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Synthesis of [1,2,4]triazolo[1,5-*a*]pyrazines as adenosine A_{2A} receptor antagonists

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Abstract—Potent and selective antagonists of the adenosine A_{2A} receptor often contain a nitrogen-rich fused-ring heterocyclic core. Replacement of the core with an isomeric ring system has previously been shown to improve target affinity, selectivity, and in vivo activity. This paper describes the preparation, by a novel route, of A_{2A} receptor antagonists containing the [1,2,4]triazolo[1,5-*a*]pyrazine nucleus, which is isomeric with the [1,2,4]triazolo[1,5-*c*]pyrimidine core of a series of known A_{2A} antagonists with in vivo activity in animal models of Parkinson's disease.

Membrane-spanning G-protein coupled receptors (GPCRs) figure prominently in the regulation of a wide array of signal transduction pathways, and are frequent and well-validated targets for therapeutic intervention. Adenosine receptors, a class of GPCRs, are integral components of signaling cascades that control a range of physiological and cellular responses.[1] Four different adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) have been biochemically and pharmacologically characterized and their regulation may offer a means to control immunological, cardiovascular, renal, or neurological responses for therapeutic benefit.[2] The identification of small-molecule agonists and antagonists of adenosine receptors has helped us to elucidate further the biochemical roles of the individual receptor subtypes and evaluate them as targets for drug discovery.[3]

The A_{2A} receptor is highly expressed in the nigrostriatum (basal ganglia) where it is co-localized with dopamine D2 receptors on striatopallidal output neurons.[4] Several pharmacological studies suggest that A_{2A} receptor antagonists have potential for use, in combination with existing therapies,[5] in the treatment of Parkinson's disease (PD) and may also exhibit neuroprotective effects.[6]

The locomotor deficits that are characteristic of PD, such as bradykinesias, tremor, and rigidity, have their origins in the progressive destruction of dopamine (DA)-producing neurons in the nigrostriatum. During the early stages of the disease, symptomatic relief may be provided by replenishing DA through administration of L-dopa.[7] However, the effectiveness of L-dopa is gradually diminished by the continual depletion of DA neurons, which results in the eventual manifestation of motor and psychological side-effects.[8]

Due, in part, to its interaction with the D2 receptor, antagonism of the A_{2A} receptor is regarded as the means to attenuate the motor fluctuations associated with decreased responsiveness to L-dopa.[9] In an early clinical demonstration of this effect, administration of low doses of theophylline led to a significant improvement in symptoms of patients with PD.[10] More recently, the A_{2A}-selective xanthine derivative KW-6002 (**1**) has been found to reverse motor disability in MPTP-lesioned primates when combined with L-dopa. KW-6002 is currently under clinical evaluation as an antiparkinsonian agent[11] (see Fig. 1).

Non-xanthine antagonists of the A_{2A} receptor often take the form of nitrogen-containing fused bicyclic or tricyclic systems that lack the agonism-conferring ribose moiety of adenosine but feature hydrophobic substituents that

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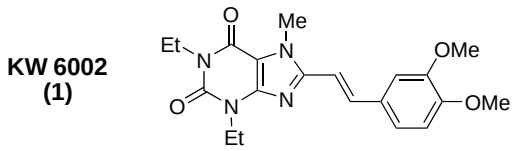


Figure 1. Xanthine-derived A_{2A} receptor antagonist (KW-6002).

impart selectivity.[12] Compounds of this type, such as SCH 58261 (**2**, A_{2a} $K_i = 2.3$ nM) and ZM241385 (**3**, A_{2a} $K_i = 0.3$ nM) and their homologs, are among the most selective A_{2A} antagonists prepared to date.[13] Despite their promising properties, however, the clinical application of **2** and **3** and their derivatives has been curtailed by their poor bioavailability (see Fig. 2).

As part of our ongoing interest in the design and synthesis of adenosine receptor antagonists, we undertook a program to develop selective A_{2A} antagonists for treatment of PD.[14] We have recently demonstrated that the nature of the polynitrogen heterocyclic core can dramatically influence A_{2A} receptor potency and selectivity, and compounds having a general structure **4** (Fig. 3) exhibited good oral activity in an animal model of PD.[15] Other researchers in the field have reported that the isomeric 5- and 8-amino[1,2,4]triazolo[1,5-*a*]pyridines act as potent A_{2A} antagonists when functionalized at the 6- and 7-positions, respectively.[16]

Based on these observations, the isomeric triazolo[1,5-*a*]pyrazine nucleus (**5**), in which the critical hydrogen bond donor and acceptor groups of **2**, **3**, and **4** are preserved, was pursued as a template from which to construct a series of A_{2A} antagonists.

A retrosynthetic strategy (Fig. 4) was envisaged in which a series of substituted derivatives (**5**) could be obtained from 2-aryl substituted bromides (**6**) by employing a commercially available 2-amino-3,5-dibromopyrazine (**7**).

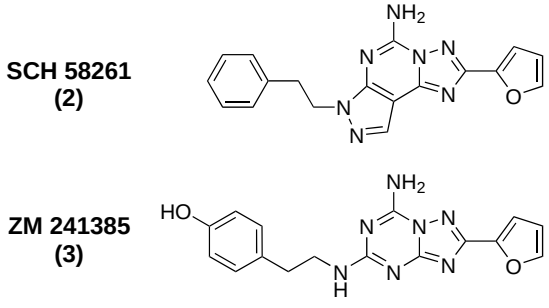


Figure 2. Selective non-xanthine A_{2A} receptor antagonists.

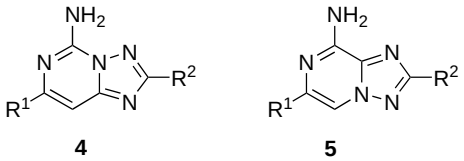


Figure 3. [1,2,4]Triazolo[1,5-*c*]pyrimidines (**4**) and isomeric [1,2,4]-triazolo[1,5-*a*]pyrazines (**5**).

Although the requisite bromides (**6**) can be obtained by *N*-amination of **7** with mesitylenesulfonyl hydroxylamine ($MtsONH_2$), followed by cyclo-condensation with the appropriate aldehyde,[17] we desired a route that did not rely on the use of thermally labile aminating reagents[18] (see Fig. 5).

The synthesis of fused triazoles via oxidative cyclization of *N*-2-pyrazinylacetamide, obtained by treatment of 2-aminopyrazine with $AlCl_3$ in acetonitrile,[19] has been reported.[20] In a similar fashion, treatment of a mixture of **7** and furonitrile in toluene with $AlCl_3$ afforded amidine **8a**. Oxidative cyclization of the crude preparation with $Pb(OAc)_4$ provided **9a** in 40% isolated yield. In the course of determining the scope and limitations of the new method, it was observed that the conditions employed in the conversion of **7**–**8** were ineffective when applied to other nitriles (Fig. 6).

Changing the solvent from toluene to 1,2-dichloroethane resulted in a more efficient conversion in the case

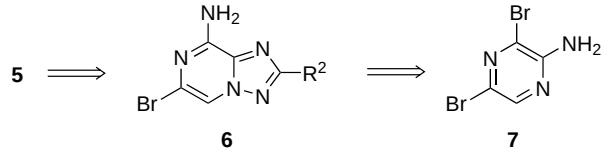


Figure 4. Retrosynthetic approach to [1,2,4]triazolo[1,5-*a*]pyrazines.

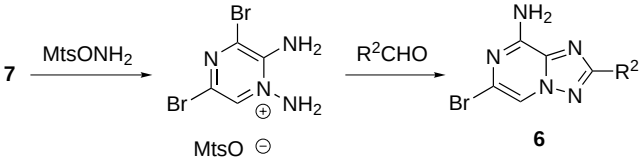
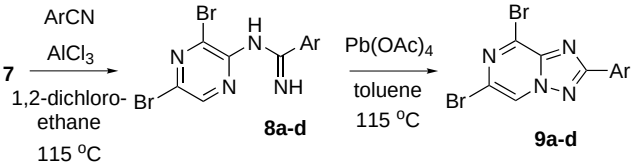


Figure 5. Synthetic route to [1,2,4]triazolo-[1,5-*a*]pyrazines.



Ar	8 (% yield)	9 (% yield)
2-furanyl	**8a (70)**	**9a (40)**
2-thienyl	**8b (68)**	**9b (45)**
phenyl	**8c (26)**	**9c (nd)**
3-fluorophenyl	**8d (30)**	**9d (46)**

Figure 6. Synthesis of [1,2,4]triazolo[1,5-*a*]pyrazines by oxidative amidine cyclization.

of the original example (**8a**) and enabled the preparation of several other derivatives (**8b–d**) in yields ranging from 26% to 70%.[21]

The effect of $AlCl_3$ on amidine formation was also studied and in the case of **8b** it was found that one equivalent of the Lewis acid was optimal; the use of 0.5 or two equivalents caused a significant reduction in yield. The resulting amidines (**8a**,**b**,**d**) were then subjected to oxidative cyclization to afford the desired triazolo[1,5-*a*]pyrazines (**9a**,**b**,**d**) in yields ranging from 40% to 46%.[22]

We employed 2-furyl derivative **9a** as a starting material for our initial series of compounds to allow a direct comparison of the receptor binding data between the new series and classes of compounds in which the furan ring has been found to confer A_{2A} receptor affinity and selectivity (Fig. 7).

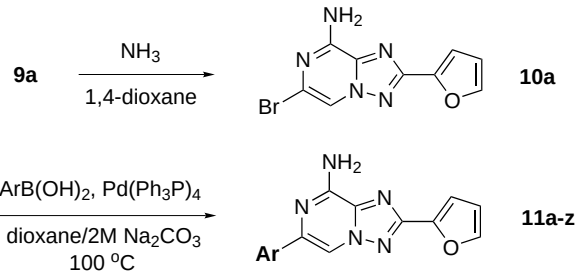


Figure 7. Synthesis of [1,2,4]triazolo[1,5-*a*]pyrazines **11a–z** by oxidative amidine cyclization.

Table 1. A_{2A} receptor binding affinities of Suzuki adducts **11a–z**, as determined in a radioligand binding assay[a]

Compound	Ar	A_{2A} (rat): K_i (nM)
11a	*m*-*i*Pr-Ph	680
11b	*m*-H_2N-Ph	630
11c	*p*-H_2N-Ph	>1000
11d	*m*-AcNH-Ph	160
11e	*m*-Ac-Ph	75
11f	*m*-NO_2-Ph	460
11g	*p*-NO_2-Ph	>1000
11h	*m*-Me_2N-Ph	140
11i	*p*-Me_2N-Ph	>1000
11j	*m*-EtO_2C-Ph	370
11k	*p*-EtO_2C-Ph	>1000
11l	*m*-CN-Ph	160
11m	*p*-CN-Ph	>1000
11n	*m*-F_3C-Ph	220
11o	*p*-F_3C-Ph	>1000
11p	*m*-$HOCH_2$-Ph	470
11q	*p*-$HOCH_2$-Ph	>1000
11r	*m*-MeO-Ph	270
11s	*m*-$MeSO_2$-Ph	110
11t	*m*-H_2NCO-Ph	110
11u	*m*-HO_2C-Ph	>1000
11v	3-Pyridyl	460
11w	2-Furanyl	400
11x	1-Naphthyl	420
11y	8-Quinolyl	250
11z	5-Pyrimidyl	360

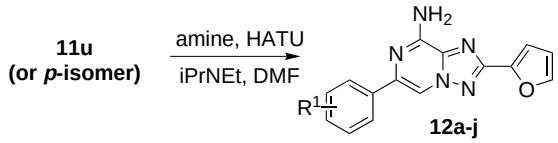
[a] A_{2A} receptor enriched membranes were prepared from rat brain homogenates and employed in binding assays using [^{3}H]ZM-241385 as radioligand and SCH-58261 (A_{2A} K_i = 37 nM) as a control. K_i values were calculated from binding curves generated in a single experiment from the mean of three determinations per concentration, with variation in individual values of <15%.

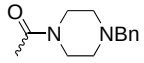
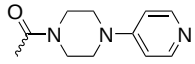
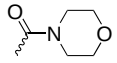
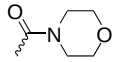
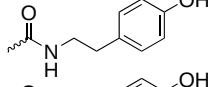
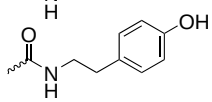
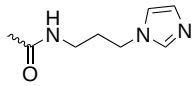
Suzuki coupling of **10a**, obtained by treatment of **9a** with NH_3/dioxane, with a series of commercially available arylboronic acids allowed the rapid assembly of a series of biaryls (**11a–z**), which were evaluated for their ability to bind to rat A_{2A} receptor in a radioligand binding assay (Table 1).

In this collection of compounds, those containing *m*-substituted phenyl rings exhibited the highest A_{2A} activity, with K_i values for the best compounds in the range of 100–500 nM. Bi- and heterocyclic derivatives (**11v–z**) exhibited comparable potency.

Based on the promising in vitro activity of members of our initial adducts, we chose amide **11t** as a starting point for further elaboration of this series. A collection of amides (Table 2, **12a–j**) was prepared from acid **11u** (and the corresponding *p*-isomer, not shown) to

Table 2. Synthesis of amides **12a–j** and their affinities toward rat A_{2A} and A_1 receptors, as measured in a radioligand binding assay[a]



Compound	R^1	*m* or *p*	A_{2A} (rat) K_i (nM)	A_1 (rat) K_i (nM)
12a	CONH-*i*Pr	*m*	140	290
12b	$CONMe_2$	*m*	70	120
12c	$CONEt_2$	*m*	1	41
12d		*m*	680	>250
12e		*m*	730	3
12f		*m*	240	<250
12g		*p*	19	1800
12h		*m*	180	>250
12i		*p*	830	36
12j		*p*	330	>250

[a] A_{2A} receptor binding measured as given in Table 1. A_1 receptor containing membranes were prepared from rat cerebral cortex. [^{3}H]DPCPX was used as radioligand and A_1 K_i values were calculated from binding curves generated in a single experiment from the mean of three determinations per concentration, with variation in individual values of <15%. SCH-58261 (2) used as a control (A_{2A} K_i = 37 nM, A_1 K_i = 390 nM).

determine the effect of further substitution on selectivity and potency.

The in vitro binding data (Table 2) indicated that simple alkyl substitution, as with *N*,*N*-diethylamide **12c** (A_{2A} K_i = 1 nM), could enhance the A_{2A} affinity but concomitantly conferred significant potency toward the A_1 receptor (A_1 K_i = 40 nM), relative to monosubstituted compounds **12a**,**b**.

We subsequently prepared a series of compounds lacking the benzene ring to determine if the amide substituents in Table 2 conferred similar in vitro activity when directly linked to the heterocyclic core. Palladium-mediated carbonylation of bromide **10a** was employed to prepare ester **13** (Fig. 8). Hydrolysis of **13** to acid **14**, followed by amine coupling, afforded the desired amides (**15a**–**i**).

The in vitro binding data for this class of compounds offer a clearer picture of the distinct SAR at the A_{2A} and A_1 receptors (Table 3). Secondary amides having an aromatic group at the terminus of an alkyl chain (**15b**–**f**) exhibit better potency than tertiary amides derived from cyclic amines (**15g**–**i**).

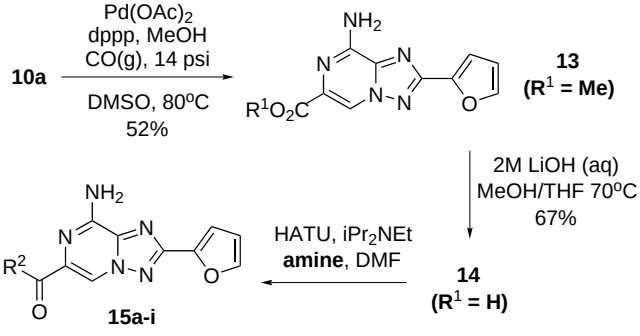
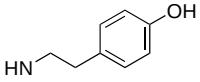
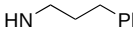
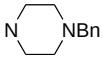
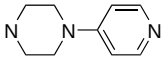


Figure 8. Synthesis of amides **15a**–**i** by Pd-catalyzed carbonylation.

Table 3. Affinities of **15a**–**i** toward rat A_{2A} and A_1 receptors, as measured in radioligand binding assays[a]

Compound	R^2	A_{2A} (rat) K_i (nM)	A_1 (rat) K_i (nM)
15a	HNBu	1	120
15b		2	14
15d		27	160
15e		1	320
15f		28	240
15g		>250	>250
15h		>250	>250
15I		>250	>250

[a] Refer to Tables 1 and 2 for details regarding rat membrane based radioligand binding assays.

Compound **15e**, which incorporates the amino-ethylphenol unit from ZM241385 (**3**), exhibited high affinity (A_{2A} K_i = 1 nM) and impressive selectivity (300-fold) against the A_1 receptor.

In summary, we have described the synthesis of a series of triazolo[1,5-*a*]pyrazine-derived A_{2A} receptor antagonists by a novel route. The compounds obtained through this route represent potential leads for the development of analogs with good in vitro potency and selectivity. Further details of our efforts to optimize these properties through side-chain modifications will be made in due course.

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21. Representative procedure for the synthesis of amidine derivatives (**8**): *N*-(3,5-dibromo-pyrazin-2-yl)-thiophene-2-carboxamidine (**8b**). A stirred solution of 3,5-dibromo-pyrazin-2-ylamine (**7**, 500 mg, 1.98 mmol), thiophene-2-carbonitrile (260 μL, 2.04 mmol), and $AlCl_3$ (232 mg, 1.74 mmol) in dichloroethane (5 ml) was heated at 115 °C overnight. The mixture was allowed to cool to rt and diluted with water (5 mL). After 30 min, the resulting precipitate was collected and purified by column chromatography (SiO_2, THF as eluent) to afford 488 mg (68%) of **8b** as a yellow solid, mp 250 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6); δ 7.22 (t, J = 3.9 Hz, 1H), 7.83 (dd, J = 4.8 Hz, 1H), 7.99 (d, J = 4.1 Hz, 1H), 8.49 (s, 1H), 8.88 (br s, 1H); MS: m/z = 361 (MH+).
22. Representative procedure for the synthesis of dibromo-[1,2,4]triazolo[1,5-*a*]pyrazines (**9**): 6,8-dibromo-2-furan-2-yl-[1,2,4]triazolo[1,5-*a*]pyrazine (**9a**). A mixture of compound **8a** (47 g, 0.14 mol), $Pb(OAc)_4$ (95% purity, 160 g, 0.34 mol), and toluene (940 ml) was heated at reflux for 2 h. The reaction mixture was allowed to cool to rt and concentrated in vacuo. The resulting residue was purified by flash chromatography on silica using EtOAc in hexanes (1:5 to 1:3) as eluent to afford **9a** as a yellow solid (19.2 g, 40%), mp 250 °C (dec). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.60 (dd, 1H), 7.33 (d, 1H), 7.62 (d, 1H), 8.63 (s, 1H); MS: m/z = 343 (MH+).