Synthesis and Biological Effects of Novel 2-Amino-3-naphthoylthiophenes as Allosteric Enhancers of the A₁ Adenosine Receptor

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The current study describes the synthesis and biological evaluation of a novel series of 2-amino-3-naphthoylthiophenes, with variable modifications at the 4- and 5-position of the thiophene as well as the naphthoyl ring. Allosteric enhancer activity was measured in several ways: (1) evaluating the effect on forskolin-stimulated cAMP accumulation in the presence of an A₁-adenosine agonist (CPA) in Chinese hamster ovary (CHO) cells expressing the cloned human A₁-adenosine receptor (hA₁AR); (2) ability of these compounds to displace the binding of [³H]DPCPX, [³H]ZM 241385, and [³H]MRE 3008F20 to the ligand binding site of CHO cells expressing the hA₁, hA₂ₐ, and hA₃ adenosine receptors, respectively; (3) effect on the binding of [³H]CCPA to membranes from CHO cells expressing hA₁AR, to rat brain and human cortex membrane preparations containing native adenosine A₁ receptors; (4) kinetics of the dissociation of [³H]CCPA from CHO-hA1 membranes. The pharmacological assays compared the various activities to that of the reference compound PD 81,723 (compound **1**). Several compounds appeared to be better than PD 81,723 to enhance the effect of CPA (and thus reduce cAMP content) in the CHO:hA₁ assay. The effect of these compounds at a concentration of 10 μM was slightly greater than that of the same concentration of the PD 81,723 and substantially greater than that of PD 81,723 when responses to 1 μM of each compound were compared. These include compounds **23**, **25−29**, **31−34**, **38**, **39**, **43**, and **58**. Cycloalkylthiophenes tended to be more potent then their 4,5-dimethyl analogues, and in the series of cycloalkylthiophenes, tetrahydrobenzo[*b*]thiophene derivatives appeared to be more potent than the dihydrocyclopentadien[*b*]thiophene counterparts. Some of the most potent compounds were tested at a concentration of 10 μM for their affinity as competitors to the antagonist binding site of CHO cells expressing hA₁, hA₂ₐ, and hA₃ adenosine receptors. None inhibited binding at the hA₂ₐ-AR, but most of them inhibited binding to the hA₁AR to varying extents (0−19%) as well as to the hA₃AR to a substantial degree (0−57%). At a concentration of 10μM, the compounds **31**, **34**, **37**, **38**, and **39** were more active than PD 81,723 to increase the binding of [³H]CCPA to CHO:hA₁, human brain and rat cortex membranes. Compound **37** was the most active compound increasing the binding to CHO:hA₁, human brain, and rat cortex membranes by 149, 43, and 27%, respectively (51, 15, and 22%, respectively, for PD 81,723). A good correlation was found between the increments [³H]CCPA binding to A₁ receptors expressed in different systems. Unlike the effect on agonist binding, the tested compounds did not increase the binding of the antagonist [³H]DPCPX on hCHO-A₁ membranes. Ligand dissociation studies revealed that two compounds (**22** and **39**) were more potent than **1** to slow the dissociation of [³H]CCPA from CHO:hA₁AR membranes. No clear-cut structure−activity relationship can be observed based on data from the functional assay, but we have identified several compounds, in particular **37** and **39**, which appeared to be more potent than **1** and that may be selected for further development.

Introduction

Allosteric effects are observed when there are interactions between two binding processes that occur simultaneously or sequentially: the binding of one ligand affects the binding of another ligand. The flexible nature of the interaction between the receptors and various allosteric modulators, together with the potential for subtype selectivity, make allosteric sites attractive for therapeutic intervention.[1] In the case of the GABA_A receptor, which is a transmitter-gated ion channel, the benzodiazepines acting on an allosteric site on the receptor showed substantial therapeutic effects and acceptable side effects.[2]

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Adenosine is an ubiquitous autocoid with multiple effects, which exerts its actions on the human body interacting with four different P_1-purinoreceptor subtypes classified as A_1, A_{2A}, A_{2B}, and A_3.[3] These receptor subtypes belong to the superfamily of G-protein-coupled receptors and are widely distributed throughout the body.[4] Extracellular adenosine, as a breakdown product of ATP, protects tissues from ischemic damage by lowering oxygen demand and increasing oxygen supply. Agents that increase the activation of A_1-adenosine receptor in response to adenosine would be useful in conditions characterized by a localized oxygen deficit, such as angina, myocardial infarction, and stroke.[5]

The adenosine A_1 receptor is coupled to Gi/Go protein signal transduction pathways and may mediate a number of biochemical processes such as the activation of several types of K^+ channels, inactivation of N-, P-, and Q-type Ca^{2+} channels, inhibition of adenylyl cyclase,[6] and activation of phospholipase C.

A variety of adenosine-mediated effects occurs via the A_1 adenosine receptors, highly and extensively expressed in the central nervous system (CNS), and in other tissues such as kidney, lung, bladder, and heart.[4] Thus, it would be of great therapeutic importance to have compounds that are able to enhance the activation of A_1-adenosine receptors by the endogenous ligand, adenosine, within specific target tissues. Such an opportunity of intervention is provided by the concept of allosteric modulation of G-protein-coupled receptors (GPCRs). This concept, investigated also in other classes of GPCRs, such as the muscarinic,[1b,1d] adrenergic,[7] D_2 dopamine,[8] 5-HT,[9] tachykinin NK-1 and NK-2,[10] angiotensin AT_1,[11] P_{2Y},[12] and oxytocin[13] receptors, is also demonstrated for the adenosine A_1 receptor.[14a,b]

Allosteric enhancers of the action of adenosine are believed to stabilize a conformation of the A_1-adenosine receptor that has a high affinity for agonists. This effect is manifested as a slowing of the rate of dissociation of agonist from the receptor.[14a] In addition, an allosteric enhancer appears to stabilize an active conformation of the receptor even in the absence of an agonist. Thus, in cells with A_1-adenosine receptors that are active spontaneously in the absence of an agonist, such as the chinese hamster ovary (CHO) cells used in our study, an allosteric enhancer may increase the number of receptors that are active at any given time, and thereby cause a change in cell function.[14c,d]

The currently available allosteric enhancers of agonist binding to the A_1 adenosine receptor have several nonspecific actions. These nonspecific actions include antagonism of the A_1 adenosine receptor [14a,b] and inhibition of the activity of adenylyl cyclase.[14d]

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.[14] Among the compounds tested by Bruns, it was demonstrated that PD 81,723 (2-amino-4,5-dimethylthien-3-yl)-[3-(trifluoromethyl)phenyl]methanone (**1**) represents a specific and selective allosteric enhancer of agonist binding to the A_1 receptor, with the best ratio of enhancement to antagonistic action at this receptor.[14b]

PD 81,723 showed to enhance agonist binding and the functional activation of the A_1 receptor in both brain and cardiovascular tissues.[15] While the exact molecular mechanism(s) through which PD 81,723 exerts its allosteric actions remain unknown, the available data indicate that PD 81,723 functions to stabilize a high affinity or agonist-preferring state of the A_1 receptor.[1c,14b,16]


1; PD 81,723
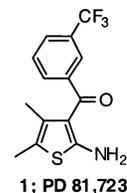
To study the role of various substitutions on the phenyl ring and the importance of the 4,5-dimethyl group on the thienyl ring, Baraldi,[17] IJzerman,[18] and Tranberg[19] have described the synthesis and biological evaluation of mostly novel PD 81,723 analogues. It was evident from previous SAR studies that substitution with electron-withdrawing substituents, such as chlorine and trifluoromethyl, on the benzoyl moiety at the 3-position of the thiophene ring resulted in higher enhancement activity.[17−19]

The purpose of our investigation was to synthesize and evaluate the allosteric enhancer activity of a new series of 52 derivatives of PD 81,723 (compounds **22**−**73** and **77**), where various modifications both on the naphthalene ring and on the 4- and 5-position of the thiophene system occurred, and to establish the structural requirements and the structure−activity relationship for enhancement of the action of an agonist at the human A_1-adenosine receptor. Previous studies by Bruns indicated that the addition of a fused ring on the thiophene provided improved allosteric enhancer activity. We sought to examine if this trend would also be observed with the more complex naphthoyl derivatives. Moreover, in the same series of 2-amino-3-benzoylthiophenes,[14a,b] the receptor environment neighboring the benzoyl binding site is lipophilic, and for this reason the substituents at the 4-position on the naphthalene were varied from H to groups with a different degree of lipophilicity (CH_3, CH_3O, Cl, Br, and I), and at 6-position from H to Cl. For substituents at the 4- and 5-position of the thiophene, we chose two methyl (obtaining 4,5-dimethylthiophenes) and an alkylene linkage between the 4- and 5-position, which varied from three to four methylenes to yield the 4,5,6,7-tetrahydrobenzo[*b*]thiophene and 5,6-dihydro-4*H*-cyclopentadien[*b*]thiophene derivatives, respectively. Finally, by the introduction of a nitrogen atom into the 6 position of the tetrahydrobenzo[*b*]thiophene ring we had the 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine derivatives. Among the synthesized compounds, some appeared superior to PD 81,723 in their enhancing activity.

Chemistry

The formation of 2-amino-3-benzoylthiophenes from the base-catalyzed condensation of carbonyl compounds and nitriles is achieved by the Gewald reaction.[20] This method was used for the synthesis of our new 2-amino-3-naphthoylthiophene derivatives **22**−**73** and **77** (Schemes 1−4) wherein the appropriate carbonyl com-

Scheme 1[a]
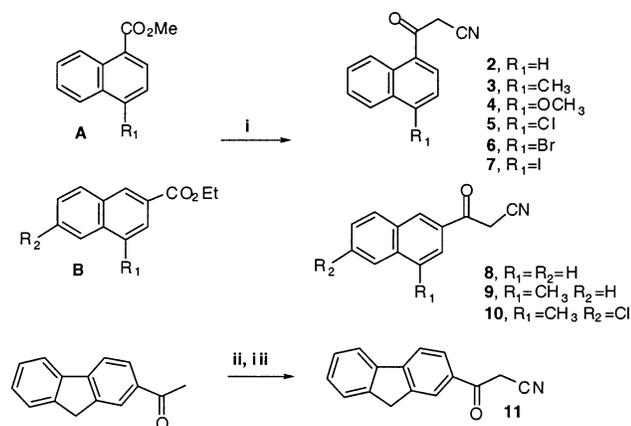
[a] Reagents and conditions. i: CH_3CN, NaH, toluene 90 °C, 18 h; ii: Br_2, AcOH, 1 h, rt; iii: KCN, EtOH/water, 2 h, rt.

Scheme 2[a]
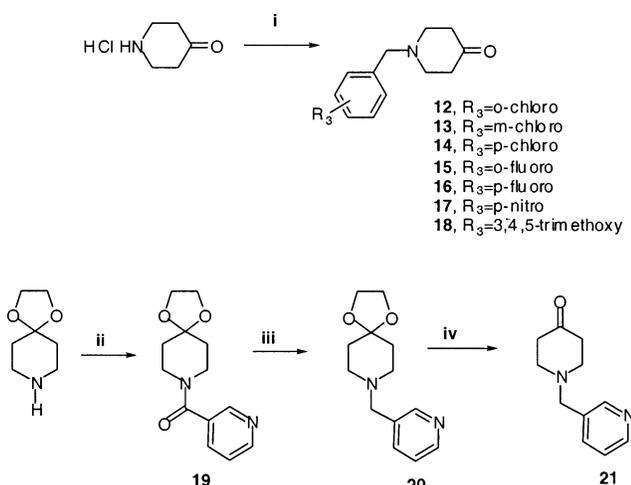
[a] Reagents and conditions. i: Benzyl chloride derivatives, DCM, TEA, reflux; ii: nicotinoyl chloride, DCM, TEA, reflux; iii: $LiAlH_4$, THF; iv: 10% HCl THF/water, reflux.

pounds were reacted with naphthoylacetonitrile derivatives **2–11** and sulfur in ethanol in the presence of morpholine, according to the route shown in Scheme 3. Only two compounds (**43** and **46**) of this series were reported previously by Tranberg.[19] The β-ketonitrile derivatives **2–10** (Scheme 1) were synthesized by condensation of the acetonitrile anion[21] with the appropriate substituted naphthoates with general formula **A**[22] and **B**.[23] The derivative **11** was synthesized starting from the commercially available 2-acetylfluorene. This was achieved by converting it to the corresponding acetyl bromide with bromine in acetic acid, followed by treatment with potassium cyanide.

The appropriate carbonyl compounds were commercially available except for the 4-benzylcyclohexanone,[24] 2-(4-methoxyphenyl)-[1,3]dithian-5-one,[25] *N*-[(3-pyridyl)methyl]-4-piperidone **21**, and the alkylated piperidones **12–18**.[26] The synthesis of these latter two series of compounds is described in Scheme 2. The preparation of alkylated piperidones **12–18** was performed following the procedure of IJzerman,[18a] which consists of the reaction of the commercially available hydrochloric salt of 4-piperidone with the appropriate substituted benzyl chloride. For the synthesis of *N*-[(3-pyridyl)methyl]-4-piperidone **21**, acylation of 4-piperidone ethylene acetal

with nicotinoyl chloride produced the amide **19** in good yield. Reduction with excess lithium aluminum hydride yielded the amine **20** that was finally transformed to the desired piperidone **21** after hydrolysis in aqueous HCl.

The synthesis of compounds **73** and **77** is shown in Scheme 4. The compound **73** was obtained by the Gewald procedure applied to β-ketonitrile **2** and 2-(4-methoxyphenyl)-[1,3]dithian-5-one **74**.[25] Compound **77** was synthesized by a three-step synthesis, starting from compound **34**. Acetylation of the amino group using a mixture of acetic anhydride and pyridine gave **75**. The subsequent dehydrogenation with Pd/C with heating furnished the benzo[*b*]thiophene derivative **76**, which was transformed by saponification into the desired product **77**.

The structures of the synthesized compounds and the yields of the syntheses are presented in Tables 1 and 2. The range of yields was 35–67%.

Results and Discussion

The purpose of our investigation was to assess a series of new compounds in which the 3-, 4-, and 5-positions of the thiophene ring were varied and to determine which among them were potentially allosteric enhancers of the action of adenosine to activate the human A_1-adenosine receptor. The reference compound for comparison was **1** (PD 81,723), and assays of allosteric enhancement were performed using Chinese hamster ovary (CHO) cells stably transfected to express the recombinant human A_1-adenosine receptors. Activation of these receptors causes an inhibition of the activity of adenylyl cyclase and a reduction of cAMP content of CHO cells. Allosteric enhancement was measured as the ability of the compounds **22–73** and **77** at four different concentrations (0.01, 0.1, 1, and 10 μM) to reduce the cAMP content of CHO:hA_1 cells. The results are shown in Table 3. It is important to note that compounds **70–73** were synthesized and biologically evaluated in their racemic forms.

The effect of each tested compound on cAMP content was presented as a percentage of the value of cAMP content in the absence of drug (control, 100%). A decrease of cAMP content is indicated in Table 3 as a negative percentage change of cAMP content from control (absence of compound) in the presence of the tested compound. Compounds with the potential to be allosteric enhancers of activation of human A_1-adenosine receptors decrease the content of cAMP in CHO cells expressing human A_1-adenosine receptors. Receptors in an active conformation in CHO:hA_1 cells cause a detectable inhibition of adenylyl cyclase activity. Allosteric enhancers are thought to stabilize the active conformation of the A_1-adenosine receptors, leading to a reduction in the cAMP content of the cells, whereas an increase of cAMP content is consistent with reduced activity of these receptors (i.e., receptor antagonism). Allosteric enhancers cannot be distinguished from agonists by use of this functional assay alone; then this assay assessed the overall effect for each tested compound as enhancer or antagonist at the A_1-adenosine receptor.

Among the synthesized compounds, the derivatives **22**, **29**, **35**, **37**, **46**, and **56** were comparable to PD 81,723 as allosteric enhancers, whereas the compound

Scheme 3[a]
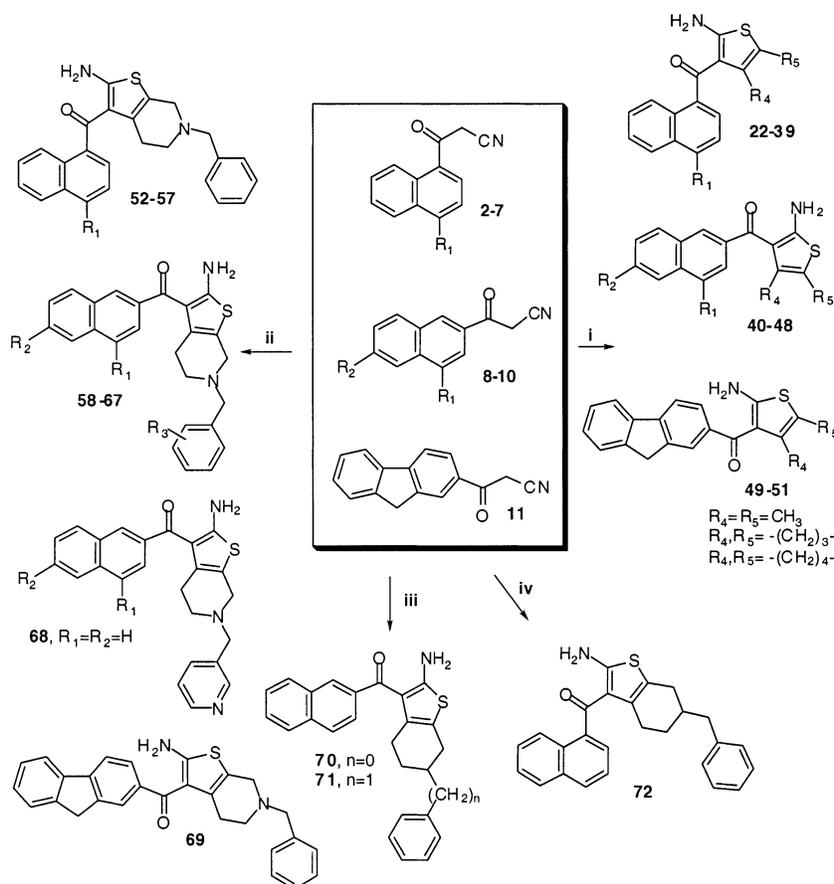
[a] Reagents and conditions. i: 2-Butanone (compounds with $R_4 = R_5 = CH_3$), cyclopentanone [compounds with R_4, $R_5 = -(CH_2)_3-$], cyclohexanone [compounds with R_4, $R_5 = -(CH_2)_4-$], S_8, morpholine, EtOH, 70 °C for 1 h then 18 h at rt; ii: *N*-benzylpiperidone or compounds **12−18** or **21**, S_8, morpholine, EtOH, 70 °C for 1 h then 18 h at rt; iii: compound **8**, 4-benzylcyclohexanone or 4-phenylcycloexanone, S_8, morpholine, EtOH, 70 °C for 1 h then 18 h at rt; iv: compound **1**, 4-benzylcyclohexanone, S_8, morpholine, EtOH, 70 °C for 1 h then 18 h at rt.

77 showed enhancement activity only at high concentration (10 μM). Several molecules appeared to be more potent than PD 81,723 as allosteric enhancers in the CHO:hA$_1$ assay. These include compounds **23**, **25−29**, **31−34**, **38**, **39**, **43**, and **58**. These latter compounds meet the following criteria: (1) they cause a decrease of cAMP content of CHO:hA$_1$ cells by more than 40% when present at a concentration of 10 μM; (2) they cause a decrease of cAMP content of at least 22% at a concentration of 1 μM; (3) they do not increase the content of cAMP of CHO:hA$_1$ cells at any concentration; (4) the response to the compound is concentration dependent.

Several compounds in the synthesized series appear to have activity as allosteric enhancer at a concentration of 10 μM, and most of these compounds have significant (i.e., causing ≥10% decrease of cAMP) activity at a concentration of 1 μM. Very few of the tested compounds increased the cAMP content of CHO:hA$_1$ cells (indicated as a positive change of cAMP content in Table 3). In contrast, DPCPX, the prototype antagonist (which is actually an inverse agonist of CHO:hA$_1$ receptors) increased the cAMP content of cells by 69% at a concentration of 1 μM (data not shown). It thus appears that none of the tested compounds was active (at 1 μM of concentration) as an inverse agonist of the human A1-adenosine receptor as well as DPCPX.

In the series of seven derivatives characterized by the presence of the same unsubstituted 1-naphthoyl moiety at the 3-position of the thiophene ring (compounds **22**, **28**, **34**, **52**, **72**, **73**, and **77**) and which differ in the C4- and C5-substituents, it appeared that the most potent compounds had a three- and four-carbon methylene linkage between the 4- and 5-position of the thiophene (compounds **28** and **34**, respectively). The presence of a methyl group in both of these positions (compound **22**) decreased slightly the potency at 10 μM, while compound **52**, which possess an *N*-benzyltetrahydropyrido-[*b*]thiophene system, showed activity as an allosteric enhancer at low concentrations (0.01−1 μM) and antagonistic activity at the highest concentration tested (10 μM). The insertion of two sulfur atoms and a *p*-methoxyphenyl ring at the 5-, 7-, and 6-position, respectively, of compound **34**, furnished compound **73** that does not demonstrate enhancement activity at any concentration. We have also introduced a benzyl group at the 7-position of the tetrahydrobenzo[*b*]thiophene ring of compound **34**, obtaining the derivative **72**. The effect of the introduction of this lipophilic substituent is detrimental for the allosteric enhancement activity. In fact, compound **72** is less active than **34** at any concentration studied.

Several chemically different substituents at the 4-position of the 1-naphthoyl moiety were investigated, and these modifications will alter the electronic, steric, and lipophilic features of this residue. In our first series of compounds, several groups having different lipophilic

Table 1. Physical and Synthetic Data for 2-Amino-3-aroylthiophenes **22−51**

compd	R$_1$	R$_2$	R$_4$	R$_5$	mp, °C	yield[a], %	formula[b]	anal
22	H		CH$_3$	CH$_3$	195−197	38	C$_{17}$H$_{15}$NOS	C, H, N
23	CH$_3$		CH$_3$	CH$_3$	171−173	59	C$_{18}$H$_{17}$NOS	C, H, N
24	OCH$_3$		CH$_3$	CH$_3$	138−140	39	C$_{18}$H$_{17}$NO$_2$S	C, H, N
25	Cl		CH$_3$	CH$_3$	175−177	48	C$_{17}$H$_{14}$ClNOS	C, H, N
26	Br		CH$_3$	CH$_3$	152−154	32	C$_{17}$H$_{14}$BrNOS	C, H, N
27	I		CH$_3$	CH$_3$	142−144	46	C$_{17}$H$_{14}$INOS	C, H, N
28	H		-(CH$_2$)$_3$-		255−257	89	C$_{18}$H$_{15}$NOS	C, H, N
29	CH$_3$		-(CH$_2$)$_3$-		233−235	56	C$_{19}$H$_{17}$NOS	C, H, N
30	OCH$_3$		-(CH$_2$)$_3$-		214−215	71	C$_{19}$H$_{17}$NO$_2$S	C, H, N
31	Cl		-(CH$_2$)$_3$-		258−260	71	C$_{18}$H$_{14}$ClNOS	C, H, N
32	Br		-(CH$_2$)$_3$-		210−212	67	C$_{18}$H$_{14}$BrNOS	C, H, N
33	I		-(CH$_2$)$_3$-		253−255	68	C$_{18}$H$_{14}$INOS	C, H, N
34	H		-(CH$_2$)$_4$-		137−140	73	C$_{19}$H$_{17}$NOS	C, H, N
35	CH$_3$		-(CH$_2$)$_4$-		211−212	79	C$_{20}$H$_{19}$NOS	C, H, N
36	OCH$_3$		-(CH$_2$)$_4$-		227−229	66	C$_{20}$H$_{19}$NO$_2$S	C, H, N
37	Cl		-(CH$_2$)$_4$-		179−181	68	C$_{19}$H$_{16}$ClNOS	C, H, N
38	Br		-(CH$_2$)$_4$-		176−178	68	C$_{19}$H$_{16}$BrNOS	C, H, N
39	I		-(CH$_2$)$_4$-		180−182	69	C$_{19}$H$_{16}$INOS	C, H, N
40	H	H	CH$_3$	CH$_3$	255−257	43	C$_{17}$H$_{15}$NOS	C, H, N
41	CH$_3$	H	CH$_3$	CH$_3$	120−122	35	C$_{18}$H$_{17}$NOS	C, H, N
42	CH$_3$	Cl	CH$_3$	CH$_3$	145−147	39	C$_{18}$H$_{16}$ClNOS	C, H, N
43	H	H	-(CH$_2$)$_3$-		178−180	50	C$_{18}$H$_{15}$NOS	C, H, N
44	CH$_3$	H	-(CH$_2$)$_3$-		65−67	38	C$_{19}$H$_{17}$NOS	C, H, N
45	CH$_3$	Cl	-(CH$_2$)$_3$-		195−197	55	C$_{19}$H$_{16}$ClNOS	C, H, N
46	H	H	-(CH$_2$)$_4$-		95−97	78	C$_{19}$H$_{17}$NOS	C, H, N
47	CH$_3$	H	-(CH$_2$)$_4$-		70−71	61	C$_{20}$H$_{19}$NOS	C, H, N
48	CH$_3$	Cl	-(CH$_2$)$_4$-		157−159	62	C$_{20}$H$_{18}$ClNOS	C, H, N
49			CH$_3$	CH$_3$	134−136	44	C$_{20}$H$_{17}$NOS	C, H, N
50			-(CH$_2$)$_3$-		176−178	68	C$_{21}$H$_{17}$NOS	C, H, N
51			-(CH$_2$)$_4$-		146−149	66	C$_{22}$H$_{19}$NOS	C, H, N

[a] Yield of synthesized compounds after purification by column chromatography. [b] All compounds were analyzed for C, H,N: analytical results were within 0.4% of theoretical value.

Scheme 4[a]
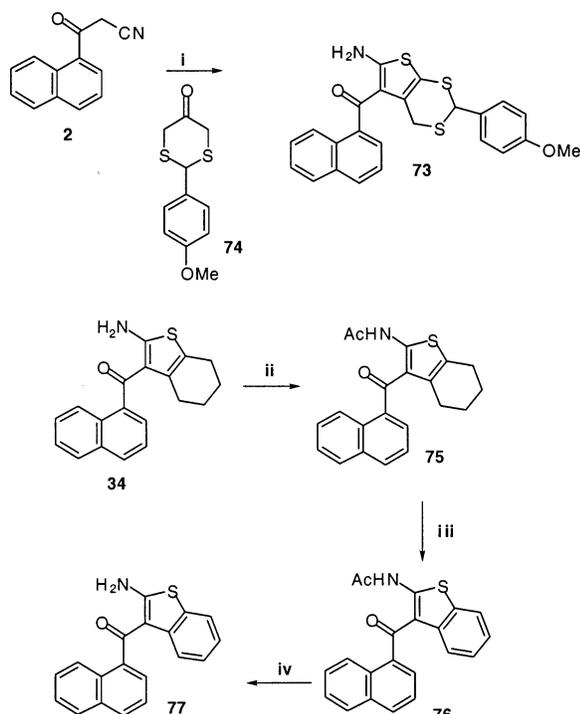
[a] Reagents and conditions. i: Ketone **74**, S$_8$, morpholine, EtOH, 70 °C for 1 h then 18 h at rt; ii: Ac$_2$O, pyridine , reflux; iii: 10%Pd/C moistened with a 50% of water, heating; iv: KOH, EtOH, reflux, 2 h.

character (CH$_3$, OCH$_3$, Cl, Br, I) were introduced. Starting from the derivatives **22**, **28**, **34**, and **52**, by the introduction of a lipophilic and electron-releasing methyl group at the 4-position of the naphthoyl ring, to obtain the derivatives **23**, **29**, **35**, and **53**, respectively, the allosteric enhancing activity was increased for compound **23** but decreased for the derivatives **29** and **35** with respect to the reference compounds **28** and **34**, respectively, and was substantially unchanged for the derivative **53**. However, compound **23** was more active both than PD 81,723 and the **22** counterparts at any concentration.

In the series of derivatives **23**, **29**, **35**, and **53**, the replacement of the methyl with a less lipophilic and more electron-releasing moiety (a methoxy group), to form derivatives **24**, **30**, **36**, and **54**, reduced allosteric enhancer activity. For these latter compounds, the reduction in activity may be attributed both to steric and electronic factors, as we have observed previously for the methoxybenzoyl counterparts.[17] In fact the methoxy group has an angularity component, i.e., part of the methoxy group can extend significantly above or below the plane of the naphthalene ring, and this introduces a unique steric parameter.

Otherwise, the substitution of the methyl with substituents showing similar electronic effects and different lipophilic characters (e.g., Cl, Br, and I) resulted in improved activity. It is noteworthy that although the introduction of a chlorine at the 4-position of the

Table 2. Physical and Synthetic Data for 2-Amino-3-aroylthiophenes **52**−**67**
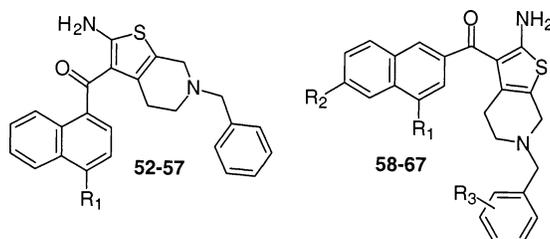
compd	R_1	R_2	R_3	mp, °C	yield[a], %	formula[b]	anal
52	H			178−180	65	$C_{25}H_{22}N_2OS$	C, H, N
53	CH_3			62−64	82	$C_{26}H_{24}N_2OS$	C, H, N
54	OCH_3			75−77	67	$C_{26}H_{24}N_2O_2S$	C, H, N
55	Cl			78−80	64	$C_{25}H_{21}ClN_2OS$	C, H, N
56	Br			68−70	63	$C_{25}H_{21}BrN_2OS$	C, H, N
57	I			110−112	59	$C_{25}H_{21}IN_2OS$	C, H, N
58	H	H	H	174−177	64	$C_{25}H_{22}N_2OS$	C, H, N
59	H	H	o-chloro	169−171	68	$C_{25}H_{21}ClN_2OS$	C, H, N
60	H	H	m-chloro	68−70	83	$C_{25}H_{21}ClN_2OS$	C, H, N
61	H	H	p-chloro	105−108	68	$C_{25}H_{21}ClN_2OS$	C, H, N
62	H	H	o-fluoro	183−185	70	$C_{25}H_{21}FlN_2OS$	C, H, N
63	H	H	p-fluoro	94−96	69	$C_{25}H_{21}FlN_2OS$	C, H, N
64	H	H	p-nitro	76−78	68	$C_{25}H_{21}N_3O_3S$	C, H, N
65	H	H	3,4,5-trimethoxy	127−129	89	$C_{29}H_{29}N_2O_4S$	C, H, N
66	CH_3	H	H	73−75	52	$C_{26}H_{24}N_2OS$	C, H, N
67	CH_3	Cl	H	148−149	32	$C_{26}H_{23}ClN_2OS$	C, H, N

[a] Yield of synthesized compounds after purification by column chromatography. [b] All compounds were analyzed for C, H, N: analytical results were within 0.4% of theoretical value.

Table 3. Percentage Change in CHO Cell CAMP Content in Presence of Compounds **22**−**67**, **73**, and **77**

compound	change in cAMP content from control (mean ± SEM)[a] concentration of compounds				compound	change in cAMP content from control (mean ± SEM)[a] concentration of compounds			
	0.01 μM	0.1 μM	1 μM	10 μM		0.01 μM	0.1 μM	1 μM	10 μM
PD 81,723	−1 ± 2	−7 ± 2	−13 ± 1	−50 ± 1	**48**	0 ± 2	1 ± 3	−11 ± 5	−13 ± 2
22	−0.6 ± 2	−3 ± 3	6 ± 5	−45 ± 2	**49**	−4 ± 5	−17 ± 3	−10 ± 5	−19 ± 6
23	−5 ± 1	−8 ± 3	−32 ± 2	−51 ± 4	**50**	−3 ± 5	−15 ± 3	−6 ± 5	−9 ± 6
24	9 ± 6	3 ± 10	−5 ± 9	−38 ± 9	**51**	7 ± 6	6 ± 5	7 ± 4	3 ± 3
25	12 ± 4	−18 ± 5	−47 ± 6	−56 ± 2	**52**	−21 ± 3	−7 ± 4	−25 ± 4	19 ± 2
26	−8 ± 4	−1 ± 4	−2 ± 3	−60 ± 3	**53**	0 ± 3	−7 ± 4	−13 ± 3	−34 ± 1
27	−5 ± 6	−3 ± 10	−19 ± 10	−63 ± 5	**54**	0 ± 4	11 ± 12	3 ± 8	−12 ± 5
28	−0.6 ± 3	−6 ± 1	−29 ± 4	−60 ± 1	**55**	3 ± 4	−1 ± 4	−7 ± 6	−21 ± 5
29	9 ± 4	4 ± 3	−17 ± 4	−48 ± 2	**56**	18 ± 2	−5 ± 2	−12 ± 1	−42 ± 3
30	−6 ± 3	−3 ± 4	−8 ± 4	−35 ± 3	**57**	−6 ± 3	1 ± 6	−6 ± 4	−13 ± 5
31	−10 ± 2	−15 ± 4	−27 ± 2	−55 ± 2	**58**	−11 ± 4	−12 ± 3	−18 ± 3	−60 ± 4
32	3 ± 3	19 ± 5	−14 ± 5	−67 ± 3	**59**	−10 ± 4	−20 ± 4	−10 ± 5	−17 ± 5
33	−6 ± 3	−8 ± 3	−22 ± 4	−75 ± 1	**60**	9 ± 8	−12 ± 4	7 ± 3	−3 ± 3
34	−11 ± 4	−15 ± 4	−22 ± 3	−52 ± 3	**61**	−6 ± 6	−8 ± 3	−21 ± 3	−6 ± 4
35	17 ± 5	13 ± 5	−21 ± 1	−45 ± 5	**62**	−3 ± 4	−3 ± 5	−10 ± 6	−31 ± 4
36	5 ± 4	−5 ± 4	−3 ± 3	−17 ± 1	**63**	−11 ± 6	−9 ± 5	−2 ± 4	−8 ± 3
37	−11 ± 3	3 ± 3	−25 ± 4	−44 ± 2	**64**	8 ± 6	12 ± 9	26 ± 13	26 ± 12
38	−6 ± 3	−10 ± 2	−29 ± 2	−72 ± 1	**65**	−3 ± 4	−1 ± 3	−10 ± 7	5 ± 5
39	−3 ± 4	−4 ± 4	−24 ± 5	−67 ± 2	**66**	0 ± 5	2 ± 10	0 ± 7	−16 ± 10
40	−1 ± 3	−4 ± 3	−8 ± 3	−28 ± 3	**67**	−1 ± 3	1 ± 4	−5 ± 3	−9 ± 6
41	4 ± 6	−3 ± 5	−9 ± 6	−22 ± 2	**68**	−8 ± 2	−12 ± 3	41 ± 12	56 ± 14
42	0 ± 5	0 ± 5	−18 ± 4	−24 ± 6	**69**	4 ± 7	−7 ± 7	8 ± 6	−1 ± 5
43	−19 ± 3	−14 ± 4	−15 ± 3	−51 ± 3	**70**	−8 ± 7	5 ± 3	31 ± 8	33 ± 13
44	0 ± 3	−7 ± 4	−13 ± 3	−34 ± 1	**71**	7 ± 5	10 ± 4	−9 ± 3	28 ± 10
45	4 ± 3	−14 ± 3	−6 ± 2	−17 ± 1	**72**	−8 ± 3	−7 ± 4	−14 ± 2	−21 ± 3
46	−13 ± 4	−24 ± 3	−28 ± 2	−42 ± 3	**73**	18 ± 6	16 ± 5	4 ± 5	6 ± 5
47	−7 ± 5	−9 ± 5	−8 ± 4	−20 ± 5	**77**	17 ± 5	9 ± 17	1 ± 8	−37 ± 6

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

1-naphthoyl moiety (compounds **25**, **31**, **37**, and **55**) had not a profound effect on their activity with respect to the 4-unsubstituted derivatives, the increase of the size of the halogen atom from chlorine to bromine (derivatives **26**, **32**, **38**, and **56**) to end with indole (compounds **27**, **33**, **39**, and **57**) caused a large increase of the allosteric enhancer activity, especially when a lipophilic substituent (methyl or cycloalkyl moiety) was present in 4- and 5-position of the thiophene ring.

These findings suggest that the introduction of a lipophilic halogen or methyl group at the 4-position of the naphthalene ring were preferred for enhancing activity, whereas a more hydrophilic group such as the methoxy was not favorable. The results indicated sig-

nificant qualitative direct correlation between the allosteric enhancer activity and the lipophilicity of the substitution at the 4-position of the 1-naphthoyl moiety, where the compounds characterized by the presence of substituents (Cl, Br, I) with higher lipophilic character were among the most potent as A_1 adenosine allosteric enhancers.

The electronic effect of the substituents is not relevant for biological activity, since substituents different in this respect (e.g., methyl and chlorine) confer similar enhancing effects (cf. **23** and **25**, **29** and **31**, **35** and **37**, **53** and **55**). This was confirmed comparing substituents showing similar electronic effect, i.e., chlorine with bromine and iodine (**25** vs **26** and **27**, **31** vs **32** and **33**, **37** vs **38** and **39**, **55** vs **56** and **57**).

To determine if changes in activity were correlated with physicochemical properties of the substituents, we have analyzed the allosteric enhancement obtained at 10 μM of concentration of each tested compound, using electronic (σ_p), lipophilic (π), and steric (molar refractivity, MR) parameters.[28] Unfortunately, a quantitative structure–activity analysis (QSAR) was not feasible for each of these parameters (r^2 for the regressions of enhancement activity on σ_p, π and MR parameters were <0.1 and were not significant, data not shown).

Molecules with the same substituents at different positions and which are characterized by the same lipophilicity have studied. This is the case of two groups of isomer derivatives, where the first group is constituted by the thiophene derivatives substituted in 3-position with the 1-naphthoyl and 2-naphthoyl moieties (compounds **22**, **28**, **34**, **52**, **72** and **40**, **43**, **46**, **58**, **71**, **77**, respectively). The second group included the derivatives modified on the naphthoyl ring and corresponding to 4-methyl-1-naphthoyl and 4-methyl-2-naphthoyl thiophene derivatives (compounds **23**, **29**, **35**, **53** and **41**, **44**, **47**, **66**, respectively).

In the first group of molecules, comparing the activities of derivatives bearing the same substituent on 4- and 5-position of the thiophene ring (**22** vs **40**, **28** vs **43**, **34** vs **46**, **52** vs **58**, **72** vs **71**), with the exception of the compound **58**, at high concentrations (10 μM) the derivatives with the 1-naphthoyl moiety are generally more potent than the corresponding analogues with the 2-naphthoyl substituent. This is confirmed also for the second group of derivatives, where with the exception of the compound **66**, the molecules which possess the 4-methyl-1-naphthoyl moiety (**23**, **29**, and **35**) are more potent then the analogues characterized by the presence of the 4-methyl-2-naphthoyl moiety (**41**, **44**, and **47**). Therefore, because molecules with equivalent lipophilicity may have very different levels of activity, we can conclude that lipophilicity is a necessary condition but not the only determining parameter for the allosteric enhancer activity.

In the series of 2-naphthoyl derivatives, only two compounds (**43** and **58**) are more active than PD 81,-723. For the derivative **58**, the introduction of different electron-withdrawing, electron-donating and hydrophobic substituents in the phenyl ring of the *N*-benzyl moiety (compounds **59**–**65**) was responsible for a marked decrease in their activity as A_1 allosteric enhancers. Only compounds **59** and **62**, which have the 2-fluoro and 2-chloro substituent, respectively, on the benzyl group

retained activity. The 4-substituted benzyls (**61** and **63**–**65**) were inactive. Starting from **58**, removal of the nitrogen from the 6-position of the tetrahydropyrido[*b*]-thiophene system, to yield **71**, resulted in a loss of allosteric enhancer activity, and this finding suggests that the nitrogen atom at the 6-position is required for the activity of compound **58**. In contrast, for compound **52** which showed a weak allosteric enhancer activity with respect to the isomer **58**, removal of the nitrogen from the 6-position has the effect to furnish a compound (**72**), which showed moderate enhancer potency at high concentration (10 μM). For the same compound **58**, the phenyl ring of the *N*-benzyl group has been replaced by a bioisosteric pyridine ring (compound **68**), Our objective behind introducing this polar bioisosteric moiety was to decrease the lipophilicity of **58** without compromising activity. Unfortunately, the compound **68** exhibited reduced potency at any concentration when compared to the counterpart **58**, and this result indicated that the exchange of the phenyl ring in the *N*-benzyl part of the molecule with a nitrogen-containing heterocycle did not result in a good bioisosteric replacement, and it was not tolerated in terms of allosteric enhancer activity at A_1 adenosine receptor.

In the series of derivatives which possess the 4-methyl-2-naphthoyl moiety (**40**, **44**, **47**, and **66**), the introduction of a lipophilic chloro atom at the 6-position of the naphthalene moiety did not improve the allosteric enhancer potency, which indicates an unfavorable steric interaction originating from the presence of 6-chloro substitution in compounds **42**, **45**, **48**, and **67**.

An increase of the lipophilic bulk at the 3-position of the thiophene ring by the introduction of a tricyclic 2-fluorenoyl moiety (compounds **49**–**51** and **69**) led to a substantial decline in potency.

A series of selected compounds which appeared to be better than (**28**, **31**, **32**–**34**, **38**, **39**, **43**, and **58**) or comparable (**22**, **30**, **37**, **40**, **42**, **44**, **46**, **62**, and **77**) to PD 81,723 as allosteric enhancers in the CHO:hA_1 assay were tested at a concentration of 10 μM for their affinity for subtypes of adenosine receptors. The ability of these compounds to displace the binding of [³H]DPCPX, [³H]-ZM 241385, and [³H]MRE 3008F20 to the ligand binding site of CHO:hA_1, CHO:hA_{2A}, and CHO:hA_3 adenosine receptors, respectively, is shown in Table 4. None inhibited binding at the hA_{2A}AR, but most of the subset inhibited binding to the hA_1AR to some extent (0–19%) and to the hA_3AR to a substantial degree (as high as 57%).

The prototype enhancer PD 81,723 did not inhibit the binding of a radiolabeled antagonist to A_1 and A_{2A} receptors, but it reduced by 21% the binding of [³H]MRE 3008F20 to the A_3 receptor. The derivatives **22** and **32** showed the same behavior, whereas compounds **28**, **33**, **42**, **44**, and **46** bound only to the A_1 receptor. For only two compounds (**40** and **31**) it was possible to achieve a good separation between enhancing activity and binding to the orthosteric site. Compound **31** was more active than PD 81,723 in its enhancing activity, and at the same time was devoid of activity on A_1, A_{2A} and A_3 receptors. All other compounds inhibited [³H]MRE 3008F20 binding to A_3 receptor in a manner which is better (**34**, **37**–**39**, **43**, and **83**) or comparable (**77** and **58**) to PD 81,723.

Table 4. Inhibition Activity of a Selected Number of Enhancer Compounds

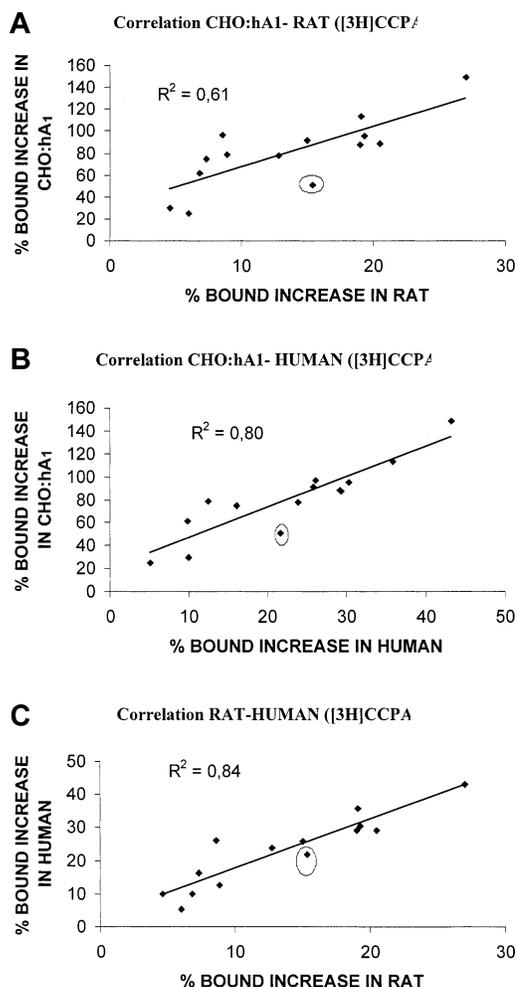| compound	% inhibition A_1[a]	% inhibition A_{2A}[b]	% inhibition A_3[c]
PD 81,723	0 ± 0	0 ± 0	21.0 ± 1.8
22	0 ± 0	0 ± 0	18.0 ± 2.3
28	8.0 ± 2.8	0 ± 0	0 ± 0
30	19.0 ± 1.3	0 ± 0	57 ± 2.9
31	0 ± 0	0 ± 0	0 ± 0
32	0 ± 0	0 ± 0	22.0 ± 4.6
33	3.3 ± 0.5	0 ± 0	0 ± 0
34	2.0 ± 1.4	0 ± 0	34.5 ± 3.7
37	4.8 ± 0.8	0 ± 0	30.5 ± 3.1
38	12.0 ± 1.4	3.0 ± 1.9	48.5 ± 6.3
39	15.3 ± 1.6	3.0 ± 1.7	48.0 ± 4.4
40	0 ± 0	0 ± 0	0 ± 0
42	4.0 ± 1.0	0 ± 0	0 ± 0
43	14.5 ± 1.8	0 ± 0	54.0 ± 8.9
44	6.0 ± 1.2	0 ± 0	0 ± 0
46	3.3 ± 0.2	0 ± 0	0 ± 0
58	2.5 ± 0.3	0 ± 0	22.0 ± 5.1
62	3.0 ± 0.7	0 ± 0	0 ± 0
77	13.5 ± 1.6	0 ± 0	19 ± 1.6

[a] Inhibition activity was expressed as percent displacement value ($\pm$STD, $n = 3$) of 1 nM of [³H]DPCPX by 10 μM of tested compound. [b] Inhibition activity was expressed as percent displacement value ($\pm$STD, $n = 3$) of 2 nM of [³H]ZM 241385 by 10 μM of tested compound. [c] Inhibition activity was expressed as percent displacement value ($\pm$STD, $n = 3$) of 2 nM [³H]MRE 3008F20 by 10 μM of tested compound.

Finally, CHO cells expressing the human adenosine A_1 receptor (Coho₁), and rat brain and human cortex membrane preparations containing native adenosine A_1 receptors were used to evaluate the effect of selected allotter enhancers on the binding of the radiolabel led agonist [³H]CCPA to A_1 receptors.

At higher concentrations some compounds produced inhibition of [³H]CCPA specific binding (data not shown). It should be mentioned, however, that the limited solubility of the tested compounds precluded the recording of a full concentration-effect curve. Figure 1 A-C shows the correlation between increases of [³H]CCPA binding to A_1 receptors in CHO:hA_1 and rat cortex membranes, CHO:hA_1 and human brain membranes and rat cortex and human brain membranes, respectively, caused by 10 μM concentration of a selected series of tested compounds. A good correlation was found between the increments [³H]CCPA binding to A_1 receptors expressed in different systems. Compound **37** at 10 μM was the most active compound increasing the binding of [³H]CCPA to CHO:hA_1, human brain, and rat cortex membranes by 149, 43, and 27%, respectively. Unlike the effect on agonist binding, the tested compounds did not increase the binding of the antagonist [³H]DPCPX on CHO:hA1 membranes. The results of these experiments demonstrated that our tested compounds significantly enhanced agonist, but not antagonist binding to the recombinant human A_1 adenosine receptors.

Ligand dissociation studies were conducted in an attempt to further elucidate putative mechanism(s) underlying the effects of the tested compounds to enhance A_1 agonist binding to hCHO-A1 membranes. Comparison of the dissociation rates ($t_{1/2}$ values) revealed that tested compounds (10 μM) significantly slowed the dissociation of [³H]CCPA from hCHO-A1 membranes (Table 5).

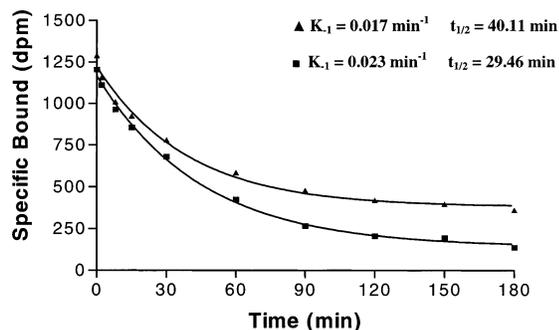Figure 2 shows a typical dissociation curve: the dissociation of [³H]CCPA was significantly decreased by

A Correlation CHO:hA1- RAT ([3H]CCP*А*

B Correlation CHO:hA1- HUMAN ([3H]CCP*А*

C Correlation RAT-HUMAN ([3H]CCPA

Figure 1. Comparison between % of bound increase values of [³H]CCPA binding to CHO:hA_1 and rat cortex (A), CHO:hA_1 and human brain (B), and rat cortex and human brain (C) membranes. The circle indicates PD 81,723 (compound **1**).

Table 5. Enhancer's Effects on [³H]CCPA Dissociation from A_1 Adenosine Receptors Transfected in CHO Cells[a]

| compound (10 μM)	% $t_{1/2}$ increase	% K_{-1} decrease
PD 81,723	43 ± 3	30 ± 3
22	85 ± 9	46 ± 4
28	0 ± 0	0 ± 0
31	43 ± 3	30 ± 3
32	4 ± 1	4 ± 1
33	41 ± 2	29 ± 2
34	29 ± 3	23 ± 2
37	36 ± 4	27 ± 2
38	43 ± 3	30 ± 3
39	60 ± 5	38 ± 4
43	42 ± 4	29 ± 3
46	10 ± 1	9 ± 1

[a] The results are the average of three experiments at 10 μM concentration of each tested compound.

37, as shown by the 36% increase in $t_{1/2}$. This particular behavior is of great importance because it allows us to analyze the enhancing activity of the tested compounds more directly through its influence on the dissociation rate of [³H]CCPA agonist for the A_1 adenosine receptor. Our data are in agreement with previous reports showing that the dissociation of agonist from the receptor was, in the presence of enhancer compounds, significantly decreased.[14,15b,18b,19,27]

To determine whether our compounds potentiate agonist binding to A_1 receptors, saturation-binding

Figure 2. Typical dissociation curve of [^{3}H]CCPA from A_1 adenosine receptors in CHO cells in the presence (▲) and absence (■) of enhancer **37** (10 μM).
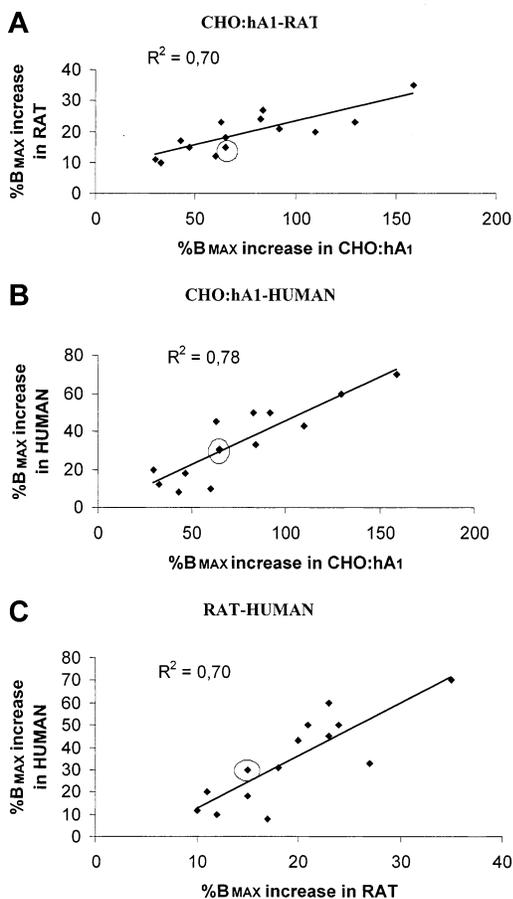
Figure 3. Comparison between % of B_{MAX} increase values of [^{3}H]CCPA binding to CHO:hA$_1$ and rat cortex (A), CHO:hA$_1$ and human brain (B), and rat cortex and human brain (C) membranes. The circle indicates PD 81,723 (compound **1**).

experiments were performed using membranes prepared from CHO:hA$_1$, rat cortex, and human brain. In the current study PD 81,723 increased the B_{MAX} of the agonist [^{3}H]CCPA to recombinant human A$_1$ adenosine receptors in CHO cells membranes and to A$_1$ receptors in human brain and rat cortex membranes.

Figure 3 A–C shows the correlation between increments in the maximum specific binding (B_{MAX}) of [^{3}H]-CCPA binding to A$_1$ receptors in CHO:hA1 and rat cortex membranes, CHO:hA1 and human brain membranes, and rat cortex and human brain membranes, respectively. A good correlation was found between the increments in B_{MAX} of [^{3}H]CCPA binding to A$_1$ receptors expressed in different systems. The B_{MAX} of the A$_1$

receptor agonist [^{3}H]CCPA increased up to 159% (in CHO:hA1 membranes) in the presence of **37** (10 μM). The K_D value of [^{3}H]CCPA was not significantly affected by the allosteric enhancers. In contrast to the effect of our tested compounds on agonist binding, the B_{MAX} and K_D of the A$_1$ antagonist [^{3}H]DPCPX was not significantly different in the absence and presence of test compounds (data not shown).

Conclusions

We have identified novel allosteric enhancers of agonist binding to adenosine A$_1$ receptors. Some of the compounds proved superior to the reference enhancer PD 81, 723. With the exception of compounds **32** and **38**, the tetrahydrobenzo[*b*]thiophene derivatives appeared to be more potent than the dihydrocyclopentadien[*b*]thiophene counterparts.

In the series of 4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine derivatives **52**−**69**, the only compound, which showed significant allosteric enhancer activity at any concentration, was derivative **58**. In the series of compounds **58**−**65**, which bear the 2-naphthoyl moiety and different benzyl derivatives on position 6, the benzyl moiety was the best substituent whereas the substitution of the benzene with a pyridine caused a loss of enhancing activity (compound **68**). Indeed, only the unsubstituted *N*-benzyl derivative **58** possessed an activity superior to PD 81,723 at any concentration. The lack of activity of **71** (which does not contain the ring nitrogen) supports the importance of the tetrahydropyridyl ring nitrogen for the allosteric enhancer activity.

Of all compounds synthesized for this study, only two (**43** and **46**) were synthesized and studied by Tranberg[19] (see Table 3). Our results are consistent with the results reported by Tranberg, even though different assays and different sources of adenosine receptors were used in these studies. However, it should be noted that our assay of effects of putative enhancers on cAMP content of CHO cells expressing human A$_1$-adenosine receptors is not a specific assay of allosteric enhancement of agonist binding. Our assay does not directly measure the interaction between receptor activation and G-protein activation, and our observations may be complicated by drug actions not related to enhancement, such as cell toxicity. However, the effect of a tested compound in the intact cell cAMP assay used in this study may be a more useful predictor of the effect of the compound in vivo than a binding assay that more specifically assesses allosteric enhancement. It is intended that these derivatives may be of help in better understanding adenosine receptor function. We propose to continue this study focusing our efforts on the evaluation of their enhancing activity at A$_{2A}$ and A$_3$[29] adenosine receptors expressed in CHO cells by means of radioligand binding studies performed with the agonists [^{3}H]CGS 21680 and [^{125}I]AB-MECA for A$_{2A}$ and A$_3$, respectively.

Experimental Section

Abbreviations. ADA, adenosine deaminase; [^{3}H]DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; [^{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]CHA, N^6-[adenine-2,8-3*H*]cyclohexyladenosine; [^{3}H]CCPA, 2-chloro-N^6-cyclopentyladenosine; [^{3}H]ZM 241385, [2-3*H*](4-(2-[7-amino-

2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)-phenol); *R*-PIA, *R*-N^6-(2-phenylisopropyl)adenosine, CHO, Chinese hamster ovary.

Chemistry. Materials and Methods. Cyclopentanone, cyclohexanone, 2-butanone, 2-acetylfluorene, 4-phenylcyclohexanone, and 4-benzylpiperidone are commercially available, and they have been purchased from Aldrich. ^{1}H NMR spectra were recorded on a Bruker AC 200 spectrometer. Chemical shifts (δ) are given in ppm upfield from tetramethylsilane as internal standard, and the spectra were recorded in appropriate deuterated solvents indicated in the procedure. Melting points (mp) were determined on a Buchi-Tottoli apparatus and are uncorrected. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. Elemental analyses were conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara. All reactions were carried out under an inert atmosphere of dry nitrogen, unless otherwise described. Standard syringe techniques were applied for transferring dry solvents. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F_{254} Merk plates) and visualized with aqueous $KMnO_4$. Flash chromatography was performed using 230−400 mesh silica gel and the solvent system indicated in the procedure. All commercially available compounds were used without further purification. Organic solutions were dried over anhydrous Na_2SO_4. Dioxane was distilled from calcium hydride, and dry DMF was distilled from calcium chloride and stored over molecular sieves (3 Å). In high-pressure hydrogenation experiments, a Parr shaker on a high-pressure autoclave was used.

General Procedure for the Synthesis of Compounds 15 and 18. To a suspension of 4-piperidone hydrochloride (10 mmol) in CH_2Cl_2 (20 mL) cooled at 0 °C were added the appropriate substituted benzyl chloride (11 mmol) and TEA (22 mmol). The mixture was heated at reflux overnight, diluted with CH_2Cl_2 (20 mL), and then washed with water and brine. The organic layer was separated, dried (Na_2SO_4), and evaporated to give a residue, which was purified by column chromatography on silica gel using EtOAc−petroleum ether (1:1, v/v) as eluent.

1-(2-Fluorobenzyl)-4-piperidone (15). Yield 56%, oil. ^{1}H NMR (CDCl$_3$) δ: 2.46 (t, 4H, $J = 6.2$ Hz), 2.79 (t, 4H, $J = 6.0$ Hz), 3.71 (s, 2H), 7.05 (t, 1H, $J = 7.2$ Hz), 7.11 (t, 1H, $J = 7.2$ Hz), 7.26 (m, 1H), 7.39 (t, 1H, $J = 7.4$ Hz).

1-(3,4,5-Trimethoxybenzyl)-4-piperidone (18). Yield 63%, mp 102−104 °C. ^{1}H NMR (CDCl$_3$) δ: 2.46 (t, 4H, $J = 6.2$ Hz), 2.75 (t, 4H, $J = 6.0$ Hz), 3.55 (s, 2H), 3.84 (s, 3H), 3.88 (s, 6H), 6.60 (s, 2H).

Synthesis of 1-Nicotinoyl-4-piperidone Ethylene Acetal (19). 4-Piperidone ethylene acetal (1.43 g., 10 mmol) was dissolved into 15 mL of dry methylene chloride solution with triethylamine (7 mL, 50 mmol). Nicotinoyl chloride (1.42 g, 10 mmol) dissolved in dry methylene chloride (10 mL) was then added to the solution in a dropwise manner. The solution was stirred under nitrogen overnight. The organic phase was washed with water (5 mL), brine, dried over Na_2SO_4, and finally concentrated under vacuo to give a crude product which was then purified by flash chromatography over a silica gel column (eluent EtOAc−petroleum ether 1:1, v/v), to yield **19** as a white solid. Yield 82% (1.92 g.), mp = 113−114 °C. ^{1}H NMR (CDCl$_3$) δ: 1.68 (m, 2H), 1.77 (m, 2H), 3.50 (m, 2H), 3.86 (m, 2H), 3.99 (s, 4H), 7.37 (dd, 1H, $J = 4.8$ and 3 Hz), 7.75 (d, 1H, $J = 7.6$ Hz), 8.66 (m, 2H).

Synthesis of 1-[(3-Pyridyl)methyl)]-4-piperidone Ethylene Acetal (20). Lithium aluminum hydride (810 mg, 21 mmol) was suspended in 20 mL of dry tetrahydrofuran (THF), and the solution was cooled in an ice bath. The compound **19** (1.17 g, 5 mmol) dissolved in 10 mL of THF was added dropwise to the cold solution. At the end of the addition, the mixture was placed at rt, and after 4 h the unreacted LiAlH$_4$ was quenched by careful addition of an excess amount of 10% NaOH solution. The solution was filtered, and the residue was repeatedly washed with an excess amount of EtOAc. The combined extract was dried and concentrated

under vacuo, and the product, as a colorless liquid, was used in the next reaction without any further purification. Yield 54% (594 mg) ^{1}H NMR (CDCl$_3$) δ: 1.74 (t, 4H, $J = 5.8$ Hz), 2.53 (t, 4H, $J = 6.2$ Hz), 3.53 (s, 2H), 3.96 (s, 4H), 7.28 (m, 1H), 7.67 (d, 1H, $J = 7.8$ Hz), 8.49 (d, 1H, $J = 4.4$ Hz), 8.54 (s, 1H).

Synthesis of 1-[(3-Pyridyl)methyl)]-4-piperidone (21). The compound **20** (920 mg, 4.18 mmol) was taken up in a mixture of THF (20 mL) and 2 N HCl (10 mL) and stirred at rt for 20 h. The solution was carefully basified with solid NaHCO$_3$, the THF was evaporated, and the remainder was partitioned between EtOAc and water. The organic layer was dried and evaporated and the residue purified by flash column chromatography (silica gel) using EtOAc−MeOH 8:2 as eluent. The compound **21** (455 mg, 57% yield) was obtained as a colorless liquid. ^{1}H NMR (CDCl$_3$) δ: 2.46 (t, 4H, $J = 6.2$ Hz), 2.76 (t, 4H, $J = 6.2$ Hz), 3.66 (s, 2H), 7.33 (dd, 1H, $J = 7.8$ and 4.8 Hz), 7.77 (d, 1H, $J = 8.0$ Hz), 8.53 (d, 1H, $J = 3.6$ Hz), 8.60 (s, 1H).

Synthesis of 2-Bromoacetylfluorene. To a solution of 2-acetylfluorene (2.08 g, 10 mmol) in 10 mL of glacial acetic acid, bromine (10 mmol, 0.51 mL) was added dropwise and the mixture stirred at room temperature for 2 h. After this time, the acetic acid was evaporated under reduced pressure at a temperature lower than 40 °C. The crude product so obtained was used for the next reaction without purification.

Synthesis of 2-Fluorenoylacetonitrile. The crude 2-bromoacetylfluorene previously prepared (2.87 g, 10 mmol) was dissolved in 95% EtOH (15 mL). A solution of potassium cyanide (3.6 g, 55 mmol), dissolved in water (10 mL), was added in one portion, and the mixture was stirred at room temperature (rt) for 24 h. The mixture was then poured onto a mixture of crushed ice and water and acidified with glacial acetic acid (pH = 5−6). The resulting solid was collected by filtration and washed with water. Yield = 83%; mp 52−155 °C. ^{1}H NMR (CDCl$_3$) δ: 3.98 (s, 2H), 4.14 (s, 2H), 7.44 (m, 2H), 7.62 (m, 1H), 7.91 (m, 3H), 8.11 (s, 1H).

General Procedure for the Synthesis of Compounds 2−10 (Step A). CH$_3$CN (8 mL, 152 mmol) was added dropwise to a suspension of the appropriate methyl ester with general formula **B** or **C** (50 mmol) and 50% NaH (50 mmol) in toluene (75 mL) under stirring at 90 °C After 24 h, the mixture was cooled at rt and the precipitate was filtered and washed with toluene (25 mL). The solid was then dissolved in water, the solution cooled at 0 °C, and the pH brought to 2 with 2 N HCl. The suspension was extracted with EtOAc (4 × 50 mL), the recombined organic layers were washed with brine, dried over Na$_2$SO$_4$, and concentrated to give the corresponding naphthoylacetonitrile **2−10** as a solid, used without any further purification for the next reaction.

2-(Naphthalene-1-carbonyl)acetonitrile (2). Yield: 78%; mp 85−86 °C. ^{1}H NMR (CDCl$_3$) δ: 4.21 (s, 2H), 7.71 (m, 2H), 7.92 (m, 1H), 8.13 (d, 1H, $J = 7.8$ Hz), 8.62 (d, 1H, $J = 8.4$ Hz), 8.76 (d, 1H, $J = 8.8$ Hz), 8.84 (d, 1H, $J = 8.4$ Hz).

2-(4-Methylnaphthalene-1-carbonyl)acetonitrile (3). Yield: 78%; mp 133−134 °C. ^{1}H NMR (CDCl$_3$) δ: 2.77 (s, 3H), 4.18 (s, 2H), 7.38 (d, 1H, $J = 7.4$ Hz), 7.66 (m, 2H), 7.79 (d, 1H, $J = 7.6$ Hz), 8.06 (d, 1H, $J = 7.4$ Hz), 8.91 (d, 1H, $J = 7.4$ Hz).

2-(4-Methoxynaphthalene-1-carbonyl)acetonitrile (4). Yield = 74%, mp 178−180 °C. ^{1}H NMR (CDCl$_3$) δ: 4.06 (s, 3H), 4.47 (s, 2H), 6.88 (d, 1H, $J = 8.2$ Hz), 7.67 (m, 2H), 8.10 (d, 1H, $J = 8.4$ Hz), 8.31 (m, 1H), 9.01 (d, 1H, $J = 8.4$ Hz).

2-(4-Chloronaphthalene-1-carbonyl)acetonitrile (5). Yield 74%; mp 123−124 °C. ^{1}H NMR (CDCl$_3$) δ: 4.19 (s, 2H), 7.73 (m, 4H), 8.38 (d, 1H, $J = 9.8$ Hz), 8.84 (d, 1H, $J = 9.8$ Hz).

2-(4-Bromonaphthalene-1-carbonyl)acetonitrile (6). Yield: 72%; mp 109 °C. ^{1}H NMR (CDCl$_3$) δ: 4.21 (s, 2H), 7.63 (m, 2H)0.7.91 (m, 2H), 8.09 (d, $J = 8.0$ Hz, 1H), 8.81 (d, $J = 8.4$ Hz, 1H).

2-(4-Iodonaphthalene-1-carbonyl)acetonitrile (7). Yield: 66%; mp 123 °C. ^{1}H NMR (CDCl$_3$) δ: 4.23 (s, 2H), 7.64

(m, 2H)0.7.90 (d, J = 7.8 Hz, 1H), 8.03 (d, J = 8.2 Hz, 1H), 8.20 (d, J = 7.8 Hz, 1H), 8.93 (d, J = 8.2 Hz, 1H).

2-(Naphthalene-2-carbonyl)acetonitrile (8). Yield: 81%; mp 124 °C. ^{1}H NMR (d_6-DMSO) δ: 4.91 (s, 2H), 7.67 (m, 2H), 8.03 (m, 4H), 8.61 (s, 1H).

2-(4-Methylnaphthalene-2-carbonyl)acetonitrile (9). Yield: 67%; mp 136 °C.^{1}H NMR (CDCl$_3$) δ: 2.73 (s, 3H), 4.46 (s, 2H), 7.48 (s, 1H), 7.57 (m, 2H), 7.83 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H).

2-(4-Methyl-6-chloronaphthalene-2-carbonyl)acetonitrile (10). Yield: 78%; mp 201 °C.1H NMR (CDCl$_3$) δ: 2.61 (s, 3H), 4.55 (s, 2H), 7.49 (m, 1H), 7.87 (m, 2H), 7.97 (s, 1H), 8.35 (s, 1H).

General Procedure for the Synthesis of Compounds 22−67 (Step B). A mixture of aroylacetonitrile (5 mmol, prepared by Step A), appropriate ketone (5 mmol), morpholine (0.44 mL, 5 mmol), and sulfur (164 mg, 5 mmol) was heated at 70° C for 1 h and then stirred at room temperature for 20 h. At the end of this period, the solvent was evaporated under reduced pressure and the residue diluted with ethyl acetate. After washing with water, the organic layer was dried, filtered, and then evaporated. The crude product was purified by flash column chromatography and then recrystallized from petroleum ether.

2-Amino-4,5-dimethylthiophen-3-yl)naphthalen-1-yl-methanone (22). 2-Butanone, 2-(naphthalen-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **22**. IR (KBr) cm^{-1}: 3358, 3242, 1576, 1426, 1282, 1253, 781; ^{1}H NMR (CDCl$_3$): δ 1.16 (s, 3H), 2.07 (s, 3H), 7.17 (bs, 2H), 7.49 (m, 4H), 7.89 (m, 3H).

(2-Amino-4,5-dimethylthiophen-3-yl)(4-methylnaphthalen-1-yl)methanone (23). 2-Butanone, 2-(4-methylnaphthalen-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **23**. IR (KBr) cm^{-1}: 3368, 1576,; 1429, 1256, 759. ^{1}H NMR (CDCl$_3$) δ: 1.17 (s, 3H), 2.06 (s, 3H), 2.73 (s, 3H), 7.04 (bs, 2H), 7.31 (m, 2H), 7.53 (m, 2H), 7.93 (d, 1H, J = 7.8 Hz), 8.05 (d, 1H, J = 7.8 Hz).

(2-Amino-4,5-dimethylthiophen-3-yl)(4-methoxynaphthalen-1-yl)methanone (24). 2-Butanone, 2-(4-methoxynaphthalen-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **24**. IR (KBr) cm^{-1}: 3354, 1582, 1416, 1242, 1087, 763. ^{1}H NMR (CDCl$_3$) δ: 1.27 (s, 3H), 2.07 (s, 3H), 4.05 (s, 3H), 6.83 (m, 3H), 7.37 (d, 1H, J = 8 Hz), 7.48 (m, 2H), 7.97 (m, 1H), 8.31 (m, 1H).

(2-Amino-4,5-dimethylthiophen-3-yl)(4-chloronaphthalen-1-yl)methanone (25). 2-Butanone, 2-(4-chloronaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **25**. IR (KBr) cm^{-1}: 3248, 1575, 1427, 1252, 965, 760. ^{1}H NMR (CDCl$_3$) δ: 2.06 (s, 9H), 7.19 (bs, 2H), 7.58 (m, 4H), 7.90 (d, 1H, J = 7.6 Hz), 8.34 (d, 1H, J = 7.6 Hz).

(2-Amino-4,5-dimethylthiophen-3-yl)(4-bromonaphthalen-1-yl)methanone (26). 2-Butanone, 2-(4-bromonaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **26**. IR (KBr) cm^{-1}: 3241, 1575, 1426, 1251, 781. ^{1}H NMR (CDCl$_3$) δ: 2.06 (s, 3H), 2.18 (s, 3H), 7.44 (m, 1H), 7.52 (m, 4H), 7.86 (m, 1H), 7.98 (m, 1H), 8.44 (m, 1H).

(2-Amino-4,5-dimethylthiophen-3-yl)(4-iodonaphthalen-1-yl)methanone (27). 2-Butanone, 2-(4-iodonaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **27**. IR (KBr) cm^{-1}: 3044, 1673, 1592, 1415, 1300, 1251, 773. ^{1}H NMR (CDCl$_3$) δ: 2.18 (s, 6H), 7.62 (m, 3H), 7.87 (m, 2H), 7.94 (d, 1H, J = 8.2 Hz), 8.43 (d, 1H, J = 8.6 Hz), 9.08 (d, 1H, J = 8.6 Hz).

2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophen-3-yl)-naphthalen-1-ylmethanone (28). Cyclopentanone, 2-(naphthalen-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **28**. IR (KBr) cm^{-1}: 3328, 3102, 2853, 1582,

1444, 1290, 1255, 1032, 780; ^{1}H NMR (CDCl$_3$): δ 1.28 (m, 2H), 1.87 (t, 2H, J = 7.0 Hz), 2.54 (t, 2H, J = 7.2 Hz), 7.41 (d, 1H, J = 6.4 Hz), 7.59 (m, 3H), 7.73 (d, 1H, J = 8.8 Hz), 8.02 (d, 2H, J = 8.6 Hz), 8.73 (bs, 2H).

(2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophen-3-yl)-(4-methylnaphthalen-1-yl)methanone (29). Cyclopentanone, 2-(4-methylnaphthalen-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **29**. IR (KBr) cm^{-1}: 3354, 1583, 1435, 1290, 1256, 1019, 835. ^{1}H NMR (CDCl$_3$) δ: 1.51 (m, 2H), 1.93 (t, 2H, J = 7.6 Hz), 2.58 (t, 2H, J = 7.6 Hz), 2.73 (s, 3H), 7.19 (m, 2H), 7.27 (m, 2H), 7.49 (m, 2H), 7.90 (d, 1H, J = 7.6 Hz), 8.03 (d, 1H, J = 7.6 Hz).

2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophen-3-yl-(4-methoxynaphtha-len-1-yl)methanone (30). Cyclopentanone, 2-(4-methoxynaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **30**. IR (KBr) cm^{-1}: 3351, 3243, 2852, 1581, 1433, 1261, 1242, 1162, 1091, 1023, 822, 762, 712; ^{1}H NMR (CDCl$_3$): δ 1.59 (m, 2H), 1.96 (m, 2H), 2.59 (t, 2H, J = 7.6 Hz), 4.04 (s, 3H), 6.79 (d, 1H, J = 8 Hz), 7.12 (bs, 2H), 7.34 (d, 1H, J = 8 Hz), 7.48 (m, 2H), 7.89 (m, 1H), 8.30 (m, 1H).

2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophen-3-yl-(4-chloronaphthalen-1-yl)methanone (31). Cyclopentanone, 2-(4-chloronaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **31**. IR (KBr) cm^{-1}: 3348, 3229, 3119, 1571, 1443, 1359, 1272, 1253, 1195, 962, 835, 787, 759; ^{1}H NMR (CDCl$_3$): δ 1.06 (m, 2H), 1.84 (t, 2H, J = 7.2 Hz), 2.52 (m, 2H), 7.37 (d, 1H, J = 6.8 Hz), 7.71 (m, 4H), 8.24 (d, 1H, J = 8.8 Hz), 8.70 (bs, 2H).

2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophen-3-yl-(4-bromonaphthalen-1-yl)methanone (32). Cyclopentanone, 2-(4-bromonaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **32**. IR (KBr) cm-1: 3333, 3110, 2851, 1576, 1443, 1289, 1254, 1032, 804, 780; ^{1}H NMR (CDCl$_3$): δ 1.29 (m, 2H), 1.81 (t, 2H, J = 6.8 Hz), 3.31 (m, 2H), 7.34 (m, 1H), 7.53 (m, 4H), 7.71 (m, 1H), 7.95 (m, 1H), 8.64 (m, 1H).

2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophen-3-yl)-(4-iodonaphthalen-1-yl)methanone (33). Cyclopentanone, 2-(4-iodonaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **33**. IR (KBr) cm^{-1}: 3329, 3221, 3106, 2852, 1581, 1444, 1290, 1254, 780; ^{1}H NMR (CDCl$_3$): δ 1.28 (m, 2H), 1.81 (t, 2H, J = 7 Hz), 3.34 (m, 2H), 7.36 (d, 1H, J = 6.8 Hz), 7.54 (m, 2H), 7.66 (d, 1H, J = 9.0 Hz), 7.97 (d, 2H, J = 8.6 Hz), 8.65 (bs, 2H).

(2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)naphthalen-1-ylmethanone (34). Cyclohexanone, 2-(naphthalen-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound. IR (KBr) cm-1: 3335, 3235, 3124, 1559, 1430, 1290, 781; ^{1}H NMR (CDCl$_3$): δ 1.29 (m, 4H), 1.50 (t, 2H, J = 6.0 Hz), 2.44 (t, 2H, J = 6.2 Hz), 7.47 (m, 4H), 7.85 (m, 4H), 8.28 (d, 1H, J = 6.4 Hz).

(2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)(4-methylnaphthalen-1-yl)methanone (35). Cyclohexanone, 2-(4-methylnaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **35**. IR (KBr) cm^{-1}: 3370, 1567, 1433, 1415, 1256, 756. ^{1}H NMR (CDCl$_3$) δ: 1.29 (m, 2H), 1.43 (m, 2H), 1.61 (t, 2H, J = 6 Hz), 2.46 (t, 2H, J = 6 Hz), 2.74 (s, 3H), 7.21 (m, 3H), 7.30 (m, 2 H), 7.51 (m, 2H), 7.93 (d, 1H, J = 7.8 Hz), 8.06 (d, 1H, J = 7.8 Hz).

2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophen-3-yl)(4-methoxynaphthalen-1-yl)methanone (36). Cyclohexanone, 2-(4-iodonaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **36**. IR (KBr) cm^{-1}: 3383, 3275, 2928, 1578, 1436, 1323, 1245, 1093, 733; ^{1}H NMR (CDCl$_3$): δ 1.28 (m, 4H), 1.48 (t, 2H, J = 7.4 Hz), 2.35 (t, 2H, J = 6 Hz),

3.98 (s, 3H), 6.78 (d, 1H, J = 8 Hz), 7.21 (d, 1H, J = 8 Hz), 7.40 (m, 2 H), 7.79 (m, 1H), 8.19 (m, 3H).

2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(4-chloronaphthalen-1-yl)methanone (37). Cyclohexanone, 2-(4-chloronaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **37**. IR (KBr) cm^{-1}: 3356, 3253, 2928, 1573, 1428, 1286, 1254, 1132, 941, 787, 760; ^{1}H NMR (CDCl$_3$): δ 1.31 (m, 4H), 1.60 (t, 2H, J = 6.4 Hz), 2.41 (t, 2H, J = 6.4 Hz), 7.29 (m, 3H), 7.56 (m, 3 H), 7.91 (d, 1H, J = 8 Hz), 8.31 (d, 1H, J = 6.8 Hz).

2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(4-bromonaphthalen-1-yl)methanone (38). Cyclohexanone, 2-(4-bromonaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **38**. IR (KBr) cm^{-1}: 3336, 3234, 2932, 1579, 1558, 1427, 1290, 1253, 1129, 780; ^{1}H NMR (CDCl$_3$): δ 1.29 (m, 4H), 1.59 (t, 2H, J = 6 Hz), 2.44 (t, 2H, J = 6 Hz), 7.26 (m, 2H), 7.48 (m, 3 H), 7.89 (m, 3H).

2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(4-iodonaphthalen-1-yl)methanone (39). Cyclohexanone, 2-(4-iodonaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **39**. IR (KBr) cm^{-1}: 3336, 3234, 3123, 2933, 1579, 1558, 1429, 1290, 1254, 1130, 780; ^{1}H NMR (CDCl$_3$): δ 1.28 (m, 4H), 1.59 (t, 2H, J = 6.4 Hz), 2.42 (t, 2H, J = 6.2 Hz), 7.26 (m, 2H), 7.49 (m, 3 H), 8.87 (m, 3H).

(2-Amino-4,5-dimethylthiophen-3-yl)naphthalen-2-yl-methanone (40). 2-Butanone, 2-(naphthalene-2-carbonyl)-acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **40**. IR (KBr) cm^{-1}: 3391, 2922, 1560, 1424, 1263, 1154, 782, 761; ^{1}H NMR (CDCl$_3$): δ 1.55 (s, 3H), 2.16 (s, 3H), 6.42 (bs, 2H), 7.54 (m, 2 H), 7.68 (m, 1H), 7.82 (d, 1H, J = 8.4 Hz), 7.87 (m, 3H).

(2-Amino-4,5-dimethylthiophen-3-yl)(4-methylnaph-thalen-2-yl)methanone (41). Butanone, 2-(4-methylnaph-thalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **41**. IR (KBr) cm^{-1}: 3431, 1555, 1428, 1277, 1027, 758. ^{1}H NMR (CDCl$_3$) δ: 1.58 (s, 3H), 2.18 (s, 3H), 2.74 (s, 3H), 6.37 (bs, 2H), 7.53 (s, 1H), 7.57 (m, 2H), 7.87 (m, 2H), 8.07 (d, 1H, J = 8.2 Hz).

(2-Amino-4,5-dimethylthiophen-3-yl)(6-chloro-4-methylnaphthalen-2-yl)methanone (42). 2-Butanone, 2-(4-methyl-6-chloronaphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **42**. IR (KBr) cm^{-1}: 3362, 1577, 1424, 1165, 863. ^{1}H NMR (CDCl$_3$) δ: 1.54 (s, 3H), 2.15 (s, 3H), 2.69 (s, 3H), 6.56 (bs, 2H), 7.48 (d, 1H, J = 8.8 Hz), 7.54 (s, 1H), 7.82 (m, 2H), 8.00 (s, 1H).

2-Amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophen-3-yl)-naphthalen-2-ylmethanone (43). Cyclopentanone, 2-(naph-thalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **43**. IR (KBr) cm^{-1}: 3341, 3240, 2926, 1561, 1436, 1285, 1039, 760, 742; ^{1}H NMR (CDCl$_3$) δ: 1.60 (m, 2H), 2.09 (m, 2H), 2.68 (m, 2H), 6.96 (bs, 2H), 7.53 (t, 2H, J = 4.4 Hz), 7.60 (d, 1H, J = 6.4 Hz), 7.89 (m, 4H).

(2-Amino-5,6-dihydro-4H-cyclopenta[*b*]thiophen-3-yl)-(4-methylnaphthalen-2-yl)methanone (44). Cyclopentanone, 2-(4-methylnaphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **44**. IR (KBr) cm^{-1}: 3447, 1560, 1430, 1289, 1157, 1037, 746. ^{1}H NMR (CDCl$_3$) δ: 1.61 (m, 2H), 2.09 (m, 2H), 2.66 (t, 2H, J = 6 Hz), 2.73 (s, 3H), 6.94 (bs, 2H), 7.48 (s, 1H), 7.56 (m, 2H), 7.81 (s, 1H), 7.88 (d, 1H, J = 8.0 Hz), 8.03 (d, 1H, J = 8.0 Hz).

(2-Amino-5,6-dihydro-4H-cyclopenta[*b*]thiophen-3-yl)-(6-chloro-4-methylnaphthalen-2-yl)methanone (45). Cyclopentanone, 2-(4-methyl-6-chloronaphthalene-2-carbonyl)-acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **45**. IR (KBr) cm^{-1}: 3405, 1589, 1428, 1292, 1162, 880. ^{1}H NMR

(CDCl$_3$) δ: 1.61 (m, 2H), 2.05 (m, 4H), 2.68 (s, 3H), 6.97 (bs, 2H), 7.48 (m, 2 H), 7.79 (m, 2H), 7.99 (s, 1H).

2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)naph-thalen-2-ylmethanone (46). Cyclohexanone, 2-(naphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **46**. IR (KBr) cm^{-1}: 3392, 2929, 1560, 1424, 1292, 1128, 783; ^{1}H NMR (CDCl$_3$): δ 1.43 (m, 4H), 1.75 (m, 2H), 2.53 (t, 2H, J = 6.2 Hz), 7.53 (m, 3H), 7.62 (d, 1H, J = 8.4 Hz), 7.89 (m, 5H).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(4-methylnaphthalen-2-yl)methanone (47). Cyclohexanone, 2-(4-methylnaphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **47**. IR (KBr) cm^{-1}: 3421, 2922, 1560, 1430. ^{1}H NMR (CDCl$_3$) δ: 1.45 (m, 2H), 1.73 (m, 2H), 1.84 (m, 2H), 2.54 (t, 2H, J = 6.0 Hz), 2.73 (s, 3H), 6.63 (bs, 2H), 7.48 (s, 1H), 7.57 (m, 2H), 7.82 (s, 1H), 7.90 (d, 1H, J = 8.0 Hz), 8.03 (d, 1H, J = 8.0 Hz).

2-Amino-4,5,6,7-tetrahydro-benzo[*b*]thiophen-3-yl)(6-chloro-4-methylnaphthalen-2-yl)methanone (48). Cyclohexanone, 2-(4-methyl-6-chloronaphthalene-2-carbonyl)aceto-nitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **48**. IR (KBr) cm^{-1}: 3259, 1559, 1430, 1294, 1090, 787. ^{1}H NMR (CDCl$_3$) δ: 1.44 (m, 2H), 1.75 (m, 4H), 2.53 (d, 2H, J = 7.2 Hz), 2.69 (s, 3H), 6.73 (bs, 2H), 7.48 (m, 2 H), 7.80 (m, 2H), 7.98 (s, 1H).

(2-Amino-4,5-dimethylthiophen-3-yl)(9*H*-fluoren-2-yl)-methanone (49). Butanone, 2-(fluorene-2-carbonyl)acetoni-trile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **49**. IR (KBr) cm^{-1}: 3360, 2910, 2830, 1660, 1530, 1280, 740; ^{1}H NMR (CDCl$_3$) δ: 1.16 (s, 3H), 2.07 (s, 3H), 3.86 (s, 2H), 6.82 (bs, 2H), 7.32 (m, 3H), 7.52 (d, 1H, J = 8 Hz), 7.56 (d, 1H, J = 7.8 Hz), 7.78 (t, 2H, J = 7.8 Hz).

(2-Amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophen-3-yl)-(9*H*-fluoren-2-yl)methanone (50). Cyclopentanone, 2-(fluo-rene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **50**. IR (KBr) cm^{-1}: 3400, 2928, 2846, 1685, 1560, 1430, 1268, 737; ^{1}H NMR (CDCl$_3$) δ: 2.15 (m, 4H), 2.69 (m, 2H), 3.94 (s, 2H), 6.86 (bs, 2H), 7.36 (m, 3H), 7.54 (d, 1H, J = 8 Hz), 7.58 (d, 1H, J = 7.8 Hz), 7.89 (t, 2H, J = 7.8 Hz).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(9*H*-fluoren-2-yl)methanone (51). Cyclohexanone, 2-(fluorene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **51**. IR (KBr) cm^{-1}: 3428, 2927, 1560, 1431, 1267, 1228, 1139, 760; ^{1}H NMR (CDCl$_3$) δ: 1.48 (m, 2H), 1.72 (m, 2H), 1.86 (t, 2H, J = 6 Hz), 2.53 (t, 2H, J = 6 Hz), 3.94 (s, 2H), 6.53 (bs, 2H), 7.37 (m, 2H), 7.55 (t, 1H, J = 7.2 Hz), 7.70 (s, 2H), 7.81 (t, 2H, J = 7.8 Hz).

(2-Amino-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyri-din-3-yl)naphthalen-1-ylmethanone (52). 1-Benzyl-4-pip-eridone, 2-(naphthalen-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **52**. IR (KBr) cm^{-1}: 3435, 1576, 1426, 1358, 1253, 784; ^{1}H NMR (CDCl$_3$) δ: 1.53 (m, 2H), 2.30 (m, 2H), 3.38 (s, 2H), 3.54 (s, 2H), 7.43 (m, 11 H), 7.88 (m, 3H).

(2-Amino-6-benzyl-4,5,6,7-tetrahydro-thieno[2,3-*c*]py-ridin-3-yl)(4-methylnaphthalen-1-yl)methanone (53). *N*-Benzyl-4-piperidone, 2-(4-methylnaphthalene-1-carbonyl)-acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **53**. IR (KBr) cm^{-1}: 3250, 1734, 1574, 1430, 1357, 1255, 698. ^{1}H NMR (CDCl$_3$) δ: 1.56 (m, 2H), 2.26 (bs, 2H), 2.71 (s, 2H), 2.74 (s, 3H), 3.36 (s, 2H), 3.51 (s, 2H), 7.29 (m, 9H), 7.50 (m, 2H), 7.88 (d, 1H, J = 7.6 Hz), 7.99 (d, 1H, J = 7.6 Hz).

(2-Amino-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyri-din-3-yl)(4-methoxynaphthalen-2-yl)methanone (54). *N*-Benzyl-4-piperidone, 2-(4-methoxynaphthalene-1-carbonyl)-acetonitrile, morpholine, and sulfur were reacted according to

the procedure of Step B to afford the desired compound **54**. IR (KBr) cm^{-1}: 3369, 1578, 1432, 1240, 1091, 769. ^{1}H NMR (CDCl$_3$) δ: 1.65 (bs, 2H), 2.32 (bs, 2H), 3.37 (s, 2H), 3.53 (s, 2H), 4.03 (s, 3H), 6.77 (d, 1H, J = 8 Hz), 7.10 (bs, 2H), 7.33 (m, 5H), 7.35 (d, 1H, J = 8 Hz), 7.47 (m, 2H), 7.90 (m, 1H), 8.28 (m, 1H).

(2-Amino-6-benzyl-4,5,6,7-tetrahydro-thieno[2,3-c]pyridin-3-yl)(4-chloronaphthalen-1-yl)methanone (55). *N*-Benzyl-4-piperidone, 2-(4-chloronaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **55**. IR (KBr) cm^{-1}: 3369, 1569, 1430, 1358, 1131, 698. ^{1}H NMR (CDCl$_3$) δ: 2.46 (t, 2H, J = 5.4 Hz), 2.75 (t, 2H, J = 5.4 Hz), 3.34 (s, 2H), 3.52 (s, 2H), 7.34 (m, 7 H), 7.56 (m, 4H), 7.87 (d, 1H, J = 7.8 Hz), 8.33 (d, 1H, J = 7.8 Hz).

(2-Amino-6-benzyl-4,5,6,7-tetrahydro-thieno[2,3-c]pyridin-3-yl)(4-bromonaphthalen-1-yl)methanone (56). *N*-Benzyl-4-piperidone, 2-(4-bromonaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **56**. IR (KBr) cm^{-1}: 3255, 1570, 1424, 1248, 1130, 783. ^{1}H NMR (CDCl$_3$) δ: 2.52 (t, 2H, J = 5.2 Hz), 2.78 (t, 2H, J = 5.2 Hz), 3.36 (s, 2H), 3.52 (s, 2H), 7.31 (m, 7 H), 7.48 (m, 4H), 7.85 (d, 1H, J = 7.8 Hz), 8.36 (d, 1H, J = 7.8 Hz).

(2-Amino-6-benzyl-4,5,6,7-tetrahydro-thieno[2,3-c]pyridin-3-yl)(4-iodonaphthalen-1-yl)methanone (57). *N*-Benzyl-4-piperidone, 2-(4-iodonaphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **57**. IR (KBr) cm^{-1}: 3108, 1581, 1433, 1359, 1048, 783. ^{1}H NMR (CDCl$_3$) δ: 1.55 (m, 2H), 2.27 (m, 2H), 3.35 (s, 2H), 3.51 (s, 2H), 7.29 (m, 7 H), 7.45 (m, 4H), 7.86 (d, J = 6.8 Hz).

2-Amino-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-3-yl)naphtha-len-2-ylmethanone (58). 1-Benzyl-4-piperidone, 2-(naphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **58**. IR (KBr) cm^{-1}: 3415, 3313, 2926, 1578, 1458, 1407, 1358, 1128, 749; ^{1}H NMR (CDCl$_3$) δ: 1.94 (t, 2H, J = 5.2 Hz), 2.41 (t, 2H, J = 5.8 Hz), 3.42 (s, 2H), 3.60 (s, 2H), 6.79 (bs, 2H), 7.31 (m, 4H), 7.53 (m, 2H), 7.62 (dd, 1H, J = 9.6 and 1.4 Hz), 7.87 (m, 5H).

[2-Amino-6-(2-chlorobenzyl)-4,5,6,7-tetrahydrothieno-[2,3-c]pyridin-3-yl]naphthalen-2-ylmethanone (59). 1-(2-Chlorobenzyl)-4-piperidone, 2-(naphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **59**. IR (KBr) cm^{-1}: 3419, 3317, 2918, 2792, 1600, 1578, 1461, 1410, 1359, 1282, 1135, 984, 781, 759; ^{1}H NMR (CDCl$_3$) δ: 1.96 (t, 2H, J = 5.4 Hz), 2.49 (t, 2H, J = 5.6 Hz), 3.53 (s, 2H), 3.73 (s, 2H), 6.60 (bs, 2H), 7.19 (m, 4H), 7.46 (d, 1H, J = 6.6 Hz), 7.52 (t, 2H, J = 6.4 Hz), 7.61 (d, 1H, J = 8.4 Hz), 7.88 (m, 2H), 7.99 (s, 1H).

[2-Amino-6-(3-chlorobenzyl)-4,5,6,7-tetrahydrothieno-[2,3-c]pyridin-3-yl]naphthalen-2-ylmethanone (60). 1-(3-Chlorobenzyl)-4-piperidone, 2-(naphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **60**. IR (KBr) cm^{-1}: 3270, 1575, 1523, 1357, 1285, 1129, 780. ^{1}H NMR (CDCl$_3$) δ: 1.94 (t, 2H, J = 5.6 Hz), 2.41 (t, 2H, J = 5.6 Hz), 3.46 (s, 2H), 3.53 (s, 2H), 6.81 (bs, 2H), 7.26 (m, 4H), 7.33 (s, 1H), 7.56 (m, 3H), 8.15 (m, 3H).

[2-Amino-6-(4-chlorobenzyl)-4,5,6,7-tetrahydrothieno-[2,3-c]pyridin-3-yl]naphthalen-2-ylmethanone (61). 1-(4-Chlorobenzyl)-4-piperidone, 2-(naphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **61**. IR (KBr) cm^{-1}: 3392, 2963, 1715, 1577, 1423, 1262, 1088, 1016, 800; ^{1}H NMR (CDCl$_3$) δ: 1.93 (t, 2H, J = 5.6 Hz), 2.43 (t, 2H, J = 5.8 Hz), 3.42 (s, 2H), 3.56 (s, 2H), 6.80 (bs, 2H), 7.26 (s, 4H), 7.59 (m, 3H), 7.92 (m, 4H).

[2-Amino-6-(2-fluorobenzyl)-4,5,6,7-tetrahydrothieno-[2,3-c]pyridin-3-yl]naphthalen-2-ylmethanone (62). 1-(2-Fluorobenzyl)-4-piperidone, 2-(naphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the

procedure of Step B to afford the desired compound **62**. IR (KBr) cm^{-1}: 3420, 3316, 2793, 1579, 1458, 1358, 1285, 1228, 1131, 762; ^{1}H NMR (CDCl$_3$) δ: 1.95 (t, 2H, J = 5.6 Hz), 2.45 (t, 2H, J = 5.4 Hz), 3.49 (s, 2H), 3.66 (s, 2H), 6.61 (bs, 2H), 7.08 (m, 3H), 7.31 (m, 1H), 7.35 (d, 1H, J = 7.2 Hz), 7.54 (t, 2H, J = 4.2 Hz), 7.63 (d, 1H, J = 8.6 Hz), 7.86 (m, 2H), 7.97 (s, 1H).

[2-Amino-6-(4-fluorobenzyl)-4,5,6,7-tetrahydrothieno-[2,3-c]pyridin-3-yl]naphthalen-2-ylmethanone (63). 1-(4-Fluorobenzyl)-4-piperidone, 2-(naphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **63**. IR (KBr) cm^{-1}: 3401, 2928, 1577, 1508, 1424, 1263, 1221, 1130, 824; ^{1}H NMR (CDCl$_3$) δ: 1.96 (t, 2H, J = 5.4 Hz), 2.38 (t, 2H, J = 5.6 Hz), 3.42 (s, 2H), 3.56 (s, 2H), 6.81 (bs, 2H), 6.98 (t, 2H, J = 8.8 Hz), 7.27 (t, 2H, J = 6.2 Hz), 7.56 (m, 2H), 7.90 (m, 5H).

[2-Amino-6-(4-nitrobenzyl)-4,5,6,7-tetrahydrobenzo[b]-thiophen-3-yl]naphthalen-2-ylmethanone (64). 1-(4-Nitrobenzyl)-4-piperidone, 2-(naphthalene-2-carbonyl)-acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **64**. IR (KBr) cm^{-1}: 3422, 1577, 1518, 1424, 1344, 858, 740. ^{1}H NMR (CDCl$_3$) δ: 1.94 (t, 2H, J = 5.4 Hz), 2.42 (t, 2H, J = 5.4 Hz), 3.45 (s, 2H), 3.68 (s, 2H), 6.80 (bs, 2H), 7.58 (m, 5H), 7.91 (m, 4H), 8.15 (d, 2H, J = 8.6 Hz).

[2-Amino-6-(3,4,5-trimethoxybenzyl)-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-3-yl]naphthalen-2-ylmethanone (65). 1-(3,4,5-Trimethoxybenzyl)-4-piperidone, 2-(naphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **65**. IR (KBr) cm-1: 3394, 2933, 2831, 1578, 1421, 1358, 1233, 1125, 1005, 782; ^{1}H NMR (CDCl$_3$) δ: 1.93 (t, 2H, J = 5.6 Hz), 2.40 (t, 2H, J = 5.6 Hz), 3.46 (s, 2H), 3.52 (s, 2H), 3.81 (s, 3H), 3.83 (s, 6H), 6.53 (s, 2H), 6.62 (bs, 2H), 7.53 (t, 2H, J = 4 Hz), 7.62 (d, 1H, J = 8.8 Hz), 7.87 (m, 3H), 7.98 (s, 1H).

(2-Amino-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-3-yl)(4-methylnaphthalen-2-yl)methanone (66). *N*-Benzyl-4-piperidone, 2-(4-methylnaphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **66**. IR (KBr) cm^{-1}: 3441, 1638, 1455, 1324, 1167, 986. ^{1}H NMR (CDCl$_3$) δ: 2.47 (t, 2H, J = 6 Hz), 2.71 (t, 2H, J = 6 Hz), 2.74 (s, 3H), 3.71 (m, 2H), 3.75 (s, 2H), 6.85 (bs, 2H), 7.31 (m, 5H), 7.46 (s, 1H), 7.56 (m, 2H), 7.82 (s, 1H), 7.87 (d, 1H, J = 7.8 Hz), 8.02 (d, 1H, J = 7.8 Hz).

(2-Amino-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-3-yl)(6-chloro-4-methylnaphthalen-2-yl)methanone (67). *N*-Benzyl-4-piperidone, 2-(4-methyl-6-chloronaphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **67**. IR (KBr) cm^{-1}: 3377, 1561, 1438, 1364, 1298, 1246, 733. ^{1}H NMR (CDCl$_3$) δ: 1.91 (t, 2H, J = 5.4 Hz), 2.41 (t, 2H, J = 5.4 Hz), 2.68 (s, 3H), 3.43 (s, 2H), 3.62 (s, 2H), 6.82 (bs, 2H), 7.28 (m, 5 H), 7.47 (m, 2H), 7.80 (m, 2H), 7.99 (s, 1H).

(2-Amino-6-pyridin-3-ylmethyl-4,5,6,7-tetrahydrothieno-[2,3-c]pyridin-3-yl)naphthalen-2-ylmethanone (68). *N*-(3-Nicotinyl)-4-piperidone, 2-(naphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **68**. Yield 57%, mp 189−190 °C. IR (KBr) cm^{-1}: 3370, 1599, 1580, 1458, 1230, 1022, 783. ^{1}H NMR (CDCl$_3$) δ: 1.98 (t, 2H, J = 5.2 Hz), 2.44 (t, 2H, J = 5.8 Hz), 3.49 (s, 2H), 3.66 (s, 2H), 6.84 (bs, 2H), 7.26 (d, 2H, J = 7.8 Hz), 7.56 (m, 3H), 7.72 (d, 1H, J = 7.6 Hz), 7.91 (m, 4H), 8.53 (s, 1H).

(2-Amino-5,6-dihydro-4H-cyclopenta[b]thiophen-3-yl)-(9H-fluoren-2-yl)methanone (69). Cyclopentanone, 2-(fluorene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **69** as yellow solid. Yield: 68%; mp 197−200 °C (petroleum ether). IR (KBr) cm^{-1}: 3400, 2928, 2846, 1685, 1560, 1430, 1268, 737; ^{1}H NMR (CDCl$_3$) δ: 2.15 (m, 4H), 2.69

(m, 2H), 3.94 (s, 2H), 6.86 (bs, 2H), 7.36 (m, 3H), 7.54 (d, 1H, $J = 8$ Hz), 7.58 (d, 1H, $J = 7.8$ Hz), 7.89 (t, 2H, $J = 7.8$ Hz).

(2-Amino-6-phenyl-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)naphthalen-2-ylmethanone (70). 4-Phenylcyclohexanone, 2-(naphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **70**. Yield 78%; mp 119−120 °C. IR (KBr) cm^{-1}: 3402, 1560, 1439, 1245, 760. ^{1}H NMR (CDCl$_3$) δ: 1.63 (m, 1H), 1.72 (d, 2H, $J = 10.4$ Hz), 2.75 (m, 2H), 2.96 (m, 2H), 7.21 (bs, 2H), 7.23 (m, 6H), 7.55 (dd, 1H, $J = 8.4$ and 4.6 Hz), 7.63 (d, 1H, $J = 7.6$ Hz), 7.89 (m, 3H), 7.99 (s, 1H).

(2-Amino-6-benzyl-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl]naphthalen-2-ylmethanone (71). 4-Benzylcyclohexanone, 2-(naphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **71**. Yield: 60%; mp 153 °C (petroleum ether). IR (KBr) cm^{-1}: 3419, 3315, 2912, 1596, 1568, 1456, 1282, 1130, 781, 749, 699. ^{1}H NMR (CDCl$_3$) δ: 1.04 (m, 2H), 1.62 (m, 2H), 1.81 (m, 1H), 2.19 (m, 2H), 2.59 (d, 2H, $J = 7.4$ Hz), 6.67 (bs, 2H),7.19 (m, 4H), 7.56 (m, 4H), 7.88 (m, 4H).

(2-Amino-6-benzyl-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)naphthalen-2-ylmethanone (72). 4-Benzylcyclohexanone, 2-(naphthalene-2-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **72** as white solid. Yield: 61%; mp 69−70 C (petroleum ether). IR (KBr) cm^{-1}: 3369, 2914, 1569, 1424, 1285, 1252, 782; ^{1}H NMR (CDCl$_3$): δ 1.17 (m, 2H), 1.27 (m, 1H), 1.56 (m, 1H), 1.81 (m, 1H), 2.19 (m, 2H), 2.49 (d, 2H, $J = 7$ Hz), 7.03 (bs, 2H), 7.29 (m, 6H), 7.48 (m, 5H), 7.88 (d, 1H, $J = 8.8$ Hz).

[2-Amino-6-(4-methoxyphenyl)-4*H*-1,5,7-trithia-inden-3-yl]naphthalen-1-ylmethanone (73). 2-(4-Methoxyphenyl)-1,3-dithian-5-one, 2-(naphthalene-1-carbonyl)acetonitrile, morpholine, and sulfur were reacted according to the procedure of Step B to afford the desired compound **73** as a yellow solid. Yield: 77%; mp 132−134 C (petroleum ether). IR (KBr) cm^{-1}: 3412, 1607, 1578, 1509, 1420, 1303, 1254, 1176, 1112, 1029, 778; ^{1}H NMR (CDCl$_3$): δ 2.17 (s, 2H), 3.77 (s, 3H), 3.81 (s, 1H), 6.78 (d, 2H, $J = 8.6$ Hz), 7.28 (bs, 2H), 7.29 (m, 2H), 7.51 (m, 5 H), 7.93 (m, 2H).

Preparation of (2-Acetylamino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)naphthalen-1-ylmethanone (75). Pyridine (10 drops) was added to a stirred solution of **34** (1.6 g, 5.2 mmol) in Ac$_2$O (12 mL) at room temperature. The solution was refluxed for 2 h, poured in water, and extracted with EtOAc (50 mL). The extract was washed successively with saturated aqueous NaHCO$_3$, water, and brine, dried over Na$_2$SO$_4$, and concentrated under vacuo. Recrystallization from petroleum ether afforded **75** as a yellow solid. Yield = 88%, mp 234 °C. ^{1}H NMR (CDCl$_3$): δ 1.34 (m, 4H), 1.61 (t, 2H, $J = 6.0$ Hz), 2.36 (s, 3H), 2.62 (t, 2H, $J = 6.2$ Hz), 7.51 (m, 4H), 7.92 (m, 4H), 12.3 (s, 1H).

(2-Acetylaminobenzo[*b*]thiophen-3-yl)naphthalen-1-ylmethanone (76). A mixture of **75** (621 mg, 1.8 mmol), 10% Pd−C (50% wet, 1.4 g), and CHCl$_3$ (20 mL) was stirred at room temperature for 10 min, and then the solvent was evaporated. The resulting powder was heated at 130 °C for 20 h, cooled to rt, and extracted with EtOAc. The insoluble solids were filtered out, and the filtrate was concentrated under vacuo. Recrystallization from petroleum ether afforded 76 as a yellow solid. Yield = 60%, mp 134 °C. ^{1}H NMR (CDCl$_3$) δ: 2.44 (s, 3H), 6.30 (d, 1H, $J = 8.4$ Hz), 6.86 (t, 1H, $J = 7.4$ Hz), 7.14 (t, 1H, $J = 7.4$ Hz), 7.51 (m, 4H), 7.71 (d, 1H, $J = 7.8$ Hz), 7.95 (t, 1H, $J = 6.6$ Hz), 7.95 (d, 1H, $J = 7.8$ Hz), 12.8 (s, 1H).

(2-Aminobenzo[*b*]thiophen-3-yl)naphthalen-1-ylmethanone (77). A mixture of **76** (160 mg, 0.464 mmol), 1 N aqueous NaOH (0.5 mL, 0.5 mmol), and EtOH (10 mL) was refluxed for 5 h and then concentrated in vacuo. The residue was diluted with EtOAc, washed successively with water and brine, dried over Na$_2$SO$_4$, and evaporated in vacuo. The residue was purified by flash chromatography on silica gel using EtOAc−petroleum ether (2:8, v/v) as eluent. Recrystallization from petroleum ether furnished **77** as a yellow solid. Yield = 78%; mp 162−164 °C. IR (KBr) cm^{-1}: 3351, 1587, 1466, 1420,

1287, 1240, 1201, 781. ^{1}H NMR (CDCl$_3$) δ: 6.03 (d, 1H, $J = 7.8$ Hz), 6.74 (t, 1H, $J = 7.4$ Hz), 6.95 (t, 1H, $J = 7.4$ Hz), 7.50 (m, 5H), 7.78 (m, 2H), 7.96 (m, 3H).

Biological Materials. [^{3}H]DPCPX (specific activity, 112 Ci/mmol) and [^{3}H]CCPA (specific activity, 55 Ci/mmol) were obtained from NEN Research Products (Boston, MA); [^{3}H]ZM 241385 (specific activity, 17 Ci/mmol) was obtained from Tocris Cookson (Bristol, UK); [^{3}H]MRE 3008F20 (specific activity 67 Ci/mmol) was obtained from Amersham International (Buckinghamshire, UK). CHO cells transfected with the human recombinant A1 adenosine receptor (hCHO-A1) were obtained by Prof. K. N. Klotz, University of Würzburg. Human cerebral cortex was obtained from the University of Ferrara, Medicina Legale Section, with approval of human tissue use protocol. Rat cerebral cortex was harvested from adult male Wistar rats. All tissue culture reagents were obtained from Sigma.

Biological Methods. Cyclic AMP Accumulation in CHO Cells. Chinese hamster ovary cells expressing human recombinant A$_1$-adenosine receptors (CHO:hA$_1$ cells) at a density of approximately 8000 fmol/mg protein were prepared as previously described,[14c] and aliquots of these cells at low passage numbers were frozen and stored in liquid nitrogen. Upon the arrival of a group of compounds for testing in the laboratory, an aliquot of cells was removed from liquid nitrogen storage and grown in Ham's F-12 culture medium with 10% fetal bovine serum and 0.5 mg/mL of antibiotic G-418.[30] Cells were passaged thrice weekly. For experiments, aliquots of cells were placed into 12-well culture plates with culture medium, serum, and antibiotic for 48 h, by which time the cells had grown to a confluent monolayer.

To begin an experiment, growth medium was removed from the culture plates and cells were washed once with Hanks buffered saline solution. The wash solution was then removed and replaced with fresh Hanks solution containing forskolin (1 μM), rolipram (20 μM), CPA (0.01 nM), adenosine deaminase (2 U/mL), and the allosteric enhancer to be tested. Forskolin was used to stimulate the activity of adenylyl cyclase, rolipram to inhibit cAMP phosphodiesterase, adenosine deaminase to degrade endogenous adenosine, and CPA to cause a small increase of the number of activated adenosine receptors.

After 6 min of incubation at 36 °C in the presence of drugs, the incubation solution was removed, and hydrochloric acid (final concentration, 50 mM) was added to cells to terminate drug action. The content of cAMP in acidified extracts of cells was determined by radioimmunoassay as previously described.[14c] Because the magnitude of the effects of allosteric enhancers on CHO:hA$_1$ cells changed subtly with passage number and differed slightly among different aliquots of cells, the action of tested compounds and the action of the reference compound PD 81,723 were assayed in each experiment.

Allosteric enhancement was measured as the action of a test compound at different concentrations (0.01, 0.1, 1, and 10 μM) to reduce the cAMP content of CHO:hA$_1$ cells in the presence of 0.05−0.1 nM CPA. CPA (0.05−0.1 nM) alone causes a slight reduction of cAMP content of cells by activation of A$_1$-adenosine receptors. Allosteric enhancement of the action of CPA causes a further reduction of the cAMP content of CHO:hA$_1$ cells. Because the spontaneous activity of adenosine receptors in CHO:hA$_1$ cells causes an inhibition of adenylyl cyclase activity even in the absence of an agonist,[30] antagonists of adenosine receptors increase cAMP content of cells. Therefore, compounds that increased cAMP content of cells in this study were provisionally identified as A$_1$-adenosine receptor antagonists.

Membrane Preparation from CHO-A$_1$ Cells. For membrane preparation the culture medium was removed. The cells were washed with PBS and scraped off T75 flasks in ice-cold hypotonic buffer (5 mM Tris HCl, 2 mM EDTA, pH 7.4). The cell suspension was homogenized using a Polytron, and the homogenate was spun for 10 min at 1000g. The supernatant was then centrifuged for 30 min at 100 000g. The membrane pellet was resuspended in 50 mM Tris HCl buffer pH 7.4 and incubated with 2U/ml of ADA for 30 min at 37 °C. Then the suspension was stored at −80 °C. The protein concentration

was determined according to a Bio-Rad method with bovine albumin as a standard reference.[31]

Membrane Preparation from Rat Cortex and Human Brain. Cerebral cortical tissue from each species was homogenized using a Polytron (setting 6, 20 s) in 20 volumes of ice-cold 50 mM Tris-HCl, pH 7.4. This crude membrane homogenate was then centrifuged at 48 000g for 15 min at 4 °C. The resulting pellet was resuspended in buffer containing 2 IU/ml ADA to 20 mg/mL original tissue weight and incubated at 37 °C for 30 min to remove endogenous adenosine. This membrane homogenate was recentrifuged at 48 000g for 15 min at 4 °C. The resulting membrane pellet was resuspended and recentrifuged at 48 000g for 15 min at 4 °C. The final membrane pellets were stored at −80 °C until the time of assay.

Adenosine Receptor Binding. To determine the effect of the new series of derivatives of PD 81,723 on the binding of ligands to A_1, A_{2A}, and A_3 receptors, membranes from CHO: hA_1, hA_{2A}, hA_3, rat cortex, and human brain were incubated in a buffer solution in the absence and presence of test compounds. Test agents were dissolved in DMSO and added to the assay from a 100-fold concentrated solution in DMSO. Control incubations also contained 1% DMSO. Bound and free radioactivities were separated by filtering the assay mixture through Whatman GF/B glass-fiber filters using a Micro-Mate 196 cell harvester (Packard Instrument Company). The filter-bound radioactivity was counted on Top Count Microplate Scintillation Counter (efficiency 57%) with Micro-Scint 20.

Binding of 1 nM [^{3}H]CCPA to A_1 receptors in CHO:hA_1, rat, and human brain membranes in the absence and presence of increasing concentrations of test compounds was carried out in triplicate at 25 °C for 90 min in 50 mM Tris-HCl, pH 7.4. Nonspecific binding was defined as binding in the presence of 1 μM R-PIA.

Saturation. Saturation binding experiments of [^{3}H]CCPA (0.05 to 10 nM) to A1 receptors expressed in CHO:hA_1, rat, and human brain membranes were performed in triplicate at 25 °C for 90 min in 50 mM Tris-HCl, pH 7.4, in the absence and presence of test compounds. Nonspecific binding was defined as binding in the presence of 1 μM R-PIA.

Competition Assay. Competition experiments of 1 nM [^{3}H]-DPCPX to CHO:hA_1 membranes were performed incubating membranes (100 μg of protein/assay) at 25 °C for 150 min. Competition experiments were performed in duplicate in a final volume of 250 μL in test tubes containing 50 μM Tris HCl buffer, pH 7.4 and 100 μL of membranes and at least six to eight different concentrations of the tested compounds. Nonspecific binding was defined as the binding in the presence of 1 μM DPCPX and was about 25% of total binding.

Competition experiments of 2 nM [^{3}H]ZM241385 to CHO:hA_{2A} membranes were performed incubating membranes (100 μg of protein/assay) at 4 °C for 60 min. Competition experiments were performed in duplicate in a final volume of 250 μL in test tubes containing 50 μM Tris HCl buffer, 10 μM MgCl$_2$, pH 7.4 and 100 μL of membranes and at least six to eight different concentrations of the tested compounds. Nonspecific binding was defined as the binding in the presence of 1 μM ZM241385 and was about 30% of total binding.

Competition experiments of 2 nM [^{3}H]MRE 3008F20 to CHO:hA_3 membranes were performed incubating membranes (100 μg of protein/assay) at 4 °C for 150 min. Competition experiments were performed in duplicate in a final volume of 250 μL in test tubes containing 50 μM Tris HCl buffer, 10 μM MgCl$_2$, 1 mM EDTA, pH 7.4 and 100 μL of membranes and at least six to eight different concentrations of the tested compounds. Nonspecific binding was defined as the binding in the presence of 1 μM MRE 3008F20 and was about 30% of total binding.

[^{3}H]CCPA Kinetic Dissociation Assay. Kinetic dissociation experiments of 1.5 nM [^{3}H]CCPA to CHO:hA_1 membranes were performed preassociating [^{3}H]CCPA with the A1 receptor for 2 h in the absence of test agents under the same condition as the standard [^{3}H]CCPA binding assay. After the 2 h preassociation dissociation was initiated by addition of R-PIA

(1 μM) and test agents (10 μM). Specific binding was then measured at 2−180 min after the addition of R-PIA (1 μM) and test agents (10 μM).

Data Analysis. All values are expressed as mean ± SEM of three independent experiments. Inhibitory binding constants, K_i, were calculated from the IC$_{50}$ values according to the Cheng and Prusoff equation, $K_i = IC_{50}/(1 + [C^*]/KD^*)$, where [C*] is the concentration of the radioligand and KD* its dissociation constant.[32] A weighted non linear least-squares curve fitting program LIGAND was used for computer analysis of saturation, competition, and kinetic experiments.[33] For experiments with two comparison groups, statistical analysis was performed with a two-tailed t test. Differences between group mean values were considered significant at $P \leq 0.05$.

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