Allosteric Modulation of the Adenosine A_1 Receptor. Synthesis and Biological Evaluation of Novel 2-Amino-3-benzoylthiophenes as Allosteric Enhancers of Agonist Binding†

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Novel allosteric enhancers of agonist binding to the rat adenosine A_1 receptor are described. The lead compound for the new series was PD 81,723 ((2-amino-4,5-dimethyl-3-thienyl)[3-(trifluoromethyl)phenyl]methanone), a compound previously reported by Bruns and co-workers (*Mol. Pharmacol.* **1990**, *38*, 950−958). The 4,5-dimethyl group and the benzoyl moiety were targets for further modifications, leading to series of 4,5-dialkyl (**12a**−**g**), of tetrahydrobenzo (**12h**−**u**), and of tetrahydropyridine (**13a**−**g**) derivatives. A number of compounds, in particular **12b**, **12e**, **12j**, **12n**, and **12u**, proved superior to PD 81,723. Their EC_{50} values for enhancing the binding of the adenosine A_1 receptor agonist N^6-cyclopentyladenosine to the receptor were lower, and/or their antagonistic activity on the adenosine A_1 receptor was shown to be diminished.

Introduction

Extracellular adenosine is regarded as a local hormone with profound and ubiquitous physiological effects. This nucleoside is currently known to interact with four different subtypes of adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3), all members of the large superfamily of G protein-coupled receptors.[1−3] The wide distribution of adenosine receptors offers both opportunities and drawbacks for therapeutic intervention.[4,5] As an example, A_1 adenosine receptors are found in the CNS, in heart and adipose tissue. Hence, agonists are capable of reducing free-fatty-acid levels in the blood through their interaction with adenosine A_1 receptors on fat cells. This is a useful feature in noninsulin-dependent diabetes mellitus (type II diabetes), since insulin's action is then sensitized.[6] However, the concomitant bradycardia and drop in mean arterial pressure due to interference with cardiovascular adenosine receptors are to be considered as serious side effects.[7]

Here we report our progress on a fundamentally different approach. The formation of extracellular adenosine as a breakdown product of ATP is a local phenomenon, induced by a tissue at risk, e.g., under hypoxic or anoxic conditions (heart failure, stroke, epilepsy). This adenosine production is considered to be tissue-protective.[8−10] As a consequence, compounds that would either augment the concentration of adenosine or enhance its action *locally* might have a better therapeutic profile than the agonists described above. Marketed nucleoside transport blockers such as dipyridamole and dilazep have already proven the first concept. Such compounds inhibit the intracellular uptake of extracellular adenosine, thereby effectively increasing its concentration outside the cell.[11]

We chose to focus on the second category, i.e., compounds that enhance agonist action. In the early 1990s Bruns and co-workers reported on various allosteric modulators, 2-amino-3-benzoylthiophene derivatives capable of enhancing the binding and activity of reference A_1 receptor agonists, such as N^6-cyclopentyladenosine (CPA).[12,13] One of these "allosteric modulators", PD 81,723, has been examined pharmacologically in greater detail by various independent research groups.[14,15] It was convincingly demonstrated that PD 81,723 also enhances the action of endogenous adenosine, which corroborates the concept mentioned above.[16,17] Also, long-term exposure of cells expressing the human adenosine A_1 receptor to PD 81,723 caused only little desensitization and down-regulation, which is considered to be encouraging in terms of therapeutic potential.[18]

In this study we describe the synthesis and biological evaluation of mostly novel PD 81,723 analogues. We have identified a number of compounds that appear superior to PD 81,723 in their enhancing activity.

Chemistry

The synthesis of the 2-amino-3-benzoylthiophene derivatives (**12a**−**u**, **13a**−**g**, **14**) was accomplished via the routes illustrated in Schemes 1−3. The methods used in these schemes were described previously.[19,20] The appropriate carbonyl compounds (**6a**−**c**, **7**, **9**−**11**) were reacted with benzoylacetonitrile derivatives (**4a**−**m**) and sulfur in ethanol in the presence of diethylamine to provide the products in moderate to low yields (Scheme 3). These benzoylacetonitrile derivatives (Scheme 1), when not commercially available, were synthesized by condensation of the acetonitrile anion with the appropriate substituted methyl benzoates (**1a**−

† Abbreviations: [^{3}H]DPCPX, [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine; [^{3}H]CCPA, [^{3}H]-2-chloro-N^6-cyclopentyladenosine; CH_3CN, acetonitrile; CPA, N^6-cyclopentyladenosine; HOAc, acetic acid; KO*t*Bu, potassium *tert*-butoxide; PD 81,723, (2-amino-4,5-dimethyl-3-thienyl)-[3-(trifluoromethyl)phenyl]methanone; THF, tetrahydrofuran; TLC, thin-layer chromatography.

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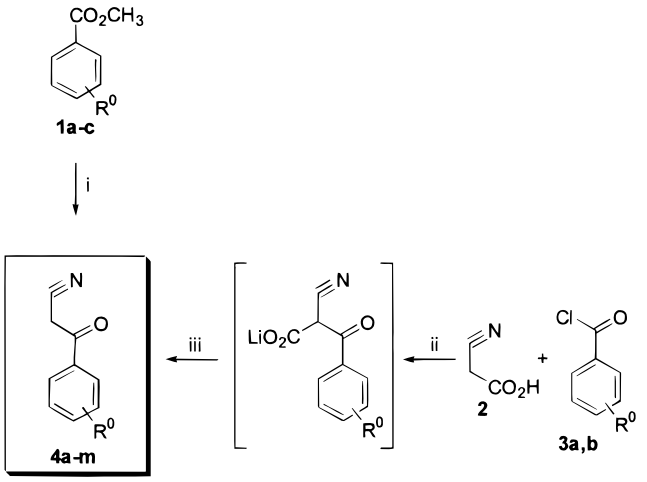
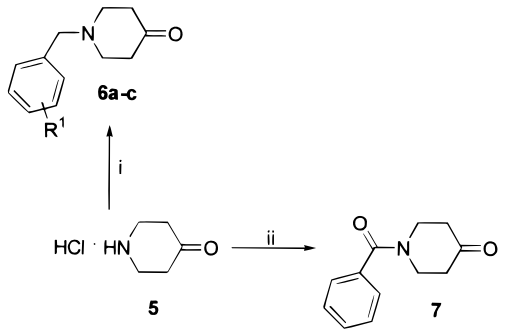
Scheme 1. Synthesis of Benzoylacetonitriles[a]



[a] Reagents and conditions: (i) KO*t*Bu, CH_3CN, 50 °C; (ii) THF, *n*-BuLi, −70 °C; (iii) 20% HCl.

Scheme 2. Synthesis of Substituted Piperidones[a]



[a] Reagents and conditions: (i) benzyl chloride derivatives (**8a**−**c**), $(CH_2Cl)_2$, Et_3N, ΔT; (ii) BzCl, CH_2Cl_2, Et_3N.

c) in moderate to low yields.[21] Unfortunately, this procedure failed for 4-nitro- and 4-iodobenzoylacetonitrile. However, the latter two benzoylacetonitriles (**4d**, **4e**) could be obtained in reasonable yield by condensation of 4-nitro- and 4-iodobenzoyl chloride (**3a**,**b**) with the dilithium salt of cyanoacetic acid (**2**).[22] The appropriate carbonyl compounds were commercially available except for the acylated and alkylated piperidones (see Scheme 2). Alkylation was effected by reaction of the hydrochloric salt of 4-piperidone (**5**) with substituted benzyl chloride (**8a**−**c**) in dichloroethane in the presence of triethylamine under reflux conditions for 16 h. After workup and distillation, products **6a**−**c** were obtained in high yields. Acylation of the hydrochloric salt of 4-piperidone (**5**) with benzoyl chloride was easily accomplished in dichloromethane, using triethylamine as a base, to afford 1-benzoyl-4-piperidone (**7**) in good yield.

All synthetic and physicochemical characteristics of the compounds are listed in Tables 1 and 2.

Biology

All compounds were tested in radioligand binding assays on adenosine A_1 receptors, both for their enhancing and antagonistic activity. The enhancement of agonist binding was demonstrated in two ways. First, displacement studies with [^{3}H]DPCPX as the radiolabeled antagonist and CPA as the displacing agonist were recorded in the absence and presence of 10 μM of a putative allosteric enhancer. In such studies the reference compound PD 81,723 and many of the analogues in the present study were capable of shifting the CPA displacement curve to the left with an approximately 2-fold affinity shift. In this experimental setup it appeared that many of the compounds at low CPA concentrations displaced some of the [^{3}H]DPCPX binding themselves. Therefore we decided to analyze the enhancing activity more directly through its influence on the dissociation rate of a radiolabeled agonist for the adenosine A_1 receptor.[13] We used [^{3}H]CCPA (2-chloro-N^6-cyclopentyladenosine) for that purpose. After 2.5 h of incubation of receptor preparation with radioligand, dissociation was induced by addition of 100 μM of CPA in the absence or presence of 10 μM of putative enhancer. After 60 min the reaction was stopped and the difference in [^{3}H]CCPA binding between the two conditions was taken as a measure for enhancing activity. In all experiments PD 81,723 was incorporated as a reference substance. Its enhancing activity served as a control and was set to 100%. Some of the more potent compounds were evaluated at different concentrations which allowed the construction of concentration−effect curves and the determination of EC_{50} values. The antagonistic effects of the compounds were assessed by their inhibition of [^{3}H]DPCPX binding at one concentration of 10 μM.

Results and Discussion

In Table 3 the biological effects of all compounds are summarized. We first examined close analogues of PD 81,723, i.e., 4,5-dialkyl substituted 2-amino-3-benzoylthiophenes. 4,5-Dimethyl substitution as in PD 81,723 and 4-ethyl,5-methyl substitution led to compounds **12a**−**g**. Of those compounds, none was a more potent enhancer than PD 81,723, although chloro substitution in either the *meta* or *para* position on the benzoyl ring led to activities comparable with the *meta* trifluoromethyl substituent of PD 81,723. In general, however, the compounds were somewhat less active as antagonists (Table 3), improving the window between enhancing and antagonistic activity. This was particularly true for **12e** and **12g**. Compound **12e** has been reported before as RS-74513-000. This PD 81,723 analogue displayed enhancing activity on a field-stimulated isolated guinea pig ileum preparation.[23] An obvious extension to these series were the "cyclized" PD 81,723 analogues **12h**−**u**. In this series we applied a "Topliss" approach[24] by varying substituents on the benzoyl ring system. Some of the compounds (**12a**, **12d**, **12h**−**j**, **12k** (PD 71,605), **12n**, **13a**, and **13e** (PD 117,975)) were also reported on by Bruns and co-workers.[12,13] On the *para* position halogen substitution was preferred over nitro (**12q**), carboxylate (**12t**), and carboxylic ester (**12s**) groups. Also, methyl and trifluoromethyl substitution on this position yielded compounds (**12r**, **12m**) with higher enhancing activity than PD 81,723. For those compounds with the same substituent on either the *meta* or the *para* position, it appeared that the *para* substitution was better (**12p** vs **12l** and **12n** vs **12k**). Interestingly, combined *meta*/*para* substitution as in the dichloro compound **12u** seemed to have an additive effect, since this derivative showed enhancing activity more pronouncedly than either the *meta* or the *para* substituted chloro derivative. In a third series of 6-benzyl substituted 2-amino-3-benzoyl-4,5,6,7-tetrahydrothieno-

Scheme 3. Synthesis of 2-Amino-3-benzoylthiophenes

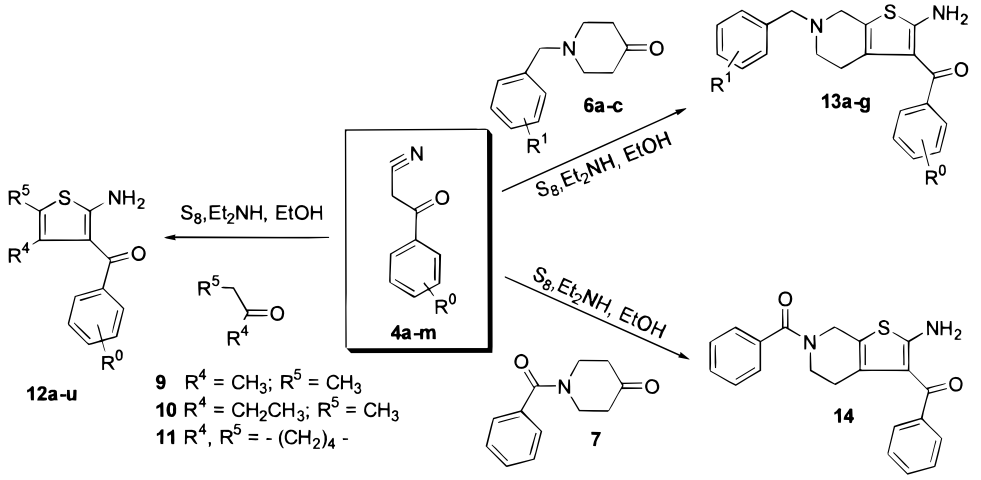
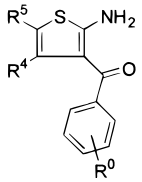


Table 1. Physical and Synthetic Data for 2-Amino-3-benzoylthiophenes **12a**−**u**



compd	R^0	R^4	R^5	mp, °C	cryst solvent[a]	yield,[b] %	formula[c]	EI-MS *m/z* [M^+]
12a[13]	H	CH_3	CH_3	133−134	a	12	$C_{13}H_{13}NOS$	232
12b	3-Cl	CH_3	CH_3	114−116	a	15	$C_{13}H_{12}ClNOS$	266
12c	4-Cl	CH_3	CH_3	123−125	a	10	$C_{13}H_{12}ClNOS$	266
12d[13]	H	CH_2CH_3	CH_3	129−131	a	17	$C_{14}H_{15}NOS$	246
12e[23]	3-CF_3	CH_2CH_3	CH_3	100−101	a	12	$C_{15}H_{14}F_3NOS$	314
12f	3-Cl	CH_2CH_3	CH_3	99−102	a	16	$C_{14}H_{14}ClNOS$	280
12g	4-Cl	CH_2CH_3	CH_3	108−110	a	21	$C_{14}H_{14}ClNOS$	280
12h[19]	H	$-(CH_2)_4-$		152−153	b	52	$C_{15}H_{15}NOS$	258
12i[20]	2-Cl	$-(CH_2)_4-$		145−147	c	10	$C_{15}H_{14}ClNOS$	292
12j[13]	3-CF_3	$-(CH_2)_4-$		122−123	c	37	$C_{16}H_{14}F_3NOS$	326
12k[13,20]	3-Cl	$-(CH_2)_4-$		114−115	a	34	$C_{15}H_{14}ClNOS$	292
12l	3-I	$-(CH_2)_4-$		160−162	a	20	$C_{15}H_{14}INOS$	384
12m	4-CF_3	$-(CH_2)_4-$		145−147	a	30	$C_{16}H_{14}F_3NOS$	326
12n[13]	4-Cl	$-(CH_2)_4-$		140−142	a	46	$C_{15}H_{14}ClNOS$	292
12o	4-Br	$-(CH_2)_4-$		152−153	a	35	$C_{15}H_{14}BrNOS$	336
12p	4-I	$-(CH_2)_4-$		150−152	c	36	$C_{15}H_{14}INOS$	384
12q	4-NO_2	$-(CH_2)_4-$		202−204	a	15	$C_{15}H_{14}N_2O_3S$	303
12r	4-CH_3	$-(CH_2)_4-$		155−156	a	25	$C_{16}H_{17}NOS$	272
12s	4-CO_2CH_3	$-(CH_2)_4-$		159−160	a	15	$C_{17}H_{17}NO_3S$	316
12t	4-CO_2H	$-(CH_2)_4-$		232−233	d	93[d]	$C_{16}H_{15}NO_3S$	302
12u	3,4-Cl	$-(CH_2)_4-$		152−154	a	40	$C_{15}H_{13}Cl_2NOS$	326

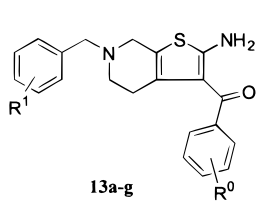
[a] Code: a, dichloromethane/*n*-hexane; b, toluene/*n*-hexane; c, ethanol; d, H_2O. [b] Yields were not optimized. [c] All compounds were analyzed for C, H, N; analytical results were within 0.4% of theoretical values. [d] Prepared from **12s**.

[2,3-*c*]pyridines (see also Table 2), other potent enhancers were identified. The two phenyl rings in this class of compounds allow combined structural variations of which we explored only chloro substitution in this study. Again, on the benzoyl moiety, 3,4-dichloro substitution was most favored (**13g** vs **13a** and **13e**). The benzyl moiety was best substituted with a *meta* chloro substituent (**13b** vs **13a**, **13c** and **13d**). A benzoyl rather than a benzyl moiety as in **14** provided only little enhancing activity.

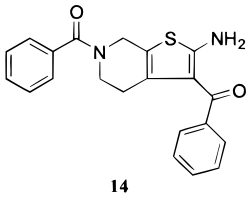
EC_{50} values were determined for the more potent compounds. In our hands PD 81,723 had an EC_{50} value of 15 μM, quite comparable to the value of 10 μM reported before.[13] It should be mentioned, however, that its limited solubility precluded the recording of a full concentration−effect curve (see also Figure 1). This was easily achieved for the more potent compounds, of which **12j**, **12m**, **12p**, and **12u** proved to be 3-fold more potent than PD 81,723 (Table 3 and Figure 1). These materials proved to have the same efficacy, i.e., they achieved the same maximal effect.

Subsequently, we analyzed the antagonistic behavior of the compounds in a simple binding assay with [^{3}H]-DPCPX as the radioligand and the individual compounds at one single concentration of 10 μM (Table 3). PD 81,723 displaced approximately 40% of the radioligand, which on further analysis of more concentrations yielded a K_i value of 11 μM. All other compounds in the present study showed some antagonistic behavior too, although to varying extents. Apparently, it is possible to achieve some separation between enhancing and antagonistic activity. In this respect, a few compounds warrant further discussion. The close PD 81,723 analogues **12b** and **12e** are equipotent to PD 81,723 in their

Table 2. Physical and Synthetic Data for 2-Amino-3-benzoyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridines **13a**−**g** and **14**



13a-g



14

compd	R^0	R^1	mp, °C	cryst solvent[a]	yield,[b] %	formula[c]	EI-MS *m/z* [M^+]
13a[13]	H	H	178−180	a	50	$C_{21}H_{20}N_2OS$	349
13b	H	3-Cl	152−153	a	30	$C_{21}H_{19}ClN_2OS$	383
13c	H	4-Cl	148−149	a	18	$C_{21}H_{19}ClN_2OS$	383
13d	H	3,4-Cl	186−188	a	44	$C_{21}H_{18}Cl_2N_2OS$	417
13e[13]	4-Cl	H	144−146	b	38	$C_{21}H_{19}ClN_2OS$	383
13f	4-Cl	3,4-Cl	100−102	c	45	$C_{21}H_{17}Cl_3N_2OS$	451
13g	3,4-Cl	H	85−87	a	50	$C_{21}H_{18}Cl_2N_2OS$	417
14			233−235	a	40	$C_{21}H_{18}N_2O_2S$	363

[a] Code: a, ethanol; b, ether/*n*-hexane; c, 2-propanol. [b] Yields were not optimized. [c] All compounds were analyzed for C, H, N; analytical results were within 0.4% of theoretical values.

Table 3. Enhancing and Antagonistic Activity of Compounds **12a**−**u**, **13a**−**g**, and **14**

compd	% enhancement[a]	EC_{50} (μM)[b]	% antagonism[c]
PD 81,723	100.0	14.7 (±1.4)	39.5 (±4.3)
12a	8.0 (±5.5)		13.9 (±3.2)
12b	80.3 (±19.0)		18.7 (±4.1)
12c	93.5 (±31.6)		41.0 (±6.1)
12d	31.4 (±4.4)		12.6 (±3.0)
12e	111.9 (±10.5)		5.4 (±10.9)
12f	29.9 (±7.5)		22.3 (±2.0)
12g	97.2 (±24.9)		19.8 (±12.2)
12h	47.2 (±4.6)		34.7 (±5.8)
12i	72.5 (±19.3)		35.1 (±3.4)
12j	122.0 (±19.3)	6.0 (±1.0)	32.2 (±8.4)
12k	92.7 (±6.4)	10.5 (±0.9)	50.6 (±4.9)
12l	113.4 (±18.4)		65.8 (±0.9)
12m	131.2 (±11.0)	4.7 (±0.9)	56.7 (±3.9)
12n	122.8 (±14.6)	6.8 (±0.2)	39.9 (±5.5)
12o	128.2 (±18.3)	16.4 (±0.3)	41.6 (±3.7)
12p	154.7 (±21.0)	4.5 (±0.2)	64.4 (±8.1)
12q	33.9 (±21.7)		19.5 (±2.5)
12r	137.2 (±20.9)	34.9 (±1.4)	30.3 (±2.9)
12s	43.9 (±8.9)		28.9 ($n = 1$)
12t	28.6 (±3.3)		nd
12u	151.0 (±24.2)	6.2 (±0.9)	34.7 (±4.5)
13a	52.8 (±36.6)		67.1 (±5.5)
13b	105.7 (±27.3)	15.1 ($n = 1$)	80.1 (±1.2)
13c	69.0 (±23.5)		52.2 (±2.5)
13d	57.0 (±35.8)		4.0 (±2.0)
13e	132.4 (±21.0)	11.3 (±1.1)	60.9 (±0.1)
13f	106.5 (±30.6)	32.0 (±1.1)	45.7 (±2.1)
13g	173.6 (±37.5)	9.2 (±1.4)	51.0 (±0.0)
14	13.8 (±27.3)		71.8 (±2.1)
theophylline	14.9 (±7.5)		56.2 (±5.1)

[a] Enhancing activity by 10 μM of test compound is expressed as percent decrease (±SEM) in [^{3}H]CCPA dissociation over control (0%) and that of 10 μM PD 81,723 (100.0%, $n = 3$). [b] EC_{50} for enhancing activity (±SEM, $n = 3$). [c] Antagonistic activity is expressed as percent displacement (±SEM, $n = 3$−5) of 0.4 nM of [^{3}H]DPCPX by 10 μM of test compound. nd: not determined.

enhancing activity, but have less antagonistic activity (Table 3). Compounds **12j**, **12n**, and **12u** are more potent enhancers than PD 81,723, but have comparable antagonistic activity (Table 3). For reasons of comparison, data for theophylline, a classical competitive adenosine receptor antagonist, were also incorporated in Table 3. It had negligible enhancing activity while showing modest antagonistic activity at 10 μM, comparable to PD 81,723 and derivatives.

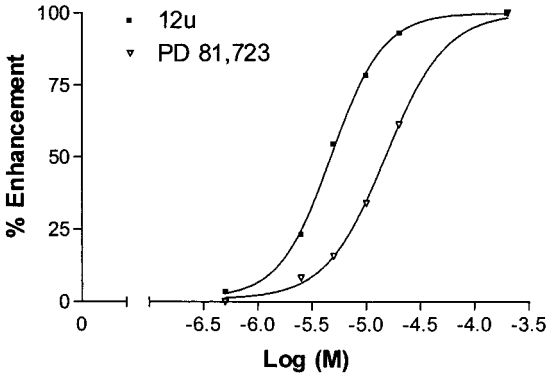


Figure 1. Concentration−effect curves for derivative **12u** and PD 81,723 from one representative experiment. Enhancement of 100% is equivalent to the maximum decrease in [^{3}H]CCPA dissociation by the highest concentration of **12u**.

A quantitative structure−activity analysis (QSAR) was not feasible due to solubility problems that prevented the determination of some EC_{50} values. However, a more qualitative approach suggests that lipophilicity is of prime importance on the benzoyl ring system of series **12** and **13**. Lipophilic *meta* and/or *para* substituents such as halogen are preferred for enhancing activity, whereas more hydrophilic groups such as nitro (**12q**) and carboxylate (**12t**) are not favorable. Electronic influences are probably small, since substituents different in this respect (e.g., methyl and chloro) have similar enhancing effects (cf. **12n** and **12r**).

In conclusion, we have identified novel allosteric enhancers of agonist binding to adenosine A_1 receptors. Some of the compounds proved superior to the reference material PD 81,723. It is hoped that these derivatives may be of help in better understanding adenosine receptor function. It is tempting to speculate that allosteric modulation of G protein-coupled receptors is a more generally valid concept. This prospect seems realistic, since examples for other receptors such as muscarinic,[25] α_2-adrenergic,[26] and serotonin[27] are already at hand. In that case, the development of new chemical entities with a mechanism of action fundamentally different from "classical" agonists and antagonists is anticipated.

Experimental Section

Chemicals and Solvents. All chemicals and solvents used were commercially available unless stated otherwise. [^{3}H]-DPCPX ([^{3}H]-8-cyclopentyl-1,3-dipropyl-xanthine, 120 Ci/mmol, antagonist) and [^{3}H]CCPA (2-chloro-N^6-[^{3}H]-cyclopentyladenosine, 30 Ci/mmol, agonist) were purchased from NEN, and N^6-cyclopentyladenosine (CPA, agonist) was purchased from RBI.

Chromatography. Thin-layer chromatography (TLC) was carried out using aluminum sheets (20 cm × 20 cm) with silica gel F_{254} from Merck. Spots were visualized under UV light (254 nm). Column chromatography was performed on columns of silica gel 60 (Merck 230−400 mesh).

Instruments and Analyses. Elemental analyses were performed for C, H, and N (Microanalytical Laboratory, Department of Chemistry, University College, Dublin, Ireland). ^{1}H NMR spectra were measured at 200 MHz with a JEOL JNM-FX 200 spectrometer equipped with a PG 200 computer operating in the Fourier transform mode. Chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard.

All high-resolution mass spectra were measured on a Finnigan MAT900 mass spectrometer equipped with a direct insertion probe for EI experiments (70 eV with resolution 1000).

Melting points were determined in a Büchi capillary melting point apparatus and are uncorrected.

General Procedure for the Synthesis of Benzoylacetonitrile Derivatives.[21,22] **Method A.** To a mixture of 50 mmol of methyl benzoate derivative **1a**−**c** and 62 mmol of acetonitrile was added 45 mmol of KO*t*Bu. The suspension was heated at 50 °C for 4 h. Water (25 mL) was added, and this solution was washed with CH_2Cl_2. The aqueous phase was acidified with concentrated HCl and extracted with CH_2Cl_2 (3×). The combined organic layers were washed with 10% $NaHCO_3$, dried ($MgSO_4$), and evaporated to give a solid.

Method B. A solution of cyanoacetic acid (**2**, 2 equiv) was dissolved in THF and cooled to −70 °C. To the mixture was added *n*-BuLi (4 equiv), and the reaction temperature was then allowed to rise to 0 °C. The mixture was stirred for 0.5 h at 0 °C and then recooled to −70 °C, and a solution of acid chloride **3a,b** (1 equiv) in THF was added. After being stirred for 1 h, the mixture was allowed to gradually come to room temperature over a period of 1 h. Then aqueous HCl (20%) was added, and the solution was extracted with CH_2Cl_2 (2×). The combined organic layers were washed with 10% $NaHCO_3$, dried ($MgSO_4$), and evaporated to give a solid. After purification by column chromatography the products were used in the next step.

4-Trifluoromethylbenzoylacetonitrile (4a) was prepared according to method A, starting from methyl 4-trifluoromethylbenzoate in a yield of 15%: ^{1}H NMR ($CDCl_3$) δ 3.25 (bs, 2H, CH_2), 7.21−8.09 (m, 4H, H_{arom}).

4-Bromobenzoylacetonitrile (4b) was prepared according to method A, starting from methyl 4-bromobenzoate in a yield of 27%: ^{1}H NMR ($CDCl_3$) δ 2.91 (s, 2H, CH_2), 7.85−8.25 (m, 4H, H_{arom}).

4-Methylbenzoylacetonitrile (4c) was prepared according to method A, starting from methyl 4-methylbenzoate in a yield of 30%: ^{1}H NMR ($CDCl_3$) δ 1.61 (s, 3H, CH_3), 4.05 (s, 2H, CH_2), 7.29−7.83 (m, 4H, H_{arom}).

4-Nitrobenzoylacetonitrile (4d) was prepared according to method B, starting from 4-nitrobenzoyl chloride in a yield of 51%: ^{1}H NMR ($CDCl_3$) δ 4.01 (bs, 2H, CH_2), 7.69−7.94 (m, 4H, H_{arom}).

4-Iodobenzoylacetonitrile (4e) was prepared according to method B, starting from 4-iodobenzoyl chloride in a yield of 20%: ^{1}H NMR ($CDCl_3$) δ 4.85 (bs, 2H, CH_2), 7.54−7.90 (m, 4H, H_{arom}).

General Procedure for the Synthesis of Substituted 1-Benzyl-4-piperidones 6a−c. To a mixture of 50 mmol of 4-piperidone monohydrate mono hydrochloric acid (**5**) and 50 mmol of benzyl chloride derivative **8a**−**c** in dichloroethane (50 mL) was added Et_3N (14 mL). After the mixture was refluxed for 16 h, CH_2Cl_2 (50 mL) was added. The mixture was washed with water (20 mL), dried ($MgSO_4$), and evaporated. The residual oil was distilled (oil pump, 0.05−0.1 mmHg) to give pure product.

1-(3-Chlorobenzyl)-4-piperidone (6a) was prepared as described above in a yield of 85% (oil pump, 0.05−0.1 mmHg, 145 °C): ^{1}H NMR ($CDCl_3$) δ 2.42−2.48 (m, 4H, 2 × CH_2), 2.71−2.77 (m, 4H, 2 × CH_2), 3.60 (bs, 2H, CH_2Ph), 7.25−7.38 (m, 4H, H_{arom}).

1-(4-Chlorobenzyl)-4-piperidone (6b) was prepared as described above in a yield of 80% (oil pump, 0.05−0.1 mmHg, 140−150 °C): ^{1}H NMR ($CDCl_3$) δ 2.42−2.45 (m, 4H, 2 × CH_2), 2.68−2.71 (m, 4H, 2 × CH_2), 3.56 (bs, 2H, CH_2Ph), 7.29−7.32 (m, 4H, H_{arom}).

1-(3,4-Dichlorobenzyl)-4-piperidone (6c) was prepared as described above in a yield of 75% (oil pump, 0.05−0.1 mmHg, 175 °C): ^{1}H NMR ($CDCl_3$) δ 2.43−2.48 (m, 4H, 2 × CH_2), 2.71−2.74 (m, 4H, 2 × CH_2), 3.57 (bs, 2H, CH_2Ph), 7.23−7.49 (m, 3H, H_{arom}).

1-Benzoyl-4-piperidone (7). To a mixture of 4-piperidone mono hydrate mono hydrochloric acid (**5**, 3.9 g, 25 mmol) and benzoyl chloride (2.95 mL, 25 mmol) in CH_2Cl_2 (50 mL) was added Et_3N (10 mL). After the mixture was stirred for 16 h, CH_2Cl_2 (50 mL) was added. The mixture was washed with water (20 mL), dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography to afford **7** in 80%: MS *m*/*z* 204 $[MH]^+$.

General Procedure for the Synthesis of 2-Amino-3-benzoylthiophenes 12a−u, 13a−g, and 14.[19,20] To a suspension of 5 mmol of carbonyl compound, 5 mmol of benzoylacetonitrile derivative, and 5.05 mmol of sulfur in 1.5 mL of ethanol was added 1 mL of Et_2NH. The mixture was stirred for 2 h at 50 °C. The mixture was cooled to room temperature. If the product crystallized from the crude reaction mixture, the precipitate was collected and recrystallized from the appropriate solvent. When no crystallization occurred, the mixture was evaporated and crystallized from the appropriate solvent, after column chromatography purification.

(2-Amino-4,5-dimethyl-3-thienyl)(phenyl)methanone (12a)[13] was prepared as described above, starting from methyl ethyl ketone and benzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.54 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 6.40 (bs, 2H, NH_2), 7.39−7.54 (m, 5H, H_{arom}).

(2-Amino-4,5-dimethyl-3-thienyl)(3-chlorophenyl)methanone (12b) was prepared as described above, starting from methyl ethyl ketone and 3-chlorobenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 6.75 (bs, 2H, NH_2), 7.30−7.49 (m, 4H, H_{arom}).

(2-Amino-4,5-dimethyl-3-thienyl)(4-chlorophenyl)methanone (12c) was prepared as described above, starting from methyl ethyl ketone and 4-chlorobenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.55 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 7.35−7.49 (m, 6H, NH_2, H_{arom}).

(2-Amino-4-ethyl-5-methyl-3-thienyl)(phenyl)methanone (12d)[13] was prepared as described above, starting from 3-pentanone and benzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 0.71 (t, 3H, CH_3), 2.09 (q, 2H, CH_2), 2.16 (s, 3H, CH_3), 6.10 (bs, 2H, NH_2), 7.30−7.65 (m, 5H, H_{arom}).

(2-Amino-4-ethyl-5-methyl-3-thienyl)[3-(trifluoromethyl)phenyl]methanone (12e)[23] was prepared as described above, starting from 3-pentanone and 3-trifluoromethylbenzoylacetonitrile: ^{1}H NMR (CDCl3) δ 0.68 (t, 3H, CH_3), 2.00 (q, 2H, CH_2), 2.16 (s, 3H, CH_3), 6.55 (bs, 2H, NH_2), 7.54−7.80 (m, 4H, H_{arom}).

(2-Amino-4-ethyl-5-methyl-3-thienyl)(3-chlorophenyl)-methanone (12f) was prepared as described above, starting from 3-pentanone and 3-chlorobenzoylacetonitrile: ^{1}H NMR (CDCl3) δ 0.71 (t, 3H, CH_3), 2.08 (q, 2H, CH_2), 2.13 (s, 3H, CH_3), 6.40 (bs, 2H, NH_2), 7.30−7.60 (m, 4H, H_{arom}).

(2-Amino-4-ethyl-5-methyl-3-thienyl)(4-chlorophenyl)-methanone (12g) was prepared as described above, starting from 3-pentanone and 4-chlorobenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 0.71 (t, 3H, CH_3), 2.09 (q, 2H, CH_2), 2.16 (s, 3H, CH_3), 6.00 (bs, 2H, NH_2), 7.36−7.55 (m, 4H, H_{arom}).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(phenyl)methanone (12h)[19] was prepared as described above, starting from cyclohexanone and benzoylacetonitrile: mp 155−153 °C (lit.[19] 155 °C); ^{1}H NMR ($CDCl_3$) δ 1.40−1.62 (m, 2H, CH_2), 1.68−1.80 (m, 4H, 2 × CH_2), 2.48−2.55 (m, 2H, CH_2), 6.65 (bs, 2H, NH_2), 7.38−7.60 (m, 5H, H_{arom}).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(2-chlorophenyl)methanone (12i) was prepared as described above, starting from cyclohexanone and 2-chlorobenzoylacetonitrile: mp 145−147 °C (lit.[20] 155−157 °C); ^{1}H NMR ($CDCl_3$) δ 1.44−1.71 (m, 6H, 3 × CH_2), 2.44−2.49 (m, 2H, CH_2), 7.21−7.37 (m, 4H, H_{arom}).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)[3-(trifluoromethyl)phenyl]methanone (12j) was prepared as described above, starting from cyclohexanone and 3-trifluoromethylbenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.41−1.63 (m, 2H, CH_2), 1.68−1.79 (m, 4H, 2 × CH_2), 2.47−2.54 (m, 2H, CH_2), 7.00 (bs, 2H, NH_2), 7.48−7.72 (m, 4H, H_{arom}).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(3-chlorophenyl)methanone (12k)[13,20] was prepared as described above, starting from cyclohexanone and 3-chlorobenzoylacetonitrile: mp 114−115 °C (lit.[20] 108−110 °C); ^{1}H NMR ($CDCl_3$) δ 1.42−1.58 (m, 2H, CH_2), 1.70−1.87 (m, 4H, 2 × CH_2), 2.47−2.55 (m, 2H, CH_2), 6.88 (bs, 2H, NH_2), 7.31−7.44 (m, 4H, H_{arom}).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(3-iodophenyl)methanone (12l) was prepared as described above, starting from cyclohexanone and 3-iodobenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.47−1.82 (m, 6H, 3 × CH_2), 2.49−2.55 (m, 2H, CH_2), 6.80 (bs, 2H, NH_2), 7.14−7.79 (m, 4H, H_{arom}).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)[4-(trifluoromethyl)phenyl]methanone (12m) was prepared as described above, starting from cyclohexanone and 4-trifluoromethylbenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.48−1.50 (m, 2H, CH_2), 1.69−1.76 (m, 4H, 2 × CH_2), 2.48 (m, 2H, CH_2), 6.91 (bs, 2H, NH_2), 7.54−7.69 (m, 4H, H_{arom}).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(4-chlorophenyl)methanone (12n)[13] was prepared as described above, starting from cyclohexanone and 4-chlorobenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.47−1.52 (m, 2H, CH_2), 1.71−1.81 (m, 4H, 2 × CH_2), 2.47−2.54 (m, 2H, CH_2), 6.71 (bs, 2H, NH_2), 7.34−7.45 (m, 4H, H_{arom}).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(4-bromophenyl)methanone (12o) was prepared as described above, starting from cyclohexanone and 4-bromobenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.46−1.54 (m, 2H, CH_2), 1.67−1.83 (m, 4H, 2 × CH_2), 2.46−2.51 (m, 2H, CH_2), 6.80 (bs, 2H, NH_2), 7.33−7.55 (m, 4H, H_{arom}).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(4-iodophenyl)methanone (12p) was prepared as described above, starting from cyclohexanone and 4-iodobenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.41−1.87 (m, 6H, 3 × CH_2), 2.43−2.52 (m, 2H, CH_2), 6.82 (b, 2H, NH_2), 7.17−7.75 (m, 4H, H_{arom}).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(4-methylphenyl)methanone (12q) was prepared as described above, starting from cyclohexanone and 4-methylbenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.45−1.50 (m, 2H, CH_2), 1.70−1.91 (m, 4H, 2 × CH_2), 2.38 (s, 3H, CH_3), 2.47−2.54 (m, 2H, CH_2), 6.05−6.60 (b, 2H, NH_2), 7.16−7.42 (m, 4H, H_{arom}).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(4-nitrophenyl)methanone (12r) was prepared as described above, starting from cyclohexanone and 4-nitrobenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 0.85−1.16 (m, 6H, 3 × CH_2), 1.88−2.00 (m, 2H, CH_2), 6.43 (bs, 2H, NH_2), 7.01−7.69 (m, 4H, H_{arom}).

Methyl 4[(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)carbonyl]benzoate (12s) was prepared as described above, starting from cyclohexanone and methyl 4-(2-cyanoacetyl-)benzoate: ^{1}H NMR ($CDCl_3$) δ 1.41−1.47 (m, 2H, CH_2), 1.68−1.73 (m, 4H, 2 × CH_2), 2.45−2.51 (m, 2H, CH_2), 3.94 (s, 3H, OCH_3), 7.12 (bs, 2H, NH_2), 7.49−8.10 (m, 4H, H_{arom}).

4-[(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)-carbonyl]benzoic Acid (12t). To a solution of methyl 4-[(2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)carbonyl]-benzoate (**12s**, 160 mg, 0.5 mmol) in MeOH (2 mL) was added KOH (1 M, 5 mL). The mixture was stirred for 0.5 h at 50 °C. Then MeOH was evaporated, and aqueous HCl (1 M) was added until the product precipitated. Now the mixture was stirred at 0 °C for 0.5 h and filtered. The resulting solid was dried to afford **12t** (140 mg, 93%): ^{1}H NMR (CD_3OD) δ 1.40−1.49 (m, 2H, CH_2), 1.64−1.70 (m, 4H, 2 × CH_2), 2.42−2.48 (m, 2H, CH_2), 7.43−8.09 (m, 4H, H_{arom}).

(2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)(3,4-dichlorophenyl)methanone (12u) was prepared as described above, starting from cyclohexanone and 3,4-dichlorobenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.46−1.54 (m, 2H, CH_2), 1.67−1.83 (m, 4H, 2 × CH_2), 2.45−2.51 (m, 2H, CH_2), 7.11 (bs, 2H, NH_2), 7.27−7.7.56 (m, 3H, H_{arom}).

(2-Amino-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-3-yl)(phenyl)methanone (13a)[13] was prepared as described above, starting from 1-benzyl-4-piperidone and benzoylacetonitrile: ^{1}H NMR (DMSO-d_6) δ 1.71 (m, 2H, CH_2CH_2N), 2.34 (m, 2H, CH_2CH_2N), 3.27 (m, 2H, NCH_2), 3.53 (m, 2H, CH_2-Ph), 7.27−7.44 (m, 10H, H_{arom}), 8.20 (bs, 2H, NH_2).

(2-Amino-6-(3-chlorobenzyl)-4,5,6,7-tetrahydrothieno-[2,3-*c*]pyridin-3-yl)(phenyl)methanone (13b) was prepared as described above, starting from 1-(3-chlorobenzyl)-4-piperidone and benzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.90−1.96 (m, 2H, CH_2CH_2N), 2.43−2.49 (m, 2H, CH_2CH_2N), 3.42 (bs, 2H, NCH_2), 3.59 (bs, 2H, CH_2Ph), 6.80 (bs, 2H, NH_2), 7.19−7.51 (m, 9H, H_{arom}).

(2-Amino-6-(4-chlorobenzyl)-4,5,6,7-tetrahydrothieno-[2,3-*c*]pyridin-3-yl)(phenyl)methanone (13c) was prepared as described above, starting from 1-(4-chlorobenzyl)-4-piperidone and benzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.89−1.91 (m, 2H, CH_2CH_2N), 2.42−2.47 (m, 2H, CH_2CH_2N), 3.40 (bs, 2H, NCH_2), 3.57 (bs, 2H, CH_2Ph), 6.80 (bs, 2H, NH_2), 7.24−7.47 (m, 9H, H_{arom}).

(2-Amino-6-(3,4-dichlorobenzyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-3-yl)(phenyl)methanone (13d) was prepared as described above, starting from 1-(3,4-dichlorobenzyl)-4-piperidone and benzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.92−1.95 (m, 2H, CH_2CH_2N), 2.43−2.48 (m, 2H, CH_2CH_2N), 3.40 (bs, 2H, NCH_2), 3.55 (bs, 2H, CH_2Ph), 6.79 (bs, 2H, NH_2), 7.19−7.47 (m, 8H, H_{arom}).

(2-Amino-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-3-yl)(4-chlorophenyl)methanone (13e)[13] was prepared as described above, starting from 1-benzyl-4-piperidone and 4-chlorobenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.90−1.96 (m, 2H, CH_2CH_2N), 2.46−2.51 (m, 2H, CH_2CH_2N), 3.41 (bs, 2H, NCH_2), 3.61 (bs, 2H, CH_2Ph), 6.79 (bs, 2H, NH_2), 7.27−7.45 (m, 9H, H_{arom}).

(2-Amino-6-(3,4-dichlorobenzyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-3-yl)(4- chlorophenyl)methanone (13f) was prepared as described above, starting from 1-(3,4-dichlorobenzyl)-4-piperidone and 4-chlorobenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.93−1.97 (m, 2H, CH_2CH_2N), 2.45−2.48 (m, 2H, CH_2CH_2N), 3.38 (bs, 2H, NCH_2), 3.56 (bs, 2H, CH_2-Ph), 6.86 (bs, 2H, NH_2), 7.15−7.48 (m, 7H, H_{arom}).

(2-Amino-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-3-yl)(3,4-dichlorophenyl)methanone (13g) was prepared as described above, starting from 1-benzyl-4-piperidone and 3,4-dichlorobenzoylacetonitrile: ^{1}H NMR ($CDCl_3$) δ 1.92−1.96 (m, 2H, CH_2CH_2N), 2.44−2.47 (m, 2H, CH_2CH_2N), 3.39 (bs, 2H, NCH_2), 3.61 (bs, 2H, CH_2Ph), 7.16−7.55 (m, 10H, NH_2, H_{arom}).

(2-Amino-6-benzoyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-3-yl)(phenyl)methanone (14) was prepared as described above, starting from 1-benzoyl-4-piperidone and benzoylacetonitrile: ^{1}H NMR (DMSO-d_6) δ 1.79−1.82 (m, 2H, CH_2CH_2N), 3.12−3.57 (m, 2H, CH_2CH_2N), 4.31−473 (m, 2H, NCH_2), 7.18−7.42 (m, 10H, H_{arom}), 8.22 (bs, 2H, NH_2).

Binding Studies. The adenosine A_1 binding assays were carried out on membranes of rat brain cortex. Membranes were prepared according to a method described previously,[28] except that the membranes were incubated with 2 U/mL ADA at 37 °C before storage.[29] Protein concentrations were measured with the BCA method.[30]

1. Displacement Assay. The assays were performed as originally described.[31] Rat brain cortex membranes (30 μg/mL) were incubated for 1 h at 25 °C in Tris/HCl buffer (50 mM, pH 7.4) in the presence of 0.4 nM [^{3}H]DPCPX (K_d 0.28 nM[29]) and 10 μM test compound. Nonspecific binding was determined in the presence of 10 μM CPA.

2. Dissociation Assay.[13] Rat brain cortex membranes were preincubated for 2.5 h with 0.5 nM [^{3}H]CCPA after which displacement was initiated by 100 μM CPA, in the presence or absence of 10 μM of test compound. The amount of [^{3}H]-CCPA still bound to the receptor was measured after 1 h.

Data Analysis. EC_{50} values for enhancing activity were calculated with Prism (Graph Pad, San Diego, CA).

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