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Synthesis of 2-amino-5-benzoyl-4-(2-furyl)thiazoles as adenosine A_{2A} receptor antagonists

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ABSTRACT

The discovery and synthesis of a series of 2-amino-5-benzoyl-4-(2-furyl)thiazoles as adenosine A_{2A} receptor antagonists from a small-molecule combinatorial library using a high-throughput radioligand-binding assay is described. Antagonists were further characterized in the A_{2A} binding assay and an A_1 selectivity assay. Selected examples exhibited excellent affinity for A_{2A} and good selectivity versus the A_1 receptor.

Adenosine is a modulator of multiple physiological processes, including cardiovascular, neurological and respiratory functions. Adenosine mediates its effects through the specific G-protein-coupled receptors (GPCR's) A_1, A_{2A}, A_{2B} and A_3. A_{2A} antagonists have been shown to produce an increase in locomotor activity, a decrease of neuroleptic-induced catalepsy, and a decrease of MPTP-induced hypomotility. These observations support therapeutic use of A_{2A} antagonists for neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease.

Parkinson's disease is a neurodegenerative disorder characterized by loss of motor coordination manifested as tremor and rigidity of the limbs and trunk.[1] These symptoms are due to the deterioration and loss of dopaminergic neurons in the pars compacta region of the substantia nigra, which result in a decrease of dopamine in the striatum.[2] Restoration of motor activity is achieved by treatment with L-3,4-dihydroxyphenylalanine (L-Dopa).[3,4] L-Dopa treats the symptoms of Parkinson's disease but does not arrest or reverse the neurodegeneration of dopaminergic neurons. The finding that the adenosine A_{2A} receptor is primarily located in the striatum[5] and is co-expressed with the dopamine D_2 receptor[6,7] support a role for A_{2A} in motor activity. Stimulation of the A_{2A} receptor has been found to induce sedation and catalepsy, and inhibits the motor-stimulating effects of dopamine receptor agonists.[8] A_{2A} antagonists synergize with D_2 agonists to stimulate locomotor activity.[9] The effects of A_{2A} antagonists have also been reported to afford neuroprotection in animal models of Parkinson's disease.[10,11] Studies with A_{2A} and/or D_2 knockout mice support these observations.[12]

The in vivo efficacy of a variety of small-molecule A_{2A} antagonists in animal models of Parkinson's disease has established the potential pharmaceutical utility for this class of compounds.[13–22] The A_{2A} antagonists Istradefylline (KW-6002)[17] and Privadenant (SCH-420814)[20] (Fig. 1) are currently the subject of clinical trials.

High-throughput screening of an extensive set of ECLiPS™ (Encoded Combinatorial Libraries on Polymeric Support)[23] libraries (90 Libraries, >4 million compounds) employing an A_{2A} radioligand-binding assay resulted in the identification of a number of active compound series. One particular library of ~18,000 compounds, based on a 2-aminothiazole core structure, elicited a set of 2-amino-5-benzoyl-4-(2-furyl)thiazoles **7** as potent A_{2A} antagonists.

The solid phase synthesis of the 2-aminothiazole library was conducted using 250 μm polystyrene beads (**1**) in conjunction with an acid-cleavable linker. The resin **1** was acylated with the acid-cleavable linker, 4-(4′-formyl-3′-methoxy)phenoxybutyric acid, to provide aldehyde **2** (Scheme 1).[24] Generation of a resin-bound secondary amine **3** was achieved by reductive alkylation of a series of primary amines (R^1NH_2) with **2**. Reaction of **3** with a diverse set of acylisothiocyanates gave *N*-acyl thiourea **4**. This was followed by *S*-alkylation with a series of α-bromoketones and subsequent cyclization to give resin-bound 2-aminothiazoles **5**. Cleavage of **5** from

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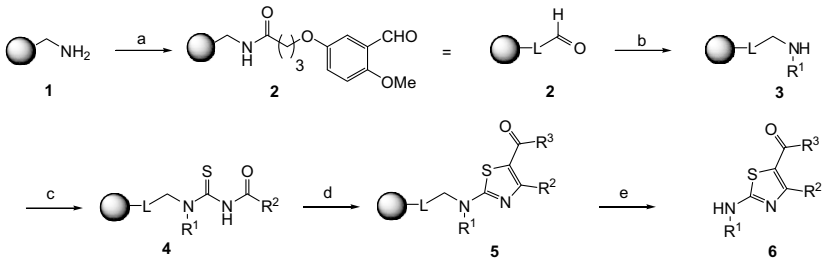


Scheme 1. Reagents and conditions: (a) 4-(4′-formyl-3′-methoxy)phenoxybutyric acid, DIC, HOBt monohydrate, CH_2Cl_2, DMF, 25 °C; (b) R^1NH_2, $Na(OAc)_3BH$, 1,2-dichloroethane, 25 °C; (c) $R^2C(O)NCS$, CH_2Cl_2, 25 °C; (d) $R^3C(O)CH_2Br$, AcOH, DMF, 25 °C; (e) TFA, CH_3CN, 25 °C.

the solid support with trifluoroacetic acid provided 2-aminothiazoles **6**.

A_{2A} active compounds identified from screening the 2-aminothaizole library exhibited conserved structural features at the R^2 and R^3 positions, but were tolerant of multiple substituents and functionalities at the R^1 position. The frequency of the synthons in the active compounds at the three different diversity positions (R^1–R^3) is shown in Figure 2. Of the possible 47 components incorporated at the R^1 position, 22 of the R^1 substituents were present in the active compound set. In contrast, only one of the possible 12 components at the R^2 position (R^2 component #9 = 2-furanyl) and one of the possible 32 components at the R^3 position (R^3 component #8 = phenyl) were identified from the screen. These results provided a low molecular weight template (**7**) for further development (Fig. 3).

The 2-aminothiazole **7a** identified in the library screen was resynthesized on solid phase according to Scheme 1 and confirmed A_{2A} activity with a K_i of 67 nM (Fig. 3). Functional antagonism was demonstrated through evaluation in a human calcium-mobilization assay using HEK-293 cells co-expressing human A_{2A} receptor and G_{qi} protein.

To facilitate further exploration of the role of the R^1 substituent, a solution-phase synthesis was devised to generate analogs. A similar synthetic strategy to that used on solid phase was employed, utilizing 2-furoyl isothiocyanate and α-bromoacetophenone to build the heterocycle. To ensure cyclization of the *S*-alkylated *N*-acylthiourea occurred to give the desired 2-aminothiazole product, the primary amines **8** under investigation were initially protected via reductive alkylation with 2,4-dimethoxybenzaldehyde to provide **9** (Scheme 2). *N*-Acylthiourea **10** was formed by reaction of **9** with 2-furoyl isothiocyanate. 2-Aminothiazole formation along with subsequent dimethoxybenzyl removal was conducted via reaction of **10** with α-bromoacetophenone in AcOH/DMF.

Direct *N*-acylthiourea formation via reaction of 2-furoyl isothiocyanate with a primary amine **8** effectively provides **11** (Scheme 3). However, cyclization through reaction with α-bromoacetophenone results in the formation of the heterocycle **12** as opposed to the desired isomeric 2-aminothiazole **7**.[25] Compounds of type **12** displayed no binding activity at the A_{2A} receptor.

Examples of 2-amino-5-benzoyl-4-(2-furyl)thiazoles **7** synthesized utilizing the solution-phase synthesis (Scheme 2) and

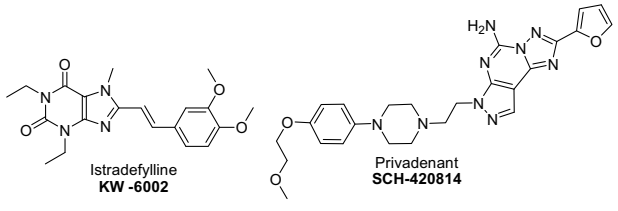


Figure 1. A_{2A} antagonists KW-6002 and SCH-4020814.

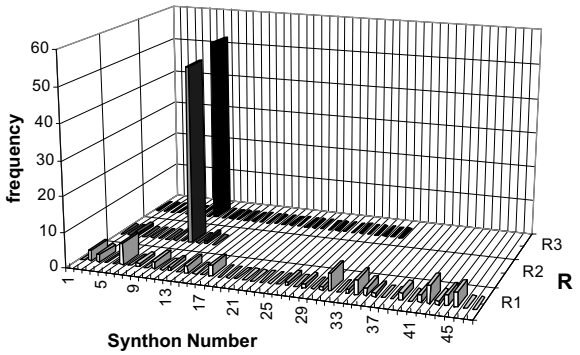


Figure 2. Synthon frequency analysis for the combinatorial variables identified in the active compound set from A_{2A} screening of the 2-aminothiazole library.

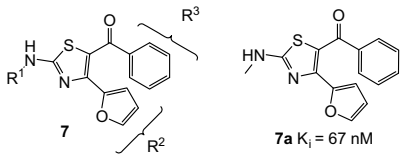
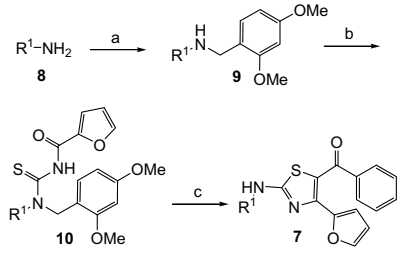
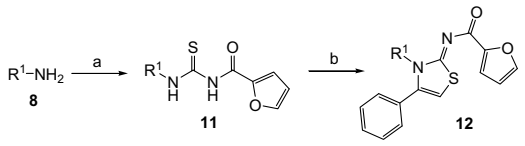


Figure 3. General template for 2-aminothiazole A_{2A} antagonists.



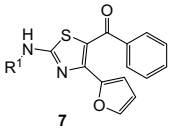
Scheme 2. Reagents and conditions: (a) 2,4-dimethoxybenzaldehyde, $Na(OAc)_3BH$, 1,2-dichloroethane, 25 °C; (b) 2-furoyl isothiocyanate, CH_2Cl_2, 25 °C; (c) α-bromoacetophenone 5%AcOH/DMF, 90 °C.



Scheme 3. Reagents and conditions: (a) 2-furoyl isothiocyanate, CH_2Cl_2, 25 °C; (b) α-bromoacetophenone, 5%AcOH/DMF, 90 °C.

incorporating a selection of substituents at the 2-position are detailed in Table 1. Activity at the human A_{2A} receptor was examined

Table 1
Secondary 2-amino-5-benzoyl-4-(2-furyl)thiazole A_{2A} antagonists



7

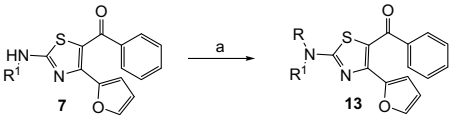
Compound	R^1	hA_{2A} binding $K_i \pm SD$[a] (nM)	hA_1 binding $K_i \pm SD$[b] (nM)	hA_1/hA_{2A} ratio
7a	Methyl	67 ± 3	3580 ± 260	54
7b	*i*-Butyl	89 ± 37	1280 ± 160	14
7c	2-Methoxyethyl	195 ± 91	6780 ± 1800	35
7d	2-Methoxypropyl	154 ± 29	3970 ± 440	26
7e	2-Acylaminoethyl	281 ± 9	2420 ± 110	9
7f	1-(Acetyl)-piperidine-4-yl	455 ± 268	1240 ± 515	3
7g	1-(2-Fluorobenzoyl)-piperidine-4-yl	105 ± 22	3710 ± 640	35
7h	3,4-Difluorobenzyl	64 ± 13	>7500	>120
7i	2-Furfuryl	43 ± 3	1460 ± 310	35
7j	2-Thiophenemethyl	54 ± 6	2990 ± 130	56
7k	(3,4-Methylenedioxy)phenethyl	61 ± 23	3500 ± 870	58
7l	2-Thiophenethyl	12 ± 2	>6700	>580
7m	Phenylpropyl	105 ± 30	6300 ± 655	60

[a] $K_i \pm SD$ determined by competition binding of [^{3}H]SCH-58261.[28]
[b] $K_i \pm SD$ determined by competition binding of [^{3}H]DPCPX.[29]

in the same radioligand-binding assay as used for the initial screen. Activity at the human A_1 receptor was also measured using an appropriate radioligand-binding assay.[29] Selectivity versus the A_1 receptor is desirable based on potential adverse cardiovascular side-effects associated with A_1 antagonism.[26] Analogs display good overall A_{2A} antagonist activity, showing a high degree of flexibility for the substituents at the 2-amino position. Hydrophobic substituents are well tolerated providing compounds exhibiting K_i values < 100 nM. Aliphatic examples include the methyl (**7a**) and isobutyl (**7b**) analogs, displaying K_i values between 70 and 90 nM. Analogs incorporating arylalkyl and heteroarylalkyl substituents (**7h–m**) are also shown to be favorable, with the 2-thiophenethyl analog (**7l**) exhibiting a K_i value of 12 nM. The inclusion of more polar functionality such as ether-based components (**7c, d**) or *N*-acetylethylene (**7e**) and *N*-acetyl-piperidine-4-yl-based (**7f**) amino substituents result in less potent compounds. Activity at the A_1 receptor was consistently in the micromolar range, providing a range of selectivity ratios when compared with A_{2A} binding. Interestingly, the arylalkyl and heteroarylalkyl-based analogs provide the highest selectivity ratios, with the 2-thiophenethyl example (**7l**) exceeding 500-fold selectivity versus the A_1 receptor.

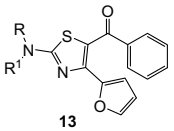
Three analogs (**7a, 7i**, and **7l**) incorporating methyl, furfuryl and 2-thiophenethyl at the 2-amino position, respectively, were selected to examine the effect of alkylation of the secondary amino group of the 2-amino thiazole. Alkylation was conducted with either methyl iodide or benzyl bromide using polystyrene-bound *N*-phenyl-tris(dimethylamino)imino phosphorane (BEMP)[27] as a base to provide **13** (Scheme 4).

Alkylation of **7a** and **7l** resulted in dramatic losses in A_{2A} activity, suggesting either a key role for the hydrogen-bond donor of the secondary amino group, or an adverse steric consequence resulting from the alkylation (Table 2). In contrast, alkylation of the furfuryl example **7i** resulted in a moderate decrease in activity for the methylated analog **13c** and maintenance of activity for the benzylated analog **13d**. In addition, **13d** exhibited no binding activity at the A_1 receptor at concentrations up to 10 μM. The unexpected A_{2A} activity of **13d** likely stems from the presence of the furfuryl group



Scheme 4. Reagents and conditions: (a) alkyl halide (R–X), polystyrene-bound BEMP, CH_3CN, 25 °C.

Table 2
Tertiary 2-amino-5-benzoyl-4-(2-furyl)thiazole A_{2A} antagonists



13

Compound	R^1	R	hA_{2A} binding $K_i \pm SD$[a] (nM)	hA_1 binding $K_i \pm SD$[b] (nM)	hA_1/hA_{2A} ratio
7a	Methyl	H	67 ± 3	3580 ± 260	54
13a	Methyl	Methyl	6818 ± 2370	>10,000	>1.5
13b	Methyl	Benzyl	2798 ± 1340	9460 ± 330	3.4
7i	2-Furfuryl	H	43 ± 3	1460 ± 310	35
13c	2-Furfuryl	Methyl	160 ± 72	3420 ± 210	21
13d	2-Furfuryl	Benzyl	88 ± 38	>10,000	>115
7l	2-Thiophenethyl	H	12 ± 2	>6700	>580
13e	2-Thiophenethyl	Methyl	8170 ± 5100	>10,000	>1.2
13f	2-Thiophenethyl	Benzyl	7140 ± 5710	>10,000	>1.4

[a] $K_i \pm SD$ determined by competition binding of [^{3}H]SCH-58261.[28]
[b] $K_i \pm SD$ determined by competition binding of [^{3}H]DPCPX.[29]

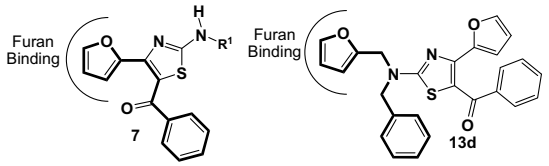


Figure 4. Potential for alternative binding of **13d** versus **7**.

at R^1 and the resulting pseudo symmetry of the molecule. The presence of the furan heterocycle within the 2-amino substituent may allow the antagonist to adopt an alternative binding conformation whereby the furfuryl heterocycle binds in the site adopted by the 4-furan group of the other analogs (Fig. 4).

In summary, potent small-molecule antagonists of the A_{2A} receptor displaying a good selectivity versus the A_1 receptor have been identified. Specifically, **7l** is an A_{2A} antagonist with a K_i of 12 nM and displays >500-fold binding selectivity versus A_1. In addition, **13d** shows maintenance of good A_{2A} binding activity (K_i < 100 nM) with no associated A_1 activity up to concentrations of 10 μM. The results presented provide a low molecular weight template for further development of an A_{2A} antagonist.

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28. *A_{2A} binding assay:* Membranes from HEK-293 cells expressing recombinant human A_{2A} receptor (0.04 mg/mL), yttrium oxide wheat germ-agglutinin-coated SPA beads (4 mg/mL), 0.01 mg/mL adenosine deaminase and 2 nM [^{3}H]SCH58261 were incubated with test compounds (1% DMSO final) in 1× PBS + 10 mM $MgCl_2$ overnight following centrifugation at 1000 rpm for 2 min. Signal was detected using the Viewlux CCD Imager. Assays were performed in duplicate and compounds were tested at least two times. The data were fit to a one-site competition binding model for IC_{50} determination using the program GraphPad Prism (GraphPad Software, Inc., San Diego, CA) and K_i values were calculated using the Cheng–Prusoff equation.[30]
29. *A_1 binding assay:* Membranes from CHO-K1 cells expressing recombinant human A_1 receptor (0.04 mg/mL), wheat germ-agglutinin-coated SPA beads (2 mg/mL), 0.01 mg/mL adenosine deaminase and 2 nM [^{3}H]DPCPX were incubated with test compounds (1% DMSO final) in 1× PBS + 10 mM $MgCl_2$. for 2 h following centrifugation at 1000 rpm for 1 min. Signal was detected using the Microbeta Trilux. Assays were performed in duplicate and compounds were tested at least two times. The data were fit to a one-site competition binding model for IC_{50} determination using the program GraphPad Prism (GraphPad Software, Inc., San Diego, CA) and K_i values were calculated using the Cheng–Prusoff equation.[30]
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