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## Synthesis of 2-amino-5-benzoyl-4-(2-furyl)thiazoles as adenosine A<sub>2A</sub> 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<sub>2A</sub> receptor antagonists from a small-molecule combinatorial library using a high-throughput radioligand-binding assay is described. Antagonists were further characterized in the A<sub>2A</sub> binding assay and an A<sub>1</sub> selectivity assay. Selected examples exhibited excellent affinity for A<sub>2A</sub> and good selectivity versus the A<sub>1</sub> receptor.

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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<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub>. A<sub>2A</sub> 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<sub>2A</sub> 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.<sup>1</sup> 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.<sup>2</sup> Restoration of motor activity is achieved by treatment with L-3,4-dihydroxyphenylalanine (L-Dopa).<sup>3,4</sup> 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<sub>2A</sub> receptor is primarily located in the striatum<sup>5</sup> and is co-expressed with the dopamine D<sub>2</sub> receptor<sup>6,7</sup> support a role for A<sub>2A</sub> in motor activity. Stimulation of the A<sub>2A</sub> receptor has been found to induce sedation and catalepsy, and inhibits the motor-stimulating effects of dopamine receptor agonists.<sup>8</sup> A<sub>2A</sub> antagonists synergize with D<sub>2</sub> agonists to

stimulate locomotor activity.<sup>9</sup> The effects of A<sub>2A</sub> antagonists have also been reported to afford neuroprotection in animal models of Parkinson's disease.<sup>10,11</sup> Studies with A<sub>2A</sub> and/or D<sub>2</sub> knockout mice support these observations.<sup>12</sup>

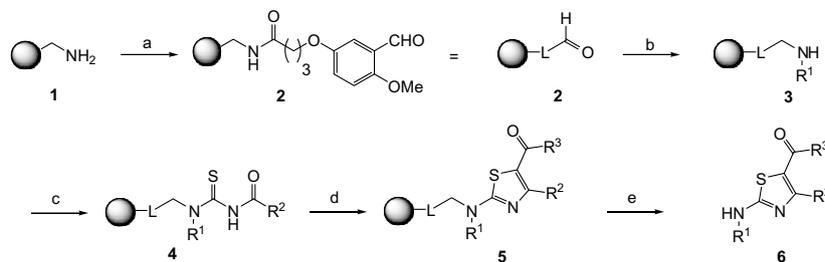
The in vivo efficacy of a variety of small-molecule A<sub>2A</sub> antagonists in animal models of Parkinson's disease has established the potential pharmaceutical utility for this class of compounds.<sup>13–22</sup> The A<sub>2A</sub> antagonists Istradefylline (KW-6002)<sup>17</sup> and Privadenant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

High-throughput screening of an extensive set of ECLiPS<sup>TM</sup> (Encoded Combinatorial Libraries on Polymeric Support)<sup>23</sup> libraries (90 Libraries, >4 million compounds) employing an A<sub>2A</sub> 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<sub>2A</sub> 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).<sup>24</sup> Generation of a resin-bound secondary amine **3** was achieved by reductive alkylation of a series of primary amines (R<sup>1</sup>NH<sub>2</sub>) 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<sub>2</sub>Cl<sub>2</sub>, DMF, 25 °C; (b) R<sup>1</sup>NH<sub>2</sub>, Na(OAc)<sub>3</sub>BH, 1,2-dichloroethane, 25 °C; (c) R<sup>2</sup>C(O)NCS, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C; (d) R<sup>2</sup>C(O)CH<sub>2</sub>Br, AcOH, DMF, 25 °C; (e) TFA, CH<sub>3</sub>CN, 25 °C.

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

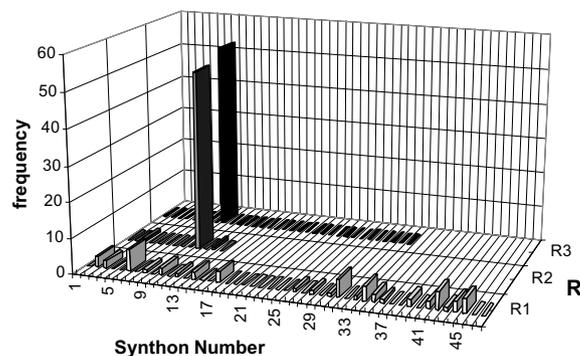
A<sub>2A</sub> active compounds identified from screening the 2-aminothiazole library exhibited conserved structural features at the R<sup>2</sup> and R<sup>3</sup> positions, but were tolerant of multiple substituents and functionalities at the R<sup>1</sup> position. The frequency of the synthons in the active compounds at the three different diversity positions (R<sup>1</sup>–R<sup>3</sup>) is shown in Figure 2. Of the possible 47 components incorporated at the R<sup>1</sup> position, 22 of the R<sup>1</sup> substituents were present in the active compound set. In contrast, only one of the possible 12 components at the R<sup>2</sup> position (R<sup>2</sup> component #9 = 2-furanyl) and one of the possible 32 components at the R<sup>3</sup> position (R<sup>3</sup> 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<sub>2A</sub> activity with a K<sub>i</sub> 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<sub>2A</sub> receptor and G<sub>qi</sub> protein.

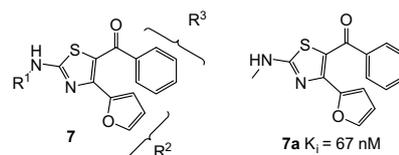
To facilitate further exploration of the role of the R<sup>1</sup> substituent, a solution-phase synthesis was devised to generate analogs. A similar synthetic strategy to that used on solid phase was employed, utilizing 2-furoyl isothiocyanate and α-bromoacetophenone to build the heterocycle. To ensure 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**.<sup>25</sup> Compounds of type **12** displayed no binding activity at the A<sub>2A</sub> receptor.

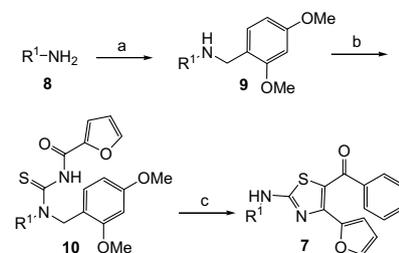
Examples of 2-amino-5-benzoyl-4-(2-furyl)thiazoles **7** synthesized utilizing the solution-phase synthesis (Scheme 2) and



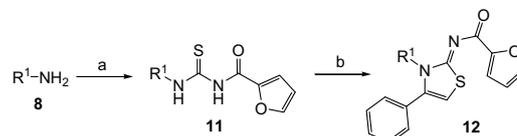
**Figure 2.** Synthon frequency analysis for the combinatorial variables identified in the active compound set from A<sub>2A</sub> screening of the 2-aminothiazole library.



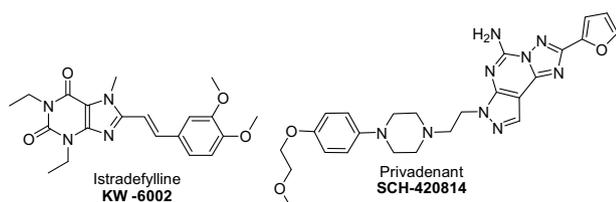
**Figure 3.** General template for 2-aminothiazole A<sub>2A</sub> antagonists.



**Scheme 2.** Reagents and conditions: (a) 2,4-dimethoxybenzaldehyde, Na(OAc)<sub>3</sub>BH, 1,2-dichloroethane, 25 °C; (b) 2-furoyl isothiocyanate, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C; (c) α-bromoacetophenone 5%AcOH/DMF, 90 °C.



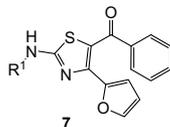
**Scheme 3.** Reagents and conditions: (a) 2-furoyl isothiocyanate, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C; (b) α-bromoacetophenone, 5%AcOH/DMF, 90 °C.



**Figure 1.** A<sub>2A</sub> antagonists KW-6002 and SCH-4020814.

incorporating a selection of substituents at the 2-position are detailed in Table 1. Activity at the human A<sub>2A</sub> receptor was examined

**Table 1**  
Secondary 2-amino-5-benzoyl-4-(2-furyl)thiazole A<sub>2A</sub> antagonists



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

<sup>a</sup> K<sub>i</sub> ± SD determined by competition binding of [<sup>3</sup>H]SCH-58261.<sup>28</sup>

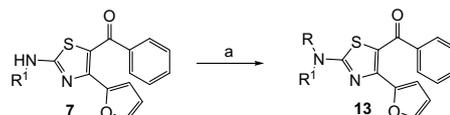
<sup>b</sup> K<sub>i</sub> ± SD determined by competition binding of [<sup>3</sup>H]DPCPX.<sup>29</sup>

in the same radioligand-binding assay as used for the initial screen. Activity at the human A<sub>1</sub> receptor was also measured using an appropriate radioligand-binding assay.<sup>29</sup> Selectivity versus the A<sub>1</sub> receptor is desirable based on potential adverse cardiovascular side-effects associated with A<sub>1</sub> antagonism.<sup>26</sup> Analogs display good overall A<sub>2A</sub> 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<sub>i</sub> values < 100 nM. Aliphatic examples include the methyl (**7a**) and isobutyl (**7b**) analogs, displaying K<sub>i</sub> 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<sub>i</sub> value of 12 nM. The inclusion of more polar functionality such as ether-based components (**7c, d**) or *N*-acetylene (**7e**) and *N*-acetyl-piperidine-4-yl-based (**7f**) amino substituents result in less potent compounds. Activity at the A<sub>1</sub> receptor was consistently in the micromolar range, providing a range of selectivity ratios when compared with A<sub>2A</sub> 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<sub>1</sub> receptor.

Three analogs (**7a, 7i, and 7l**) incorporating methyl, furfuryl and 2-thiophenethyl at the 2-amino position, respectively, were

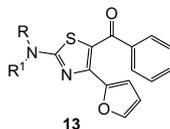
selected to examine the effect of alkylation of the secondary amino group of the 2-amino thiazole. Alkylation was conducted with either methyl iodide or benzyl bromide using polystyrene-bound *N*-phenyl-tris(dimethylamino)imino phosphorane (BEMP)<sup>27</sup> as a base to provide **13** (Scheme 4).

Alkylation of **7a** and **7l** resulted in dramatic losses in A<sub>2A</sub> 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<sub>1</sub> receptor at concentrations up to 10 μM. The unexpected A<sub>2A</sub> 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<sub>3</sub>CN, 25 °C.

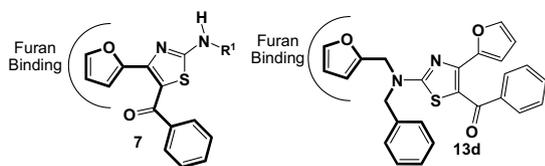
**Table 2**  
Tertiary 2-amino-5-benzoyl-4-(2-furyl)thiazole A<sub>2A</sub> antagonists



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

<sup>a</sup> K<sub>i</sub> ± SD determined by competition binding of [<sup>3</sup>H]SCH-58261.<sup>28</sup>

<sup>b</sup> K<sub>i</sub> ± SD determined by competition binding of [<sup>3</sup>H]DPCPX.<sup>29</sup>



**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, **71** 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  $\mu$ M. The results presented provide a low molecular weight template for further development of an  $A_{2A}$  antagonist.

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- $A_{2A}$  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  $1 \times$  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.<sup>30</sup>
- $A_1$  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  $1 \times$  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.<sup>30</sup>
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