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Derivatives of the Triazoloquinazoline Adenosine Antagonist (CGS15943) Are Selective for the Human A_3 Receptor Subtype

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Abstract

The adenosine antagonist 9-chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-*c*]quinazolin-5-amine (CGS15943) binds to human A_3 receptors with high affinity (K_i = 14 nM), while it lacks affinity at rat A_3 receptors. Acylated derivatives of the 5-amino group and other modifications were prepared in an effort to provide A_3 subtype selectivity. Affinity was determined in radioligand binding assays at rat brain A_1 and A_{2A} receptors using [^{3}H]-(*R*)-PIA ([^{3}H]-(*R*)-N^6-(phenylisopropyl)-adenosine) and [^{3}H]CGS 21680 ([^{3}H]-2-[[4-(2-carboxy ethyl)phenyl]ethylamino]-5′-(*N*-ethylcarbamoyl)adenosine), respectively. Affinity was determined at cloned human A_3 receptors using [^{125}I]AB-MECA (N^6-(4-amino-3-iodobenzyl)-5′-(*N*-methylcarbamoyl)adenosine). A series of straight chain alkyl amides demonstrated that the optimal chain length occurs with the 5-*N*-propionyl derivative, **3**, which had a K_i value of 7.7 nM at human A_3 receptors, and was 40- and 14-fold selective vs rat A_1 and A_{2A} receptors, respectively. The 5-*N*-benzoyl derivative, **10**, displayed K_i values of 680 and 273 nM at rat A_1 and A_{2A} receptors, respectively, and 3.0 nM at human A_3 receptors. A 5-*N*-phenylacetyl derivative, **12**, was 470-fold selective for human A_3 vs rat A_1 receptors with a K_i value of 0.65 nM. A conjugate of Boc-γ-aminobutyric acid was also prepared but was nonselective. Conversion of the 5-amino group of CGS15943 to an oxo function resulted in lower affinity but 15-fold selectivity for human A_3 receptors.

Introduction

Four subtypes of adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) have been cloned and studied pharmacologically.[1] Since the recent discovery of the adenosine A_3 receptor and the cloning of its species homologues,[2] much effort has been made in order to characterize the receptor biochemically and pharmacologically. Principally, the development of selective agonist ligands[3] has aided in this effort. The A_3 receptor mediates processes of inflammation,[2] hypotension,[4] and mast cell degranulation.[5] This receptor apparently also has a role in the central nervous system. The A_3 selective agonist IB-MECA induces behavioral depression[6]

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and upon chronic administration protects against cerebral ischemia.[7] A_3 selective agonists at high concentrations were also found to induce apoptosis in HL-60 human leukemia cells.[8] These and other findings have made the A_3 receptor a promising therapeutic target.[3] Selective antagonists for the A_3 receptor are sought as potential antiinflammatory or possibly antiischemic agents in the brain.[9–11]

Thousands of analogues of the classical adenosine antagonists, the xanthines, have been prepared, resulting in high degrees of selectivity for A_1 or A_{2A} receptor subtypes.[12–14] However, the xanthines have not provided fruitful leads for A_3 receptor antagonists, since they are generally much weaker in binding at the A_3 subtype than at A_1 or A_{2A} subtypes.[9,10] Thus, the search for A_3 receptor antagonists has been directed toward the screening of libraries to identify structurally novel leads.[11,15–18] Diverse classes of heterocycles, non-xanthine adenosine antagonists, have already been found to bind to A_1 and A_{2A} receptors and more recently to A_3 adenosine receptors. We recently reported that two chemical classes, the flavonoids[16] and dihydropyridines,[18] are amenable to modification, resulting in ligands selective for the recently discovered A_3 receptor. The flavonoid **I** (MRS 1067)[17] and the dihydropyridine derivative **II** (MRS 1097)[18] (Figure 1) are selective human A_3 receptor antagonists.

The non-xanthine adenosine antagonist **1** (CGS15943, 9-chloro-2-(2-furanyl) [1,2,4]triazolo[1,5-*c*]quinazolin-5-amine)[19,20] is very potent and slightly selective at human brain A_{2A} receptors with a K_i value of 1.7 nM.[21,29] It had been under development as an antiasthmatic agent, until unanticipated skin sensitivity was observed.[12] This antagonist was reported to be inactive in binding at rat A_3 receptors.[10] Nevertheless, in light of the considerable species variability in affinity of non-adenosine derivatives at A_3 receptors, the affinity of **1** (CGS15943) was determined at cloned human A_3 receptors and was found to be relatively high. In this study we have prepared a series of derivatives of **1** and found that *N*-acylation greatly enhances affinity and selectivity for human A_3 receptors.

Results

Synthesis.

The structures of the triazoloquinazoline derivatives tested for affinity in radioligand binding assays at adenosine receptors are shown in Table 1. Several of the derivatives, *e.g.* the bromo derivative, **13**, and the 5-oxo derivative, **15**, had been reported previously by Francis et al.[19] to bind to A_1 and A_{2A} receptors. The 5-oxo derivative[19] was prepared in the present study by an alternate route using an acid treatment of commercially available **1** (Figure 2). Various *N*-acyl derivatives were synthesized from **1** using standard acylation methodologies (Figure 2). Compound **13**[19,20] was prepared by first protecting the amino group as the *tert*-butyloxycarbonyl derivative, followed by bromination using *N*-bromosuccinimide and acidic deprotection. The yields and chemical characterization of these compounds are reported in Table 2.

Binding at Adenosine Receptors.

K_i values were determined in radioligand binding assays at rat cortical A_1 receptors vs [^{3}H]-(*R*)-PIA ([^{3}H]-(*R*)-*N*^{6}-(phenylisopropyl)adenosine) or rat striatal A_{2A} receptors vs [^{3}H]CGS 21680 ([^{3}H]-2-[[4-(2-carboxyethyl)phenyl]ethylamino]-5′-(*N*-ethylcarbamoyl)adenosine.[22,23] Affinity at cloned human A_3 receptors expressed in HEK-293 cells[24] was determined using [^{125}I]AB-MECA (*N*^{6}-(4-amino-3-[^{125}I]iodobenzyl)-5′-(*N*-methyl carbamoyl)adenosine).[25] In this study, affinities at adenosine receptors from different species are compared. The species differences in ligand affinity for triazoloquinazolines at A_1 and A_{2A} receptors are minor, whereas those at A_3 receptors are significant (Figure 1).[2,26] Thus, our ratios of affinity are indicative of selectivities in human, but not rat tissue.

The primary amine and 2-furanyl moieties in **1** have been considered most important for binding to rat A_1 and rat A_{2A} receptors.[19] In an effort to investigate the effects on binding at human A_3 receptors, various structural modifications of these groups were made. The structure–activity relationships (SAR) for binding at adenosine receptors indicated that a variety of *N*-acyl groups are tolerated at the 5-amino position (Table 1). As with the parent compound **1**,[19] the affinity of the *N*-acylated derivatives (**2**–**12**) at A_{2A} receptors tended to equal or surpass the affinity at A_1 receptors. The substituent R_1 was varied from acetyl to valeryl in a homologous series (**2**–**5**). Also several acyl congeners containing either bulky *tert*-butyl or derivatized aromatic moieties were synthesized to investigate steric and electrostatic effects on the competitive binding at each of the adenosine receptors.

Among the straight acyl chain derivatives, the *N*-propionyl derivative, **3**, had the highest binding affinity (K_i = 7.7 nM) and selectivity (40-fold vs rat A_1, 14-fold vs rat A_{2A}) at human A_3 receptors. Within this series, the affinity at A_3 receptors varied only 3-fold with varying chain lengths, while at A_{2A} receptors a greater variation was observed. The affinities of compounds **6** and **7** indicated that bulky groups at position R_1 were less well tolerated than straight alkyl chains in human A_3 receptor binding. The pivaloyl derivative, **6**, was 32-fold less potent at A_3 receptors than the propionyl derivative, **3**, from which it differed only in the presence of two methyl groups at a branched carbon. The change from an amide, **6**, to the corresponding urethane, **7**, had no effect on affinity at A_1 and A_{2A} receptors, while the affinity at A_3 receptors was enhanced ~3-fold. The competitive binding curves of compounds **3**, **6**, and **7** at human A_3 receptors are shown in Figure 3A.

A Boc-γ-amino butyric acid conjugate, **8**, of **1** was slightly selective for human A_3 receptors. The removal of the Boc group resulting in an acyl chain containing the primary amine functionality, **9**, which is positively charged at pH 7.4, reduced potency at human A_3 receptors, while increasing potency at rat A_1 and rat A_{2A} receptors.

Among aromatic acyl derivatives, the *N*-benzoyl derivative, **10**, showed higher binding affinity (K_i = 3.0 nM) at the human A_3 receptor than **1**, while affinities at rat A_1 and rat A_{2A} receptors were significantly decreased. At rat A_3 receptors,[9] compound **10** was much less potent (data not shown), with only 23% of radioligand binding displaced at 3 μM. Substitution of the phenyl ring of the *N*-benzoyl group with a 3-iodo substituent, **11**, caused a marked reduction in affinity only at human A_3 receptors. However, the less sterically

hindered *N*-phenylacetyl compound, **12**, showed yet higher affinity (K_i = 0.65 nM) and selectivity (470-fold vs rat A_1 receptors) for human A_3 receptors than **10**. The competitive binding curves of compound **12** at rat A_1 and A_{2A} receptors and at human A_3 receptors are shown in Figure 3B.

Two 5-bromofuranyl derivatives were prepared. Compound **13**, which was used as the synthetic precursor in the preparation of tritiated **1**[20] and reported to be much less potent at rat A_1 and rat A_{2A} receptors,[19] showed appreciable potencies at human A_3 receptors. Compound **14** was prepared with an expectation of improving selectivity and affinity with respect to compounds **10** and **13**. Unfortunately, this combination of functionalities displayed a dramatic loss of binding activity at all three adenosine receptor subtypes.

Finally, two 5-oxo compounds were also prepared and tested. Compound **15** was previously reported to be much less potent than **1** at A_1 and A_{2A} receptors,[19] and we found it to be 15–17-fold A_3 receptor selective. However, compound **16**, the N^6-propyl derivative of **15** displaced radioligand binding with lower affinity at the A_3 adenosine receptor subtype.

Discussion

Until the present there have been few reports of leads for the development of selective antagonists for the A_3 receptor,[17,18] especially having high potency. We have screened chemical libraries[11] and known adenosine receptor ligands[10] for potential A_3 receptor antagonists. Initially we reported that the triazoloquinazoline **1**, which was reported as the first potent, non-xanthine, but nonselective adenosine A_1 and A_{2A} receptor antagonist, did not have appreciable binding activity at rat A_3 receptors.[10] Nevertheless, in this study, it proved to be highly potent at human A_3 receptors (K_i = 13.8 nM), suggesting that it may serve as a lead compound in this species. This finding encouraged us to investigate structure–activity relationships of **1** derivatives for the development of highly potent and selective antagonists at human but not rat A_3 receptors. There are dramatic species differences[2,26] in the affinity of antagonists binding at A_3 receptors. The overall identity of protein sequences between the A_3 adenosine receptors of rat and human was reported as 72%,[1,2] which is relatively low, consistent with the high variability in antagonist affinity.

The present findings indicated that certain less polar derivatives of **1** displayed increased affinity at the human A_3 receptor, and this enhanced binding affinity could be distinguished from effects at rat A_1 and rat A_{2A} receptors. Compound **13** and **15**, which were previously reported to have poor binding affinities at rat A_1 and A_{2A} receptors, showed binding affinities to human A_3 receptors in the sub-micromolar range; thus some degree of selectivity was present. The most significant findings in the present study are that compounds **10** and **12**, containing the *N*-benzoyl and *N*-phenylacetyl substituents, respectively, are highly potent and selective for human A_3 receptors. A competitive binding assay of **10** showed high affinity at human A_3 receptors (K_i = 3.0 nM), with 220-fold selectivity vs rat A_1 receptors and 90-fold selectivity vs rat A_{2A} receptors. Compound **12** displayed a K_i value of 0.65 nM, with 470-fold selectivity vs rat A_1 receptor and 80-fold selectivity vs rat A_{2A} receptors. The displacement of radioligand binding at rat A_3 receptors

by **10** and **12** was only 23% (3 μM) and 12% (1 μM), respectively, showing that there are marked differences of affinities between human A_3 and rat A_3 receptors.

Compound **14**, the benzoyl derivative of compound **13**, however, had greatly diminished affinity at adenosine receptors; thus the A_3 receptor selectivity enhancing effects induced by *N*-acylation and by 5-bromination are not additive.

N-Acylations with various alkanecarboxylic acids resulted in less selective ligands than the aryl carboxylic acid derivatives. A structure–activity relationship analysis of various length of acyl chain substituents (**2–7**) resulted in the interesting finding that compound **3** displayed the optimum acyl chain length for A_3 receptor binding.

There appears to be a hydrophobic pocket in the receptor in the vicinity of the N^5-amino group, since hydrophobic *N*-acyl and *N*-aryl substituents could enhance potency. In contrast, removal of the hydrophobic Boc group from **8** resulted in the positively charged congener **9** that displayed a lower affinity at A_3 receptors. According to molecular modeling,[15] in the receptor bound states the N^6 region of adenosine corresponds to the position of N^5 of **1**. In the present study, the introduction of an aryl ring in that region enhanced selectivity, as was found in the case of N^6-benzylad-enosine derivatives.[3] Compound **11** was synthesized to test further the hypothesis of overlap with the N^6 region of adenosine, since the N^6-3-iodobenzyl substituent, vs unsubstituted N^6-3-benzyl, has been shown to enhance A_3 receptor affinity and selectivity. Nevertheless, in the present series, the iodo substituent offered no advantage. N^5 is also proposed to correspond to the N3-position of xanthines,[15] at which large hydrophobic substituents are tolerated,[10] consistent with our findings.

The selective ligands introduced here should be useful as antagonists in characterizing and probing the physiological role of human A_3 receptors. They may also provide affinity probes such as radioligands for human A_3 receptors. These selective agents must now be studied in functional assays.

Experimental Section

Materials.

Compound **1**, (*R*)-PIA, and 2-chloroadenosine were purchased from Research Biochemicals International (Natick, MA). All acylating agents were obtained from Aldrich (St. Louis, MO).

Synthesis.

Proton nuclear magnetic resonance spectroscopy was performed on a Varian GEMINI-300 spectrometer and spectra were taken in DMSO-d_6 or $CDCl_3$. Unless noted, chemical shifts are expressed as ppm downfield from tetramethylsilane. Chemical-ionization (CI) mass spectrometry was performed with a Finnigan 4600 mass spectrometer and electron-impact (EI) mass spectrometry with a VG7070F mass spectrometer at 6 kV. Elemental analysis was performed by Atlantic Microlab Inc. (Norcross, GA) or Galbraith Laboratories, Inc. (Knoxville, TN). All melting points were determined with a Unimelt capillary melting point apparatus (Arthur H. Thomas Co., PA) and were uncorrected. All triazoloquinone derivatives

showed one spot on TLC (MK6F silica, 0.25 mm, glass backed, Whatman Inc., Clifton, NJ). Where needed evaluation of purity was done on a Hewlett-Packard 1090 HPLC system using OD-5–60 C18 analytical column (150 mm × 4.6 mm, Separation Methods Technologies, Inc., Newark, DE) in two different linear gradient solvent systems. One solvent system (A) was 0.1 M TEAA/CH_3CN, 30:70 to 10:90, in 20 min with flow rate 1 mL/min. The other (B) was H_2O/MeOH, 40:60 to 10:90, in 20 min with flow rate 1 mL/min. Peaks were detected by UV absorption using a diode array detector.

General Procedure for Preparation of 5-*N*-Acyl Derivatives of 1.

Method A (Symmetrical Anhydride).—To a stirred solution of **1** (10 mg, 0.035 mmol), anhydride (0.105 mmol) and (dimethylamino)pyridine (0.5 mg, 0.004 mmol) in 1.5 mL of anhydrous DMF was added triethylamine (73 μL, 0.525 mmol) at room temperature. The mixture was stirred for 48 h and then evaporated to dryness under reduced pressure. The residue was purified by preparative silica gel TLC (CH_2Cl_2/MeOH, 50:1 ~ 75:1) to afford the desired compounds (**2**–**5**, **7**, and **10**).

Method B (Acid Chloride).—To a stirred solution of **1** (10 mg, 0.035 mmol) and anhydrous pyridine (40 μL, 0.5 mmol) in 1.5 mL of anhydrous CH_2Cl_2 was added acyl chloride (0.105 mmol) at 0 °C. The mixture was stirred at room temperature for 24–48 h and then treated with same procedure as method A for purification of the desired compounds (**6** and **12**).

Method C (Carbodiimide).—A solution of **1** (10 mg, 0.035 mmol), the carboxylic acid component (0.210 mmol), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (41 mg, 0.210 mmol), 1-hydroxybenzotriazole (28 mg, 0.210 mmol), 4-(dimethylamino)pyridine (0.5 mg, 0.004 mmol), and triethylamine (74 μL, 0.530 mmol) in 2 mL of anhydrous DMF/CH_2Cl_2 (1:1 v/v) was stirred at room temperature for 48 h. The mixture was treated with same procedure as method A for purification of desired compounds (**8** and **11**).

5-(Acetylamino)-9-chloro-2-(2-furanyl)[1,2,4]triazolo-[1,5-*c*]quinazoline (2): ^{1}H NMR ($CDCl_3$) δ 2.78 (3H, s, CH_3CO), 6.63–6.65 (1H, m, H-4′), 7.30 (1H, d, J = 2.9, H-3′),7.68 (1H, broad s, H-5′), 7.73 (1H, dd, J = 1.9, 8.8, H-8), 7.86 (1H, d, J = 8.8, H-7), 8.48 (1H, d, J = 2.9, H-10), 8.99 (NH, broad s).

9-Chloro-2-(2-furanyl)-5-(*n*-propionylamino)[1,2,4]-triazolo[1,5-*c*]quinazoline (3): ^{1}H NMR ($CDCl_3$) δ 1.34 (3H, t, J = 7.8, CH_3CH_2CO), 3.10 (2H, q, J = 7.8, CH_3CH_2CO), 6.63–6.65 (1H, m, H-4′), 7.31 (1H, d, J = 3.9, H-3′), 7.68 (1H, d, J = 1.9, H-5′), 7.73 (1H, dd, J = 2.0, 8.8, H-8), 7.88 (1H, d, J = 8.8, H-7), 8.48 (1H, d, J = 2.0, H-10), 9.01 (NH, broad s).

5-(*n*-Butyrylamino)-9-chloro-2-(2-furanyl)[1,2,4]triazolo-[1,5-*c*]quinazoline (4): ^{1}H NMR ($CDCl_3$) δ 1.10 (3H, t, J = 7.4, 7.3, CH_3CH_2CH_2CO), 1.84–1.91 (2H, m, CH_3CH_2CH_2CO), 3.03 (2H, t, J = 7.4, 7.3, CH_3CH_2CH_2CO), 6.63–6.65 (1H, m, H-4′), 7.31 (1H, d, J = 3.4, H-3′), 7.68 (1H, d, J = 1.8, H-5′), 7.73 (1H, dd, J = 2.3, 8.8, H-8), 7.88 (1H, d, J = 8.8, H-7), 8.48 (1H, d, J = 2.3, H-10), 8.97 (NH, broad s).

9-Chloro-2-(2-furanyl)-5-(*n*-pentanoylamino)[1,2,4]-triazolo[1,5-*c*]quinazoline (5): ^{1}H NMR ($CDCl_3$) δ 1.01 (3H, t, J = 6.8, 7.8, CH_3CH$_2$CH$_2$CH$_2$CO), 1.48–1.55 (2H, m, CH$_3$CH_2CH$_2$CH$_2$CO), 1.77–1.85 (2H, m, CH$_3$CH$_2$CH_2CH$_2$CO), 3.05 (2H, t, J = 7.82, 6.8, CH$_3$CH$_2$CH$_2$CH_2CO), 6.63–6.65 (1H, m, H-4′), 7.31 (1H, d, J = 2.9, H-3′), 7.68 (1H, broad s, H-5′), 7.73 (1H, dd, J = 2.0, 8.8, H-8), 7.88 (1H, d, J = 8.8, 7-H), 8.48 (1H, d, J = 2.9, 10-H), 8.98 (NH, broad s).

9-Chloro-2-(2-furanyl)-5-[(trimethylacetyl)amino][1,2,4]-triazolo[1,5-*c*]quinazoline (6): ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s, (CH_3)$_3$CCO), 6.63–6.65 (1H, m, H-4′), 7.31 (1H, d, J = 3.4, H-3′), 7.68–7.69 (1H, m, H-5′), 7.74 (1H, dd, J = 2.5, 8.9, H-8), 8.01 (1H, d, J = 8.9, H-7), 8.49 (1H, d, J = 2.5, H-10), 9.39 (NH, broad s).

5-[(*tert*-Butoxycarbonyl)amino]-9-chloro-2-(2-furanyl)-[1,2,4]triazolo[1,5-*c*]quinazoline (7): ^{1}H NMR ($CDCl_3$) δ 1.63 (9H, s, (CH_3)$_3$COCO), 6.65–6.66 (1H, m, H-4′), 7.35 (1H, d, J = 3.5, H-3′), 7.69 (1H, broad s, H-5′), 7.74 (1H, dd, J = 2.6, 8.9, H-8), 8.01 (1H, d, J = 8.9, H-7), 8.49 (1H, d, J = 2.6, H-10), 8.56 (NH, broad s).

5-[[4-[(*tert*-Butoxycarbonyl)amino]butyryl]amino]-9-chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-*c*]quinazoline (8): ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s, (CH_3)$_3$COCONH), 2.03 (2H, pen, J = 6.8, NHCH$_2$CH_2CH$_2$CO), 3.13 (2H, t, J = 6.8, 7.3, NHCH$_2$CH$_2$CH_2CO), 3.32 (2H, q, J = 6.4, NHCH_2CH$_2$CH$_2$-CO), 4.81 (NH, broad s), 6.63–6.65 (1H, m, H-4′), 7.27–7.32 (1H, m, H-3′), 7.67–7.68 (1H, m, H-5′), 7.71–7.75 (1H, m, H-8), 7.91 (1H, d, J = 8.8, H-7), 8.48 (1H, d, J = 2.0, H-10), 9.15 (NH, broad s).

5-[(4-aminobutyryl)amino]-9-chloro-2-(2-furanyl)[1,2,4]-triazolo[1,5-*c*]quinazoline (9).—A solution of **8** (3 mg, 6.4 μmol) in 1 mL of 50% TFA in CH_2Cl_2 was left to stand for 10 min and evaporated under reduced pressure. Crystallization of the residue with MeOH/CH_2Cl_2 gave 3.2 mg of **9** (83% as TFA salt) as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.87–1.97 (2H, m, H$_2$NCH$_2$CH_2CH$_2$CO), 2.82 (2H, t, J = 7.32, H$_2$NCH$_2$-CH$_2$CH_2CO), 2.88–2.94 (2H, m, H$_2$NCH_2CH$_2$CH$_2$CO), 6.78–6.80 (1H, m, H-4′), 7.34 (1H, d, J = 3.05, H-3′), 7.77 (NH_2, broad s), 7.93–8.02 (3H, m), 8.40 (1H, d, J = 1.2, H-10), 11.17 (NH, s).

5-(Benzoylamino)-9-chloro-2-(2-furanyl)[1,2,4]triazolo-[1,5-*c*]quinazoline (10): ^{1}H NMR ($CDCl_3$) δ 6.64–6.66 (1H, m, H-4′), 7.35 (1H, d, J = 2.9, H-3′), 7.61–7.68 (3H, m), 7.68–7.70 (1H, m, H-5), 7.77 (1H, dd, J = 2.0, 8.8, H-8), 8.04–8.10 (3H, m), 8.52 (1H, d, J = 2.0), 9.75 (NH, broad s).

9-Chloro-2-(2-furanyl)-5-[(3-iodobenzoyl)amino][1,2,4]-triazolo[1,5-*c*]quinazoline (11): ^{1}H NMR ($CDCl_3$) δ 6.64–6.66 (1H, m, H-4′), 7.30–7.36 (3H, m), 7.70 (1H, broad s, H-5′), 7.77 (1H, dd, J = 2.0, 10.7, H-8), 7.92–8.10 (2H, broad m), 8.39 (1H, m), 8.52 (1H, d, J = 2.0, hH-10), 9.62 (NH, broad s).

9-Chloro-2-(2-furanyl)-5-[(phenylacetyl)amino][1,2,4]-triazolo[1,5-*c*]quinazoline (12): ^{1}H NMR ($CDCl_3$) δ 4.38 (2H, s, CH_2CO), 6.62–6.65 (1H, m, H-4′), 7.24–7.26

(1H, m, H-3′), 7.35–7.44 (5H, m), 7.67–7.68 (1H, m, H-5′), 7.74 (1H, dd, *J* = 2.2, 9.0, H-8), 7.93 (1H, d, *J* = 8.8, H-7), 8.49 (1H, m, H-10), 9.10 (NH, broad s).

5-Amino-2-[2-(5-bromofuranyl)]-9-chloro[1,2,4]triazolo-[1,5-*c*]quinazoline (13): A solution of **7** (0.01 g, 0.026 mmol) and *N*-bromosuccinimide (0.005 g, 0.028 mmol) in 2 mL of AcOH/$CHCl_3$ (1:1) was stirred for 1 h at room temperature. The mixture was poured into 10 mL of saturated $NaHCO_3$ solution, and the product was extracted with 10 mL of $CHCl_3$ three times. The combined $CHCl_3$ solution was washed with brine, dried over anhydrous Na_2SO_4, and evaporated to dryness under reduced pressure. The residue was purified by preparative silica gel TLC ($CHCl_3$/MeOH, 80:1) to afford 2-[2-(5-bromofuranyl)]-5-[(*tert*-butoxycarbonyl)amino]-9-chloro[1,2,4]-triazolo[1,5-*c*]quinazoline (0.012 g, 99%) as a white solid: MS (CI, NH_3) 466 ($M^+ + 1$); ^{1}H-NMR ($CDCl_3$) δ 1.63 (9H, s, $(CH_3)_3$-OCO), 6.58 (1H, d, *J* = 3.6, H-4′), 7.29 (1H, d, *J* = 3.6, H-3′), 7.73 (1H, dd, *J* = 2.3, 8.8, H-8), 7.98 (1H, d, *J* = 9.0, H-7), 8.46 (1H, d, *J* = 2.3, H-10), 8.53 (NH, broad s). To a solution of this compound in 2 mL of CH_2Cl_2 was added TFA (0.05 mL, 0.67 mmol), and the mixture was stirred for 2 h at room temperature. The reaction mixture was treated with same workup procedure above. A preparative silica gel TLC (*n*-hexane/$CHCl_3$/MeOH, 1:1:0.1) of the crude product gave **13** (4.5 mg, 48%) as a white solid: MS (CI, NH_3) 366 ($M^+ + 1$), 383 ($M^+ + 18$); ^{1}H-NMR ($CDCl_3$) δ 5.94 (NH_2, broad s), 6.56 (1H, d, *J* = 3.8, H-4′), 7.25 (1H, d, *J* = 3.8, H-3′), 7.63–7.65 (2H, m, H-8 + H-7), 8.41 (1H, d, *J* = 2.1, H-10).

5-(Benzoylamino)-2-[2-(5-bromofuranyl)]-9-chloro[1,2,4]-triazolo[1,5-*c*]quinazoline (14).—The furanyl group in compound **10** was brominated by the same method as above: ^{1}H-NMR ($CDCl_3$) δ 6.64–6.66 (1H, m, H-4′), 7.35 (1H, d, *J* = 2.9, H-3′), 7.61–7.68 (2H, m), 7.68–7.70 (1H, m), 7.77 (1H, dd, *J* = 2.0, 8.8, H-8), 8.04–8.10 (3H, m), 8.52 (1H, d, *J* = 2.0, H-10), 9.75 (NH, broad s); HPLC retention time: 4.6 min (>95% purity) using solvent system A, 12.5 min (>95% purity) using solvent system B.

9-Chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-*c*]quinazolin-5(6*H*)-one (15).—A solution of **1** (0.075 g, 0.263 mmol) in 8.0 mL of AcOH and 2.0 mL of H_2O in a sealed tube was heated for 72 h at 100 °C. The solution was coevaporated with toluene under reduced pressure, and the residue was purified by preparative silica gel TLC ($CHCl_3$/MeOH, 15:1) to afford **15** (0.065 g, 86%) as a white solid: mp >310 °C; MS (CI NH_3) 287 ($M^+ + 1$), 304 ($M^+ + 18$); ^{1}H-NMR (DMSO-d_6) δ 6.73–6.74 (1H, m, H-4′), 7.26 (1H, d, *J* = 3.5, H-3′), 7.46 (1H, d, *J* = 8.8, H-7), 7.77 (1H, dd, *J* = 2.5, 8.9, H-8), 7.97 (1H, s, H-5′), 8.16 (1H, d, *J* = 2.5, H-10), 12.47 (NH, s).

9-Chloro-2-(2-furanyl) 6-*n*-propyl[1,2,4]triazolo[1,5-*c*]-quinazolin-5-one (16).— To a suspension of **15** (0.021 g, 0.073 mmol) in 2 mL of anhydrous THF was added a suspension of NaH (6 mg, 60% in mineral oil, prewashed with *n*-hexane, 0.15 mmol) in 2 mL of anhydrous THF followed by HMPA (0.21 mL, 12 mmol) under N_2 atmosphere at room temperature. The mixture was stirred vigorously for 30 min (H_2 gas evolved). 1-Bromopropane (28 μL, 0.3 mmol) was added, and the reaction mixture was refluxed for 6 h. After cooling, the precipitate was removed by filtration through a small volume of silica gel bed and the filtrate was evaporated. The residue was purified by preparative silica gel

TLC (*n*-hexane/EtOAc, 2:1) to afford **16** (0.012 g, 50%) as a white solid: 1H-NMR ($CDCl_3$) δ 1.11 (3H, t, J = 7.5, 7.5, $CH_3CH_2CH_2N$), 1.85–1.93 (2H, m, CH_3CH_2-CH_2N), 4.35 (2H, t, J = 8.0, 7.5, $CH_3CH_2CH_2N$), 6.60–6.61 (1H, m, H-4′), 7.32 (1H, d, J = 3.4, H-3′), 7.37 (1H, d, J = 9.3, H-7), 7.66 (1H, broad s, H-5′), 7.68 (1H, dd, J = 2.39, 9.0, H-8), 8.52 (1H, d, J = 2.5, H-10); HPLC retention time 3.5 min (>95% purity) using solvent system A, 9.1 min (>95% purity) using solvent system B.

Pharmacology: Radioligand Binding Studies.

Binding of [^{3}H]-(*R*)-PIA to A_1 receptors from rat cerebral cortex membranes and of [^{3}H]CGS 21680 to A_{2A} receptors from rat striatal membranes was performed as described previously.[6,8] Adenosine deaminase (3 units/mL) was present during the preparation of the brain membranes, in a preincubation of 30 min at 30 °C and during the incubation with the radioligands.

Binding of [^{125}I]AB-MECA in membranes prepared from HEK-293 cells stably expressing the human A_3 receptor (Receptor Biology, Inc., Baltimore, MD), or from CHO cells stably expressing the rat A_3 receptor, was as described.[17,25] The assay medium consisted of a buffer containing 50 mM Tris, 10 mM Mg^{2+}, and 1 mM EDTA, at pH 8.0. The glass incubation 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. Nonspecific binding was determined in the presence of 200 μM NECA.

All nonradioactive compounds were initially dissolved in DMSO and diluted with buffer to the final concentration, where the amount of DMSO never exceeded 2%.

Incubations 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 buffer each.

At least five different concentrations of competitor, spanning 3 orders of magnitude adjusted appropriately for the IC_{50} of each compound, were used. IC_{50} values, calculated with the nonlinear regression method implemented in the InPlot program (Graph-PAD, San Diego, CA), were converted to apparent K_i values using the Cheng-Prusoff equation[27] and K_d values of 1.0 nM, 14 nM for [^{3}H]-(*R*)-PIA and [^{3}H]CGS 21680, and 0.59 nM for binding of [^{125}I]AB-MECA at human A_3 receptors, respectively. Most Hill coefficients of tested compounds were in the range 0.8–1.1.

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Abbreviations:

AcOH	acetic acid
Boc	*tert*-butoxycarbonyl

CGS 21680	2-[[4-(2-carboxyethyl)phenyl]ethylamino]-5′-*N*-(ethylcarbamoyl)adenosine
CGS15943	9-chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-*c*]quinazolin-5-amine
CHO cells	Chinese hamster ovary cells
CI	chemical ionization
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
EDTA	ethylenediaminetetraacatate
HEK cells	human embryonic kidney cells
HMPA	hexamethylphosphotriamide
[^{125}I]AB-MECA	[^{125}I]-N^6-(4-amino-3-iodobenzyl)-5′-(*N*-methylcarbamoyl)adenosine
K_i	equilibrium inhibition constant
MS	mass spectrum
NECA	(*N*-ethylcarbamoyl)adenosine
(*R*)-PIA	(*R*)-N^6-(phenylisopropyl)adenosine
SAR	structure-activity relationship
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
Tris	tris(hydroxymethyl)amino-methane

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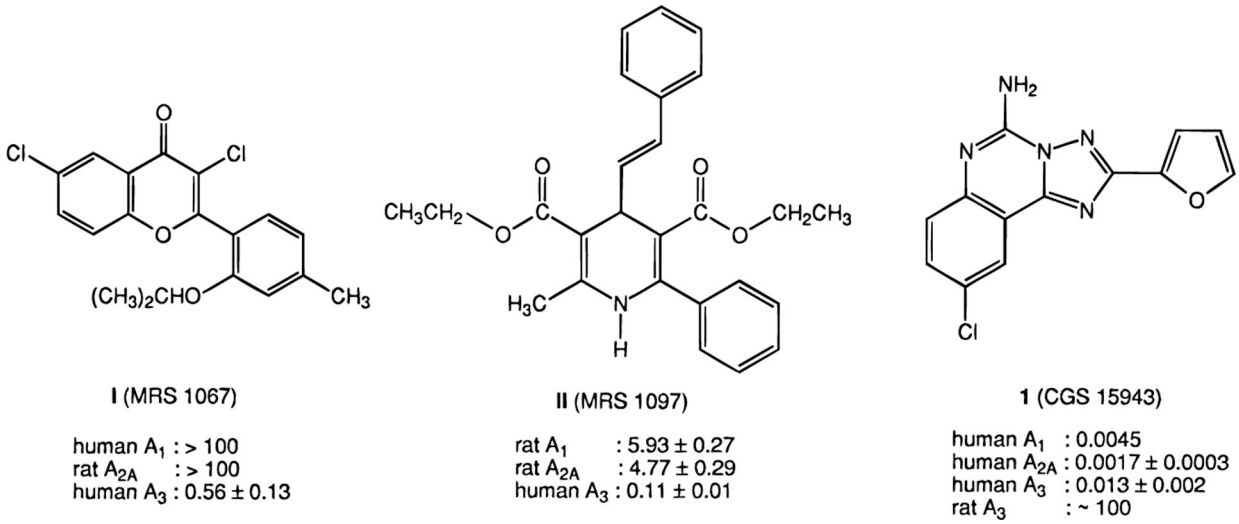

Figure 1.

Structures and affinities (K_i value in μM) of novel A_3 adenosine receptor antagonists the flavonoid **I** (MRS 1067),[17] the dihydopyridine **II** (MRS 1097),[18] and the nonselective antagonist triazoloquinazoline **1** (CGS15943).[19,28,29]

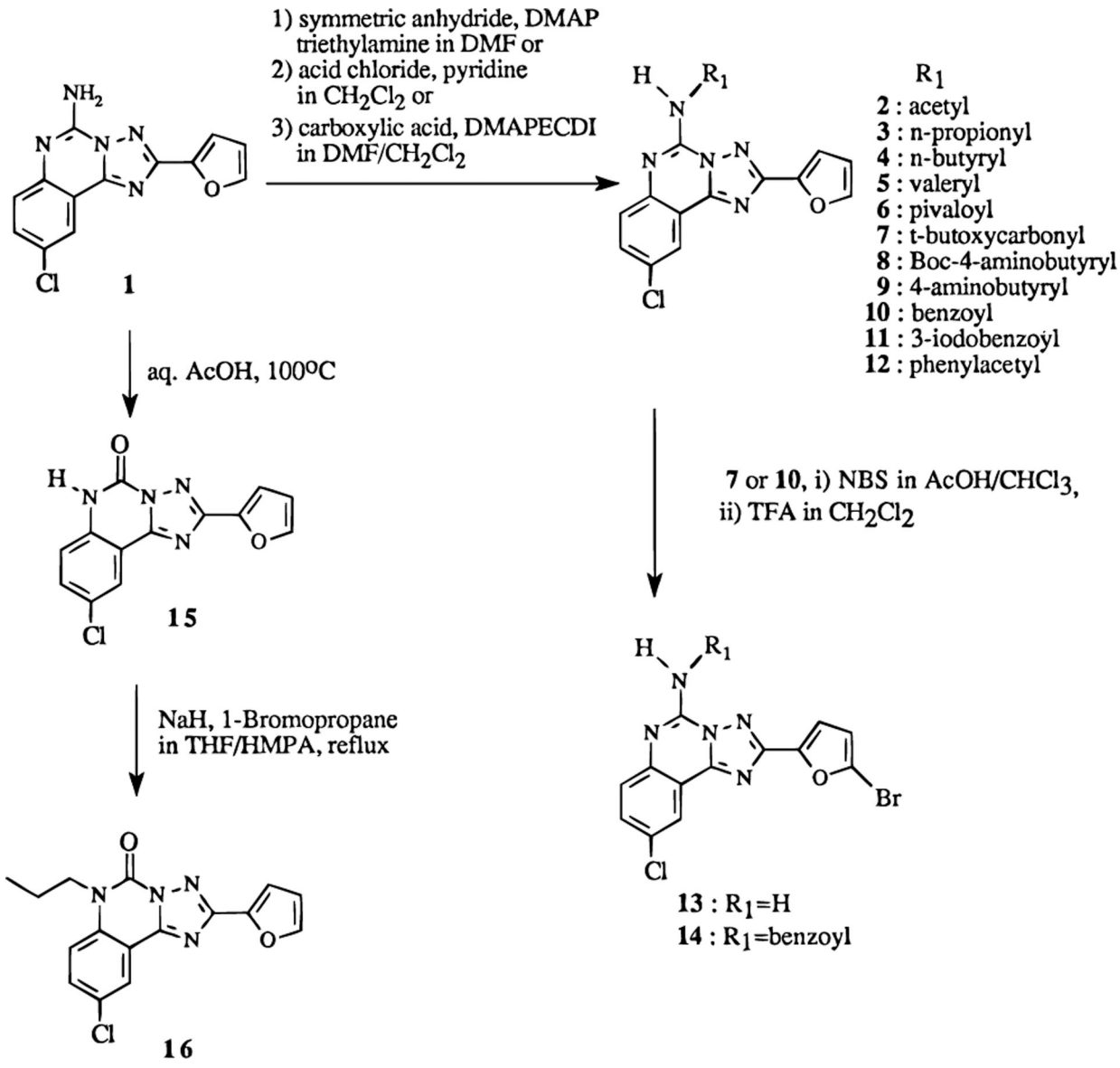

Figure 2.

Synthesis of *N*-acylated and various other derivatives of **1** (CGS15943).

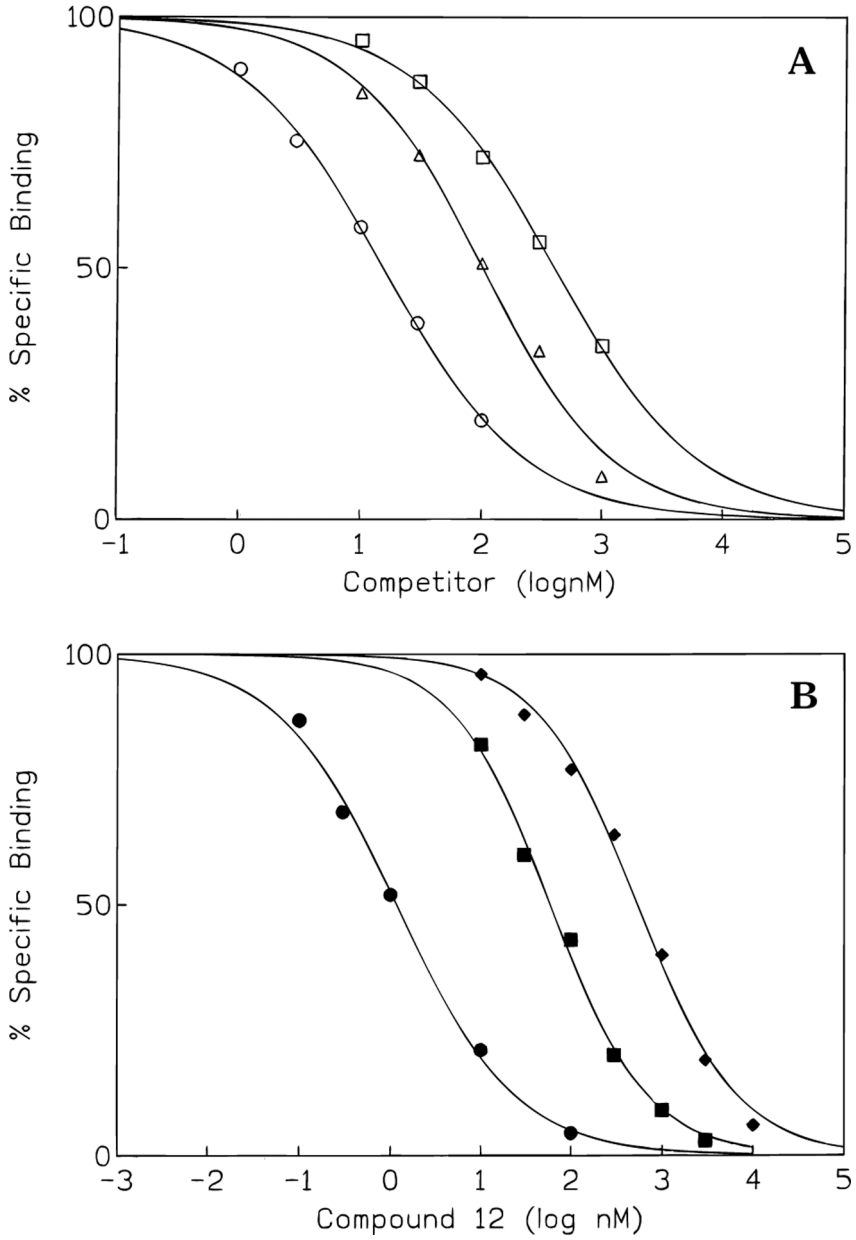

Figure 3.

Representative competition curves comparing inhibition of binding at adenosine receptors. (A) Inhibition by compounds **3** (circles), **6** (squares), and **7** (triangles) of [^{125}I]AB-MECA binding at human A_3 receptors, expressed in HEK-293 cells. (B) Inhibition by compound **12** of [^{125}I]AB-MECA binding at human A_3 receptors (filled circles, transfected HEK-293 cell membranes, n_H = 0.75), [^{3}H]-(*R*)-PIA binding at rat brain A_1 (filled diamonds, nH) = 0.89), and [^{3}H]CGS 21680 binding at A_{2A} (filled squares, n_H = 0.84) receptors (in brain membranes).

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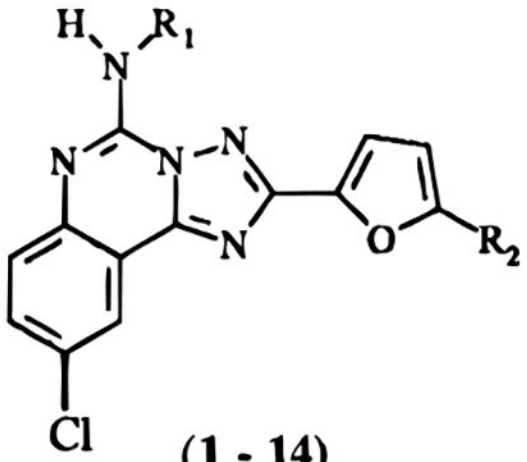
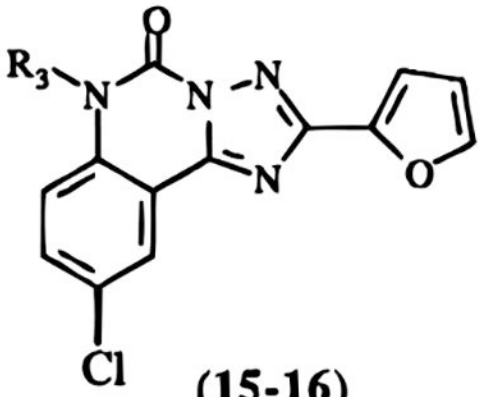
Table 1.

Affinities of Triazoloquinazoline Derivatives in Radioligand Binding Assays at A_1, A_{2A}, and A_3 Receptors

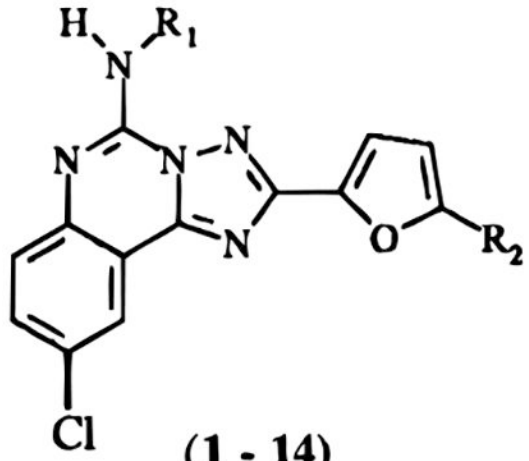


(1 - 14)

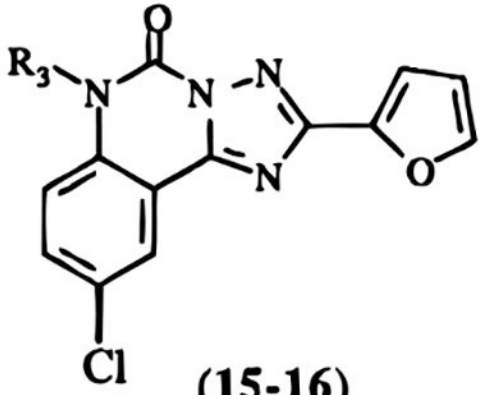


(15-16)

				K_i (nM), IC_{50} (nM), or % inhibition				
compd	**R_1**	**R_2**	**R_3**	**rA_1**[a]	**rA_{2A}**[b]	**hA_3**[c]	**rA_1/hA_3**	**rA_{2A}/hA_3**
1 (CGS15943)	H	H		21 ± 3[d]	3.3 ± 1.7[d]	13.8 ± 2.4		
2	$COCH_3$	H		52.2 ± 2.6	36.3 ± 3.2	13.9 ± 2.5	3.8	2.6
3 (MRS1186)	$COCH_2CH_3$	H		283 ± 42	106 ± 30	7.66 ± 3.03	40	14
4	$CO(CH_2)_2CH_3$	H		32.5 ± 9.4	37.6 ± 5.6	14.6 ± 2.8	2.2	2.6
5	$CO(CH_2)_3CH_3$	H		28.9 ± 3.7	48.8 ± 10.5	21.5 ± 6.2	1.3	2.3
6	$COC(CH_3)_3$	H		205 ± 20	88.8 ± 20.5	244 ± 6	0.84	0.36
7	$COOC(CH_3)_3$	H		190 ± 16	92.0 ± 8.0	82.5 ± 23.3	2.3	1.1
8	$CO(CH_2)_3$NH-Boc	H		91.3 ± 13.2	135 ± 38	32.9 ± 1.7	2.8	4.1
9	$CO(CH_2)_3NH_2$	H		11.3 ± 2.4	14.4	80.8 ± 7.4	0.14	0.18
10 (MRS1177)	COPh	H		680 ± 82	273 ± 42	3.03 ± 1.73	220	90
11	CO(3-I-Ph)	H		200	310 ± 151	23.8 ± 5.2	8.4	13
12 (MRS1220)	$COCH_2$Ph	H		305 ± 51	52.0 ± 8.8	0.65 ± 0.25	470	80



(1 - 14)



(15-16)

				K_i (nM), IC_{50} (nM), or % inhibition				
compd	**R_1**	**R_2**	**R_3**	**rA_1[a]**	**rA_{2A}[b]**	**hA_3[c]**	**rA_1/hA_3**	**rA_{2A}/hA_3**
13	H	Br		1570[d]	531[d]	64.0 ± 13.1		
14	COPh	Br		<10%[e]	<10%[e]	856 ± 156		
15			H	3950 ± 250	4380	260 ± 87	15	17
16			$(CH_2)_2CH_3$	1850 ± 420	6960	1813 ± 720	1.0	3.8

[a] Displacement of specific [^{3}H]-(*R*)-PIA binding in rat brain membranes, expressed as K_i ± SEM in nM (n = 3–5).

[b] Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes, expressed as K_i ± SEM in nM (n = 3–6).

[c] Displacement of specific [^{125}I]AB-MECA binding at human A_3 receptors expressed in HEK-293 cells, in membranes, expressed as K_i ±SEM in nM (n = 3–4).

[d] IC_{50} values from Francis et al.[19]

[e] Percent displacement of specific binding at concentration 1 μM.

Table 2.

Yields and Chemical Characterization of Triazoloquinazoline Derivatives

compd no.	method[a]	% yield	mp (°C)	MS	formula	analysis
2	A	96	245	CI: 328	$C_{15}H_{10}cN_5O_2C1\cdot0.16CH_2C1_2$	C,H,N
3	A	84	224	CI: 342	$C_{16}H_{12}N_5O_2Cl$	C,H,N
4	A	96	232	CI: 356	$C_{17}H_{14}N_5O_2Cl\cdot0.2MeOH$	C,H,N
5	A	73	220	FAB: 370	$C_{18}H_{16}N_5O_2Cl\cdot0.1EtOAc$	C,H,N
6	B	70	200	CI: 370	$C_{18}H_{16}N_5O_2Cl$	C,H,N
7	A	98	285	CI: 386	$C_{18}H_{16}N_5O_3Cl$	C,H,N
8	C	49	209	CI: 471	$C_{22}H_{23}N_6O_4Cl\cdot0.11CH_2Cl_2$	C,H,N
9		83	252	FAB: 371	$C_{17}H_{15}N_6O_2Cl\cdot C_2H_1O_2F_3\cdot0.15CH_2Cl_2$	C,H,N
10	A	73	239	CI: 390	$C_{20}H_{12}N_5O_2Cl\cdot0.6CH_2Cl_2$	C,H,N
11	C	50	243	CI: 515	$C_{20}cH_{11}N_5O_2ClI\cdot0.75MeOH$	C,H,N
12	B	35	233	EI: 403	$C_{21}H_{14}N_5O_2Cl\cdot0.38EtOAc$	C,H,N
14		28	266	FAB: 469	$C_{20}H_{11}N_5O_2BrCl$	b
16		50	178	EI: 328	$C_{16}H_{13}N_50_2C1$	b

[a] A, symmetric anhydride method; B, acid chloride method; C, carbodiimide method.

[b] High-resolution mass in EI or FAB^+ mode (*m/z*) determined to be within acceptable limits. **14**: calcd, 467.9863; found, 467.9871. **16**: calcd, 328.0727; found, 328.0726.