JOURNAL OF ENZYME INHIBITION AND MEDICINAL CHEMISTRY, 2017
VOL. 32, NO. 1, 850–864
https://doi.org/10.1080/14756366.2017.1334648

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RESEARCH PAPER

Design, synthesis and evaluation of 2-aryl benzoxazoles as promising hit for the A_{2A} receptor

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ABSTRACT

The development of adenosine A_{2A} receptor antagonists has received much interest in recent years for the treatment of neurodegenerative diseases. Based on docking studies, a new series of 2-arylbenzoxazoles has been identified as potential A_{2A}R antagonists. Structure-affinity relationship was investigated in position 2, 5 and 6 of the benzoxazole heterocycle leading to compounds with a micromolar affinity towards the A_{2A} receptor. Compound **F1**, with an affinity of 1 μm, presented good absorption, distribution, metabolism and excretion properties with an excellent aqueous solubility (184 μm) without being cytotoxic at 100 μm. This compound, along with low-molecular weight compound **D1** ($K_i = 10$ μm), can be easily modulated and thus considered as relevant starting points for further hit-to-lead optimisation.

ARTICLE HISTORY
Received 24 March 2017
Revised 10 May 2017
Accepted 18 May 2017

KEYWORDS
Benzoxazole; A_{2A} receptor; solubility; neurodegenerative disease

Introduction

Alzheimer's (AD) and Parkinson's diseases (PD), currently the most important neurodegenerative pathologies, are characterised by a progressive neuronal death[1]. Current therapies are restricted to symptomatic interventions and do not prevent progressive neuronal loss. Therefore, novel therapeutic solutions are needed and one of the promising strategies consists of targeting the adenosine A_{2A} receptor.

Epidemiological studies have shown that people consuming regularly over a lifetime caffeine-based beverages are substantially less likely to develop PD and AD[2,3]. Caffeine exerts its biological effects primarily by antagonising adenosine receptors (GPCRs). The adenosine A_{2A} receptor subtype, one of the four adenosine receptors, was shown in multiple studies to be responsible for the neuroprotective effects of caffeine in experimental models of AD and PD[4–6]. Besides, many A_{2A} antagonists have been synthesised over the past few years and some showed promising results in managing cognitive dysfunction in both diseases[7]. For example, antagonists (Figure 1) such as Istradefylline (KW-6002), Preladenant (SCH 4208[8]) and Tozadenant (SYN 115)[9] have been investigated clinically in PD with promising results, especially Istradefylline which has been approved in Japan as an adjunct to L-DOPA therapy[10,11]. Less research work has been undertaken regarding AD but it is now well established that A_{2A} antagonists lead to the improvement of spatial memory accompanied by the decrease of Aβ amyloid burden, Tau hyperphosphorylation and neurotoxicity[6,12].

Therefore, developing A_{2A} antagonists constitutes a promising therapeutic strategy for the treatment of both AD and PD. However, although many antagonists have been developed so far, constant drawbacks remain such as high toxicity and poor solubility[7,8,13,14]. These important limitations have obstructed the development of drugs targeting this receptor. Therefore, one of the main challenges regarding A_{2A} antagonist development is to improve solubility and lower toxicity while keeping good affinity at A_{2A} receptor. Selectivity parameters are now more debated since studies have highlighted the therapeutic potential of dual A_1/A_{2A} antagonists[15] as well as a non-selective ligand proven by caffeine, for neurodegenerative disease.

With the aim of developing novel A_{2A} antagonists with good water solubility, we designed a series of benzoxazoles bearing a protonable amine function (Figure 2(A)). We first focused on a diffuse hydrophobic zone located at the top of the active site that is generally occupied by an aryl group in well-known antagonists (Figure 2(B)). Because of the presence of an acidic cluster (Glu169, Asp170) in this pocket, a tertiary amine which can allow for an ionic interaction could be a good alternative to an aryl group (Figure 2(C)). The present work describes the medicinal chemistry approach leading to a series of 2-arylbenzoxazoles which best compounds display micromolar affinity towards the A_{2A} receptor and good water solubility.

Methods

Chemistry

All reagents and solvents were purchased and used without further purification. Reactions were monitored by TLC performed on MachereyeNagel Alugram® Sil 60/UV254 sheets (thickness 0.2 mm). Some purification of products was carried out by column

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Supplemental data for this article can be accessed here.

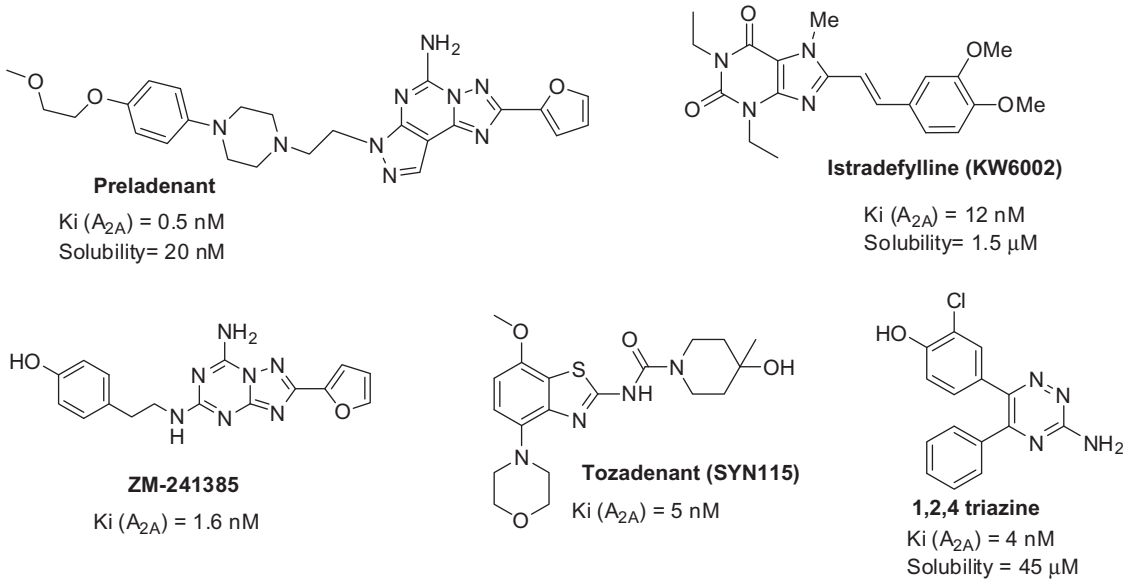


Figure 1. Selective adenosine A_{2A} receptor antagonists.

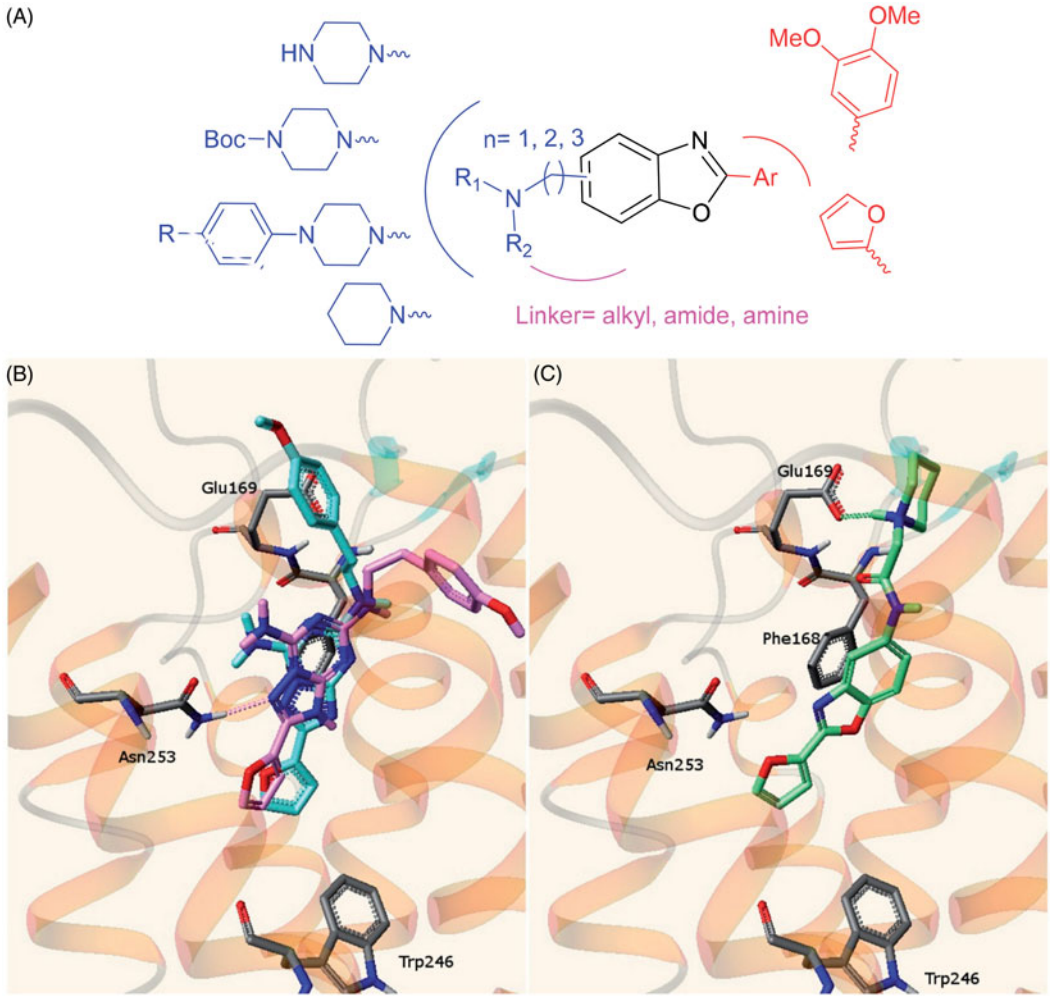


Figure 2. Molecular modelling-guided design. (A) Representation of various modulations around the benzoxazole scaffold. (B) Predicted binding mode of ZM-241385 in the apoA2AR-T4E pocket (dark) compared with the X-ray binding mode (gray). (C) Putative binding mode of compound **F1** in the apoA2AR-T4E pocket.

chromatography using MachereyeNagel silica gel (230e400 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 (^{1}H) or 75 MHz (^{13}C). 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 × 4.6 mm). A gradient starting from 98% H_2O/formate buffer 5 mm (pH 3.8) and reaching 100% CH_3CN/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. FT-IR spectra were recorded on a Thermo Nicolet Avatar 320 FT-IR spectrometer. The purity of final compounds was verified by high-pressure liquid chromatography (HPLC) columns: C4 Interchrom UPTISPHERE. Analytical HPLC was performed on a Shimadzu LC-2010AHT system equipped with a UV detector set at 254 and 215 nm. Compounds were dissolved in 50 ml acetonitrile and 950 ml buffer A and injected into the system. The following eluent systems were used: buffer A (H_2O/TFA, 100:0.1) and buffer B (CH_3CN/H_2O/TFA, 80:20:0.1). HPLC retention times (HPLC tR) 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.

General procedure for the synthesis of amide (2a–2d)

To a solution of acid (17.8 mmol) in DCM (100 ml) at 0 °C were added thionyl chloride (71.4 mmol) and 5 drops of DMF. This solution was stirred for 3 h at reflux, cooled to room temperature and concentrated *in vacuo*. The residue was diluted in 50 ml of EtOAc and added to a solution of aminophenol (16.6 mmol) and Et_3N (35.6 mmol) in 35 ml of EtOAc at 0 °C. The reaction mixture was stirred at room temperature overnight, hydrolysed with water and extracted twice with EtOAc. An acid–base workup with saturated $NaHCO_3$ and 1 M HCl solution was performed and the organic layer was concentrated *in vacuo*. The solid was then suspended in a mixture of EtOH/H_2O (250 ml/10 ml) and NaOH was added (54 mmol). The reaction mixture was stirred at reflux for 4 h, cooled to room temperature, acidified slowly with 6 M HCl up to acidic pH. Resulting solid was filtered, washed with water and recrystallised in an appropriate solvent.

N-(2-Hydroxy-5-methylphenyl)furan-2-carboxamide (2a): The title compound was prepared from 2-furoic acid and 4-methyl-2-aminophenol to afford **2a** as a beige solid (80%): mp 186 °C (acetonitrile). ^{1}H NMR (300 MHz, [D$_6$]DMSO): 9.76 (br s, 1H, OH), 9.09 (br s, 1H, NH), 7.92 (m, 1H, H$_{5'}$), 7.72 (m, 1H, H$_6$), 7.28 (dd, 1H, H$_4$, J = 2.2 Hz and J = 8.4 Hz), 6.79 (m, 2H, H$_3$ and H$_{3'}$), 6.70 (dd, 1H, H$_{4'}$, J = 1.7 Hz and J = 3.5 Hz), 2.21 (s, 3H, CH$_3$). IR (ν, cm^{-1}): 3380 (NH), 2750–3100 (OH), 1640 (C = O). LC-MS (ESI) *m/z* found: 218 $[M+H]^+$.

N-(2-Hydroxy-5-methylphenyl)-3,4-dimethoxybenzamide (***2b***): The title compound was prepared from 3,4-dimethoxybenzoic acid and 4-methyl-2-aminophenol to afford **2b** as a white solid (82%): mp 164 °C (acetonitrile). ^{1}H NMR (300 MHz, CDCl$_3$): 9.47 (br s, 1H, OH), 9.41 (br s, 1H, NH), 7.61 (dd, 1H, H$_4$, J = 2.0 Hz and J = 8.4 Hz), 7.55 (d, 1H, H$_6$, J = 2.0 Hz), 7.42 (m, 1H, H$_{2'}$), 7.07 (d, 1H, H$_3$, J = 8.4 Hz), 6.83 (dd, 1H, H$_{6'}$, J = 1.7 Hz and J = 8.3 Hz), 6.79 (d, 1H, H$_{5'}$, J = 8.3 Hz), 3.84 (s, 3H, OMe), 3.82 (s, 3H, OMe), 2.22 (s, 3H, CH$_3$). IR (ν, cm^{-1}): 3368 (NH), 3182–2854 (OH), 1653 (C = O). LC-MS (ESI) *m/z* found: 288 $[M+H]^+$.

N-(2-Hydroxy-4-methylphenyl)furan-2-carboxamide (2c): The title compound was prepared from furoic acid and 5-methyl-2-aminophenol to afford **2c** as a brown solid (72%): mp 170 °C (acetonitrile). ^{1}H NMR (300 MHz, CDCl$_3$): 8.85 (br s, 1H, OH), 8.34 (br s, 1H, NH), 7.50 (m, 1H, H$_{5'}$), 7.28 (m, 1H, H$_{3'}$), 7.10 (d, 1H, H$_6$, J = 8.1 Hz), 6.87 (m, 1H, H$_3$), 6.72 (m, 1H, H$_5$), 6.58 (dd, 1H, H$_{4'}$, J = 1.7 Hz and J = 3.5 Hz), 2.30 (s, 3H, CH$_3$). IR (ν, cm^{-1}): 3388 (NH), 3182–2854 (OH), 1649 (C = O).

N-(2-Hydroxy-5-nitro-phenyl) furan-2-carboxamide (***2d***): The title compound was prepared from furoic acid and 4-nitro-2-aminophenol to afford **2d** as a yellow solid (84%): mp 165 °C (methanol). ^{1}H NMR (300 MHz, CDCl$_3$): 9.77 (br s, 1H, OH), 9.13 (br s, 1H, NH), 8.79 (d, 1H, H$_6$, J = 1.9 Hz), 8.25 (dd, 1H, H$_4$, J = 1.9 Hz and J = 8.6 Hz), 8.07 (m, 1H, H$_{5'}$), 7.81 (d, 1H, H$_{3'}$, J = 3.5 Hz), 7.32 (d, 1H, H$_3$, J = 8.6 Hz), 6.78 (dd, 1H, H$_{4'}$, J = 1.7 Hz and J = 3.5 Hz). IR (ν, cm^{-1}): 3376 (NH), 3000–2500 (OH), 1640 (C = O).

General procedure for the synthesis of benzoxazole (3a–3d)

A suspension of amide ***2*** (2.21 mmol) and TsOH (5.53 mmol) was refluxed in toluene (150 ml) equipped with a Dean–Stark apparatus until complete dissolution for 17 h. The solution was then cooled to room temperature, hydrolysed with water and basified with a 6 M solution of NaOH up to basic pH (10–12). The organic layer was separated, dried over K_2CO_3 and concentrated *in vacuo*. Solid was suspended from the appropriate solvent and filtered.

2-(Furan-2-yl)-5-methyl-1,3-benzoxazole (***3a***): The title compound was prepared from amide **2a** to afford **3a** as a beige solid (82%): mp 64 °C (petroleum ether). ^{1}H NMR (300 MHz, CDCl$_3$): 7.67 (m, 1H, H$_{5'}$), 7.54 (m, 1H, H$_{3'}$), 7.44 (d, 1H, H$_7$, J = 8.3 Hz), 7.25 (m, 1H, H$_4$), 7.17 (m, 1H, H$_6$), 6.70 (dd, 1H, H$_{4'}$, J = 1.7 Hz and J = 3.5 Hz), 2.49 (s, 3H, CH$_3$). LC-MS (ESI) *m/z* found: 200 $[M+H]^+$.

2-(3,4-Dimethoxyphenyl)-5-methyl-1,3-benzoxazole (3b): The title compound was prepared from amide **2b** to afford **3b** as a beige solid (80%): mp 136 °C (diethyl ether). ^{1}H NMR (300 MHz, CDCl$_3$): 7.83 (dd, 1H, H$_{6'}$, J = 1.9 Hz and J = 8.4 Hz), 7.75 (d, 1H, H$_{2'}$, J = 1.9 Hz), 7.53 (m, 1H, H$_6$), 7.43 (d, 1H, H$_{5'}$, J = 8.4 Hz), 7.13 (m, 1H, H$_4$), 6.98 (d, 1H, H$_7$, J = 8.4 Hz), 4.02 (s, 3H, OMe), 3.97 (s, 3H, OMe), 2.48 (s, 3H, CH$_3$). LC-MS (ESI) *m/z* found: 270 $[M+H]^+$.

2-(Furan-2-yl)-6-methyl-1,3-benzoxazole (3c): The title compound was prepared from amide **2c** to afford **3c** as a beige solid (68%): mp 54 °C (diethyl ether). ^{1}H NMR (300 MHz, CDCl$_3$): 8.04 (m, 1H, H$_{5'}$), 7.61 (d, 1H, H$_4$, J = 8.1 Hz), 7.40 (d, 1H, H$_{3'}$, J = 3.5 Hz), 7.38 (m, 1H, H$_7$), 7.27–7.18 (m, 1H, H$_5$), 6.79 (dd, 1H, H$_{4'}$, J = 1.7 Hz and J = 3.5 Hz), 2.44 (s, 3H, CH$_3$). LC-MS (ESI) *m/z* found: 200 $[M+H]^+$.

2-(Furan-2-yl)-5-nitro-1,3-benzoxazole (3d): The title compound was prepared from amide **2b** to afford **3d** as a yellow solid (82%): mp 182 °C (diethyl ether). ^{1}H NMR (300 MHz, CDCl$_3$): 8.62 (d, 1H, H$_4$, J = 2.1 Hz), 8.34 (dd, 1H, H$_6$, J = 2.1 Hz and J = 8.4 Hz), 7.75 (d, 1H, H$_7$, J = 8.4 Hz), 7.68 (m, 1H, H$_{5'}$), 7.39 (d, 1H, H$_{3'}$, J = 3.5 Hz), 6.68 (dd, 1H, H$_{4'}$, J = 1.7 Hz and J = 3.5 Hz). IR (ν, cm^{-1}): 1519 (Ar-NO$_2$). LC-MS (ESI) *m/z* found: 231 $[M+H]^+$.

Methyl 2-[2-(3,4-dimethoxyphenyl)-1,3-benzoxazol-5-yl] acetate (3e): To a suspension of methyl 2-(3-amino-4-hydroxyphenyl) acetate (1.6 g, 8.83 mmol) in T_3P (solution in EtOAc) (4.2 g, 13.3 mmol), were added 3,4-dimethoxybenzoic acid (1.61 g, 8.83 mmol) and DIPEA (1.46 ml, 8.83 mmol). The reaction mixture was heated overnight at 120 °C, cooled to room temperature, suspended in water and extracted three times with EtOAc. Combined organic layers were washed 1 M NaOH solution, dried over $MgSO_4$ and concentrated *in vacuo*. Solid was recrystallised from EtOH to afford compound **(3e)** as a beige solid (1.59 g, 55%): mp 110 °C. ^{1}H NMR (300 MHz, CDCl$_3$): 7.82 (dd, 1H, H$_{6'}$, J = 8.4 Hz and J = 1.8 Hz), 7.74 (d, 1H, H$_4$, J = 1.8 Hz), 7.64 (m, 1H, H$_{2'}$), 7.49 (d, 1H, H$_7$, J = 8.4 Hz), 7.24 (dd, 1H, H$_6$, J = 8.4 Hz and J = 1.8 Hz), 6.98 (d, 1H, H$_{5'}$, J = 8.4 Hz), 4.01 (s, 3H, OMe), 3.95 (s, 3H, OMe), 3.75 (s, 2H, CH$_2$),

3.70 (s, 3H, CO_2Me). IR (ν, cm^{-1}): 1730 (ester C = O). LC-MS (ESI) *m/z* found: 328 $[M+H]^+$.

General procedure for the synthesis of compound (4a–4c)

To a solution of compound **(3a–3c)** (20.1 mmol) in CCl_4 (150 ml) was added *N*-bromosuccinimide (NBS) (24.1 mmol) and benzoyl peroxide (1.41 mmol) and the reaction mixture was refluxed under a halogen lamp (230 W). After 3 h and 30 min stirring, the mixture was cooled to room temperature and the succinimide was filtered off. Then, the solution was concentrated *in vacuo*, and the solid was suspended in diethyl ether and filtered.

5-(Bromomethyl)-2-(furan-2-yl)-1,3-benzoxazole (4a): The title compound was prepared from benzoxazole **3a** to afford **4a** as a beige solid (70%): mp 130 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.77 (m, 1H, $H_{5'}$), 7.67 (m, 1H, $H_{3'}$), 7.54 (d, 1H, H_7, $J = 8.4$ Hz), 7.42 (dd, 1H, H_6, $J = 1.8$ Hz and $J = 8.4$ Hz), 7.30 (m, 1H, H_4), 6.64 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 4.64 (s, 2H, CH_2). LC-MS (ESI) *m/z* found: 278 $[M+H]^+$, 280 $[M+H]^+$.

6-(Bromomethyl)-2-(furan-2-yl)-1,3-benzoxazole (***4b***): The title compound was prepared from benzoxazole **3b** to afford **4b** as a brown solid (65%): mp 122 °C, ^{1}H NMR (300 MHz, $CDCl_3$): 7.72–7.68 (m, 2H, $H_{5'}$ and H_4), 7.60 (m, 1H, $H_{7'}$), 7.40 (dd, 1H, H_5, $J = 8.2$ Hz and $J = 1.6$ Hz), 7.30 (dd, 1H, $H_{3'}$, $J = 0.7$ Hz and $J = 3.5$ Hz), 6.63 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 4.64 (s, 2H, CH_2). IR (ν, cm^{-1}): 750 (C-Br).

5-(Bromomethyl)-2-(3,4-dimethoxyphenyl)-1,3-benzoxazole (***4c***): The title compound was prepared from benzoxazole **3c** to afford **4c** as a beige solid (75%): mp 140 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.86 (dd, 1H, $H_{6'}$, $J = 1.9$ Hz and $J = 8.4$ Hz), 7.76 (m, 2H, H_4 and $H_{2'}$), 7.53 (d, 1H, H_7 or $H_{5'}$, $J = 8.5$ Hz), 7.39 (dd, 1H, H_6, $J = 1.7$ Hz and $J = 8.4$ Hz), 7.00 (d, 1H, H_7 or $H_{5'}$, $J = 8.4$ Hz), 4.65 (s, 2H, CH_2), 4.02 (s, 3H, OMe), 3.99 (s, 3H, OMe). IR (ν, cm^{-1}): 750 (C-Br).

General procedure for the synthesis of compound (A1–A3 and A5–A8)

To a solution of amine (2.05 mmol) in acetone (15 ml) were added compound (**4a–4c**) (1.87 mmol) and Et_3N (2.05 mmol). The reaction mixture was refluxed 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 $MgSO_4$ and concentrated *in vacuo* to give a solid which was then purified.

2-(Furan-2-yl)-5-(piperidin-1-ylmethyl)-1,3-benzoxazole hydrochloride (***A1***): The title compound was prepared from compound **4a** and piperidine. Solid was suspended in diethyl ether with HCl gas, concentrated *in vacuo* and recrystallised from acetonitrile to afford **A1** as a white solid (74%): mp >300 °C. ^{1}H NMR (300 MHz, $[D_6]$DMSO): 12.43 (br s, 1H, NH^+), 8.02 (m, 1H, H_6), 7.75 (m, 1H, $H_{5'}$), 7.70 (m, 1H, H_4), 7.66 (d, 1H, H_7, $J = 8.1$ Hz), 7.32 (d, 1H, $H_{3'}$, $J = 3.5$ Hz), 6.65 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 4.26 (d, 2H, CH_2, $J = 5.0$ Hz), 3.47 (m, 2H, $H_{piperidine}$), 2.61 (m, 2H, $H_{piperidine}$), 2.33 (m, 2H, $H_{piperidine}$), 1.92–1.80 (m, 3H, $H_{piperidine}$), 1.35 (m, 1H, $H_{piperidine}$). ^{13}C NMR (75 MHz, $[D_6]$DMSO): 156.5 (C), 151.0 (C), 146.3 (CH), 142.1 (C), 142.0 (C), 129.1 (CH), 125.1 (C), 122.9 (CH), 115.3 (CH), 112.5 (CH), 111.8 (CH), 60.9 (CH_2), 52.3 (2 CH_2), 22.5 (2 CH_2), 22.1 (CH_2). LC-MS (ESI) *m/z* found: 283 $[M+H]^+$. HPLC: C_4 column: $t_R = 16.2$ min, purity 98%.

2-(Furan-2-yl)-5-[(4-phenylepiperazin-1-yl)methyl]-1,3-benzoxazole hydrochloride (***A2***): The title compound was prepared from compound **4a** and phenylpiperazine. Solid was suspended in diethyl ether with HCl gas, concentrated *in vacuo* and recrystallised from ethanol to afford **A2** as a white solid (65%): mp >300 °C. ^{1}H NMR (300 MHz, $[D_6]$DMSO): 13.12 (br s, 1H, NH^+), 8.04 (m, 1H, $H_{5'}$), 7.83 (m, 1H, H_6), 7.68 (m, 2H), 7.33 (d, 1H, $H_{3'}$, $J = 3.5$ Hz), 7.30–7.25 (m, 2H), 6.98–6.89 (m, 3H), 6.66 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 4.37 (s, 2H, CH_2), 3.75–3.52 (m, 6H, $H_{piperazine}$), 3.06 (m, 2H, $H_{piperazine}$). ^{13}C NMR (75 MHz, $[D_6]$DMSO): 156.0 (C), 150.6 (C), 150.0 (C), 148.0 (CH), 141.9 (C), 141.7 (C), 129.6 (2 CH), 129.4 (CH), 127.5 (C), 123.5 (CH), 120.4 (CH), 116.4 (2 CH), 116.2 (CH), 113.4 (CH), 111.6 (CH), 58.7 (CH_2), 50.6 (2 CH_2), 45.7 (2 CH_2). LC-MS (ESI) *m/z* Found: 360 $[M+H]^+$. HPLC: C_4 column: $t_R = 15.5$ min, purity >99%.tert-Butyl4-{[2-(Furan-2-yl)-1,3-benzoxazol-5-yl]methyl}piperazine-1-carboxylate (***A3***): The title compound was prepared from compound **4a** and boc-piperazine. Solid was recrystallised from methanol to afford **A3** as a white solid (83%): mp 128 °C (methanol). ^{1}H NMR (300 MHz, $CDCl_3$): 7.69 (m, 1H, $H_{5'}$), 7.67 (m, 1H, H_4), 7.49 (d, 1H, H_7, $J = 8.3$ Hz), 7.34 (dd, 1H, H_6, $J = 1.5$ Hz and $J = 8.3$ Hz), 7.26 (m, 1H, $H_{3'}$), 6.62 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 3.63 (s, 2H, CH_2), 3.44 (t, 4H, $H_{piperazine}$, $J = 4.9$ Hz), 2.42 (t, 4H, $H_{piperazine}$, $J = 3.5$ Hz), 1.45 (s, 9H, (3 CH_3). NMR ^{13}C ($CDCl_3$, δ ppm): 163.0 (C), 155.6 (C), 154.0 (C), 149.8 (C), 147.7 (C), 141.9 (C), 141.8 (CH), 132.2 (CH), 127.9 (C), 121.5 (CH), 115.8 (CH), 113.3 (CH), 111.2 (CH), 79.6 (2 CH_2), 60.8 (CH_2), 51.9 (2 CH_2), 26.5 (3 CH_3). IR (ν, cm^{-1}): 1679 (C = O). LC-MS (ESI) *m/z* found: 328 $[M\text{-}tBu+H]^+$, 284 $[M+H]^+$. HPLC: C_4 column: $t_R = 14.7$ min, purity >99%.

4-(4-{[2-(Furan-2-yl)-1,3-benzoxazol-5-yl]methyl}piperazin-1-yl)phenol (***A5***): The title compound was prepared from compound **4a** and 4-piperazinephenol. Solid was purified by flash chromatography using DCM/EtOAc (9/1) to afford **A5** as a white solid (83%): mp 212 °C. ^{1}H NMR (300 MHz, $[D_6]$DMSO): 8.79 (br s, 1H, OH), 8.07 (m, 1H, $H_{5'}$), 7.71 (m, 2H, H_7 and H_4), 7.46 (d, 1H, $H_{3'}$, $J = 3.5$ Hz), 7.41 (dd, 1H, H_6, $J = 1.2$ Hz and $J = 8.4$ Hz), 6.82 (dd, 1H, $H_{4'}$, $J = 1.8$ Hz and $J = 3.5$ Hz), 6.78 (d, 2H, H_{phenyl}, $J = 8.7$ Hz), 6.63 (d, 2H, H_{phenyl}, $J = 8.7$ Hz), 3.64 (s, 2H, CH_2), 2.96 (m, 4H, $H_{piperazine}$), 2.55 (m, 4H, $H_{piperazine}$). ^{13}C NMR (75 MHz, $[D_6]$DMSO): 155.4 (C), 151.3 (C), 149.2 (C), 147.6 (CH), 144.7 (C), 142.1 (C), 141.7 (C), 135.9 (C), 126.9 (CH), 120.2 (CH), 118.2 (2 CH), 115.9 (2 CH), 115.5 (CH), 113.3 (CH), 110.8 (CH), 62.2 (CH_2), 53.2 (2 CH_2), 50.5 (2 CH_2). IR (ν, cm^{-1}): 3195–2780 (OH). LC-MS (ESI) *m/z* found: 376 $[M+H]^+$. HPLC: C_4 column: $t_R = 10.2$ min, purity 99% C_{18} column: $t_R = 14.3$ min, purity >99%.

2-(Furan-2-yl)-5-({4-[4-(2-methoxyethoxy)phenyl]piperazin-1-yl}methyl)-1,3-benzoxazole (***A6***): The title compound was prepared from compound **4a** and 1-[4-(2-methoxyethoxy)phenyl]piperazine. Solid was recrystallised from ethanol to afford **A6** as a white solid (33%): mp 123 °C. RMN ^{1}H (300 MHz, $[D_6]$DMSO): 8.08 (m, 1H, $H_{5'}$), 7.72 (m, 2H, H_7 and H_4), 7.46 (dd, 1H, $H_{3'}$, $J = 0.7$ Hz and $J = 3.5$ Hz), 7.40 (dd, 1H, H_6, $J = 1.4$ Hz and $J = 8.4$ Hz), 6.88–6.79 (m, 5H, $H_{4'}$, H_{phenyl}), 3.99 (m, 2H, OCH_2), 3.64 (s, 2H, CH_2), 3.61 (m, 2H, CH_2O), 3.29 (s, 3H, OCH_3), 3.01 (m, 4H, $H_{piperazine}$), 2.54 (m, 4H, $H_{piperazine}$). RMN ^{13}C (75 MHz, $[D_6]$DMSO): 155.4 (C), 152.5 (C), 149.2 (CH), 147.5 (C), 145.9 (C), 142.1 (C), 141.7 (C), 135.9 (C), 126.1 (C), 119.9 (CH), 118.1 (2 CH), 115.4 (2 CH), 114.1 (CH), 112.3 (CH), 110.2 (CH), 71.2 (CH_2), 67.7 (CH_2), 60.8 (CH_2), 53.4 (2 CH_2), 50.6 (2 CH_2), 33.7 (CH_3). LC-MS (ESI) *m/z* found: 434 $[M+H]^+$. HPLC: C_4 column: $t_R = 16.1$ min, purity >99%.

2-(Furan-2-yl)-6-(piperidin-1-ylmethyl)-1,3-benzoxazole hydrochloride (***A7***): The title compound was prepared from compound **4c** and piperidine. Solid was suspended in diethyl ether with HCl gas, concentrated *in vacuo* and recrystallised from acetonitrile to afford **A7** (276 mg, 68%): mp >300 °C. ^{1}H NMR (300 MHz, $[D_6]$DMSO): 11.08 (br s, 1H, NH^+), 8.16–8.10 (m, 2H, H_7 and $H_{5'}$), 7.82 (d, 1H, H_4, $J = 8.1$ Hz), 7.63 (m, 1H, H_5), 7.51 (d, 1H, $H_{3'}$, $J = 3.2$ Hz), 6.83 (m, 1H, $H_{4'}$), 4.38 (m, 2H, CH_2), 3.28 (m, 2H, $H_{piperidine}$), 2.83 (m, 2H, $H_{piperidine}$), 1.76–1.66 (m, 5H, $H_{piperidine}$),

1.35 (m, 1H, $H_{piperidine}$). ^{13}C NMR (75 MHz, [D_6]DMSO): 156.1 (C), 149.9 (C), 148.0 (CH), 142.4 (C), 141.7 (C), 129.0 (C), 127.8 (CH), 120.1 (CH), 116.3 (CH), 114.4 (CH), 113.4 (CH), 59.1 (CH_2), 52.0 (2 CH_2), 22.6 (2 CH_2), 21.9 (CH_2). LC-MS (ESI) *m/z* found: 283 $[M+H]^+$. HPLC: C_4 column: $t_R = 15.7$ min, purity 96%.

2-(3,4-Dimethoxyphenyl)-5-(piperidin-1-ylmethyl)-1,3-benzoxazole hydrochloride (***A8***): The title compound was prepared from compound **4b** and piperidine. Solid was suspended in diethyl ether with HCl gas, concentrated *in vacuo* and recrystallised from acetonitrile to afford **A8** as a white solid (200 mg, 60%): mp >300 °C, ^1H NMR (300 MHz, [D_6]DMSO): 12.31 (br s, 1H, NH^+), 7.90 (dd, 1H, $H_{6'}$, $J = 1.6$ Hz and $J = 8.4$ Hz), 7.82 (dd, 1H, H_6, $J = 1.9$ Hz and $J = 8.4$ Hz), 7.78 (d, 1H, $H_{2'}$, $J = 1.6$ Hz), 7.71 (d, 1H, H_4, $J = 1.9$ Hz), 7.62 (d, 1H, $H_{5'}$, $J = 8.4$ Hz), 6.97 (d, 1H, H_7, $J = 8.4$ Hz), 4.27 (d, 2H, CH_2, $J = 5.1$ Hz), 3.99 (s, 3H, OMe), 3.96 (s, 3H, OMe), 3.45 (m, 2H, $H_{piperidine}$), 2.64 (m, 2H, $H_{piperidine}$), 2.34 (m, 2H, $H_{piperidine}$), 1.86 (m, 3H, $H_{piperidine}$), 1.35 (m, 1H, $H_{piperidine}$). ^{13}C NMR (75 MHz, [D_6]DMSO): δ 164.4 (C), 152.4 (C), 151.5 (C), 149.3 (C), 142.6 (C), 128.4 (CH), 124.7 (C), 122.5 (CH), 121.5 (C), 119.0 (CH), 111.5 (CH), 111.1 (CH), 110.1 (CH), 60.9 (CH_2), 56.1 (CH_3), 56.1 (CH_3), 52.6 (2 CH_2), 22.5 (2 CH_2), 22.0 (CH_2). LC-MS (ESI) *m/z* found: 353 $[M+H]^+$. HPLC: C_4 column: $t_R = 14.3$ min, purity 99%.

Synthesis of 2-(Furan-2-yl)-5-(piperazin-1-ylmethyl)-1,3-benzoxazole (***A4***): A solution of compound **(A3)** (100 mg, 0.35 mmol) diluted in 5 ml of DCM with TFA (2 ml, 26 mmol) was stirred for 3 h at room temperature, hydrolysed with water and basified with 1 M solution of NaOH up to basic pH and extracted three times with DCM. Combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Solid was suspended in diethyl ether with a drop of ethanol and filtered to afford compound **(A4)** as a beige solid (60%): mp 192 °C. ^1H NMR (300 MHz, [D_6]DMSO): 8.08 (m, 1H, $H_{5'}$), 7.74–7.71 (m, 2H, H_4 and H_7), 7.46 (dd, 1H, $H_{3'}$, $J = 0.7$ Hz and $J = 3.5$ Hz), 7.38 (dd, 1H, H_6, $J = 1.6$ Hz and $J = 8.3$ Hz), 6.83 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 3.65 (s, 2H, CH_2), 3.45–3.39 (m, 4H, $H_{piperazine}$), 2.43 (m, 4H, $H_{piperazine}$). ^{13}C NMR (75 MHz, [D_6]DMSO): 155.4 (C), 149.3 (C), 147.6 (CH), 142.0 (C), 141.7 (C), 135.2 (C), 127.0 (CH), 120.3 (CH), 115.6 (CH), 113.3 (CH), 110.9 (CH), 61.9 (CH_2), 50.6 (2 CH_2), 44.1 (2 CH_2). LC-MS (ESI) *m/z* found: 284 $[M+H]^+$. HPLC: C_4 column: $t_R = 15.8$ min, purity 99%.

2-[2-(Furan-2-yl)-1,3-benzoxazol-5-yl]acetonitrile (***5***): To a solution of 5-(bromomethyl)-2-(3,4-dimethoxyphenyl)-1,3-benzoxazole **(4a)** (1.23 g, 3.53 mmol) in a mixture of EtOH (56 ml) and H_2O (15 ml) was added KCN (1.15 g, 17.7 mmol). After one night stirring at reflux, the reaction mixture was cooled to room temperature and concentrated *in vacuo*. Solid was suspended in water and extracted three times with EtOAc. Combined organic layer were dried over $MgSO_4$ and concentrated *in vacuo*. Solid was then recrystallised in methanol to afford (**5**) as a beige solid (582 mg, 56%): mp 140 °C. ^1H NMR (300 MHz, $CDCl_3$): 7.71–7.70 (m, 2H, $H_{5'}$ and $H_{3'}$), 7.58 (d, 1H, H_7, $J = 8.3$ Hz), 7.36–7.31 (m, 2H, H_4 and H_6), 6.64 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 3.90 (s, 2H, CH_2). IR (ν, cm^{-1}): 2240 (CN). LC-MS (ESI) *m/z* found: 225 $[M+H]^+$.

2-[2-(Furan-2-yl)-1,3-benzoxazol-5-yl] acetic acid (***6***): A solution of compound **(5)** (1.1 g, 4.91 mmol) in a mixture of AcOH (5.5 ml), H_2SO_4 (5.5 ml) and H_2O (5.5 ml) was refluxed for 17 h. After cooling to room temperature, cold water was added and the solution was extracted with EtOAc. The organic layer was extracted twice with a saturated $NaHCO_3$ solution, and then the aqueous layer was acidified with 1 M HCl solution and extracted twice with EtOAc. Combined organic layer were dried over $MgSO_4$ and concentrated *under vacuum*. Solid was suspended in diethyl ether and filtered to afford compound **(6)** as a white solid (702 mg, 60%): mp 203 °C. ^1H NMR (300 MHz, $CDCl_3$): 12.38 (br s, 1H, OH), 8.07 (m, 1H, $H_{5'}$), 7.68 (d, 1H, H_7, $J = 8.4$ Hz), 7.66 (m, 1H, $H_{3'}$), 7.45 (m, 1H, H_4), 7.32 (dd, 1H, H_6, $J = 1.8$ Hz and $J = 8.4$ Hz), 6.80 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 3.72 (s, 2H, CH_2). IR (ν, cm^{-1}): 1710 (acid C = O). LC-MS (ESI) *m/z* found: 244 $[M+H]^+$.

General procedure for the synthesis of amide (7a–7d)

To a solution of acid **(6)** (1.5 mmol) in toluene (4 ml) was added at 0 °C $SOCl_2$ (5.97 mmol). The mixture was refluxed during 1 h, cooled to room temperature and concentrated *in vacuo*. The oil was then diluted with EtOAc (26 ml) and added dropwise to a solution of amine (1.6 mmol) and Et_3N (2.25 mmol) in EtOAc (30 ml) while being stirred and cooled in an ice bath. After 1 h and 30 min stirring at room temperature, the mixture was hydrolysed with water, extracted twice with EtOAc and combined organic layers were washed with a saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$ and concentrated *in vacuo* to give a solid which was suspended in diethyl ether and filtered.

2-[2-(Furan-2-yl)-1,3-benzoxazol-5-yl]-1-(piperidin-1-yl)ethan-1-one (***7a***): The title compound was prepared from compound **6** and piperidine to afford **7a** as a white solid (76%): mp 112 °C. ^1H NMR (300 MHz, $CDCl_3$): 8.16 (d, 1H, $H_{5'}$, $J = 1.7$ Hz), 7.71 (m, 2H, H_4 and H_7), 7.68 (m, 1H, H_6), 7.34 (d, 1H, $H_{3'}$, $J = 3.5$ Hz), 6.81 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 3.78 (s, 2H, CH_2), 3.39 (m, 4H, $H_{piperidine}$), 1.68 (m, 4H, $H_{piperidine}$), 1.47 (m, 2H, $H_{piperidine}$). IR (ν, cm^{-1}): 1640 (C = O).

2-[2-(Furan-2-yl)-1,3-benzoxazol-5-yl]-1-(4-phenylpiperazin-1-yl)ethan-1-one (***7b***): The title compound was prepared from compound **6** and phenylpiperazine to afford **7b** as a white solid (78%): mp 128 °C. ^1H NMR (300 MHz, $CDCl_3$): 7.68 (m, 1H, $H_{5'}$), 7.63 (m, 1H, $H_{3'}$), 7.52 (d, 1H, H_7, $J = 8.4$ Hz), 7.32–7.23 (m, 4H, H_{phenyl}), 6.89–6.87 (m, 3H, H_4, H_6 and H_{phenyl}), 6.63 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 3.91 (s, 2H, CH_2), 3.83 (t, 2H, $H_{piperazine}$, $J = 4.6$ Hz), 3.66 (t, 2H, $H_{piperazine}$, $J = 4.6$ Hz), 3.16 (t, 2H, $H_{piperazine}$, $J = 4.6$ Hz), 3.02 (t, 2H, $H_{piperazine}$, $J = 4.6$ Hz). IR (ν, cm^{-1}): 1640 (C = O).

2-[2-(Furan-2-yl)-1,3-benzoxazole-5-yl]-1-(morpholine-4-yl) ethan-1-one (***7c***): The title compound was prepared from compound **6** and morpholine to afford **7c** as a white solid (57%): mp 118 °C. ^1H NMR (300 MHz, $CDCl_3$): 7.67 (m, 1H, $H_{5'}$), 7.59 (m, 1H, $H_{3'}$), 7.52 (d, 1H, H_7, $J = 8.4$ Hz), 7.27 (m, 2H, H_4 and H_6), 6.63 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 3.85 (s, 2H, CH_2), 3.66 (m, 4H, $H_{morpholine}$), 3.50 (m, 4H, $H_{morpholine}$). IR (ν, cm^{-1}): 1630 (C = O).

2-[2-(Furan-2-yl)-1,3-benzoxazol-5-yl]-1-{4-[4-(2-methoxyethoxy)-phenyl]piperazin-1-yl}ethan-1-one (***7d***): The title compound was prepared from compound **6** and 1-[4-(2-methoxyethoxy)phenyl]piperazine to afford **7d** as a beige solid (73%): mp 114 °C. ^1H NMR (300 MHz, [D_6]DMSO): 7.67 (m, 1H, $H_{5'}$), 7.62 (m, 1H, $H_{3'}$), 7.51 (d, 1H, H_7, $J = 8.4$ Hz), 7.29 (m, 2H, H_6 and H_4), 6.84 (m, 4H, H_{phenyl} and H_{phenyl}), 6.63 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 4.05 (t, 2H, CH_2O, $J = 4.6$ Hz), 3.89 (s, 2H, CH_2), 3.81 (t, 2H, $H_{piperazine}$, $J = 4.7$ Hz), 3.71 (t, 2H, CH_2O, $J = 4.6$ Hz), 3.63 (t, 2H, $H_{piperazine}$, $J = 4.7$ Hz), 3.43 (s, 3H, OCH_3), 3.02 (t, 2H, $H_{piperazine}$, $J = 4.7$ Hz), 2.88 (t, 2H, $H_{piperazine}$, $J = 4.7$ Hz). IR (ν, cm^{-1}): 1630 (C = O).

General procedure for the synthesis of compound (B1–B4)

To a solution of amide (1.06 mmol) **(7a–7d)** in THF (5 ml) was added $LiAlH_4$ (2.65 mmol). After 1 h stirring at room temperature, water (0.1 ml), 1 M NaOH solution (0.1 ml) and water (0.3 ml) were added successively to get a white mineral solid which was filtered off and washed with EtOAc (50 ml). Organic layer was then washed with water, brine solution, dried over $MgSO_4$ and concentrated *in vacuo* to afford a solid which was then purified.

2-(Furan-2-yl)-5-[2-(piperidine-1-yl) ethyl]-1,3-benzoxazole hydrochloride (***B1***): The title compound was prepared from amide **7a**.

Solid was suspended in diethyl ether with HCl gas, concentrated *in vacuo* and recrystallised from acetonitrile to afford **B1** as a white solid (10%): mp >300 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): 10.53 (br s, 1H, NH$^+$), 8.0 (m, 1H, H$_{5'}$), 7.74 (d, 1H, H$_7$, J = 8.4 Hz), 7.69 (m, 1H, H$_4$), 7.46 (d, 1H, H$_{3'}$, J = 3.5 Hz), 7.33 (dd, 1H, H$_6$, J = 3.4 and J = 8.4 Hz), 6.82 (dd, 1H, H$_{4'}$, J = 1.7 Hz and J = 3.5 Hz), 3.52–3.48 (m, 2H, C*H*$_2$CH$_2$), 3.33–3.19 (m, 4H, H$_{piperidine}$), 2.93–2.90 (m, 2H, CH$_2$C*H*$_2$), 1.80–1.70 (m, 5H, H$_{piperidine}$), 1.43–1.39 (m, 1H, H$_{piperidine}$). ^{13}C NMR (75 MHz, [D$_6$]DMSO): 155.5 (C), 149.0 (C), 147.7 (CH), 142.0 (C), 141.9 (C), 134.9 (C), 126.8 (CH), 120.1 (CH), 115.7 (CH), 113.3 (CH), 111.3 (CH), 57.2 (CH$_2$), 52.5 (2 CH$_2$), 29.7 (CH$_2$), 22.8 (2 CH$_2$), 21.9 (CH$_2$). LC-MS (ESI) *m/z* found: 297 [M + H]$^+$. HPLC: C$_4$ column: t$_R$ = 16.3 min, purity >99%.

2-(Furan-2-yl)-5-[2-(4-phenylpiperazin-1-yl) ethyl]-1,3-benzoxazole (***B2***): The title compound was prepared from amide **7b**. Solid was recrystallised from ethanol to afford **B2** as a white solid (12%): mp 160 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): 8.06 (m, 1H, H$_{5'}$), 7.65 (m, 1H, H$_4$), 7.56 (1H, d, H$_7$, J = 8.2 Hz), 7.43 (d, 1H, H$_{3'}$, J = 3.5 Hz), 7.31 (dd, 1H, H$_6$, J = 1.3 Hz and J = 8.2 Hz), 7.22–7.17 (m, 2H, H$_{phenyl}$), 6.92 (d, 2H, H$_{phenyl}$, J = 7.9 Hz), 6.80 (dd, 1H, H$_{4'}$, J = 1.7 Hz and J = 3.5 Hz), 6.78–6.73 (m, 1H, H$_{phenyl}$), 3.12 (m, 4H, H$_{piperazine}$), 2.88 (t, 2H, C*H*$_2$CH$_2$, J = 7.3 Hz), 2.64–2.57 (m, 6H, CH$_2$C*H*$_2$ and H$_{piperazine}$). ^{13}C NMR (75 MHz, [D$_6$]DMSO): 155.2 (C), 151.5 (C), 148.5 (C), 147.5 (CH), 141.7 (C), 142.1 (C), 138.2 (C), 129.4 (CH), 126.9 (CH), 120.0 (CH), 119.2 (CH), 115.8 (CH), 115.4 (CH), 113.2 (CH), 110.8 (CH), 60.4 (CH$_2$), 53.1 (2 CH$_2$), 48.7 (2 CH$_2$), 33.0 (CH$_2$). LC-MS (ESI) *m/z* found: 374 [M + H]$^+$. HPLC: C$_4$ column: t$_R$ = 15.8 min, purity >99%.

2-(Furan-2-yl)-5-[2-(morpholin-4-yl) ethyl]-1,3-benzoxazole hydrochloride (***B3***): The title compound was prepared from amide **7c**. Solid was suspended in diethyl ether with HCl gas, concentrated *in vacuo* and recrystallised from acetonitrile to afford **B3** as a white solid (80%): mp >300 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): 11.36 (br s, 1H, NH$^+$), 8.06 (m, 1H, H$_{5'}$), 7.73–7.67 (m, 2H, H$_7$ and H$_4$), 7.43 (m, 1H, H$_{3'}$), 7.31 (m, 1H, H$_6$), 7.80 (m, 1H, H$_{4'}$), 3.92–3.82 (m, 4H, C*H*$_2$C*H*$_2$), 3.31–3.18 (m, 8H, H$_{morpholine}$). LC-MS (ESI) *m/z* found: 299 [M + H]$^+$. HPLC: C$_4$ column: t$_R$ = 14.8 min, purity 99%.

2-(Furan-2-yl)-5-(2-{4-[4-(2-methoxyethoxy)phenyl]piperazin-1-yl}ethyl)-1,3-benzoxazole (***B4***): The title compound was prepared from amide **7d**. Solid was suspended recrystallised from acetonitrile to afford **B4** as a beige solid (73%): mp >300 °C. ^{1}H NMR (300 MHz, CDCl$_3$): 7.67 (m, 1H, H$_{5'}$), 7.62 (m, 1H, H$_{3'}$), 7.48 (d, 1H, H$_7$, J = 8.3 Hz), 7.27 (m, 2H, H$_6$ and H$_4$), 7.22 (m, 1H, H$_{phenyl}$), 6.90 (m, 4H, H$_{phenyl}$), 6.63 (dd, 1H, H$_{4'}$, J = 1.7 Hz and J = 3.5 Hz), 4.09 (t, 2H, OCH$_2$, J = 4.6 Hz), 3.73 (t, 2H, MeOC*H*$_2$, J = 4.6 Hz), 3.45 (s, 3H, OMe), 3.15 (m, 4H, H$_{piperazine}$), 2.98 (m, 2H, C*H*$_2$CH$_2$), 2.73 (m, 6H, H$_{piperazine}$ and CH$_2$C*H*$_2$). ^{13}C NMR (75 MHz, CDCl$_3$): 155.5 (C), 152.9 (C), 148.8 (C), 145.9 (C), 145.7 (CH), 142.7 (C), 141.9 (C), 137.3 (C), 126.1 (CH), 119.9 (CH), 118.1 (2 CH), 115.4 (2 CH), 114.1 (CH), 112.3 (CH), 110.2 (CH), 71.2 (CH$_2$), 67.7 (CH$_2$), 60.8 (CH$_2$), 59.2 (CH$_2$), 53.4 (2 CH$_2$), 50.6 (2 CH$_2$), 33.7 (CH$_3$). LC-MS (ESI) *m/z* found: 448 [M + H]$^+$. HPLC: C$_4$ column: t$_R$ = 15.2 min, purity 99%.

General procedure for the synthesis of acid (8a–8b)

A solution of dimethyl malonate (9.06 mmol) in acetone (33 ml) with K$_2$CO$_3$ (13.6 mmol) was stirred for 20 min at room temperature. Then compound **(4a, 4c)** (4.53 mmol) was added and the mixture was stirred at reflux for 2 h, cooled to room temperature and concentrated *in vacuo*. Solid was suspended in water and extracted twice with EtOAc. Combined organic layer were dried over MgSO$_4$ and then concentrated *in vacuo*. The solid was suspended in H$_2$O (7 ml) and NaOH (18.1 mmol) was added. The mixture was stirred overnight at 40 °C and then washed with EtOAc. The aqueous layer was acidified with 6 M HCl solution up to acid pH (1–3) and the formed solid was filtered. Crude was heated in DMF (5 ml) at 80 °C for 3 h, cooled to room temperature, hydrolysed with water and acidified with 1 M HCl solution and extracted three times with EtOAc. Combined organic layers were washed with brine, dried over MgSO$_4$ and concentrated *in vacuo*. Solid was suspended in diethyl ether and filtered.

3-[2-(Furan-2-yl)-1,3-benzoxazol-5-yl] propanoic acid (***8a***): The title compound was prepared from compound **4a** to afford **8a** as a white solid (43%): mp 207 °C. ^{1}H NMR (300 MHz, CDCl$_3$): 12.19 (br s, 1H, OH), 8.05 (m, 1H, H$_{5'}$), 7.66–7.62 (m, 2H, H$_6$ and H$_4$), 7.42 (d, 1H, H$_{3'}$, J = 3.5 Hz), 7.28 (d, 1H, H$_7$, J = 8.2 Hz), 6.79 (m, 1H, H$_{4'}$), 2.94 (t, 2H, C*H*$_2$CH$_2$, J = 7.5 Hz), 2.59 (t, 2H, CH$_2$C*H*$_2$, J = 7.5 Hz). IR (ν, cm^{-1}): 1686 (C = O). LC-MS (ESI) *m/z* found: 258 [M + H]$^+$.

3-[2-(3,4-Dimethoxyphenyl)-1,3-benzoxazol-5-yl] propanoic acid (***8b***): The title compound was prepared from compound **4c** to afford **8b** as a white solid (47%): mp 182 °C. ^{1}H NMR (300 MHz, CDCl$_3$): 12.14 (br s, 1H, OH), 7.77 (dd, 1H, H$_{6'}$, J = 2.0 Hz and J = 8.4 Hz), 7.67–7.60 (m, 3H, H$_4$, H$_{2'}$ and H$_{5'}$), 7.25 (dd, 1H, H$_6$, J = 1.6 Hz and J = 8.3 Hz), 7.17 (d, 1H, H$_7$, J = 8.3 Hz), 3.88 (s, 3H, OMe), 3.86 (s, 3H, OMe), 2.94 (t, 2H, CH$_2$CH$_2$, J = 7.7 Hz), 2.59 (t, 2H, CH$_2$CH$_2$, J = 7.7 Hz). IR (ν, cm^{-1}): 1710 (C = O).

Synthesis of compounds (9a–9c)

Same procedure as described for compounds (7a–7d) has been used.

3-(2-(Furan-2-yl)-1,3-benzoxazol-5-yl)-1-(4-phenylpiperazin-1-yl)propan-1-one (***9a***): The title compound was prepared from compound **8a** and phenylpiperazine to afford **9a** as a white solid (55%): mp 164 °C. ^{1}H NMR (300 MHz, CDCl$_3$): 7.66 (m, 1H, H$_{5'}$), 7.58 (m, 1H, H$_{3'}$), 7.48 (d, 1H, H$_7$, J = 8.3 Hz), 7.29–7.23 (m, 4H, H$_{phenyl}$), 6.91–6.87 (m, 3H, H$_4$, H$_6$ and H$_{phenyl}$), 6.62 (dd, 1H, H$_{4'}$, J = 1.7 Hz and J = 3.5 Hz), 3.57 (m, 2H, H$_{piperazine}$, J = 5.7 Hz), 3.55 (m, 2H, H$_{piperazine}$), 3.17–3.11 (m, 4H, C*H*$_2$CH$_2$ and H$_{piperazine}$), 3.04 (m, 2H, H$_{piperazine}$), 2.73 (t, 2H, CH$_2$C*H*$_2$, J = 7.7 Hz). IR (ν, cm^{-1}): 1658 (C = O).

3-[2-(3,4-Dimethoxyphenyl)-1,3-benzoxazole-5-yl]-1-(piperidin-1-yl)propan-1-one (***9b***): The title compound was prepared from compound **8b** and piperidine to afford **9b** as a white solid (66%): mp 130 °C. ^{1}H NMR (300 MHz, CDCl$_3$): 7.85 (dd, 1H, H$_{6'}$, J = 8.4 Hz and J = 1.9 Hz), 7.75 (d, 1H, H$_{2'}$, J = 1.9 Hz), 7.56 (m, 1H, H$_4$), 7.58 (d, 1H, H$_{7'}$, J = 8.4 Hz), 7.21 (dd, 1H, H$_6$, J = 1.5 Hz and J = 8.1 Hz), 7.17 (d, 1H, H$_7$, J = 8.4 Hz), 4.02 (s, 3H, OMe), 3.97 (s, 3H, OMe), 3.57 (t, 1H, CH$_2$C*H*$_2$, J = 5.6 Hz), 3.35 (t, 2H, H$_{piperidine}$, J = 5.6 Hz), 3.10 (t, 2H, C*H*$_2$CH$_2$, J = 7.7 Hz), 2.67 (t, 2H, CH$_2$C*H*$_2$, J = 7.7 Hz), 1.68–1.47 (m, 6H, H$_{piperidine}$). IR (ν, cm^{-1}): 1625 (C = O).

3-(2-(3,4-Dimethoxyphenyl)-1,3-benzoxazol-5-yl)-1-(4-phenylpiperazin-1-yl)propan-1-one (***9c***): The title compound was prepared from compound **8b** and phenylpiperazine to afford **9c** as a white solid (54%): mp 160 °C. ^{1}H NMR (300 MHz, CDCl$_3$): 7.85 (dd, 1H, H$_{6'}$, J = 2.0 Hz and J = 8.5 Hz), 7.75 (d, 1H, H$_{2'}$, J = 2.0 Hz), 7.60 (d, 1H, H$_4$, J = 1.2 Hz), 7.47 (d, 1H, H$_7$, J = 8.3 Hz), 7.28–7.20 (m, 3H, H$_6$ and H$_{phenyl}$), 6.99 (d, 1H, H$_{5'}$, J = 8.5 Hz), 6.87–6.82 (m, 3H, H$_{phenyl}$), 4.02 (s, 3H, OMe), 3.98 (s, 3H, OMe), 3.79 (m, 2H, H$_{piperazine}$), 3.55 (m, 2H, H$_{piperazine}$), 3.16–3.11 (m, 4H, C*H*$_2$CH$_2$ and H$_{piperazine}$), 3.03 (m, 2H, H$_{piperazine}$), 2.74 (t, 2H, CH$_2$C*H*$_2$, J = 7.4 Hz). IR (ν, cm^{-1}): 1635 (C = O).

Synthesis of compounds (C1–C3)

Same procedure as described for compounds (**B1–B4**) has been used.

2-(Furan-2-yl)-5-(3-(4-phenylpiperazin-1-yl)propyl)-1,3-benzoxazole hydrochloride (***C1***): The title compound was prepared from

compound **9a**. Solid was suspended in diethyl ether with HCl gas, concentrated *in vacuo* and recrystallised from acetonitrile to afford **C1** as a white solid (12%): mp >300 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 11.00 (br s, 1H, NH^+), 8.07 (m, 1H, $H_{5'}$), 7.71–7.67 (m, 2H, H_4 and $H_{3'}$), 7.44 (m, 1H, H_6), 7.32–7.24 (m, 3H, H_7 and H_{phenyl}), 6.98 (m, 2H, H_{phenyl}), 6.81 (m, 2H, H_{phenyl} and $H_{4'}$), 3.76 (m, 2H), 3.57 (m, 2H), 3.12 (m, 6H), 2.80 (m, 2H, $CH_2CH_2CH_2$), 2.14 (m, 2H, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, [D_6]DMSO): 155.3 (C), 150.1 (C), 148.7 (C), 147.6 (CH), 142.0 (C), 141.9 (C), 138.3 (C), 129.6 (2 CH), 126.9 (CH), 120.4 (CH), 119.6 (CH), 116.4 (2 CH), 115.5 (CH), 113.3 (CH), 111.0 (CH), 55.5 (CH_2), 51.1 (2 CH_2), 45.9 (2 CH_2), 32.4 (CH_2), 25.6 (CH_2). LC-MS (ESI) *m/z* found: 388 $[M+H]^+$. HPLC: C_4 column: $t_R = 15.8$ min, purity 98%.

2-(3,4-Dimethoxyphenyl)-5-[3-(piperidin-1-yl)propyl]-1,3-benzoxazole hydrochloride (***C2***): The title compound was prepared from compound **9b**. Solid was suspended in diethyl ether with HCl gas, concentrated *in vacuo* and recrystallised from ethyl acetate to afford **C2** as a white solid (72%): mp 214 °C. ^{1}H NMR (300 MHz, [D_6]DMSO): 10.20 (br s, 1H, NH^+), 7.77 (dd, 1H, $H_{6'}$, $J = 2.0$ Hz and $J = 8.4$ Hz), 7.68 (d, 1H, $H_{5'}$, $J = 8.4$ Hz), 7.66–7.63 (m, 2H, $H_{2'}$ and H_4), 7.21 (dd, 1H, H_6, $J = 1.5$ Hz and $J = 8.3$ Hz), 7.17 (d, 1H, H_7, $J = 8.3$ Hz), 3.88 (s, 3H, OMe), 3.86 (s, 3H, OMe), 3.38 (m, 2H, $CH_2CH_2CH_2$), 2.99 (m, 2H, $CH_2CH_2CH_2$), 2.76 (m, 2H, $CH_2CH_2CH_2$), 2.08 (m, 2H, $H_{piperidine}$), 1.75–1.65 (m, 5H, $H_{piperidine}$), 1.35 (m, 1H, $H_{piperidine}$). ^{13}C NMR (75 MHz, [D_6]DMSO): 163.2 (C), 152.5 (C), 149.5 (C), 149.3 (C), 142.4 (C), 137.8 (C), 125.9 (C), 121.3 (CH), 119.2 (CH), 119.2 (CH), 112.4 (CH), 110.9 (CH), 110.2 (CH), 56.2 (CH_3), 56.1 (CH_3), 55.9 (CH_2), 52.4 (2 CH_2), 32.5 (CH_2), 25.6 (CH_2), 22.9 (2 CH_2), 21.9 (CH_2). LC-MS (ESI) *m/z* found: 381 $[M+H]^+$. HPLC: C_4 column: $t_R = 17.3$ min, purity 98%.

2-(3,4-Dimethoxyphenyl)-5-[3-(4-phenylpiperazin-1-yl)propyl]-1,3-benzoxazole hydrochloride (***C3***): The title compound was prepared from compound **9c**. Solid was suspended in diethyl ether with HCl gas, concentrated *in vacuo* and recrystallised from ethanol to afford **C3** as a white solid (73%): mp 194 °C. ^{1}H NMR (300 MHz, [D_6]DMSO): 10.81 (br s, 1H, NH^+), 7.77 (dd, 1H, $H_{6'}$, $J = 1.8$ Hz and $J = 8.4$ Hz), 7.70–7.65 (m, 3H, H_4, H_6 and H_7), 7.29–7.22 (m, 3H, $H_{2'}$ and H_{phenyl}), 7.17 (d, 1H, $H_{5'}$, $J = 8.4$ Hz), 6.98 (m, 2H, H_{phenyl}), 6.87–6.82 (m, 1H, H_{phenyl}), 3.88 (s, 3H, OMe), 3.86 (s, 3H, OMe), 3.77 (m, 2H, $CH_2CH_2CH_2$), 3.56 (m, 2H, $H_{piperazine}$), 3.11 (m, 6H, $H_{piperazine}$), 2.79 (t, 2H, $CH_2CH_2CH_2$, $J = 7.4$ Hz), 2.13 (m, 2H, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, [D_6]DMSO): 163.2 (C), 152.5 (C), 150.1 (C), 149.5 (C), 149.3 (C), 142.4 (C), 137.8 (C), 129.6 (2 CH), 125.9 (CH), 121.3 (CH), 120.4 (C), 119.3 (CH), 119.2 (CH), 116.4 (2 CH), 112.4 (CH), 110.9 (CH), 110.2 (CH), 56.2 (CH_2), 56.1 (CH_3), 55.5 (CH_3), 51.1 (2 CH_2), 45.9 (2 CH_2), 32.5 (CH_2), 25.7 (CH_2). LC-MS (ESI) *m/z* found: 458 $[M+H]^+$. HPLC: C_4 column: $t_R = 16.6$ min, purity >99%.

***2-(2-(3,4-dimethoxyphenyl)-1,3-benzoxazol-5-yl)ethanol* (10).** To a solution of compound **(3e)** (1 g, 3.06 mmol) in THF (10 ml) was added $LiAlH_4$ (340 mg, 9.2 mmol). After 1 h stirring at room temperature, water (0.34 ml), 1 M NaOH solution (0.34 ml) and water (1.02 ml) were added successively until get a white solid which was filtered off and washed with EtOAc (60 ml). Organic layer was washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. Solid was recrystallised in acetonitrile to afford compound **(10)** as a beige solid (603 mg, 86%): mp 210 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 9.13 (br s, 1H, OH) 7.86 (dd, 1H, $H_{6'}$, $J = 1.8$ Hz and $J = 8.4$ Hz), 7.76 (d, 1H, $H_{2'}$, $J = 1.8$ Hz), 7.61 (m, 1H, H_4), 7.50 (d, 1H, H_7, $J = 8.4$ Hz), 7.24 (dd, 1H, H_6, $J = 1.2$ Hz and $J = 8.4$ Hz), 7.00 (d, 1H, $H_{5'}$, $J = 8.4$ Hz), 4.03 (s, 3H, OMe), 3.98 (s, 3H, OMe), 3.92 (m, 2H, CH_2CH_2), 3.64 (t, 2H, CH_2CH_2, $J = 6.6$ Hz). IR (ν, cm^{-1}): 3400 (OH). LC-MS (ESI) *m/z* found: 300 $[M+H]^+$.

***2-(2-(3,4-Dimethoxyphenyl)-1,3-benzoxazol-5-yl)ethyl methanesulfonate* (11).** To a solution of compound **(10)** (800 mg, 2.37 mmol) in DCM (20 ml) with Et_3N (0.49 ml, 3.55 mmol) at 0 °C, was added dropwise mesyl chloride (0.28 ml, 3.55 mmol). After 4 h stirring at room temperature, mixture was hydrolysed with water and extracted twice with DCM. Combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Solid was suspended in diethyl ether and filtered to afford compound **(11)** as a beige solid (895 mg, 100%): mp 112 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.85 (dd, 1H, $H_{6'}$, $J = 8.4$ Hz and $J = 2.0$ Hz), 7.76 (d, 1H, $H_{4'}$, $J = 2.0$ Hz), 7.61 (d, 1H, H_4, $J = 1.6$ Hz), 7.51 (d, 1H, H_7, $J = 8.3$ Hz), 7.21 (dd, 1H, H_6, $J = 8.3$ Hz and $J = 1.6$ Hz), 7.00 (d, 1H, $H_{5'}$, $J = 8.4$ Hz), 4.47 (t, 2H, CH_2CH_2, $J = 6.8$ Hz), 4.02 (s, 3H, OMe), 3.97 (s, 3H, OMe), 3.18 (t, 2H, CH_2CH_2, $J = 6.9$ Hz), 2.88 (s, 3H, CH_3). LC-MS (ESI) *m/z* found: 378 $[M+H]^+$.

General procedure for the synthesis of compound (B5–B6)

To a solution of compound **11** (0.79 mmol) in DMF (8 ml), were added K_2CO_3 (1.46 mmol) and amine (1.03 mmol). After overnight stirring at 80 °C, the reaction mixture was cooled, hydrolysed with water and extracted three times with EtOAc. Combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to afford a solid which was then purified.

2-(3,4-Dimethoxyphenyl)-5-(2-(piperidin-1-yl)ethyl)-1,3-benzoxazole hydrochloride (***B5***): The title compound was prepared from compound **11** and piperidine. Solid was suspended in diethyl ether with HCl gas, concentrated *in vacuo* and recrystallised from ethanol to afford **B5** as a white solid (25%): mp 260 °C. ^{1}H NMR (300 MHz, [D_6]DMSO): 10.63 (br m, 1H, NH^+), 7.79 (dd, 1H, $H_{6'}$, $J = 8.4$ Hz and $J = 2.0$ Hz), 7.73 (d, 1H, H_7, $J = 8.3$ Hz), 7.68 (d, 1H, $H_{2'}$, $J = 1.3$ Hz), 7.66 (d, 1H, H_4, $J = 2.0$ Hz), 7.31 (dd, 1H, H_6, $J = 8.4$ Hz and $J = 1.7$ Hz), 7.18 (d, 1H, $H_{5'}$, $J = 8.6$ Hz), 3.89 (s, 3H, OMe), 3.87 (s, 3H, OMe), 3.50 (m, 2H, CH_2CH_2), 3.27–3.20 (m, 4H, CH_2CH_2 and $H_{piperidine}$), 2.92 (m, 2H, $H_{piperidine}$), 1.82–1.70 (m, 5H, $H_{piperidine}$), 1.42 (m, 1H, $H_{piperidine}$). ^{13}C NMR (75 MHz, [D_6]DMSO): 163.4 (C), 152.5 (C), 149.5 (C), 149.3 (C), 142.4 (C), 137.8 (C), 125.9 (C), 121.3 (CH), 119.2 (CH), 119.2 (CH), 112.4 (CH), 110.9 (CH), 110.2 (CH), 57.3 (CH_2), 56.2 (CH_3), 56.1 (CH_3), 52.4 (2 CH_2), 29.7 (CH_2), 22.8 (2 CH_2), 21.9 (CH_2). LC-MS (ESI) *m/z* found: 367 $[M+H]^+$. HPLC: C_4 column: $t_R = 16.1$ min, purity 99%.

2-(3,4-Dimethoxyphenyl)-5-[2-(4-phenylpiperazin-1-yl)ethyl]-1,3-benzoxazole (***B6***): The title compound was prepared from compound **11** and phenylpiperazine. Solid was recrystallised from ethanol to afford **B6** as a white solid (28%): mp 140 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.85 (dd, 1H, $H_{6'}$, $J = 2.0$ Hz and $J = 8.4$ Hz), 7.76 (d, 1H, $H_{2'}$, $J = 2.0$ Hz), 7.61 (m, 1H, H_4), 7.48 (d, 1H, $H_{5'}$, $J = 8.4$ Hz), 7.31–7.26 (m, 2H, H_7 and H_{phenyl}), 7.20 (dd, 1H, H_6, $J = 1.5$ Hz and $J = 8.3$ Hz), 7.01–6.85 (m, 4H, H_{phenyl}), 4.03 (s, 3H, OMe), 3.98 (s, 3H, OMe), 3.27–3.24 (t, 4H, $H_{piperazine}$, $J = 4.8$ Hz), 3.01–2.96 (m, 2H, CH_2CH_2), 2.75–2.71 (m, 6H, CH_2CH_2 and $H_{piperazine}$). ^{13}C NMR (300 MHz, $CDCl_3$): 163.4 (C), 152.0 (C), 151.3 (C), 149.4 (C), 149.2 (C), 142.5 (C), 136.9 (C), 129.1 (2 CH), 125.5 (CH), 121.1 (CH), 119.9 (C), 119.7 (CH), 119.4 (CH), 116.1 (2 CH), 111.0 (CH), 110.0 (2 CH), 60.9 (CH_2), 56.1 (CH_3), 56.0 (CH_3), 53.3 (2 CH_2), 49.2 (2 CH_2), 33.7 (CH_2). LC-MS (ESI) *m/z* found: 444 $[M+H]^+$. HPLC: C_4 column: $t_R = 15.7$ min, purity 99%.

***2-(Furan-2-yl)-1,3-benzoxazol-5-amine* (D1).** To a solution of 2-(furan-2-yl)-5-nitro-1,3-benzoxazole (3.07 g, 13.3 mmol) **(3d)** in

EtOH (130 ml) were added Pd/C (10%, 100 mg) and hydrazine monohydrate (0.78 ml, 16 mmol). The mixture was heated at 70 °C for 3 h, cooled to room temperature, the Pd/C was filtered off and the filtrate was concentrated *in vacuo*. Crude was suspended in water and extracted three times with EtOAc. Combined organic layer were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. Solid was recrystallised from hexane to afford compound **(D1)** as grey solid (2.16 g, 81%): mp 112 °C. ^{1}H NMR (300 MHz, [D_6]DMSO): 8.01 (m, 1H, $H_{5'}$), 7.40 (d, 1H, H_7, $J = 8.7$ Hz), 7.34 (d, 1H, $H_{3'}$, $J = 3.5$ Hz), 6.84 (d, 1H, H_4, $J = 2.1$ Hz), 6.77 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 6.66 (dd, 1H, H_6, $J = 2.1$ Hz and $J = 8.7$ Hz), 5.16 (br s, 2H, NH_2). ^{13}C NMR (75 MHz, [D_6]DMSO): 154.9 (C), 147.3 (C), 147.0 (CH), 142.6 (C), 142.5 (C), 142.4 (C), 114.4 (CH), 113.6 (CH), 113.1 (CH), 110.9 (CH), 103.0 (CH). IR (ν, cm^{-1}): 3424 (NH_2). LC-MS (ESI) *m/z* found: 201 $[M + H]^+$. HPLC: C_4 column: $t_R = 14.2$ min, purity 98%.

N-(4-(Benzyloxy)phenethyl)-2-(furan-2-yl)-1,3benzoxazol-5-amine (12). To a solution of compound **D1** (800 mg, 4 mmol) in DMF (15 ml) were added 1-(benzyloxy)-4-(2-bromoethyl)benzene (1.4 g, 4.8 mmol)[16] and K_2CO_3 (828 mg, 5.99 mmol). The reaction mixture was stirred at 80 °C overnight, cooled to room temperature, hydrolysed with water, acidified with 1 M HCl solution, and extracted with EtOAc three times. Combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give a yellow oil. Purification by flash chromatography was realised with EtOAc/Cyclohexane as solvent (1/9 up to 3/7) to give the product as a yellow oil which was suspended in diethyl ether and filtered to afford compound **12** as a pale brown solid (430 mg, 26%): mp 184 °C. ^{1}H NMR (75 MHz, [D_6]DMSO, *J* Hz): 8.10 (m, 1H, $H_{5'}$), 7.75 (m, 1H), 7.58 (m, 1H), 7.46–7.26 (m, 7H), 7.21 (d, 2H, H_{phenyl}, $J = 8.7$ Hz), 6.96 (d, 2H, H_{phenyl}, $J = 8.7$ Hz), 6.83 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 5.06 (s, 2H, CH_2), 3.52 (t, 2H, CH_2CH_2, $J = 7.5$ Hz), 2.99 (t, 2H, CH_2CH_2, $J = 7.5$ Hz). LC-MS (ESI) *m/z* found: 411 $[M + H]^+$.

4-(2-{[2-(Furan-2-yl)-1,3-benzoxazol-5-yl]amino}ethyl)phenol (E2). A solution of compound **12** (740 mg, 1.66 mmol) in MeOH (15 ml) with Pd/C (50 mg) under H_2 atmosphere was stirred at 25 °C for overnight. Pd/C was filtered off and the filtrate was concentrated *in vacuo*. Solid was recrystallised in CH_3CN to afford compound **E2** as a yellow crystal (104 mg, 18%): mp 174 °C. ^{1}H NMR (300 MHz, [D_6]DMSO): 9.16 (br s, 1H, OH), 8.01 (m, 1H, $H_{5'}$), 7.43 (d, 1H, H_7, $J = 8.7$ Hz), 7.33 (d, 1H, $H_{3'}$, $J = 3.5$ Hz), 7.07 (d, 2H, H_{phenyl}, $J = 8.4$ Hz), 6.80 (m, 1H, H_4), 6.77 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 6.69 (d, 2H, H_{phenyl}, $J = 8.4$ Hz), 6.71 (m, 1H, H_6), 5.73 (br t, 1H, $J = 5.6$ Hz, NH), 3.24–3.17 (m, 2H, $J = 6.9$ Hz, CH_2CH_2), 2.75 (t, 2H, $J = 7.6$ Hz, CH_2CH_2). ^{13}C NMR (75 MHz, [D_6]DMSO): 156.1 (C), 154.9 (C), 147.7 (C), 147.0 (CH), 142.8 (C), 142.5 (C), 142.2 (C); 130.3 (C), 130.0 (2 CH), 115.5 (2 CH), 114.4 (CH), 113.1 (CH), 112.8 (CH), 111.1 (CH), 100.2 (CH); 46.1 (CH_2), 34.4 (CH_2). IR (ν, cm^{-1}): 3340 (OH). LC-MS (ESI) *m/z* found: 321 $[M + H]^+$. HPLC: C_4 column: $t_R = 14.8$ min, purity 97%.

2-(Furan-2-yl)-N-(2-(piperidin-1-yl)ethyl)-1,3 benzoxazol-5-amine hydrochloride (E1). To a solution of compound **D1** (800 mg, 4 mmol) in DMF (16 ml) were added K_2CO_3 (1.7 g, 12 mmol) and *N*-2-chloroethyl piperidine hydrochloride (1471 mg, 7.99 mmol). The reaction mixture was stirred at 70 °C overnight, cooled to room temperature, hydrolysed with water and extracted three times with EtOAc. Combined organic layer were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography was performed with DCM/EtOH/NH_3 (90/10/1) as a solvent. The obtained yellow oil was suspended in diethyl ether with HCl gas to formed a solid which was filtered to afford compound **E1** as a beige solid (11 mg, 8%): mp 169 °C. ^{1}H NMR (300 MHz, [D_6]DMSO): 10.69 (br m, 1H, NH^+), 8.04 (m, 1H, $H_{5'}$), 7.55 (d, 1H, H_7, $J = 8.8$ Hz), 7.38 (m, 1H, $H_{3'}$), 7.09 (m, 1H, H_4), 6.88 (dd, 1H, H_6, $J = 1.9$ Hz and $J = 8.8$ Hz), 6.79 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 5.37 (br m, 1H, NH), 3.57 (t, 2H, CH_2CH_2, $J = 6.3$ Hz), 3.49–3.46 (m, 2H, $H_{piperidine}$), 3.24 (t, 2H, CH_2CH_2, $J = 6.2$ Hz), 2.90 (m, 2H, $H_{piperidine}$), 1.79 (m, 5H, $H_{piperidine}$), 1.39 (m, 1H, $H_{piperidine}$). ^{13}C NMR (75 MHz, [D_6]DMSO): 155.3 (C), 147.2 (CH), 145.0 (C), 143.6 (C), 142.7 (C), 124.3 (C), 114.9 (CH), 114.1 (CH), 113.2 (CH), 111.5 (CH), 102.6 (CH), 54.5 (CH_2), 52.7 (2 CH_2), 39.2 (CH_2), 22.8 (2 CH_2), 21.8 (CH_2). LC-MS (ESI) *m/z* found: 312 $[M + H]^+$. HPLC: C_4 column: $t_R = 15.9$ min, purity 99%.

2-Bromo-N-(2-(furan-2-yl)-1,3-benzoxazol-5-yl)acetamide (***13***): To a solution of compound **D1** (210 mg, 1.05 mmol) and Et_3N (0.18 ml, 1.26 mmol) in DCM (10 ml) at 0 °C was added dropwise a solution of bromoacetyl bromide (0.11 ml, 1.26 mmol) diluted in DCM (5 ml). The reaction mixture was stirred at room temperature for 2 h, hydrolysed with water and extracted twice with DCM. Combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The solid was suspended in diethyl ether and filtered to afford compound **13** as a white solid (249 mg, 74%): mp 203 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 8.27 (br s, 1H, NH), 7.96 (m,1H, $H_{5'}$), 7.70–7.69 (m, 1H, H_4), 7.56–7.50 (m, 2H, H_7 and H_6), 7.30 (dd, 1H, $H_{3'}$, $J = 0.7$ Hz and $J = 3.5$ Hz), 6.39 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 4.08 (s, 2H, CH_2). IR (ν, cm^{-1}): 3246 (NH), 1643 (C = O).

***General procedure for the synthesis of compounds* (F1–F3)**

To a solution of compound **13** (0.59 mmol) in acetone (5 ml) were added K_2CO_3 (0.88 mmol) and the amine (0.65 mmol). The reaction mixture was refluxed for 2 h, cooled to room temperature and concentrated *in vacuo*. The crude was suspended in water and extracted three times with EtOAc. Combined organic layer were washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to afford a solid which was then purified.

N-[2-(Furan-2-yl)-1,3-benzoxazol-5-yl]-2-(piperidin-1-yl)acetamide hydrochloride (***F1***): The title compound was prepared from compound **13** and piperidine. Solid was suspended in diethyl ether with HCl gas, concentrated *in vacuo* and recrystallised from ethanol to afford **F1** as a white solid (57%): mp >300 °C. ^{1}H NMR (300 MHz, [D_6]DMSO): 11.26 (br s, 1H, NH^+), 9.99 (br s, 1H, NH), 8.17 (m, 1H, $H_{5'}$), 8.08 (m, 1H, H_4), 7.76 (d, 1H, H_7, $J = 8.8$ Hz), 7.58 (dd, 1H, H_6, $J = 1.9$ Hz and $J = 8.8$ Hz), 7.46 (d, 1H, $H_{3'}$, $J = 3.5$ Hz), 6.82 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 4.18 (s, 2H, CH_2), 3.50–3.47 (m, 2H, $H_{piperidine}$), 3.10 (m, 2H, $H_{piperidine}$), 1.80–1.67 (m, 5H, $H_{piperidine}$), 1.40 (m, 1H, $H_{piperidine}$). ^{13}C NMR (75 MHz, [D_6]DMSO): 163.4 (C), 156.0 (C), 147.7 (CH), 146.6 (C), 141.9 (C), 141.8 (C), 135.9 (C), 118.2 (CH), 115.8 (CH), 113.3 (CH), 111.4 (CH), 110.8 (CH), 57.6 (CH_2), 53.4 (2 CH_2), 22.7 (2 CH_2), 21.6 (CH_2). LC-MS (ESI) *m/z* found: 326 $[M + H]^+$. HPLC: C_4 column: $t_R = 15.1$ min, purity 98%.

N-[2-(Furan-2-yl)-1,3-benzoxazol-5-yl]-2-(4-phenylpiperazin-1-yl)acetamide (***F2***): The title compound was prepared from compound **13** and phenylpiperazine. Solid was recrystallised from ethanol to afford **F2** as a white solid (48%): mp 174 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 9.26 (br s, 1H, NH^+), 8.02 (m, 1H, H_4), 7.68–7.67 (m, 1H, $H_{5'}$), 7.56 (dd, 1H, H_6, $J = 2.0$ Hz and $J = 8.8$ Hz), 7.51 (d, 1H, H_7, $J = 8.6$ Hz), 7.3–7.29 (m, 3H, H_{phenyl} and $H_{3'}$), 6.98–6.88 (m, 3H, H_{phenyl} and H_{phenyl}), 6.63 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 3.30 (t, 4H, $H_{piperazine}$, $J = 4.9$ Hz), 3.26 (s, 2H, CH_2), 2.83 (t, 4H, $H_{piperazine}$, $J = 4.9$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 168.2 (C), 156.2 (C),

152.9 (C), 146.9 (C), 145.8 (CH), 142.5 (C), 142.2 (C), 134.9 (C), 129.2 (2 CH), 120.2 (CH), 117.8 (CH), 116.3 (2 CH), 114.5 (CH), 112.3 (CH), 111.2 (CH), 110.5 (CH), 62.0 (CH_2), 50.6 (2 CH_2), 49.5 (2 CH_2). LC-MS (ESI) *m/z* found: 403 $[M+H]^+$. HPLC: C_4 column: $t_R = 14.7$ min, purity >99%.tert-Butyl-4-({[2-(furan-2-yl)-1,3-benzoxazol-5-yl]carbamoyl}methyl)piperazine-1-carboxylate (***F3***): The title compound was prepared from compound **13** and *tert*-butyl piperazine-1-carboxylate. Solid was recrystallised from ethanol to afford **F3** as a white solid (90%): mp 186 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 9.15 (br s, 1H, NH), 7.98 (d, 1H, H_4, $J = 2.0$ Hz), 7.68–7.67 (m, 1H, $H_{5'}$), 7.55 (dd, 1H, H_6, $J = 2.0$ Hz and $J = 8.6$ Hz), 7.50 (d, 1H, H_7, $J = 8.6$ Hz), 7.28 (m, 1H, $H_{3'}$), 6.62 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 3.54 (t, 4H, $H_{piperazine}$, $J = 4.9$ Hz), 3.19 (s, 2H, CH_2), 2.60 (t, 4H, $H_{piperazine}$, $J = 4.9$ Hz), 1.48 (s, 9H, 3 CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 167.9 (C), 156.2 (C), 154.6 (C), 147.0 (C), 145.9 (CH), 142.5 (C), 142.2 (C), 134.8 (C), 117.8 (CH), 114.5 (CH), 112.3 (CH), 111.2 (CH), 110.5 (CH), 80.1 (CH_2), 62.1 (2 CH_2), 53.3 (2 CH_2), 28.4 (3 CH_3). IR (μ, cm^{-1}): 1684 (C = O), 1673 (C = O). LC-MS (ESI) *m/z* found: 371 $[M\text{-}tBu+H]^+$, 427 $[M+H]^+$. HPLC: C_4 column: $t_R = 15.1$ min, purity 99%.

N-(2-(Furan-2-yl)-1,3-benzoxazol-5-yl)-2-(piperazin-1-yl)acetamide dihydrochloride (F4): To a solution of **(F3)** (180 mg, 0.422 mmol) in MeOH (10 ml) was added 6 M HCl (1.41 ml, 8.44 mmol) and the reaction mixture was stirred at 40 °C for 15 h. The precipitated product was filtered and washed with diethyl ether to afford compound **(F4)** as a white solid (121 mg, 72%): mp 192 °C. ^{1}H NMR (300 MHz, $[D_6]$DMSO): 11.16 (br s, 1H, NH), 9.88 (br s, 2H, NH_2^+), 8.16 (d, 1H, H_4, $J = 1.8$ Hz), 8.07 (m, 1H, $H_{5'}$), 7.75 (d, 1H, H_7, $J = 8.8$ Hz), 7.59 (dd, 1H, H_6, $J = 1.8$ Hz and $J = 8.8$ Hz), 7.45–7.44 (m, 1H, $H_{3'}$), 6.81 (dd, 1H, $H_{4'}$, $J = 1.7$ Hz and $J = 3.5$ Hz), 4.22 (s, 2H, CH_2), 3.57 (s, 4H, $H_{piperazine}$, $J = 4.9$ Hz), 3.43 (s, 4H, $H_{piperazine}$, $J = 4.9$ Hz). ^{13}C NMR (75 MHz, $[D_6]$DMSO): 163.7 (C), 155.9 (C), 147.7 (CH), 146.5 (C), 141.9 (C), 141.8 (C), 135.9 (C), 118.2 (CH), 115.7 (CH), 113.3 (CH), 111.4 (CH), 110.8 (CH), 57.6 (CH_2), 49.1 (4 CH_2). LC-MS (ESI) *m/z* found: 327 $[M+H]^+$. HPLC: C_4 column: $t_R = 14.9$ min, purity 99%.

Molecular docking

Molecular docking was performed using Gold suite v5.2[17] within the Hermes v1.6 GUI (CCDC©). Thus after adding hydrogens, water molecules were deleted and docking was performed in a 10 Å around co-crystallised ligands then ligands deleted. Early termination of three docking solutions within 1.5 Å was set up in order to highlight ligands converging towards a few binding modes.

Pharmacological assays

Displacement binding assays

Radioligands were obtained from the following source: $[^3H]$-ZM241385 from IsoBio (10–50 Ci/ml), [3H]-DPCPX from PerkinElmer (Waltham, MA) (120 Ci/mmol), [3H]-CPX from Perkin Elmer (120 Ci/mmol), [125I]-AB-MECA from Chelatec (2200 Ci/mmol) (Saint-Herblain, France).

For A_{2A} receptor binding evaluation, the stock solution of compounds was prepared in DMSO. The final concentration of DMSO was no more than 3% for radioligand binding assay.

Competition binding curves of the A_{2A} receptor antagonist $[^3H]$-ZM241385 by the designed A_{2A} antagonists described above, were performed as before[18] in Human HEK293 A_{2A}R membranes (PerkinElmer). 0.5 ml of membranes (0.5 U of A_{2A}R) were incubated with $[^3H]$-ZM241385 (2 nm) and increasing concentrations of the designed A_{2A}R antagonists (0–600 nm) in a final volume of 300 μl in the presence of 1 U/ml of adenosine deaminase (Roche, Basel, Switzerland). All samples were assayed in duplicate. Non-specific binding was determined for each assay in the presence of the antagonist ZM-24135 (8.3 nm). Microplates were incubated for 1 h at room temperature and the reaction was stopped by vacuum filtration with a Skatron semi-automatic cell harvester with chilled incubation solution (pH 7.4, Tris 50 mm MgCl 10 mm) to filter mats 1.5 mm (Molecular Devices, Sunnyvale, CA). Three millilitres of scintillation cocktail (OptiPhase "HiSafe" 2, PerkinElmer) were added and radioactivity bound to the filters was determined after 12 h. Molecules inhibited binding by ≥30% at 10 μm were submitted to K_i evaluation. This percentage was calculated using Excel 2013 as a ratio of data with ligands to data without ligands. Data were analysed using Graph Pad Prism, version 5.01 (San Diego, CA). Inhibition constants (K_i) were calculated from the IC_{50} values by non-linear regression analysis, the Cheng and Prusoff equation and K_D value of 1.0 nm were used. Displacement reference curves were performed with ZM-24135 (0–6 nm in 6%, 40% or 60% of DMSO). No difference was observed between each concentration.

Affinity towards A_1R (human recombinant CHO cells, [3H]-DPCPX (1 nm), Cerep catalogue reference 0002, as described by Townsend-Nicholson[19]), A_{2B}R (human recombinant HEK-293 cells, [3H]-CPX (5 nm), Cerep catalogue reference 0005, as described by Stehle[20]) and A_3R (human recombinant HEK-293 cells, [125I]-AB-MECA (0.15 nm), Cerep catalogue reference 0006, as described by Salvatore[21]) was determined by CEREP laboratories. K_D values used were: 1.7 nm for [3H]-DPCPX, 65 nm for [3H]-CPX and 0.15 nm for [125I]-AB-MECA. For these three receptors, the stock solution of compounds was prepared in DMSO. The final concentration of DMSO was no more than 1%. Data were analysed using SigmaPlot® version 4.0 for Windows® (© 1997 by SPSS Inc., Chicago, IL).

Cell culture and cytotoxicity assay

The human neuroblastoma cell line (SY5Y) was cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco, Waltham, MA) supplemented with 2 mm L-glutamine, 100 mg/ml streptomycin, 100 IU/ml penicillin, 1 mm non-essential amino acids and 10% (v/v) heat-inactivated foetal bovine serum (Sigma-Aldrich, Saint-Louis, MO), and grown at 37 °C in a humidified incubator with 5% CO_2. 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 a 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.

Absorption, distribution, metabolism and excretion (ADME) assessment

Aqueous solubility (in phosphate-buffered saline, PBS, pH 7.4; incubation room temperature for 24 h as described by Lipinski[22] Eurofins Cerep SA catalogue reference G235), partition coefficient (log D, n-octanol/PBS, pH 7.4, room temperature for 60 min as described by Sangster[23]; Eurofins Cerep SA catalogue reference 0417), human plasma protein binding evaluated at 10 μm concentration for 4 h at 37 °C as described by Banker[24] (Eurofins Cerep SA catalogue reference 2194), A-B and B-A permeability coefficient evaluated at 10 μm for 40 min as described by Hidalgo[25] (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[26] (Eurofins Cerep SA catalogue reference 0607) were determined in standard assays by Eurofins Cerep SA, France www.cerep.fr).

Results and discussion

Structure-based insights

Our design was guided by molecular modelling studies which took into consideration the two structures of adenosine A_{2A} receptors co-crystallised with high-affinity A_{2A} antagonists ZM-241385 and T4E (1,2,4-triazines) in respective 3EML[27,28] and 3UZC[29] PDB entries. Although several molecular dynamics studies have shown the importance of water molecules for ligand recognition[30,31], these molecules represent a bias in molecular docking with many options like their rigid pre-docking displacement, a tolerance for moving them or simply delete them. Since T4E-bound structures show that the key water molecules in ZM-241385-bound crystal structures could be displaced by aryl substituents, we decided to avoid this experimental bias in order to benefit with the whole cavity.

In contrast with ZM-241385 bound structure, the T4E-bound one was identified as the most suitable target to predict correct docking poses of both T4E (no data show) and ZM-241385 within a 2.0 Å structural deviation in comparison with experimental co-crystallised poses (Figure 2(B)). This is due to the greater volume of T4E-bound pocket that allows to accommodate bulky di-aryl substituted triazine as well as linear ZM-241385 ligands. Consequently, apoA2AR-T4E pocket appears to be more relevant to accommodate a wide diversity of chemical structures and was therefore used to perform our docking calculations.

A set of benzoxazole-based molecules have been docked, using Gold suite v5.2 within the Hermes v1.6 GUI (CCDC©), into the apoA2AR-T4E pocket and the ones that satisfied interactions with essential amino acids[32] Phe168, Glu169, Trp246 and Asn253 were selected. ZM-241385 was shown as an example (Figure 2(B)) rather than the triazine because it is structurally closer to our benzoxazole ligands (Figure 2(C)). As illustrated in Figure 2(B), the central heterocycle of ZM-241385 interacts through a hydrogen bond with Asn253 and π-stacking with Phe168. Benzoxazole ring seems to recapitulate these interactions and could, therefore, constitute a good alternative as a central core. The furan, found in many A_{2A} antagonists, was selected to interact with Trp246 by aromatic interaction and thus made the antagonist character of our ligands. Indeed, interaction with this key amino acid is well known to lock the A_{2A} receptor[33] and more generally class-A GPCR[34] in their inactive conformation. The 3,4 dimethoxyphenyl, found on Istradefylline, was also used to create this interaction. Furthermore, different nature and size of linkers was used to bring selected amine function. Indeed, these basic functions in designed ligands (Figure 2(C)) occupy the same pocket as the phenol part of the ZM-241385 and allow not only to interact with Glu169 through an ionic interaction but also to improve solubility.

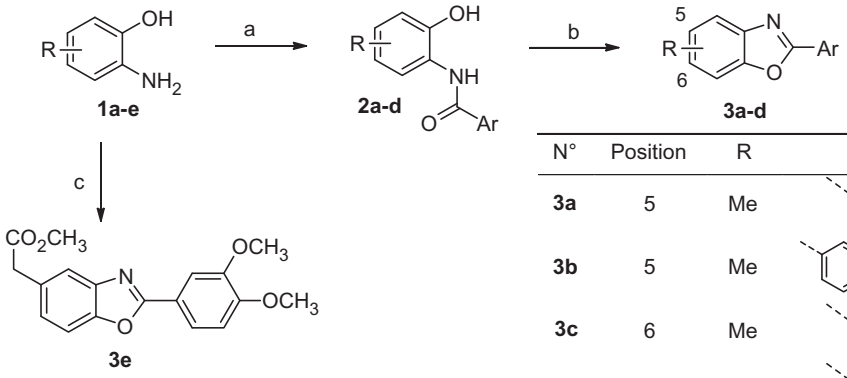
Chemistry

Synthetic routes used to prepare benzoxazole derivatives are depicted in Schemes 1–3. The first set of benzoxazoles (**3a–d**) was prepared from commercially available aminophenols using two different synthetic routes (Scheme 1). The first one led to molecules **3a–d**, in two steps. Indeed, aminophenol derivatives **1a–e** reacted with appropriate acyl chlorides to give an equimolar mixture of mono and diacyl compounds which, when treated under basic conditions, gave amides **2a–d**. Subsequent cyclisation under acidic conditions afforded benzoxazoles **3a–d** in good yield[35]. The second synthetic route allowed to transform aminophenol **1e** into compound **3e** in one step using T3P®[36].

A radical bromination of compounds **3a–c** was performed to generate the key brominated intermediates **4a–c** (Scheme 2) which allowed the introduction of the tertiary amine at different distances from the central heterocycle. Molecules **A1–8** displaying a one-methylene linker were obtained by reacting **4a–c** with various amines. Compound **A4** was synthesised from **A3** deprotection using TFA. Based on binding results (see Table 1), we focused the further synthetic effort on C-5 substituted compounds. Treatment of **4a** with potassium cyanide followed by an acidic hydrolysis gave carboxylic acid **6** which was subjected to amidification and reduction to afford target compounds **B1–4** (two-methylene linker).

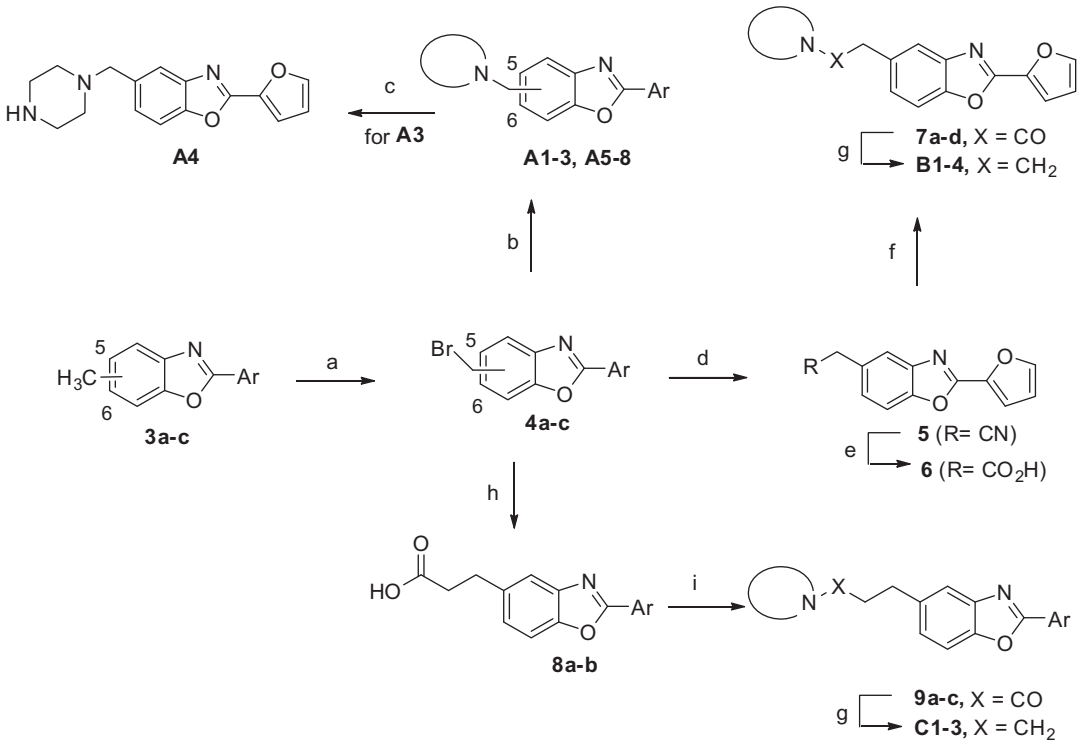
To get molecules **C1–3** (three-methylene linker), the same procedure was used starting from carboxylic acids **8a–b**, obtained by malonic substitution performed on compounds **4a–b** followed by a basic hydrolysis and then a decarboxylation reaction by heating in DMF.

To obtain molecules **B5–6** (Scheme 3), ester function of compound **3e** was first reduced to alcohol **10** using $LiAlH_4$. The latter was then activated by the action of mesyl chloride to afford molecule **11**. The classical nucleophilic substitution was then performed to give compounds **B5–6**.

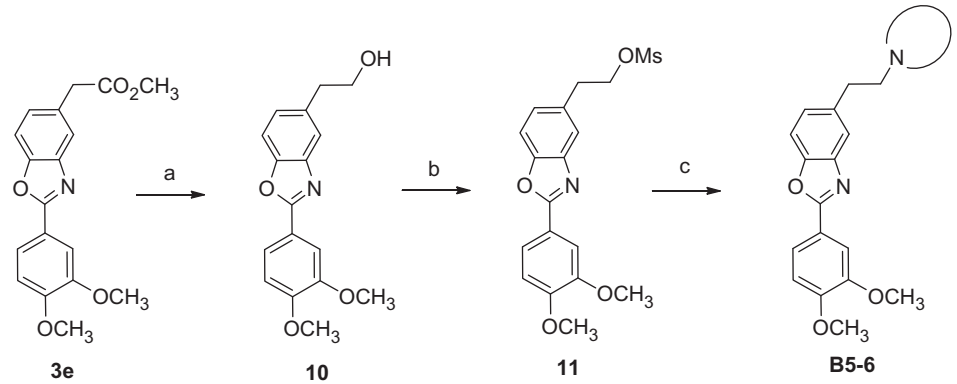


N°	Position	R	Ar
3a	5	Me	
3b	5	Me	
3c	6	Me	
3d	5	NO_2	

Scheme 1. Reagents and conditions: (a) *i)* $ArCO_2H$, $SOCl_2$, DMF, DCM, *ii)* Et_3N, EtOAc, aminophenol (**1a–d**); iii) NaOH, EtOH/H_2O, *then* 6 M HCl, 60–80% over 2 steps; (b) APTS, toluene, reflux, 70–80%, (c) T3P® (50% in EtOAc), DIPEA, 3,4-dimethoxybenzoic acid, 35%.



Scheme 2. Reagents and conditions: (a) NBS, benzoyl peroxide, CCl_4, reflux/hυ (230 W), 60–75%; (b) R_2R_1NH, Et_3N, acetone, 50–85%; (c) TFA, DCM, 60%; (d) KCN, EtOH/H_2O, 50–60%; (e) H_2O/H_2SO_4/AcOH, reflux, 60–70%; (f) *i)* $SOCl_2$, toluene; *ii)* secondary amine, Et_3N, EtOAc, 50–60%; (g) $LiAlH_4$, THF, 60–75%; (h) *i)* dimethyl malonate, K_2CO_3, acetone; *ii)* NaOH, H_2O then 6 M HCl; *iii)* DMF, reflux, 40–43%.



Scheme 3. Reagents and conditions: (a) $LiAlH_4$, THF, 86%; (b) MsCl, Et_3N, DCM, quant.; (c) secondary amine, K_2CO_3, DMF, 60 °C, 25–28%.

To get target molecules with an amide (**F1–4**) or ethylamine linker (**E1–2**), 5-nitro benzoxazole derivative (**3d**) was reduced with hydrazine hydrate in the presence of Pd/C to afford compound **D1** (Scheme 4). Nucleophilic substitution gave compounds **E1** and **12**. The latter was deprotected under H_2 atmosphere afforded **E2**. To get molecules **F1–3**, treatment of **D1** with bromoacetyl bromide gave compound **13** that was substituted with various amines. Compound **F4** was obtained from **F3** deprotection using 6 M HCl in MeOH.

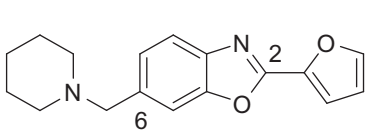
Structure-affinity relationship and early ADME studies

Affinities of benzoxazole derivatives for the human adenosine receptor were determined by a competitive radioligand displacement assay using [^{3}H]-ZM241385 as radioligand[18]. All compounds were first screened at 10 μm concentration, and K_i values were determined for those exhibiting a specific displacement superior to 35%.

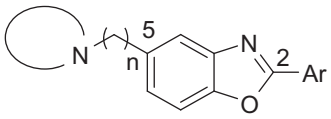
The first set of derivatives **A1–8**, allowed drawing some early SARs (Table 1). First, comparing molecules **A1–6**, piperidine emerged as the preferred amine since a dramatic decrease in affinity was observed with all other selected ones. Surprisingly, the phenylpiperazine group featured in many A_{2A} antagonists was not tolerated in our series.

Regarding the position of the protonable amine function, compounds with a chain at the C-5 position appeared to exhibit a higher affinity than a compound with a chain at the C-6 position (molecules **A1** versus **A7**). Therefore, the protonable amine was placed in the C-5 position for following molecules. Concerning the aryl in position 2 of the benzoxazole, replacing the furan of **A1** by a 3,4-dimethoxyphenyl (**A8**) totally abolished affinity. This result is in agreement with literature since the preference of the adenosine A_{2A} receptor for furan substituent is well documented even if the 3,4-dimethoxyphenyl is found on the Istradefylline molecule[7,13].

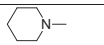
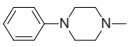
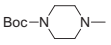
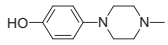
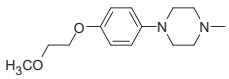
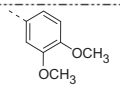
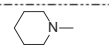
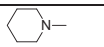
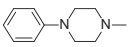
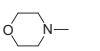
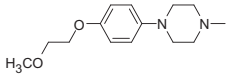
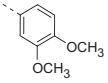
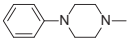
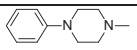
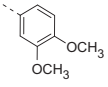
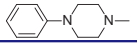
Table 1. A_{2A} receptor affinity and cytotoxicity data of compounds **A1–8**, **B1–6** and **C1–3.**



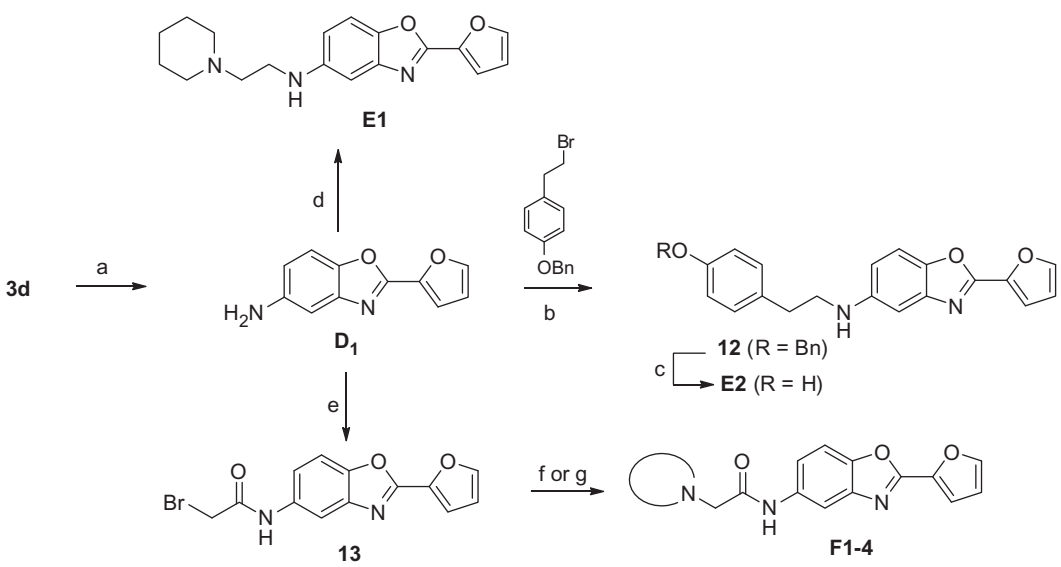
A7



A1-A6, A8, B1-B6, C1-C3

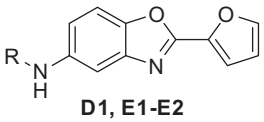
	Cpd.	Ar		Ki ± SEM (μM)[a]/ % inhib[b]	CC_{50}[c]/ % inhib[d]
	ZM - 241385			0.0013 ± 0.002	
n = 1	A1			19 ± 3.1	1%
	A2			10%	33%
	A3			15%	30%
	A4			15%	0%
	A5			11%	27%
	A6			14%	37%
	A7		-	5%	0%
	A8			18%	0%
n = 2	B1			7 ± 1.4	1%
	B2			3%	0%
	B3			11 ± 0.25	11%
	B4			10%	-
	B5			10%	40%
	B6			5%	19%
n = 3	C1			28%	-
	C2			10%	73 μM
	C3			11%	

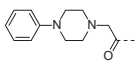
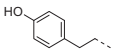
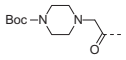
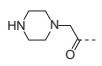
[a]Displacement of specific [^{3}H]-ZM 241385 binding to hA_{2A} receptors stably expressed in HEK293 cells. [b]Displacement percentage of specific [^{3}H]-ZM 241385 at 10 μm. [c]Compound concentration causing 50% of SY5Y cell death after 24 h treatment. [d]Percentage of dead SY5Y cells after 24 h treatment at 100 μm.



Scheme 4. Reagents and conditions: (a) hydrazine hydrate, Pd/C (10%), EtOH, 81%; (b) 1-(benzyloxy)-4-(2-bromoethyl)benzene, K_2CO_3, DMF, 70 °C, 15%; (c) H_2, Pd/C (10%), MeOH, 48%; (d) *N*-chloroethylpiperidine, K_2CO_3, DMF, 70 °C, 8%; (e) bromoacetylbromide, NEt_3, DCM, 75%; (f) for **F1–3**, secondary amine, K_2CO_3, acetone, 48–90%; (g) for **F4**, *i*) boc-piperazine, K_2CO_3, acetone, *ii*) 6 M HCl, MeOH, 72%.

Table 2. A_{2A} receptor affinity and cytotoxicity data of compounds **D1**, **E1–2** and **F1–4.**



Cpd.	R	Ki (μM)[a]/ % inhib[b]	CC_{50}[c]/ % inhib[d]	Cpd.	R	Ki (μM)[a]/ % inhib[b]	CC_{50}[c]/ % inhib[d]
D1	H	10 ± 0.5	4%	**F1**		1 ± 0.08	1%
E1		11 ± 0.02	3%	**F2**		12%	1%
E2		15%	55 μM	**F3**		25%	12%
				F4		8%	0%

[a]Displacement of specific [^{3}H]-ZM 241385 (2 nm) binding to hA_{2A} receptors stably expressed in HEK293 cells. [b]Displacement percentage of specific [^{3}H]-ZM 241385 at 10 μm. [c]Compound concentration causing 50% of SY5Y cell death after 24 h treatment. [d]Percentage of dead SY5Y cells after 24 h treatment at 100 μm.

Table 3. Preliminary ADME studies of F1.

Cpd.	Solubility (μm) PBS, pH 7.4[a]	PPB (%)[b]	Permeability Caco-2- (10^{-6} cm/s)[c] A/B	B/A	$LogD_{7.4}$[d]	HLM[e] $t_{1/2}$ (min)	Cl_{int}[f] (μl/min/mg)
F1	184	83	50	24	2.33	11	630

[a]Evaluated after 24 h stirring. [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]Determined between a mixture $PBS_{7.4}$/octanol. [e]HLM = human liver microsome. [f]Cl_{int} = Compound was tested at 0.1 μm concentration.

Throughout Table 1, a similar tendency was observed for the other analogues suggesting that the piperidine chain at C-5 and the furan at C-2 of the benzoxazole ring are the best moieties for A_{2A} receptor affinity.

Concerning the linker, the comparison between **B1** and **A1** revealed that increasing the length to two carbons is beneficial for affinity. Moreover, as can be seen from affinity data of **E1** (11 μm) and **F1** (1 μm) (Table 2), a three-atom linker is also well tolerated. When comparing the latter two compounds, rigidifying the linker by incorporating an amide in place of an amine allows an increase in affinity. Besides, **F1** was found to be the most active compound in this series with a K_i value of 1 μm. As can be seen from Figure 2(C) the three-atom linker seems necessary to allow the interaction between the piperidine and Glu169. This ligand also exhibited a slight selectivity (see Table 4 of the Supplementary Material) versus A_1 receptor (2.5-fold). Concerning the two others adenosine receptors, preliminary studies showed a probably good interaction with hA_{2B} (73% inhibition at 10 μm) but a highly selectivity over adenosine A_3 receptor (11% inhibition at 10 μm). These informations do not constitute a brake for the development of **F1** as a potential drug candidate at this "hit" identification stage.

Interestingly, replacing the protonable amine of **E1** by the aminoethyl phenol chain present in ZM-241385 (**E2**) led to a drastic drop in affinity, highlighting the importance of the tertiary amine in this series.

Finally, compound **D1** ($K_i = 10\ \mu$m), despite the lack of the protonable amine, could be considered a promising hit for future A_{2A} antagonist development. Indeed, given its low molecular weight ($MW = 200\ g.mol^{-1}$), it offers multiple possibilities for further modifications.

The most interesting compounds of this series, **F1** and **D1**, were subjected to preliminary pharmacokinetics studies (Table 3). As expected, molecule **F1** displaying a protonable amine exhibits a higher solubility (184 μm) than molecule **D1** (28 μm) in PBS solution at pH 7.4. When compared to reference A_{2A} antagonists, these two hits show a higher solubility[37–40]. Indeed, Istradefylline (KW-6002) and Preladenant (SCH 4208[8]), the two reference antagonists exhibit solubility values of 1.5 μm and 20 nm, respectively.

Moreover, **F1** displayed a good partition coefficient (log $D_{7.4}$) of 2.33 which is within the same range as reference A_{2A} antagonists currently in clinical studies[41]. These results confirm the importance of having a tertiary amine which allows a sharp increase in solubility while keeping a good log $D_{7.4}$ value. At 10 μm concentration, a correct value of permeability coefficient (Caco-2 cells, pH 6.5/7.4) is observed (50×10^{-6} cm/s). The efflux ratio ($^{B/A}/_{A/B}$) of 0.5 also suggested that **F1** was probably distributed through P-glycoprotein (P-gp), an important transporter protein found in cell throughout the body. A good human plasma protein binding (mean of 83%) was also observed compared to reference A_{2A} antagonist which expressed high PPB around 98%. Nevertheless, this compound also exhibited a high clearance and thus a short half time in human liver microsome at 0.1 μm.

Finally, no toxicity was observed for active compounds at 100 μm when tested on neuroblastoma cell lines (SY5Y, Tables 1,2).

Conclusions

Reported results showed a set of benzoxazole derivatives, diversely substituted at the C-2 and C-5 positions, as new "hits" molecules for adenosine A_{2A} receptor. Among the synthesised compounds, those featured by a furan at the C-2 position combined with a piperidine and an amide-based linker at C-5 resulted in the most active compound (**F1**) toward the $hA_{2A}R$ ($K_i = 1\ \mu$m). Furthermore, the latter presented good preliminary ADME properties with a very interesting solubility (184 μm) as well as good log $D_{7.4}$ (2.33) without cytotoxicity at 100 μm. Thus, both **F1** and **D1**, which can be easily modulated in position 7, appear to be promising starting points for further optimisation.

Acknowledgements

We express our thanks to the 300 MHz NMR facilities funded by the Region Nord-Pas de Calais (France), the Ministere de la Jeunesse, de l'Education Nationale et de la Recherche (MJENR) and the Fonds Europeens de Developpement Regional (FEDER). This work was supported by Lille 2 University, ANR "Adoratau", Comue Univ Lille Nord de France. Romain Duroux is the recipient of a fellowship from Lille 2 University.

Disclosure statement

No potential conflict of interest was reported by the authors.

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