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2-(2-Furanyl)-7-phenyl[1,2,4]triazolo[1,5-*c*]pyrimidin-5-amine analogs as adenosine A_{2A} antagonists: The successful reduction of hERG activity. Part 2

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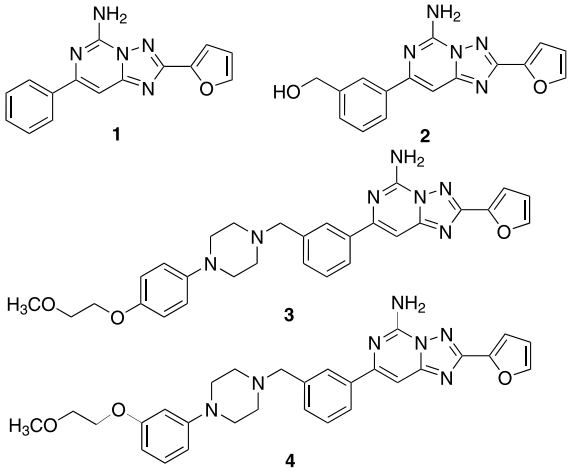
Abstract—The structure–activity relationship (SAR) exploration using 2-(2-furanyl)-7-phenyl[1,2,4]triazolo-[1,5-*c*]pyrimidin-5-amine (**1**) as a template led to the identification of a novel class of potent and selective adenosine A_{2A} receptor (AR) antagonists. However, these compounds were found to be associated with significant hERG activity. This report discusses the strategy and outcome of an expanded SAR focused on addressing the hERG liability. As a result, compounds **21** and **24** possess excellent in vitro profiles, highly promising in vivo profiles, and acceptable levels of hERG channel inhibition.

Our previous communication[1] discussing 2-(2-furanyl)-7-phenyl[1,2,4]triazolo[1,5-*c*]pyrimidin-5-amine analogs, reported several analogs (**2**–**4**) in this series which demonstrated high binding affinity for adenosine A_{2A} receptor (AR) and very good selectivity over A_1 AR. Several of these compounds displayed moderate to good anticataleptic activity (15–65% inhibition) when dosed orally at 3 mg/kg in a rat catalepsy assay. However, these analogs were found to be associated with the blockade of the hERG (human *Ether-a-go-go* Related Gene) channel. The blockade of hERG K^+ channels has been cited as a major factor in the drug-induced alteration of cardiac ventricular repolarization that is responsible for prolongation of the heart rate-corrected QT interval (QT_c). Since QT_c prolongation is believed to increase the risk of cardiac arrhythmia in patients and can lead to *torsades de points* or sudden death, it became critical to monitor these compounds for the inhibition of the hERG channel.[2]

As a result, several compounds from this series were screened for hERG activity using a standard rubidium efflux FLIPR assay at a concentration of 5 μg/mL. Compounds **3** and **4** exhibited hERG inhibition of 81% and 68%, respectively. However, compound **2** possessed acceptable hERG activity of 15% inhibition which indicates that basic nitrogen in the side chain may be responsible for the inhibition of the hERG channel. In addition, it has been reported that decreasing the pK_a of the basic amine results in lower hERG activity.[2]



Keywords: Adenosine receptor; Antagonist; Arylindenopyrimidines; hERG.
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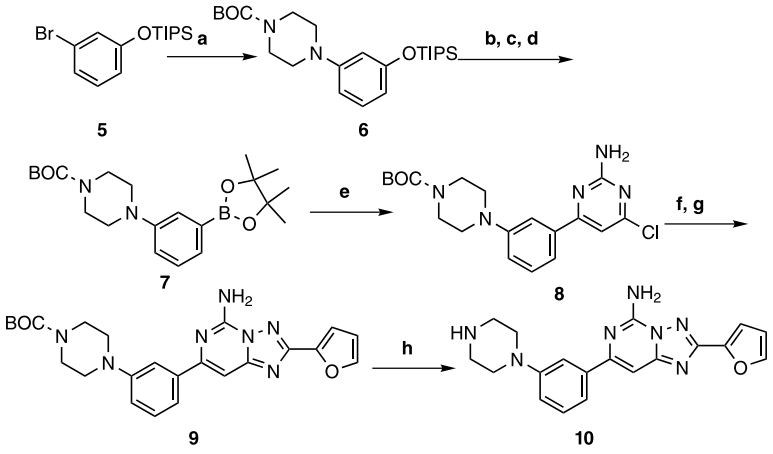
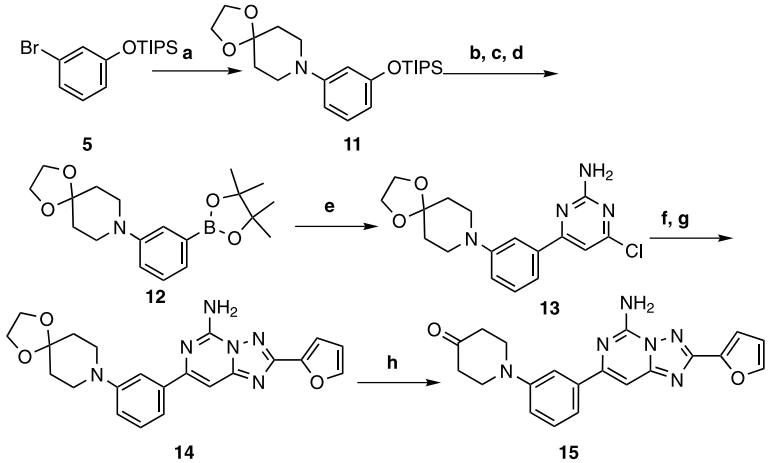
Moreover, in silico pharmacological models of the hERG channel–ligand interactions suggest that protonated amines participate in a favorable cation–π interaction with the Tyr652 residue of the hERG channel. These theories appear to support the observation that compounds in this series containing basic nitrogens exhibit higher hERG inhibitory activity than those without basic nitrogens. Consequently, SAR development was focused on reducing the basicity of the C-7 side chain.

Piperazines **10** and **16**–**20** were prepared according to the general procedure shown in Scheme 1. The palladium (Pd) catalyzed coupling of (3-bromo-phenoxy)-triisopropyl-silane (TIPS) (**5**) with 1-(^{t}butoxycarbonyl)-piperazine afforded **6**. Compound **6** was deprotected with tetrabutylammonium fluoride (TBAF), followed by conversion to the corresponding triflate and then conversion to pinacol boronic ester **7**. The palladium-catalyzed coupling between **7** and 2-amino-4,6-dichloropyrimidine produced **8**. Chloride **8** was reacted with 2-furoic hydrazide, followed by a dehydrative rearrangement[3] with *N*,*O*-bis(trimethylsilyl) acetamide (BSA) to provide **9**. Subsequent deprotection of **9** gave **10** as an advanced intermediate. Compounds **16**–**20** were prepared via subjecting **10** to various known reaction conditions to afford targets in a parallel fashion. Compounds **21** and **22** were synthesized by first, preparing the fully elaborated piperazines which were then coupled with **5**, followed by steps **b**–**g** as shown in Scheme 1. The piperidine analogs were prepared as shown in Scheme 2. The Pd-catalyzed coupling of **5** with 1,4-dioxa-8-azaspiro[4.5]decane produced **11**. Compound **11** was converted to its corresponding boronic ester **12** which was then converted to **14** via steps **e**–**g**. Deprotection of **14** provided **15** as an advanced intermediate. Compound **15** was converted to **23** and **24** under reductive amination conditions. All compounds reported herein gave satisfactory analytical results.[4]

The in vitro results of the A_{2A} AR binding assays[5] are expressed as inhibition constants (K_i, nM) and A_1/A_{2A}



Scheme 1. Reagents and conditions: (a) 1-(^{t}butoxycarbonyl)-piperazine, $Pd(OAc)_2$, $P(^tBu)_3$, NaO^tBu, toluene (80–100%); (b) TBAF, THF (90–100%); (c) Tf_2O, Et_3N, CH_2Cl_2 (80%); (d) Bis(pinacolato)diboron, $PdCl_2$(dppf), dppf, KOAc, dioxane (82%); (e) 2-amino-4,6-dichloropyrimidine, $Pd(PPh_3)_4$, K_2CO_3, CH_3CN/H_2O (50–70%); (f) 2-furoic hydrazide, BuOH; (g) BSA; (h) HCl, dioxane (73%).



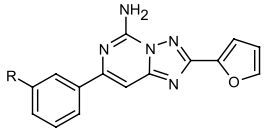
Scheme 2. Reagents and conditions: (a) 1,4-dioxa-8-azaspiro[4.5]decane, $Pd(OAc)_2$, $P(^tBu)_3$, NaO^tBu, toluene (83%); (b) TBAF, THF (89%); (c) Tf_2O, Et_3N, CH_2Cl_2 (85%); (d) Bis(pinacolato)diboron, $PdCl_2$(dppf), dppf, KOAc, dioxane (40%); (e) 2-amino-4,6-dichloropyrimidine, $Pd(PPh_3)_4$, K_2CO_3, CH_3CN/H_2O (82%); (f) 2-furoic hydrazide, BuOH; (g) BSA; (h) TFA, CH_2Cl_2 (80%).

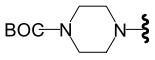
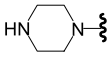
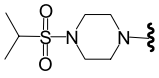
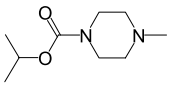
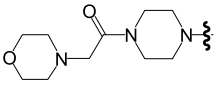
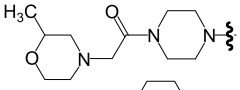
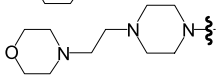
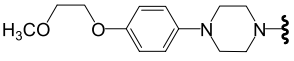
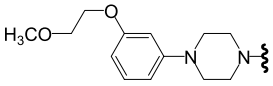
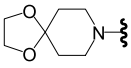
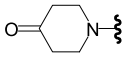
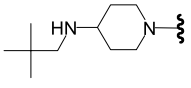
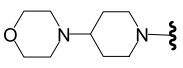
describes the selectivity over the A_1 AR. All assays were performed in duplicates and reported as mean values. These compounds were not tested against the other known adenosine receptor subtypes, A_3 and A_{2B}. The hERG activity was measured using a rubidium efflux FLIPR assay and is expressed as a percent inhibition at a concentration of 5 μg/mL.[6] The anti-cataleptic activity in the rat catalepsy assay is expressed in percent reduction of cataleptic effect relative to vehicle upon oral dosing at 3 mg/kg and determined at 1 and 4 h after the cataleptic effect was observed.

Results in Table 1 show that among the piperazine analogs, unsubstituted piperazine **10** displays the lowest selectivity over A_1 AR. Substitution of N-4 of **10** with electron withdrawing groups produced compounds **9** and **16–19** with subnanomolar binding affinities for A_{2A} AR while retaining high selectivity over A_1 AR. Methoxyethoxyphenyl substituted analog **21** was found to be more than 1000-fold selective over A_1 AR. The SAR of C-4 substituted piperidine analogs **14**, **15**, **23**, and **24** showed that a variety of substituents were tolerated at C-4 of the piperidine.

Derivatization of the basic nitrogen produced significant reduction of hERG channel inhibition in the piperazine series. This is clearly demonstrated by comparing the hERG activity of compound **3** with that of compound

Table 1. Receptor affinity, in vivo activity and hERG activity of C-7 arene analogs



Compound	R	A_{2A} K_i (nM)	A_1/A_{2A}	% Reduction of cataleptic activity (3 mg/kg) 1 h/4 h	% Inhibition of hERG (5 μg/mL)
9		0.5	745	34/43	−3
10		5.7	66	NT	NT
16		0.5	525	13/13	10
17		1.0	269	0/23	8
18		0.5	366	23/47	5
19		0.8	511	28/0	−3
20		1.3	229	23/32	13
21		1.0	1059	55/40	−4
22		2.3	154	19/46	3
14		0.4	470	45/20	8
15		1.3	133	NT	NT
23		18.0	122	10/12	9
24		0.8	116	36/59	1

21 (81% vs 13%, respectively). Slightly basic amines were also tolerated in the side chains of both piperidine **24** and piperazine **20**. The hERG activity is also reduced when an amine is sterically hindered as in compound **23**. From these limited results it is not clear if reduction of the hERG liability is due to reduced basicity or structural modifications. Further work is required to understand these results.

Having identified compounds with high-binding affinity for A_{2A} AR, several compounds from Table 1 were assessed for their pharmacokinetic profiles and anti-cataleptic activity in the rat at an oral dose of 3.0 mg/kg. Among these compounds, **21** and **24** were found to have acceptable pharmacokinetic profiles (AUC = 160 and 310 ng h/mL, respectively). Both compounds displayed potent oral anti-cataleptic activity at 1 and 4 h after dosing.

Encouraged by this result, it was decided to investigate compounds **21** and **24** in a secondary rodent model. In this assay, hypolocomotion is induced by administration of a 1 mg/kg dose of the A_{2A} agonist, CGS-21680. The antagonists are pretreated for 4 h at varying doses. Compound **24** displayed activity at 3 mg/kg (100% inhibition) and 1 mg/kg (53% inhibition) whereas **21** displayed moderate activity (61% and 59%, respectively). The reversal of CGS-21680 agonist activity affected by compounds **21** and **24** is mediated through A_{2A} AR.

In summary, we have discovered a series of A_{2A} AR antagonists which displayed excellent affinity, selectivity over the A_1 AR, and oral anti-cataleptic activity. Through modification of the side chain, the hERG activity was significantly reduced. It was also demonstrated that selected compounds **21** and **24**, upon oral administration, showed potent activity in a rodent cataleptic assay.

Catalepsy procedure: The rodent model for Parkinson's Disease is the rat catalepsy assay where the dopamine D_2 receptor antagonist, haloperidol (1 mg/kg, subcutaneous) is administered. After 30 min, rats were placed facing upward on a wire screen inclined at 60°. The time taken for the rat to move one limb was measured, with a cut-off time of 120 s. Rats showing >75 s of immobility (catalepsy) were used for subsequent A_{2A} antagonist studies. Test compounds were administered orally and catalepsy was retested at 1 and 4 h after administration. Male CD rats (Charles River Laboratories) weighing 200–240 g were used for all studies. Upon arrival at our holding facility, rats were housed four per cage, with food and water available ad libitum. Rats were maintained on a 12 h light/dark cycle (light on 07:00; lights off 19:00). All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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3. (a) Dimroth, O. *Ann.* **1909**, *364*, 183; (b) Dimroth, O. *Ann.* **1927**, *459*, 39.
4. Compound **21**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.63 (m, 2H), 7.42 (m, 3H), 7.25 (dd, 1H), 7.07 (dd, 1H), 6.90 (m, 4H), 6.59 (dd, 1H), 6.05 (br s, 2H), 4.09 (t, 2H), 3.74 (t, 2H), 3.45 (s, 3H), 3.43 (m, 4H), 3.26 (m, 4H); Compound **24**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.62 (s, 1H), 7.58 (s, 1H), 7.39 (s, 2H), 7.34 (t, 1H), 7.24 (d, 1H), 7.02 (d, 1H), 6.59 (q, 1H), 6.15 (br s, 2H), 3.83 (d, 2H), 3.73 (m, 4H), 2.80 (t, 2H), 2.59 (m, 4H), 2.34 (m, 1H), 1.97 (m, 2H), 1.68 (m, 2H).
5. Adenosine A_{2A} and A_1 binding assays: [^{3}H]SCH-58261 and [^{3}H]DPCPX binding assays for adenosine A_{2A} and A_1 receptors, respectively, were performed as described previously[7].
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