Published in final edited form as:

Drug Dev Res. 1999 December ; 48(4): 171–177. doi:10.1002/(SICI)1098-2299(199912)48:4<171::AID-DDR4>3.0.CO;2-5.

Pyran Template Approach to the Design of Novel A₃ Adenosine Receptor Antagonists

An-Hu Li, Xiao-duo Ji, Hak Sung Kim, Neli Melman, and Kenneth A. Jacobson*

Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland

Abstract

Strategy, Management and Health Policy				
Venture Capital Enabling Technology	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I–III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

A₃ adenosine receptor antagonists have potential as anti-inflammatory, anti-asthmatic, and anti-ischemic agents. We previously reported the preparation of chemical libraries of 1,4-dihydropyridine (DHP) and pyridine derivatives and identification of members having high affinity at A₃ adenosine receptors. These derivatives were synthesized through standard three-component condensation/oxidation reactions, which permitted versatile ring substitution at five positions, i.e., the central ring served as a molecular scaffold for structurally diverse substituents. We extended this template approach from the DHP series to chemically stable pyran derivatives, in which the ring NH is replaced by O and which is similarly derived from a stepwise reaction of three components. Since the orientation of substituent groups may be conformationally similar to the 1,4-DHPs, a direct comparison between the structure activity relationships of key derivatives in binding to adenosine receptors was carried out. Affinity at human A₃ receptors expressed in CHO cells was determined vs. binding of [¹²⁵I]AB-MECA (N⁶-(4-amino-3-iodobenzyl)-5'-N-methyl-carbamoyladenosine). There was no potency-enhancing effect, as was observed for DHPs, of 4-styryl, 4-phenylethynyl, or 6-phenyl substitutions. The most potent ligands in this group in binding to human A₃ receptors were 6-methyl and 6-phenyl analogs, **3a** (MRS 1704) and **4a** (MRS 1705), respectively, of 3,5-diethyl 2-methyl-4-phenyl-4*H*-pyran-3,5-dicarboxylate, which had K_i values of 381 and 583 nM, respectively. These two derivatives were selective for human A₃ receptors vs. rat brain A₁ receptors by 57-fold and 24-fold, respectively. These derivatives were inactive in binding at rat brain A_{2A} receptors, and at recombinant human A_{2B} receptors displayed K_i values of 17.3 and 23.2 μM, respectively. The selectivity, but not affinity, of the pyran derivatives in binding to the A₃ receptor subtype was generally enhanced vs. the corresponding DHP derivatives.

*Correspondence to: Dr. K.A. Jacobson, Chief, Molecular Recognition Section, LBC, NIDDK, NIH, Bldg. 8A, Rm. B1A-19, Bethesda, MD 20892-0810. kajacobs@helix.nih.gov.

Keywords

G protein-coupled receptors; structure–activity relationships; template; radioligand binding; purines

INTRODUCTION

A₃ adenosine receptors occur in the inflammatory/immune system [Beaven et al., 1994; Knight et al., 1997], the cardiovascular system [Hannon et al., 1995; Strickler et al., 1996], the renal system [Casavola et al., 1998], and the central nervous system [Jacobson et al., 1993; von Lubitz et al., 1994; Abbracchio et al., 1995; Dunwiddie et al., 1997]. Activation of the receptor is coupled to the inhibition of adenylyl cyclase, through a pertussis toxin-sensitive G protein, G_i [Palmer and Stiles, 1997]. In addition, stimulation of phospholipase C results from A₃ adenosine receptor activation, likely through the βγ subunits of G_i. A₃ adenosine receptor antagonists are sought as potential anti-inflammatory [Beaven et al., 1994], anti-glaucoma [Mitchell et al., 1999], anti-asthmatic [Knight et al., 1997], or anti-ischemic agents [von Lubitz et al., 1997].

The classical adenosine antagonists, xanthines, while potent and/or selective for A₁/A_{2A}/A_{2B} receptors, have not yet provided fruitful leads for A₃ receptor-selective antagonists, due to generally low affinity relative to the other adenosine receptor subtypes [Jacobson, 1998]. Thus, the first selective antagonists reported for adenosine A₃ receptors were nonxanthine heterocycles, such as flavonoids and 1,4-dihydropyridine (DHP) derivatives. Other classes of heterocycles have been found to block receptors selectively [Jacobson et al., 1996; van Muijlwijk-Koezen et al., 1998]. 1,4-DHPs are classically antagonists of L-type calcium channels; however, by modification of substituent groups it was possible to achieve both A₃ receptor selectivity and eliminate binding to L-type channels [Jiang et al., 1997, 1999]. Thus, the 1,4-DHP structure has been used as a template for novel ligands (Fig. 1) for adenosine and other receptors [Triggle, 1985; Coburn et al., 1988; Wong et al., 1998]. Because of the versatility of this template in the development of ligands for numerous binding sites, the DHPs have been termed a “privileged structure.”

Among the first adenosine receptor-selective DHPs was MRS 1097, **1** (Fig. 1) [Jiang et al., 1997], shown to be specific for adenosine receptors vs. many other receptors and ion channels. The 4-phenylethynyl-6-phenyl-1,4-DHP MRS 1191, **2a**, had selectivity >1,300-fold for human A₃ receptors vs. other subtypes of adenosine receptors and was a potent antagonist in functional assays [Jacobson et al., 1997]. The template approach was further extended with reports that not only could the substituent groups of the DHP be substituted with resulting increases in affinity, but the DHP ring could be oxidized, leading to pyridine derivatives as novel A₃ receptor selective antagonists [Li et al., 1999] and even methylated, leading to pyridinium salts [Xie et al., 1999] having such selectivity.

In the present study, we report a conceptual extension of this template approach (Fig. 2), leading to pyrans, which can be considered analogs of 1,4-DHPs in which oxygen replaces N-H. In parallel to the DHP series, which are prepared through condensation of three components, the pyran derivatives may be derived from reaction of three components

[Wolinsky and Hauer, 1969], although in a stepwise series of reactions. The versatility of introducing a wide variety of substituents is an advantage shared by both the pyran and DHP synthetic pathways.

MATERIALS AND METHODS

Materials and Instrumentation

Phenylpropargyl aldehyde (**11b**), benzyl acetoacetate (**10c**), ethyl benzoylacetate (**10b**), ethyl acetoacetate (**10a**), cinnamaldehyde (**11a**), benzaldehyde (**11c**), *m*-nitrobenzaldehyde (**11d**), zinc chloride were purchased from Aldrich (St. Louis, MO). Benzyl benzoylacetate (**10d**) was prepared as in our previous work [Li et al., 1998]. All other materials were obtained from commercial sources.

Proton nuclear magnetic resonance spectroscopy was performed on a Varian GEMINI-300 spectrometer and all spectra were taken in CDCl₃. 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. High-resolution EI, FAB (fast atom bombardment) mass spectrometry was performed with a JEOL SX102 spectrometer, using 6-kV Xe atoms following desorption from a glycerol matrix. Elemental analyses were performed by Atlantic Microlab (Norcross, GA).

Synthesis

General procedure for pyran syntheses (**3a–9a**, Fig. 2) [Wolinsky and Hauer, 1969]

Method A: Ethyl acetoacetate (**10a**, 2 equiv.), an aldehyde (**11a** or **11b**, 1 equiv.), and zinc chloride (0.5 equiv.) were stirred in Ac₂O-AcOH (1:1.2) at room temperature for 2 weeks. The reaction was poured into water and extracted with EtOAc. The extract was washed cautiously with saturated NaHCO₃ solution and water, dried over MgSO₄, and purified by preparative TLC (silica 60; 1,000 μm; Analtech, Newark, DE; petroleum ether-ethyl acetate (9:1)) to give pyran compound **7a** (yield: 24%) or **8a** (yield: 7%).

3,5-Diethyl (E)-2,6-dimethyl-4-((E)-styryl)-4*H*-pyran-3,5-dicarboxylate (**7a**): ¹H NMR (CDCl₃): 1.29 (t, *J* = 6.9 Hz, 6 H), 2.34 (s, 6 H), 4.12–4.30 (m, 4 H), 4.40 (d, *J* = 6.9 Hz, 1 H), 6.10 (dd, *J* = 15.6, 6.9 Hz, 1 H), 6.33 (d, *J* = 15.9 Hz, 1 H), 7.16–7.34 (m, 5 H). MS (CI/NH₃): *m/z* 374 (M⁺+NH₄), 253 (M⁺-PhCH=CH, 100%).

3,5-Diethyl 2,6-dimethyl-4-phenylethynyl-4*H*-pyran-3,5-dicarboxylate (**8a**): ¹H NMR (CDCl₃): 1.34 (t, *J* = 7.8 Hz, 6 H), 2.35 (s, 6 H), 4.16–4.39 (m, 4 H), 4.75 (s, 1 H), 7.23–7.25 (m, 3 H), 7.30–7.36 (m, 2 H). MS (CI/NH₃): *m/z* 372 (M⁺+NH₄), 355 (M⁺+1), 253 (M⁺-PhC₂).

Method B: β-Ketoester (**10a–d**, 1 equiv.), α,β-unsaturated-α-acetyl ester (**12b–d**, 1 equiv.), and zinc chloride (0.5 equiv.) were stirred in Ac₂O-AcOH (1:1.2) at room temperature for 1–4 weeks (for **3a** and **4a**: 1 week; for **5a** and **6a**: 2 weeks; for **9a**: 4 weeks). Work-up using

the same procedure described in Method A yielded compound **3a** (yield: 6%), **4a** (yield: 10%), **5a** (yield: 7%), **6a** (yield: 5%), or **9a** (yield: 7%).

3,5-Diethyl 2,6-dimethyl-4-phenyl-4*H*-pyran-3,5-dicarboxylate (**3a**): ^1H NMR (CDCl_3): 1.20 (t, $J = 7.8$ Hz, 6 H), 2.36 (s, 6 H), 4.03–4.14 (m, 4 H), 4.75 (s, 1 H), 7.13–7.24 (m, 5 H). MS (CI/NH_3): m/z 348 ($\text{M}^+ + \text{NH}_4$).

3,5-Diethyl 4,6-diphenyl-2-methyl-4*H*-pyran-3,5-dicarboxylate (**4a**): ^1H NMR (CDCl_3): 0.88 (t, $J = 7.8$ Hz, 3 H), 1.22 (t, $J = 7.8$ Hz, 3 H), 2.43 (s, 3 H), 3.88 (q, $J = 7.8$ Hz, 2 H), 4.12 (q, $J = 7.8$ Hz, 2 H), 4.88 (s, 1 H), 7.17–7.43 (m, 10 H). MS (CI/NH_3): m/z 410 ($\text{M}^+ + \text{NH}_4$, base), 393 ($\text{M}^+ + 1$).

3,5-Diethyl 2,6-dimethyl-4-(*m*-nitrophenyl)-4*H*-pyran-3,5-dicarboxylate (**5a**): ^1H NMR (CDCl_3): 1.20 (t, $J = 6.9$ Hz, 6 H), 2.40 (s, 6 H), 4.04–4.15 (m, 4 H), 4.87 (s, 1 H), 7.42 (t, $J = 7.8$ Hz, 1 H), 7.59 (d, $J = 7.8$ Hz, 1 H), 8.04 (d, $J = 7.8$ Hz, 1 H), 7.11 (t, $J = 2.1$ Hz, $J = 7.8$ Hz, 1 H). MS (EI): m/z 375 (M^+), 253 ($\text{M}^+ - \text{C}_6\text{H}_4\text{NO}_2$, base).

5-Benzyl 3-ethyl 2,6-dimethyl-4-(*m*-nitrophenyl)-4*H*-pyran-3,5-dicarboxylate (**6a**): ^1H NMR (CDCl_3): 1.19 (t, $J = 7.8$ Hz, 3 H), 2.38 (s, 3 H), 2.41 (s, 3 H), 4.03–4.14 (m, 2 H), 4.86 (s, 1 H), 5.07 (AB, $J = 12.6$ Hz, 2 H), 7.16 (m, 1 H), 7.30–7.44 (m, 4 H), 7.50 (m, 1 H), 7.58 (d, $J = 7.8$ Hz, 1 H), 8.00 (d, $J = 8.1$ Hz, 1 H), 8.11 (s, 1 H). MS (EI): m/z 437 (M^+), 346 ($\text{M}^+ - \text{CH}_2\text{Ph}$), 315 ($\text{M}^+ - \text{C}_6\text{H}_4\text{NO}_2$), 91 ($^+\text{CH}_2\text{Ph}$).

5-Benzyl 3-ethyl 2-methyl-6-phenyl-4-phenyl-ethynyl-4*H*-pyran-3,5-dicarboxylate (**9a**): ^1H NMR (CDCl_3): 1.33 (t, $J = 7.8$ Hz, 3 H), 2.44 (s, 3 H), 4.13–4.29 (m, 2 H), 4.83 (s, 1 H), 5.16 (AB, $J = 11.7$ Hz, 2 H), 6.94–7.61 (m, 15 H). MS (EI): m/z 478 (M^+), 361 ($\text{M}^+ - \text{PhC}_2$), 283 ($\text{M}^+ - \text{PhC}_2 - \text{CH}_2\text{Ph}$), 91 ($^+\text{CH}_2\text{Ph}$).

Preparation of unsaturated compounds 12a–d [Chupp, 1990]—Cinnaldehyde (**11a**, 1.32 g, 10 mmol) or phenyl-propargyl aldehyde (**11b**, 1.30 g, 10 mmol) or benzaldehyde (**11c**, 1.06 g, 10 mmol) or *m*-nitrobenzaldehyde (**11d**, 1.51 g, 10 mmol) was added to 3 ml of dichloromethane containing piperidine (4.3 mg) and ethyl acetoacetate (**10a**, 1.30 g, 10 mmol). The mixture was stirred for 24 h, and external cooling was used to maintain the temperature below 30°C. The solvent was removed under vacuum and the residue was chromatographed (silica 60; petroleum ether-ethyl acetate (9:1)) to give 1.52 g of compound **12a** (yield: 62%), 0.912 g of compound **12b** (yield: 38%), 0.471 g of compound **12c** (yield: 22%), or 1.32 g of compound **12d** (yield: 50%).

Ethyl 2-acetyl-5-phenyl-penta-2,4-dienoate (**12a**): ^1H NMR (CDCl_3): 1.42 (t, $J = 6.9$ Hz, 3 H), 2.41 (s, 3 H), 4.40 (q, $J = 6.9$ Hz, 2 H), 7.14 (t, $J = 15.9, 12.9$ Hz, 1 H), 7.29 (d, $J = 14.7$ Hz, 1 H), 7.36–7.41 (m, 3 H), 7.45 (d, $J = 11.7$ Hz, 1 H), 7.50–7.53 (m, 2 H). MS (CI/NH_3): m/z 262 ($\text{M}^+ + \text{NH}_4$), 245 ($\text{M}^+ + 1$, base).

Ethyl 2-acetyl-5-phenyl-pent-2-en-4-ynoate (**12b**): ^1H NMR (CDCl_3): 1.33 (t, $J = 6.9$ Hz, 1.5 H), 1.39 (t, $J = 6.9$ Hz, 1.5 H), 2.41 (s, 1.5 H), 2.52 (s, 1.5 H), 4.30 (q, $J = 6.9$ Hz, 1 H), 4.40 (q, $J = 6.9$ Hz, 1 H), 7.04 (s, 1 H), 7.34–7.42 (m, 3 H), 7.47–7.51 (m, 2 H). MS (CI/NH_3): m/z 260 ($\text{M}^+ + \text{NH}_4$), 243 ($\text{M}^+ + 1$, base).

Ethyl 2-acetyl-cinnamate (**12c**): ^1H NMR (CDCl_3): 1.28 (t, $J = 7.8$ Hz, 3 H), 2.43 (s, 3 H), 4.34 (q, $J = 7.8$ Hz, 2 H), 7.40–7.42 (m, 3 H), 7.45–7.48 (m, 2 H), 7.58 (s, 1 H). MS (CI/ NH_3): m/z 236 ($\text{M}^+ + \text{NH}_4$, base), 219 ($\text{M}^+ + 1$).

Ethyl 2-acetyl-3-(*m*-nitrophenyl)-acrylate (**12d**): ^1H NMR (CDCl_3): 1.33 (t, $J = 6.9$ Hz, 3 H), 2.52 (s, 3 H), 4.30 (q, $J = 6.9$ Hz, 2 H), 7.56–7.66 (m, 2 H), 7.77 (d, 1 H, $J = 7.8$ Hz), 8.28 (d, 1 H, $J = 7.8$ Hz), 8.35 (s, 1 H). MS (CI/ NH_3): m/z 281 ($\text{M}^+ + \text{NH}_4$), 264 ($\text{M}^+ + 1$, base).

Synthesis of 1,4-DHP derivatives—Compounds **4b–7b** and **9b** were prepared as reported [Li et al., 1998; Harper et al., 1999].

3,5-Diethyl 2,6-dimethyl-4-phenyl-1,4-dihydro-pyridine-3,5-dicarboxylate (**3b**): A mixture of ethyl 3-aminocrotonate (129 mg, 1.00 mmol) and benzaldehyde (106 mg, 1.00 mmol) and ethyl acetoacetate (94 mg, 1.00 mmol) was dissolved in 3 ml of absolute ethanol. The mixture was sealed in a capped Pyrex tube, and heated to 90–95°C with stirring for 40 h. The resulting mixture was cooled to room temperature and evaporated in vacuo. A mixture of petroleum ether/ethyl acetate (15/1, 5 ml) was added to this residue. After stirring for 1 h at 90°C, the mixture was cooled to room temperature, and stirring continued for 2 h. The fluorescent solid was collected by suction filtration to give 207 mg of the desired product, 3,5-diethyl 2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (62.8% yield): mp 156–157°C; ^1H -NMR (CDCl_3) δ 1.22 (t, $J = 6.9$ Hz, 6H), 2.34 (s, 6H), 4.01 (m, 4H), 5.00 (s, 1H), 5.58 (s, br, 1H), 7.06–7.40 (m, 5H); MS (CI/ NH_3): m/z 347 ($\text{M}^+ + \text{NH}_4$); HRMS calcd for $\text{C}_{19}\text{H}_{23}\text{N}_1\text{O}_4$ 329.1627, found 329.1614.

3,5-Diethyl 2,6-dimethyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate (**8b**): A mixture of ethyl 3-aminocrotonate (129 mg, 1.00 mmol) and phenylpropargyl aldehyde (130 mg, 1.00 mmol) and ethyl acetoacetate (94 mg, 1.00 mmol) was dissolved in 3 ml of absolute ethanol. The mixture was sealed in a capped Pyrex tube, and heated to 90–95°C with stirring for 40 h. The resulting mixture was cooled to room temperature and a white precipitate was collected by suction filtration and washed with cold ethanol (3 ml $\times$ 2) to yield 200 mg of the desired 3,5-diethyl 2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate. (57.0% yield): mp 193–194°C; ^1H -NMR (CDCl_3) δ 1.33 (t, $J = 6.9$ Hz, 6H), 2.34 (s, 6H), 4.26 (m, 4H), 4.99 (s, 1H), 5.67 (bs, 1H), 7.20–7.44 (m, 5H); MS (CI/ NH_3): m/z 354 (MH^+); HRMS calcd for $\text{C}_{21}\text{H}_{23}\text{NO}_4$ 353.1627, found 353.1615.

Pharmacology

Radioligand binding studies—Binding of [^3H]*R*- N^6 -phenylisopropyladenosine ([^3H]*R*-PIA) to A_1 receptors from rat cerebral cortex membranes and of [^3H]-2-[4-[(2-carboxyethyl)phenyl]-ethylamino]-5'-*N*-ethylcarbamoyladenine ([^3H]CGS 21680) to A_{2A} receptors from rat striatal membranes was performed as described previously [Schwabe and Trost, 1980; Jarvis et al., 1989]. Adenosine deaminase (3 IU/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. Nonspecific binding was determined in the presence of 10 μM (A_1 receptors) or 20 μM (A_{2A} receptors) 2-chloroadenosine.

Binding of [125 I]N⁶-(4-amino-3-iodobenzyl)-5'-N-methylcarbamoyladenosine ([125 I]AB-MECA) to membranes prepared from HEK-293 cells stably expressing the human A₃ receptor, clone HS-21a (Receptor Biology, Beltsville, MD) or to membranes prepared from CHO cells stably expressing the rat A₃ receptor was performed at 4°C, as described previously [Olah et al., 1994]. The assay medium consisted of a buffer containing 10 mM Mg²⁺, 50 mM Tris, 3 units/ml adenosine deaminase, and 1 mM EDTA, at pH 8.0. The glass incubation tubes contained 100 μ l of the membrane suspension (0.3 mg protein/ml, stored at -80°C in the same buffer), 50 μ l of [125 I]AB-MECA (final concentration 0.3 nM), and 50 μ l of a solution of the proposed antagonist. Nonspecific binding was determined in the presence of 100 μ M N⁶-phenylisopropyladenosine (NECA). Binding at human A₃ receptors expressed in HEK-293 cells using [3 H]ZM241,385 as radioligand was measured as described previously [Ji and Jacobson, 1999].

All nonradioactive compounds were initially dissolved in DMSO and diluted with assay medium to the final concentration. The amount of DMSO never exceeded 1%.

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 three orders of magnitude adjusted appropriately for the IC₅₀ of each compound, were used. IC₅₀ 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 [Cheng and Prusoff, 1973] and K_d values of 1.0 nM ([3 H]R-PIA); 15.5 nM ([3 H]CGS 21680); 33.6 nM ([3 H]ZM241,385); 0.59 nM and 1.46 nM ([125 I]AB-MECA at human and rat A₃ receptors, respectively).

RESULTS AND DISCUSSION

Synthesis

The route used to prepare the pyran derivatives [Wolinsky and Hauer, 1969] is shown in Figure 2. Yields and characterization of the pyrans tested as A₃ receptor antagonists are described in Table 1. The structures of the pyran derivatives and the corresponding 1,4-dihydropyridines, prepared using published procedures [Jiang et al., 1997; Li et al., 1998], for comparison of binding affinities are shown in Table 2. The relatively low yields in the syntheses of pyrans suggest that methodological improvements are needed. The 3-nitro group (**5**, **6**) is included for analogy to the common L-type calcium channel blocker, nitrendipine [Harper et al., 1999].

Pharmacology

K_i values for both novel pyran derivatives (**3a–9a**) and the corresponding dihydropyridines (**3b–9b**) were determined in radioligand binding assays at adenosine receptors. The pyran derivatives generally displaced binding at A₁ and A_{2A} receptors in rat brain membranes weakly, using as radioligands [3 H]R-PIA [Schwabe and Trost, 1980] and [3 H]CGS 21680 [Jarvis et al., 1989], respectively. At A₁ receptors only **8a** was more potent in binding than

the corresponding dihydropyridine, **8b**. Compounds **3a** and **4a** were 15- and 1.8-fold less potent, respectively, in binding at A₁ receptors than the corresponding dihydropyridines. The K_i values of compounds **5a–7a** and **9a** at rat A₁ receptors were >100 μM, although the corresponding dihydropyridines displayed micromolar affinity. At A_{2A} receptors all of the pyran derivatives were essentially inactive (K_i >100 μM) in binding.

Binding of the radioligand [¹²⁵I]AB-MECA [Olah et al., 1994] was carried out using recombinant human A₃ adenosine receptors expressed in Chinese hamster ovary (CHO) cells. The most potent ligands among pyran derivatives in binding to human A₃ receptors were 6-methyl and 6-phenyl analogs, **3a** (MRS 1704) and **4a** (MRS 1705), respectively, of 3,5-diethyl 2-methyl-4-phenyl-4*H*-pyran-3,5-dicarboxylate, which had K_i values of 381 and 583 nM, respectively. These two derivatives were selective vs. binding at rat brain A₁ receptors by 57-fold and 24-fold, respectively. Compounds **3a** and **4a** were inactive in binding at rat brain A_{2A} receptors and at recombinant human A_{2B} receptors displayed K_i values of 17.3 ± 3.1 and 23.2 ± 5.4 μM, respectively [Ji and Jacobson, 1999]. Other pyran derivatives, e.g., **5a–7a**, displayed A₃ receptor affinities similar to the affinities of the corresponding DHP derivatives and were inactive at rat brain A₁ and A_{2A} receptors. Thus, the selectivity, but not affinity, of the pyran derivatives in binding to the A₃ receptor subtype was generally enhanced vs. DHP derivatives.

The presence of a 6-phenyl substituent in the pyrans, which is potency-enhancing in the DHP derivatives, either had no effect (**4a**) or reduced (**9a**) the A₃ receptor affinity. Similarly, there was no potency-enhancing effect, as has been observed for DHPs, with 4-styryl or 4-phenylethynyl substitutions, in comparison to the 4-phenyl substitution.

The chirality of the asymmetrically substituted pyran derivatives, e.g., **4a**, **6a**, and **9a**, was ignored in this study, and only racemic compounds were prepared and tested in binding assays. For the binding of 1,4-DHPs to recombinant human A₃ adenosine receptors, their chirality was explored [Jiang et al., 1999]. Specifically, the 4*S*-isomer of MRS 1191, **2b**, was found to be 35-fold more potent in binding than the antipode. To further test the hypothesis that pyrans and DHPs bind to a common region of the receptor, it would be useful to compare the stereoselectivity of binding of the respective classes of compounds.

In conclusion, we have conceptually extended the approach of using dihydropyridines as a template for the design of ligands for G protein-coupled receptors through the replacement of the heterocyclic 1-amino group with an oxygen atom. We have demonstrated that the 1-hydrogen of the dihydropyridines is not required for recognition of this class of antagonists by the human A₃ receptor, for example, through a potential hydrogen bond with the receptor protein. The lack of the requirement for such a hydrogen bond was predicted in molecular modeling studies [Moro et al., 1998]. It remains to be determined if such bonding is required at other receptor binding sites, such as α-adrenergic receptors, at which DHPs have been found to bind. Since the selectivity of the derivatives for the A₃ receptor has been enhanced, the pyran strategy, based on existing structure–activity relationships (SAR) of DHP derivatives, may be considered as a general means of extending the template approach to an analogous but chemically distinct series. Nevertheless, the correspondence of SAR for pyran

and DHP derivatives is not strictly parallel and is dependent on which functional groups are present.

Acknowledgments

We thank Dr. Stefano Moro (NIDDK) for helpful discussions.

Abbreviations

[¹²⁵I]AB-MECA	[¹²⁵ I]N ⁶ -(4-amino-3-iodo-benzyl)-5'-N-methylcarbamoyladenine
CGS 21680	2-[4-[(2-carboxyethyl)phenyl]ethyl-amino]-5'-N-ethylcarbamoyladenine
CHO cells	Chinese hamster ovary cells
DMSO	dimethylsulfoxide
HEK cells	human embryonic kidney cells
IB-MECA	N ⁶ -(3-iodobenzyl)-5'-N-methylcarbamoyladenine
K_i	equilibrium inhibition constant
MRS	1191 3-ethyl 5-benzyl 2-methyl-6-phenyl-4-phenylethynyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate
R-PIA	R-N ⁶ -phenylisopropyladenine
SAR	structure–activity relationship
THF	tetrahydrofuran
Tris	tris(hydroxymethyl)-aminomethane
ZM241	, 385 4-(2-[7-Amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazinyl-amino]ethyl)phenol

References

- Abbraccio MP, Brambilla R, Ceruti S, Kim HO, von Lubitz DKJE, Jacobson KA, Cattabeni F. G-protein-dependent activation of phospholipase C by adenosine A₃ receptors in rat brain. *Mol Pharmacol*. 1995; 48:1038–1045. [PubMed: 8848003]
- Beaven MA, Ramkumar V, Ali H. Adenosine-A₃ receptors in mast-cells. *Trends Pharmacol Sci*. 1994; 15:13–14. [PubMed: 8140652]
- Casavola V, Di Sole F, Guerra L, Deellis L, Reshkin SJ, Jacobson KA. Adenosine A₃-receptor increases cytosolic calcium concentration via calcium influx in A6 cells. *Drug Devel Res*. 1998; 43:62.
- Cheng YC, Prusoff WH. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC₅₀) of an enzyme reaction. *Biochem Pharmacol*. 1973; 22:3099–3108. [PubMed: 4202581]
- Chupp JP. Derivation of fluorine-containing pyridine dicarboxylates. IV. N-substituted DHP derivatives. *J Heterocycl Chem*. 1990; 27:1697–1704.
- Coburn RA, Wierzbica M, Suto MJ, Solo AJ, Triggie AM, Triggie DJ. 1,4-Dihydropyridine antagonist activities at the calcium channel: a quantitative structure-activity relationship approach. *J Med Chem*. 1988; 31:2103–2107. [PubMed: 2846838]

- Dunwiddie TV, Diao L, Kim HO, Jiang J-I, Jacobson KA. Activation of hippocampal adenosine A₃ receptors produces a heterologous desensitization of A₁ receptor mediated responses in rat hippocampus. *J Neurosci*. 1997; 17:607–614. [PubMed: 8987783]
- Hannon JP, Pfannkuche HJ, Fozard JR. A role for mast cells in adenosine A₃ receptor-mediated hypotension in the rat. *Br J Pharmacol*. 1995; 115:945–952. [PubMed: 7582525]
- Harper JL, Li A-H, Jacobson KA, Daly JW. Dihydropyridines as inhibitors of capacitative calcium entry. *Biochem Pharmacol*. 1999 submitted.
- Jacobson KA. A₃ adenosine receptors: novel ligands and paradoxical effects. *Trends Pharmacol Sci*. 1998; 19:184–191. [PubMed: 9652191]
- Jacobson KA, Nikodijevic O, Shi D, Gallo-Rodriguez C, Olah ME, Stiles GL, Daly JW. A role for central A₃-adenosine receptors: mediation of behavioral depressant effects. *FEBS Lett*. 1993; 336:57–60. [PubMed: 8262217]
- Jacobson MA, Chakravarty PK, Johnson RG, Norton R. Novel selective non-xanthine selective A₃ adenosine receptor antagonists. *Drug Dev Res*. 1996; 37:131.
- Jacobson KA, Park KS, Jiang J-I, Kim Y-C, Olah ME, Stiles GL, Ji XD. Pharmacological characterization of novel A₃ adenosine receptor-selective antagonists. *Neuropharmacology*. 1997; 36:1157–1165. [PubMed: 9364471]
- Jarvis MF, Schutz R, Hutchison AJ, Do E, Sills MA, Williams M. [³H]CGS 21680, an A₂ selective adenosine receptor agonist directly labels A₂ receptors in rat brain tissue. *J Pharmacol Exp Ther*. 1989; 251:888–893. [PubMed: 2600819]
- Ji XD, Jacobson KA. Use of the triazolotriazine [³H]ZM241385 as a radioligand at recombinant human A_{2B} adenosine receptors. *Drug Design Discov*. 1999; 16:217–226.
- Jiang JL, van Rhee AM, Chang L, Patchornik A, Evans P, Melman N, Jacobson KA. Structure activity relationships of 4-phenylethynyl-6-phenyl-1,4-dihydropyridines as highly selective A₃ adenosine receptor antagonists. *J Med Chem*. 1997; 40:2596–2608. [PubMed: 9258367]
- Jiang JL, Li A-H, Jang SY, Chang L, Melman N, Moro S, Ji X-D, Lobkovsky EB, Clardy JC, Jacobson KA. Chiral resolution and stereospecificity of 4-phenylethynyl-6-phenyl-1,4-dihydro-pyridine derivatives as selective A₃ adenosine receptor antagonists. *J Med Chem*. 1999; 42:3055–3065. [PubMed: 10447949]
- Knight D, Zheng X, Rocchini C, Jacobson MA, Bai T, Walker B. Adenosine A₃ receptor stimulation inhibits migration of human eosinophils. *J Leukoc Biol*. 1997; 62:465–468. [PubMed: 9335316]
- Li A-H, Moro S, Melman N, Ji XD, Jacobson KA. Structure activity relationships and molecular modeling of 3,5-diacyl-2,4-dialkylpyridine derivatives as selective A₃ adenosine receptor antagonists. *J Med Chem*. 1998; 41:3186–3201. [PubMed: 9703464]
- Li A-H, Moro S, Forsyth N, Melman N, Ji XD, Jacobson KA. Synthesis, CoMFA analysis, and receptor docking of 3,5-diacyl-2,4-dialkylpyridine derivatives as selective A₃ adenosine receptor antagonists. *J Med Chem*. 1999; 42:706–721. [PubMed: 10052977]
- Mitchell CH, Peterson-Yantorno K, Carre DA, McGlinn AM, Coca-Prados M, Stone RA, Civan MM. A₃ adenosine receptors regulate Cl⁻ channels of nonpigmented ciliary epithelial cells. *Am J Physiol*. 1999; 276:C659–C666. [PubMed: 10069993]
- Moro S, Li A-H, Jacobson KA. Molecular modeling studies of human A₃ adenosine receptor antagonists: structural homology and receptor docking. *J Chem Inf Comput Sci*. 1998; 38:1239–1248. [PubMed: 9845970]
- Olah ME, Gallo-Rodriguez C, Jacobson KA, Stiles GL. [¹²⁵I]AB-MECA, a high affinity radioligand for the rat A₃ adenosine receptor. *Mol Pharmacol*. 1994; 45:978–982. [PubMed: 8190112]
- Palmer TM, Stiles GL. Structure-function analysis of inhibitory adenosine receptor regulation. *Neuropharmacology*. 1997; 36:1141–1147. [PubMed: 9364469]
- Schwabe U, Trost T. Characterization of adenosine receptors in rat brain by (–) [³H]N⁶-phenylisopropyladenosine. *Naunyn Schmiedebergs Arch Pharmacol*. 1980; 313:179–187. [PubMed: 6253840]
- Strickler J, Jacobson KA, Liang BT. Direct preconditioning of cultured chick ventricular myocytes: novel functions of cardiac adenosine A_{2A} and A₃ receptors. *J Clin Invest*. 1996; 98:1773–1779. [PubMed: 8878427]

- Triggle, DJ. Drugs acting on ion channels and membranes. In: Emmett, JC., editor. Comprehensive medicinal chemistry. Vol. 3. London: Pergamon Press; 1985. p. 1047-1099.
- van Muijlwijk-Koezen JE, Timmerman H, Link R, Van der Goot H, IJzerman AP. A novel class of adenosine A₃ receptor ligands. II. Structure affinity profile of a series of isoquinoline and quinazoline compounds. *J Med Chem.* 1998; 41:3994–4000. [PubMed: 9767637]
- von Lubitz DKJE, Lin RCS, Popik P, Carter MF, Jacobson KA. Adenosine A₃ receptor stimulation and cerebral ischemia. *Eur J Pharmacol.* 1994; 263:59–67. [PubMed: 7821362]
- von Lubitz DKJE, Lin RCS, Jacobson KA. Adenosine A₃ receptor antagonists and protection cerebral ischemic damage in gerbils. *Soc Neurosci Abstr* 745.16, 23. 1997:1924.
- Wolinsky J, Hauer HS. Substituted γ -pyrans. *J Org Chem.* 1969; 34:3169–3174.
- Wong WC, Chiu G, Wetzel JM, Marzabadi MR, Nagarathnam D, Wang D, Fang J, Miao SW, Hong X, Forray C, Vaysse PJ, Branchek TA, Gluchowski C, Tang R, Lepor H. Identification of a dihydropyridine as a potent α_1 adrenoceptor-selective antagonist that inhibits phenylephrine-induced contraction of the human prostate. *J Med Chem.* 1998; 41:2643–2650. [PubMed: 9651170]
- Xie R, Li A-H, Ji XD, Melman N, Olah ME, Stiles GL, Jacobson KA. Selective adenosine A₃ receptor agonists: water soluble 3,5-diacetylpyridinium salts and their oxidative generation from dihydropyridine precursors. *J Med Chem.* 1999; 42:4232–4238. [PubMed: 10514293]

Fig. 1.
 Structures of selective racemic (**1** and **2a**) and chiral (**2b**) 1,4-DHPs which act as potent and selective A₃ adenosine receptor antagonists.

Reagents: a) $\text{ZnCl}_2, \text{Ac}_2\text{O}, \text{AcOH}$; b) piperidine.

Fig. 2.

Synthesis of substituted pyran derivatives through condensation of three components, in a single reaction (**A**, to form symmetrically substituted pyrans) and by a stepwise reaction sequence (**B**).

TABLE 1

Yields and Analysis of 4H-Pyran and 1,4-Dihydropyridine Derivatives

No	Formula	Analysis	Yield (%)
3a	C ₁₉ H ₂₂ O ₅ .0.1C ₇ H ₈	C,H,N	6
3b	C ₁₉ H ₂₃ NO ₄	C,H,N	63
4a	C ₂₄ H ₂₄ O ₅	C,H,N	10
5a	C ₁₉ H ₂₁ NO ₇	HRMS *	7
6a	C ₂₄ H ₂₃ NO ₇	HRMS *	5
7a	C ₂₁ H ₂₄ O ₅ .0.15CDCl ₃	C,H,N,	24
8a	C ₂₁ H ₂₂ O ₅ .0.1CDCl ₃	C,H,N	7
8b	C ₂₁ H ₂₃ NO ₄	C,H,N	57
9a	C ₃₁ H ₂₆ O ₅	HRMS *	7

* Compounds were shown to be pure on analytical TLC (silica gel 60, 250 μm) with EtOAc-petroleum ether = 10:90 (v/v): Compound 5a: R_f = 0.36; Compound 6a: R_f = 0.38; Compound 9a: R_f = 0.29.

TABLE 2

Affinities of Pyran Derivatives and Corresponding DHPs in Radioligand Binding Assays at A₁, A_{2A}, and A₃ Receptors^{a-c}

R ₄	R ₅	R ₆	Pyran derivative compound	rA ₁ ^d	hA ₃ ^c	DHP derivative compound	rA ₁ ^d	hA ₃ ^d
Ph	CH ₂ CH ₃	CH ₃	3a MRS1704	21.9 ± 2.2	0.381	3b	1.49 ± 0.78	6.13 ± 1.75
Ph	CH ₂ CH ₃	Ph	4a MRS1705	14.2 ± 1.2	0.583 ± 0.164	4b ^d	8.03 ± 2.05	1.71 ± 0.36
3-NO ₂ -Ph	CH ₂ CH ₃	CH ₃	5a MRS1752	>100	2.27 ± 0.63	5b ^d	3.34 ± 2.17	2.51 ± 0.15
3-NO ₂ -Ph	CH ₂ Ph	CH ₃	6a MRS1753	>100	3.50 ± 2.20	6b	18.3 ± 1.9	1.59 ± 0.41
styryl	CH ₂ CH ₃	CH ₃	7a MRS1703	>100	1.83 ± 0.47	7b ^d	5.93 ± 0.27	0.108 ± 0.012
Ph-C≡C-	CH ₂ CH ₃	CH ₃	8a MRS1702	10.7 ± 2.8	1.86 ± 0.68	8b	>100	>10
Ph-C≡C-	CH ₂ Ph	Ph	9a MRS1751	>100	20.2 ± 8.3	9b ^d	40.1 ± 7.5	0.0314 ± 0.0028

^aDisplacement of specific [³H]R-PIA binding in rat brain membranes, expressed as K_i ± SEM in μM (n = 3–5)

^bDisplacement of specific [³H]CGS 21680 binding to A_{2A} receptors in rat striatal membranes. Compounds 3a–9a (pyran derivatives) and DHP 8b were all determined to have K_i ≥ 100 μM at rat A_{2A} receptors. Compound 3b had a K_i value of 20.9 ± 3.8 μM at rat A_{2A} receptors.

^cDisplacement of specific [¹²⁵I]AB-MECA binding at human A₃ receptors expressed in HEK cells, in membranes, expressed as K_i ± SEM in μM (n = 3–4).

^dValues from Jiang et al., 1998 (7b), 1997 (4b, 9b); van Rhee et al., 1996 (5b).