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Synthesis and Biological Activities of Flavonoid Derivatives as A_3 Adenosine Receptor Antagonists

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Abstract

A broad screening of phytochemicals has demonstrated that certain flavone and flavonol derivatives have a relatively high affinity at A_3 adenosine receptors, with K_i values of ≥1 μM (Ji et al. *J. Med. Chem*. **1996**, *39*, 781–788). We have further modified the flavone structure to achieve a degree of selectivity for cloned human brain A_3 receptors, determined in competitive binding assays versus [^{125}I]AB-MECA [*N*^{6}-(4-amino-3-iodobenzyl)adenosine-5′-(*N*-methyluronamide)]. Affinity was determined in radioligand binding assays at rat brain A_1 and A_{2A} receptors using [^{3}H]-*N*^{6}-PIA ([^{3}H]-(*R*)-*N*^{6}-phenylisopropyladenosine) and [^{3}H]CGS21680 [[^{3}H]-2-[[4-(2-carboxyethyl)phenyl]ethylamino]-5′-(*N*-ethylcarbamoyl)adenosine], respectively. The triethyl and tripropyl ether derivatives of the flavonol galangin, **4**, had K_i values of 0.3–0.4 μM at human A_3 receptors. The presence of a 5-hydroxyl group increased selectivity of flavonols for human A_3 receptors. The 2′,3,4′,7-tetraethyl ether derivative of the flavonol morin, **7**, displayed a K_i value of 4.8 μM at human A_3 receptors and was inactive at rat A_1/A_{2A} receptors. 3,6-Dichloro-2′-(isopropyloxy)-4′-methylflavone, **11e**, was both potent and highly selective (~200-fold) for human A_3 receptors (K_i = 0.56 μM). Among dihydroflavonol analogues, the 2-styryl instead of the 2-aryl substituent, in **15**, afforded selectivity for human A_3 vs rat A_1 or A_{2A} receptors. The 2-styryl-6-propoxy derivative, **20**, of the furanochromone visnagin was 30-fold selective for human A_3 receptors vs either rat A_1 or A_{2A} receptors. Several of the more potent derivatives effectively antagonized the effects of an agonist in a functional A_3 receptor assay, *i.e.* inhibition of adenylyl cyclase in CHO cells expressing cloned rat A_3 receptors. In conclusion, these series of flavonoids provide leads for the development of novel potent and subtype selective A_3 antagonists.

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Introduction

The principal mechanism by which caffeine and other alkylxanthines act as physiological stimulants is by blocking the effects of the ubiquitous neuromodulator adenosine,[1] which acts as a “local hormone” within a given organ. Adenosine is produced locally in response to increased activity or stress to the system. This feedback mechanism allows the organ to compensate for the stress by decreasing energy demand (depressant activity) and increasing oxygen supply (*e.g.* by vasodilation).[2]

Extracellular adenosine activates receptors of the A_1 and A_{2A} subtypes, which depresses the action of the heart, brain, kidneys, and the immune system, as well as other systems.[3] Recently, the novel A_3 subtype of adenosine receptors has been cloned,[4,5] and its pharmacological[6–9] and regulatory characteristics[10] have been studied. Activation of A_3 receptors requires relatively high, *i.e.* pathological, concentrations of adenosine; the K_i value of adenosine has been estimated as ~1 μM at A_3 receptors versus 10 and 30 nM at A_1 and A_{2A} receptors, respectively.[11] Thus the physiological role of A_3 receptors may be very different from that of the A_1 and A_{2A} subtypes. A_3 agonists induce hypotension and promote the release of inflammatory mediators from mast cells.[8] We have introduced the first selective agonist ligands for the A_3 subtype, including the agonist IB-MECA [N^6-(3-iodobenzyl)adenosine-5′-(*N*-methyluronamide)].[9,12] IB-MECA has been shown to elicit locomotor depression,[9] and following chronic administration it acts as a cerebroprotectant in stroke and seizure models.[13]

In principle, a selective A_3 adenosine receptor antagonist should serve as a cerebroprotective, antiasthmatic, or antiinflammatory agent.[6,7,11,13] Previously, we have successfully designed specific antagonists for A_1 and A_{2A} receptors by chemically modifying the structures of caffeine and other xanthines.[3] However, at A_3 receptors xanthines are of particularly low affinity[14] and have not yet provided satisfactory chemical leads for antagonists.[15] Consequently, we have searched diverse chemical libraries for lead structures for developing A_3 antagonists.[16–19] A number of classes of non-xanthine adenosine antagonists have already been reported, including various nitrogen heterocycles and several classes of nonnitrogen heterocycles.[16] We recently reported that tetrahydrobenzothiophenones, *e.g.* ethyl 3-(benzylthio)-4,5,6,7-tetrahydro-4-oxobenzo[*c*]thiophene-1-carboxylate (BTH_4), bind to A_1 and A_{2A} adenosine receptors in the micromolar range.[17] One member of this series, 4,5-dihydro-1-(methylthio)benzo[*c*]thiophene-3-carboxylic acid hydrazide, **I** (Figure 1), bound with slight selectivity for rat A_3 receptors. Siddiqi et al.[16] have shown that folic acid (**II**), a pyridopyrimidinone (**III**), cytochalasin H (**IV**), 11-hydroxytetracarbazolenine (**V**), the adenosine uptake inhibitor dipyridamole (**VI**), certain sulfonylpiperazines (*e.g.* the protein kinase C inhibitor HA-100, **VII**), and a number of other hetero-cyclic substances displace radioligand from adenosine receptors with low affinity but with apparent selectivity for rat A_3 receptors. Recently, a broad screening of phytochemicals has demonstrated that certain flavones and flavonols, members of the larger class of phenolic natural products known as flavonoids, have a relatively high affinity at A_3 adenosine receptors, with K_i values in the micromolar range, close to that of the natural ligand adenosine.[19] The affinity of some

flavones at A_1 and A_{2A} receptors was also noted by Ares et al.[20] In the present study we have investigated the structure—activity relationships of flavonoids at adenosine receptors in an effort to develop novel A_3 adenosine antagonists. Selected analogues have been tested in a functional A_3 receptor assay, *i.e.* inhibition of adenylyl cyclase in Chinese hamster ovary (CHO) cells expressing cloned rat A_3 receptors.[4]

Results

Synthesis.

The structures of the flavonoid derivatives (including flavonols, **1**–**10**; flavones, **11**; flavanones, **12** and **13**; dihydroflavonols, **14**–**16**; and furylchromones, **17**–**24**) examined in the present study are shown in Table 1, and the chemical characterization is summarized in Table 2. In our previous study,[19] the flavonol galangin (3,5,7-trihydroxyflavone), **1**, was shown to bind to adenosine receptors nonselectively, while its trimethyl ether derivative, **2**, was shown to have enhanced affinity at A_3 receptors. Thus, we synthesized and compared in receptor binding assays the series of alkyl ethers of galangin, **2**–**4**, and other 3-substituted flavone derivatives.

The alkylation of galangin was carried out using the appropriate alkyl iodide and potassium carbonate in refluxing acetone (Figure 2), providing the completely alkylated product, **3a** or **4**. Partial alkylation leaving the 5-position hydroxyl group unreacted, to provide **3b**, was accomplished under less forceful conditions, *e.g.* alkyl bromide at room temperature. The *O*-alkylation of morin (2′,3,4′,5,7-pentahydroxyflavone) was complicated by an even lower reactivity of the 5-hydroxy group than in galangin, leading to tetra- rather than pentasubstitution (Figure 2). Thus, when ethyl sulfate was used the fully alkylated morin derivative, **8**, was obtained, while reaction with ethyl iodide provided exclusively the tetraethyl derivative, **7**. The position of the nonalkylated hydroxyl group of **3b** and **7** was determined from the NMR chemical shift. The resonance for the 5-OH proton in flavone derivatives has been reported to appear at roughly 12 ppm.[12] The downfield shift is apparently caused by the adjacent carbonyl to which it may form a hydrogen bond and thus a noncovalent six-membered ring. This proton signal is present in the spectra of **3b**, **5a**, and **7** but not **8**.

The synthetic routes to flavone derivatives have been investigated extensively and optimized.[22–25] As shown in Figure 3, a synthetic route used to form the flavone ring system was via a condensation of a 2-hydroxyacetophenone derivative, **25**, with the appropriate aldehyde, **26**, which gave rise to the 2-substituent. The condensation resulted first in a *trans*-olefin, **27**, which could be cyclized under basic oxidizing conditions to give either the 2,3-saturated dihydroflavonol (*e.g.* 3-hydroxyflavanone) analogues, **15** and **16**, or at higher temperature the corresponding dehydro derivative, **10**, a flavonol. Cinnamaldehyde (**26**, R = PhCHd=CH−) used as the second component, led to styryl substitution at the 2-position (**15**). Similarly, use of phenylpropargyl aldehyde (**26**, R = PhC≡C−) provided 2-phenylethynyl substitution of the flavonol (**10**) or dihydroflavonol (**16**). Evidence that the dihydroflavonols obtained were of the *trans* configuration were deduced from NMR coupling constants for the 2- and 3-position protons in compounds **14**-**16** of ~12 Hz,

characteristic of *trans* vicinal coupling.[26] The 3-chloro substituent was introduced in flavone derivatives (as in the preparation of **11b** and **11d**) using sulfuryl chloride in carbon tetrachloride.[37]

An alternate approach to providing olefinic substitution at the 2-position (Figure 4) was to condense a 2-methylchromone (such as the natural product visnagin, **17**)[27] with an aldehyde, **28**, under basic conditions. This method was used to prepare the 2-styryl derivative, **19**. Evidence for a *trans*-styryl group of **19** was derived from the NMR spectra with an olefinic coupling constant of 15 Hz.[26] In the presence of sodium ethoxide the 5-methoxy group readily exchanged, resulting in compound **20**. The corresponding 5-propoxy derivative, **21**, was obtained using an alternate approach (Figure 4), in which visnagin was demethylated to give **29**, followed by condensation with benzaldehyde. The 2-methyl group of visnagin could also be oxidized to an aldehyde group, as in **18**.[28]

Affinity at Adenosine Receptors.

K_i values at A_1 and A_{2A} receptors were determined in radioligand binding assays in rat brain membranes vs [^{3}H]PIA or [^{3}H]CGS 21680, respectively.[29,30] Affinity at human brain A_3 receptors expressed in HEK-293 cells[31] was determined using [^{125}I]AB-MECA [N^6-(4-amino-3-iodobenzyl)adenosine-5′-(*N*-methyluronamide)].[32] The complete set of compounds was tested at human A_3 receptors, and only selected compounds at rat A_3 receptors (stably expressed in CHO cells[4,14–16]), which would be preferred for comparison with the A_1 and A_{2A} data. We chose the human A_3 receptor for our screen for the following reasons: (1) The affinity of most known adenosine receptor antagonists is minimal at rat A_3 receptors.[14,16] (2) The human A_3, being more sensitive, allowed for a better comparison between compounds.[31] (3) The human receptor is more relevant to therapeutic need. (4) The preliminary indications are that the A_3 selectivity of flavones may be at least as great as indicated in this study when comparison is made between human receptor homologues. For example, the K_i value of galangin at human A_{2A} receptors is 4.1 ± 0.7 μM (unpublished), compared to 0.97 μM at rat A_{2A} receptors.[19] At cloned human A_1 receptors, the IC_{50} value for galangin in displacement 2 nM [^{125}I]AB-MECA is 13.6 ± 1.5 μM (unpublished), *i.e.* weaker than at rat A_1 receptors.

As we have previously reported,[19] the flavonol galangin (2-phenyl-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one), **1**, inhibited binding at three adenosine receptor subtypes with K_i values of 1–3 μM. The trialkylation of galangin (compounds **2**, **3a**, and **4**) caused an increase in affinity at human A_3 receptors, in the order OPr = OEt > OMe > OH. At rat A_1 and A_{2A} receptors the affinity either was unchanged (A_1) or varied in the order OH > OEt > OPr > OMe (A_{2A}). The most potent and "A_3 receptor selective" of the galangin ethers was the tripropyl derivative, **4**, which had a K_i value of 0.32 μM at human A_3 receptors and was 3-fold selective vs rat A_1 receptors. The presence of a free 5-hydroxyl group, in **3b**, greatly decreased A_{2A} receptor affinity, while A_3 receptor affinity was only 2-fold diminished. Morin, **5a**, was considerably less potent than galangin at all three adenosine receptor subtypes.[19] However, *O*-alkylation caused a gain in affinity, primarily at the human A_3 receptors. As previously reported,[19] pentamethylmorin, **6**, appeared to bind with some selectivity (10- and 18-fold for human A_3 vs rat A_1 and A_{2A} receptors, respectively), with

a K_i value of 2.65 μM at human A_3 receptors. The affinities of tetra- and pentaethyl ether derivatives of morin, **7** and **8**, were compared. The presence of a free 5-hydroxyl group, in 2′,3,4′,7-tetraethylmorin, **7**, greatly diminished affinity at A_1 and A_{2A} receptors and slightly enhanced affinity at A_3 receptors, relative to the pentaethyl ether derivative, **8**. Thus, compound **7** appeared to be selective for human A_3 receptors vs rat A_1 and A_{2A} receptors. A derivative of 2′,4′,5,7-tetrahydroxyflavone, **5b**, that was prenylated[33] at the 3- and 6-positions and methylated at the 7-hydroxyl group had K_i values of 9.1 and 4.6 μM at rat A_1 and human A_3 receptors, respectively.

A trimethoxyphenyl derivative of flavonol, **9**, only weakly displaced the binding of [^{125}I]AB-MECA binding at human A_3 receptors. Compound **9** also bound weakly at rat A_{2A} receptors, but with moderate affinity at rat A_1 receptors (K_i = 7.3 μM). The combination of an acetylenic substituent at the 2-position and a 6-methyl ether group (as in compound **10**) failed to significantly enhance potency or selectivity.

The binding affinities of a number of flavones (non-hydroxylated at the 3-position) were compared. Flavone itself, **11a**, was 5-fold selective for either rat A_1 or A_{2A} receptors vs human A_3 receptors. Compound **11e**, a 3,6-dichloro derivative, was particularly potent and selective for human A_3 receptors. The affinities of compound **11a** at cloned rat A_3 receptors (K_i = 5.50 ± 2.221 μM) and at human A_1 receptors (at 100 μM, only 35.0 ± 3.8% displacement of the binding of 2 nM [^{125}I]AB-MECA) were also measured. Thus, this flavone derivative is ~20-fold selective for rat A_3 vs rat A_1 receptors, and ~200-fold selective for human A_3 vs human A_1 receptors. This compound, obtained from a chemical library of the National Cancer Institute (NCI), was found by Edwards et al.[34] to lack potential as an anticancer agent. It was selected in this study for its combination of substituent groups at 3- and 2′-positions.[19] The source of the selectivity apparently resided with 3,6-dichloro substitution, as indicated by the selectivity of compound **11d**, which had a K_i value of 0.741 μM at human A_3 receptors. This compound was >100-fold selective for human A_3 receptors vs rat A_1 receptors and 73-fold selective vs rat A_{2A} receptors. The presence of trimethyl substitution of the phenyl ring in **11f** did not eliminate selectivity, although the affinity at A_3 receptors was somewhat diminished. Curiously, either 3-chloro or 6-chloro substitution alone (**11b** or **11c**) did not result in increased potency or selectivity for human A_3 receptors. 3-Chloroflavone, **11b**, was more potent than the 6-chloro analogue, **11c**; however, it did not exceed the affinity of unsubstituted flavone, **11a**.

A number of derivatives of flavanone, **12**, showed moderate affinity at adenosine receptors. Within a series of 2,3-saturated derivatives,[35] a 2′-hydroxyl group enhanced the adenosine receptor affinity (*e.g.* the flavanone derivative **13a**). Compound **13b**, 4′-hydroxyflavanone, was less potent in binding to all three subtypes of adenosine receptors than the corresponding 2′-hydroxy analogue. Additional substitution of the A ring of 4′-hydroxyflavanone, as in sakuranetin, **13c**, enhanced affinity at human A_3 receptors. Previously we reported that reduction of the 2,3-olefinic bond of the flavonols, as in *trans*-(±)-dihydroquercetin,[19] greatly diminished affinity at adenosine receptors. Consistently, the 3-hydroxyl group of **14** greatly diminished affinity relative to **13a**.

Among dihydroflavonol analogues, the 2-styryl instead of the 2-aryl substituent, **15**, afforded >8-fold selectivity for human A_3 vs rat A_1 and A_{2A} receptors. The corresponding dihydroflavonol 2-phenylethynyl derivative, **16**, was nearly as selective as **15** (6.2-fold for human A_3 vs rat A_1 receptors).

Visnagin, **17**, a vasoactive furanochromone derivative[27,36] isolated from *Ammi visnaga*, a fruit that has been used in folk medicine for its antispasmodic properties and in the treatment of angina pectoris,[36] bound weakly to adenosine receptors. The corresponding 2-aldehyde, **18**, was a similarly weak ligand at adenosine receptors. The presence of a *trans*-styryl group at the 2-styryl position, in **19**, caused an 11-fold enhancement of A_3 affinity, with less pronounced enhancements of affinity at A_1 and A_{2A} receptors. Elongation of the alkyl group at the 6-methyl ether of **19** caused a further affinity enhancement at A_3 receptors, thus the corresponding 6-ethyl ether, **20**, was 31-fold selective for human A_3 receptors vs rat A_1 receptors, with a K_i value of 1.16 μM. The selectivity of **20** for human A_3 vs rat A_{2A} receptors was 29-fold. Maximal selectivity was obtained with **20**, since the corresponding 6-propyloxy derivative, **21**, was less selective. The 6-ethoxy and 6-propyloxy derivatives, **20** and **21**, were 7.1- and 2.1-fold more potent, respectively, than the 6-methoxy ether, **19**. The 1-phenyl-1,3-butadiene derivative, **22**, corresponding to an olefinic homologation of compound **19**, was inactive in binding at all three adenosine receptor subtypes. The predicted enhancement of A_3 receptor affinity upon ethyl substitution (**23**) of the 6-methyl ether, by analogy to compound **20**, was insignificant.

Compound **24**, the Schiff's base equivalent of compound **19**, *i.e.* in which the α-methyne group was formalistically replaced with nitrogen, bound selectively to human A_3 (K_i 9.2 μM) vs rat A_1 receptors.

At rat A_3 receptors the affinity of flavones and flavonols, like that of many xanthines, has been shown to be weaker than at human A_3 receptors.[19] The K_i values of **1** and **2** have been shown to be 43.1 and 17.4 μM, respectively, at rat A_3 receptors. This represents a human A_3 vs rat A_3 affinity ratio of 14-fold in both cases. The K_i values of several novel derivatives in this study (Table 3) have been measured in binding at cloned rat A_3 receptors in membranes of transfected CHO cells.[18,32] The K_i value of **11e** at rat A_3 receptors was 5.50 ± 2.21 μM; thus it was roughly 20-fold selective vs rat A_1 receptors. In the rat compound **20** was only 3-fold selective for A_3 receptors. The ratios between human and rat affinities were 50-, 9.8-, and 16-fold for **3a**, **11e**, and **20**, respectively.

Functional Assay at Cloned Rat A_3 Receptors.

We examined the antagonist properties of several flavonoid derivatives in a functional assay at A_3 receptors, *i.e.* inhibition of adenylyl cyclase in CHO cells expressing cloned rat A_3 receptors. Among the derivatives chosen, compounds **11e**, **15**, and **20** were subtype selective in binding, and compound **3a** was very potent at human A_3 receptors although nonselective. Adenylyl cyclase was inhibited by IB-MECA over the concentration range of 10^{-9}–10^{-4} M in transfected CHO cells. The maximal extent of inhibition was 50–60% with an IC_{50} of approximately 0.1 μM, as reported.[12] In Table 3 the concentration of IB-MECA used was either 0.1 or 1.0 μM, *i.e.* 1- or 10-fold the IC_{50} for the inhibition of rat A_3 receptor

mediated inhibition of adenylyl cyclase, respectively. Compound **3a** at a concentration of 50 μM nearly completely abolished the inhibitory effect of 0.1 μM IB-MECA on adenylyl cyclase (Table 3). Compound **11e** (Figure 5) also reversed the inhibition of adenylyl cyclase induced by IB-MECA at several concentrations. Compounds **15** or **16** at concentrations as high as 50 μM showed no significant effect on potency or maximal effect of agonist-induced inhibition of adenylyl cyclase.

Discussion

The affinity of xanthines at A_3 receptors, although species dependent, is generally extremely weak relative to their affinity at other subtypes.[14] Thus, the naturally occurring xanthines do not act as antagonists at A_3 receptors, as they do at the other subtypes. It is possible that certain members of another large family of natural products, the flavonoids, which are even more wide-spread in the human diet than the xanthines,[38] may serve in this capacity as A_3 receptor antagonists.[19] More than 4000 flavonoids have already been isolated and identified from vascular plants,[38] and they display a very wide range of biological activities. For example, flavonoids are used therapeutically to improve circulation and integrity of blood vessels[40] and to suppress inflammation.[41] They have been proposed as a potential treatment for diabetes, allergic reactions, infections, and other disorders.[42] Even antineoplastic activities have been claimed for flavonoids.[34] Many of these reported biological effects have not yet been fully explained mechanistically, although many mammalian enzymes and other proteins regulating intracellular biochemical processes, such as protein tyrosine kinases,[23,24] are known to be affected by flavonoids.[43,44] Flavones also inhibit phosphodiesterases and HIV integrase.[48,49] Flavones have been studied in relation to the biological activity of adenosine[45] and adenosine deaminase, which is inhibited at high concentrations of certain flavones.[46]

In several recent reports, flavones were found to bind to adenosine receptors,[19,20] and certain derivatives have substantial affinity at the A_3 receptor subtype.[19] We identified flavones and flavonols as moderately potent adenosine receptor ligands as a result of screening a natural products library. Ares et al.[20] independently discovered the affinity of flavones at A_1 and A_{2A} receptors in an investigation of the known gastroprotective effects of the substances,[47] which yet lack a mechanistic explanation.

The most selective compounds for human A_3 receptors identified in this study were compounds **7** and **11d-f**, which were all essentially inactive at A_1 and A_{2A} receptors. The presence of a 5-hydroxyl group, as in **3b** and **7**, increased selectivity of flavonols for human A_3 receptors. The most potent compounds in binding to A_3 receptor (in order of increasing K_i value) were compounds **4**, **3a** > **11e**, **11d** ≥ **3b** ≥ **20**, **2** > **6**. The degree of selectivity achieved was as high as 200-fold for compound **11e** (Table 1). Since flavonoids are known to have affinity for many other proteins, it will be necessary to screen these derivatives in many other biochemical assays and at other receptors in order to establish true selectivity.

Conformational insights may be obtained from the present results. Compound **11e** was highly selective for the human A_3 receptor. In this derivative both the 3- and 2′-positions are highly substituted; thus there is likely steric hindrance to free rotation of the phenyl ring

and assumption of a planar geometry. The potency and selectivity were similar to compound **11d**, which was not as highly hindered sterically. Thus, we may conclude that a coplanar geometry between the two ring systems in the flavonols is not required.

Functional antagonism was indicated for derivatives, compounds **3a** and **11e**. It will be necessary to study the pharmacological effects in greater detail, in order to determine that other sites of interaction, for example at the level of G-proteins and second messengers, are not involved in the observed biological effect on adenylyl cyclase. It is likely that only flavonoids having K_i values of ≤1 μM at human A_3 receptors will be useful as pharmacological probes.

In conclusion, these series of flavonoids provide leads for the development of novel potent and selective A_3 adenosine receptor antagonists,[14] although more detailed pharmacological characterization is required. The present findings indicate that the chemical modification of flavones at the 2- (with styryl groups), 3- (with hydroxyl or chloro), 6- (with chloro), and 2′-positions (with sterically bulky groups) may prove fruitful in this regard. The 2,3-position double bond appears to be required for recognition by human A_3 receptors when both a 2-phenyl substituent and a 3-hydroxyl group are present. However the double bond is not an absolute requirement, since flavanone derivatives **13a–c**, which lack the 3-hydroxyl group, displayed moderate affinity but not selectivity. Another means of restoring affinity in the reduced flavonol derivatives was to introduce a styryl or phenylethynyl group at the 2-position (compounds **15** and **16**). Additional stucture–activity studies will be needed in order to design flavonoid analogues that are highly A_3 receptor selective across species.

Experimental Section

Materials.

Compounds **1**, **11a**, **12**, and **17** were obtained from Fluka, Ronkonoma, NY, or from Aldrich, St. Louis, MO. Compounds **13a**, **13c**, and **14** were obtained from Apin Chemicals, Ltd., Oxon, U.K. Compounds **5a** and **13b** were obtained from K+K Laboratories, Jamaica, NY. Compounds **5b** (NSC #241010-z), **9** (NSC #78634-f), **11e** (NSC #74899-t), and **11f** (NSC #74931-f) were obtained from NCI (Bethesda, MD).

Synthesis.

Proton nuclear magnetic resonance spectroscopy was performed on a Varian GEMINI-300 spectrometer and spectra were taken in DMSO-d_6. Electron-impact mass spectrometry was performed with a VG7070F mass spectrometer at 6 kV. Elemental analysis was performed by Atlantic Microlabs, Inc. (Norcross, GA).

3,5,7-Trimethoxyflavone (2).

Galangin (27 mg, 0.1 mmol) was dissolved in dry acetone (stored over K_2CO_3, 20 mL), solid potassium carbonate (0.5 g) and dimethyl sulfate (1 mL) were added, and the mixture was refluxed for 4 h and then cooled to room temperature. The solution was filtered and evaporated, water (20 mL) and concentrated ammonium hydroxide (2 mL) were added, and the solution was extracted with ethyl acetate (15 mL × 2). The solvent was evaporated,

and the residue was recrystallized from methanol/water to give 21 mg of product (67%): ^{1}H-NMR (DMSO-d_6) δ 3.73 (s, 3H, 3-OCH_3), 3.86 (s, 3H, 7-OCH_3), 3.86 (s, 3H, 5-OCH_3), 6.48 (s, 1H, 8-H), 6.79 (s, 1H, 6-H), 7.99 (m, 3H, Ar), 8.02 (m, 2H, Ar); MS (CI/NH_3) *m*/*z* 313 (MH^+, base).

3,5,7-Triethoxyflavone (3a).

Galangin (30 mg, 0.11 mmol) was dissolved in dry acetone (45 mL), solid potassium carbonate (0.5 g) and iodoethane were added, and the mixture was refluxed overnight. The solution was filtered and evaporated, water (20 mL) and concentrated ammonia (1 mL) were added, and the solution was extracted with ethyl acetate. The ethyl acetate was evaporated, and the crude mass was purified on a preparative silica TLC plate to give 12 mg of 3,5,7-triethoxy-flavone: mp 111–114 °C; mass (FAB) *m*/*z* 355 (M^+ + 1, base); ^{1}H-NMR ($CDCl_3$) δ 1.33 (t, 3H, CH_3), 1.55 (t, 3H, *J* = 7 Hz, CH_3), 1.62 (t, 3H, *J* = 7 Hz, CH_3), 4.1–4.25 (m, 6H, CH_2), 6.4 (s, 1H), 6.55 (s, 1H), 7.5–7.6 (m, 3H), 8.1–8.2.

3,7-Diethoxy-5-hydroxyflavone (3b).

Galangin (27 mg, 0.1 mmol) was dissolved in dry acetone (20 mL), solid potassium carbonate (0.5 g) and bromoethane (1 mL) were added, and the mixture was stirred overnight, at room temperature. The solution was filtered and evaporated, water (20 mL) and concentrated ammonium hydroxide (2 mL) were added, and the solution was extracted with ethyl acetate (20 mL × 2). The solution was dried, and the solvent was evaporated. The residue was purified by preparative TLC plate (silica, ethyl acetate/petroleum ether (2:8) to give 21 mg of product (64%): ^{1}H-NMR ($CDCl_3$) δ 1.34 (t, 3H, *J* = 7.3 Hz, 3-CH_3), 1.48 (t, 3H, *J* = 7.1 Hz, 7-CH_3), 4.14 (m, 4H, 2 × OCH_2), 6.38 (s, 1H, 6-H), 6.47 (s, 1H, 8-H), 12.64 (s, 1H, 5-OH); MS (CI/NH_3) *m*/*z* 327 (MH^+, base).

3,5,7-Tripropyloxyflavone (4).

Galangin (30 mg, 0.11 mmol) was dissolved in dry acetone (45 mL), solid potassium carbonate (0.5 g) and 1-iodopropane were added, and the mixture was refluxed overnight. The solution was filtered and evaporated, water (20 mL) and concentrated ammonia (1 mL) were added, and the solution was extracted with ethyl acetate. The ethyl acetate was evaporated, and the crude mass was purified on a preparative TLC plate (silica) to give 25 mg (63%) of 3,5,7-tripropyloxyflavone: mp 90–93 °C; mass spectra (CI/NH_3) *m*/*z* 397 (M^+ + 1, base); ^{1}H-NMR ($CDCl_3$) δ 0.85–1.2 (m, 9H), 1.6–2.1 (m, 6H), 3.9–4.1 (m, 6H), 6.32 (s, 1H), 6.5 (s, 1H), 7.4–7.5 (m, 3H), 8.04–8.12 (m, 2H).

2′,3,4′,5,7-Pentakis(methyloxy)flavone (6).

Morin (120 mg, 0.4 mmol) was dissolved in dry acetone (80 mL). Solid potassium carbonate (2 g) and iodomethane (2 mL) were added, and the mixture was refluxed overnight and then cooled to room temperature. The solution was filtered and evaporated, and the residue was separated by preparative TLC plates (silica, ethyl acetate) to give 80 mg of product (55%); ^{1}H-NMR ($CDCl_3$) δ 3.86, 3.88, 3.91, 3.93, 4.02 (5s, 15H, 5 × OCH_3), 6.79 (3m, 3H, phenyl), 6.65 (m, 1H, 8-H), 7.42 (m, 1H, 6-H); MS (EI) *m*/*z* 372 (M^+), 371 $(M - H)^+$, 341 $(M - OCH_3)^+$.

2′,4′,3,7-Tetraethoxy-5-hydroxymorin (7).

Compound **7** was synthesized according to the above procedure for **6**, except using iodoethane. The product, **7**, was separated on a preparative TLC plate with petroleum ether/ethyl acetate and displayed a mass (FAB) *m*/*z* 415 ($M^+ + 1$, base): ^{1}H-NMR ($CDCl_3$) δ 1.15 (t, 3H, CH_3), 1.35 (t, 3H, CH_3), 1.45 (dt, 6H, CH_3), 3.9–4.2 (m, 8H, CH_2), 6.33 (d, 1H), 6.6 (m, 3H), 7.4 (d, 1H).

2′,3,4′,5,7-Pentaethoxyflavone (8).

Morin (302 mg, 1 mmol) was dissolved in dry acetone (80 mL), solid potassium carbonate (5 g) and diethyl sulfate (5 mL) were added, and the mixture was refluxed overnight and then cooled to room temperature. The solution was filtered and evaporated, water (50 mL) and concentrated ammonium hydroxide (10 mL) were added, and the solution was extracted with ethyl acetate (40 mL × 2). The solution was dried, and the solvent was evaporated. The residue was crystallized from ethyl acetate and petroleum ether (1:9) to give 335 mg of product (76%): ^{1}H-NMR ($CDCl_3$) δ 1.12 (t, 3H, J = 7.1 Hz, CH_3), 1.32 (t, 3H, J = 7.1 Hz, CH_3), 1.45 (2t, overlap, 6H, 2 × CH_3), 1.56 (t, 3H, J = 7.1 Hz, CH_3), 4.10 (m, 10 H, 5 × OCH_2),6.32 (d, 1H, J = 1.2 Hz, 2-phenyl), 6.39 (s, 1H, 2-phenyl), 6.53 (s, 1H, 2-phenyl), 6.58 (s, 1H, 6-H), 7.37 (d, 1H, 8-H); MS (CI/NH_3) *m*/*z* 443 (MH^+, base).

2-(Phenylethynyl)-3-hydroxy-6-methoxyflavone (10).

1-(2-Hydroxy-5-methylphenyl)-5-phenylpenta-2-en-4-yn-1-one (compound **27b**, 60 mg, 0.2 mmol) was dissolved in ethanol (1.5 mL). Sodium hydroxide (1 N, 0.4 mL) and hydrogen peroxide (0.5 g) were added, and the mixture was heated at 90 °C for 35 min. The cooled mixture was diluted with water (5 mL) and acidified with hydrochloric acid (1 N). A precipitate was collected and recrystallized from methanol: mass (CI/NH_3) *m*/*z* 293 ($M^+ + 1$, base); ^{1}H-NMR ($CDCl_3$) δ 3.95 (s, 3H, CH_3), 6.92 (s, 1H), 7.25–8.0 (m, 7H).

3-Chloroflavone (11b).

Sulfuryl chloride (85 mg, 0.55 mL) in 2 mL of carbon tetrachloride was added dropwise to a solution of flavone (111 mg, 0.5 mmol) in 5 mL of carbon tetrachloride. The mixture was stirred overnight. The solution was diluted with CH_2Cl_2 (5 mL), washed with water (10 mL × 2), saturated sodium bicarbonate solution (10 mL × 2), and brine (10 mL × 2), and dried over sodium sulfate. The solvent was evaporated, and the residue was purified on a preparative TLC plate (silica, ethyl acetate/petroleum ether, 2:8) to give 80 mg of product (63%): ^{1}H-NMR ($CDCl_3$) δ 7.46–7.58 (m, 5H, 2-C_6H_5), 7.74 (m, 1H, 7-H), 7.94 (m, 2H, 5,6-H), 8.33 (dd, 1H, J = 7.8, 2.0 Hz, 8-H); MS (EI) *m*/*z* 256 (M^+, base), 228 $(M - CO)^+$, 221 $(M - Cl)^+$; mp 121 °C.

6-Chloroflavone (11c).

Bromine (185 mg, 1.15 mmol) was added a solution of 2-hydroxy-5-chlorochalcone (258 mg, 1.0 mmol) in acetic acid (10 mL). After the reaction was stirred at ambient temperature for 3 h, 1% aqueous sodium bisulfite solution (20 mL) was added slowly. The resulting precipitate was filtered off, washed with water, and suspended in ethanol (10 mL); potassium hydroxide (200 mg, 3.5 mmol) dissolved in water (3.5 mL) was added; and stirring was

continued overnight. The reaction mixture was acidified with 2 N HCl. The precipitate formed was filtered off and washed with water to give 105 mg of residue, which was recrystallized from EtOH/H_2O to give 85 mg of product (33%): ^{1}H-NMR ($CDCl_3$) δ 6.85 (s, 1H, 3-H), 7.54–7.69 (m, 5H, 2-C_6H_5), 7.93 (m, 2H, 5, 7-H), 8.22 (d, 1H, J = 2.7 Hz, 8-H); MS (CI/NH_3) m/z 257 (MH^+, base) and 259 ($M + 2H^+$) in 3:1 ratio; mp 170–172 °C.

3,6-Dichloroflavone (11d).

Sulfuryl chloride (28 μL, 0.28 mmol) in 2 mL of carbon tetrachloride was added dropwise to a solution of 6-chloroflavone (65 mg, 0.25 mmol) in 5 mL of carbon tetrachloride. The mixture was stirred overnight. The solution was diluted with CH_2Cl_2 (5 mL), washed with water (5 mL × 2), saturated sodium bicarbonate solution (5 mL × 2), and brine (5 mL × 2), and dried over sodium sulfate. The solvent was evaporated, and the residue was purified on a preparative TLC plate (silica, ethyl acetate/petroleum ether, 2:8) to give 8 mg of product (10%): ^{1}H-NMR ($CDCl_3$) δ 7.49–7.70 (m, 5H, 2-C_6H_5), 7.91 (m, 2H, 5,7-H), 8.28 (d, 1H, J = 2.6 Hz, 8-H); MS (EI) m/z 290, 292, 294 (M^+:($M + 2^+$):($M + 4^+$) = 9:6:1); mp 184–186 °C.

trans-2-Styryl-3-hydroxy-6-methoxyflavanone (15).

2′-Hydroxy-5′-methoxyacetophenone (**25**, 4.1 g, 22 mmol) and cinnamaldehyde (3.35 g, 25 mmol) were dissolved in minimal methanol (2.5 mL). Concentrated sodium hydroxide (12.5 mL, 50%) was added, and the mixture was kept on ice for 8 h. The resulted solid was suspended in water and acidified using HCl (4 N). The oil that separated was dissolved in ethanol and crystallized from ethanol/water to give 1-(2-hydroxy-5-methylphenyl)-5-phenylpenta-2,4-dien-1-one (1.2 g) as a red-brown powder: mass (CI/NH_3) m/z 281 (M^+ + 1, base); ^{1}H-NMR ($CDCl_3$) δ 3.9 (s, 3H, CH_3), 6.95–7.8 (m, 12H). The above compound (170 mg, 0.6 mmol) was dissolved in an mixture of ethanol (3.5 mL) and acetone (4 mL). Sodium hydroxide (1 N, 1 mL) and hydrogen peroxide (1 mL, 35%) were added, and the solution was stirred for 6 h at room temperature. The mixture was precipitated by adding water and HCl and purified on prep TLC (petroleum ether/ethyl acetate) to give compound **15**: mp 160–163 °C; mass (CI/NH_3) m/z 297 (M^+ + 1,base); ^{1}H-NMR ($CDCl_3$) δ 3.9 (s, 3H, CH_3), 4.45 (d, J = 12 Hz, 1H), 4.8 (m, 1H), 6.55 (dd, 1H), 6.9–7.6 (m, 9H).

trans-2-(Phenylethynyl)-3-hydroxy-6-methoxyflavanone (16).

2′-Hydroxy-5′-methoxyacetophenone and phenylpropargyl aldehyde were condensed according to the procedure for the preparation of compound **15** to give 1-(2-hydroxy-5-methylphenyl)-5-phenylpenta-2-en-4-yn-1-one: mass (CI/NH_3) m/z 279 (M^+ + 1, base); ^{1}H-NMR ($CDCl_3$) δ 3.9 (s, 3H, CH_3), 7.0 (d, 1H), 7.1–7.6 (m, 9H). The above compound was allowed to react with hydrogen peroxide to give compound **16** as a white powder: mp 135–138 °C; mass (EI) m/z 295 (M+1), 150 (base); ^{1}H-NMR ($CDCl_3$) δ 3.85 (s, 3H, OCH_3), 4.6 (d, J = 12 Hz, 1H), 5.09 (d, J = 12 Hz, 1H), 7.0–7.6 (m, 8H).

4-Methoxy-7-formyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one (18)

4-Methoxy-7-formyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one (18) was synthesized from visnagin (4-methoxy-7-methyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one) according to ref 28.

4-(Methyloxy)-7-*trans*-styrylvisnagin (19) and 4-(Ethyloxy)-7-*trans*-styrylvisnagin (20).

Visnagin (160 mg, 0.7 mmol) and benzaldehyde (120 mg, 1.1 mmol) were dissolved in ethanol (4 mL). Sodium ethoxide (20% in ethanol, 0.5 mL) was added, and the mixture was stirred for 10 min at 80 °C. Disappearance of the starting material was accompanied by formation of two products, R_f 0.7 and 0.8 in ethyl acetate. Both were separated on preparative TLC plates (ethyl acetate) to give compound **19**: mp 175–178 °C; mass (CI/NH_3) m/z 319 (M^+ + 1, base); ^{1}H-NMR ($CDCl_3$) δ 4.2 (s, 3H, OCH_3), 6.23 (s, 1H), 6.75 (d, J = 15 Hz, 1H), 7.05 (s, 1H), 7.3–7.8 (m, 8H).

Compound **20**: mp 148–151 °C; mass (CI/NH_3) m/z 333 (M^+ + 1, base); ^{1}H-NMR ($CDCl_3$) δ 1.55 (t, 3H, CH_3), 4.4 (q, 2H, OCH_2), 6.23 (s, 1H), 6.78 (d, J = 15 Hz, 1H), 7.0 (s, 1H), 7.35–7.7 (m, 8H).

4-(Propyloxy)-7-*trans*-styrylvisnagin (21).

A mixture of visnagin (2 g, 8.7 mmol), potassium iodide (10 g), and acetic acid (50 mL) was refluxed for 7 h. After cooling, the precipitate was removed by filtration, and the filtrate was evaporated under reduced pressure, and coevaporated with toluene (10 mL × 2). The residue was crystallized from ethanol to give 1.62 g (81%) of demethylation product of visnagin: ^{1}H-NMR ($CDCl_3$) δ 2.40 (s, 3H, 7-CH_3), 6.12 (s, 1H, 6-H), 7.00 and 7.62 (2d, 2H, J = 2.9 Hz, 3-H and 2H), 7.05 (s, 1H, 9-H), 13.6 (s, 1H, 4-OH). The mixture of demethylated visnagin (430 mg, 2 mmol), iodopropane (3 mL), and potassium carbonate (5 g) in dry acetone (80 mL) was refluxed overnight. The solid was removed by filtration, the solvent was evaporated, water (50 mL) and concentrated ammonium hydroxide (15 mL) were added, and the solution was extracted with ethyl acetate (40 mL × 2). The organic layer was dried over sodium sulfate, and the solvent was evaporated. The residue was purified by preparative TLC plates (silica, ethyl acetate/petroleum ether, 4:6) to give 465 mg (90%) of 4-(propyloxy)visnagin (**30**): ^{1}H-NMR ($CDCl_3$) δ 1.10 (t, 3H, J = 7.5 Hz, CH_3), 1.94 (m, 2H), 2.33 (s, 3H, 7-CH_3), 4.23 (t, 2H, J = 6.7 Hz, 4-OCH_2), 6.03 (s, 1H, 6-H), 6.97 and 7.60 (2d, 2H, J = 2.7 Hz, 3-H and 2-H), 7.27 (s, 1H, 9-H). 4-(Propyloxy)visnagin (86 mg, 0.3 mmol) and benzaldehyde (50 mg, 0.5 mmol) were dissolved in ethanol (2 mL). While stirring, sodium ethoxide (20% in ethanol, 0.3 mL) was added, and the mixture was stirred for 15 min at 80 °C. The solvent was evaporated, the residue was partitioned between ice water (20 mL) and ethyl acetate (20 mL), the aqueous layer was extracted with ethyl acetate (20 mL), and the combined organic layer was dried over sodium sulfate. The solvent was evaporated, and the residue was separated by preparative TLC plate (silica, ethyl acetate/petroleum ether, 4:6) to give 26 mg of the desired product (25%): ^{1}H-NMR ($CDCl_3$) δ 1.07 (t, 3H, J = 7.5 Hz, CH_3), 1.97 (m, 2H), 4.25 (t, 2H, J = 6.7 Hz, 4-OCH_2), 6.21 (s, 1H, 6-H), 6.78 (d, 2H, J = 14 Hz, 3-H), 7.00 (s, 1H, 9-H), 7.33 (m, 8H); MS (CI/NH_3) m/z 347 (MH^+, base).

4-Methoxy-7-(4-phenyl-1,3-pentadienyl)-5*H*-furo[3,2-*g*][1]benzopyran-5-one (22)and 4-Ethoxy-7-(4-phenyl-1,3-pentadienyl)-5*H*-furo[3,2-*g*][1]benzopyran-5-one (23).

Compound **22** was prepared by dissolving visnagin and cinnamaldehyde in ethanol in the presence of sodium ethoxide according to the above procedure for **19** and **20**: mp 162–165

°C; mass (EI) *m*/*z* 344 (M^+, base); ^{1}H-NMR ($CDCl_3$) δ 4.2 (s, 3H, OCH_3), 6.15 (s, 1H), 6.33 (d, *J* = 15 Hz, 1H), 6.95 (s, 1H), 7.0–7.6 (m, 10H).

Compound **23** was also isolated from the above reaction: mass (EI) *m*/*z* 358 (M^+), 343 (M – 15, base); ^{1}H-NMR ($CDCl_3$) δ 1.55 (t, 3H, CH_3), 4.4 (q, 2H, OCH_2), 6.15 (s, 1H), 6.35 (d, *J* = 15 Hz, 1H), 7.4 (d, *J* = 15 Hz, 1H), 6.9–7.6 (m, 10H).

4-Methoxy-7-formyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one Aniline Schiff base (24).

Compound **18** (400 mg, prepared according to ref 28) and aniline (0.5 mL) were dissolved in toluene and stirred overnight at 25 °C. The course of the reaction was followed using analytical TLC (silica, $CHCl_3$/MeOH, 20:1). The solvent was evaporated, and the excess aniline was removed under high vacuum. The product (R_f ~ 0.7) was purified using preparative TLC (silica, ethyl acetate): mass (CI/NH_3) *m*/*z* 320 (M^+ + 1, base).

Pharmacology. Radioligand Binding Studies.

The radioligand binding data for the novel compounds were determined as described previously.[17,18] Membranes prepared from HEK-293 cells stably expressing the human A_3 receptor were obtained from Receptor Biology, Inc. (Baltimore, MD). CHO cells stably expressing the rat A_3 adenosine receptor were grown in F-12 medium containing 10% fetal bovine serum and penicillin/streptomycin (100 units/mL and 100 μg/mL, respectively) at 37 °C in a 5% CO_2 atmosphere, and membrane homogenates were prepared as reported.[18] Binding of [^{125}I]-N^6-(4-amino-3-iodobenzyl)adenosine-5′-(*N*-methyluronamide) ([^{125}I]AB-MECA) to rat A_3 receptors in stably transfected CHO cell membranes was performed as described.[32] Assays at human A_3 receptors were performed in a buffer containing 50 mM Tris, 10 mM Mg^{2+}, and 1 mM EDTA at pH 8.0. The glass tubes contained 100 μL of the membrane suspension (0.3 mg of protein/mL, stored at −80 °C in the same buffer), 50 μL of [^{125}I]AB-MECA (final concentration 0.3 nM), and 50 μL of a solution of the proposed antagonist. All nonradioactive compounds were initially dissolved in DMSO and diluted with buffer to the final concentration, where the amount of DMSO never exceeded 1%. Duplicate incubations were carried out for 1 h at 25 °C[19] and were terminated by rapid filtration over Whatman GF/B filters, using a Brandell cell harvester (Brandell, Gaithersburg, MD). The tubes were rinsed three times with 3 mL of buffer each. The radioactivity on the filters was determined with a Beckman 5500B γ-counter. Nonspecific binding was determined in the presence of 100 μM N^6-phenylisopropyladenosine [(*R*)-PIA].

Binding of [^{3}H]-(*R*)-PIA to A_1 receptors from rat cortical membranes and of [^{3}H]CGS 21680 to A_{2A} receptors from rat striatal membranes was performed as described previously.[29,30]

Adenosine deaminase (3 units/mL) was present during the preparation of the brain membranes, in which an incubation of 30 min at 30 °C is carried out, and during the incubation with the radioligands. At least five different concentrations spanning 3 orders of magnitude, adjusted appropriately for the IC_{50} of each compound, were used. IC_{50} values, computer-generated using the nonlinear regression method implemented in the InPlot program (Graph-PAD, San Diego, CA), were converted to apparent K_i values using K_d

values of 1.0 and 14 nM for [^{3}H]PIA and [^{3}H]CGS 21680 binding, respectively, applying the Cheng–Prusoff equation.[39] A K_d value of 0.59 nM for [^{125}I]AB-MECA binding at human A_3 receptors was assumed.[19]

Adenylyl cyclase activity was measured in membranes from CHO cells stably expressing the rat A_3 receptor, prepared as above, using a previously reported method.[4,12] The method involved addition of [α-^{32}P]ATP to membranes in the presence of forskolin to stimulate adenylyl cyclase and papaverine as a phosphodiesterase inhibitor. The reaction was terminated by addition of a stop solution containing 20 000 cpm/mL [^{3}H]cyclic AMP. The total radiolabeled cyclic AMP was isolated on columns of Dowex 50 ion exchange resin and alumina. Maximal inhibition of adenylyl cyclase activity corresponded to 40–50% of total activity under conditions of stimulation (typically 6–8-fold) in the presence of 1 μM forskolin. IC_{50} values were calculated using InPlot (GraphPad, San Diego, CA).

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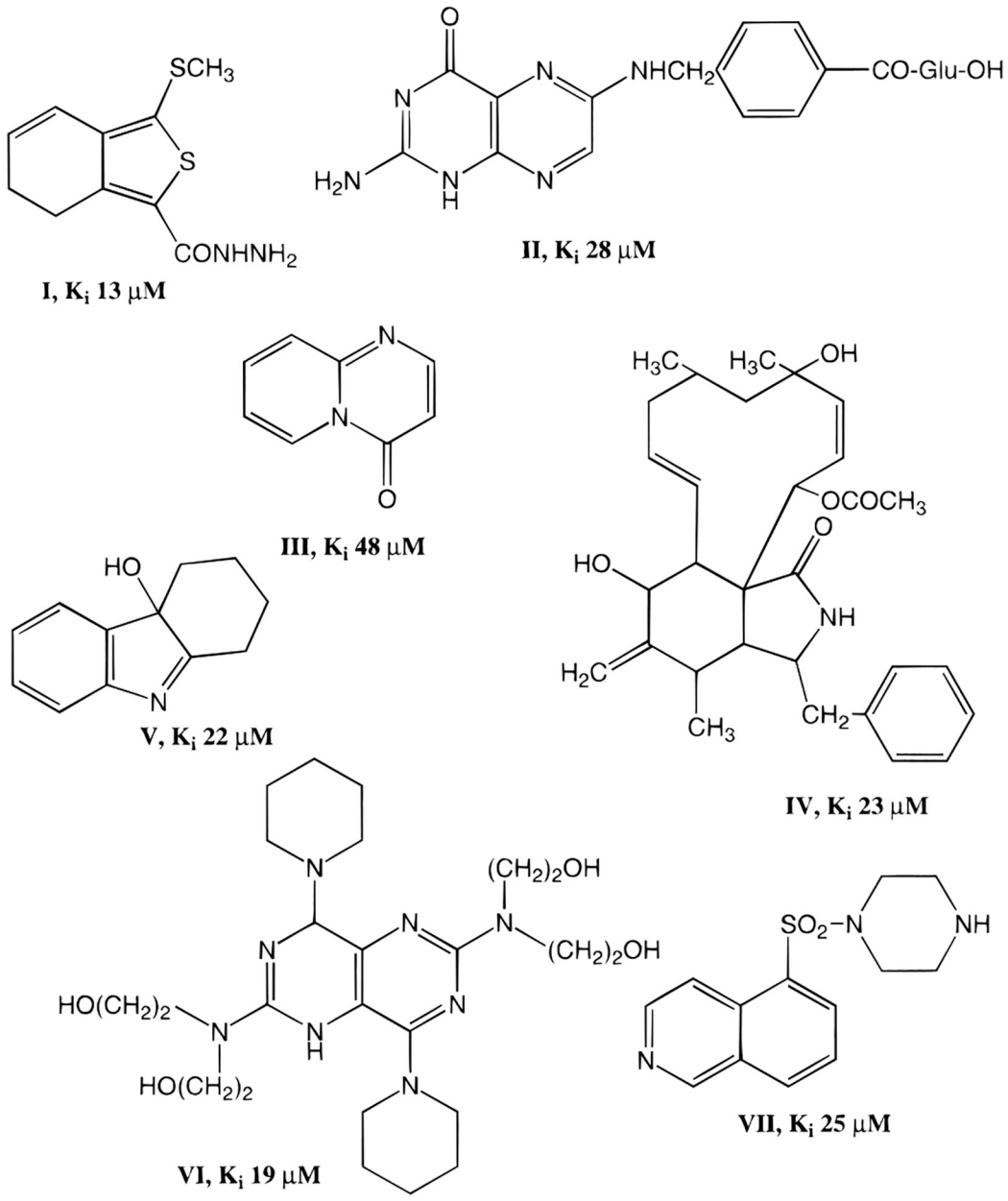

Figure 1.

Substances that have selectivity in binding to rat A_3 vs A_1 and A_{2A} adenosine receptors. K_i values shown are in competition for [^{125}I]AB-MECA binding in membranes from CHO cells expressing the cloned rat A_3 receptors.[16,17]

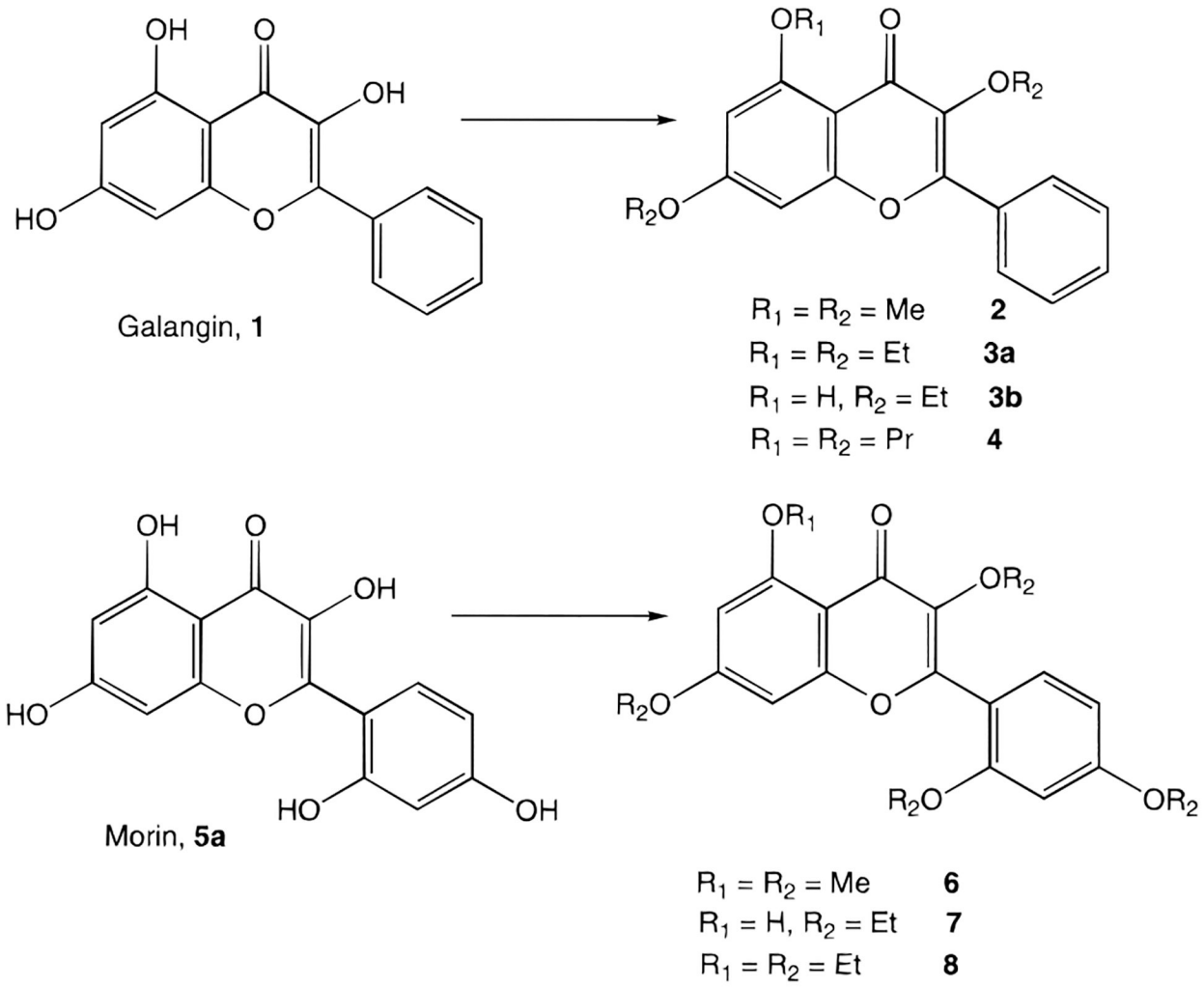

Figure 2.

Synthesis of alkylated derivatives of galangin and morin. Reagents: **2**, **3a**, **4**, and **7**, R-I; **3b**, Et-Br; **6** and **8**, R_2-SO_4.

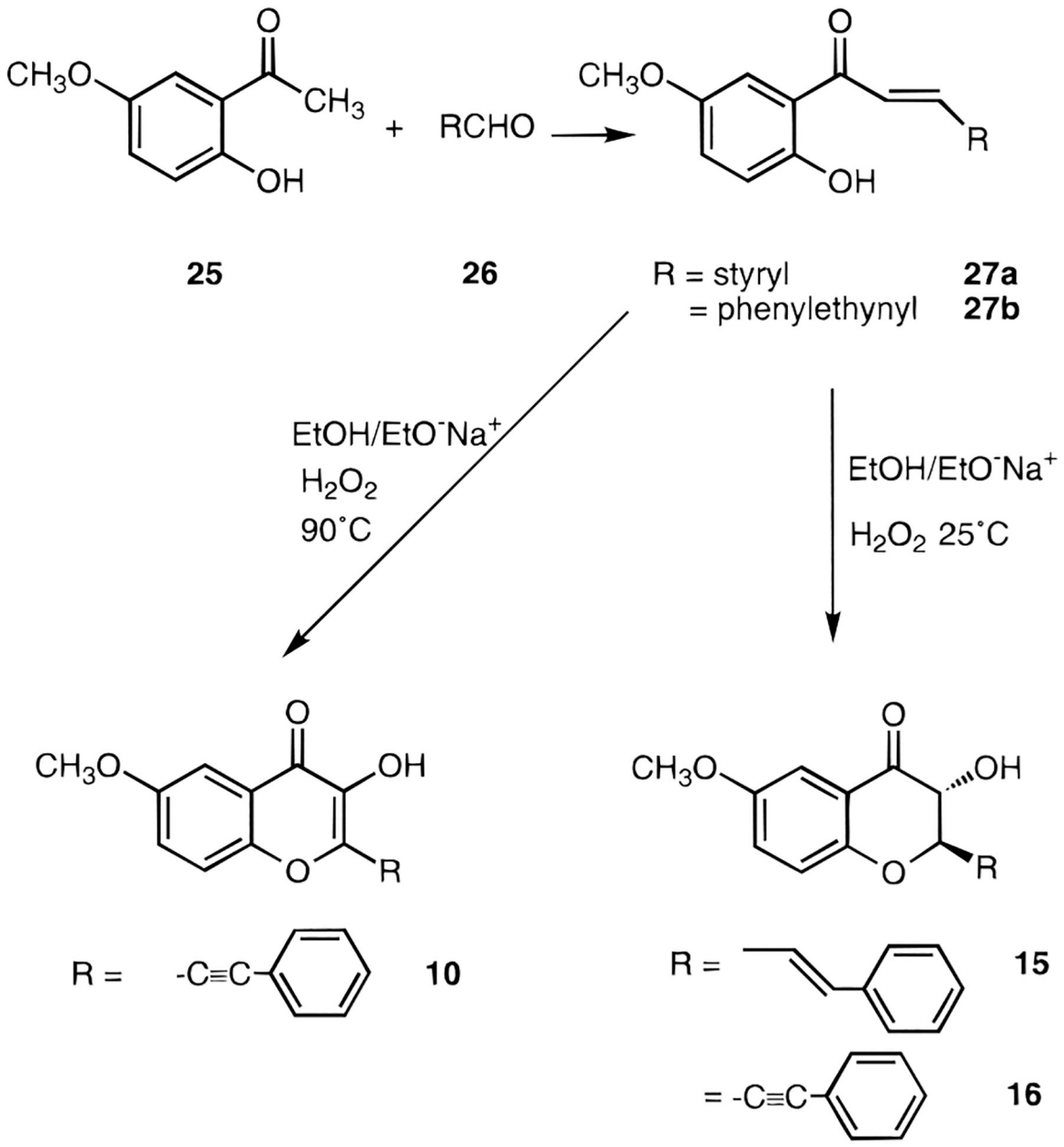

Figure 3.
Synthesis of 2-phenylethynyl and 2-styryl derivatives of 4-methoxyfuranochromone.

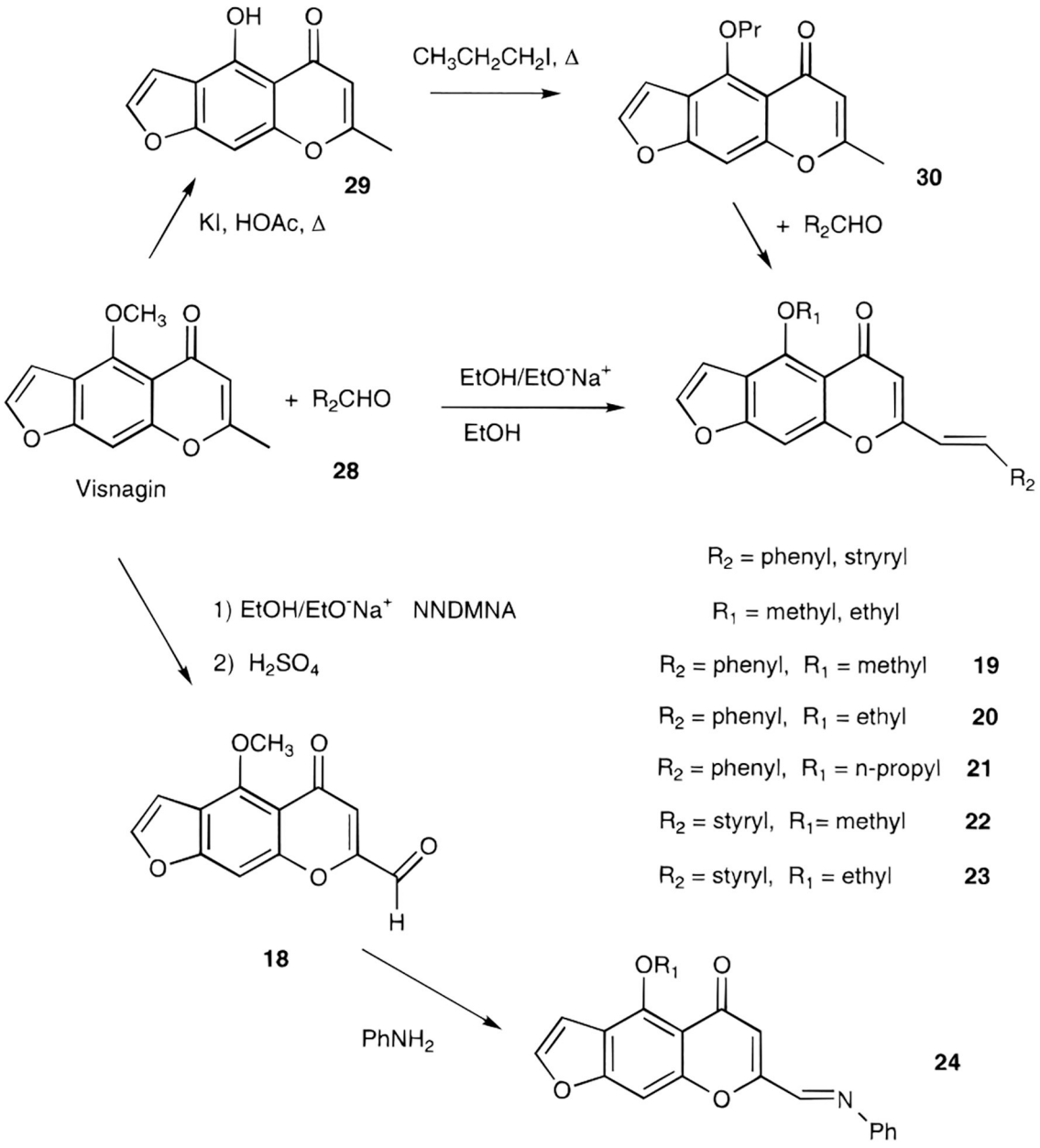

Figure 4.
Chemical derivatization of visnagin and alkoxy substitution at the 5-position.

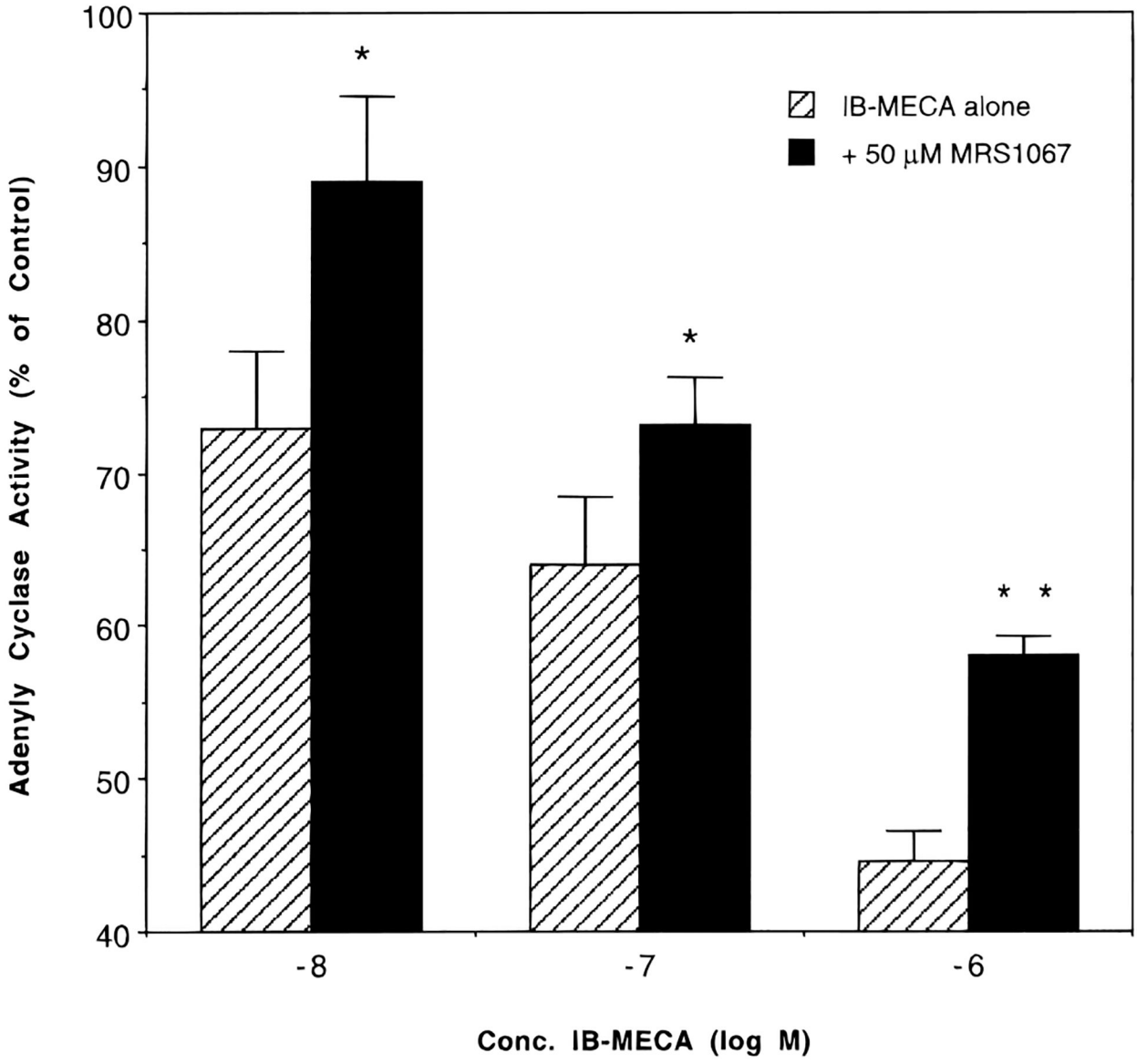

Figure 5.

Inhibition of adenylate cyclase in membranes from CHO cells stably transfected with rat A_3 receptors elicited by the agonist N^6-(3-iodobenzyl)adenosine-5′-(*N*-methyluronamide) (IB-MECA), alone in the presence of 50 μM compound **11e** (K_i = 5.50 ± 2.21 μM at rat A_3 receptors). The assay was carried out as described in Experimental Section in the presence of 1 μM forskolin. Each data point is shown as mean ± S.E.M. for three determinations. *p < 0.05, **p < 0.001 (t test).

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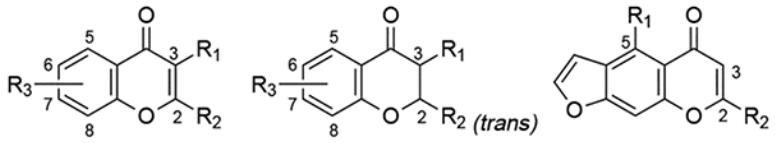
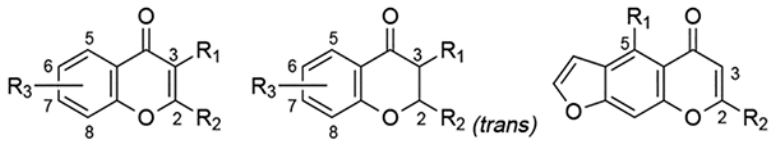
Table 1.

Affinities of Flavonoid Derivatives in Radioligand Binding Assays at A_1, A_{2a}, and A_3 Receptors[a–c]



compound	R_1	R_2	R_3	K_i (μM) or % inhibition[c]			rA_1/hA_3
				rA_1	rA_{2A}	hA_3	
1[f] (galangin)	OH	Ph	5,7-$(OH)_2$	0.863 ± 0.092	0.966 ± 0.164	3.15 ± 0.85	0.27
2[f] (MRS928)	OMe	Ph	5,7-$(OMe)_2$	0.509 ± 0.049	6.45 ± 1.48	1.21 ± 0.30	0.42
3a (MRS1041)	OEt	Ph	5,7-$(OEt)_2$	0.603 ± 0.022	3.31 ± 1.24	0.364 ± 0.067	1.7
3b (MRS1093)	OEt	Ph	5-OH-7-OEt	1.89 ± 0.56	63.8 ± 18.8	0.748 ± 0.402	2.5
4 (MRS1042)	OPr	Ph	5,7-$(OPr)_2$	1.10 ± 0.20	3.22 ± 1.31	0.317 ± 0.089	3.5
5a[f] (morin)	OH	2′,4′-$(OH)_2$Ph	5,7-(OH)2	13.8 ± 3.1	17.3 ± 4.1	34 ± 4% (10^{-4})	~0.1
5b	$CH_2CH=C(CH_3)_2$	2′,4′-$(OH)_2$Ph	5-OH-6-CH=CHCH-$(CH_3)_2$-7-OMe	9.09 ± 0.91	*d* (10^{-4})	4.59 ± 1.69	2.0
6[f] (MRS923)	OMe	2′,4′-$(OMe)_2$Ph	5,7-$(OMe)_2$	27.6 ± 7.5	46.7 ± 2.7	2.65 ± 0.72	10
7 (MRS1063)	OEt	2′,4′-$(OEt)_2$Ph	5-OH-7-OEt	*d* (10^{-4})	*d* (10^{-4})	4.83 ± 1.40	>40
8 (MRS1086)	OEt	2′,4′-$(OEt)_2$Ph	5,7-$(OEt)_2$	32.3 ± 9.1	26.8	7.27 ± 1.88	4.4
9	OH	2′,4′,6′-$(OMe)_3$Ph	H	7.32 ± 1.36	*d* (10^{-4})	50.1 ± 7.8	0.15
10 (MRS1072)	OH	CtCPh	6-OCH_3	34 ± 9% (10^{-4})	*d* (10^{-4})	24.0 ± 9.7	~8
11a[f] (flavone)	H	Ph	H	3.28 ± 0.92	3.45 ± 1.16	16.9 ± 3.8	0.19
11b (MRS1132)	Cl	Ph	H	2.48 ± 0.72	25.1 ± 5.4	11.5 ± 3.7	0.22
11c (MRS1131)	H	Ph	6-Cl	28 ± 6% (10^{-5})	*d* (10^{-4})	32 ± 5% (10^{-4})	
11d (MRS1088)	Cl	Ph	6-Cl	15% (10^{-4})	54.5 ± 26.3	0.741 ± 0.325	>100
11e (MRS1067)	Cl	2′-*i*-PrO-4′-MePh	6-Cl	36 ± 12% (10^{-4})	19 ± 9% (10^{-4})	0.561 ± 0.129	~200
11f (MRS1089)	Cl	2′,4′,6′-Me_3Ph	6-Cl	37 ± 5% (10^{-4})	*d* (10^{-4})	5.24 ± 0.52	~20
12 (flavanone)	H	Ph	H	32.0 ± 4.8	nd	50.1 ± 27.1	0.64



compound	R_1	R_2	R_3	K_i (μM) or % inhibition[c]			rA_1/hA_3
				rA_1	rA_{2A}	hA_3	
13a	H	2′-OHPh	H	2.64 ± 0.56	17.6 ± 2.1	6.07 ± 1.43	0.43
13b	H	4′-OHPh	H	11.5 ± 3.7	35% (10^{-4})	42.8 ± 10.5	0.27
13c[f] (sakuranetin)	H	4′-OHPh	5-OH-7-OMe	8.18 ± 2.53	35.6 ± 13.0	3.40 ± 0.18	2.4
14[e]	OH	2′-OHPh	H	91.9 ± 0.3	*d* (10^{-4})	27 ± 3% (10^{-4})	<1
15[e] (MRS1061)	OH	CH=CHPh	6-OMe	*d* (10^{-4})	*d* (10^{-4})	21.1 ± 9.9	>8
16[e] (MRS1062)	OH	C≡CPh	6-OMe	50.3 ± 17.0	*d* (10^{-4})	8.17 ± 0.43	6.2
17 (visnagin)	OCH_3	CH_3		36 ± 3% (10^{-4})	42.4 ± 11.9	60.0 ± 17.8	~2
18	OCH_3	CHO		*d* (10^{-4})	25 ± 8% (10^{-4})	88.9 ± 27.1	>2
19 (MRS1065)	OCH_3	CH=CHPh		32.6 ± 10.5	11.5 ± 1.3	8.28 ± 2.69	0.37
20 (MRS1066)	OC_2H_5	CH=CHPh		35.6 ± 12.7	33.8 ± 14.8	1.16 ± 0.45	31
21 (MRS1084)	$O(CH_2)_2CH_3$	CH=CHPh		40.0 ± 3.5	49.0	3.95 ± 1.98	10
22 (MRS1070)	OCH_3	CH=CHCH=CHPh		*d* ($10^{-4)}$	*d* (10^{-4})	*d* (10^{-4})	
23 (MRS1071)	OC_2H_5	CH=CHCH=CHPh		*d* (10^{-4})	167 ± 92	45.5 ± 10.3	>4
24 (MRS1078)	OCH_3	CH=NPh		*d* (10^{-4})	*d* (10^{-4})	9.18 ± 2.56	>20

[a] Displacement of specific [^{3}H]PIA binding in rat brain membranes, expressed as K_i ± SEM in μM (n = 3–5), or as a percentage of specific binding displaced at 100 μM.

[b] Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes, expressed as K_i ± SEM in μM (n = 3–6), or as a percentage of specific binding displaced at 100 μM.

[c] Displacement of specific [^{125}I]AB-MECA binding in the presence of 10 mM Mg^{2+} at human A_3 receptors expressed in HEK293 cells, in membranes, expressed as K_i = SEM in μM (n = 2–5), or as a percentage of specific binding displaced at 100 μM.

[d] Displacement of ≤10% of specific binding at the indicated concentrated (M).

[e] 2,3-*trans*.

[f] Values from ref 19.

[g] The systematic name for compounds **17–24** is different from the numbering scheme used in this table and in Results section.

nd = not determined.

Table 2.

Physical Characterization of Flavone, Flavanol, and Flavanone Derivatives

compd no.	mp (°C)	MS	formula	anal.
2	201	313 (CI)	$C_{18}H_{16}O_5 \cdot 0.75H_2O$	C,H
3a	111–114	355 (FAB)	$C_{21}H_{22}O_5$	C,H
3b	100–101	337 (CI)	$C_{19}H_{18}O_5$	C,H
4	90–93	397 (CI)	$C_{24}H_{28}O_5$	C,H
6	153–156	373 (CI)	$C_{20}H_{20}O_7$	C,H
7	122–123	415 (FAB)	$C_{23}H_{26}O_7$	C,H
8	115–116	443 (CI)	$C_{25}H_{30}O_7$	C,H
10	oil	293 (CI)	$C_{18}H_{12}O_4$	C,H
11b	121	256 (EI)	$C_{15}H_9O_2Cl \cdot 0.25EtOH$	C,H
11c	170–172	257 (CI)	$C_{15}H_9O_2Cl \cdot O.1H_2O$	C,H
11d	184–186	290 (EI)	$C_{15}H_8O_2Cl_2$	*a*
15	160–163	297 (CI)	$C_{18}H_{16}O_4 \cdot 0.25H_2O$	C,H
16	135–138	294 (EI)	$C_{18}H_{14}O_4$	*a,b*
19	175–178	319 (CI)	$C_{20}H_{14}O_4 \cdot 0.25H_2O$	C,H
20	148–151	333 (CI)	$C_{21}H_{16}O_4$	C,H
21	132	347 (CI)	$C_{22}H_{18}O_4$	C,H
22	162–165	344 (EI)	$C_{22}H_{16}O_4 \cdot 0.5H_2O$	C,H
23	162–165	358 (EI)	$C_{23}H_{18}O_4$	C,H
24	150–154	320 (CI)	$C_{19}H_{14}NO_4$	C,H

[a] High-resolution mass in FAB^+ mode *m*/*z* determined to be within acceptable limits. **11d**: calcd, 289.9901; found, 289.9893. **16**: calcd, 294.0892; found, 294.0889.

[b] C: calcd, 73.46; found, 74.30. H: calcd, 4.80; found, 5.27.

Table 3.

Inhibition of Binding of [^{125}I]AB-MECA at Cloned Rat A_3 Receptors and Effects of Flavone Derivatives (50 μM) on the A_3 Agonist Elicited Inhibition of Adenylyl Cyclase

		% inhibition of adenylyl cyclase[a]	
compd[b]	**K_i (μM) or % displacement**	**IB-MECA (10^{-7})**	**IB-MECA (10^{-6})**
control		23.7 ± 5.1	35.3 ± 7.2
3a	18.2 ± 5.6	2.2 ± 2.2	19.1 ± 7.4
15	43 ± 3% (100 μM)	25.9 ± 2.4	39.2 ± 3.0
20	12.3 ± 6.6	21.2 ± 3.9	40.9 ± 3.7

[a]Assayed in membranes from CHO cells stably expressing the rat A3 receptor using a previously reported method[4,12] in the presence of 1 μM forskolin (n = 3).

[b]For plot of results for compound **11e**, refer to Figure 5.