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Antagonists of the adenosine A_{2A} receptor based on a 2-arylbenzoxazole scaffold: Investigation of the C5- and C7-positions to enhance affinity

Romain Duroux^a, Laurence Agouridas^a, Nicolas Renault^b, Jamal El Bakali^a, Christophe Furman^b, Patricia Melnyk^a, Saïd Yous^{a*}

^a Univ. Lille, Inserm, CHU Lille, UMR-S 1172 - JPArc - Centre de Recherche Jean-Pierre AUBERT Neurosciences et Cancer, F-59000 Lille, France

^b Univ. Lille, Inserm, CHU Lille, U995 - LIRIC - Lille Inflammation Research International Center, F-59000 Lille, France

*Corresponding author. "Onco and Neurochemistry", Jean-Pierre Aubert Research Center, UMR-S1172, Faculté de Pharmacie (Université Lille), 3, rue du Prof. Laguesse, BP 83, 59006 Lille Cedex, France. E-mail: said.yous@univ-lille2.fr

Abstract

We have recently reported a series of 2-furoyl-benzoxazoles as potential A_{2A} adenosine receptor (A_{2A}R) antagonists. Two hits were identified with interesting pharmacokinetic properties but were found to bind the hA_{2A}R receptor in the micromolar-range. Herein, in order to enhance affinity toward the hA_{2A}R, we explored the C5- and C7-position of hits **1** and **2** based on docking studies. These modifications led to compounds with nanomolar-range affinity (e.g. **6a**, K_i = 40 nM) and high antagonist activity (e.g. **6a**, IC₅₀ = 70.6 nM). Selected compounds also exhibited interesting *in vitro* DMPK (Drug, Metabolism and Pharmacokinetics) properties including high solubility and low cytotoxicity. Therefore, the benzoxazole ring appears as a highly effective scaffold for the design of new A_{2A} antagonists.

Keywords: benzoxazole, A_{2A} receptor, DMPK, neurodegenerative disease

1. Introduction

Targeting the adenosine A_{2A} receptor ($A_{2A}R$) has emerged as a promising strategy for the treatment of both Alzheimer's (AD) and Parkinson's diseases (PD) [1]. This receptor, one of the four adenosine receptors with A_1 , A_{2B} and A_3 , is coupled to the stimulatory G protein [2]. Interest for $A_{2A}R$ emerged with epidemiological studies showing that people consuming regularly caffeine-based beverages over a lifetime are substantially less likely to develop these two diseases [3-4]. Indeed, in experimental models of AD and PD [5-7], caffeine exerts neuroprotective effects notably by controlling the glutamate excitotoxicity and the microglia-mediated neuroinflammation [8]. Besides, many $A_{2A}R$ antagonists have been discovered over the past few years (Fig. 1). For example, Istradefylline (KW-6002) was approved in Japan for the treatment of PD [9-11] and acts by boosting dopaminergic signaling, reducing thereby motor deficits. Preladenant [9], has also been investigated clinically but was discontinued due to a lack of efficacy. Regarding AD, it is now well established that $A_{2A}R$ antagonists lead to the improvement of spatial memory associated with the decrease of $A\beta$ amyloid burden, Tau hyperphosphorylation and neurotoxicity [7, 12].

However, although many A_{2A} antagonists display high potency, constant drawbacks remain such as poor solubility and synthetic tractability and high toxicity [8, 13-15]. These drawbacks have limited the development of potential drugs targeting this receptor. Therefore, the main challenge regarding A_{2A} antagonist development is to improve their pharmacokinetic properties and especially their solubility.

We have recently reported a series of 2-arylbenzoxazole derivatives [16] as $A_{2A}R$ antagonists. Two hits were identified from this study as shown in figure 2 (compound **1** and **2**). These ligands display promising pharmacokinetic properties but bind the *hA* $A_{2A}R$ in the micromolar-range. The present work describes the structural investigation of benzoxazole scaffold to improve binding affinity while keeping good pharmacokinetic properties.

2. Results and discussion

2.1. Molecular modeling-guided design

Putative binding mode of **2** (Fig. 3A) showed that benzoxazole scaffold interacts through an aromatic interaction with Phe168 and a hydrogen bond with Asn253. The furan forms a hydrogen bond with Asn253 and interacts with Trp246 and His250 through aromatic interactions [16]. Furthermore, the piperidine moiety of compound **2** creates an additional interaction with Glu169. This putative binding mode suggests that introducing a substituent at the C-7 position of the benzoxazole ring would improve affinity by exploring an unexplored hydrophobic pocket (Fig. 3A & 3C). This pocket delimited by Ala63, Val84, Ile274 and His278 is occupied by the 2-chlorophenol of reference triazine T4E (PBD: 3UZC, Fig 3B) [17] and not by compound **2** (Fig. 3A). Therefore, we evaluated the impact of the introduction of an aromatic ring at the C-7 position of both benzoxazoles **1** (compounds **6a-i**, Fig. 2) and **2** (compounds **11-17**, Fig. 2 & 3C). As an alternative, we introduced a primary amine at this position instead of the aromatic group in order to interact with Asn253, inducing a different orientation of the benzoxazole ring (compound **21**, Fig. 3D). We also investigated the nature of the tertiary amine, as well as the length of the linker between the amide function and the tertiary amine.

2.2. Chemistry

All designed compounds have been synthesized as summarized in Schemes 1-2. Treatment of commercially available 2-amino-6-chloro-4-nitrophenol with 2-furoyl chloride (Scheme 1) provided an equimolar mixture of mono and diacyl compounds which, after alkaline hydrolysis, gave amide **3**. Cyclisation under acidic conditions using PTSA afforded benzoxazole **4**. Compounds **5a-h** and **5i** were obtained respectively *via* Suzuki [18] and Buchwald [19] coupling. Finally, the nitro group was reduced with hydrazine hydrate in the presence of Raney Nickel to afford amines **6a-i**.

Amidation of these 5-amino-benzoxazoles, gave bromoalkyle amides **7-10** that were substituted with various tertiary amines to afford final compounds **11-17**.

To synthesize molecule **21** and its positional isomer **25** (Scheme 2), a Buchwald-Hartwig coupling was performed on benzoxazole **4** using benzophenone imine in order to generate protected exocyclic amine (**18**). The nitro function of **18** was then reduced with hydrazine hydrate in the presence of Raney Nickel and directly *N*-acylated using bromoacetyl bromide to get **19**. Classical nucleophilic substitution was then performed to give compound **20** followed by amine deprotection under acidic conditions to afford compound **21**.

To get compound **25**, similar conditions were used starting from amine deprotection of **18** under acidic conditions. Treatment of **22** with bromoacetyl bromide followed by nucleophilic substitution with piperidine and nitro group reduction afforded **25**.

2.3. Structure-Affinity Relationship studies

Affinities of benzoxazole derivatives for the *hA_{2A}R* were determined by a competitive radioligand displacement assay using [³H]-ZM241385 [20].

Firstly, comparing molecules **1** (*K_i* = 10 μM) and **6a** (*K_i* = 40 nM) on the one hand and **2** (*K_i* = 1 μM) and **11** (*K_i* = 210 nM) on the other hand, showed that adding an aromatic ring at C-7 position was beneficial for *A_{2A}* affinity (table 1). To a lesser extent, introducing an amine at the C-7 position of the benzoxazole of compound **2** (**21**, *K_i* = 480 nM) also improved affinity. Interestingly, the positional isomer of the latter (compound **25**) displayed no affinity for the *hA_{2A}R* (*K_i* > 10 μM).

Regarding the ring at C-7 position, replacement of the furan (**6a**, *K_i* = 40 nM) generally led to a loss of affinity. Indeed, while thiophene (**6b**, *K_i* = 71 nM), 3,4-dimethoxyphenyl (**6e**, *K_i* = 90 nM) were well tolerated, other substituents like 4-trifluoromethylphenyl (**6f**), 4-methylphenyl (**6h**) or morpholine (**6i**) totally abolished affinity.

First optimizations around **11** ($K_i = 210$ nM) were focused on the linker length between the tertiary amine and the amide at C-5 position. Increasing the length of the linker from one methylene group to two (**12**, $K_i = 81$ nM) or three (**13**, $K_i = 98$ nM) allowed an improvement of affinity. Based on these results, modulation of the tertiary amine of **12** was realized keeping the two-methylene linker. Replacement of the piperidine with a morpholine had no significant impact on affinity (**14**, $K_i = 90$ nM) while the more hydrophobic 1,2,4 tetrahydroisoquinoline (THQ) (**15**, $K_i = 30$ nM) allowed a moderate improvement of affinity. Noteworthy is the sharp loss of affinity when replacing the piperidine of **12** with the *N'*-(4-(2-methoxyethoxy)phenyl)-piperazinyl (PP) group of Preladenant (**16**, $K_i > 10$ μ M). Finally, whereas replacing the furan in C-7 position of **6a** by a 3,4-dimethoxyphenyl (**6e**) did not significantly affect affinity, the same modification on compound **15** ($K_i = 81$ nM) led to a dramatic loss of affinity (**17**, $K_i > 10$ μ M).

In order to confirm that these new compounds behave as functional antagonists at the $hA_{2A}R$, representative compounds **6a**, **11** and **21** were evaluated using the $GTP\gamma^{35}S$ binding assay (table 2). Experiments were performed as previously described [21]. Briefly, this technique, not often used to assess functional activity at the $A_{2A}R$, was developed in our lab using the same $hA_{2A}R$ membranes as for the competitive radioligand displacement assay. Under the assay conditions, reference $A_{2A}R$ agonist CGS241680 increased $GTP\gamma^{35}S$ binding to HEK293- $A_{2A}R$ membranes, in a concentration-dependent manner ($EC_{50} = 283$ nM). The decrease of $GTP\gamma^{35}S$ binding induced by CGS241680 (1 μ M), was quantified in the presence of increasing concentrations (10 nM - 100 μ M) of selected compounds (**6a**, **11** and **21**, table 2). ZM241385 was used as a control antagonist ($IC_{50} = 80.8$ nM). In this assay, the new compounds behaved as antagonists with **6a** displaying a potency ($IC_{50} = 70.6$ nM) in the same range as reference ZM-241385.

2.4 Preliminary *in vitro* DMPK evaluation

Compounds **2**, **6a**, **12**, **14**, **15**, were profiled for in vitro metabolic stability, plasma protein binding, aqueous solubility and intestinal absorption (table 3). Cytotoxicity was also evaluated on neuroblastoma cell line (SY5Y).

At 10 μM concentration, compounds **12**, **14** and **15** significantly bind plasma proteins whereas **6a** and **2** showed a slightly better unbound fractions. A lower permeability value was observed for molecules with a basic center (**12**, **14**, **15**) compared to **6a** which exhibits a high permeability value (P_{app} of 77×10^{-6} cm/s). Interestingly, adding a second furan in position 7 improves metabolic stability ($t_{1/2} > 41$ min) in human liver microsomes (*e.g.* compare **2** with **12**). Except for **12**, a low cytotoxicity is observed for this series. Finally, with the exception of **15**, molecules with a protonable amine (**2**, **12** and **14**) exhibit a high aqueous solubility (≥ 165 μM) which is a considerable advantage as compared to many A_{2A} antagonists (reported solubility for Preladenant and KW6002 is 1.5 μM and 20 nM, respectively) [22-24].

3. Conclusion

The present work deals with the optimization of a new series of benzoxazoles as A_{2A} antagonists. Modulation on the C-7 position of the benzoxazole ring by adding a furan significantly improved binding affinity of **1** and **2** toward $hA_{2A}R$. Compound **6a**, displayed the highest binding affinity for $hA_{2A}R$ ($K_i = 40$ nM) with high antagonist activity ($IC_{50} = 70.6$ nM). Moreover, addition of a tertiary amine-based chain at the C-5 position resulted in ligands with interesting DMPK properties, especially a high solubility, while keeping a good affinity (*e.g.* **12**: $K_i = 81$ nM). Overall, the data presented here show that the benzoxazole ring is a highly effective scaffold for the design of new A_{2A} antagonists.

4. Experimental sections

4.1. Chemistry

All reagents and solvents were purchased and used without further purification. Reactions were monitored by TLC performed on Macherey-Nagel Alugram® Sil 60/UV254 sheets (thickness 0.2 mm). Some purification of products was carried out by column chromatography using Macherey-Nagel silica gel (230-400 mesh). Melting points were determined on a BÜCHI B-540 apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance 300 spectrometer operating at 300 MHz (¹H) or 75 MHz (¹³C). Chemical shifts are in parts per million (ppm) and were referenced to the residual proton peaks in deuterated solvents. Mass spectra were recorded with an LCMS (Waters Alliance Micromass ZQ 2000). LCMS analysis was performed using a Waters XBridge C18 column (5 µm particle size column, dimensions 50 mm x 4.6 mm). A gradient starting from 98% H₂O/formate buffer 5 mM (pH 3.8) and reaching 100% CH₃CN/ formate buffer 5 mM (pH 3.8) within 4 min at a flow rate of 2 mL/min was used followed by a return to the starting conditions within 1 min. The purity of final compounds was verified by two types of high pressure liquid chromatography (HPLC) columns: C18 Interchrom UPTISPHERE and C4 Interchrom UPTISPHERE. Analytical HPLC was performed on a Shimadzu LC-2010AHT system equipped with a UV detector set at 254 nm and 215 nm. Compounds were dissolved in 50 mL acetonitrile and 950 mL buffer B, and injected into the system. The following eluent systems were used: buffer A (H₂O/TFA, 100:0.1) and buffer B (CH₃CN/H₂O/TFA, 80:20:0.1). HPLC retention times (HPLC t_R) were obtained at a flow rate of 0.2 mL/min for 35 min using the following conditions: a gradient run from 100% of buffer A over 1 min, then to 100% of buffer B over the next 30 min.

4.2. *N*-(3-Chloro-2-hydroxy-5-nitrophenyl)furan-2-carboxamide (**3**).

To a solution of 2-furoic acid (3.4 g, 0.03 mol) in DCM (90 mL) was added SOCl₂ (4.7 mL, 0.06 mol) dropwise and 4 drops of DMF. The mixture was refluxed for 12 h, cooled to room

temperature and concentrated *in vacuo*. The obtained residue was diluted in EtOAc (30 mL) and added dropwise to a solution of 2-amino-4-chloro-6-nitrophenol (3.76 g, 0.02 mol) and Et₃N (5.5 mL, 0.04 mol) in EtOAc (100 mL) at 0 °C. After 2 h stirring at room temperature, the mixture was hydrolyzed with water and extracted twice with EtOAc. Combined organic layers were washed with 1 M HCl solution, NaHCO₃, dried over MgSO₄ and concentrated *in vacuo*. Solid was suspended in H₂O/EtOH (150/20 mL) and NaOH (2.4 g, 0.06 mol) was added. The mixture was heated at 70 °C for 3 h, cooled to room temperature, acidified with 6 M HCl solution up to acid pH, filtered, washed with water and diethyl ether to afford a yellow solid (5.5 g, 97%): mp >300 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 9.73 (br s, 1H), 8.78 (br s, 1H), 8.60 (d, 1H, *J* = 2.7 Hz), 7.14 (d, 1H, *J* = 2.7 Hz), 7.99 (m, 1H), 7.37 (dd, 1H, *J* = 0.7 Hz and *J* = 3.5 Hz), 6.74 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz). ¹³C NMR (DMSO-*d*₆, 75 MHz): δ 156.9 (C), 152.8 (C), 147.2 (C), 146.7 (CH), 139.2 (C), 127.6 (C), 122.2 (CH), 121.5 (C), 118.3 (CH), 116.3 (CH), 113.0 (CH). LC-MS (ESI) *m/z* found: 283 [M+H]⁺.

4.3. 7-Chloro-2-(furan-2-yl)-5-nitro-1,3-benzoxazole (**4**).

A mixture of **3** (5.2 g, 0.018 mol) and PTSA (10.5 g, 0.054 mol) in toluene (130 mL) was refluxed overnight with a Dean-Stark apparatus. The solution was cooled to room temperature and the organic layer was washed twice with 1 M NaOH solution, dried over K₂CO₃ and concentrated *in vacuo*. The resulting solid was then recrystallization from EtOAc to afford a yellow solid (3.4 g, 71%): mp 146 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.52 (d, 1H, *J* = 2.7 Hz), 8.33 (d, 1H, *J* = 2.7 Hz), 7.78 (m, 1H), 7.45 (dd, 1H, *J* = 0.7 Hz and *J* = 3.5 Hz), 6.71 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 157.9 (C), 150.5 (C), 147.4 (CH), 145.8 (C), 142.9 (C), 140.9 (C), 121.5 (CH), 117.2 (CH), 116.7 (C), 114.7 (CH), 112.8 (CH). LC-MS (ESI) *m/z* found: 265 [M+H]⁺.

4.4. General procedure for the synthesis of compounds **5a-5g**.

A mixture of **4** (500 mg, 1.89 mmol), arylboronic acid (2.83 mmol), K₃PO₄ (2 M, 3.78 mmol), palladium diacetate (8.5 mg, 0.038 mmol) and PPh₃ (19.8 mg, 0.075 mmol) in dioxane (8 mL) was degassed and then heated in a sealed tube at 110 °C for 4 h. After cooling to room temperature, the mixture was suspended in water and extracted three times with EtOAc. Combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. The obtained solid was then recrystallized from EtOH.

4.4.1. 2,7-bis-(Furan-2-yl)-5-nitro-1,3-benzoxazole (**5a**).

Yield 65%; Beige solid mp 222 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.67 (d, 1H, *J* = 2.1 Hz), 8.47 (d, 1H, *J* = 2.1 Hz), 7.77 (m, 1H), 7.66 (m, 1H), 7.45 (m, 1H), 7.26 (m, 1H), 6.71 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz) 6.64 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz). ¹³C NMR (75 MHz, CDCl₃): δ 157.7 (C), 148.3 (C), 146.9 (CH), 146.4 (C), 146.1 (C), 143.8 (CH), 142.6 (C), 141.4 (C), 116.3 (CH), 115.9 (CH), 115.7 (C), 113.9 (CH), 112.7 (CH), 112.4 (CH), 111.9 (CH). LC-MS (ESI) *m/z* found: 297 [M+H]⁺.

4.4.2. 2-(Furan-2-yl)-5-nitro-7-(thiophen-2-yl)-1,3-benzoxazole (**5b**).

Yield 42%; Beige solid mp 206 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.51 (d, 1H, *J* = 2.1 Hz), 8.48 (d, 1H, *J* = 2.1 Hz), 8.20 (m, 1H), 8.06 (dd, 1H, *J* = 1.1 Hz and *J* = 3.7 Hz), 7.88 (dd, 1H, *J* = 1.1 Hz and *J* = 3.7 Hz), 7.69 (dd, 1H, *J* = 0.6 Hz and *J* = 3.6 Hz), 7.33 (dd, 1H, *J* = 3.7 Hz and *J* = 5.1 Hz) 6.91 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 157.9 (C), 149.4 (C), 148.8 (CH), 146.1 (C), 142.9 (C), 141.1 (C), 134.9 (C), 129.4 (CH), 129.2 (CH), 129.0 (CH), 119.1 (C), 117.8 (CH), 117.7 (CH), 114.1 (CH), 113.7 (CH). LC-MS (ESI) *m/z* found: 313 [M+H]⁺.

4.4.3. 2-(Furan-2-yl)-5-nitro-7-phenyl-1,3-benzoxazole (**5c**).

Yield 63 %; Pale yellow solid mp 193 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.58 (d, 1H, *J* = 2.2 Hz), 8.45 (d, 1H, *J* = 2.2 Hz), 8.16 (m, 1H), 8.01-7.98 (m, 2H), 7.65-7.57 (m, 3H), 7.56-7.51 (m, 1H), 6.87 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 157.9 (C), 151.2 (C), 148.6 (CH), 146.1 (C), 143.0 (C), 141.2 (C), 133.3 (C), 129.9 (CH), 129.7 (2 CH), 128.8 (2 CH), 125.2 (C), 119.9 (CH), 117.6 (CH), 114.7 (CH), 113.6 (CH). LC-MS (ESI) *m/z* found: 307 [M+H]⁺.

4.4.4. 2-(Furan-2-yl)-5-nitro-7-(pyridin-3-yl)-1,3-benzoxazole (**5d**).

Yield 54%; White solid mp 246 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 9.21 (m, 1H), 8.74 (m, 1H), 8.66 (d, 1H, *J* = 2.2 Hz), 8.74 (m, 1H), 8.57 (d, 1H, *J* = 2.2 Hz), 8.16 (m, 1H), 7.66-7.63 (m, 2H), 6.87 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 157.9 (C), 153.1 (C), 150.6 (C), 149.4 (CH), 148.7 (C), 146.2 (C), 136.4 (C), 134.8 (CH), 133.3 (CH), 129.1 (2 CH), 124.7 (C), 120.3 (CH), 117.8 (CH), 115.5 (CH), 113.7 (CH). LC-MS (ESI) *m/z* found: 308 [M+H]⁺.

4.4.5. 7-(3,4-Dimethoxyphenyl)-2-(furan-2-yl)-5-nitro-1,3-benzoxazole (**5e**).

Yield 51%; Beige solid mp 176 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.50 (m, 1H), 8.43 (m, 1H), 8.15 (m, 1H), 7.57-7.55 (m, 3H), 7.16 (d, 1H, *J* = 8.4 Hz), 6.86 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 3.89 (s, 3H), 3.85 (s, 3H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 157.7 (C), 150.9 (C), 150.3 (C), 149.5 (C), 148.6 (CH), 146.1 (C), 142.8 (C), 141.2 (C), 125.6 (C), 125.3 (C), 121.5 (CH), 119.3 (CH), 117.3 (CH), 113.9 (CH), 113.6 (CH), 112.6 (CH), 112.0 (CH), 56.1 (CH₃), 56.0 (CH₃). LC-MS (ESI) *m/z* found: 367 [M+H]⁺.

4.4.6. 2-(Furan-2-yl)-5-nitro-7-(4-(trifluoromethyl)phenyl)-1,3-benzoxazole (**5f**).

Yield 38%; White solid mp 218 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.64 (m, 1H), 8.53 (m, 1H), 8.22 (d, 2H, *J* = 7.8 Hz), 8.16 (m, 1H), 7.97 (d, 2H, *J* = 7.8 Hz), 7.63 (m, 1H), 6.86 (m, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 157.9 (C), 151.3 (C), 148.7 (CH), 146.1 (C), 143.1 (C), 141.1 (C), 137.3 (C), 130.1 (C), 129.7 (2 CH), 126.6 (CH), 126.5 (CH), 123.5 (C), 122.7 (C), 120.3 (CH), 117.8 (CH), 115.6 (CH), 113.7 (CH). LC-MS (ESI) *m/z* found: 375 [M+H]⁺.

4.4.7. 2-(Furan-2-yl)-5-nitro-7-(*p*-tolyl)-1,3-benzoxazole (**5h**).

Yield 41%; White solid mp 178 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.55 (d, 1H, *J* = 2.2 Hz), 8.41 (d, 1H, *J* = 2.2 Hz), 8.16 (m, 1H), 7.88 (d, 2H, *J* = 8.1 Hz), 7.60 (d, 1H, *J* = 3.1 Hz), 7.43 (d, 2H, *J* = 8.1 Hz), 6.86 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 2.41 (s, 3H, Me). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 157.8 (C), 151.1 (C), 148.6 (CH), 146.1 (C), 142.9 (C), 141.2 (C), 139.6 (C), 130.4 (C), 130.3 (2 CH), 128.6 (2 CH), 125.2 (C), 119.5 (CH), 117.5 (CH), 114.4 (CH), 113.6 (CH), 21.3 (CH₃). LC-MS (ESI) *m/z* found: 321 [M+H]⁺.

4.4.8. 2-(Furan-2-yl)-7-morpholino-5-nitro-1,3-benzoxazole (**5i**).

A mixture of **4** (400 mg, 1.51 mmol), palladium diacetate (10.2 mg, 0.045 mmol), BINAP (37.6 mg, 0.061 mmol), cesium carbonate (985 mg, 3.02 mmol) and morpholine (0.2 mL, 2.27 mmol) in toluene (7 mL) was degassed and heated in a sealed tube for 3 h. The reaction was cooled to room temperature, hydrolyzed with water and extracted with DCM. Organic layer was washed with water, dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was suspended in EtOAc and then filtered to afford a yellow solid (253 mg, 53%): mp 214 °C. ¹H NMR (300 MHz, CDCl₃): δ 8.19 (d, 1H, *J* = 2.1 Hz), 7.73 (m, 1H), 7.70 (d, 1H, *J* = 2.1 Hz), 7.36 (d, 1H, *J* = 3.5 Hz), 7.67 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 3.99 (t, 4H, *J* = 4.7 Hz), 3.49 (t, 4H, *J* = 4.7 Hz). ¹³C NMR (75 MHz, CDCl₃): δ 156.6 (C), 146.6 (CH), 146.4 (C), 144.2 (C), 143.0

(C), 141.5 (C), 136.6 (C), 115.7 (CH), 112.6 (CH), 108.2 (CH), 106.7 (CH), 66.6 (2 CH₂), 49.2 (2 CH₂). LC-MS (ESI) m/z found: 316 [M+H]⁺.

4.5. General procedure for the synthesis of compounds (**6a-6g**).

To a solution of **5** (1.01 mmol) in EtOAc (20 mL) was added Raney Nickel and hydrazine monohydrate (1.52 mmol). The mixture was stirred for 30 min at room temperature, catalyst was then filtered off and the mixture was hydrolyzed with water and extracted three times with EtOAc. Combined organic layers were washed with brine, dried over MgSO₄ and concentrated *in vacuo*. Solid was then recrystallized from a mixture of diethyl ether/petroleum ether (10/1).

4.5.1 2,7-Di(furan-2-yl)benzoxazol-5-amine (**6a**).

Yield 74%; Beige solid mp 160 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.05 (m, 1H), 7.86 (m, 1H), 7.51 (d, 1H, *J* = 3.3 Hz), 7.13 (d, 1H, *J* = 3.3 Hz), 7.04 (d, 1H, *J* = 2.0 Hz), 6.82-6.79 (m, 2H), 6.71 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 5.28 (br s, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 155.2 (C), 148.5 (C), 147.5 (C), 147.2 (CH), 143.7 (CH), 143.3 (C), 142.3 (C), 137.7 (C), 115.1 (CH), 114.7 (C), 113.2 (CH), 112.8 (CH), 110.1 (CH), 107.6 (CH), 102.4 (CH). LC-MS (ESI) m/z found: 267 [M+H]⁺. HPLC: C4 column: t_R = 13.6 min, purity >99%; C18 column: t_R = 21.8 min, purity >99%.

4.5.2. 2-(Furan-2-yl)-7-(thiophen-2-yl)-1,3-benzoxazol-5-amine (**6b**).

Yield 17%; Beige solid mp 158 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.07 (m, 1H), 7.77 (dd, 1H, *J* = 1.0 Hz and *J* = 3.6 Hz), 7.68 (dd, 1H, *J* = 0.9 Hz and *J* = 5.1 Hz), 7.46 (d, 1H, *J* = 3.5 Hz), 7.26 (dd, 1H, *J* = 3.6 Hz and *J* = 5.0 Hz), 6.97 (d, 1H, *J* = 2.0 Hz), 6.91 (m, 2H), 5.27 (br s, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 155.2 (C), 147.6 (C), 147.2 (CH), 143.5 (C), 142.4 (C), 138.6 (C), 138.0 (C), 128.9 (CH), 126.8 (2 CH), 118.0 (C), 115.0 (CH), 113.2 (CH), 110.0 (CH), 102.5 (CH). LC-MS (ESI) m/z found: 283 [M+H]⁺. HPLC: C4 column: t_R = 14.1 min, purity >99%; C18 column: t_R = 22.7 min, purity 95%.

4.5.3. 2-(Furan-2-yl)-7-phenyl-1,3-benzoxazol-5-amine (**6c**).

Yield 82%; White solid mp 182 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.03 (m, 1H), 7.85-7.82 (m, 2H), 7.58-7.53 (m, 2H), 7.44 (m, 1H), 7.38 (dd, 1H, *J* = 0.7 Hz and *J* = 3.5 Hz), 6.89-6.78 (m, 3H), 5.24 (br s, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 155.1 (C), 147.6 (C), 147.0 (CH), 143.5 (C), 142.4 (C), 140.0 (C), 135.8 (C), 129.4 (2 CH), 128.5 (CH), 128.1 (2 CH), 124.4 (C), 114.7 (CH), 113.1 (CH), 112.1 (CH), 102.6 (CH). LC-MS (ESI) *m/z* found: 277 [M+H]⁺. HPLC: C4 column: *t*_R = 14.05 min, purity >99%; C18 column: *t*_R = 22.9 min, purity >99%.

4.5.4. 2-(Furan-2-yl)-7-(pyridin-3-yl)-1,3-benzoxazol-5-amine (**6d**).

Yield 70%; White solid mp 176 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 9.03 (d, 1H, *J* = 1.8 Hz), 8.63 (dd, 1H, *J* = 1.8 Hz and *J* = 4.7 Hz), 8.22-8.18 (m, 1H), 8.03 (m, 1H), 7.60-7.56 (m, 1H), 7.39 (d, 1H, *J* = 3.5 Hz), 6.95 (d, 1H, *J* = 2.0 Hz), 6.90 (d, 1H, *J* = 2.0 Hz), 6.79 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 5.28 (br s, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 155.3 (C), 149.5 (C), 148.8 (CH), 147.8 (CH), 147.2 (CH), 143.6 (C), 142.3 (C), 140.0 (C), 135.5 (CH), 131.6 (C), 124.5 (CH), 121.1 (C), 115.0 (CH), 113.2 (CH), 111.9 (CH), 103.3 (CH). LC-MS (ESI) *m/z* found: 278 [M+H]⁺. HPLC: C4 column: *t*_R = 14.88 min, purity >99%; C18 column: *t*_R = 17.07 min, purity >99%.

4.5.5. 7-(3,4-Dimethoxyphenyl)-2-(furan-2-yl)-1,3-benzoxazol-5-amine (**6e**).

Yield 35%; White solid mp 172 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.02 (d, 1H), 7.40-7.34 (m, 3H), 7.14-7.11 (m, 1H), 6.91 (m, 1H), 6.80 (m, 2H), 5.17 (br s, 2H), 3.86 (s, 3H), 3.83 (s, 3H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 155.0 (C), 149.3 (C), 149.2 (C), 147.5 (C), 147.0 (CH), 143.4 (C), 142.5 (C), 139.9 (C), 128.2 (C), 124.3 (C), 120.6 (CH), 114.6 (CH), 113.1 (CH), 112.6 (CH), 111.7 (2 CH), 102.0 (CH), 56.1 (CH₃), 56.0 (CH₃). LC-MS (ESI) *m/z* found: 337 [M+H]⁺. HPLC: C4 column: *t*_R = 14.2 min, purity >99%; C18 column: *t*_R = 22.1 min, purity 95%.

4.5.6. 2-(Furan-2-yl)-7-(4-(trifluoromethyl)phenyl)-1,3-benzoxazol-5-amine (**6f**).

Yield 33%; Beige solid mp 206 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.05 (m, 3H), 7.91 (d, 2H, *J* = 8.2 Hz), 7.41 (d, 1H, *J* = 3.3 Hz), 6.98 (d, 1H, *J* = 1.9 Hz), 6.92 (d, 1H, *J* = 1.9 Hz), 6.79 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 5.30 (br s, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 155.3 (C), 147.8 (CH), 147.2 (CH), 143.6 (C), 142.3 (C), 140.0 (C), 139.8 (C), 128.9 (CH), 128.6 (C), 126.5 (C), 126.4 (CH), 126.3 (CH), 122.9 (C), 122.7 (C), 115.0 (CH), 113.2 (CH), 112.1 (CH), 103.6 (CH). LC-MS (ESI) *m/z* found: 345 [M+H]⁺. HPLC: C4 column: *t*_R = 16.1 min, purity 98%; C18 column: *t*_R = 26.03 min, purity >99%.

4.5.7. 7-(4-Fluorophenyl)-2-(furan-2-yl)-1,3-benzoxazol-5-amine (**6g**).

Same procedure as describe for compound **5** was used starting from **4** (400 mg, 1.51 mmol), K₃PO₄ (641 mg, 2M, 3.02 mmol), 4-fluorophenylboronic acid (317 mg, 2.27 mmol), palladium diacetate (2%, 6.79 mg, 0.030 mmol) and triphenylphosphine (4%, 15.9 mg, 0.061 mmol) in dioxane (7 mL). Final solid was suspended with cold diethyl ether and filtered to afford a beige solid which was directly engaged in next step. Solid was suspended in EtOAc (17 mL) and Raney Ni (70 mg) was added followed by hydrazine hydrate (0.073 mL, 1.5 mmol). The mixture was stirred for 2 h at room temperature and then the catalyst was filtered off. The reaction mixture was hydrolyzed with water and then extracted three times with ethyl acetate. Combined organic layers were dried over MgSO₄, and evaporated *in vacuo* to afford a solid which was recrystallized using diethyl ether and petroleum ether (10/1), to afford **6g** as a beige solid (70 mg, 24%). mp 173 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.04 (m, 1H), 7.85 (dd, 2H, *J* = 5.6 Hz and *J* = 8.7 Hz), 7.42-7.36 (m, 3H), 6.89 (d, 1H, *J* = 1.8 Hz), 6.84 (d, 1H, *J* = 1.8 Hz), 6.79 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 5.23 (br s, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 164.0 (C), 160.7 (C), 155.1 (C), 147.7 (CH), 147.1 (C), 143.4 (C), 142.4 (C), 139.9 (C), 132.2 (CH), 123.3 (C), 130.1 (CH), 116.5 (CH), 116.2 (CH), 114.8 (CH), 113.1 (CH), 112.0 (CH),

102.6 (CH). LC-MS (ESI) m/z found: 295 [M+H]⁺. HPLC: C4 column: t_R = 14.3 min, purity >99%; C18 column: t_R = 23.5 min, purity >99%.

4.5.8. 2-(Furan-2-yl)-7-(p-tolyl)-1,3-benzoxazol-5-amine (**6h**).

Yield 65%; White solid mp 182 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.03 (m, 1H), 7.71 (m, 2H), 7.36 (m, 3H), 6.89-6.78 (m, 3H), 5.20 (br s, 2H), 2.38 (s, 3H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 155.1 (C), 147.6 (C), 147.0 (CH), 143.4 (C), 142.5 (C), 140.0 (C), 137.9 (C), 132.8 (C), 130.0 (2 CH), 129.8 (2 CH), 124.3 (C), 114.7 (CH), 113.1 (CH), 111.8 (CH), 102.3 (CH), 21.3 (CH₃). LC-MS (ESI) m/z found: 291 [M+H]⁺. HPLC: C4 column: t_R = 14.9 min, purity 98%; C18 column: t_R = 24.2 min, purity >99%.

4.5.9. 2-(Furan-2-yl)-7-morpholino-1,3-benzoxazol-5-amine (**6i**).

Yield 27%; Pale yellow solid mp 158 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.00 (m, 1H), 7.34 (d, 1H, *J* = 3.1 Hz), 6.75 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 6.39 (d, 1H, *J* = 1.8 Hz), 6.17 (d, 1H, *J* = 1.8 Hz), 5.01 (br s, 2H), 3.80 (t, 4H, *J* = 4.4 Hz), 3.80 (t, 4H, *J* = 4.4 Hz). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 154.0 (C), 147.7 (C), 146.8 (C), 143.8 (CH), 142.5 (C), 137.0 (C), 133.7 (C), 114.3 (CH), 113.0 (CH), 100.5 (CH), 95.4 (CH), 66.5 (2 CH₂), 49.6 (2 CH₂). LC-MS (ESI) m/z found: 286 [M+H]⁺. HPLC: C4 column: t_R = 7.95 min, purity 98%; C18 column: t_R = 19.4 min, purity >99%.

4.6. General procedure for the synthesis of compounds **7-10**.

To a solution of **6a** or **6e** (0.676 mmol) and K₂CO₃ (2.03 mmol) in EtOAc/H₂O (10/3 mL) at 0 °C was added dropwise bromoalkanoyl halide (0.88 mmol) diluted in EtOAc (3 mL). After 1 h stirring at room temperature, water was added and the mixture was extracted three times with EtOAc. Combined organic layers were washed with 1 M HCl solution, brine, dried over MgSO₄ and concentrated *in vacuo*. Resulting solid was suspended in diethyl ether and filtered to afford derivatives **7-10**.

4.6.1. 2-Bromo-*N*-(2,7-di(furan-2-yl)-1,3-benzoxazol-5-yl)acetamide (**7**).

Yield 63 %; Beige solid mp 244 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.67 (br s, 1H), 8.11 (m, 1H), 7.97-7.94 (m, 3H), 7.64 (d, 1H, *J* = 3.0 Hz), 7.26 (d, 1H, *J* = 3.0 Hz), 6.85 (m, 1H), 6.77 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 4.09 (s, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 165.5 (C), 156.2 (C), 155.9 (C), 147.4 (CH), 144.5 (CH), 142.6 (C), 141.7 (C), 141.6 (C), 136.3 (C), 116.4 (CH), 114.9 (C), 113.4 (CH), 113.1 (CH), 112.2 (CH), 111.3 (CH), 109.5 (CH), 62.4 (CH₂). LC-MS (ESI) *m/z* found: 387, 389 [M+H]⁺.

4.6.2. 3-Bromo-*N*-(2,7-di(furan-2-yl)-1,3-benzoxazol-5-yl)propanamide (**8**).

Yield 39%; Beige solid mp 250 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.35 (br s, 1H), 8.11 (m, 1H), 8.00-7.98 (m, 2H), 7.93 (m, 1H), 7.64 (dd, 1H, *J* = 0.6 Hz and *J* = 3.6 Hz), 7.25 (dd, 1H, *J* = 0.6 Hz and *J* = 3.6 Hz), 6.85 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 6.76 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 3.77 (t, 2H, *J* = 6.3 Hz), 3.00 (t, 2H, *J* = 6.4 Hz). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 168.8 (C), 156.0 (C), 147.8 (CH), 147.6 (C), 144.3 (CH), 142.6 (C), 141.8 (C), 141.1 (C), 137.2 (C), 116.1 (CH), 114.7 (C), 113.3 (CH), 113.0 (CH), 111.9 (CH), 111.0 (CH), 109.0 (CH), 41.3 (CH₂), 29.6 (CH₂). LC-MS (ESI) *m/z* found: 401, 403 [M+H]⁺.

4.6.3. 4-Bromo-*N*-(2,7-di(furan-2-yl)-1,3-benzoxazol-5-yl)butanamide (**9**).

Yield 36%; Pale brown mp 275 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.26 (br s, 1H), 8.11 (m, 1H), 8.00-7.98 (m, 2H), 7.93 (m, 1H), 7.64 (dd, 1H, *J* = 3.0 Hz), 7.25 (dd, 1H, *J* = 3.0 Hz), 6.85 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 6.76 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 3.62 (t, 2H, *J* = 6.6 Hz), 2.55 (t, 2H, *J* = 7.2 Hz), 2.20-2.13 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 168.6 (C), 156.6 (C), 148.3 (CH), 147.7 (C), 144.5 (CH), 142.9 (C), 142.3 (C), 141.2 (C), 137.3 (C), 116.1 (CH), 114.7 (C), 113.5 (CH), 113.9 (CH), 112.2 (CH), 111.1 (CH), 109.0 (CH), 32.3 (CH₂), 28.6 (CH₂), 24.5 (CH₂). LC-MS (ESI) *m/z* found: 415, 417 [M+H]⁺.

4.6.4. 3-Bromo-*N*-(7-(3,4-dimethoxyphenyl)-2-(furan-2-yl)-1,3-benzoxazol-5-yl)propanamide (**10**).

Yield 42%; White solid mp 173 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.30 (br s, 1H), 8.08 (m, 2H), 7.73 (d, 1H, *J* = 1.9 Hz), 7.45-7.42 (m, 3H), 7.16 (m, 1H), 6.82 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 3.88 (s, 3H), 3.84 (s, 3H), 3.75 (m, 2H), 3.00 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 168.8 (C), 155.9 (C), 149.6 (CH), 149.4 (C), 147.6 (CH), 143.5 (C), 142.6 (C), 142.0 (C), 137.2 (C), 127.3 (C), 124.5 (CH), 120.8 (C), 115.8 (CH), 115.6 (CH), 113.3 (CH), 112.7 (CH), 111.7 (CH), 108.8 (CH), 56.1 (CH₃), 56.0 (CH₃), 55.7 (CH₂), 29.6 (CH₂). LC-MS (ESI) *m/z* found: 471, 473 [M+H]⁺.

4.7. General procedure for the synthesis of compounds **11-17**.

To a solution of appropriate compound **7-10** (0.387 mmol) in acetonitrile (20 mL) was added Et₃N (0.775 mmol) and appropriate amine (0.426 mmol). The solution was refluxed for 6 h and then concentrated *in vacuo*. Crude was suspended in water and extracted three times with EtOAc. Combined organic layers were dried over MgSO₄ and concentrated *in vacuo* to give a solid which was then purified.

4.7.1. *N*-(2,7-Di(furan-2-yl)-1,3-benzoxazol-5-yl)-2-(piperidin-1-yl)acetamide hydrochloride (**11**).

The title compound was prepared from **7** and piperidine. Solid was suspended in acetonitrile with HCl(g), concentrated *in vacuo* and recrystallized from ethanol to afford **11**. Yield 78%; White solid mp > 300 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 11.39 (br s, 1H), 10.03 (br s, 1H), 8.12 (m, 1H), 8.05 (m, 1H), 8.03 (m, 1H), 7.94 (m, 1H), 7.64 (d, 1H, *J* = 3.4 Hz), 7.27 (d, 1H, *J* = 3.4 Hz), 6.85 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 6.77 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 4.21 (s, 2H), 3.50 (m, 2H), 3.12 (m, 2H), 1.18 (m, 4H), 1.68 (m, 1H), 1.42 (m, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 163.6 (C), 156.2 (C), 148.0 (C), 147.4 (CH), 144.5 (CH), 142.6

(C), 141.7 (C), 141.6 (C), 136.3 (C), 116.4 (CH), 114.9 (C), 113.4 (CH), 113.1 (CH), 112.2 (CH), 111.3 (CH), 109.5 (CH), 57.6 (CH₂), 53.5 (2 CH₂), 22.7 (2 CH₂), 21.6 (CH₂). LC-MS (ESI) m/z found: 392 [M+H]⁺. HPLC: C4 column: t_R = 15.9 min, purity >99%; C18 column: t_R = 24.5 min, purity >99%.

4.7.2. *N*-(2,7-Di(furan-2-yl)-1,3-benzoxazol-5-yl)-3-(piperidin-1-yl)propanamide (**12**).

The title compound was prepared from **8** and piperidine and recrystallized from EtOAc to afford **12**. Yield 61%; White solid mp 268 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.40 (br s, 1H), 8.11 (m, 1H), 7.98 (d, 1H, *J* = 1.8 Hz), 7.95 (d, 1H, *J* = 1.8 Hz), 7.93 (d, 1H, *J* = 1.2 Hz), 7.62 (d, 1H, *J* = 3.0 Hz), 7.24 (d, 1H, *J* = 3.0 Hz), 6.84 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 6.76 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 2.63 (t, 2H, *J* = 6.3 Hz), 2.50 (m, 2H, *J* = 6.4 Hz), 2.40 (m, 4H), 1.50 (m, 4H), 1.39 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 171.0 (C), 156.0 (C), 147.8 (CH), 147.6 (C), 144.3 (CH), 142.5 (C), 141.8 (C), 141.0 (C), 137.5 (C), 116.2 (CH), 114.7 (C), 113.3 (CH), 113.0 (CH), 111.8 (CH), 111.0 (CH), 109.9 (CH), 54.8 (CH₂), 54.1 (2 CH₂), 34.6 (CH₂), 26.0 (2 CH₂), 24.4 (CH₂). LC-MS (ESI) m/z found: 406 [M+H]⁺. HPLC: C4 column: t_R = 16.47 min, purity >99%; C18 column: t_R = 24.75 min, purity >99%.

4.7.3. *N*-(2,7-Di(furan-2-yl)-1,3-benzoxazol-5-yl)-4-(piperidin-1-yl)butanamide (**13**).

The title compound was prepared from **9** and piperidine. Solid was recrystallized from EtOAc to afford **13**. Yield 43 %; White solid mp 274 °C (EtOAc). ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.14 (br s, 1H), 8.11 (m, 1H), 8.00 (m, 1H), 7.95 (m, 1H), 7.92 (m, 1H), 7.62 (d, 1H, *J* = 3.0 Hz), 7.24 (d, 1H, *J* = 3.0 Hz), 6.84 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 6.76 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 2.35-2.25 (m, 8H), 1.78-1.73 (m, 2H), 1.46 (m, 4H), 1.36 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 171.9 (C), 155.9 (C), 147.8 (CH), 147.7 (CH), 144.3 (C), 142.5 (C), 141.9 (C), 140.9 (C), 137.7 (C), 116.1 (CH), 114.6 (C), 113.3 (CH), 112.9 (CH), 111.9 (CH), 110.9 (CH), 108.9 (CH), 58.5 (CH₂), 54.5 (2 CH₂), 35.0 (CH₂), 26.0 (2 CH₂), 24.6 (CH₂),

22.7 (CH₂). LC-MS (ESI) m/z found: 420 [M+H]⁺. HPLC: C4 column: t_R = 17.06 min, purity 98%; C18 column: t_R = 25.22 min, purity 98%.

4.7.4. *N*-(2,7-Di(furan-2-yl)-1,3-benzoxazol-5-yl)-3-morpholinopropanamide (**14**).

The title compound was prepared from **8** and morpholine. Solid was recrystallized from EtOAc to afford **14**. Yield 72%; White solid mp 130 °C (EtOAc). ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.28 (br s, 1H), 8.11 (m, 1H), 7.98 (d, 1H, *J* = 1.9 Hz), 7.95 (d, 1H, *J* = 1.9 Hz), 7.93 (m, 1H), 7.62 (d, 1H, *J* = 3.4 Hz), 7.24 (d, 1H, *J* = 3.4 Hz), 6.84 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 6.76 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 3.57 (t, 4H, *J* = 4.5 Hz), 2.65 (t, 2H, *J* = 7.0 Hz), 2.50 (t, 2H, *J* = 6.7 Hz), 2.40 (m, 4H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 170.8 (C), 156.0 (C), 147.8 (CH), 147.7 (C), 144.3 (CH), 142.5 (C), 141.9 (C), 141.0 (C), 137.5 (C), 116.1 (CH), 114.7 (C), 113.3 (CH), 113.0 (CH), 111.8 (CH), 111.0 (CH), 109.0 (CH), 66.7 (2 CH₂), 54.0 (CH₂), 53.5 (2 CH₂), 34.5 (CH₂). LC-MS (ESI) m/z found: 406 [M-H]⁺. HPLC: C4 column: t_R = 14.22 min, purity 99%; C18 column: t_R = 23.86 min, purity >99%.

4.7.5. *N*-(2,7-Di(furan-2-yl)-1,3-benzoxazol-5-yl)-3-(3,4-dihydroisoquinolin-2(1H)-yl)propanamide hydrochloride (**15**).

The title compound was prepared from **8** and 1,2,3,4-tetrahydroisoquinoline. Solid was suspended in acetonitrile with HCl_(g), concentrated *in vacuo* and recrystallized from ethanol to afford **15**. Yield 39%; White solid mp 256 °C (EtOH). ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.85 (br s, 1H), 10.72 (br s, 1H), 8.11 (m, 1H), 8.04 (m, 1H), 8.02 (m, 1H), 7.94 (d, 1H), 7.64 (m, 1H, *J* = 3.4 Hz), 7.28-7.23 (m, 5H), 6.85 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz) 6.77 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 4.59-4.40 (m, 2H), 3.74-3.66 (m, 4H), 3.24-3.08 (m, 4H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 170.8 (C), 155.9 (C), 147.8 (CH), 147.7 (C), 144.3 (CH), 142.5 (C), 141.9 (C), 141.0 (C), 137.6 (C), 135.2 (C), 134.5 (C), 128.9 (C), 126.8 (CH), 126.4 (CH), 125.9 (CH), 116.1 (CH), 114.7 (CH), 113.3 (CH), 113.0 (CH), 111.8 (CH), 110.9 (CH), 108.9 (CH),

55.7 (CH₂), 54.1 (CH₂), 50.7 (CH₂), 35.0 (CH₂), 29.2 (CH₂). LC-MS (ESI) m/z found: 454 [M+H]⁺. HPLC: C4 column: t_R = 16.86 min, purity >99%; C18 column: t_R = 26.16 min, purity >99%.

4.7.6. *N*-(2,7-Di(furan-2-yl)-1,3-benzoxazol-5-yl)-3-(4-(4-(2-ethoxyethoxy)phenyl)piperazin-1-yl)propanamide (**16**).

The title compound was prepared from **8** and 1-[4-(2-methoxyethoxy)phenyl]piperazine. Solid was suspended in acetonitrile with HCl_(g), concentrated *in vacuo* and recrystallized from acetonitrile to afford **16**. Yield 35%; White solid mp 185 °C (Acetonitrile). ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.34 (br s, 1H), 8.11 (m, 1H), 8.0 (d, 1H, *J* = 1.6 Hz), 7.97 (d, 1H, *J* = 1.6 Hz), 7.92 (d, 1H, *J* = 1.1 Hz), 7.63 (d, 1H, *J* = 3.3 Hz), 7.24 (d, 1H, *J* = 3.3 Hz), 6.88-6.78 (m, 5H), 6.76 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 3.98 (t, 2H, *J* = 1.7 Hz), 3.60 (t, 2H, *J* = 1.7 Hz), 3.28 (s, 3H), 3.02 (m, 4H), 2.71 (m, 2H), 2.63-2.52 (m, 6H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 170.8 (C), 156.0 (C), 152.5 (C), 147.8 (CH), 147.7 (C), 145.9 (C), 144.3 (CH), 142.5 (C), 141.9 (C), 141.0 (C), 137.5 (C), 117.7 (2 CH), 116.1 (CH), 115.4 (2 CH), 114.7 (C), 113.3 (CH), 113.0 (CH), 111.8 (CH), 110.9 (CH), 108.9 (CH), 71.0 (CH₂), 67.5 (CH₂), 58.6 (CH₃), 54.3 (2 CH₂), 53.0 (CH₂), 50.0 (2 CH₂), 34.8 (CH₂). LC-MS (ESI) m/z found: 557 [M+H]⁺. HPLC: C4 column: t_R = 16.65 min, purity >99%; C18 column: t_R = 26.14 min, purity >99%.

4.7.7. 3-(3,4-Dihydroisoquinolin-2(1H)-yl)-*N*-(7-(3,4-dimethoxyphenyl)-2-(furan-2-yl)-1,3-benzoxazol-5-yl)propanamide hydrochloride (**17**).

The title compound was prepared from **10** and 1,2,3,4-tetrahydroisoquinoline. Solid was suspended in acetonitrile with HCl_(g), concentrated *in vacuo* and recrystallized from ethanol to afford **17**. Yield 34%; Beige solid mp 258 °C (EtOH). ¹H NMR (300 MHz, Acetone-*d*₆): δ 10.94 (br s, 1H), 10.70 (br s, 1H), 8.11 (m, 2H), 7.83 (m, 1H), 7.46-7.44 (m, 3H), 7.28-7.18 (m, 5H), 6.84 (m, 1H), 4.55-4.41 (m, 2H), 3.89 (s, 3H), 3.87 (s, 3H), 3.77 (m, 1H), 3.57 (m, 2H),

3.24-3-11 (m, 5H). ^{13}C NMR (75 MHz, Acetone- d_6): δ 168.4 (C), 155.9 (C), 149.7 (CH), 149.4 (CH), 147.7 (C), 143.5 (C), 142.6 (C), 142.0 (C), 137.2 (C), 131.8 (C), 129.0 (2 CH), 128.2 (C), 127.3 (C), 127.1 (2 CH), 124.4 (C), 120.8 (CH), 116.0 (C), 115.7 (CH), 113.4 (CH), 112.7 (CH), 111.7 (CH), 109.0 (CH), 56.1 (2 CH₃), 52.6 (CH₂), 51.6 (CH₂), 49.3 (CH₂), 31.2 (CH₂), 25.6 (CH₂). LC-MS (ESI) m/z found: 522 [M-H]⁺. HPLC: C4 column: t_R = 16.91 min, purity >99%; C18 column: t_R = 26.53 min, purity >99%.

4.8. *N*-(diphenylmethylene)-2-(furan-2-yl)-5-nitro-1,3-benzoxazol-7-amine (**18**).

In a sealed tube, a mixture of **4** (1.2 g, 4.53 mmol), benzophenone imine (1.14 mL, 6.8 mmol), palladium diacetate (30.5 mg, 0.136 mmol), triphenylphosphine (71 mg, 0.272 mmol), BINAP (112 mg, 0.181 mmol) and cesium carbonate (2.95 g, 9.07 mmol) in toluene (20 mL) was degassed and then heated at 90 °C for 3 h, cooled to room temperature and diluted with EtOAc. Precipitate was filtered off and organic layer was washed with water, 1 M HCl solution and then combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. Solid was suspended in diethyl ether and filtered to afford a yellow solid (1.37 g, 75%): mp 216 °C. ^1H NMR (300 MHz, DMSO- d_6): δ 8.22 (d, 1H, J = 2.4 Hz), 8.10 (m, 1H), 7.81-7.78 (m, 2H), 7.67 (d, 1H, J = 2.4 Hz), 7.65-7.62 (m, 1H), 7.57-7.53 (m, 3H), 7.27 (m, 5H), 6.82 (dd, 1H, J = 1.7 Hz and J = 3.5 Hz). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.6 (C), 157.4 (C), 148.6 (CH), 145.6 (2 C), 144.5 (C), 142.4 (C), 140.9 (C), 138.1 (C), 136.0 (CH), 135.6 (CH), 132.6 (CH), 130.0 (2 CH), 129.1 (2 CH), 128.8 (2 CH), 128.7 (2 CH), 117.5 (CH), 113.6 (2 CH), 110.7 (CH). LC-MS (ESI) m/z found: 410 [M+H]⁺.

4.9. 2-Bromo-*N*-(7-((diphenylmethylene)amino)-2-(furan-2-yl)-1,3-benzoxazol-5-yl)acetamide (**19**).

To a solution of **18** (200 mg, 0.489 mmol) in EtOAc (20 mL) was added Raney Ni (70 mg) and hydrazine hydrate (0.036 mL, 0.733 mmol). The mixture was stirred for 30 min at room

temperature, catalyst was then filtered off and the mixture was concentrated *in vacuo*. Crude was diluted in EtOAc/H₂O (40 mL/8 mL) with K₂CO₃ (135 mg, 0.977 mmol) at 0 °C and the bromoacetyl bromide (0.064 mL, 0.733 mmol) diluted in EtOAc (40 mL) was added dropwise. After 1 h stirring at room temperature, aqueous layer was extracted three times with EtOAc. Combined organic layer were dried over MgSO₄ and concentrated *in vacuo*. Solid was suspended in diethyl ether and filtered to afford a white solid (208 mg, 85%): mp 201 °C. ¹H NMR (300 MHz, DMSO-*d*₆): 9.68 (br s, 1H), 8.01 (m, 1H), 7.77-7.74 (m, 2H), 7.72 (d, 1H, *J* = 1.8 Hz), 7.61-7.50 (m, 3H), 7.38 (d, 1H, *J* = 3.3 Hz), 7.27-7.18 (m, 5H), 7.10 (d, 1H, *J* = 1.8 Hz), 6.75 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 4.02 (s, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): 171.7 (C), 169.0 (C), 155.4 (C), 147.5 (2 C), 142.2 (C), 141.8 (C), 138.6 (C), 136.5 (CH), 136.1 (C), 135.3 (C), 132.1 (CH), 129.7 (2 CH), 129.6 (CH), 129.1 (2 CH), 128.7 (2 CH), 128.5 (2 CH), 115.6 (CH), 113.2 (CH), 110.3 (CH), 105.6 (CH), 63.1 (CH₂), 54.5 (2 CH₂), 25.9 (2 CH₂), 24.0 (CH₂).

4.10. *N*-(7-((Diphenylmethylene)amino)-2-(furan-2-yl)-1,3-benzoxazol-5-yl)-2-(piperidin-1-yl)acetamide (**20**).

To a solution of **19** (200 mg, 0.517 mmol) in acetone (18 mL) were added piperidine (0.102 mL, 1.03 mmol) and Et₃N (0.1 mL, 1.03 mmol). Reaction mixture was refluxed for 6 h, cooled to room temperature and concentrated *in vacuo*. Solid was suspended in water and extracted three times with EtOAc. Combined organic layers were dried over MgSO₄ and concentrated *in vacuo* to give a solid which was suspended in diethyl ether and filtered to afford a white solid (80 mg, 52%); mp 194 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 9.68 (br s, 1H), 8.01 (m, 1H), 7.77-7.74 (m, 2H), 7.72 (d, 1H, *J* = 1.8 Hz), 7.61-7.50 (m, 3H), 7.38 (d, 1H, *J* = 3.3 Hz), 7.27-7.18 (m, 5H), 7.10 (d, 1H, *J* = 1.8 Hz), 6.75 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 3.04 (s, 2H), 2.43 (m, 4H), 1.55 (m, 4H), 1.39 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 171.7 (C), 169.0 (C), 155.4 (C), 147.5 (2 C), 142.2 (C), 141.8 (C), 138.6 (C), 136.5 (CH), 136.1 (C), 135.3 (C),

132.1 (CH), 129.7 (2 CH), 129.6 (CH), 129.1 (2 CH), 128.7 (2 CH), 128.5 (2 CH), 115.6 (CH), 113.2 (CH), 110.3 (CH), 105.6 (CH), 63.1 (CH₂), 54.5 (2 CH₂), 25.9 (2 CH₂), 24.0 (CH₂). LC-MS (ESI) m/z found: 505 [M+H]⁺.

4.11. *N*-(7-Amino-2-(furan-2-yl)-1,3-benzoxazol-5-yl)-2-(piperidin-1-yl)acetamide dihydrochloride (**21**).

A solution of **20** (356 mg, 0.706 mmol) in a mixture of THF (7 mL) and 4 M HCl_(aq) (1.5 mL, 6 mmol) was stirred for 30 min at room temperature. The precipitate formed was filtered and washed with EtOAc to afford a white solid (220 mg, 83%); mp >300 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.90 (br s, 1H), 10.00 (br s, 1H), 8.05 (m, 1H), 7.40 (m, 1H), 7.34 (m, 1H), 6.93 (m, 1H), 6.80 (m, 1H), 5.30 (br s, 3H), 4.14 (s, 2H), 3.47 (m, 2H), 3.08 (m, 2H), 1.79-1.67 (m, 5H), 14.40 (m, 1H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 163.1 (C), 154.0 (C), 147.2 (2 C), 142.3 (CH), 136.4 (C), 135.7 (C), 132.8 (C), 114.9 (CH), 113.2 (CH), 103.7 (CH), 99.1 (CH), 57.6 (CH₂), 53.4 (2 CH₂), 22.6 (2 CH₂), 21.6 (CH₂). LC-MS (ESI) m/z found: 341 [M+H]⁺. HPLC: C4 column: t_R = 13.9 min, purity >99%; C18 column: t_R = 19.4 min, purity 99%.

4.12. 2-(Furan-2-yl)-5-nitro-1,3-benzoxazol-7-amine (**22**).

A suspension of **18** (585 mg, 1.43 mmol) in a mixture of 4 M HCl_(aq) (10 mL, 40 mmol) and THF (20 mL) was refluxed for 30 min. Mixture was cooled to room temperature and concentrated *in vacuo* to afford a solid which was suspended in EtOAc and filtered to afford a yellow solid (265 mg, 76%); mp 258 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.12 (m, 1H), 7.75 (d, 1H, *J* = 2.4 Hz), 7.55 (d, 1H, *J* = 2.4 Hz), 7.47 (dd, 1H, *J* = 0.6 Hz and *J* = 3.2 Hz), 6.85 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 6.36 (br s, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 156.6 (C), 148.0 (CH), 146.5 (C), 142.1 (C), 141.7 (C), 141.6 (C), 134.6 (C), 116.4 (CH), 113.5 (CH), 105.2 (CH), 102.5 (CH). LC-MS (ESI) m/z found: 246 [M+H]⁺.

4.13. 2-Bromo-*N*-(2-(furan-2-yl)-5-nitro-1,3-benzoxazol-7-yl)acetamide (**23**).

To a solution of **22** (400 mg, 1.63 mmol) with K₂CO₃ (563 mg, 4.08 mmol) in a mixture of EtOAc (100 mL) and water (30 mL) at 0 °C was added dropwise bromoacetyl bromide (0.17 mL, 1.96 mmol) diluted in EtOAc (30 mL). After 30 min stirring at room temperature, aqueous layer was extracted three times with EtOAc. Combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. Solid was recrystallized from EtOH to afford a beige solid (248 mg, 42%); mp 262 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.25 (br s, 1H), 8.76 (d, 1H, *J* = 2.7 Hz), 8.40 (d, 1H, *J* = 2.7 Hz), 8.17 (m, 1H), 7.58 (m, 1H), 6.88 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 4.18 (s, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 172.2 (C), 157.2 (C), 148.6 (CH), 145.5 (C), 145.3 (C), 142.5 (C), 141.0 (C), 123.3 (C), 117.5 (CH), 113.7 (CH), 113.6 (CH), 111.5 (CH), 62.1 (CH₂). LC-MS (ESI) *m/z* found: 366, 368 [M+H]⁺.

4.14. *N*-(2-(Furan-2-yl)-5-nitro-1,3-benzoxazol-7-yl)-2-(piperidin-1-yl)acetamide (**24**).

To a solution of **23** (248 mg, 0.677 mmol) in acetone (8 mL) was added piperidine (0.074 mL, 0.75 mmol) and Et₃N (0.12 mL, 0.88 mmol) and the mixture was heated at reflux for 1 h, cooled to room temperature and concentrated *in vacuo*. Solid was suspended in water and extracted three times with EtOAc. Combined organic layers were dried over MgSO₄ and concentrated *in vacuo* to afford a pale yellow solid (170 mg, 68%); mp 252 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.37 (br s, 1H), 8.86 (d, 1H, *J* = 2.7 Hz), 8.38 (d, 1H, *J* = 2.7 Hz), 8.18 (m, 1H), 7.52 (m, 1H), 6.85 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 3.25 (s, 2H), 2.54 (t, 4H, *J* = 9.9 Hz), 1.62 (m, 4H), 1.46 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 170.1 (C), 157.1 (C), 148.7 (CH), 145.6 (C), 144.7 (C), 142.4 (C), 141.1 (C), 123.5 (C), 117.3 (CH), 113.7 (CH), 112.6 (CH), 111.3 (CH), 62.3 (CH₂), 54.5 (2 CH₂), 26.2 (2 CH₂), 23.9 (CH₂). LC-MS (ESI) *m/z* found: 371 [M+H]⁺.

4.15. *N*-(5-Amino-2-(furan-2-yl)-1,3-benzoxazol-7-yl)-2-(piperidin-1-yl)acetamide (**25**).

To a suspension of **24** (155 mg, 0.419 mmol) in EtOAc (20 mL) was added Raney Ni (15 mg) and hydrazine monohydrate (0.04 mL, 0.837 mmol). The mixture was stirred at room temperature for 1 h and catalyst was filtered off. Organic layer was washed with water, brine, dried over MgSO₄ and concentrated *in vacuo*. Solid was recrystallized from acetonitrile to afford a yellow solid (100 mg, 70%); mp 260 °C. ¹H NMR (300 MHz, CDCl₃): δ 9.89 (br s, 1H), 7.68 (d, 1H, *J* = 2.7 Hz), 7.67 (m, 1H), 7.17 (d, 1H, *J* = 2.7 Hz), 6.77 (m, 1H), 6.32 (dd, 1H, *J* = 1.7 Hz and *J* = 3.5 Hz), 3.78 (br s, 2H), 3.16 (s, 2H), 2.63 (t, 4H, *J* = 9.9 Hz), 2.75 (m, 4H), 1.57 (m, 2H). ¹³C NMR (75 MHz, CDCl₃): δ 169.3 (C), 155.6 (C), 145.6 (CH), 144.9 (C), 142.8 (C), 142.7 (C), 134.2 (C), 122.7 (C), 113.6 (CH), 112.2 (CH), 104.8 (CH), 100.1 (CH), 62.6 (CH₂), 54.9 (2 CH₂), 26.6 (2 CH₂), 23.7 (CH₂). LC-MS (ESI) *m/z* found: 341 [M+H]⁺. HPLC: C4 column: *t_R* = 13.9 min, purity >99%; C18 column: *t_R* = 19.4 min, purity 99%.

4.16. Molecular Docking

Molecular docking was performed using Gold suite v5.2 [25] within the Hermes v1.6 GUI (CCDC[®]). Thus after adding hydrogen atoms, water molecules were deleted and docking was performed in a 10 Å around co-crystallized ligands then ligands deleted. Early termination of 3 docking solutions within 1.5 Å was set up in order to highlight ligands converging toward a few binding modes.

4.17. *In vitro* testing

4.17.1. Displacement binding assays

Compounds of interest are selected by a competition-binding assay at 10⁻⁵ M giving a displacement of the reference compound ([³H]-ZM24135) greater than 50%.

Stock solutions of the compounds were prepared in DMSO and further diluted with the binding buffer to the desired concentration. Final DMSO concentrations in the assay were less than 0.1%. Briefly [³H]-ZM24135 (2 nM final) as radioligand for the *hA_{2A}* receptor, was added to

4,5 μg of membranes (PerkinElmer) resuspended in 300 μL (final volume) binding buffer (50 mM Tris, 10 mM MgCl_2 , 0.2 UI/mL of adenosine deaminase, pH 7.4) [20]. After 1 h at room temperature, the incubation was stopped and the solutions were rapidly filtered over Unifilter-96 GF/B glass fiber pre-soaked in binding buffer on a Filtermate Unifilter 96-Harvester (PerkinElmer) and washed 10x times with of ice-cold binding buffer. The radioactivity on the filters was measured using TopCount NXT™ Microplate Scintillation Counter (PerkinElmer) using 30 μL of MicroScint™ 40 (PerkinElmer) after 30 min resting. The nonspecific binding was determined in the presence of 5 mM ZM-24135.

4.17.2. [^{35}S]-GTPS binding assay

The [^{35}S]GTP γ S binding assay was carried out as described previously [21] with minor modifications. HEK293- $\text{A}_{2\text{A}}\text{R}$ membranes were pre-incubated in triplicate, in 96-well plates, at 25°C in 250 μL final volume of 50 mM Tris-HCl buffer (pH 7.4) containing 0.2 nM [^{35}S]GTP γ S, 10 μM GDP, 5 mM MgCl_2 , 1 mM EDTA, 1 mM dithiothreitol, 100 mM NaCl, 0,5% bovine serum albumin and 0.2 UI/ml adenosine deaminase with various concentrations of CGS241680 (Emax determination) or with the designed $\text{A}_{2\text{A}}\text{R}$ antagonists (10 min pre-incubation) and 1 μM of CGS241680 (IC_{50} determination, inhibition versus response). The incubation was stopped by a rapid filtration over Unifilter-96 GF/B glass fiber and washed 10x times with an ice-cold binding buffer. The nonspecific binding was measured in the presence of 100 μM Gpp(NH)p. Radioactivity was quantified using a TopCount scintillation counter.

Data Analysis.

K_i and IC_{50} values were determined by nonlinear regression analysis performed using the GraphPad prism 5.0 program (GraphPad Software, San Diego).

4.17.3. Cell culture and cytotoxicity assay

The human neuroblastoma cell line (SY5Y) was cultured in DMEM (Dulbecco's Modified Eagle Medium) (Gibco) supplemented with 2 mM L-glutamine, 100 mg/ml streptomycin, 100 IU/mL penicillin, 1 mM non-essential amino acids and 10% (v/v) heatinactivated fetal bovine serum (Sigma Aldrich), and grown at 37 °C in a humidified incubator with 5% CO₂. Cells were seeded at 2000 cells per well onto 96-well plates in DMEM medium. Cells were starved for 24 h to obtain synchronous cultures, and were then incubated in culture medium that contained various concentrations of test compounds, each dissolved in less than 0.1% DMSO. After 72 h of incubation, cell growth was estimated by the colorimetric MTT (thiazolyl blue tetrazolium bromide) assay.

4.17.4 ADME assessment

Aqueous solubility (in phosphate-buffered saline, PBS, pH 7.4; incubation room temperature for 24h as described by Lipinski [26] Eurofins Cerep SA catalogue reference G235), human plasma protein binding evaluated at 10 µM concentration for 4 h at 37 °C as described by Banker [27] (Eurofins Cerep SA catalogue reference 2194), A-B and B-A permeability coefficient evaluated at 10 µM for 40 min as described by Hidalgo [28] (Papp, Caco-2 cells, pH 6.5/7.4; Eurofins Cerep SA catalogue reference G228), metabolic stability in human liver microsomes evaluated at 0.1 µM concentration for 0, 15, 30, 45, 60 min at 37 °C as described by Obach [29] (Eurofins Cerep SA catalogue reference 0607) were determined in standard assays by Eurofins Cerep SA, France www.cerep.fr).

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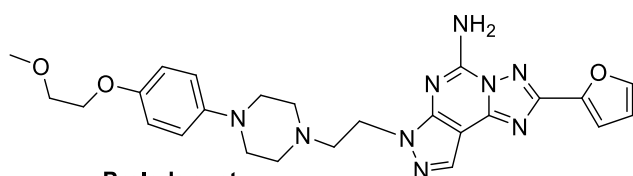
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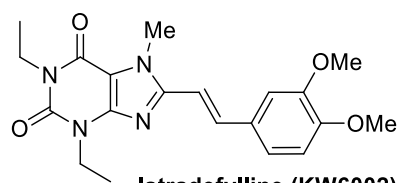
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Figure 1. Representative A_{2A} receptor antagonists



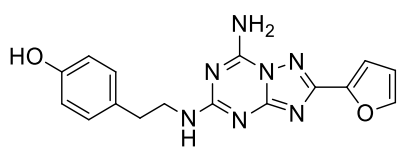
Preladenant

$K_i (A_{2A}) = 0.5 \text{ nM}$



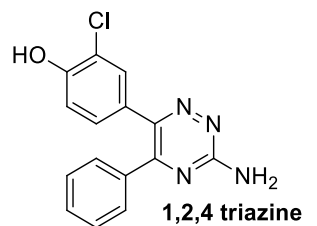
Istradefylline (KW6002)

$K_i (A_{2A}) = 12 \text{ nM}$



ZM-241385

$K_i (A_{2A}) = 1.6 \text{ nM}$



1,2,4 triazine

$K_i (A_{2A}) = 4 \text{ nM}$

Figure 2. Structural modifications of hits **1** and **2** to develop new A_{2A}R antagonists

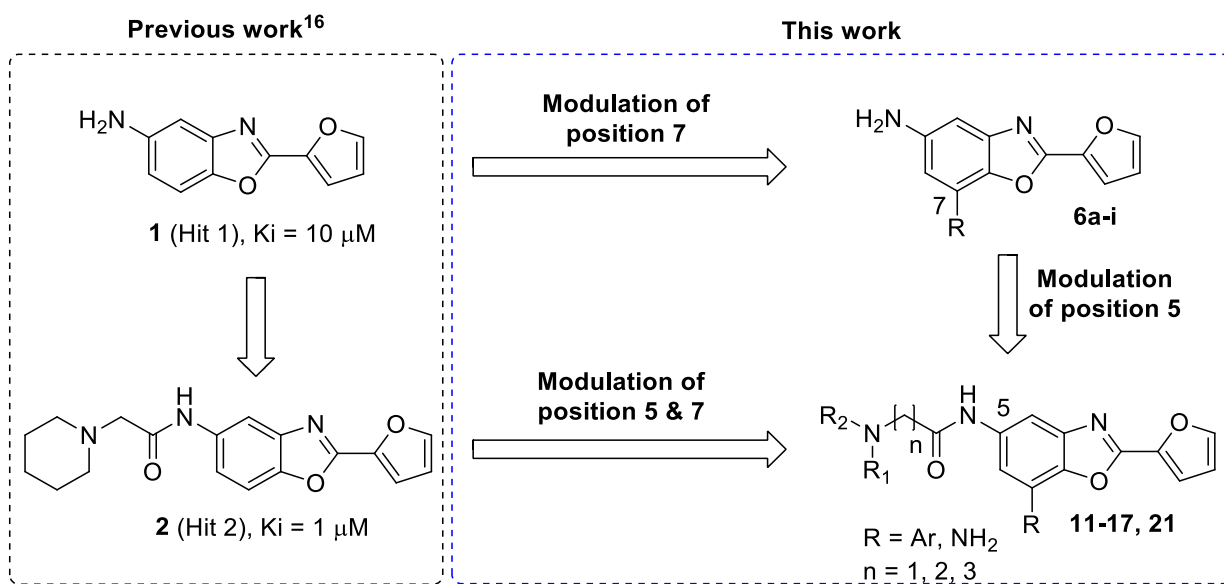
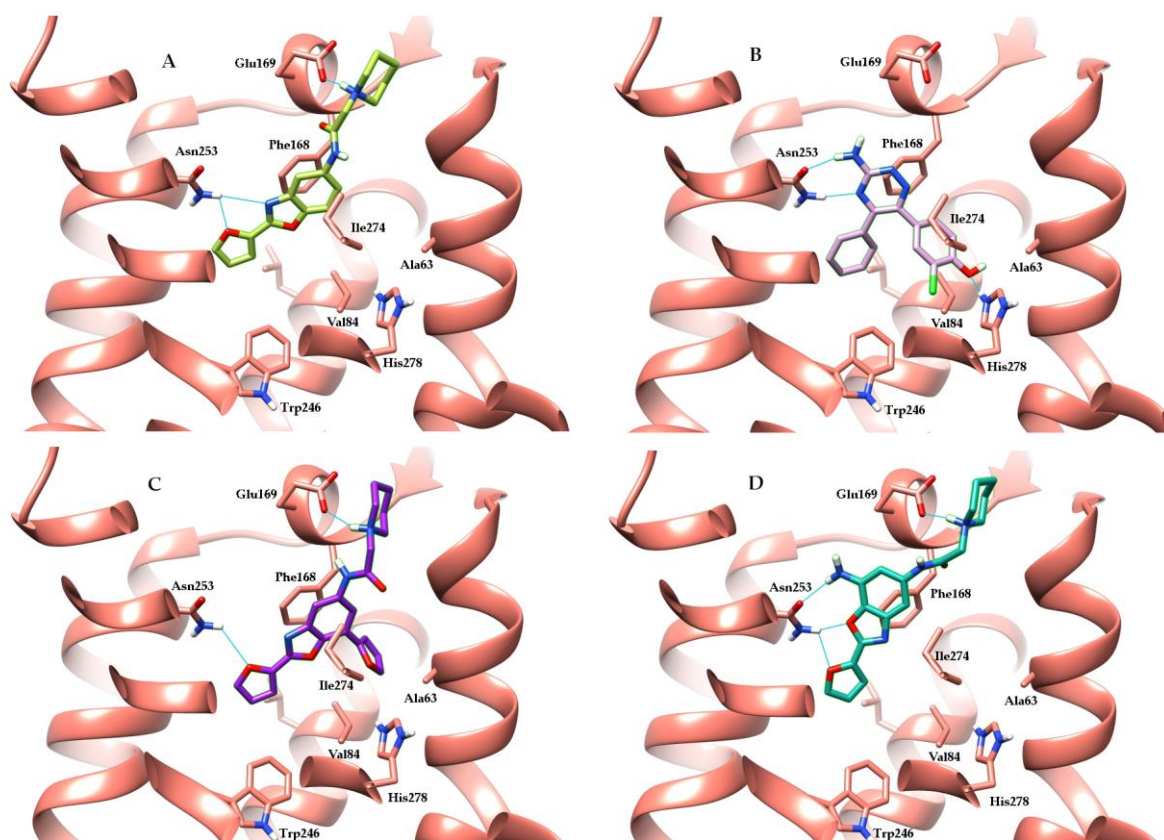
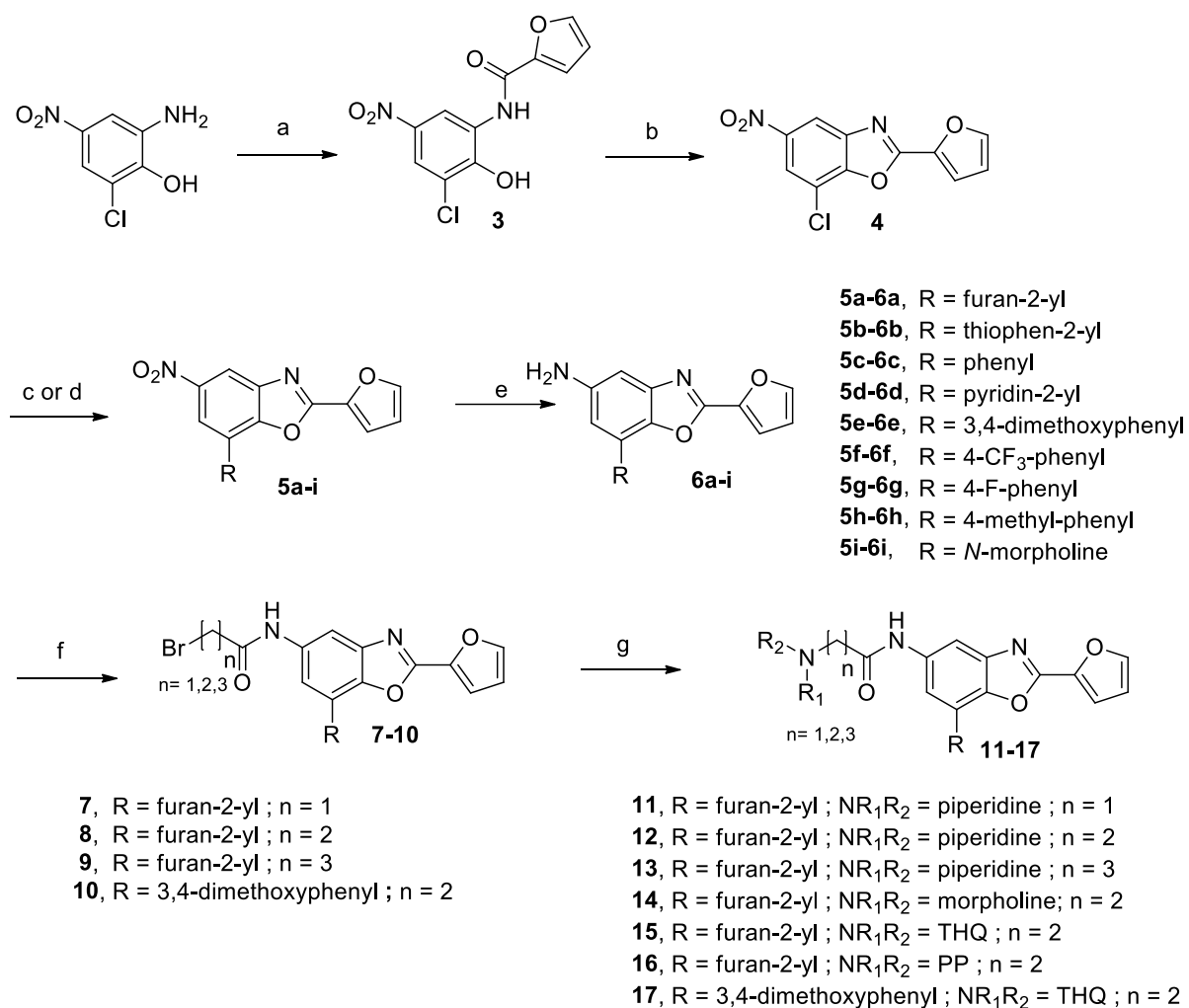


Figure 3. Molecular modelling-guided design. (A) Predicted binding mode of **2** in the apoA_{2A}R-T4E pocket; (B) X-ray structure of reference triazine T4E bound to A_{2A} receptor; Predicted binding mode of **11** (C) and **21** (D) in the apoA_{2A}R-T4E pocket.

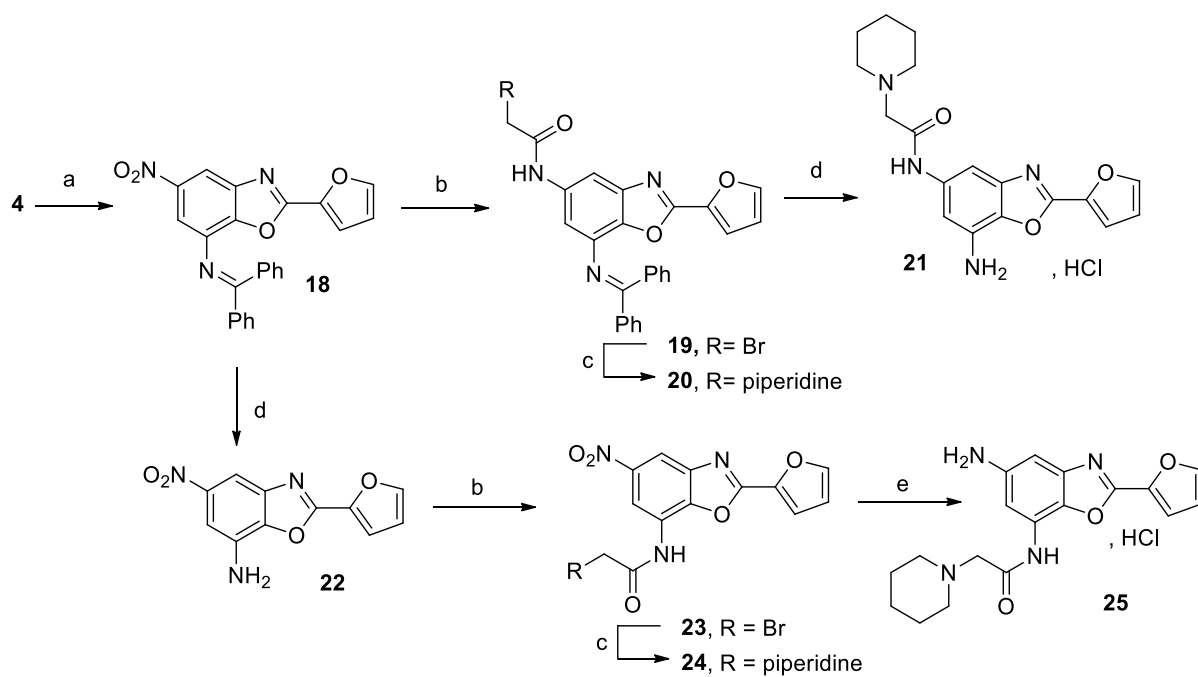


Scheme 1^a. Synthesis of compounds **6a-i** and **11-17**



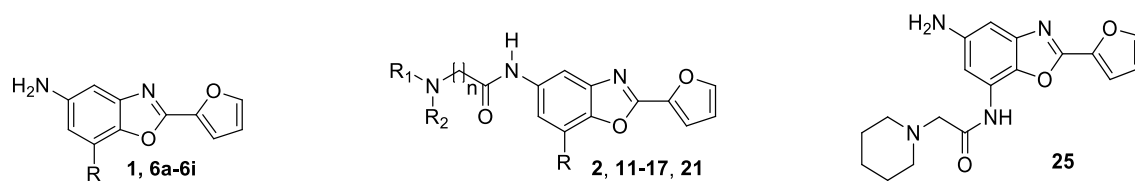
^a *Reagents and conditions:* (a) i) Furoic acid, SOCl₂, CH₂Cl₂, ii) Et₃N, EtOAc, iii) NaOH, H₂O/EtOH, then 6 N HCl, 97%; (c) PTSA, toluene, reflux, 71%; (c) RB(OH)₂, K₃PO₄, Pd(OAc)₂, PPh₃, dioxane/H₂O, 24-63%; (d) morpholine, Pd(OAc)₂, BINAP, Cs₂CO₃, 53%; (e) H₂NNH₂.H₂O, Raney Ni, EtOAc, 17-82%; (f) Br(CH₂)_nCOX (X = Cl, Br), K₂CO₃, EtOAc/H₂O, 40-50%; (g) HNR₁R₂, K₂CO₃, acetone, 35-70%.

Scheme 2^a. Synthesis of compounds **21** and **25**.



^a *Reagents and conditions:* (a) Benzophenone imine, Pd(OAc)₂, BINAP, Cs₂CO₃, toluene; 75%; (b) *i*) NH₂NH₂·H₂O, Raney Ni, EtOAc, 55-70%; *ii*) bromoacetyl bromide, K₂CO₃, EtOAc/H₂O, 65%; (c) piperidine, K₂CO₃, acetone, 75%; (d) HCl_(g), THF, 85%; (e) NH₂NH₂·H₂O, Raney Ni, 70%.

Table 1: Binding affinity for synthesized compounds 1, 2, 6a-i, 11-17, 21 and 25



Cpd.	R	hA_{2A} Ki (nM) ^a	Cpd.	R	NR ₁ R ₂	n	hA_{2A} Ki (nM) ^a
1	H	10000 ± 500	2	H		1	1000 ± 80
6a		40 ± 6	11			1	210 ± 30
6b		71 ± 3	12			2	81 ± 15
6c		202 ± 12	13			3	98 ± 13
6d		135 ± 17	14			2	90 ± 5
6e		90 ± 5	15			2	30 ± 2
6f		> 10000	16			2	> 10000
6g		438 ± 35	17			2	> 10000
6h		> 10000	21	NH ₂		1	480 ± 40
6i		> 10000	25	-	-	-	> 10000
			ZM-241385	/	/	-	1.3 ± 0.2

^a Ki values were obtained from nonlinear analysis of competition curves using [³H]-ZM241385 as radioligand and are expressed as mean ± SEM.

Table 2: Binding affinities and functional activities at *hA_{2A}R* for selected compounds

Cpd.	<i>hA_{2A}</i> K_i (nM)^a	³⁵GTPγS IC₅₀ (nM)^b
ZM-241385	1.3 ± 0.2	80.8 ± 7.5
6a	40 ± 6	70.6 ± 3.8
11	210 ± 30	486 ± 29
21	480 ± 40	250 ± 16

^a K_i values were obtained from nonlinear analysis of competition curves using [³H]-ZM241385 as radioligand and are expressed as mean ± SEM. ^b Effect of tested compounds on GTPγ³⁵S recruitment by *hA_{2A}R* stably expressed in HEK293 cells treated with CGS21680 (1 μM).

Table 3: Preliminary pharmacokinetics profiles of leading compounds

Cpd.	Solubility (μM) ^a	PPB (%) ^b	Permeability		clogP ^d	HLM ^e t _{1/2} (min)	Cl _{int} ^f ($\mu\text{L}/\text{min}$ /mg)	Cytotox. IC ₅₀ (μM) ^g or % I _{10μM} ^h
			A/B	B/A				
2	184	83	50	24	3.25	11	630	0%
6a	31	90	77	37	2.49	> 60	< 115	40
12	165	98	2.3	1.2	4.02	41	172	9
14	173	98	11	1.6	2.96	> 60	< 115	77
15	8	99.9	0.2	0	4.50	> 60	< 115	37%

^a Evaluated after 24 h stirring in PBS (pH 7.4). ^b PPB = plasma protein binding. Compound was tested at 10 μM concentration. ^c Permeability = Compound was tested at 10 μM concentration at pH 6.5/7.4. ^d Calculated by Molinspiration. ^e HLM = Human liver microsomes. ^f Cl_{int} = Compound was tested at 0.1 μM concentration. ^g Compound concentration causing 50% of SY5Y cell death after 24 h treatment. ^h Percentage of dead SY5Y cells after 24 h treatment at 10 μM