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# New insight into adenosine receptors selectivity derived from a novel series of [5-substituted-4-phenyl-1,3-thiazol-2-yl] benzamides and furamides<sup>\*</sup>

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#### A R T I C L E I N F O

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#### 1. Introduction

Over the past few decades, much attention has been focused on the development of ligands for adenosine receptors subtypes, since there is significant potential to address dysfunction in range of diseases and to design, drug like candidates in wide range of therapeutic areas. However, there is a need for the specificity or significant selectivity towards individual receptor subtypes. The ubiquitous tissue distribution of adenosine receptors is largely responsible for the broad variety of effects produced by adenosine throughout several organ systems. Adenosine receptors are classified into three types, namely A<sub>1</sub>, A<sub>2</sub> and A<sub>3</sub>; the A<sub>2</sub> receptors are in turn sub-classified into two subtypes, A<sub>2A</sub> and A<sub>2B</sub>. Adenosine receptor signalling is diverse and occurs through inhibition or stimulation of adenylyl cyclase, activation of phospholipase C (PLC),

### ABSTRACT

A series of [5-substituted-4-phenyl-1,3-thiazol-2-yl] benzamide and furamide analogues were investigated in radioligand binding studies at adenosine receptor subtypes with an aim to obtain potent and selective adenosine receptor ligands. Benzamide and furamide linked to thiazole was found to be crucial for high adenosine receptor affinity. The most potent compound indentified in this study was **5d** with low nanomolar affinity for all four adenosine receptor subtypes. Compounds **5a** and **5g** showed moderate selectivity for A<sub>2A</sub> adenosine receptors. Molecular docking versus all four human adenosine receptors combined with membrane molecular dynamics studies were performed to rationalise the peculiar selectivity profile of **5d** antagonist.

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Ca<sup>2+</sup>-signaling and the mitogen-activated protein kinases (MAPK) pathway [1].

Activation of the A<sub>1</sub>AR mediates an inhibition of adenylyl cyclase through activation of pertussis toxin-sensitive  $G_{i/o}$  proteins [2,3] and results in increased activity of PLC [4,5]. High and intermediate levels of A<sub>1</sub> Adenosine receptor expression were found in the brain, heart, adipose tissue, stomach, vas deferens, testis, spleen, kidney, aorta, liver, eye and bladder [6]. A<sub>1</sub> agonists may find application in various diseases and disorders such as stroke, epilepsy, migraine, pain, cardiac ischemia, arrhythmias, while antagonists may be useful in conditions such as cognitive disorders and oedema [7].

Activation of the  $A_{2A}$  AR increases adenylyl cyclase activity mediated by  $G_s$  as the major G-protein associated with  $A_{2A}$ . The  $A_{2A}$ AR is also known to act through  $G_{olf}$  [8] in the striatum and was shown to activate the PLC pathway in rat artery [9].

The  $A_{2A}$  adenosine receptors are highly expressed in the striatum, nucleus accumbens, and olfactory tubercle [6]. High and intermediate expression levels were also found in immune cells, heart, lung and blood vessels. The therapeutic implications of  $A_{2A}$ 







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Adenosine receptor agonists result from cardiovascular effects such as vasodilation, tachycardia (central effects), hypotension (peripheral effects), and platelet aggregation. Additional therapeutic indications for agonists may be respiratory disorders, rheumatoid arthritis, inflammation, wound healing, and sepsis, while antagonists are discussed as treatment in Parkinson's disease, neuronal protection in ischemia, Huntington's disease and migraine [7].

The  $A_{2B}$  adenosine receptor is positively coupled to both adenylyl cyclase and PLC [10–13]. Inhibition of  $A_{2B}$  ARs may be useful in diarrhoea, diabetes and asthma.

The A<sub>3</sub> ARs are coupled to the classical second-messenger pathways such as inhibition of adenylyl cyclase [14], stimulation of PLC [15] and calcium mobilization [16–19]. A protective effect on cardiac cells was shown to be mediated through the activation of  $K_{ATP}$ channels [20]. A<sub>3</sub> adenosine receptor activation may find applications in stroke, lung injury (asthma and COPD), cardiac ischemia, rheumatoid arthritis and cancer [21]. The blockade of A<sub>3</sub> adenosine receptor is useful in glaucoma, asthma and renal failure [7].

One of the hottest topics in targeting GPCRs is to define the selectivity profile of both agonists and antagonists against the different receptor subtypes. The understanding at the molecular level of the receptor-ligand requirements to improve the selectivity profiles can be achieved integrating structure activity relationship with computational studies. In fact, we have previously shown that the decoration of 2-aminothiazole scaffold, with an aroylamino moiety at the 2 position and an aroyl moiety at the 5 position respectively, shifts the selectivity profile versus the human A<sub>1</sub> receptor [22]. With the aim to obtain better rationalize for the observed this selectivity profile shift, we designed a novel series of [5-substituted-4-phenyl-1,3-thiazol-2-yl] benzamide and furamide derivatives. Interestingly, we have identified compound 5d with lower nanomolar antagonist against all four adenosine receptor subtypes. Compound 5d represents a very special chemical entities among all known adenosine receptor antagonists, because it hedges in its structure the most critical chemical features essential for the recognition of all four adenosine receptor subtypes. Molecular docking versus all four human adenosine receptors combined with membrane molecular dynamics studies have been performed to rationalise the peculiar selectivity profile of this novel antagonist.

#### 2. Results and discussion

The synthesis of the 2-aminothiazole derivatives was performed according to procedures by Rajappa et al. [23]. Initially, various benzoyl and 2-furanoyl isothiocyanates were synthesised using the method reported by Preston Reeves W. et al. [24] where in a mixture of benzoyl or 2-furanoyl chloride, benzene and tetra butyl ammonium bromide was stirred at room temperature, followed by addition of 33% potassium thiocyanate solution drop-wise. *N*,*N*-diethyl benzamidine was prepared by treating benzonitrile at 0-30 °C with anhydrous aluminium chloride followed by addition

of diethyl amine. These isothiocyanates were utilized further for the synthesis of various amidinothioureas (**3a–d**, Scheme 1) by treating with *N*,*N*-diethyl benzamidine in a suitable solvent at conditions (10-25 °C). These amidinothioureas (**3a–d**) were then reacted with substituted halomethylene compounds to get the desired 2-aminothiazole derivatives (Scheme 1).

Several halomethyl compounds were synthesized for the purpose of diverse substitutions at 5 position on the 2-aminothiazole core ring. The compound (A, Fig. 1) had selectivity towards  $A_3$  adenosine receptor. This compound **1** incorporates a 4-pyridyl which has a hydrogen bond acceptor feature at the 5-position of the 2-aminothiazole scaffold. The importance of this feature was reinforced by the benzoyl (B, Fig. 1) in our own work [22]. The lone pair of electrons on the carbonyl oxygen simulates the nitrogen pair of the pyridyl candidates. The bioisosteric equivalence of pyridyl with the benzoyl is well established [25].

In order to evaluate whether additional hydrogen bond acceptor feature at the 5-position would have beneficial effect on biological activity profile, The pyridyl in the compound (A, Fig. 1) was replaced with 4-amino-6-dimethylamino-2-chloromethyl[1,3,5]triazine, 2-chloromethyl-5,6-dimethyl-thieno[2,3-d]pyrimidin-4-ylamine and the 2-chloromethyl-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyr-imidin-4-yl amine fragments. Further, to retain the fragments of carbonyl and pyridyl nitrogen as hydrogen bond acceptors respectively in single molecule at 5-position, various analogues were synthesized with 2-pyridoyl, 3-pyridoyl and 4-pyridoyl at the 5-position of the 2-aminothiazole scaffold.

The triazine ring was constructed using an anti-diabetic drug metformin hydrochloride as the starting material where metformin hydrochloride was treated with chloroethyl acetate in dry methanol under basic conditions [26] to furnish chloromethyl-2,4disubstituted triazine (Scheme 2).

The thieno[2,3-*d*]pyrimidines were synthesised using the method reported by Dave et al. [27]; where the 2-amino-5,6-dimethyl-thiophen-3-carbonitrile was obtained using ethyl-methylketone, malononitrile, sulphur and morpholine as base using Gewald synthesis and later was cyclised to thienopyrimidines using strong acidic conditions with chloroacetonitrile to yield compound **13** (Scheme 3).

Similarly, synthesis of 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3d]pyrimidin-4-yl amine was carried out using Gewald reaction with cyclohexanone, malononitrile and sulphur followed by cyclization with chloroacetonitrile in presence of acid resulted in compound **16** (Scheme 4).

#### 3. Structure activity relationships

#### 3.1. Benzamide derivatives

The activity profiles of 2-aminothiazole analogues are outlined in Table 1. Comparison of the activity profile at the receptor



Scheme 1. Synthesis of 2-aminothiazole derivatives (4a-4k, 5a-5i).



Fig. 1. Some potent and selective adenosine receptor ligands with 2-aminothiazole scaffold.

subtypes seem to indicate that hetero-aryl/aroyl substitution lead to better active and selective compounds compared to aroyl substitutions (2, Fig. 1). Here benzoyl substituent was replaced by 2pyridoyl and 3-pyridoyl substituents (4a-4b). The 2-pyridoyl had moderate affinity, while the 3-pyridoyl analogue showed no binding in concentrations up to 10 µM. The 2-pyridoyl and 3-pyridoyl analogues showed limited A2A selectivity (5.7 and 4 fold over A<sub>1</sub> and 3.4 and 2 fold over A<sub>3</sub>, respectively), further no measurable affinity for A<sub>2B</sub> receptors was observed. This implies that a 2-pyridoyl or 4-pyridoyl substituent is well tolerated by the all receptor subtypes and found more selective towards A2A receptor subtypes. Replacing the pyridoyl ring with amino-triazine 4c-4e produced ligands with good activity but with loss of selectivity for the receptor subtypes. However, the triazinesubstituted thiazoles can be used as lead compounds for further structural modification. The dimethylthieno-pyrimidin-2-yl-4amine substitution at 5 position of the thiazole gave encouraging activity as these analogues **4f-4h** showed medium to strong affinity and selectivity towards the adenosine A<sub>3</sub> receptors. However, tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-2-yl-4-amine analogues 4i-4k showed low affinity. The low binding affinity in the case of the tetrahydro-benzo[4,5]thieno[2,3-d]pyrimidin-2-yl-4amine could possibly be due to an unfavourable steric bulk (Table 1).

#### 3.2. Furamide derivatives

Furan is a structural fragment which can be considered as a bioisostere of the corresponding benzamido compounds. The furan group is present in block buster drugs like ranitidine, furosemide or cefuroxime and is also widely used as a building block for adenosine receptor antagonists like CGS 15943, SCH 58261 or MRE 3008F20 [28]. Furthermore, the furan heterocycles with the 2-amino substituent is found to be an important pharmacophore for selective  $A_{2A}$  receptor antagonists [29]. Based on this report, we replaced the benzoyl substitution at 2 position of 2-aminothiazole (Table 2) to 2-furanoyl and assessed the effect of different substituents at the 5 position of the 2-aminothiazole.

The results for the furamido compounds are summarised in Table 2. In general, the replacement of the benzamido substituent



Scheme 2. Synthesis of halomethylene triazine derivatives.

at the 2-position produced ligands with slightly higher affinity for the adenosine receptor subtypes. The 2-pyridoyl substitution in 5 position leads to ligand **5a** with good affinity at the A<sub>2A</sub> adenosine receptor ( $K_i = 74.7 \text{ nM}$ ) and with some selectivity towards A<sub>1</sub> and A<sub>3</sub> (5 and 13 fold, respectively) (Table 2). The 3-pyridoyl at 5 position binds generally with lower affinity as the 2-pyridoyl. Compound 5d with a 4-pyridyl is striking as it shows sub-nanomolar affinity for the A<sub>1</sub> adenosine receptor with an about 11 and 16 fold selectivity versus the A<sub>2A</sub> and A<sub>3</sub> receptor, respectively. In addition, it also shows very high  $A_{2B}$  affinity ( $K_i = 2.8$  nM) which distinguishes it as one of the most potent non-xanthine antagonists at this receptor subtype. The respective 2-pyridyl 5c exhibits much lower affinity at all receptor subtypes (30 fold at A2A to 480 fold at the  $A_1$ ). The 5-substitution with a thieno [2,3-d] pyrimidine produced compound **5e** with low affinity at A<sub>3</sub> receptors ( $K_i = 746$  nM) and some selectivity over  $A_1$  (16 fold),  $A_{2A}$  (8 fold) and  $A_{2B}$  (>13 fold) receptors. Some A2A selectivity was observed for the compound bearing a diamino triazine in 5 position 5g with an  $A_{2A} K_i$  of 179 nM it is 3 fold, 20 fold and 5 fold selective compared to the A<sub>1</sub>, A<sub>2A</sub> and A<sub>3</sub> adenosine receptor, respectively. The 5 position substitution with a thieno[2,3-*d*]pyrimidin-4-one **5h** and quinazolin-4-one **5i** resulted in compounds with marginal affinity.

Molecular modelling studies were carried out to rationalize the unique potency and selectivity profiles provided by derivative **5d**, suggesting us that the incorporation of the 4-pyridyl moiety in the furamido-thiazole scaffold guarantees the presence of all critical chemical features essential for the recognition of the four adenosine receptor subtypes. As detailed into the Supplementary information section, starting from the crystallographic structure of the human A2A adenosine receptor co-crystallized with the 6-(2,6-dimethylpyridin-4-yl)-5-phenyl-1,2,4-triazin-3-amine (PDB ID: 3UZA) [30], we have constructed using homology modeling technique the homologous A<sub>1</sub>, A<sub>2B</sub> and A<sub>3</sub> receptors. We decided to select this specific crystallographic structure as a template because we found an interesting chemical similarity between the 6-(2,6dimethylpyridin-4-yl)-5-phenyl-1,2,4-triazin-3-amine and our 5d antagonist (furan-2-carboxylic acid (4-phenyl-5-pyridin-4-yl-thiazol-2-yl)-amide).

Molecular docking studies have been performed using a very well consolidated protocol, previously reported [31], and deeply described in the Supplementary information section. Interestingly, we found energetically favourable poses of **5d** in all four adenosine receptor subtypes with a very conserved binding motif. In order to provide a direct comparison between selected compounds and all the human adenosine receptors interaction patterns, key residues involved in binding with the considered ligands along with a quantitative estimate of the occurring ligand—protein interactions have been reported as "Interaction Energy Fingerprints" (hereby indicated as IEFs), and are graphically displayed either as histograms or as 3D colour maps.

The individual electrostatic and hydrophobic contributions to the interaction energy of each receptor residue with **5d** ligand have been calculated and the corresponding results were collected in Fig. 2, whereas the IEFs of hA<sub>2A</sub> AR with **5a**, **5b**, **5c** and **5d** have been reported in Fig. S1 (Supporting information) Consistently with their structural similarity, **5d** shares the same binging orientation observed for the 6-(2,6-dimethylpyridin-4-yl)-5-phenyl-1,2,4triazin-3-amine antagonist co-crystallized with the human A<sub>2A</sub> receptor [30]. In particular, the furamido moiety of the 5-substituted-4-phenyl-1,3-thiazol-2-yl derivatives is directed towards the extracellular side of all human adenosine receptors as shown for compound **5d** in Fig. 2. The phenyl ring in 4 position is directed towards the conserved His6.52 and Trp6.48, interacting with the hydrophobic side-chains of Leu3.33 and Val3.32 (Val87, Val84 and Val85 in A<sub>1</sub>, A<sub>2</sub>A and A<sub>2B</sub> ARs, respectively) or Leu3.32



**Scheme 3.** Synthesis of thieno[2,3-*d*]pyrimidine derivatives.

(Leu90 in A<sub>3</sub> AR). The substituted aminothiazole ring makes favourable hydrophobic interactions also with Leu6.51 (Leu250, Leu249 and Leu246 in A<sub>1</sub>, A<sub>2A</sub> and A<sub>3</sub> ARs respectively) or Val6.51 (Val250 in A<sub>2B</sub> AR). An additional  $\pi$ – $\pi$  stacking interaction occurs between the conserved Phe168 side chain (A<sub>2A</sub> AR), located in the second extracellular loop of adenosine receptors, and the aminothiazole scaffold.

Focusing our attention on the "non selective" profile of compound **5d**, the 4-pyridyl ring attached in 4 position of the 2aminothiazole scaffold is directed towards the very conserved His7.43. The endocyclic nitrogen atom of pyridine can accept a hydrogen bond from the previously cited residue side chain. Additional important hydrogen bonding interactions are present between the nitrogen atoms of the substituted 2-aminothiazole core and the conserved Asn6.55.

The low nanomolar affinity of compound **5d** for all four human adenosine receptor subtypes can be rationalized observing the peculiar hydrophobic and electrostatic interaction pattern between the selected furamide and the receptor subtypes reported in Fig. 2. The conserved Asn6.55 and His7.43 are responsible of providing most of the electrostatic interactions with the cited ligand. A dramatic loss in potency is shown when the hydrogen bonding interaction with His7.43 is abolished. Docking results showed that 2pyridyl derivatives at 4 position of the 2-aminothiazole ring are able to establish a strong hydrogen bonding interaction with the conserved Asn6.55 side chain in all human adenosine receptor subtypes, but are unable to adopt the correct geometrical conformation to create an additional hydrogen bond with the conserved His7.43. As proof of concept, the 2-pyridyl at 5 position (compound 5c) consistently reduces the binding affinities against all adenosine receptors subtypes (Table 2). In fact, the analysis of the IEF, associated to the predicted binding mode of compound 5c with A2A AR and reported in Fig. S1, shows that His7.43 does not play a significantly role in ligand binding.

Moreover, the molecular docking studies of compound **5a** and **5b** emphasize the importance of how a critical molecular engineering, of the aminothiazole scaffold, is a key point to obtain potent and non-selective ARs antagonists. The analysis of the predicted binding conformations of compound **5a** and **5b** highlighted that both nitrogen atoms, which are part of the 2-aminothiazole aromatic ring, make hydrogen bonding interactions with the conserved Asn6.55 side chain. Beside maintaining the same scaffold orientation and a similar pattern of ligand-receptor hydrophobic contacts, as shown in Fig. S1 for  $A_{2A}$  AR, the carbonyl spacer, that connects the pyridine ring and the 2-aminothiazole core, prevent the cited-above compounds to adopt the correct geometrical orientation in order to create an additional hydrogen bond with the conserved His278. In fact, the pyridoyl moiety of compound **5a** and **5b**, which is not involved in any hydrogen bonding interaction

within the residues that constitutes the binding pocket of ARs models (Fig. S1), is directed towards a cleft formed by Val3.32, Ile2.64 and Ala2.61 in  $A_1$ ,  $A_{2A}$  and  $A_{2B}$  ARs, or by Leu3.32, Val2.64 and Ala2.61 in  $A_3$  AR.

Additionally, the time-dependent stability of the receptorligand interaction network has been addressed using membrane molecular dynamics simulations. Dynamic "Interaction Energy Fingerprints" between compound **5d** and all adenosine receptor subtypes are graphically displayed as 3D colour maps in Fig. 3. The best docking poses of compound 5d in all adenosine receptor subtypes (Fig. 2) were used as starting point for the simulations. Moreover, as detailed into the Supplementary information, the individual electrostatic and hydrophobic contributions to the interaction energy of each receptor residue with 5d ligand have been calculated over the nanoseconds time scale trajectories, every 100 ps after the equilibration period of each built system has been reached, as shown in Fig. 3 per each receptor. Globally, the highaffinity compound 5d showed to have a modest fluctuation from the original starting position (r.m.s.d. < 2.5 Å). The deep analysis of Fig. 3 shows a persistent and strong electrostatic contribution of Asp253 and His278 to the 5d binding energetics in all human adenosine receptors. Additionally, in A1, A2A and A2B receptors the recruitment of the polar Glu169 through a water-mediated hydrogen-bonding interaction to the amide moiety of 5d increases its stability of interaction with the orthosteric binding pockets. In addition to the electrostatic contribution, a cooperative and stable network of hydrophobic interactions contributes to the stabilization of **5d** into all adenosine receptor subtypes, as shown in Figs. 2 and 3. It is worth to underline the interaction between 5d and the conserved Phe, located in the second extracellular loop of adenosine receptor subtypes (Phe171, Phe168. Phe173 and Phe168 in A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub>, respectively), with Leu6.51 (Leu250, Leu249 and Leu246 in A<sub>1</sub>, A<sub>2A</sub> and A<sub>3</sub>, and mutated in valine in A<sub>2B</sub>) and Ile7.39 (Ile274, Phe274. Phe276 and Phe268 in A1, A2A, A2B and A3, respectively). An intense hydrophobic contribution of Val169 in A<sub>3</sub> AR, clearly visible in Fig. 3. Moreover, favourable hydrophobic interactions are found to be persistent with an additional contribution is due to the favourable contact between the conserved F168 side chains (A2A AR), located Adenosine Receptors, and the aminothiazole ring. Compound 5b interacts with the hydrophobic sidechains of the conserved Leu3.33 and Val3.32 (Val87, Val84 and Val85 in A1, A2A and A2B ARs respectively) or Leu3.32 (Leu90 in A3 AR) and with His6.52 and Trp6.48.

### 4. Conclusion

A series of 24 [5-substituted-4-phenyl-1,3-thiazol-2-yl] benzamides and furamides derivatives was synthesized and pharmacologically evaluated at the adenosine receptors subtypes  $A_1$ ,  $A_{2A}$ ,  $A_{2B}$ 



Scheme 4. Synthesis of 5,6,7,8-tetrahydrobenzothieno[2,3-d]pyrimidine derivatives.

#### Table 1

Benzamide compounds with biological activities (4a-4k).



Compound	R <sup>1</sup>	R <sup>2</sup>	<i>K<sub>i</sub></i> (nM) (95% confidence limits)				
			hA <sub>1</sub>	hA <sub>2A</sub>	hA <sub>2B</sub> <sup>a</sup>	hA <sub>3</sub>	
4a 4b	4-OMe-C <sub>6</sub> H <sub>4</sub> 4-OMe-C <sub>6</sub> H <sub>4</sub>	2-Pyridoyl 3-Pyridoyl	688 (614–769) >10,000	128 (87.9–186) >10,000	>10,000 >10,000	201 (166–243) >10,000	
4c	4-OMe-C <sub>6</sub> H <sub>4</sub>	NH2 N N N	10,400 (7310–14,800)	2010 (1730–2340)	>10,000	2640 (1.970–3530)	
4d 4e	$\begin{array}{l} \text{4-Me-}C_6H_4\\ C_6H_5 \end{array}$	I	3630 (2890–4570) 801 (695–923)	562 (469–673) 327 (264–405)	>30,000 >10,000	816 (517–1290) 567 (452–712)	
4f 4g 4h	4-OMe-C <sub>6</sub> H <sub>4</sub> 4-Me-C <sub>6</sub> H <sub>4</sub> C <sub>6</sub> H <sub>5</sub>	H <sub>2</sub> N S N S <sup>55</sup>	>100,000 >30,000 7240 (6380–8220)	>30,000 >30,000 4870 (4050–5860)	>10,000 >10,000 >10,000	2470 (2090–2910) 1380 (798–2400) 676 (599–762)	
4i	4-OMe-C <sub>6</sub> H <sub>4</sub>	H <sub>2</sub> N N	>10,000	>10,000	>10,000	>10,000	
4j 4k	4-Me-C <sub>6</sub> H <sub>4</sub> C <sub>6</sub> H <sub>5</sub>	- <sub>S</sub> - N s	>10,000 >10,000	>10,000 >10,000	>10,000 >10,000	>10,000 >10,000	

Data are expressed as geometric means, with 95% confidence limits in parentheses.

<sup>a</sup> Inhibition of NECA-stimulated adenylyl cyclase activity. N.T., not tested.

and A<sub>3</sub>. Notably, we have identified compound **5d** as a low nanomolar antagonist versus all four adenosine receptor subtypes. The added value of this novel potent and non-selective antagonist is to incorporate in its structure all common chemical features for the recognition of all four adenosine receptor binding sites. Moreover, **5d** could represent a special building block to design novel potent and selective antagonists, in particular human against the A<sub>2B</sub> receptors. Molecular docking against all four human adenosine receptors combined with membrane molecular dynamics studies have been performed to rationalise the peculiar selectivity profile of this novel antagonist.

### 5. Experimental section

#### 5.1. General methods

Melting points are uncorrected. <sup>1</sup>H NMR (400 MHz) and <sup>13</sup>C NMR (75 MHz) spectra were measured in CDCl<sub>3</sub>, CD<sub>3</sub>OD or DMSO- $d_6$ , and chemical shifts are reported in parts per million ( $\delta$ ) downfield from tetramethylsilane as internal standard.

#### 5.2. Synthesis

#### 5.2.1. 1-Benzoyl-3-[1-diethylamino-1-phenyl-meth-(E)-ylidene]thiourea [**3a**]

The titled compound was synthesised by adding to a stirred solution of benzoyl isothiocyanate (0.0282 mol) in 5 ml toluene, *N*,*N*-diethyl-benzamidine (0.0282 mol) at -5 to 0 °C. The solution was stirred for 3 h at ambient temperature and the separated solid was filtered and washed with hexane and dried to yield **3a** as yellow crystals, %Yield: 56, LCMS: (M + 1): 340, Molecular Formula  $-C_{19}H_{21}N_{3}OS$ , m.p. = 110–112 °C. *Rf*: 0.35 (Dichloromethane). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm = 1.1–1.3 (t, 3H, –N–CH<sub>2</sub>–CH<sub>3</sub>); 1.4–1.6 (t, 3H, –N–CH<sub>2</sub>–CH<sub>3</sub>); 3.2–3.4 (q, 2H, –N–CH<sub>2</sub>–CH<sub>3</sub>); 3.6–3.8

(q, 2H,  $-N-CH_2-CH_3$ ); 7.2–7.4 (m, 10H,  $C_6H_5$ , CO– $C_6H_5$ ), 9 (s, 1H, NH).

# 5.2.2. 1-[1-Diethylamino-1-phenyl-meth-(E)-ylidene]-3-(4-methyl-benzoyl)-thiourea[**3b**]

The titled compound was synthesised by adding to a stirred solution of *p*-methyl benzoyl isothiocyanate (0.0282 mol) in 5 ml toluene, with *N*,*N*-diethyl-benzamidine (0.0282 mol) at -5 to 0 °C. The solution was stirred for 3 h at ambient temperature and the separated solid was filtered and washed with hexane and dried to yield adducts **3b** as pale yellow crystals %Yield: 56, LCMS: (M + 1) at 354, Molecular Formula – C<sub>20</sub>H<sub>23</sub>N<sub>3</sub>OS, m.p. = 118–119 °C. *R*<sub>f</sub>: 0.44 (Toluene:EtOAc::8:2).

# 5.2.3. 1-[1-Diethylamino-1-phenyl-meth-(E)-ylidene]-3-(4-methoxy-benzoyl)-thiourea [**3c**]

The titled compound was synthesised by adding to a stirred solution of *p*-methoxy benzoyl isothiocyanate (0.0282 mol) in 5 ml toluene, *N*,*N*-diethyl benzamidine (0.0282 mol) at -5 to 0 °C. The solution was stirred for 3 h at ambient temperature and the separated solid was filtered and washed with hexane and dried to yield adduct **3c** as light yellow crystals. %Yield: 58, LCMS: (M + 1): 371, Molecular Formula – C<sub>20</sub>H<sub>23</sub>N<sub>3</sub>O<sub>2</sub>S. m.p. = 123 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  ppm = 1.1–1.3 (t, 3H, –N–CH<sub>2</sub>–CH<sub>3</sub>); 1.4–1.6 (t, 3H, –N–CH<sub>2</sub>–CH<sub>3</sub>); 3.2–3.4 (q, 2H, –N–CH<sub>2</sub>–CH<sub>3</sub>); 3.6–3.8 (q, 2H, –N–CH<sub>2</sub>–CH<sub>3</sub>); 3.9 (s, 3H, –OCH<sub>3</sub>), 6.90–7.0 (d, 2H, Ar); 7.3–7.5 (m, 5H, –C<sub>6</sub>H<sub>5</sub>); 7.7–7.8 (d, 2H, Ar); 9 (s, 1H, NH).

# 5.2.4. 1-[1-Diethylamino-1-phenyl-meth-(Z)-ylidine]-3-(furan-2-carbonyl)-thiourea [**3d**]

To a solution of Furoyl isothiocyanate (0.0654 mol) in 5% ethyl acetate in Petroleum ether (60–80 °C), cooled to -5 to 0 °C, neat *N*,*N*diethyl benzamidine (0.0654 mol) was added drop wise such that the temperature does not go beyond 5 °C. The solution was stirred

#### Table 2

Furamide compounds with biological activities (5a-5i).



Compound	R <sup>2</sup>	$K_i$ (nM) (95% confidence limits)					
		hA <sub>1</sub>	hA <sub>2A</sub>	hA <sub>2B</sub> <sup>a</sup>	hA <sub>3</sub>		
5a 5b 5c 5d	2-Pyridoyl 3-Pyridoyl 2-Pyridyl 4-Pyridyl	406 (356–462) 177 (141–223) 276 (227–337) 0.58 (0.47–0.71)	74.7 (62.6–89.1) 552 (358–852) 193 (168–222) 6.65 (4.69–9.43)	9110 (7800–10,700) >10,000 841 (792–893) 2.78 (2.40–3.23)	951 (754–1200) 1870 (1700–2070) 2040 (1690–2450) 9.36 (8.90–9.85)		
5e	H <sub>2</sub> N SNN s <sup>s<sup>5</sup></sup>	12,000 (9650–14,900)	5950 (3390–10,400)	>10,000	746 (516–1080)		
5f	H <sub>2</sub> N S	>10,000	14,400 (9550–21,700)	>10,000	36,600 (24,000–56,000)		
5g	NH2 N N N N S	536 (451–638)	179 (156–205)	8000 (6710–9540)	918 (803–1050)		
5h		>10,000	>100,000	>10,000	>10,000		
5i	NH NH	>10,000	8780 (4500–17,200)	>10,000	3930 (2460–6280)		

Data are expressed as geometric means, with 95% confidence limits in parentheses.

<sup>a</sup> Inhibition of NECA-stimulated adenylyl cyclase activity.

vigorously for 3 h. The bright yellow precipitate was filtered off under suction and washed with 10 ml hexane. The solid was air-dried. Yield obtained is 56%. (LC-MS): (M + 1) at 330 and (M + 23) at 352, Molecular Formula –  $C_{17}H_{19}N_{3}O_{2}S$ , m.p. = 109 °C.  $R_{f}$ : 0.31 (Toluene:E-tOAc::8:2). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm = 1.19–1.25 (t, 3H, – NCH<sub>2</sub>CH<sub>3</sub>), 1.38–1.42 (t, 3H, –NCH<sub>2</sub>CH<sub>3</sub>), 3.33–3.40 (q, H, –NCH<sub>2</sub>CH<sub>3</sub>), 3.63–3.70 (q, H, –NCH<sub>2</sub>CH<sub>3</sub>), 6.53–6.55 (q, 1H, Furoyl), 7.24–7.27 (t, 1H, Ar–H), 7.39–7.49 (m, 6H, C6H5–, Furoyl), 9.19 (s, 1H, –NH).

# 5.2.5. Synthesis of N-[5-substituted-4-phenyl-1,3-thiazol-2-yl] benzamides (**4a**–**l**)

5.2.5.1. Method A: synthesis of 5-pyridoylanalogues. The thiourea adducts (0.0027 mol) were reacted with respective substituted pyridoyl bromides in equimolar ratio in acetonitrile at 70 °C for 24 h and the precipitated product was filtered under suction and recrystallised from ethyl acetate.

5.2.5.2. Method B: synthesis of triazines/thienopyridine analogues. The thiourea derivatives (0.0027 mol) were dissolved in 5 ml of methanol, to this chloromethyl compound (Schemes 2 and 3) (0.0027 mol) dissolved in 5 ml methanol was added, the resulting solution was stirred at 70 °C temperature for 24 h. The precipitated compound was filtered, washed with 2 ml methanol was air-dried. The compounds were purified by column chromatography.

Alternatively the thiourea derivatives (0.0011 mol) were dissolved in 5 ml *N*,*N*-dimethyl formamide, to this was added chloromethyl compounds (0.0011 mol) dissolved in 5 ml dimethyl formamide and the reaction stirred for 48 h at room temperature. The reaction then was slowly poured in 3% sodium bicarbonate solution under constant swirling condition. The product was filtered under suction, washed with water and air-dried. The compounds were purified by column chromatography.

### 5.2.6. 4-Methoxy-N-[4-phenyl-5-(pyridine-2-carbonyl)-thiazol-2yl]-benzamide [4a]

The titled compound was synthesised employing method C by reacting **3c** (0.0027 mol) with 2-bromo-1-(pyridin-2-yl)ethanone (0.0027 mol) in DMF at room temperature under stirred condition for 24 h %Yield: 58, LCMS:  $(M + H)^+$  at 416 & (M + 23) at 438, HRMS calcd for C<sub>23</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>S  $[M + H]^+$ , 416.0985, found 416.0994. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3+</sub>  $d_6$  DMSO)  $\delta$  ppm: 3.83 (s, 3H, –OCH<sub>3</sub>), 6.73–8.06 (m, 13H, C<sub>6</sub>H<sub>5</sub>, CO–C<sub>5</sub>H<sub>4</sub>N, pOCH<sub>3</sub>–H<sub>4</sub>–CO), 12.42 (s, 1H, –NHCO). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3+</sub>  $d_6$  DMSO)  $\delta$  ppm:56.12, 113.14, 118.10, 123.56, 125.86, 128.32, 130.46, 133.81, 134.56, 136.72, 145.62, 148.56, 150.04, 152.65, 156.34 m.p. = 203–5 °C. *R*<sub>f</sub>: 0.66 (Toluene:Acetonitrile::8:2).

#### 5.2.7. 4-Methoxy-N-[4-phenyl-5-(pyridine-3-carbonyl)-thiazol-2yl]-benzamide [**4b**]

The titled compound was synthesised employing method C by reacting **3c** (0.0027 mol) with 2-bromo-1-(pyridin-3-yl)ethanone (0.0027 mol) in DMF at room temperature under stirred condition for 24 h %Yield: 60, LCMS: (M + 1) at 416 & (M + 23) at 438, HRMS calcd for C<sub>23</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>S [M + H]<sup>+</sup>, calcd 416.0985, found 416.1780. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3+</sub>  $d_6$  DMSO)  $\delta$  ppm: 3.80 (s, 3H, -OCH<sub>3</sub>), 6.73–8.06 (m, 13H, C<sub>6</sub>H<sub>5</sub>, CO-C<sub>5</sub>H<sub>4</sub>N, 4-OCH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>-CO), 12.57 (s,



**Fig. 2.** Panel A: Predicted binding mode of compound **5d** (white sticks) in comparison with a 1,2,4-triazin-3-amine derivative (orange sticks) co-crystallized into the binding pocket of the human A<sub>2A</sub> adenosine receptor (PDB ID: 3UZA). The view of TM7 is partially omitted. Side chains of the amino acids in contact with compound **5d** are highlighted (gray sticks). Hydrogen atoms are not displayed. Panel B: Electrostatic interaction energy (kcal/mol) and hydrophobic interaction scores (arbitrary hydrophobic units) between each amino acid located into the binding pocket of all human adenosine receptors and the selected docked pose of compound **5d**. Electrostatic interaction energy values and hydrophobic interaction scores are plotted in blue bars and yellow bars, respectively. Ballesteros—-Weinstein residue numbers are reported. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 3.** Panel A: Per residue dynamic electrostatic interaction energy map and per residue dynamic hydrophobic interaction score map. The individual electrostatic and hydrophobic contributions to the interaction energy of each receptor residue with compound **5d** have been calculated analysing molecular dynamics simulations trajectories. Residue numbering and simulation time (ns) are plotted in the *X* and *Y* axes, respectively. Electrostatic energy values are expressed in kcal/mol, while hydrophobic scores are expressed in arbitrary hydrophobic units. Panel B: The predicted binding mode of compound **5d** into the binding pocket of all human adenosine receptors. The side chains of some amino acids in contact with compound **5d** are highlighted (gray sticks). Hydrogen atoms are not displayed.

1H, -NHCO), <sup>13</sup>C NMR (75 MHz,  $CDCl_{3+} d_6$  DMSO)  $\delta$  ppm: 56.05, 113.00, 118.03, 123.46, 125.78, 128.32, 129.37, 130.34, 133.79, 134.28, 136.70, 145.57, 148.48, 149.97, 152.61, 156.26, 156.90, 161.37, 188.24. m.p. = 294-6 °C. *R*<sub>f</sub>: 0.50 (Toluene:Acetonitrile::8:2).

# 5.2.8. N-(5-(4-Amino-6-(dimethylamino)-1,3,5-triazin-2-yl)-4-phenylthiazol-2-yl)-4-methoxybenzamide [**4**c]

The titled compound was synthesised using method B, the thiourea derivative 3c (0.0027 mol) was dissolved in 5 ml of

methanol, to this 11 (0.0027 mol) dissolved in 5 ml methanol was added, the resulting solution was stirred at 70 °C temperature for 24 h %Yield: 37, LCMS: (M + 1) at 448, HRMS calcd for  $C_{22}H_{21}N_7O_2S$  [M + H]<sup>+</sup>, 448.1477, found 448.1483. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 2.64, 2.96 (s, 6H, 2× CH<sub>3</sub>), 3.85 (s, 3H, -OCH<sub>3</sub>), 6.81 (s, 2H, NH<sub>2</sub> broad), 7.07–8.15 (m, 9H, Ar–H), 12.69 (s, 1H, -NHCO). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 35.52, 56.04, 125.24, 127.57, 128.41, 128.86, 129.25, 130.46, 132.29, 133.27, 135.29, 136.56, 159.24, 165.20,

166.02, 166.59, 166.96. m.p. = 268-70 °C.  $R_f$ : 0.44 (Toluene:Acetonitrile::8:2).

# 5.2.9. N-(5-(4-Amino-6-(dimethylamino)-1,3,5-triazin-2-yl)-4-phenylthiazol-2-yl)-4-methylbenzamide [**4d**]

The titled compound was synthesised using method B, the thiourea derivative **3b** (0.0027 mol) was dissolved in 5 ml of methanol, to this **11** (0.0027 mol) dissolved in 5 ml methanol was added, the resulting solution was stirred at 70 °C temperature for 24 h %Yield: 39, LCMS: (M + 1) at 432 & (M + 23) at 454, HRMS calcd for C<sub>22</sub>H<sub>21</sub>N<sub>7</sub>OS [M + H]<sup>+</sup>, 432.1528, found 448.1538. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 2.39 (s, 3H, –CH<sub>3</sub>), 2.64, 2.96 (s, 6H, – N(CH<sub>3</sub>)<sub>2</sub>), 6.82 (s, 1H, –NH<sub>2</sub> broad), 7.37–8.05 (m, 9H, Ar), 12.74 (s, 1H, –NHCO), <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 22.4, 35.96, 125.39, 127.76, 128.56, 128.96, 129.46, 130.35, 132.03, 133.56, 135.34, 136.76, 159.35, 165.29, 166.07, 166.61, 167.01. m.p. = 258–60 °C. *R<sub>f</sub>*: 0.41 (Toluene:Acetonitrile::8:2).

# 5.2.10. N-[5-(4-Amino-6-dimethylamino-[1,3,5]triazin-2-yl)-4-phenyl-thiazol-2-yl]-benzamide [**4e**]

The titled compound was synthesised using method B, the thiourea derivative **3a** (0.0027 mol) was dissolved in 5 ml of methanol, to this **11** (0.0027 mol) dissolved in 5 ml methanol was added, the resulting solution was stirred at 70 °C temperature for 24 h. %Yield: 39, LCMS: (M + 1) at 418 & (M + 23) at 440, Molecular Formula –  $C_{21}H_{19}N_7OS$ , m.p. = 160 °C.  $R_f$ : 0.38 (Toluene:Acetonitrile::8:2). HRMS calcd for  $C_{22}H_{19}N_7OS$  [M + H]<sup>+</sup>, 418.1366, found 418.1359. <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ )  $\delta$  ppm = 2.6 and 2.93 (2s, 6H, N–[CH<sub>3</sub>]<sub>2</sub>); 6.75 (s, 1H, –NH, 7.31–8.13 (m, 10H, –Ar–H, – C<sub>6</sub>H<sub>5</sub>–CO)). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 35.83, 125.86, 127.71, 128.27, 128.73, 129.07, 130.34, 132.28, 133.22, 136.46, 159.19, 165.16, 165.92, 166.57, 166.76.

### 5.2.11. N-[5-(4-Amino-5,6-dimethyl-thieno[2,3-d]pyrimidin-2-yl)-4-phenyl-thiazol-2-yl]-4-methoxy-benzamide [4f]

The titled compound was synthesised employing method C by reacting **3c** (0.0027 mol) with **15** (0.0027 mol) in DMF at room temperature under stirred condition for 48 h. %Yield: 58, LCMS: (M + 1) at 488, and (M + 18) at 510, Molecular Formula –  $C_{25}H_{21}N_5O_2S_2$ , m.p. = 283–5 °C. *Rf*: 0.53 (Toluene:EtOAc::8:2). HRMS calcd for  $C_{25}H_{21}N_5O_2S_2$  [M + H]<sup>+</sup>, 488.1131, found 488.1340. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 2.23 (s, 3H, –CH<sub>3</sub>), 2.42 (s, 3H, –CH<sub>3</sub>), 3.8 (s, 3H, –OCH<sub>3</sub>), 6.55 (s, 1H, –NH<sub>2</sub> broad), 6.73–7.89 (m, 9H, Ar), 12.19 (s, 1H, –NHCO), <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 13.49, 14.25, 56.05, 112.87, 114.83, 117.15, 124.02, 125.32, 126.56, 127.78, 128.56, 130.16, 130.25, 136.31, 145.72, 147.91, 148.14, 152.26, 156.54, 158.27, 168.03.

### 5.2.12. N-[5-(4-Amino-5,6-dimethyl-thieno[2,3-d]pyrimidin-2-yl)-4-phenyl-thiazol-2-yl]-4-methyl-benzamide [**4g**]

The titled compound was synthesised employing method C by reacting **3b** (0.0027 mol) with **15** (0.0027 mol) in DMF at room temperature under stirred condition for 48 h. %Yield: 56, LCMS: (M + 1) at 472, Molecular Formula –  $C_{25}H_{21}N_5OS_2$ , m.p. = 310 °C.  $R_f$ : 0.61 (Toluene:Acetonitrile::8:2). HRMS for  $C_{25}H_{21}N_5OS_2$  [M + H]<sup>+</sup>, calcd 472.1187, found 472.1179. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm = 2.42–2.52 (3s, 9H, –CH<sub>3</sub>); 7.33–7.87 (m, 9H, –Ar–H, p– H<sub>3</sub>C–C<sub>6</sub>H<sub>4</sub>–CO); 9.61 (s, 1H, –NH). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 13.56, 14.24, 21.23, 112.98, 114.52, 117.16, 123.93, 125.56, 126.70, 127.90, 128.98, 129.97, 130.28, 136.52, 144.71, 145.93, 148.05, 152.39, 156.63, 158.24, 168.70.

### 5.2.13. N-[5-(4-Amino-5,6-dimethyl-thieno[2,3-d]pyrimidin-2-yl)-4-phenyl-thiazol-2-yl]-benzamide [**4h**]

The titled compound was synthesised employing method C by reacting **3a** (0.0027 mol) with **15** (0.0027 mol) in DMF at room

temperature under stirred condition for 48 h %Yield: 54, LCMS: (M + 1) at 458, m.p. = 257–9 °C. *R*<sub>f</sub>: 0.59 (Toluene:Acetoni-trile::8:2). HRMS calcd for C<sub>24</sub>H<sub>19</sub>N<sub>5</sub>OS<sub>2</sub> [M + H]<sup>+</sup>458.1025, found 458.1030. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 1.93 (s, 3H, –CH<sub>3</sub>), 2.11 (s, 3H, –CH<sub>3</sub>), 6.63 (s, 1H, –NH<sub>2</sub> broad), 6.75–7.46 (m, 5H, Ar–H), 7.56–8.45 (m, 5H, Ar–H), 12.55 (s, 1H, –NHCO), <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 12.96, 13.93, 112.56, 113.98, 117.56, 124.07, 125.65, 126.86, 127.92, 128.83, 130.04, 130.32, 136.58, 145.09, 146.15, 148.56, 152.45, 156.73, 158.83, 168.93.

# 5.2.14. N-[5-(4-Amino-5,6,7,8-tetrahydro-benzo[4,5]-thieno[2,3-d] pyrimidin-2-yl)-4-phenyl-thiazol-2-yl]-4-methoxy-benzamide [4i]

The titled compound was synthesised employing method C by reacting **3c** (0.0027 mol) with **18** (0.0027 mol) in DMF at room temperature under stirred condition for 48 h %Yield: 56, LCMS: (M + 2) at 515 & (M + 23) at 537, m.p.  $\geq$  310 °C.  $R_f$ : 0.71 (Tolue-ne:Acetonitrile::7:3). HRMS calcd for  $C_{27}H_{23}N_5O_2S_2$  [M + H]<sup>+</sup> 513.1287, found 513.1293. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 12.87 (s, 1H, -NHCO), 6.87–8.16 (m, 9H, Ar–H), 6.87 (s, 2H, NH<sub>2</sub> broad), 3.86 (s, 3H, -CH<sub>3</sub>), 2.88 (t, 2H, -CH<sub>2</sub>), 2.75 (t, 2H, -CH<sub>2</sub>), 1.79 (s, 4H, 2× -CH<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 22.22, 22.92, 25.02, 25.76, 56.05, 114.47, 118.24, 123.95, 128.92, 129.10, 130.93, 131.42, 132.26, 133.35, 134.40, 147.87, 150.00, 158.93, 159.32, 163.46, 165.40.

# 5.2.15. N-[5-(4-Amino-5,6,7,8-tetrahydro-benzo[4,5]-thieno[2,3-d] pyrimidin-2-yl)-4-phenyl-thiazol-2-yl]-4-methyl-benzamide [4j]

The titled compound was synthesised employing method C by reacting **3b** (0.0027 mol) with **18** (0.0027 mol) in DMF at room temperature under stirred condition for 48 h. %Yield: 51, LCMS: (M + 2) at 499, m.p.  $\geq 310$  °C.  $R_f$ : 0.73 (Toluene:Acetonitrile::7:3). HRMS calcd for  $C_{27}H_{23}N_5O_2S_2$   $[M + H]^+$  497.1338, found 497.1344. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 1.80 (s, 4H,  $-2 \times CH_2$ ), 2.39 (s, 3H,  $-CH_3$ ), 2.72 (s, 2H,  $-CH_2$ ), 2.91 (s, 2H,  $-CH_2$ ), 6.68 (s, 2H,  $-NH_2$  broad), 7.36–8.07 (m, 9H, Ar–H), 12.65 (s, 1H, -NHCO), <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 22.01, 22.97, 25.46, 25.76, 114.56, 118.65, 124.15, 129.07, 129.46, 131.06, 131.96, 132.46, 133.53, 134.52, 148.07, 150.24, 158.68, 158.85, 159.36, 163.52, 165.57.

# 5.2.16. N-[5-(4-Amino-5,6,7,8-tetrahydro-benzo[4,5]-thieno[2,3-d] pyrimidin-2-yl)-4-phenyl-thiazol-2-yl]-benzamide [**4k**]

The titled compound was synthesised employing method C by reacting **3a** (0.0027 mol) with **18** (0.0027 mol) in DMF at room temperature under stirred condition for 48 h. %Yield: 53, LCMS: (M + 2) at 485 & (M + 23) at 507, Molecular Formula –  $C_{26}H_{21}N_5OS_2$ , m.p.  $\geq 310$  °C.  $R_{f}$ : 0.71 (Toluene:Acetonitrile::7:3). HRMS calcd for  $C_{26}H_{21}N_5OS_2$  [M + H]<sup>+</sup> 483.1188, found 483.1180. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 1.78 (s, 4H,  $-2 \times$  CH<sub>2</sub>), 2.33 (t, 2H, - CH<sub>2</sub>), 2.71 (t, 2H, -CH<sub>2</sub>), 6.59 (s, 1H, -NH<sub>2</sub> broad), 6.78–7.81 (m, 10H, Ar–H), 12.60 (s, 1H, -NHCO). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 21.96, 23.01, 25.23, 25.56, 114.48, 118.56, 124.01, 129.04, 129.46, 131.04, 131.56, 132.46, 133.43, 134.45, 148.02, 150.15, 158.78, 158.97, 159.36, 163.50, 165.42.

# 5.2.17. Furan-2-carboxylic-acid [4-phenyl-5-(pyridine-2-carbonyl)-thiazol-2-yl]-amide [**5a**]

To a solution of 1 part of **3d** in 15 parts dimethyl formamide was added 1 part of 2-bromo-1-(pyridin-2-yl)ethanone at room temperature and the solution stirred for 24 h. Reaction mixture was poured in 50 parts of water with shaking, pure product separated as a solid was filtered and dried. %Yield: 58, (LC-MS): (M + 1) at 376 and (M + 23) at 398, m.p. =  $172-4 \degree C. R_{f}$ : 0.71 (Toluene:ACN::7:3). HRMS calcd for C<sub>20</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>S [M + H]<sup>+</sup> 375.0672, found 375.0608. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 6.78–7.5 (d & m 3H Furoyl–H), 7.6–7.9 (5H, Ar–H), 8.1–8.8 (m, 5H, C<sub>5</sub>H<sub>4</sub>N), 12.79 (s, 1H, –NHCO). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 111.51, 115.46, 121.27, 121.02,

126.27, 127.96, 128.43, 130.17, 132.92, 133.45, 137.42, 144.18, 148.24, 149.56, 153.25, 154.52, 162.71, 164.38, 188.37.

# 5.2.18. Furan-2-carboxylic acid [4-phenyl-5-(pyridine-3-carbonyl)-thiazol-2-yl]-amide [**5b**]

To a solution of 1 part of **3d** in 15 parts dimethyl formamide was added 1 part of 2-bromo-1-(pyridin-3-yl)ethanone at room temperature and the solution stirred for 24 h. Reaction mixture was poured in 50 parts of water with shaking, pure product separated as a solid was filtered and dried. %Yield: 52, (LC-MS): (M + 1) at 376 and (M + 23) at 398, Molecular Formula - C<sub>20</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>S, m.p. = 240–4 °C. *R<sub>f</sub>*: 0.44 (Toluene:ACN::8:2). HRMS calcd for C<sub>20</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>S [M + H]<sup>+</sup> 375.0672, found 375.0680. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 6.78–7.5 (d & m 3H Furoyl–H), 7.6–7.9 (5H, Ar–H), 8.1–8.8 (m, 5H, C<sub>5</sub>H<sub>4</sub>N), 12.85 (s, 1H, –NHCO), <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 111.42, 115.76, 124.42, 127.66, 128.51, 129.28, 130.37, 132.95, 133.42, 136.63, 144.17, 148.42, 151.82, 155.15, 155.27, 162.71, 164.35, 189.46.

# 5.2.19. Furan-2-carboxylic acid (4-phenyl-5-pyridin-2-yl-thiazol-2-yl)-amide [**5c**]

To a solution of 1 part of **3d** in 15 parts isopropyl alcohol was added 1 part of 2-chloromethyl pyridine at 80 °C for 24 h and the precipitated product was filtered off under suction and dried. % Yield: 48, (LC-MS): (M + 1) at 348, (M + 23) at 370 m.p. = 233 °C. *R<sub>f</sub>*: 0.42 (Toluene:ACN::8:2). HRMS calcd for C<sub>19</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>S [M + H]<sup>+</sup> 347.0722, found 347.0728. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 6.89–7.5 (d & m 3H, Furoyl–H), 7.6–7.9 (5H, Ar–H), 8.1–8.8 (m, 5H, C<sub>5</sub>H<sub>4</sub>N), 12.75 (s, 1H, –NHCO), <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 110.96, 114.26, 117.96, 124.14, 124.66, 127.43, 128.95, 129.42, 133.28, 137.56, 144.15, 148.37, 149.42, 151.93, 154.92, 162.91, 164.57.

# 5.2.20. Furan-2-carboxylic acid (4-phenyl-5-pyridin-4-yl-thiazol-2-yl)-amide [5d]

To a solution of 1 part of **3d** in 15 parts isopropyl alcohol was added 1 part of 4-chloromethyl pyridine at 80 °C for 24 h and the precipitated product was filtered off under suction and dried. % Yield: 45, (LC-MS): (M + 1) at 348, Molecular Formula – C<sub>19</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>S, m.p. = 291–3 °C. *R<sub>f</sub>*: 0.25 (Toluene:ACN::8:2). HRMS calcd for C<sub>19</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>S [M + H]<sup>+</sup> 347.0722, found 347.0723. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 6.9–7.4 (d & m 3H, Furoyl–H), 7.6–7.9 (5H, Ar–H), 8.2–8.8 (m, 5H, C<sub>5</sub>H<sub>4</sub>N), 12.81 (s, 1H, –NHCO). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 111.56, 115.52, 117.85, 118.72, 127.73, 128.91, 129.48, 133.41, 143.52, 145.91, 148.47, 150.27, 155.29, 162.91, 164.48.

# 5.2.21. Furan-2-carboxylic acid [5-(4-amino-5,6-dimethyl-thieno [2,3-d]pyrimidin-2-yl)-4-phenyl-thiazol-2-yl]-amide [**5e**]

To a solution of 1 part of **3d** in 15 parts dimethyl formamide was added 1 part of 15 at room temperature and the solution stirred for 48 h. Reaction mixture was added in 50 parts of water with shaking, product separated as a solid was filtered and dried. %Yield: 38, (LC-MS): (M + 1) at 448, m.p. = 175–8 °C. *Rf*: 0.17 (Toluene:EtOAc::8:2). HRMS calcd for C<sub>22</sub>H<sub>17</sub>N<sub>5</sub>O<sub>2</sub>S<sub>2</sub> [M + H]<sup>+</sup> 448.0818, found 448.0824. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 2.36 (s, 3H, –CH<sub>3</sub>), 2.40 (s, 3H, –CH<sub>3</sub>), 6.76–8.05 (m, 8H, Furoyl–H, Ar–H), 12.99 (s, 1H, –NHCO), <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 10.56, 13.17, 110.96, 114.37, 115.76, 117.86, 127.83, 128.94, 129.47, 131.49, 133.28, 136.17, 144.29, 146.71, 148.59, 154.81, 158.91, 162.47, 163.15, 164.72.

### 5.2.22. Furan-2-carboxylic acid [5-(4-amino-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrim-idin-2-yl)-4-phenyl-thiazol-2-yl]amide [**5f**]

To a solution of 1 part of **3d** in 15 parts dimethyl formamide was added 1 part of **18** at room temperature and the solution stirred for 48 h. Reaction mixture was added in 50 parts of water with shaking,

product separated as a solid was filtered and dried. %Yield: 42, (LC-MS): (M + 2) at 475, Molecular Formula –  $C_{24}H_{17}N_5O_2S_2$ , m.p.  $\geq$  310 °C. *Rf*: 0.35 (Toluene:ACN::8:2). HRMS calcd for  $C_{24}H_{19}N_5O_2S_2$  [M + H]<sup>+</sup>473.0974, found 473.0980. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 2.07 (m, 4H, 2× –CH<sub>2</sub>), 2.55 (t, 2H, –CH<sub>2</sub>), 2.73 (t, 2H, –CH<sub>2</sub>), 6.53 (s, 1H, –NH<sub>2</sub> broad), 6.78–7.56 (m, 5H, Ar–H), 7.87–8.45 (m, 3H, Furoyl–H), 12.5 (s, 1H, –NHCO), <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  ppm: 23.26, 23.91, 25.18, 111.59, 113.48, 115.47, 118.27, 127.41, 127.95, 128.73, 129.48, 133.29, 137.56, 144.26, 145.82, 148.38, 154.92, 158.73, 162.48, 162.91, 164.72.

### 5.2.23. N-(5-(4-Amino-6-(dimethylamino)-1,3,5-triazin-2-yl)-4-phenylthiazol-2-yl)furan-2-carboxamide [**5g**]

To a solution of 1 part of **3d** in 15 parts methanol was added 1 part of **11** in methanol and reacted at 70 °C for 24 h. The precipitated product obtained was filtered off under suction and air-dried. %Yield: 54, (LC-MS): (M + 1) at 408 and (M + 23) at 430 m.p. = 250-2 °C.  $R_f$ : 0.15 (Toluene:EtOAc::8:2). <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ ) [ppm] = 2.62 and 2.96 (2s, 6H,  $-N(CH_3)_2$ ), 6.75–6.82 (broad s, 2H,  $-NH_2$ ) (m, 8H, Furoyl–H, Ar–H), 12.82 (s, 1H, -NH), HRMS calcd for C<sub>19</sub>H<sub>17</sub>N<sub>7</sub>O<sub>2</sub>S [M + H]<sup>+</sup> 408.1158, found 408.1164. <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 40.27, 80.15, 11.92, 115.7, 117.71, 128.34, 129.18, 129.45, 133.27, 144.29, 148.47, 154.78, 161.49, 162.95, 164.58, 167.2, 170.45.

# 5.2.24. Furan-2-carboxylic acid [5-(5,6-dimethyl-4-oxo-3,4-dihydro-thieno[2,3-d]pyrimidin-2-yl)-4-phenyl-thiazol-2-yl]-amide [**5h**]

To a solution of 1 urea derivative (F1) in 15 parts isopropyl alcohol was added 1 part of 2-(chloromethyl)-5,6-dimethylthieno [2,3-*d*]pyrimidin-4(3*H*)-one at 80 °C for 24 h and then reaction mixture was concentrated and to it water was added to get the precipitate which was filtered off under suction and dried. %Yield: 38, (LC-MS): (M + 1) at 449 and (M + 23) at 471, Molecular Formula – C<sub>22</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub>S<sub>2</sub>, m.p.  $\geq$  310 °C. *R<sub>f</sub>*: 0.33 (Toluene:ACN::8:2). HRMS calcd for C<sub>22</sub>H<sub>16</sub>N<sub>4</sub>O<sub>3</sub>S<sub>2</sub> [M + H]<sup>+</sup> 449.0664, found 449.0658. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 2.26 (s, 3H, –CH<sub>3</sub>), 2.43 (s, 3H, –CH<sub>3</sub>), 6.78–7.82 (m, Furoyl–H 8H, Ar–H), 12.17 (s, 1H, –NHCO), 12.74 (s, 1H, –NHCO), <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 10.27, 10.58, 111.56, 115.97, 118.74, 127.57, 129.59, 131.91, 133.15, 134.29, 144.48, 148.59, 154.63, 156.10, 156.78, 161.38, 162.72, 164.27.

### 5.2.25. Furan-2-carboxylic acid [5-(4-oxo-3,4-dihydro-quinazolin-2-yl)-4-phenyl-thiazol-2-yl]-amide [**5i**]

To a solution of urea derivative (F1) in 15 parts isopropyl alcohol was added 1 part of 2-(chloromethyl)quinazolin-4(3*H*)-one at 80 °C for 24 h and the precipitated product was filtered off under suction and dried. %Yield: 42, (LC-MS): (M + 1) at 415 and (M + 23) at 437, m.p. > 310 °C. *R*<sub>f</sub>: 0.73 (Toluene:ACN::7:3). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) [ppm] = 6.76-6.78 (q 1H, Furoyl–H), 7.38–7.61 (m, 6H, C6H5–, Furoyl–H), 8.06–8.12 (d, 2H, Ar–H), 7.69–7.72 (d, 2H, Ar–H), 7.76–7.77 (d, 2H, Ar–H), 7.82–7.87 (q, 1H, Ar–H), 12.17 and 13.08 (2s, 2H, –NH). HRMS calcd for C<sub>22</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub>S [M + H]<sup>+</sup> 414.0781, found 408.0787. <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 111.72, 115.45, 120.96, 126.57, 126.88, 127.17, 127.56, 128.95, 129.46, 132.57, 133.00, 133.78, 144.18, 144.95, 148.45, 154.78, 156.27, 161.28, 162.94, 164.58.

#### 6. Biological methods

Receptor-radioligand binding studies were performed as previously described by Klotz et al. [32]. The membranes for radioligand binding were prepared from CHO cells stably transfected with human adenosine receptor subtypes in a two-step procedure. In a first low-speed step ( $1000 \times g$ ) cell fragments and nuclei were removed. The crude membrane fraction was sedimented from the supernatant at  $100,000 \times g$ . The membrane pellet was resuspended in the buffer used for the respective binding experiments, frozen in liquid nitrogen and stored at -80 °C. For the measurement of adenylyl cyclase activity only one high-speed centrifugation of the homogenate was used. The resulting crude membrane pellet was re-suspended in 50 mM Tris/HCl, pH 7.4 and immediately used for the cyclase assay.

For agonist radioligand binding at A<sub>1</sub> adenosine receptors 1 nM [<sup>3</sup>H]CCPA was used, whereas 30 nM [<sup>3</sup>H]NECA were used for A<sub>2A</sub> receptors. For A<sub>3</sub> adenosine receptors the newly developed high-affinity agonist [<sup>3</sup>H]HEMADO [33] at a concentration of 1 nM was used.

Non-specific binding of [<sup>3</sup>H]CCPA was determined in the presence of 1 mM theophylline, in the case of  $[^{3}H]$ NECA and  $[^{3}H]$ HEMADO 100 µM R-PIA was used. K<sub>i</sub>-values from competition experiments were calculated with the program SCTFIT [34].

Radioligand binding at A<sub>2B</sub> adenosine receptors is problematic as no high-affinity ligand is available for this subtype. Therefore, inhibition of NECA-stimulated adenylyl cyclase activity was determined as a measurement of affinity of compounds. EC<sub>50</sub>-values from these experiments were converted to K<sub>i</sub>-values with the Cheng and Prusoff [35] equation.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.03.020.

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