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Discovery of 2-aminoimidazopyridine adenosine A_{2A} receptor antagonists

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ABSTRACT

A novel series of adenosine A_{2A} receptor antagonists was identified by high-throughput screening of an encoded combinatorial compound collection. The initial hits were optimized for A_{2A} binding affinity, A_1 selectivity, and in vitro microsomal stability generating orally available 2-aminoimidazo[4,5-*b*]pyridine-based A_{2A} antagonist leads.

Adenosine mediates various biological functions through its interaction with a family of G-protein-coupled receptors.[1] To date, four subtypes of adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) have been identified and each exhibits a unique expression pattern and physiological role.[1] Adenosine receptor subtype A_{2A}, located in the striatum of the brain, contributes to the regulation of motor activity by modulating dopamine D_2 receptor activation.[2] Hence, inhibition of the interaction of adenosine with the A_{2A} receptor may provide a potential treatment for Parkinson's disease.[3] Notably, A_{2A} antagonists such as xanthine KW-6002,[4] pyrazolotriazolopyrimidine SCH420814,[5] and triazolopyrimidine V2006/BIIB-014[6] have advanced to human clinical trials for motor-related disorders. In addition, chemotypes such as thiazolopyrimidines,[7] benzofurans,[8] and 6-furanyl-4-carboxamidylpyrimidines[9] have been recently disclosed as A_{2A} antagonists. In our efforts, high-throughput screening of an extensive encoded combinatorial compound collection[10] (~4.5 million compounds screened) also identified multiple active chemotypes. We have recently reported the discovery of substituted aminothiazoles[11] and trisubstituted purinones[12] as A_{2A} receptor antagonists identified through this screening campaign. Herein, the optimization of a third chemotype, based on a 2-aminoimidazopyridine scaffold, identified via this high-throughput screen is discussed.

The initial hit, benzimidazole **1a**, was an active A_{2A} antagonist (46 nM) but lacked microsomal stability (11% remaining after 0.5 h treatment with human liver microsomes).[13] Intriguingly, the hit lacked a furan moiety,[14] a common feature of non-xanthine A_{2A} antagonists. Hence, a hit to lead effort was initiated with the goal of converting this active in vitro hit into an orally available, brain penetrant lead molecule. In addition, selectivity against the A_1 receptor subtype was considered desirable in light of the potential for cardiovascular or renal effects associated with antagonism of the A_1 subtype.[15]

Benzimidazoles **1a**–**1h** were synthesized via the solid-phase route outlined in Scheme 1. Acid-cleavable linker 4-(4′-formyl-3′-methoxy)phenoxy butyric acid[16] was coupled to amino-TentaGel generating resin **6**. Reductive amination with 3-methoxybenzylamine followed by HATU-mediated coupling of 4-fluoro-3-nitrobenzoic acid produced intermediate **8**. Subsequent fluorine displacement, nitro reduction, and cyclization with cyanogen bromide yielded resin-bound aminobenzimidazole **11**, a key intermediate which enabled access to various R^3 substitutions on the 2-amino group of the benzimidazole core.

The unsubstituted aminobenzimidazole **1b** (generated via direct cleavage of resin-bound intermediate **11**) exhibited a dramatic drop in binding affinity with the A_{2A} receptor (Table 1). Additionally, analogs containing smaller alkyl secondary amides or tertiary amides in this R^3 position were inactive (data not shown). Hence, a focus was placed on optimizing the substitution of the benzoyl amide.

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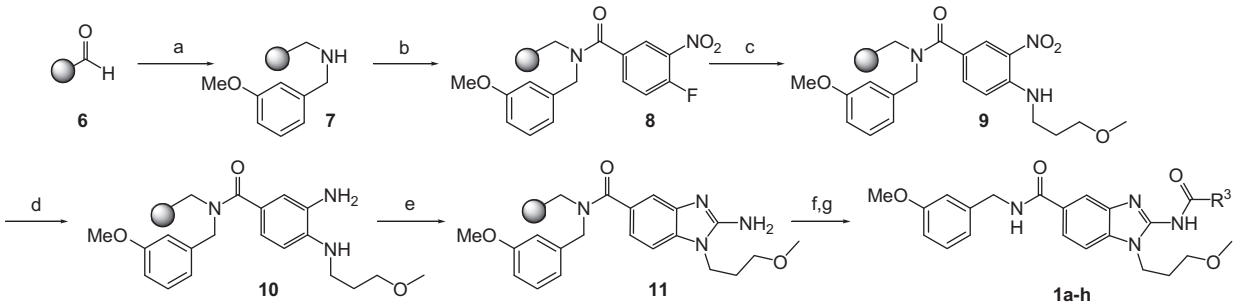
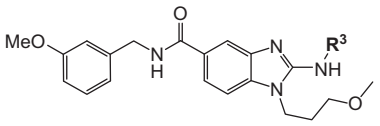


Scheme 1. Solid-phase synthesis of analogs **1a**–**1h**. Initial resin **6** = TentaGel resin harboring an acid-cleavable 4-(4′-formyl-3′-methoxy)phenoxybutyric acid linker.[17] Reagents and conditions: (a) 3-methoxybenzylamine, $Na(OAc)_3BH$, DCE, rt; (b) 3-nitro-4-fluorobenzoic acid, HATU, DIEA, DMF, rt; (c) 3-methoxypropylamine, DMF, rt; (d) $SnCl_2$, DMF, rt; (e) BrCN, DMF, rt; (f) substituted benzoic acid, HATU, DIEA, DMF, rt; (g) 50% TFA/DCM (v/v), rt.

Table 1
SAR for R^3 2-aminobenzimidazole analogs

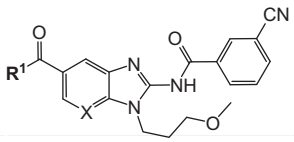


Compds	R^3	Human A_{2A} binding K_i (nM)[16]
1a	3-Cyanobenzoyl	46
1b	H	6770
1c	3-Fluorobenzoyl	210
1d	3-Chlorobenzoyl	439
1e	3-Trifluoromethylbenzoyl	>10,000
1f	3-Methoxybenzoyl	2380
1g	2-Chlorobenzoyl	4710
1h	4-Cyanobenzoyl	1670

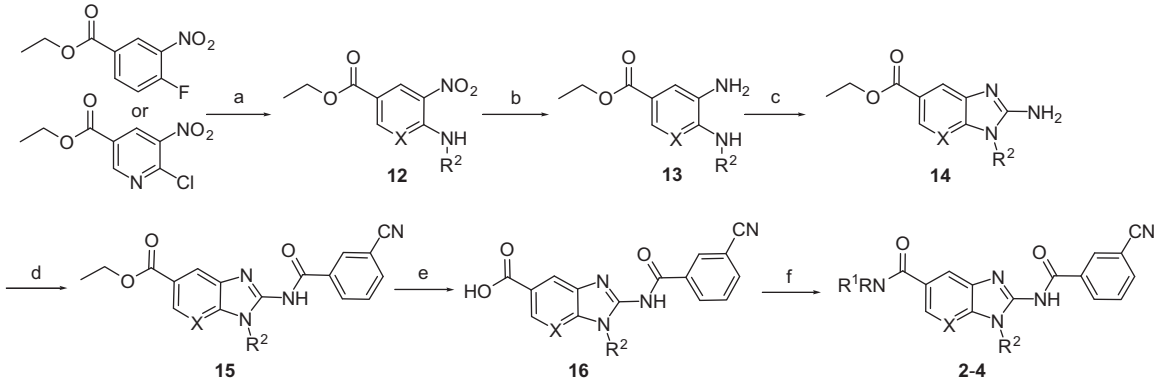
As is demonstrated in Table 1, alternative electron-withdrawing substituents such as 3-fluoro (**1c**) and 3-chloro (**1d**) suffered five or 10-fold reductions in binding affinity, respectively, when compared to the 3-cyano parent (**1a**). The more lipophilic electron-withdrawing 3-trifluoromethyl group (**1e**) was inactive in the assay. Substitution with an electron-donating methoxy group on the 3-position of the benzamide also produced a weakly active analog (**1f**). Relocation of the electron-withdrawing group to either the 2-position (**1g**) or 4-position (**1h**) on the ring also led to a drop in affinity.

Similarity between the 2-aminobenzamidazole core of these compounds and the 2-aminobenzothiazole core of previously disclosed A_{2A} antagonists[18] was noted. However, various benzoyl substitution patterns were allowed on the previously disclosed 2-aminobenzothiazole scaffold.[18] In contrast, as indicated in Table 1, the 3-cyano substitution on this 2-aminobenzamidazole (**1a**)

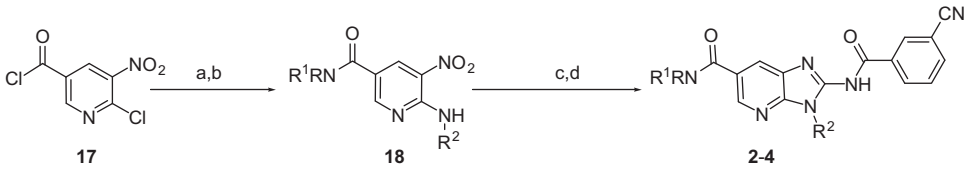
Table 2
Effect of central ring system on in vitro microsome stability



Compds	R^1	X	Human A_{2A} binding K_i (nM)	Liver microsome stability (% remaining-0.5 h)[13]	
				Rat	Human
2a	*n*-Butylamino	CH	48	38%	27%
2b	*n*-Butylamino	N	40	73%	95%
2c	Piperidinyl	CH	48	16%	67%
2d	Piperidinyl	N	9.6	36%	90%



Scheme 2. Solution-phase synthesis route for analogs **2**–**4**. Reagents and conditions: (a) R^2NH_2, THF, rt; (b) $Na_2S_2O_4$, $NaHCO_3$, 1,4-dioxane/H_2O; rt; (c) BrCN, MeOH, rt; (d) 3-cyanobenzoic acid, EDC, HOBt, DCM, rt; (e) LiOH, 1,4-dioxane, H_2O, 80 °C; (f) R^1RNH, EDC, HOBt, DCM, rt.



Scheme 3. Alternative route for analogs **2–4**. Reagents and conditions: (a) R^1RNH, DIEA, DCM; rt; (b) R^2NH_2, THF, rt; (c) $Na_2S_2O_4$, $NaHCO_3$, 1,4-dioxane/H_2O, rt; (d) 3-cyanobenzoyl isothiocyanate[18], DCM, EDC, Et_3N, rt.

uniquely provided high binding affinity. This 3-cyanobenzoyl group was held constant through further optimization efforts.

A limited set of examples indicated increased microsomal stability when the benzimidazole core was replaced with an imidazo[4,5-*b*]pyridine core (Table 2; compare **2a** to **2b** and **2c** to **2d**). Since this change did not negatively affect binding affinity and the potential for added microsomal stability was desirable, the imidazopyridine series was the main focus moving forward.

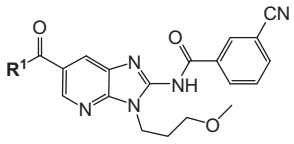
A solution-phase synthetic route (Scheme 2) was employed for the generation of imidazopyridine analogs aimed at understanding the SAR of the R^1 and R^2 moieties. The appropriate *ortho*-nitro, halogen-substituted benzoic ester was reacted with an amine to generate intermediate **12**. Nitro reduction to diamine **13**, cyclization with cyanogen bromide to **14**, and coupling with 3-cyanobenzoic acid produced ester **15**. Hydrolysis yielded acid **16** which was converted to the analogs **2–4** via carbodiimide-mediated amide formation. Alternatively, as outlined in Scheme 3, acid chloride **17** could be reacted with two sequential amines to generate intermediate **18**. Nitro reduction and cyclization with 3-cyanobenzoyl isothiocyanate[19] yielded the desired analogs **2–4**.

As suggested by the human A_{2A} binding affinity of compounds in Table 2, elimination of the original R^1 3-methoxybenzyl moiety was tolerated allowing a reduction of the molecular weight. While simplification to the primary amide was not allowed (Table 3; **3a**), analog **2d** suggested that tertiary amides might be preferred, thereby removing one undesired hydrogen bond donor from this potential CNS molecule. As demonstrated in Table 3, other tertiary amides were indeed tolerated. While the highly simplified dimethylamide analog **3b** had less affinity than **1a**, slightly larger alkyl amides were more tightly bound (**3c** and **3d**).

An in vitro cAMP functional assay was employed to further characterize the activity of these analogs in rat cells (Table 3).[20] Sterically crowded R^1 alkylamides (as in analogs **3e–3g**) imparted enhanced activity in this cAMP functional assay. In addition, these sterically crowded R^1 analogs demonstrated improved selectivity against the A_1 receptor subtype.

The compounds discussed thus far have harbored a fixed 3-methoxypropyl moiety at the *N*3-position of the imidazole ring. Changes to this R^2 position were explored while maintaining a simple *N*-piperidinylamide in the R^1 position (Table 4; **4a–4j**).

Table 3
SAR for R^1 2-aminoimidazopyridine analogs



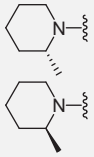
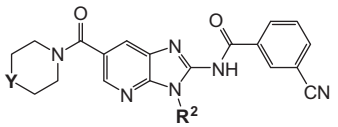
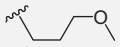
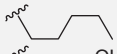
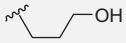
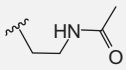
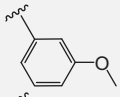
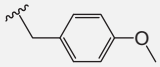
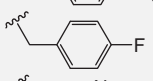
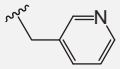
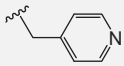
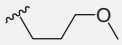
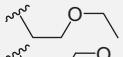
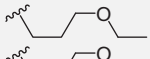
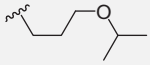
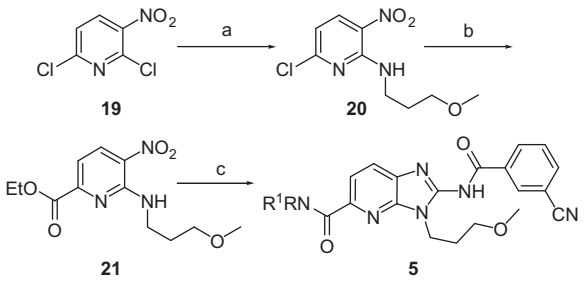
Compds	R^1	Human A_{2A} binding K_i (nM)	Rat A_{2A} cAMP K_i (nM)[19]	A_1/A_{2A} ratio
3a		2430	ND	ND
3b		123	ND	30
3c		25	24	48
3d		16	59	97
3e		13	12	237
2d		9.6	52	93
3f		7.7	6.4	234
3g		11	14	120

Table 4
SAR for R^2 2-aminoimidazopyridine analogs



Compds	Y	R^2	Human A_{2A} binding K_i (nM)	A_1/A_{2A} ratio
2d	CH_2		9.6	93
4a	CH_2		147	22
4b	CH_2		904	3
4c	CH_2		51	10
4d	CH_2		179	10
4e	CH_2		37	>46
4f	CH_2		325	2
4g	CH_2		1220	7
4h	CH_2		2590	3
4i	CH_2		233	4
4j	CH_2		35	39
4k	O		92	61
4l	O		3300	1.8
4m	O		1100	9
4n	O		>10,000	–



Scheme 4. Synthesis of analogs **5a**–**5d**. Reagents and conditions: (a) 3-methoxypropylamine, DIEA, THF, rt, 83%; (b) CO, EtOH, Et_3N, $PdCl_2(PPh_3)_2$, 70 °C, 37%; (c) procedures outlined in Scheme 3.

Truncation of the longer 3-methoxypropyl chain (**2d**) to a simplified methyl group (**4a**) resulted in a 15-fold drop in binding affinity for the A_{2A} receptor. Replacement of the oxygen in the methoxypropyl group with a methylene (**4b**) caused an even sharper drop in affinity. However, the hydroxypropyl analog **4c** exhibited only a minor loss of affinity for the receptor. Hence, it was hypothesized that the oxygen of this chain served as a hydrogen bond acceptor with the A_{2A} receptor. Replacement with a different functional group capable of accepting a hydrogen bond, for instance an acetamide (**4e**), was tolerated lending support to the hypothesis. The aromatic analogs in this series (**4f**–**4j**) also support the requirement for a hydrogen bond acceptor. *Para*-fluorobenzyl analog **4h** is a comparatively weak binder of the A_{2A} receptor in relation to the pyridinylmethyl analogs **4i** and **4j**. The location of the hydrogen bond accepting moiety is important since the 3-pyridylmethyl analog **4i** is sixfold less tightly bound then the 4-pyridylmethyl analog **4j**. Additionally, the 3-methoxyphenyl analog **4f** is fourfold more tightly bound than the 4-methoxybenzyl analog **4g**. Surprisingly, the 3-methoxybenzyl analog (not shown) was inactive in the assay.

A series of relatively conservative modifications to the 3-methoxypropyl R^3 group was tested while maintaining a *N*-morpholino amide in the R^1 position (**4k**–**4n**). When compared to the 3-methoxypropyl analog **4k**, relocation of the oxygen closer to the imidazopyridine core (**4l**) was detrimental to the binding affinity. Extension of the methoxy group to an ethoxy (**4m**) or isopropoxy group (**4n**) was also not tolerated.

Clearly, the 3-methoxypropyl chain (**2d**) was providing an optimal electronic and steric display to the A_{2A} receptor. In addition, none of the changes outlined in Table 4 enhanced selectivity against the A_1 receptor subtype.

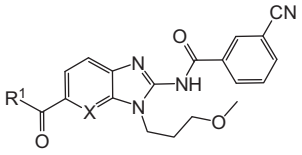
Since the R^1 amide proved to be the most tolerant toward change, the possibility of relocating this functional group from the 6-position to the 5-position of the imidazopyridine was explored. The synthesis of these analogs (**5a**–**5d**) is outlined in Scheme 4. Treatment of 2,6-dichloro-3-nitropyridine (**19**) with 3-methoxypropylamine led to intermediate **20**. Palladium-catalyzed carbonylation[21] generated ester **21** which was converted to the desired analogs via the steps outlined in Scheme 3.

In general the SAR for the 5-substituted amides was similar to the 6-position amide analogs (Table 5). Again, small alkyl tertiary amides were preferred at R^1 with the *N*-methyl-*N*-isopropylamide derivative **5d** exhibiting high activity in both the A_{2A} binding and functional assays. The 5-carboxamido-substituted analogs were generally less stable to microsomes than their 6-substituted counterparts. However, analog **5c** was particularly notable since it maintained moderate stability in the liver microsome assay.

Selected compounds which exhibited activity in the cAMP functional assay, A_1 selectivity, and in vitro microsomal stability were tested for brain levels in rat after oral dosing.[22] A comparison of two example analogs is provided in Table 6. Analog **5c** exhibited the best overall profile of desired in vitro activity, good plasma exposure, and modest brain levels after oral dosing in the rat.

In summary, a novel class of A_{2A} antagonists has been discovered via a high-throughput screen. These analogs represent the third chemotype of A_{2A} antagonists identified from an encoded combinatorial compound collection.[11,12] Initial optimization of this series led to the synthesis of 2-aminoimidazopyridine **5c**, an orally available A_{2A} antagonist (K_i = 12 nM) which exhibits 80-fold selectivity against the A_1 receptor and activity in an in vitro cAMP accumulation assay.

Table 5
SAR for 5-carboxamidoimidazopyridine analogs



Compds	R^1	X	hA_{2A} binding K_i (nM)	rA_{2A} cAMP K_i (nM)	A_1/A_{2A} ratio	Liver microsome stability (% remaining-0.5 h)	
						Rat	Human
5a		CH	3.1	16	183	19%	36%
5b		CH	17	85	131	12%	19%
5c		N	12	23	80	52%	52%
5d		N	2.5	4.3	88	14%	25%

Table 6
Brain levels in rat at 1 and 4 h (po; 30 mpk).[22]

Compds	Plasma level (ng/mL) 1 h	Plasma level (ng/mL) 4 h	Brain level (ng/g) 1 h	Brain level (ng/g) 4 h	Brain/plasma ratio 1 h/4 h
3c	4360	2290	480	210	0.11/0.09
5c	4630	2380	1050	450	0.23/0.19

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20. A rat functional assay was chosen to support in vivo rat model studies. Rat pheochromocytoma (PC-12) cells (ATCC, CRL-1721) were maintained in 1× Ham's F12 medium (Invitrogen) supplemented with 10% horse serum (Invitrogen), 2% FBS (Invitrogen), and 1× Penn/Strep (Invitrogen) in rat tail collagen (BD Biosciences)-treated tissue culture flasks. cAMP was quantified using the Perkin Elmer LANCE™ cAMP 384 kit. On the day of the assay, 5000 PC-12 cells were pre-incubated with compound for 15 min and then stimulated with 50 nM CGS-21680 for 15 min in a final volume of 18 μL. Assay was conducted in 1× HBSS (Invitrogen), 5 mM HEPES (Invitrogen), 0.05% BSA, (Sigma), 50 μM rolipram (Sigma), pH 7.4 buffer at 37 °C/5% CO_2. Final DMSO concentration was 0.5%. Following stimulation, the cells were lysed with cAMP detection solution provided with the assay kit. Signal was allowed to develop for 1 hour and detected using the EnVision™ Multiplate Reader or the Victor™ Multilabel counter (Perkin Elmer). Assays were performed in duplicate and compounds were tested a minimum of 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.
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22. Procedure for the rat brain level determination: male Sprague–Dawley rats were used for all studies. Rats were acclimated to the vivarium for 5–7 days before each study. On the day before the study, the rats were fasted overnight (18–20 h) but allowed free access to water. The next day, each rat was weighed and dosed (based on the individual animal's body weight) via oral intubation with test compounds suspended in 0.5%MC. One, 2 or 4 h after compound administration rats (two/group) were euthanized by CO_2 asphyxiation. Blood was collected by cardiac puncture and plasma prepared. Brain tissue was collected following organ perfusion to remove any excess blood. The brain tissue was homogenized on ice in cold PBS. The plasma and homogenized brain tissue were stored frozen until the time of analysis using LC/MS/MS. Standards curves, quality control and appropriate blank samples were included and all samples were analyzed. Results were expressed as the brain/plasma ratio (ng/g) at the specified time.