Synthesis and Biological Evaluation of 2-Amino-3-(4-Chlorobenzoyl)-4-[*N*-(Substituted) Piperazin-1-yl]Thiophenes as Potent Allosteric Enhancers of the A_1 Adenosine Receptor

Romeo Romagnoli,*[,†] Pier Giovanni Baraldi,*[,†] Maria Dora Carrion,[†] Carlota Lopez Cara,[†] Olga Cruz-Lopez,[†] Maria Antonietta Iaconinoto,[†] Delia Preti,[†] John C. Shryock,[‡] Allan R. Moorman,[§] Fabrizio Vincenzi,[||] Katia Varani,[||] and Pier Andrea Borea[||]

Dipartimento di Scienze Farmaceutiche, Università di Ferrara, Ferrara, Italy, Department of Medicine, University of Florida, Gainesville, Florida, King Pharmaceuticals Inc., Research and Development, 4000 CentreGreen Way, Suite 300, Cary, North Carolina, Dipartimento di Medicina Clinica e Sperimentale, Sezione di Farmacologia, Università di Ferrara, Ferrara, Italy

Received May 19, 2008

The synthesis and evaluation of a series of 2-amino-3-(4-chlorobenzoyl)-4-[4-(alkyl/aryl)piperazin-yl]thiophene derivatives as allosteric enhancers of the A_1-adenosine receptor are described. The nature of substituents on the phenyl ring tethered to the piperazine seem to exert a fundamental influence on the allosteric enhancer activity, with the 4-chlorophenyl **8f** and 4-trifluoromethyl **8j** derivatives being the most active compounds in binding (saturation and displacement experiments) and functional cAMP studies.

Introduction

Adenosine is an ubiquitous autocoid with multiple effects, exerting its actions on the human body by interacting with four different P_1-purinoreceptor subtypes classified as A_1, A_{2A}, A_{2B}, and A_3.[1] Pharmacological agents that increase the activation of A_1-adenosine receptors in response to adenosine would be useful in conditions characterized by a localized oxygen deficit such as angina, myocardial infarction, and stroke.[2] Compounds that are able to enhance the activity of the A_1-adenosine receptors by the endogenous ligand within specific tissues may have potential therapeutic advantages over nonendogenous agonists.. Such an opportunity for intervention is provided by the concept of allosteric modulation of G protein coupled receptors (GPCRs[a]).[3]

Allosteric enhancers of the action of adenosine are believed to stabilize the conformation of A_1-adenosine receptors that has a high affinity for agonists. This effect is manifested as a slowing of the rate of dissociation of agonist from the receptor.[4] In addition, allosteric enhancers appear to stabilize an active receptor conformation, even in the absence of an agonist. Thus, in cells expressing A_1-adenosine receptors such as Chinese hamster ovary (CHO) cells, an allosteric enhancer may increase the number of receptors in an active conformation and thereby cause a change in cell functions.[5,6] Bruns and co-workers reported that 2-amino-3-benzoylthiophene derivatives are capable both of enhancing the binding and activity of reference A_1 receptor agonists, such as N^6-cyclopentyladenosine (CPA), to the A_1 adenosine receptor and, usually at higher concentrations, of acting as competitive antagonists at the same receptor.[5−7] Many papers demonstrated that (2-amino-4,5-dimethylthien-3-yl)-[3-(trifluoromethyl)phenyl]-methanone (PD 81,723, compound **1**) represents a specific and selective allosteric enhancer of the A_1 receptors, with the best ratio of enhancement to antagonistic action at this receptor.[7−9] Until now, PD 81,723 was considered as a lead compound for the development of new series.

It was evident from detailed structure−activity analyses performed by several groups[10] that electron-withdrawing substituents, such as 4-chloro and 3-trifluoromethyl, on the benzoyl moiety at the 3-position of the thiophene ring resulted in higher enhancement activity. It has been shown that large hydrophobic groups at the 4-position of the thiophene ring and small substituents (H and CH_3) at the 5-position increased allosteric enhancing activity.[7,10]

The purpose of our investigation was to synthesize and evaluate the allosteric enhancer activity of a new series of derivatives, characterized by a common 2-amino-3-(4-chlorobenzoyl) thiophene core, where various alkyl or aryl piperazine moieties are attached to a methylene at the 4-position of the thiophene ring. Our synthetic efforts in this series focused mainly on the phenyl ring attached to the piperazine. To obtain a general sense of how the activity was affected by the electronic character of this phenyl group, a series of representative electron-withdrawing groups (F, Cl, CN, CF_3) and electron-donating groups (CH_3, 3,4-methylenedioxy) were introduced as substituents at the para-position.

Chemistry

The approach taken for the preparation of compounds **8a**−**l** is shown in Scheme 1. The treatment of the 2,5-dimethyl-(1,4)dithiane-2,5-diol (the dimer of α-mercaptoacetone) in ethanol with triethylamine (2.2 equiv), followed by the addition of 3-(4-chlorophenyl)-3-oxopropanenitrile, furnished the 2-amino-3-(4-chlorobenzoyl)-4-methylthiophene **2**. Compound **2** was converted almost quantitatively to the phthalimido derivative **3** using phthalic anhydride in acetic acid, then brominated in the 5-position with NBS in benzene to give the 5-bromo thiophene derivative **4**. Subsequent reaction with NBS in CCl_4 resulted in side chain bromination and the formation of the 4-bromomethyl-5-bromo thiophene analogue **5**, used as a common intermediate

* To whom correspondence should be addressed. For R.R.: phone, 39-(0)532455303; fax, 39-(0)532455953; E-mail: rmr@unife.it. For P.G.B.: phone, 39-(0)532455293; fax, 39-(0)532-455953; E-mail: baraldi@unife.it.

† Dipartimento di Scienze Farmaceutiche, Università di Ferrara.

‡ Department of Medicine, University of Florida.

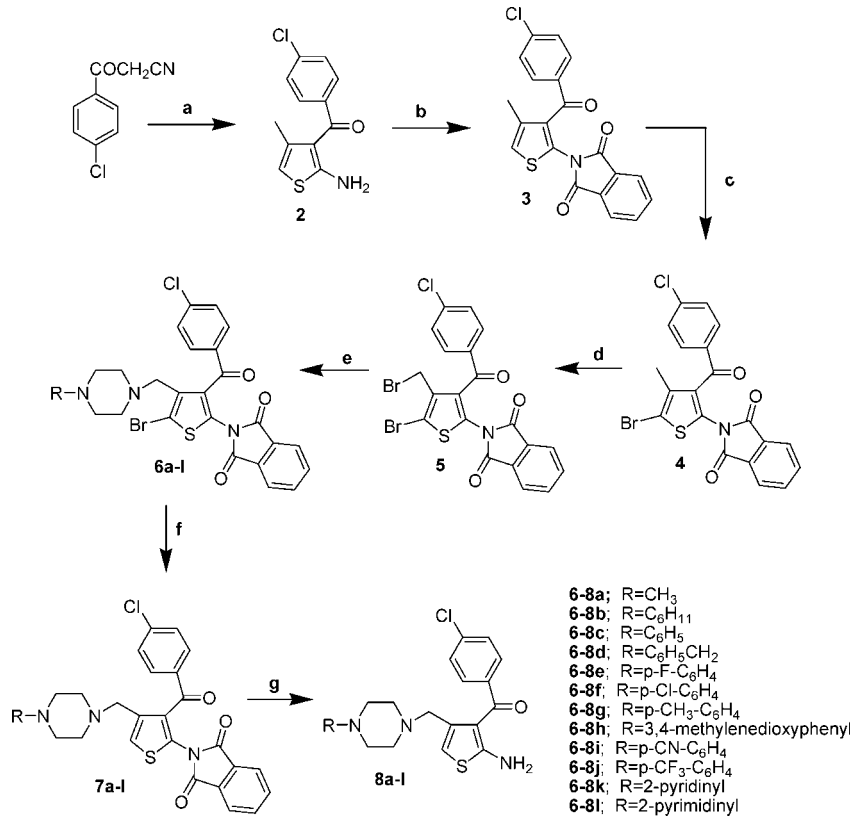
§ King Pharmaceuticals Inc., Research and Development.

|| Dipartimento di Medicina Clinica e Sperimentale, Sezione di Farmacologia, Università di Ferrara.

[a] Abbreviations: GPCRs, G protein coupled receptors; [^{3}H]DPCPX, [^{3}H]1,3-dipropyl-8-cyclopentyl-xanthine; [^{3}H]MRE 3008F20, [^{3}H]5-*N*-(4-methoxyphenylcarbamoyl)amino-8-propyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-c]pyrimidine; [^{3}H]CCPA, [^{3}H]2-chloro-N^6-cyclopentyladenosine; [^{3}H]ZM 241385, [^{3}H](4-(2-[7-amino-2-(2-furyl)[1,2.4]triazolo[2,3-*a*]-[1,3,5]triazin-5-ylamino]ethyl)phenol); CPA, N^6-cyclopentyladenosine; CHO, Chinese hamster ovary; cAMP, adenosine 3′,5′-cyclic monophosphate; AEs, allosteric enhancers; NBS, *N*-bromosuccinimide.

10.1021/jm800586p CCC: $40.75 © 2008 American Chemical Society
Published on Web 08/26/2008

Scheme 1[a]



[a] Reagents. (a) 2,5-dimethyl-[1,4]dithiane-2,5-diol, TEA, EtOH (yield 73%); (b) phthalic anhydride, AcOH, (yield 88%); (c) NBS, C_6H_6, (yield 88%); (d) NBS, CCl_4, (yield 905); (e) *N*-alkyl/aryl piperazine, K_2CO_3, CH_2Cl_2; (f) H_2, 10% Pd/C, DMF; (g) NH_2NH_2, EtOH.

Table 1. Effect of Compounds **8a**−**l** and PD 81,723 in cAMP Assays on hA_1CHO Cells

	% change in cAMP production (mean ± SEM)[a] concentration of compounds			
compd	0.01 μM	0.1 μM	1.0 μM	10 μM
PD 81,723	−8 ± 5	−3 ± 6	−31 ± 6	−57 ± 2
8a	−3 ± 11	+3 ± 0	−1 ± 4	−16 ± 4
8b	+5 ± 5	+3 ± 7	−13 ± 4	−52 ± 3
8c	−4 ± 12	−26 ± 15	−62 ± 11	−78 ± 4
8d	+3 ± 4	+4 ± 2	−30 ± 11	−78 ± 4
8e	+3 ± 7	−19 ± 3	−63 ± 4	−77 ± 2
8f	+10 ± 4	−48 ± 14	−65 ± 4	−78 ± 2
8g	+10 ± 14	+3 ± 12	−22 ± 7	−66 ± 4
8h	−15 ± 3	−13 ± 6	−23 ± 9	−34 ± 9
8i	+12 ± 12	−8 ± 12	−54 ± 0	−72 ± 3
8j	−10 ± 7	−53 ± 8	−84 ± 7	−87 ± 5
8k	+9 ± 4	−5 ± 6	−46 ± 14	−69 ± 1
8l	+9 ± 6	+3 ± 9	−25 ± 4	−69 ± 6

[a] The results are the average of six experiments at each of four concentrations of tested compound.

Table 2. Antagonist Activity of Compounds **8a**−**l**

compd	% inhibition A_1[a]	% inhibition A_{2A}[b]	% inhibition A_3[c]
PD 81,723	0 ± 0	0 ± 0	21 ± 2
8a	3 ± 1	0 ± 0	0 ± 0
8b	0 ± 0	0 ± 0	5 ± 1
8c	0 ± 0	0 ± 0	0 ± 0
8d	0 ± 0	0 ± 0	0 ± 0
8e	0 ± 0	0 ± 0	17 ± 2
8f	0 ± 0	3 ± 1	6 ± 1
8g	0 ± 0	0 ± 0	0 ± 0
8h	0 ± 0	0 ± 0	0 ± 0
8i	0 ± 0	0 ± 0	20 ± 3
8j	0 ± 0	0 ± 0	15 ± 2
8k	0 ± 0	4 ± 1	0 ± 0
8l	0 ± 0	0 ± 0	0 ± 0

[a] Inhibition activity is expressed as percent displacement value (±SEM, $n = 3$) of 1 nM [^{3}H]DPCPX by 10 μM of tested compounds. [b] Inhibition activity is expressed as percent displacement value (±SEM, $n = 3$) of 2 nM [^{3}H]ZM 241385 by 10 μM of tested compounds. [c] Inhibition activity is expressed as percent displacement value (±SEM, $n = 3$) of 2 nM [^{3}H]MRE 3008F20 by 10 μM of tested compounds.

for the synthesis of final compounds **8a**−**l** by a three-step synthesis. Unfortunately, all of the attempts to selectively brominate compound **3** at the 4-position failed. Compound **5** was coupled with the appropriate *N*-alkyl/arylpiperazines (2 equiv) in dichloromethane to give the derivatives **6a**−**l** in good yields, Dehalogenation by catalytic hydrogenation using 10% Pd/C furnished the 5-unsubstituted thiophenes **7a**−**l**. Treatment with ethanolic hydrazine afforded the final compounds **8a**−**l**.

Results and Discussion

Functional assays were performed using CHO cells stably transfected with the recombinant human A_1 adenosine receptors (hA_1CHO cells). In these experiments, the ability to reduce the cAMP levels in hA_1CHO cells by the novel compounds **8a**−**l** and by the reference compound (PD 81,723) at four different concentrations (0.01, 0.1, 1, and 10 μM) was measured (Table 1). The effect of each tested compound on cAMP production was reported as a percentage in comparison with the controls (100%). Novel compounds with the potential role of allosteric enhancers were able to activate human A_1 adenosine receptors showing a decrease of the cAMP production in hA_1CHO cells.

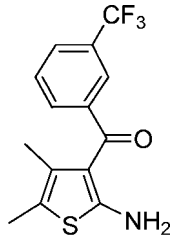
Among the synthesized compounds, only the compounds **8a** and **8h** were less active than PD 81,723. The remaining derivatives (**8c**−**g** and **8i**−**l**) showed an activity superior (from 69% to 87%) or comparable (**8b**) to PD 81,723 as allosteric

Table 3. Saturation Binding Assays in hA_1-CHO Membranes Obtained by Using [^{3}H]CCPA (A) and [^{3}H]DPCPX (B), Modulation of Enhancers (10 μM) on the CCPA Affinity (K_i) Represented by CCPA Shift in [^{3}H]DPCPX Competition Binding Experiments (C)[a]

	(A) [^{3}H]CCPA saturation binding experiments			(B) [^{3}H]DPCPX saturation binding experiments			(C) [^{3}H]DPCPX competition binding experiments	
compd	K_D (nM)	B_{max} (fmol/ mg protein)	B_{max} shift (fold of increase)	K_D (nM)	B_{max} (fmol/ mg protein)	B_{max} shift (fold of increase)	CCPA K_i (nM)	CCPA K_i shift (fold of increase)
PD 81,723	1.1 ± 0.1	673 ± 55	1.3 ± 0.1	1.8 ± 0.1	3345 ± 381	1.0 ± 0.1	9.1 ± 0.9	1.7 ± 0.2
8a	1.2 ± 0.1	570 ± 48	1.1 ± 0.1	1.7 ± 0.2	2987 ± 264	0.9 ± 0.1	11.2 ± 1.2	1.4 ± 0.1
8b	1.1 ± 0.1	622 ± 51	1.2 ± 0.1	1.9 ± 0.1	3196 ± 285	0.9 ± 0.1	10.2 ± 1.4	1.5 ± 0.1
8c	1.0 ± 0.1	3367 ± 315	6.5 ± 0.6	1.9 ± 0.2	3521 ± 367	1.0 ± 0.1	3.2 ± 0.3	4.7 ± 0.4
8d	1.1 ± 0.1	3160 ± 290	6.1 ± 0.5	1.7 ± 0.1	3418 ± 358	1.0 ± 0.1	3.5 ± 0.4	4.3 ± 0.4
8e	1.0 ± 0.1	3471 ± 326	6.7 ± 0.6	1.8 ± 0.2	3565 ± 395	1.1 ± 0.1	2.9 ± 0.3	5.2 ± 0.5
8f	1.1 ± 0.1	3626 ± 332	7.0 ± 0.6	1.8 ± 0.1	3653 ± 412	1.1 ± 0.1	2.7 ± 0.3	5.6 ± 0.5
8g	1.0 ± 0.1	2020 ± 193	3.9 ± 0.3	1.7 ± 0.1	3058 ± 269	0.9 ± 0.1	4.5 ± 0.5	3.4 ± 0.3
8h	1.1 ± 0.1	725 ± 64	1.4 ± 0.1	1.9 ± 0.2	3193 ± 256	0.9 ± 0.1	9.5 ± 0.9	1.6 ± 0.1
8i	1.1 ± 0.1	2486 ± 217	4.8 ± 0.4	1.8 ± 0.2	3264 ± 312	1.0 ± 0.1	3.8 ± 0.4	4.0 ± 0.4
8j	1.0 ± 0.1	3989 ± 377	7.7 ± 0.7	1.9 ± 0.2	3678 ± 427	1.1 ± 0.1	2.4 ± 0.2	6.3 ± 0.5
8k	1.2 ± 0.1	1813 ± 174	3.5 ± 0.3	1.7 ± 0.1	3087 ± 293	0.9 ± 0.1	6.7 ± 0.7	2.3 ± 0.2
8l	1.1 ± 0.1	1450 ± 118	2.8 ± 0.2	1.7 ± 0.2	3325 ± 288	1.0 ± 0.1	7.2 ± 0.8	2.1 ± 0.2

[a] The results are mean values (±SEM) of three independent experiments. (A) = K_D (nM), B_{max} (fmol/mg protein) and B_{max} shift obtained in [^{3}H]CCPA saturation binding experiments performed in the absence (K_D = 1.1 ± 0.1 nM, B_{max} = 515 ± 47 fmol/mg protein) and in the presence of 10 μM enhancer. (B) = K_D (nM), B_{max} (fmol/mg protein) and B_{max} shift obtained in [^{3}H]DPCPX saturation binding experiments performed in the absence (K_D = 1.8 ± 0.2 nM, B_{max} = 2950 ± 280 fmol/mg protein) and in the presence of 10 μM enhancer. (C) = K_i values of CCPA in the presence of 10 μM tested compounds and CCPA shift = K_i(CCPA)/K_i(CCPA + 10 μM enhancers) where the K_i of CCPA was 15.1 ± 1.6 nM.

Chart 1. Chemical Structure of PD 81,723



1, PD 81,723

enhancers at the highest concentration tested (10 μM). At 10-fold reduced concentration (1 μM), derivatives **8c**, **8e**−**f**, and **8i**−**k** cause a decrease of cAMP content higher than 45% (31% for PD 81,723), appearing to be considerably more active than PD 81,723. In addition, the compounds **8c**, **8e**−**f**, and **8j** caused significantly greater reductions of cAMP levels than PD 81,723 at 0.1 μM concentration. The *N*-methyl derivative (**8a**) appeared less active as an allosteric enhancer than the reference compound PD 81,723 at all concentrations tested. An increase in the lipophilicity of the alkyl group attached to N-4 position of the piperazine caused a marked decrease of cAMP production. In fact, the *N*-cyclohexyl analogue (**8b**) was more active than the *N*-methyl derivative (**8a**) at 1 and 10 μM concentration. Aromatization of the cyclohexyl group, to give the phenyl piperazine derivative **8c**, led to a substantial increase in activity. Compound **8c** had a significantly greater effect than PD 81,723 at concentrations of 0.1, 1, and 10 μM. Replacement of the phenyl moiety with a benzyl group (**8d**) was detrimental to the allosteric enhancement activity at 0.01, 0.1, and 1 μM concentrations. In fact, while **8c** and **8d** appeared to have similar efficacies at a concentration of 10 μM, this latter derivative was considerably less active than **8c** at each lower concentration studied.

As shown in Table 1, bioisosteric replacement of the phenyl ring with electron-deficient heterocycles with one nitrogen (pyridine, **8k**) or two nitrogens (pyrimidine, **8l**) led to a reduction in activity at 0.01−1.0 μM concentration, while the activity was substantially maintained at 10 μM. These derivatives were equally active at 10 μM of concentration, while **8l** was 2-fold less active than **8k** at 1 μM.

Several chemically different substituents (CH_3, 3,4-methylenedioxy, Cl, F, CN, CF_3) at the 4-position of the phenyl moiety linked to the piperazine were investigated. These modifications alter the electronic, steric, and lipophilic features of this residue. The presence of electron-withdrawing substituents led to an increase of the activity. The beneficial role of electron-withdrawing substituents was confirmed by the synthesis of the derivatives **8g** and **8h**, characterized by the presence of electron-releasing moieties on the phenyl ring. The 4-tolyl piperazine derivative (**8g**) was less active as an allosteric enhancer than the unsubstituted phenylpiperazine derivative (**8c**) at all concentrations tested, although it was similar to PD 81,723 at 1 and 10 μM of concentration. The presence of a less lipophilic and more electron-donating moiety (a 3,4-methylenedioxy group, derivative **8h**) further reduced allosteric enhancer activity. The replacement of the methyl with halogens (Cl and F) resulted in improved activity. While **8f** and **8c** showed the same allosteric enhancer activity at 1.0 and 10 μM of concentration, the compound **8f** was 2-fold more active than **8c** at 0.1 μM. By reducing the size of the halogen atom from chlorine to fluorine (derivative **8e**), the allosteric enhancer activity was maintained at 1.0 and 10 μM but led to reduced activity at lower concentration (0.1 μM) (Table 1).

Derivative **8j**, which possesses a lipohilic and strongly electron-withdrawing trifluoromethyl moiety, was the most active compound of the series. This derivative showed a similar inhibitory activity on cAMP production at 1.0 and 10 μM concentrations, reducing the cAMP levels by 84 and 87%, respectively. Interestingly, at 0.1 μM, compound **8j** showed a similar inhibitory activity as compound **8f** to reduce cAMP levels by 53 and 48%, respectively.

The ability of compounds **8a**−**l** to displace the binding of [^{3}H]DPCPX, [^{3}H]ZM241385, and [^{3}H]MRE3008F20 to hA_1CHO, hA_{2A}CHO, and hA_3CHO adenosine receptors were evaluated in competition binding experiments. None of the examined compounds significantly inhibited the specific binding of the radioligands to the hA_1, hA_{2A}, and hA_3 adenosine receptors, reaching a percentage of inhibition from 0 to 20% at 10 μM (Table 2). Binding and functional experiments demonstrated that it was possible to achieve a good separation between enhancing activity and the binding to the orthosteric site. Compound **8c** was more active than PD 81,723 in the enhancing

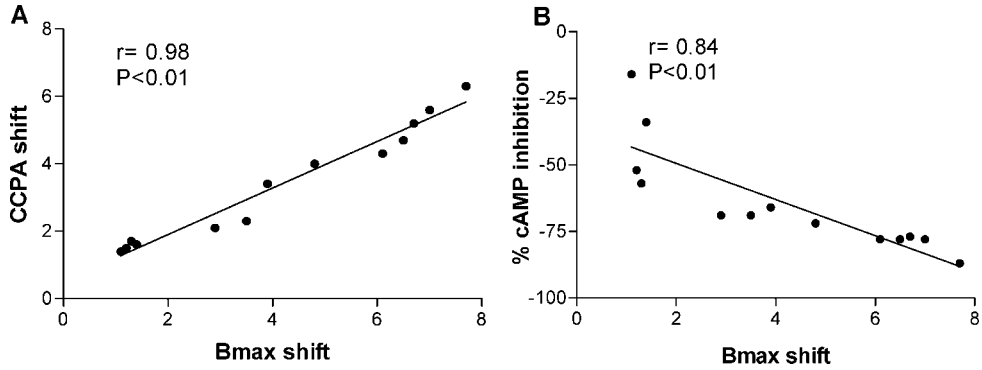


Figure 1. Comparison between binding and functional data of the examined compounds in hA_1CHO cells or membranes: (A) CCPA and B_{max} shift; (B) percentage of cAMP inhibition and B_{max} shift.

activity and at the same time was unable to bind hA_1, hA_{2A}, and hA_3 receptors, as suggested from competition binding experiments.[11]

Saturation binding experiments of the selective adenosine A_1 agonist [^{3}H]CCPA and A_1 antagonist [^{3}H]DPCPX were performed to verify if the novel compounds modified the A_1 binding parameters. From these experiments, A_1 receptor affinity (K_D), and density (B_{max}) were evaluated in the presence and in the absence of the examined compounds (PD 81,723 and **8a**−**l** at the concentration of 10 μM). No differences were found in the affinity as reported in Table 3. On the contrary, from the receptor density calculated in the presence and in the absence of enhancers, B_{max} shift was evaluated (Table 3, column A). In addition, [^{3}H]CCPA competition binding experiments were also performed with the aim to verify the specific binding (Bound shift) in the absence and in the presence of examined enhancers. In [^{3}H]CCPA saturation binding experiments, the reference compound PD 81,723 induced a B_{max} shift to human A_1 adenosine receptors of 1.3 fold. In the same experimental conditions, compounds **8c**−**f** and **8j** were significantly more potent than PD 81,723. The derivatives **8f** and **8j** were the most active compounds, causing a B_{max} shift of [^{3}H]CCPA binding to hA_1CHO cells of 7.0-fold and 7.7-fold, respectively. Analogous results were also obtained by evaluating Bound shifts: **8f** and **8j** caused a Bound shift of 6.2-fold and 6.6-fold, respectively. In addition, in [^{3}H]DPCPX saturation binding experiments, the tested compounds did not modify both affinity and receptor density in comparison with control condition (Table 3, column B). Interestingly, the presence of enhancers mediated an increase in B_{max} shift derived from [^{3}H]CCPA saturation binding experiments, suggesting the capability of novel compounds to mediate a shift from the ground state (R) to the activated state (R*) of the A_1 adenosine receptors. On the contrary, B_{max} values from [^{3}H]DPCPX saturation binding experiments was more than those obtained in [^{3}H]CCPA saturation binding experiments, suggesting that DPCPX as a typical adenosine antagonist was able to label both R and R* states of the A_1 adenosine receptors.

Table 3 also reports the derived apparent affinity (K_i) values for CCPA (column C), based on a one-state model of analysis, in the absence and in the presence of tested enhancers. This table also shows the CCPA shift representing the ratio of apparent K_i values in the absence and in the presence of the tested compounds at 10 μM concentration. In the hA_1CHO membranes, by using [^{3}H]DPCPX as radioligand, the K_i value of CCPA was 15.1 ± 1.6 nM. Interestingly, a significant decrease in the apparent K_i value was due to the presence of the putative allosteric enhancers, suggesting the increase of the high affinity binding sites. In the presence of PD 81,723, the affinity of CCPA increased of 1.7-fold. The CCPA affinity data in the presence of the derivatives **8b**−**g** and **8i**−**l** reveal that the displacement curves are shifted left, suggesting lower K_i values for CCPA. In particular, the largest affinity shift has been observed for compounds **8f** and **8j.** These molecules enhanced the apparent affinity of CCPA approximately of 5.6- and 6.3-fold, respectively (Table 3). The enhancers were able to mediate a shift of the A_1 receptors toward the high affinity state as suggested from the increase of the CCPA affinity expressed as K_i values (Table 3). Chart 1.

Figure 1 summarizes binding and functional data expressed as B_{max} and CCPA shift of the examined compounds. A very high correlation was found between the increase in B_{max} obtained from [^{3}H]CCPA saturation experiments and CCPA shift (Figure 1A). A good correlation was also found between B_{max} shift and cAMP production (Figure 1D) in the presence of examined compounds (10 μM), suggesting that the A_1 adenosine receptors revealed in binding experiments are also able to mediate an increase in functional response.

Conclusions

In conclusion, we have identified a novel series of allosteric enhancers of adenosine A_1 receptors. Some of these compounds (**8c**−**g** and **8i**−**l**) were better than the reference compound PD 81,723, with the 4-chlorophenyl **8f** and 4-trifluoromethyl **8j** derivatives being the most active compounds in binding (saturation and displacement experiments) and functional cAMP studies. The electronic effect of the substituents on the phenyl ring were important for biological activity, in particular if the compounds were tested at 1 μM concentration. Electron-withdrawing substituents at the 4-position of the phenylpiperazine moiety were preferred for enhancing activity, whereas electron-releasing groups such as the methyl and the 3,4-methylenedioxy were less favorable. The effects of compounds **8a**−**l** of functional assay (cAMP content) were consistent with their behavior in binding studies.

Experimental Section

General Procedure (A) for the Synthesis of Compounds 6a−**l.** To a stirred solution of compound **5** (900 mg, 1.6 mmol) in dry dichloromethane (5 mL) was added K_2CO_3 (1.1 equiv, 1.76 mmol, 243 mg). The mixture was cooled with a bath of ice/water, and then the appropriate N-substituted piperazine (2 equiv, 3.2 mmol) dissolved in dichloromethane (1 mL) was slowly added added in 0.5 h. The mixture was then stirred at room temperature for two hours, diluted with DCM (5 mL), and washed with water (5 mL) and then with brine (5 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo to give a brown residue that was purified by column chromatography to furnish the derivatives **6a**−**l**.

General Procedure (B) for the Synthesis of Compounds 7a−l. A solution of piperazine derivative **6a−l** (2 mmol) in DMF (20 mL), containing Et_3N (0.3 mL, 2 mmol, 1 equiv), was hydrogenated over 120 mg of 10% Pd/C at 60 psi for 3 h. The catalyst was removed by filtration, the filtrate was concentrated to give residue dissolved with dichloromethane (20 mL), washed with water (5 mL) and brine (5 mL), and dried (Na_2SO_4). The solvent was removed under reduced pressure to obtain a residue purified by column chromatography.

General Procedure (C) for the Synthesis of Compounds 8a−l. A stirred suspension of thiophene derivatives **7a−l** (0.5 mmol) and 100% hydrazine monohydrate (1.2 equiv, 0.6 mmol, 29 μL) in abs ethanol (10 mL) was refluxed for 3 h. After this time, the resulting solution was left at room temperature for 1 h. The reaction was finished after the complete solubilization of the starting material. The solvent was evaporated, and the residue was portioned between EtOAc (10 mL) and water (5 mL). The separated organic phase, washed with brine (2 mL) and dried, was then concentrated under vacuo to obtain a residue that was purified by column chromatography to give the desired products **8a−l**.

Acknowledgment. We thank King Pharmaceuticals for support of this research.

Supporting Information Available: Detailed biological protocols, synthesis and spectroscopic data for compounds **2−5** and **6−8a**,**l**, elemental analyses of compounds **8a−l**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) In our experiments, the reference compound PD 81,723 (at a concentration of 10 μM) did not inhibit [^{3}H]DPCPX binding to human A_1 receptors transfected in CHO cells. For the same reference compound, Bruns (ref 7) showed a K_i value of 11 μM obtained in competition binding experiments by using [^{3}H]DPCPX as radioligand on rat membranes. Furthermore, data performed on CHO-K1 cells stably expressing the human A_1 receptors (ref 10c) reported an inhibition of [^{3}H]DPCPX binding to human A_1 receptors by PD 81,723 only of 42 $\pm$ 7% when tested at 100 μM. We speculate that species differences in affinity binding of PD 81,723 may explain the discrepancy between the data. .

JM800586P