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Potent, selective, and orally active adenosine A_{2A} receptor antagonists: Arylpiperazine derivatives of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines

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Abstract—Antagonism of the adenosine A_{2A} receptor offers great promise in the treatment of Parkinson's disease. Employing the known pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine A_{2A} antagonist SCH 58261 as a starting point, we identified the potent and selective (vs. A1) antagonist 11 h, orally active in the rat haloperidol-induced catalepsy model. We further optimized this lead to the methoxyethoxyethyl ether **12a** (SCH 420814), which shows broad selectivity, good pharmacokinetic properties, and excellent in vivo activity.

Parkinson's disease (PD) is a very serious neurological disorder, and current methods of treatment fail to achieve long-term control. Since adenosine A_{2A} receptor antagonists have been shown to restore the deficits caused by degeneration of the striatonigral dopamine system, which is compromised by the loss of striatal neurons in this disease, A_{2A} antagonism affords a possible treatment for PD.[1] The A_{2A} antagonist KW-6002 (istradefylline) was shown to be effective in animal models of PD, and recent clinical studies demonstrated efficacy in alleviation of symptoms of the disease.[2]

Adenosine A_{2A} receptor antagonists of several structural types have been described. The earliest described are xanthines. Subsequently, non-xanthines CGS 15943[3] and CP 66,713[4] were reported. These are non-selective for the A_{2A} receptor relative to the A_1 receptor. KW-6002 is a xanthine with moderate receptor selectivity.[2] Several other bicyclic systems have been described with good potency and selectivity.[5] Beginning in 1994, reports described a series of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines, some with good selectivity.[6] The parent compound of this tricyclic series is SCH 58261,[6c] a high-affinity A_{2A} receptor antagonist (K_i = 2 nM), which shows potent in vivo activity in animal models of PD. However, SCH 58261 is only moderately selective for A_{2A} receptors over A_1 receptors, possesses very poor solubility, and fails to show activity upon oral administration. We report the discovery of high-affinity A_{2A} receptor antagonists with improved selectivity and potent oral activity through modification of the phenethyl side chain of SCH 58261.[7]

For preparation of compounds **1** (Scheme 1) we initially adopted the previously reported method,[6b] targeting key intermediate **3** that could be alkylated at the 7-position. However, this route behaved poorly in our hands, with **2** producing primarily **4**, rather than the required **3**. We developed an alternative synthesis of **3** (Scheme 2) that begins with commercially available **5**.[8] Dehydrative cyclization of **7** with N,O-bis(trimethylsilyl)acetamide (BSA) at reflux proceeded with concomitant Dimroth rearrangement[9] to afford the desired product **3**.

Keywords: Adenosine A_{2A}; Parkinson's; Catalepsy; Pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine.
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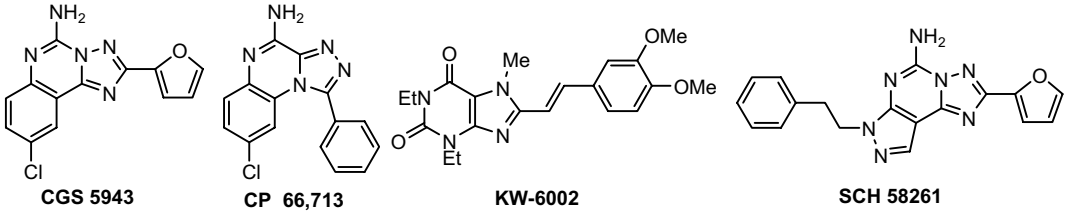
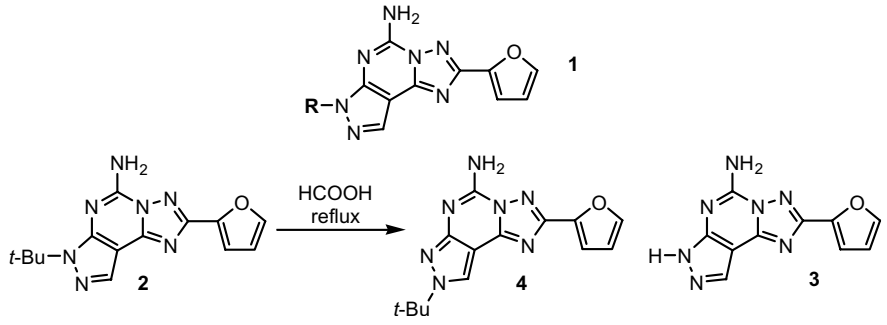
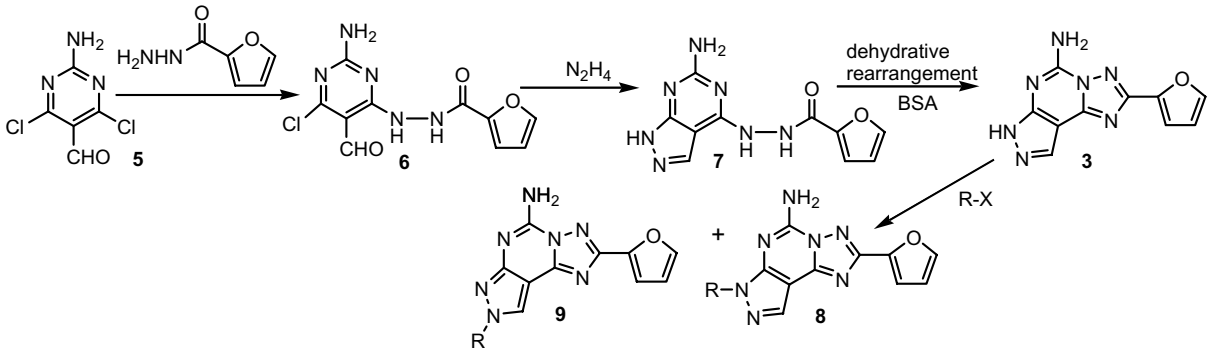


SCH 58261 prepared by alkylation of **3** from the newly developed route was identical by ^{1}H and ^{13}C NMR spectroscopy to an authentic sample prepared by the published route,[6b] confirming that the required Dimroth rearrangement had occurred. In addition, the structure of SCH 58261 was confirmed independently by X-ray crystallography,[10] rigorously establishing the structural assignments.

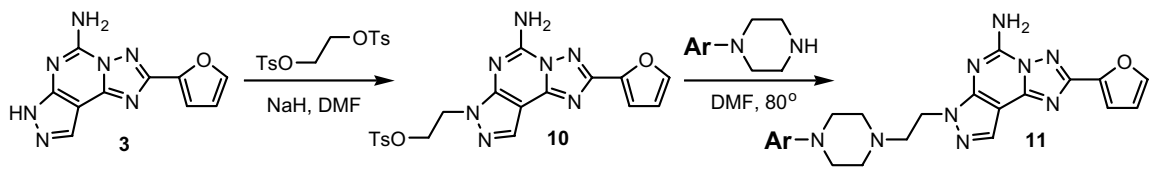
A variety of alkylated products were prepared from **3**. We found that arylpiperazine derivatives of type **11** were of particular interest. These were produced by alkylation of **3** (Scheme 3) with the bis-tosylate derived from ethylene glycol, which afforded the desired tosylate **10** as the major product, along with minor amounts of the N8 regioisomer. Amination of **10** with *N*-arylpiperazines and related amines proved very efficient and allowed



Scheme 1. Desired targets and a critical step in the previously reported synthetic route to pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines.



Scheme 2. Newly developed synthetic route to pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines.



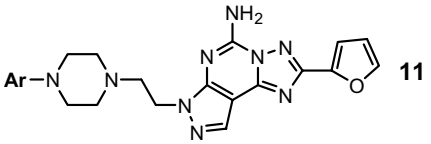
Scheme 3. Convergent synthetic route from intermediate **10**.

the separation of N7- and N8-alkylated materials to be carried out only once, at the stage of precursor **10**. Requisite *N*-arylpiperazines were typically prepared by Buchwald–Hartwig amination[11] of aryl bromides with piperazine.

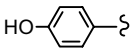
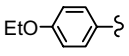
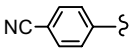
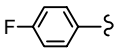
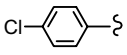
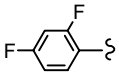
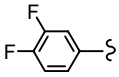
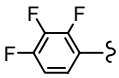
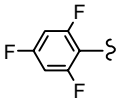
The majority of arylpiperazine derivatives that were prepared afforded high A_{2A} receptor binding affinity, with high selectivity over A_1 receptors[12] (Table 1). Affinity was assessed using radioligand competition assays as previously described;[12] SEM values were equal to or below 15% of derived K_i values. Among these, the first to demonstrate significant oral activity in the rat haloperidol-induced catalepsy assay[13] was the unsubstituted phenylpiperazine **11a**. Potent oral activity was observed at 1.0 mg/kg at 1 h post-dose, but not at 4 h. This is consistent with the observed metabolic conversion of **11a** to the *p*-hydroxyphenyl derivative **11b**, which was orally inactive at the 1.0 mg/kg dose. In an effort to identify analogs with improved duration of activity, a number of phenyl-substituted analogs **11c**–**11k** that offered the potential to block metabolism were profiled in the catalepsy assay. Of these, the 2,4-difluorophenyl derivative **11h** (SCH 412348) and the 2,4,6-trifluorophenyl derivative **11k** displayed potent anti-cataleptic activity at a dose of 1.0 mg/kg, and the activity was sustained at the 4 h time point.

While SCH 412348 and **11k** displayed potent oral activity, their solubility remained poor, and subsequent efforts were directed toward improving solubility by further modifying the phenylpiperazine substitution. Accordingly, analogs incorporating ether-linked substituents were prepared, exemplified by **12a**–**12g** (Table 2).

Table 1. Activity of arylpiperazine compounds

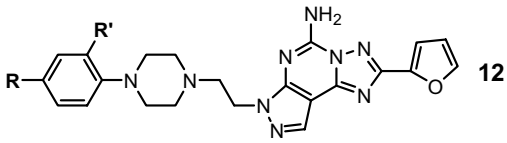


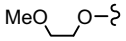
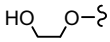
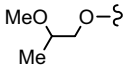
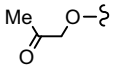
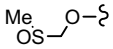
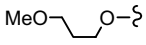
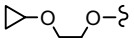
11

Compound	Ar	A_{2A} K_i[a], nM	A_1/A_{2A}[a]	Rat catalepsy, % inhibition at 1 mg/kg[b]	
				1 h	4 h
11a		1.6	343	52	0
11b		1.3	186	0	
11c		1.4	848	27	
11d		2.1	571	0	
11e		1.0	101	8	
11f		0.5	1348	11	
11g		0.6	894	20	25
11h		0.6	>1600	75	80
11i		1.4	169	45	
11j		0.6	1498	65	40
11k		1.1	319	65	75

[a] Average of duplicate determinations, human receptors.
[b] Average for $n = 3$. Maximum reduction attainable is 60–80%.

Table 2. 2-(Methoxyethoxy)phenyl and related substitution



Compound	R	R′	A_{2A} K_i[a], nM	A_1/A_{2A}[a]	Rat catalepsy, % inhibition at 1 mg/kg[b]	
					1 h	4 h
12a		H	1.1	1340	77	70
12b		F	0.4	1736	60	70
12c		H	0.6	1525	15	25
12d[c]		H	1.6	539	55	20
12e		H	0.8	427	15	10
12f[c]		H	2.2	523	30	5
12g		H	0.6	1158	8	2
12h		H	0.6	930	59	30

[a] See Table 1.
[b] See Table 1.
[c] Racemic compound.

In several cases these exhibited sub-nanomolar A_{2A} receptor binding affinity and retained excellent selectivity over A_1 receptors. Of particular interest were the methoxyethoxy derivatives **12a** and **12b**, which displayed robust oral anti-cataleptic activity that was maintained at 4 h, and **12a** (SCH 420814) was subjected to more extensive profiling.

SCH 420814 exhibits high affinity for both human and rat A_{2A} receptors, with K_i values of 1.1 and 2.5 nM, respectively. In addition, the compound is more than 1000-fold selective for human A_{2A} receptors over A_1,[14] A_{2B},[15] and A_3 receptors,[14] with K_i values at human A_1, A_{2B}, and A_3 receptors of >1000 nM, >1700 nM, and >1000 nM, respectively. In cell-based assays, SCH 420814 blocked adenylate cyclase activity stimulated by the A_{2A} agonist CGS 21680 with K_b values of 0.7 nM (rat) and 1.3 nM (human), confirming that it is an antagonist of A_{2A} receptors. The compound did not show significant binding against a panel of 59 unrelated receptors, enzymes, and ion channels.[16] In vivo, SCH 420814 dose-dependently reversed haloperidol-induced catalepsy in the rat with a MED of 0.3 mg/kg 1 h after oral administration (Fig. 1).

The solubility of SCH 420814 as the crystalline free base in water was 0.2 μM at native pH (5.1),[17] and 2 mM in 0.01 N HCl. Pharmacokinetic properties of SCH 420814 in the rat are shown in Table 3. In this species the compound demonstrated a relatively short half-life ($t_{1/2}$), moderate clearance (Cl), a high steady-state

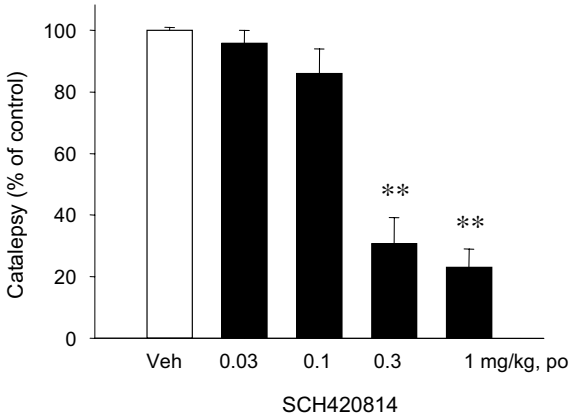


Figure 1. Dose–response of oral SCH 420814 in rat haloperidol-induced catalepsy. $^{**}P < 0.01$ versus vehicle, Dunnett's *t*-test.

Table 3. Rat pharmacokinetic properties of SCH 420814 HCl salt[a]

po dose (mg/kg)	iv dose (mg/kg)	C_{max} po (nM)	T_{max}, po (h)	AUC po (nM h)	$t_{1/2}$ iv (h)	Cl, iv (mL min^{-1} kg^{-1})	V_{ss}, iv (L kg^{-1})	F_{po} (%)
3	1	762	0.25	1560	2.1	37	2.6	57

[a] Mean values, $n = 3$ per route.

volume of distribution (V_{ss}), and a brain-to-plasma ratio of 1. When administered to rats as the hydrochloride salt, the compound was well absorbed, with a measured oral bioavailability of 57%. In several species, a major metabolite was the *O*-desmethyl compound.

In conclusion, novel, orally active phenylpiperazine adenosine A_{2A} receptor antagonists derived from SCH 58261 were identified, exemplified by SCH 412348. Optimization of the phenylpiperazine substitution resulted in identification of SCH 420814, which displays potent oral anti-cataleptic activity and favorable pharmacokinetic properties in rats. Further pharmacological characterization of SCH 412348 and SCH 420814 will be reported in due course.

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16. Includes adrenergic α_{1a}, α_{1b}, α_{2a}, α_{2b}, α_{2c}, β_1; NE uptake: AT1 and 2; bradykinin B2; CGRP; CCK1 and 2; CB1 and 2; C5a; CCR2, 3, 6, and 7; CXCR3; dopamine uptake; ET-A and B; FPR1; EGF; galanin and GALR2; glucagon; H3; IL-6; LTB4 and D4; melanocortin; motilin; muscarinic M1-M5; NK1-3; NPY1-5; prostaglandin EP1 and IP; 5-HT 1A, 2C, 6, and 7; progesterone; VIP; and V1 a.
17. A single measurement at pH 7.4 gave solubility below 10 ng/mL.