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Letters

New, Non-Adenosine, High-Potency Agonists for the Human Adenosine A_{2B} Receptor with an Improved Selectivity Profile Compared to the Reference Agonist *N*-Ethylcarboxamidoadenosine

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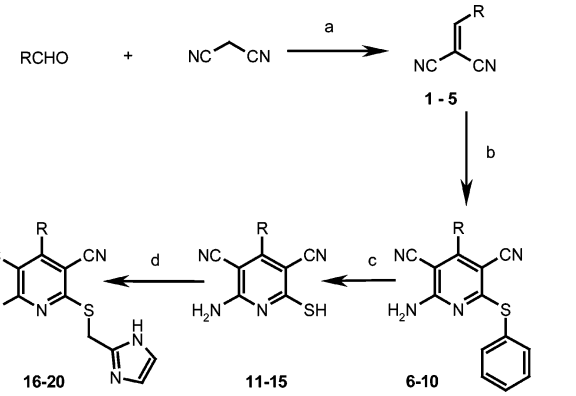
Abstract: The adenosine A_{2B} receptor is the least well characterized of the four known adenosine receptor subtypes because of the absence of potent, selective agonists. Here, we present five non-adenosine agonists. Among them, 2-amino-4-(4-hydroxyphenyl)-6-(1*H*-imidazol-2-ylmethylsulfanyl)pyridine-3,5-dicarbonitrile, **17**, LUF5834, is a high-efficacy partial agonist with EC_{50} = 12 nM and 45-fold selectivity over the adenosine A_3 receptor but lacking selectivity versus the A_1 and A_{2A} subtypes. Compound **18**, LUF5835, the 3-hydroxyphenyl analogue, is a full agonist with EC_{50} = 10 nM.

Adenosine receptors belong to the family of G-protein-coupled receptors and can be subdivided into A_1, A_{2A}, A_{2B}, and A_3 receptors. In contrast to the other three subtypes, no high-affinity adenosine analogues have so far been identified for the adenosine A_{2B} receptor.[1,2] Hence, this receptor is also known as the low-affinity receptor.[3] To date, the most potent, albeit nonselective, agonist for this receptor is *N*-ethylcarboxamidoadenosine, NECA, with affinity in the micromolar range.[4]

The adenosine A_{2B} receptor has been implied in cell proliferation and/or differentiation and in mast-cell-mediated activation of angiogenesis.[5−7] This last effect is the result of a cooperative action with the adenosine A_3 receptors.[7] A selective high-potency agonist for the adenosine A_{2B} receptor would be very useful to delineate the precise role of the adenosine A_{2B} versus the A_3 receptor in this mast-cell-mediated stimulation of angiogenesis.

Scheme 1. Synthetic Route to **16**−**20**[a]



[a] Reagents: (a) piperidine, EtOH; (b) malononitrile, thiophenol, triethylamine, EtOH; (c) (i) Na_2S, DMF, (ii) 1 M HCl; (d) 2-bromomethylimidazole, $NaHCO_3$, DMF.

Recently, Stasch et al. and Kerstin et al. reported on the synthesis of a series of substituted 2-amino-4-phenyl-6-phenylsulfanylpyridine-3,5-dicarbonitriles as agonists for adenosine receptors.[8,9] From their patent data, we deduced that certain members of this class of compounds might be interesting as agonists for the adenosine A_{2B} receptor. In this study we have synthesized five 4-phenyl-substituted 2-amino-4-phenyl-6-phenylsulfanylpyridine-3,5-dicarbonitriles. We determined their ability to activate the human adenosine A_{2B} receptor through stimulation of cAMP production in CHO cells stably expressing this receptor. In addition, we determined the affinity and efficacy of these compounds for the other three human adenosine receptors.

Compounds **16**−**20** were synthesized according to Scheme 1.[8] The aldehyde was reacted with malononitrile in the presence of a few drops of piperidine to give the intermediates (**1**−**5**) in moderate to good yields (40−83%). The pyridine ring was formed by refluxing the

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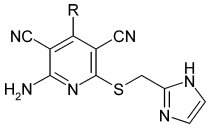
Table 1. Interaction of NECA and the Five Newly Synthesized Compounds with Human Adenosine Receptors[a]



		K_i, nM			EC_{50}, nM
compd	R	hA_1	hA_{2A}	hA_3	hA_{2B}
16	phenyl	2.4 ± 1.0	28 ± 4	171 ± 109	19 ± 7
17	*p*-OH-phenyl	2.6 ± 0.3	28 ± 4	538 ± 210	12 ± 2
18	*m*-OH-phenyl	4.4 ± 2.0	21 ± 2	104 ± 49	10 ± 3
19	*m*-OCH_3-phenyl	2.0 ± 1.0	105 ± 22	74 ± 21	34 ± 24
20	*p*-OCH_3-phenyl	7.0 ± 0.8	214 ± 37	24 ± 7.6	9 ± 3
NECA		12 (9.6−15)[12]	60 ± 10[12]	11 ± 0.8[12]	104 ± 15

[a] Radioligand binding experiments were carried out on membranes made from CHO cells stably expressing the human A_1 and from HEK293 cells stably expressing the A_{2A} and A_3 receptors with [^{3}H]DPCPX, [^{3}H]ZM241385, and [^{125}I]I-ABMECA as radioligands, respectively. To determine the ability of these compounds to activate the human A_{2B} receptor, cAMP experiments were carried out on CHO cells stably transfected with this receptor (n = 3−12). The expression level of the A_{2B} receptor on these cells amounted to approximately 300 fmol/10^6 cells.

functionalized malononitrile with another equivalent of malononitrile and an equivalent of thiophenol in ethanol and triethylamine, resulting in **6**−**10** (20−43% yield). To obtain the free thiol in the 6-position of the pyridine ring, we added 3.3 equiv of sodium sulfide in DMF at 80 °C for 2 h, resulting in quantitative yields of **11**−**15**.

2-Bromomethylimidazole was synthesized by reducing the commercially available 2-imidazole carboxaldehyde with $LiAlH_4$ in THF. The resulting alcohol function was then substituted for bromine through the action of a solution of hydrobromic acid in glacial acetic acid according to literature procedures.[10] The final step was the reaction of the free thiol with 2-bromomethylimidazole in the presence of $NaHCO_3$ in DMF at room temperature to give **16**−**20** in modest yields.

CHO cells expressing the human adenosine A_{2B} receptor were used to generate and measure cAMP production as described before.[11] For comparison, the affinity of these compounds for the human adenosine A_1, A_{2A}, and A_3 receptors stably expressed on CHO cells (A_1) or HEK293 cells (A_{2A}, A_3) was determined in radioligand binding studies with [^{3}H]DPCPX (K_D = 1.6 nM), [^{3}H]ZM241385 (K_D = 1.0 nM), and [^{125}I]I-ABMECA (K_D = 5.0 nM) as radioligands, respectively.[12] To determine whether the compounds possessed agonistic activity on the adenosine A_1, A_{2A}, and A_3 receptors, cAMP experiments were performed on CHO cells essentially as previously described.[11,12] The CHO cells expressing the human A_{2A}, A_{2B}, and A_3 receptor were provided by Dr. Steve Rees, GlaxoSmithKline, U.K. HEK293 cells expressing the human adenosine A_{2A} or the A_3 receptor were provided by Dr. J. Wang, Biogen, and Dr. K.-N. Klotz, University of Würzburg, Germany, respectively. CHO cells expressing the human adenosine A_1 receptor were provided by Dr. Andrea Townsend-Nicholson, University College of London, U.K. To analyze the data, PRISM software (GraphPad, San Diego, CA) was used.

In Table 1, the interaction of the reference compound NECA and our five newly synthesized compounds with all four human adenosine receptors is summarized. As expected, NECA was nonselective and bound not only to the A_{2B} receptor but also (with higher affinity) to the A_1, A_{2A}, and A_3 receptors. All five compounds interacted with the human adenosine A_{2B} receptor with EC_{50} ranging from 9 to 34 nM.

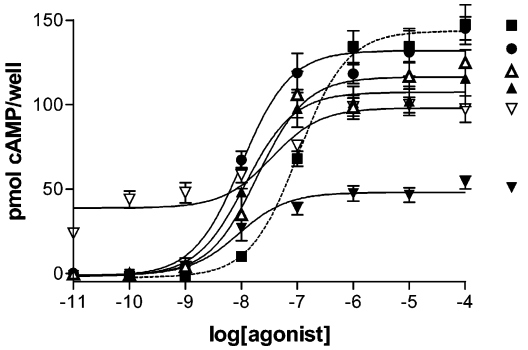


Figure 1. Stimulation of cAMP production via the human adenosine A_{2B} receptor stably expressed on CHO cells by NECA (■), **16** (△), **17** (▲), **18** (●), **19** (▽), and **20** (▼).

Table 2. Inhibition (A_1 and A_3 Receptors) and Stimulation (A_{2A} and A_{2B} Receptors) of cAMP Production by the Five Newly Synthesized Compounds Compared to Reference Agonists[a]

	efficacy,[b] %			
compd	hA_1	hA_{2A}	hA_3	hA_{2B}
16	109 ± 11	55 ± 20	84 ± 0.5	81 ± 3
17	103 ± 6	55 ± 12	23 ± 4	74 ± 2
18	112 ± 6	80 ± 6	95 ± 4	92 ± 3
19	80 ± 13	49 ± 18	39 ± 2	68 ± 3
20	46 ± 28	32 ± 5	73 ± 3	33 ± 1

[a] The production of cAMP was studied in CHO cells stably expressing the adenosine receptors (n = 3). [b] Efficacy is expressed with respect to the following reference agonists: N^6-cyclopentyladenosine (CPA), CGS21680, NECA, and 2Cl-IBMECA for the human adenosine A_1, A_{2A}, A_{2B}, and A_3 receptors, respectively. Compounds were tested at 100 times their K_i values for the A_1, A_{2A}, and A_3 receptors. To study the inhibitory effect of the agonists on the A_1 and A_3 receptors, cAMP production was stimulated with 10 μM forskolin.

Interestingly, the E_{max} value of these compounds varied greatly, indicating that substituents at the phenyl ring are important for the efficacy (Figure 1 and Table 2). The unsubstituted **16** had an efficacy of 81% compared with NECA. The para-substituted compounds had a relatively low efficacy, 74% for **17** (*p*-OH), and 33% for **20** (*p*-OCH_3), compared with NECA. Substitution on the meta position yielded compounds with efficacies of 92% for **18** (*m*-OH) and 68% for **19** (*m*-OCH_3). Overall, **18** displayed the highest efficacy of the series, 92% compared with the reference agonist NECA, combined with a low EC_{50} of 10 nM. The increased cAMP production at low concentrations of **19** was reproducible, although we have no explanation for this phenomenon. Compound **20** had a similar EC_{50} of 9 nM and was a partial agonist with an efficacy of 33% compared to NECA. In the past we have successfully synthesized potent partial agonists for the adenosine A_1, A_{2A}, and A_3 receptors.[13−15] Here, we demonstrate for the first time the synthesis of potent partial agonists for the adenosine A_{2B} receptor.

To confirm that the cAMP production in the CHO cells was due to activation of the adenosine A_{2B} receptor, we investigated whether the cAMP production could be antagonized by the potent adenosine receptor antagonist

CGS15943.[4] CGS15943 dose-dependently (0.1−10 μM) antagonized the cAMP production induced by NECA and by **16**−**20** with a pK_B of 8.3 ± 0.6 (data not shown). This value corresponds to literature data in which a pA_2 value for CGS15943 of 8.0 ± 0.3 was reported.[16] Hence, the new compounds are indeed agonists for the adenosine A_{2B} receptor.

Besides the activity of this series of compounds on the adenosine A_{2B} receptor, we also looked at the selectivity with respect to the other subtypes of adenosine receptors. In the absence of radioligand binding data on the adenosine A_{2B} receptor, this selectivity is defined as the ratio of K_i (A_1, A_{2A}, A_3) to EC_{50} (A_{2B}). The nonselective reference compound NECA prefers the G_i-coupled A_1 and A_3 receptors over the G_s-coupled A_{2A} and A_{2B} receptors. Interestingly, the new compounds provided more promising data concerning selectivity. Compound **20** for example had a 24-fold lower EC_{50} value for the adenosine A_{2B} receptor compared to its K_i value for the adenosine A_{2A} receptor. In addition, this compound was equipotent on the adenosine A_1 receptor, whereas the other compounds preferred the adenosine A_1 receptor over the adenosine A_{2B} receptor. Substantial selectivity was obtained with respect to the adenosine A_3 receptor. Compound **17** had a 45-fold lower EC_{50} value on the adenosine A_{2B} receptor compared to its affinity for the adenosine A_3 receptor.

To verify whether the new compounds also acted as agonists on the adenosine A_1, A_{2A}, and A_3 receptors, we performed cAMP studies with CHO cells expressing these receptors. The compounds were tested at a concentration of 100 times their K_i values to determine their maximal effect (Table 2). At this concentration the receptor is fully occupied, and as a result, the maximal efficacy of the compounds can be determined.

All compounds tested showed agonistic activity, at least to some extent, on all four adenosine receptors. However, strong differences between receptor subtypes were observed. Whereas the unsubstituted **16** had a high efficacy for all receptors except the A_{2A} receptor, introduction of a *m*-OH group (**18**) resulted in almost maximal efficacy on all four adenosine receptors. Substitution of the *m*-OH substituent with *m*-OCH_3 (**19**) diminished the efficacy on all four adenosine receptors. Shifting the OCH_3 group from the meta to the para position increased the efficacy on the A_3 receptor but decreased the efficacy on the A_1 and A_{2B} receptors. Finally, replacing the *p*-OCH_3 group with the *p*-OH group increased the efficacy on all adenosine receptors except for the A_3 receptor. Apparently, the substitution pattern on the phenyl ring of the newly synthesized compounds strongly affects their efficacy. In general, the adenosine A_1 and the adenosine A_{2B} receptors were activated most easily by this class of compounds. These experiments are very promising with respect to the development of selective partial agonists for each individual adenosine receptor subtype.

In summary, **18** and especially **17** had improved selectivity for the adenosine A_{2B} receptor over the A_3 receptor. In particular, the reduced affinity and the reduced efficacy of **17** for the adenosine A_3 receptor may render this ligand a suitable tool for studying the relative contributions of the A_{2B} and A_3 receptor subtypes involved in the mast-cell-mediated activation of angiogenesis. Moreover, this series of atypical non-ribose compounds provides a new structural class of agonists for the adenosine receptors, challenging the rule that adenosine receptor agonists require a more or less intact ribose function.

In conclusion, we have discovered a series of agonists for the human adenosine A_{2B} receptor. Moreover, we have identified the first partial agonist for this receptor subtype. Next to improved EC_{50} values for cAMP production compared with NECA, these new compounds also show improved selectivity over the other adenosine receptors.

Supporting Information Available: Synthetic procedures for **16**−**20** as well as ^{1}H and ^{13}C NMR, MS, and combustion analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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