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Discovery of potent and selective bicyclic A_{2B} adenosine receptor antagonists via bioisosteric amide replacement

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ABSTRACT

Several new potent and selective A_{2B} adenosine receptor antagonists have been prepared in which the aryl–amide moiety of the lead series, exemplified by **1a**, has been replaced by bioisosteric bicyclic moieties. Although the majority of compounds had generally improved microsomal stability as compared to **1a**, this was not translated into overall improvements in the pharmacokinetic profiles of a representative set of compounds.

A_{2B} receptors are responsible for the adenosine-mediated release of several inflammatory cytokines from mast cells, airway and bronchial epithelial cells, fibroblasts, smooth muscle cells, intestinal epithelial cells, monocytes and dendritic cells. Therefore A_{2B} adenosine receptor antagonists have the potential to become a new pharmacological drug class for the treatment of inflammatory conditions such as asthma.[1–4]

In a previous letter[5] we revealed that compounds of type **1**, when functionalized with appropriate substituents, are potent and selective A_{2B} adenosine receptor antagonists. Although certain compounds from this series have shown good bioavailability in pharmacokinetic studies, in general all compounds tested had short intravenous half lives. In vitro metabolism experiments performed with compounds of type **1** in rat and human microsomes, both in the presence and absence of the oxidative co-factor NADPH, indicated that, in several cases, substantial non-oxidative hydrolytic metabolism was occurring to give the corresponding 2-aminopyridine derivatives **2** (Fig. 1).

Amino pyridines of type **2** were themselves found to be weak adenosine receptor antagonists, typically losing over two orders of magnitude of potency when compared to the corresponding amide derivatives.

The potential metabolic instability of derivatives of type **1** could be a contributing factor to the short half lives as assessed in preliminary pharmacokinetic studies. It was reasoned that bicyclic derivatives of type **3** (Fig. 2), which retain the crucial hydrogen-bond donor capacity of the amide, would not be subject to the potential hydrolytic liability of the amide series and could also lead to new and novel series of A_{2B} adenosine receptor antagonists.[6]

Thus, a selection of bicylic derivatives of type **3** were prepared (Schemes 1–3) and tested (Table 1) as potential A_{2B} adenosine receptor antagonists.

Imidazolone **7** was prepared in good yield (Scheme 1) via a Curtius rearrangement of the amino acid **6** (prepared in three steps from pyridone **4**[5]). Amino acid **6** was also used in the preparation of the quinazoline dione **19** by treatment with urea at 200 °C under microwave conditions. All attempts to transform imidazolone **7** into

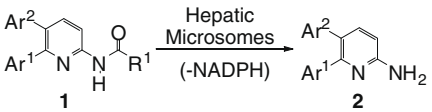

Figure 1. Non-oxidative metabolism of compounds of type **1**.

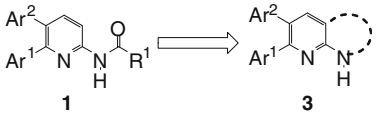

Figure 2. Proposed bicylic bioisosteres of **1**.

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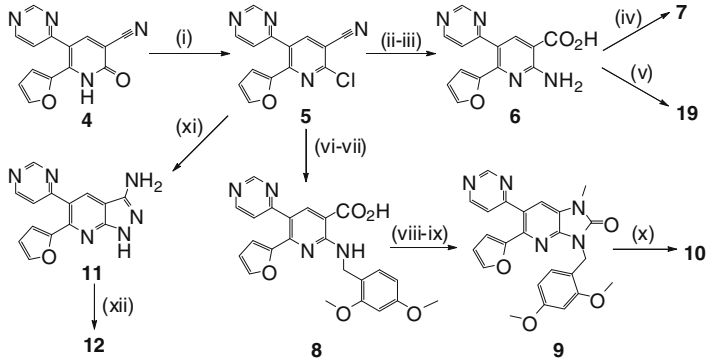
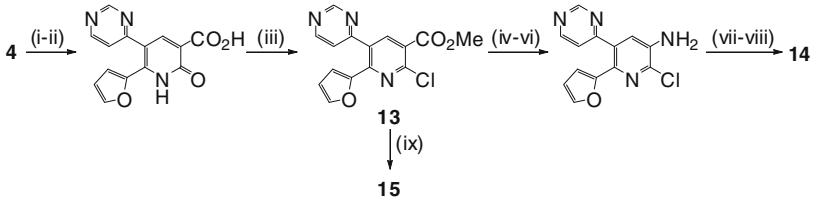
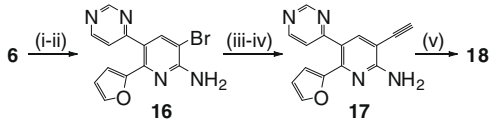


Scheme 1. Reagents and conditions: (i) $POCl_3$, reflux, 63%; (ii) Satd NH_3 in EtOH, 100 °C, 70%; (iii) NaOH (aq), EtOH, reflux, 99%; (iv) $(PhO)_2P(=O)N_3$, Et_3N, 1,4-dioxane, reflux, 56%; (v) urea, microwave, 200 °C, 58%; (vi) 2,4-dimethoxybenzylamine, Et_3N, EtOH, microwave, 175 °C, 100%; (vii) KOH, ethylene glycol, 150 °C, 93%; (viii) $(PhO)_2P(=O)N_3$, Et_3N, 1,4-dioxane, reflux, 87%; (ix) NaH, MeI, DMF, rt, 85%; (x) TFA, thioanisole, 65 °C, 32%; (xi) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 71%; (xii) $NaNO_2$ (aq), HCl (aq), AcOH, 0 °C then H_3PO_2 (aq), 0 °C, 41%.



Scheme 2. Reagents and conditions: (i) 6 M NaOH (aq), 30% H_2O_2 (aq), EtOH, H_2O, 50 °C, 80%; (ii) 10% KOH (aq), 120 °C, 100%; (iii) $POCl_3$, 120 °C then MeOH, 0 °C to rt, 66%; (iv) 2 M NaOH (aq), EtOH, rt, 98%; (v) $(PhO)_2P(=O)N_3$, Et_3N, *t*-BuOH, reflux, 86%; (vi) TFA, CH_2Cl_2, rt, 70%; (vii) CuCl, NH_3 (aq), 120 °C, 21%; (viii) $(EtO)_3CH$, AcOH, 140 °C, 43%; (ix) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 92%.



Scheme 3. Reagents and conditions: (i) Cu powder, quinoline, 230 °C, 70%; (ii) NBS, DMSO, H_2O, rt, 35%; (iii) TMS–acetylene, $PdCl_2(PPh_3)_2$, CuI, Et_3N, THF, 90 °C, 47%; (iv) K_2CO_3, MeOH, rt, 100%; (v) *t*-BuOK, NMP, 70 °C, 78%.

compound **10** via alkylation gave inseparable mixtures of isomeric products. Thus, a protecting group strategy was adopted. Nucleophilic aromatic substitution of the chloro nitrile **5** with 2,4-dimethoxybenzylamine followed by hydrolysis gave the amino acid **8** which was subjected to Curtius rearrangement and then cleanly alkylated to give **9**. Deprotection using TFA with thioanisole as a scavenger[7] gave the desired product **10**. Treatment of the chloro nitrile **5** with hydrazine gave the amino indazole **11**, which was subjected to a diazotization/reduction sequence[8] to give the unsubstituted indazole **12**. The imidazole derivative **14** was prepared in a multi-step sequence as shown in Scheme 2. Intermediate chloro ester **13** was treated with hydrazine to give **15** in good yield. Finally, the indole derivative **18** was accessed via a 5-endo-dig cyclisation[9] of the amino acetylene derivative **17** (Scheme 3), which was in turn prepared via a metal-catalyzed Sonogashira reaction[10] of the bromo derivative **16** with trimethylsilylacetylene followed by deprotection.

Biological results are given in Table 1.[11,12] Compound **1a**, a representative derivative of the afore-mentioned amide series, is included in the table as a comparison. It was gratifying to find that both the cyclic urea derivatives **7** and **10** and the quinazoline dione **19** gave comparable activity/selectivity profiles to that of the amide **1a**. Indeed, all of the bicyclic heterocycles synthesized were found to be potent A_{2B} adenosine receptor antagonists although in many cases the selectivity with respect to A_1 was found to be diminished when compared with the amide derivative **1a**.

The compounds were profiled to assess their ADME properties (Table 2). It was pleasing to note that, in rat hepatic microsomes, non-oxidative metabolism (i.e., in the absence of the co-factor NADPH) was not observed in any case with the cyclized derivatives and, in general, turnover was found to be low with the exception of the indole derivative **18**. CACO-2 flux data indicated that in all cases, except for that of compound **15**, intestinal permeability should not be a major issue with these derivatives.

The iv pharmacokinetic profiles in the rat of selected compounds are presented in Table 3. Although plasma clearance values for the cyclized compounds were, in general, lower than that seen with the amide derivative **1a**, half lives in rat remained short for all compounds tested as a result of moderate-to-high plasma clearance and low-to-moderate volumes of distribution. The furan moiety is a potential metabolic liability[13] present in all of the compounds; extra-hepatic p450-mediated metabolism of this moiety with the concomitant formation of reactive metabolites could be a contributing factor in the plasma clearance of these derivatives. Indeed, the clearance of compound **11** is higher than rat liver blood flow (even though turnover is low) which would indicate that, at least in this case, additional non-hepatic clearance mechanisms are operating.

An oral pharmacokinetic study (10 mg/kg, rat) conducted with **7** demonstrated that the compound had low bioavailability (F = 20%). Given that the CACO-2 and solubility data indicate that absorption is likely not be a major problem, the poor bioavailability could be explained by extra-hepatic and/or phase II metabolism and/or biliary excretion.

In summary, replacement of the amide moiety in the lead compound **1a** by bioisosteric bicyclic moieties led to potent and selective

Table 1
Biological activities and structures

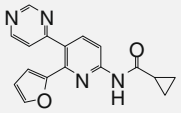
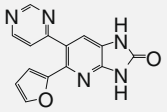
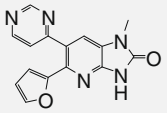
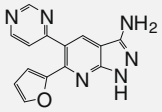
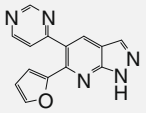
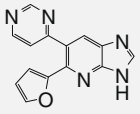
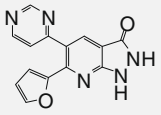
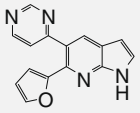
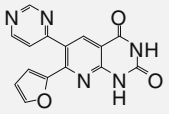
Compound	Structure	K_i (nM) or % inhibition of radioligand binding at a compound concentration of 1 μM[11]			
		hA_{2B}	hA_{2A}	hA_1	hA_3
1a		4 ± 1	239 ± 46	931 ± 121	3754 ± 866
7		6 ± 1	769 ± 190	317 ± 33	18% ± 5
10		11 ± 3	2126 ± 3	2444 ± 958	17% ± 6
11		8 ± 9	858 ± 136	357 ± 131	15% ± 5
12		23 ± 4	1656 ± 534	478 ± 25	2% ± 1
14		21 ± 2	3605 ± 2322	717 ± 302	0% ± 0
15		3 ± 1	177 ± 54	82 ± 17	2% ± 1
18		13 ± 1	619 ± 1	252 ± 75	7% ± 4
19		1 ± 0	181 ± 25	1727 ± 617	6267 ± 2322

Table 2
In vitro metabolism, CACO-2 permeability and solubility data

Compound	% Turnover[a]	P_{app}[b] (× 10^{-6} cm/s)	Thermodynamic solubility @ pH 7.4 (μg/mL)
1a	27	45	
7	15	40	48
10	8	32	
11	6	24	204
12	17	39	296
14	10	23	
15	7	3.5	
18	88	40	
19	0[c]	20	61

[a] % turnover after a 30 min incubation period at 37 °C of a 5 μM solution of test compound with rat hepatic microsomes (1 mg/mL).
[b] Passive permeability through a CACO-2 monolayer determined using 12.5 μM test compound.
[c] Poor % mass balance.

Table 3
Pharmacokinetic profiles of derivatives in rat (1 mg/kg iv)

Compound	$t_{1/2}$ terminal (h)	C_{max} (ng/mL)	Cl (L/h/kg)	$AUC_{0-\infty}$ (ng h/mL)	V_{ss} (L/kg)
1a	0.5	538	3.8	267	1.6
7	1.0	762	3.0	350	1.0
11	0.4	397	6.1	164	3.7
12	0.6	1049	1.4	736	0.8
15	0.4	1327	2.0	504	0.6
19	0.4[a]	1677	1.2	880	0.75

[a] Value refers to the half-life of the α-elimination phase where >90% of the compound is eliminated from the plasma compartment.

A_{2B} adenosine receptor antagonists which were hydrolytically stable towards liver microsomes, and that had, in general, low turnover. However the enhanced in vitro stability of the bicyclic compounds was not translated into overall improvements in the pharmacokinetic parameters of a representative set of compounds tested. Additional efforts in this area will be the subject of further communications.

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