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Brief Article

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Discovery of 7-(prolinol-*N*-yl)-2-phenylamino-thiazolo[5,4-d]pyrimidines as novel non-nucleoside partial agonists for the A_{2A} adenosine receptor: Prediction from molecular modeling

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KEYWORDS. Thiazolo[5,4-d]pyrimidines, adenosine, A_{2A} adenosine receptor agonist, non-nucleoside A_{2A} agonist, GPCR.

ABSTRACT: We describe the identification of 7-(prolinol-*N*-yl)-2-phenylamino-thiazolo[5,4-d]pyrimidines as a novel chemotype of non-nucleoside partial-agonists for the A_{2A} adenosine receptor (A_{2A}AR). Molecular-modeling indicated that the (S)-2-hydroxymethylene-pyrrolidine could mimic the interactions of agonists' ribose, suggesting that this class of compounds could have agonistic properties. This was confirmed by functional assays on the A_{2A}AR, where their efficacy could be associated to the presence of the 2-hydroxymethylene moiety. Additionally, the best compound displays promising affinity, selectivity profile and physicochemical properties.

Introduction

Adenosine (**1**, Figure 1), an endogenous purine nucleoside, is also the natural ligand for the adenosine receptors (ARs). Four distinct AR subtypes exist, designated as A_1, A_{2A}, A_{2B}, and A_3, all belonging to the G protein-coupled receptor (GPCR) superfamily. The subtypes A_1 and A_3 are coupled to G_i which inhibits adenylyl cyclase and thereby the production of cAMP from ATP, whereas A_{2A} and A_{2B} are coupled to G_s which activates this enzyme and consequently the cAMP-dependent pathways.[1, 2] All adenosine receptor subtypes are characterized by a unique pharmacological profile.[1-7]

Adenosine behaves as a high affinity agonist for the A_1, A_{2A}, and A_3 receptors (hA_1 *K*i = 310 nM, hA_{2A} *K*i = 700 nM, h_{A3} *K*i = 290 nM) and with considerably lower affinity for the A_{2B} receptor (hA_{2B} *K*i ≥ 10 μM).[5] Chemically, adenosine is a nucleoside comprising a molecule of the purine base adenine attached to a ribose moiety *via* a glycosidic bond. The unmodified adenosine has been of restricted interest in studying adenosine receptors because it is readily metabolized by a number of enzymes. The main approach being used to discover AR agonists has been the modification of adenosine itself, which has led to most of the reported A_{2A} agonists.[5, 8-11] Variations of the hydroxymethyl portion of the ribosyl unit of adenosine generated *N*-ethylcarboxamidoadenosine (NECA, **2**), which showed 35-fold higher potency (hA_{2A} *K*i = 20 nM) for A_{2A} than adenosine (**1**). However, NECA is a non-selective agonist. Its analog 2-hexynyl-NECA (HENECA, **3**), which additionally introduces modifications at the C2 position in the adenine core, exhibits high affinity at A_{2A}AR (hA_{2A} *K*i = 6.4 nM) and 10-fold selectivity over human A_1AR.[12] 2-Phenylaminoadenosine **4** (CV1808) maintains substitution at the C2 of the adenine but has an intact ribose structure, and it presents A_{2A} selectivity over the A_1 receptor (rA_{2A} *K*i = 100 nM, rA_1 *K*i = 400 nM). 4-[2-[[6-Amino-9-(*N*-ethyl-β-D-ribofuranuronamidosyl)-9*H*-purin-2-yl]amino]ethyl] benzenepropanoic acid **5** (CGS21680), another moderately A_{2A}AR-selective agonist, displays binding affinities of 27 and 19 nM at the human and rat A_{2A} receptor, respectively.[13, 14] Regadenoson (CVT3146, **6**), is an adenosine derivative bearing a *N*-pyrazole at its 2-position. This compound has been approved by the FDA as a coronary vasodilator,[15, 16] demonstrating the therapeutic potential of A_{2A} receptor agonists. The only class of non-nucleoside AR agonist are 2-amino-3,5-dicyanopyridines,[17] from which 2-((1H-imidazol-2-yl)methylthio)-6-amino-4-(3-hydroxyphenyl)pyridine-3,5-dicarbonitrile **7** (LUF5835) exerts good affinity for A_{2A} receptor.[18] The chemical structures of representative A_{2A} agonists **1-7** are shown in Figure 1.

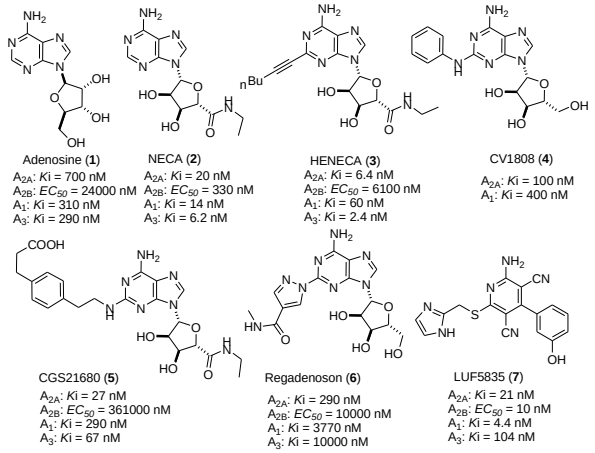


Figure 1. Structures of adenosine A_{2A} receptor agonists **1-7**.

A_{2A}AR agonists play an important role in the treatment of cerebral and cardiac ischemia, epilepsy, thrombosis, and arterial hypertension; however the drug development in this area is limited by the lack of novel scaffolds. The identification of newer

A_{2A}AR agonists has been a challenging task, as observed from the recent efforts of Jacobson and Carlsson,[19] who performed docking screens of 6.7 million commercially available molecules against active-like conformations of the A_{2A}AR to investigate whether these structures could guide the discovery of agonists. Though twenty predicted agonists were confirmed to be A_{2A}AR ligands, none of these effectively activated ARs.

In the search of safe and selective drugs that regulate this receptor, a general concern is to achieve chemical novelty and good solubility values. Thiazolo[5,4-*d*]pyrimidine is a bioisostere of purine in which the imidazole moiety is replaced by 1,3-thiazole.[20, 21] Previous studies identified thiazolo[5,4-*d*]pyrimidine derivatives as antagonists at adenosine A_{2A} receptors.[22-24] Using a computer-aided rational drug design approach, based on the A_{2A}AR crystal structures, we herein report the discovery and pharmacological characterization of 7-prolinol linked 2-phenylamino-thiazolopyrimidines as a new class of non-nucleoside A_{2A}AR partial agonists.

Results and discussion

Design of A_{2A}AR agonists. The design of the series of compounds presented here started with the consideration of the thiazolo[5,4-*d*]pyrimidine as a bioisostere of adenine. Several substituents were considered to increase binding affinity for the A_{2A} receptor. The docking studies, in both active-like and inactive conformations of the A_{2A}AR, indicated that the most common binding orientation as compared to the adenine core corresponds to a double flip of the bicyclic scaffold along the two planar axes, as opposed to the initial bioisosteric design (Figure 2).

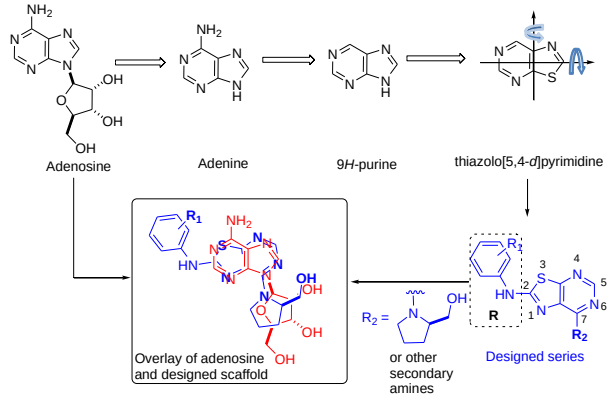


Figure 2. Design of 7-substituted thiazolo[5,4-*d*]pyrimidines as potential adenosine A_{2A} agonists. The 2-symmetry axis considered for the molecular superposition of adenosine and thiazolo[5,4-d]pyrimidine scaffolds in the A_{2A}AR binding site is shown.

According to this superposition model, the R_2 substituent overlays with the ribose of adenosine, and the R, matches the C-2 substituents in adenosine derivatives. In our case, R is a 2-arylamino mimicking the C2 of agonist **4**, and according to the docking model it occupies the same extracellular region than the C2-substituent of the crystalized agonist **5**[25] and the phenylethyl group of antagonist ZM241385 (Figure 3).[26] Pooling together the two parallel docking runs (i.e. considering the active and inactive-like conformation of the receptor), this conserved binding mode was found among the top three ranking poses in more than 78% of the compound series. In this pose, the ligand is anchored by a hydrogen bond interaction between the conserved Asn253$^{6.55}$ and N4 of the ligand scaffold, allowing the R and R_2 substituents to be positioned as described above. It has been well proven that, in the active-like structures, the binding site of the ribose corresponds to an otherwise highly hydrated region in the antagonist-bound structures. Interestingly, R_2 in many of our compounds displaces some of the water molecules present within this area in the corresponding X-ray structures. In particular, the compounds bearing a 2-hydroxymethyl pyrrolidine at R_2 (series 4) specifically displace a water molecule essential to maintain the bioactive conformation of the agonists, by consistently mediating an internal hydrogen-bond between the ribose and the adenine ring. By occupying this binding area, these compounds show an extra hydrogen bond interaction between the hydroxyl group of the ligand with the conserved His$^{7.43}$ (Figure 3). Alternatively, in some compounds the preferred docking solution presents a hydrogen bond to Ser$^{7.42}$. Our exhaustive docking exploration shows that these interactions are compatible with the active-like conformation of the receptor, and resemble the key interactions of the ribose of typical AR agonists, as revealed by X-ray crystallography,[25, 27, 28] and probed by site-directed mutagenesis experiments.[29] Based on these findings, we hypothesized that these prolinol-containing compounds (i.e., series 4, compounds **10b**, **10d**, **10g**, **10l**) could act as potential adenosine A_{2A} agonists.

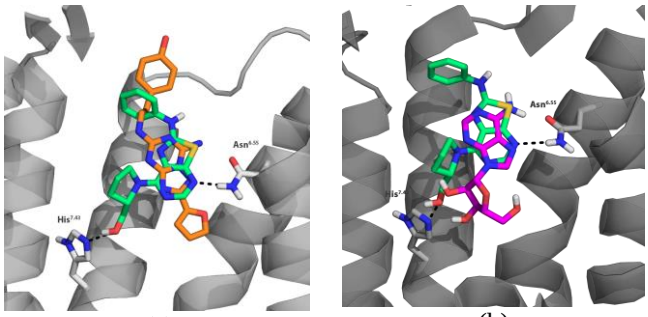
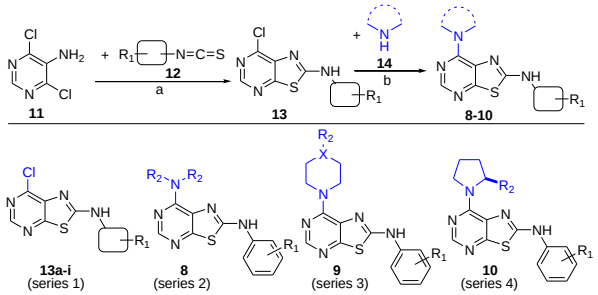


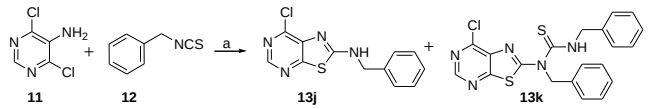
Figure 3. Binding mode proposed by molecular docking for compound **10b** on the human A_{2A}AR, superimposed with the experimental binding pose of (a) A_{2A}AR in inactive conformation, in complex with the antagonist ZM241385 (orange[26]) and (b) A_{2A}AR in the active-like conformation bound to the agonist adenosine (magenta[25]).

Synthesis. Based on the molecular modeling design, a series of 7-substituted thiazolo[5,4-*d*]pyrimidines **8-10, 13** were synthesized using the synthetic protocol as depicted in Scheme 1.[30] In order to establish structure-activity relationships, various secondary amines including morpholine, piperidine, diethylamine, dimethylamine, piperazine, *N*-alkylpiperazine, pyrrolidine and 2-hydroxymethylene pyrrolidine (prolinol) were employed for substitution at C-7 position of the thiazolo[5,4-*d*]pyrimidine scaffold. The thiazolo[5,4-d]pyrimidines **13a-l** were synthesized from 4,6-dichloro-5-aminopyrimidine (**11**) and the corresponding isothiocyanate (**12**) using KF/alumina as a catalyst with overall 52-96% isolated yield. The thiazolo[5,4-d]pyrimidine **13i** was prepared by treating **11** with benzoyl isothiocyanate. Compounds **8-10** were synthesized from 2-amino-7-chlorothiazolo[5,4-*d*]pyrimidines **13a-h** by treating with the corresponding amines **14** with overall yield of 85-98%. For synthesis of prolinol linked analogs **10b**, **10d**, **10e**, **10g**, **10j**, **10l** and **10n**, (S)-2-hydroxymethylene pyrrolidine (L-prolinol) was used. The compounds were characterized by using ^{1}H NMR, ^{13}C NMR and HR-ESIMS analysis. Four series of compounds (as depicted in Scheme 1) were prepared in order to understand the structure-activity relationships.



Scheme 1. Synthesis of 7-substituted thiazolo[5,4-d]pyrimidines **8-10**, **13**.Reagents and conditions: (a) KF/alumina (20 mol%), ACN, 60 °C, 5 h; (b) MeOH, rt, 4-5 h.

Interestingly, when benzyl isothiocyanate was reacted with 6-dichloro-5-aminopyrimidine (**11**), two products **13j** and **13k** were formed in 5% and 52% yield, respectively. The disubstituted product **13k** was formed via thioacylation of NH of the product **13j**.[20] The formation of products **13j** and **13k** is depicted in Scheme 2. The exact structures of all synthesized compounds are shown in Tables 1-4.



Scheme 2. Reaction of 6-dichloro-5-aminopyrimidine (**11**) with benzyl isothiocyanate (**12**): (a) KF/alumina (20 mol%), ACN, 60 °C, 5 h.

Adenosine receptor binding studies. Radioligand binding assays were performed for all four series of thiazolo[5,4-*d*]pyrimidines at the human A_1-, A_{2A}-, and A_3AR subtypes. Due to the lack of a suitable radioligand for A_{2B} adenosine receptors, the effect on NECA-stimulated adenylyl cyclase was determined as a measure for A_{2B} affinity. None of the compounds in this study showed detectable affinity (K_i values > 10,000 nM) for the A_{2B} subtype (data not shown). Most compounds of the series 1 bearing a 7-chloro substituent showed modest affinity for A_{2A}AR (Table 1). For the best compound **13h**, an A_{2A} K_i of 1,050 nM was determined. For thiazolo[5,4-*d*]pyrimidines containing an alkylamino substitution at C-7 position (series 2), similar results were found (Table 2) with compound **8b** showing the highest binding affinity for A_{2A}AR (K_i = 1,270 nM). In the third series, comprising a six-membered saturated *N*-heterocycle at C-7 position, a morpholine substituted analog **9a** showed good binding affinity towards adenosine A_{2A} receptor with K_i value of 333 nM, and >3,000 and 13-fold selectivity towards A_1 and A_3 receptors, respectively (Table 3). Compounds from the fourth series where the C-7 position of the thiazolo[5,4-d]pyrimidine scaffold is substituted with a five-membered saturated *N*-heterocycle, showed promising binding affinity for the adenosine A_{2A} receptor (Table 4). The most potent compound in this series is **10m** bearing a 3,4,5-tri-OMe phenylamino group at C-2 and pyrrolidine at C-7 position. Compound **10m** showed binding affinity for A_{2A}AR with K_i value of 102 nM. The binding affinity (K_i) of this compound for A_1 and A_3ARs was >100,000 and 1,280 nM, respectively, indicating its selectivity towards A_{2A}AR.

The obtained results for A_{2A}AR binding allowed to envisage a clear structure-activity relationship. The analogs substituted with electron-donating groups (EDG) at the C-2 phenylamino ring showed higher binding affinity towards A_{2A}AR than analogs with electron-withdrawing groups (EWG) at the phenyl ring. In agreement with this, compound **13h** in series 1 substituted with a 3,4,5-trimethoxy phenyl ring shows the highest affinity with a *K*i value of 1,050 nM for the A_{2A} adenosine receptor, whereas analog **13e** substituted with *p*-nitrophenyl ring is inactive (K_i > 30,000) (Table 1). Similar observations were also made in series 4, where **10m** substituted with an EDG shows better binding affinity (K_i = 102 nM) than **10h** possessing an EWG (K_i = 3,180 nM). Furthermore, among various substituents (*viz.* chloro, dialkylamino, six-membered *N*-heterocycle, and five-membered *N*-heterocycle) attempted at C-7 position, particularly the analogs bearing a five-membered *N*-heterocycle at C-7 position presented with the highest affinity. The best compound from this series is **10m** (K_i for A_{2A}AR is 102 nM). The five-membered *N*-heterocycle bearing compounds **10b** and **10l** also exhibited high A_{2A}affinity; however, they were less selective against A_1 and A_3 receptors (3-7 fold) than compound **10m** (> 100 and 12 fold, respectively).

Characterization of selected compounds in A_{2A}AR functional assay. Based on the predictions of molecular modeling, we investigated the potential presence of agonistic activity of the prolinol linked analogs. Six compounds bearing a hydroxymethylene substituent on the pyrrolidine (compounds **10b**, **10d**, **10g**, **10j**, **10l**, and **10n**) were tested in an A_{2A}AR functional assay consisting of the measurement of adenylyl cyclase activity. Their corresponding analogs without the hydroxymethylene substituent (compounds **10a**, **10c**, **10f**, **10i**, **10k** and **10m**) were also tested, the hypothesis being that they would not show agonistic activity as opposed to their hydroxymethylene substituted analogs. The results of the functional assay are shown in Figure 4.

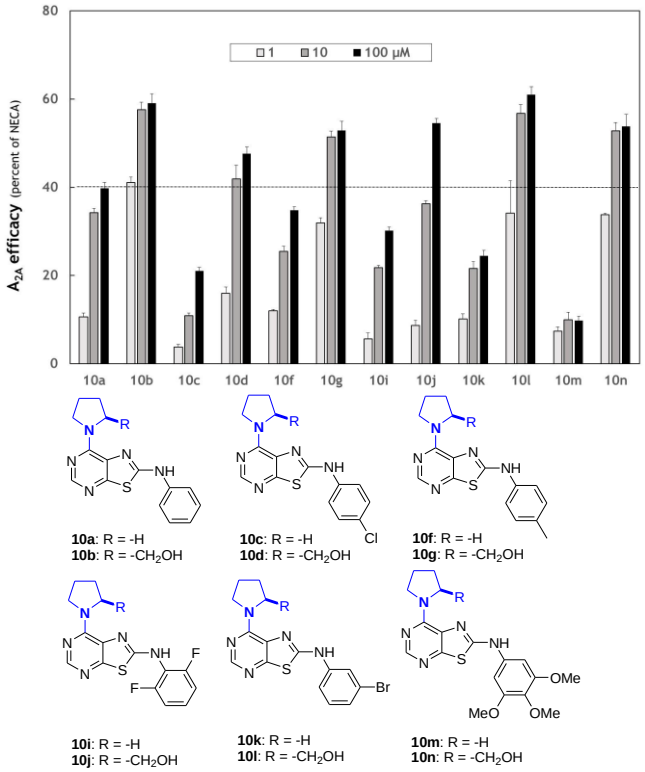


Figure 4. Agonistic activity of prolinol analogs **10b**, **10d**, **10g**, **10j**, **10l**, **10n** and their corresponding pyrrolidine (non-prolinol) analogs **10a, 10c, 10f, 10i, 10k, 10m** at A_{2A} adenosine receptors.

All predicted agonists **10b**, **10d**, **10g**, **10j**, **10l**, and **10n** showed about 50-60% efficacy at 100 µM concentration (reference value for NECA = 100%), whereas the corresponding control compounds **10a**, **10c**, **10f**, **10i**, **10k** and **10m** showed neglegible or significantly lower (10-40%) efficacy levels. Compound **10m,** with the highest A_{2A} affinity of all new ligands reported in this study, presented the lowest A_{2A} efficacy (10%, see Figure 4). However, its corresponding prolinol analog **10n**, with a 3-times lower affinity, showed >50% efficacy, substantiating the relevance of a hydroxymethylene substituent on the pyrrolidine for agonistic activity. In all the six pair of analogs (**10a**/**10b**, **10c**/**10d**, **10f**/**10g**, **10i**/**10j**, **10k**/**10l**, **10m**/**10n**) tested, the 2-hydroxymethylene substituted compounds consistently show higher efficacy than their analogs lacking this functional group. Taken together, this clearly indicated that the presence of 2-hydroxymethylene group on pyrrolidine (i.e. prolinol) is relevantly contributing to A_{2A}AR agonism. The prolinol containing compounds **10b** and **10l** were the most potent A_{2A} adenosine receptor partial agonists in this study with *K*i values of 200 and 153 nM.

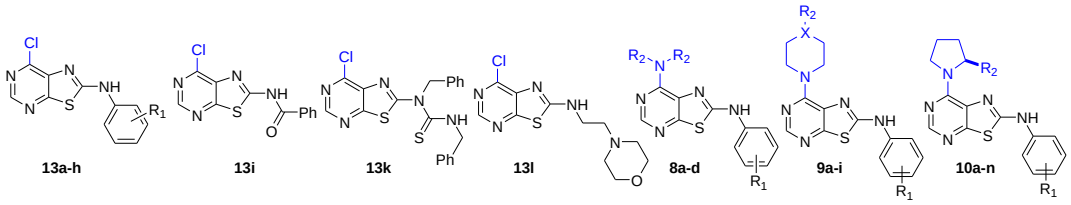


Table 1. Adenosine receptor binding affinity of 7-chloro substituted thiazolo[5,4-d]pyrimidines **13a-l** (series 1)

Entry	R_1	K_i in nM[a]		
		A_1	A_{2A}	A_3
13a	H	> 100,000	1,520 (1,130-1,760)	3,670 (3,030-4,440)
13b	4-Cl	25,000 (13,900-44,700)	2,690 (1,750-4,130)	4,940 (3,240-7,510)
13c	4-OMe	11,500 (10,200-12,900)	3,570 (2,140-5,960)	5,880 (4,980-6,950)
13d	4-Me	>100,000	4,930 (2,580-9,410)	4,640 (3,540-6,080)
13e	4-NO_2	>30,000	>30,000	7,950 (4,900-12,900)
13f	2,6-di-F	17,800 (15,400-20,600)	8,110 (7,690-8,560)	17,200 (12,500-23,700)
13g	3-Br	12,600 (9,550-16,600)	2,960 (2,600-3,370)	3,900 (2,970-5,120)
13h	3,4,5-tri-OMe	>100,000	1,050 (1,030-1,080)	2,650 (2,260-3,110)
13i	-	7,240 (5,610-9,330)	1,500 (1,420-1,590)	7,330 (5,920-9,090)
13k	-	>30,000	1,870 (1,290-2,720)	1,030 (905-1,170)
13l	-	>100,000	>100,000	>100,000

Table 2. Adenosine receptor binding affinity of 7-dialkylamino-substituted thiazolo[5,4-d]pyrimidines **8a-d** (series 2)

Entry	R_1	R_2	K_i in nM[a]		
			A_1	A_{2A}	A_3
8a	H	Et	> 100,000	2,210 (1,100-4,440)	> 30,000
8b	H	Me	> 100,000	1,270 (927-1,730)	1,280 (1,260-1,310)
8c	4-Cl	Et	16,500 (9,900-27,500)	11,200 (8,590-14,500)	3,890 (3,160-4,790)
8d	4-Cl	Me	> 100,000	>100,000	> 10,000

Table 3. Adenosine receptor binding affinity of thiazolo[5,4-d]pyrimidines **9a-i** substituted with six-membered aliphatic *N*-heterocycle at C-7 position (series 3)

Entry	R_1	X-R_2	K_i in nM[a]		
			A_1	A_{2A}	A_3
9a	-H	O	> 100,000	333 (276-402)	4,460 (2,950-6,760)
9b	-H	CH_2	> 30,000	980 (593-1,620)	12,100 (10,100-14,400)
9c	-H	N-Me	> 30,000	2,840 (2,490-3,240)	4,310 (2,600-7,150)
9d	-H	NH	> 30,000	9,090 (6,860-12,000)	> 10,000
9e	-H	-NEt	> 100,000	8,390 (8,030-8,750)	> 30,000
9f	-H		> 100,000	>100,000	> 100,000
9g	4-Cl	O	> 100,000	1,400 (1,070-1,830)	> 100,000
9h	4-Cl	CH_2	> 100,000	13,200 (9,840-17,800)	18,300 (16,500-20,300)
9i	4-Cl	N-Me	> 100,000	>100,000	> 10,000

Table 4. Adenosine receptor binding affinity of thiazolo[5,4-d]pyrimidines **10a-n** substituted with five-membered aliphatic *N*-heterocycle at C-7 position (series 4)

Entry	R_1	R_2	K_i in nM[a]		
			A_1	A_{2A}	A_3
10a	-H	H	> 30,000	351 (266-462)	8,630 (6,380-11,700)
10b	-H	-CH_2OH	555 (448-686)	200 (190-210)	978 (825-1,160)
10c	4-Cl	H	> 100,000	2,060 (1,750-2,430)	4,260 (4,020-4,500)
10d	4-Cl	-CH_2OH	629 (517-764)	466 (422-515)	934 (740-1,180)
10e	4-OMe	-CH_2OH	713 (637-799)	1,110 (743-1,660)	594 (431-819)
10f	4-Me	-H	>100,000	974 (714-1,330)	563 (360-879)
10g	4-Me	-CH_2OH	399 (363-439)	313 (273-360)	843 (685-1,040)
10h	4-NO_2	-H	>30,000	3,180 (1,850-5,440)	3,630 (2,590-5,080)
10i	2,6-Di-F	-H	2,310 (2,070-2,570)	2,260 (2,050-2,500)	3,190 (2,710-3,740)
10j	2,6-Di-F	-CH_2OH	7,970 (6,890-9,220)	3,260 (2,680-3,970)	3,420 (2,560-4,580)
10k	3-Br	-H	>100,000	245 (200-300)	1,290 (782-2,130)
10l	3-Br	-CH_2OH	530 (328-856)	153 (117-200)	1,070 (919-1,260)
10m	3,4,5-tri-OMe	-H	>100,000	102 (84-125)	1,280 (1,010-1,630)
10n	3,4,5-tri-OMe	-CH_2OH	974 (534-1,780)	330 (288-378)	1,460 (900-2,380)

[a]Shown are K_i values in nM with 95% confidence limits in parentheses

These experimental results are in line with the molecular mechanism proposed for these compounds as being (partial) agonists (see Figure 3), i.e. interactions of the hydroxyl group in prolinol-containing compounds with His7.43 (or alternatively Ser7.42) can, to some extent, stabilise the active conformation of the receptor.

Aqueous solubility and lipophilicity determination of promising compounds. The thermodynamic equilibrium solubility of selected compounds *viz*. **10b**, **10f**, **10g**, **10k**, **10l** and **10m** was investigated in water, PBS, SGF and SIF (results are shown in Table S1). Amongst these compounds, the 7-prolinol-2-phenylamino linked thiazolo[5,4-d]pyrimidine **10b** displayed

excellent solubility (4.3, 4.8, and 3.5 mg/ml, respectively) in water, PBS and SGF. Other compounds **10f**, **10k** and **10l** also showed good water solubility with solubility values >150 µg/ml. All compounds were poorly soluble in SIF. The Log P and Log D values of these compounds were also determined using the *n*-octanol-water partition coefficient method and results are listed in Table S1. In line with solubility values, compound **10b** showed better Log P and Log D values than other compounds

Conclusion

In summary, through a rational computer-aided design approach, we have discovered 7-prolinol-substituted thiazolo[5,4-d]pyrimidines as potent and selective A_{2A} receptor partial agonists. Importantly, this novel chemotype is one of the few examples of A_{2A} adenosine receptor agonists not based on the adenosine scaffold. Compounds **10b** and **10l** emerge as the most potent adenosine A_{2A}AR partial agonists reported here, with *K*i values of 200 and 153 nM, also showing moderate selectivity for A_{2A} over A_1 and A_3 receptors. In addition, compound **10b** showed excellent solubility in water, PBS and SGF. This study has demonstrated that molecular modeling predictions can be used to successfully design novel A_{2A} adenosine receptor agonists whose basic structure does not rely on the adenosine scaffold. This unique new class of A_{2A} partial agonists can be an excellent starting point in the design of novel agents for the treatment of important diseases such as cerebral and cardiac ischemia, epilepsy, thrombosis, and arterial hypertension.

Experimental section

Molecular modeling studies. The structures of A_{2A}AR in both active-like (PDB: 2YDO, in complex with adenosine)[28] and inactive conformations (PDB: 4EIY, in complex with antagonist ZM241385),[26] were prepared for docking experiments. Selected crystallographic waters occupying the binding site were retained and the fragments for crystallization purposes removed. The structures were then refined in order to model the missing loops and add protons, as described earlier.[31] Special attention was paid to the protonation state of histidine residues in the binding site, $His^{6.52}250$ and $His^{7.43}278$, which were both modeled as neutral and protonated in Hδ, to allow the characteristic interactions of agonists according to the A_{2A}-agonist crystal structures.[25, 27, 28] All compounds were built and energy minimized using Maestro software[32] and the OPLSAA force-field.[33] Each ligand was docked 20 times with default (high accuracy) genetic algorithm (GA) search parameters, using the scoring function Chemscore as implemented in GOLD[34] and allowing full flexibility for the ligand. The search sphere was centered on the side chain (CG1) of $Ile^{7.39}$ and expanded with a radius of 15 Å, thus ensuring a generous enough search space comprising the binding site experimentally. The criterion for the docking posess election was based on a combination of the Chemscore ranking and the population (convergence) of the solutions according to anr.m.s.d. clustering criteria of 1 Å.

General method for preparation of 2-amino-7-chlorothiazolo[5,4-*d*]pyrimidines 13a-k.[30] To the solution of 4,6-dichloro-5-aminopyrimidine (**11**, 164 mg, 1 mmol) in ACN (4 mL) was added KF/alumina (20 mol%) followed by isothiocyanate **12** (1 mmol). The mixture was stirred at 60 °C for 5 h, then cooled to room temperature and concentrated under reduced pressure. The resulted mixture was dissolved in 10% methanol in $CHCl_3$ and the catalyst KF/alumina was filtered and washed with acetone and then dried and reused. The filtrate was dried and washed with hexane: diethyl ether mixture to get products **13a-k** in 80-96% yield.

General method for preparation of compounds 8a-d, 9a-i and 10a-n.[30] To the solution of 2-amino-7-thiazolo[5,4-*d*]pyrimidines (**13a-k**, 0.25 mmol) in MeOH (3 mL) was added secondary amines (1.0 mmol). The mixture was stirred at room temperature for 12-24 h and then concentrated under reduced pressure. The reaction mixture was then dried under vacuum and the final products **8a-d, 9a-i** and **10a-n** were recrystallized with methanol and chloroform mixture giving 85-98% isolated yield. The spectral data of a representative compound **10b** is provided here. Spectral data of all remaining compounds is provided in supporting information.

*2-(Phenylamino)-7-[2(S)-hydroxymethyl-pyrrolidin-1-yl]-thiazolo[5,4-d]pyrimidine (**10b**).* Yield: 86%; brown solid; m.p. 232-234 ºC; ^{1}H NMR (500 MHz, DMSO-d_6, ppm): δ 10.51 (s, 1H, NH), 8.17 (s, 1H), 7.72 (d, 2H, *J* = 7.6 Hz), 7.36 (d, 2H, *J* = 7.5 Hz), 7.00 (t, 1H, *J* =7.3 Hz), 4.82 (t, 2H, *J* = 4 Hz), 3.72-3.70 (m, 2H), 2.09-1.93 (m, 5H); ^{13}C NMR (125 MHz, CD_3OD, ppm): δ 159.84 (C), 158.01 (C), 153.25 (C), 152.14 (CH), 141.77 (C), 130.14 (CH), 124.53 (C), 123.70 (CH), 119.24 (CH), 64.42 (CH_2), 64.42 (CH_2), 61.51 (CH), 30.82 (CH_2), 28.82 (CH_2); HR-ESIMS: m/z calcd 328.1228 for $C_{16}H_{17}N_5OS+H^+$ (328.1227).

Radioligand binding to hA_1, A_{2A} and A_3ARs. [^{3}H]CCPA (1 nM), [^{3}H]NECA (10 nM), and [^{3}H]HEMADO (1 nM) were utilized in radioligand binding assays to membranes prepared from CHO cells expressing recombinant hA_1, hA_{2A} and hA_3 ARs. All procedures were carried out as previously described in detail.[13, 35] Non-radioactive compounds were initially dissolved in DMSO and diluted with buffer to the final concentration where the amount of DMSO never exceeded 1%. For incubation 96-well microplates with filter bottoms (Millipore MultiScreen MAFC) were used and incubation was terminated after 3 h at 25 °C by filtration through the built-in filter bottoms. The wells were rinsed three times with 200 µL of ice-cold binding buffer. Filter plates were dried and counted in a WallacMicroBeta Counter after addition of 20 µL of scintillator. K_i values were calculated with the program Prism (GraphPad Software Inc., La Jolla, CA).

Measurement of adenylyl cyclase activity. The agonistic activity of compounds at the A_{2A}AR was determined in adenylyl cyclase experiments as described previously.[13] To study the interaction of compounds with A_{2B} adenosine receptors inhibition of NECA-stimulated adenylyl cyclase activity was measured.

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Author Contributions. S.B.B. designed all chemistry strategies, synthetic protocols, and coordinated whole study; B.S. synthesized all compounds; S.K. performed adenosine receptor binding assays; A.O. performed molecular modelling studies; V.K. and S.S.B. performed solubility and Log P determinations; S.S.B. analyzed and interpreted solubility/Log P results; K.N.K. evaluated and interpreted adenosine receptor binding data; HGT interpreted molecular modelling results; S.B.B., R.A.V., H.G.T., K.N.K. contributed to manuscript writing.

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ABBREVIATIONS

A_{2A}AR, Adenosine A_{2A} receptor; cAMP, cyclic adenosine monophosphate; CCPA, 2-chloro-N^6-cyclopentyladenosine; EDG, electron-donating groups;EWG, electron-withdrawing groups; FDA, Food and Drug Administration; GPCR, G protein-coupled receptor; HEMADO, 2-hexyn-1-yl-N^6-methyladenosine; HENECA, 2-hexynyl-*N*-ethylcarboxamidoadenosine; NECA, *N*-ethylcarboxamidoadenosine; PBS, phosphate buffer saline; SGF, simulated gastric fluid; SIF, simulated intestinal fluid.

SUPPORTING INFORMATION AVAILABLE: Experimental details and NMR spectra scans. This material is available free of charge via the Internet at http://pubs.acs.org.

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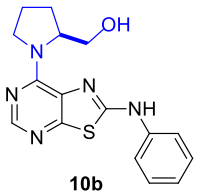
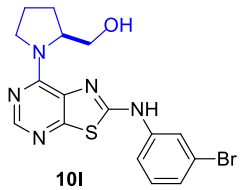
Table of Contents Graphic



10b

A_{2A}: *K*i = 200 nM
A_1: *K*i = 555 nM
A_3: *K*i = 978 nM

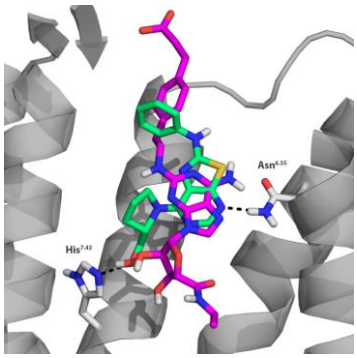
S_{water} = 4.3 mg/ml
CLogP: 3.21



10l

A_{2A}: *K*i = 153 nM
A_1: *K*i = 530 nM
A_3: *K*i = 1070 nM

S_{water} = 0.16 mg/ml
CLogP: 4.08



10b (green) with A_{2A}