Waszkielewicz, HBK-14 and HBK-15 with antidepressant-like and/or memory-enhancing properties increase serotonin levels in the hippocampus after chronic treatment in mice, Metab. Brain Dis. 32 (2017) 547–556. https://doi.org/10.1007/s11011-016-9932-9.

[35] K. Pytka, M. Głuch-Lutwin, M. Kotańska, E. Żmudzka, M. Jakubczyk, A. Waszkielewicz, P. Janiszewska, M. Walczak, HBK-15 protects mice from stress-induced behavioral disturbances and changes in corticosterone, BDNF, and NGF levels, Behav. Brain Res. 333 (2017) 54–66. https://doi.org/10.1016/j.bbr.2017.06.032.

[36] M. Kołaczkowski, P. Mierzejewski, P. Bienkowski, A. Wesołowska, A. Newman-Tancredi, Antipsychotic, antidepressant, and cognitive-impairment properties of antipsychotics: rat profile and implications for behavioral and psychological symptoms of dementia, Naunyn. Schmiedebergs Arch. Pharmacol. 387 (2014) 545–557. https://doi.org/10.1007/s00210-014-0966-4.

[37] M. Kołaczkowski, M. Marcinkowska, A. Bucki, M. Pawłowski, K. Mitka, J. Jaśkowska, P. Kowalski, G. Kazek, A. Siwek, A. Wasik, A. Wesołowska, P. Mierzejewski, P. Bienkowski, Novel Arylsulfonamide Derivatives with 5-HT6/5-HT7 Receptor Antagonism Targeting Behavioral and Psychological Symptoms of Dementia, J. Med. Chem. 57 (2014) 4543–4557. https://doi.org/10.1021/jm401895u.

[38] M. Kołaczkowski, M. Marcinkowska, A. Bucki, J. Śniecikowska, M. Pawłowski, G. Kazek, A. Siwek, M. Jastrzębska-Więsek, A. Partyka, A. Wasik, A. Wesołowska, P. Mierzejewski, P. Bienkowski, Novel 5-HT6 receptor antagonists/D2 receptor partial agonists targeting behavioral and psychological symptoms of dementia, Eur. J. Med. Chem. 92 (2015) 221–235. https://doi.org/10.1016/j.ejmech.2014.12.045.

[39] I.T. Forbes, S. Dabbs, D.M. Duckworth, A.J. Jennings, F.D. King, P.J. Lovell, A.M. Brown, L. Collin, J.J. Hagan, D.N. Middlemiss, G.J. Riley, D.R. Thomas, N. Upton, (R)-3,N-Dimethyl-N-[1-methyl-3-(4-methylpiperidin-1-yl)propyl]benzenesulfonamide: The First Selective 5-HT7 Receptor Antagonist, J. Med. Chem. 41 (1998) 655–657. https://doi.org/10.1021/jm970519e.

[40] S.M. Bromidge, M. Duckworth, I.T. Forbes, P. Ham, F.D. King, K.M. Thewlis, F.E. Blaney, C.B. Naylor, T.P. Blackburn, G.A. Kennett, M.D. Wood, S.E. Clarke, 6-Chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5- pyridyl]carbamoyl]- indoline (SB-242084): the first selective and brain penetrant 5-HT2C receptor antagonist, J.Med.Chem. 40 (1997) 3494–3496.

[41] S. Fielding, W.J. Novick, H.M. Geyer, W.W. Petko, J.C. Wilker, L. Davis, J.T. Klein, M. Cornfeldt, The preclinical antipsychotic evaluation of HRP 913, a novel benzisoxazole derivative, Drug Dev. Res. 3 (1983) 233–243. https://doi.org/10.1002/ddr.430030305.

[42] S. Kongsamut, J.E. Roehr, J. Cai, H.B. Hartman, P. Weissensee, L.L. Kerman, L. Tang, A. Sandrasagra, Iloperidone binding to human and rat dopamine and 5-HT receptors, Eur.J.Pharmacol. 317 (1996) 417–423.

[43] S.R. Marder, R.C. Meibach, Risperidone in the treatment of schizophrenia, Am. J. Psychiatry. 151 (1994) 825–835. https://doi.org/10.1176/ajp.151.6.825.

[44] D.L. al et, 3-substituted-1,2-benzisoxazoles: novel antipsychotic agents. - PubMed - NCBI, (n.d.). https://www-1ncbi-1nlm-1nih-1gov-1nb5yeie50359.hanproxy.cm-uj.krakow.pl/pubmed/?term=1356026 (accessed July 31, 2018).

[45] M. Kolaczkowski, M. Marcinkowska, A. Bucki, M. Pawlowski, A. Krukowski, R. Rusiecki, A.M. Siwek, M.A. Wolak, Sulphonamide Derivatives of Alicyclic Amines for the Treatment of Central Nervous System Diseases, WO2013001505 (A2), 03.01.2013, 2013.

[46] M. Kołaczkowski, M. Nowak, M. Pawłowski, A.J. Bojarski, Receptor-based pharmacophores for serotonin 5-HT7R antagonists-implications to selectivity, J. Med. Chem. 49 (2006) 6732–6741. https://doi.org/10.1021/jm060300c.

[47] A. Zagórska, A. Partyka, A. Bucki, M. Kołaczkowski, M. Jastrzębska-Więsek, A. Czopek, A. Siwek, M. Głuch-Lutwin, M. Bednarski, M. Bajda, J. Jończyk, K. Piska, P. Koczurkiewicz, A. Wesołowska, M. Pawłowski, Characteristics of metabolic stability and the cell permeability of 2-pyrimidinyl-piperazinyl-alkyl derivatives of 1H-imidazo[2,1-f]purine-2,4(3H,8H)-dione with antidepressant- and anxiolytic-like activities, Chem. Biol. Drug Des. 93 (2019) 511–521. https://doi.org/10.1111/cbdd.13442.

[48] D.C. Hoffman, H. Donovan, Catalepsy as a rodent model for detecting antipsychotic drugs with extrapyramidal side effect liability, Psychopharmacology (Berl.). 120 (1995) 128–133. https://doi.org/10.1007/BF02246184.

Multifunctional 6-fluoro-3-[3-(pyrrolidin-1-yl)propyl]-1,2-benzoxazoles targeting behavioral and psychological symptoms of dementia (BPSD)

Adam Bucki, Monika Marcinkowska, Joanna Śniecikowska, Agnieszka Zagórska, Marek Jamrozik, Maciej Pawłowski, Monika Głuch-Lutwin , Agata Siwek, Magdalena Jakubczyk, Karolina Pytka, Magdalena Jastrzębska-Więsek , Anna Partyka, Anna Wesołowska, Paweł Mierzejewski and Marcin Kołaczkowski

HIGHLIGHTS:

- Novel multitarget-directed ligands target non-cognitive symptoms of dementia - BPSD
- 6-fluoro-3-[3-(pyrrolidin-1-yl)propyl]-1,2-benzoxazoles – serotonin and dopamine receptors antagonists
- The most active compound displayed antipsychotic-like and mood modulating activity
- Benign safety profile and no memory deficits in the pharmacologically relevant doses
- *N*-[(3*R*)-1-[3-(6-fluoro-1,2-benzoxazol-3-yl)propyl]pyrrolidin-3-yl]-1-benzothiophene-2-sulfonamide

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Adam Bucki and Joanna Śniecikowska have received honoraria from Adamed Pharma S.A. and Marcin Kołaczkowski is an employee of Adamed Pharma S.A. The other authors report no conflict of interest and have nothing to disclose.