Communications to the Editor

Photoisomerization of a Potent and Selective Adenosine A_2 Antagonist, (*E*)-1,3-Dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine

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We have reported that 1,3,7-trialkylxanthine derivatives substituted with (*E*)-8-styryl groups act as selective A_2-antagonists in vitro and in vivo.[1] However, Jacobson et al. recently published that the degree of the A_2 selectivity of these agents might be in question.[2] Therefore, we studied potential reasons for the discrepancies with their results. Previous work indicated that photoisomerization about an ethylenic double bond is a general reaction.[3] Further, the photoinduced isomerization of the 8-styrylcaffeine derivative have been previously described.[3b] In this report we describe the result of photoisomerization (Scheme I) and binding affinity of (*E*)- and (*Z*)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (1 and 2).

Methanol solutions of 1 (KF 17837) were prepared and exposed to fluorescent lamp for varying periods. Sequential UV spectra of this solution changed very rapidly as shown in Figure 1. In contrast, no change in the UV spectrum was observed in the dark. Thus this process was dependent on photoillumination.[3] This reaction mixture was analyzed by HPLC and was found to contain 1 and a new product (retention times 7.4 and 5.5 min, respectively).[4] This product was obtained by purification on preparative HPLC in the dark.[5] Its molecular weight, determined by mass spectroscopy and elemental analysis, was identical to that of 1, thereby indicating that this product is an isomer of 1.[6] The chemical structure of the product was finally determined to be the *Z*-isomer (2) by NMR analysis (J_{10-11} = 12.7 Hz).[6]

Since dimethyl sulfoxide (DMSO) has been used in the binding assay for dissolving the compounds of limited aqueous solubility, we examined the photoliability of 1 and 2 in this solvent. Figure 2 shows the time courses of isomerization of the *E*- and *Z*-isomers in DMSO under photoillumination (fluorescent lamp, 1000 lx), respectively. Rates of photoisomerization are greatly dependent on the initial concentration of 1 or 2. At high concentration (10 mM) of substrate, photoisomerization was slow ($t_{1/2}$ = 27 h). Furthermore, crystalline 1 is stable under photoillumination. Thus we did not have any problem to obtain 1 in the usual synthetic procedures. However, at low concentration (0.1 mM), photoisomerization was very fast ($t_{1/2}$ = 0.5 h) and an equilibrium mixtures (82% *Z*-18% *E*) was eventually formed. This finding was substantiated by similarly exposing the *Z*-isomer (0.1 mM) and obtaining

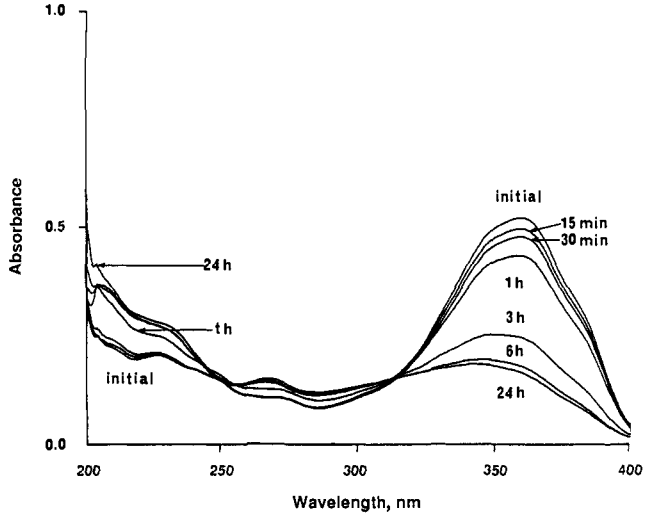
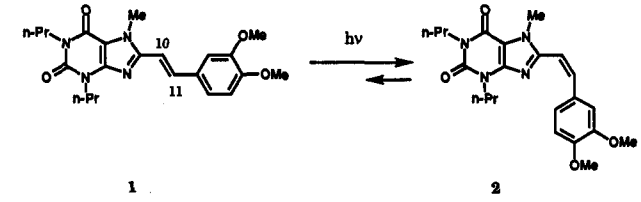


Figure 1. Sequential UV spectra of 1 in MeOH exposed to fluorescent lamp.

Scheme I



1 2

$$\log|CP - CP_\infty| \approx -(k_{obs}/2.303)t + \text{const} \quad (1)$$

the same equilibrium mixture. The change in peak areas due to the *E*- or *Z*-isomer as a function of time was subjected to pseudo-first-order analysis according to eq 1 and was shown in Figure 3. CP and CP_∞ are the percentages of the *E*-isomer at time *t* and at infinity where equilibrium mixture was obtained, respectively, and k_{obs} is the pseudo-first-order rate constant for the isomerization.[7] The k_{obs} values (0.1 mM) obtained from the decrease in the *E*-isomer (the increase in the *Z*-isomer) and from the increase in the *E*-isomer (the decrease in the *Z*-isomer) were 1.8 and 2.4 h^{-1}, respectively, and agreed approximately with each other. Further, almost the same behavior was noted with methanol as the solvent. Since we did not expect such fast photoisomerization, previous binding data for 1 could be derived from the *E*-*Z* equilibrium mixture 3. Most of our compounds are stored in DMSO solution at 0 °C for several weeks, and the binding assays are usually performed under light. Therefore, potential reason for the discrepancies with Jacobson's results[2] in the binding assay might be derived from differences in the degree of photoisomerization of 1.

The potency of these *E*- and *Z*-isomers and their equilibrium mixture (1, 2, and 3) at adenosine A_1 and A_2 receptors was determined by standard radioligand binding procedures. Adenosine A_1 binding was performed with N^6-[^{3}H]cyclohexyladenosine binding in rat forebrain membranes.[8] Adenosine A_2 receptors are further divided to A_{2a}- and A_{2b}-subtypes based on pharmacological and biochemical criteria.[9] A low-affinity A_2 site (A_{2b}) is widely distributed in the brain. In contrast, a high affinity A_2

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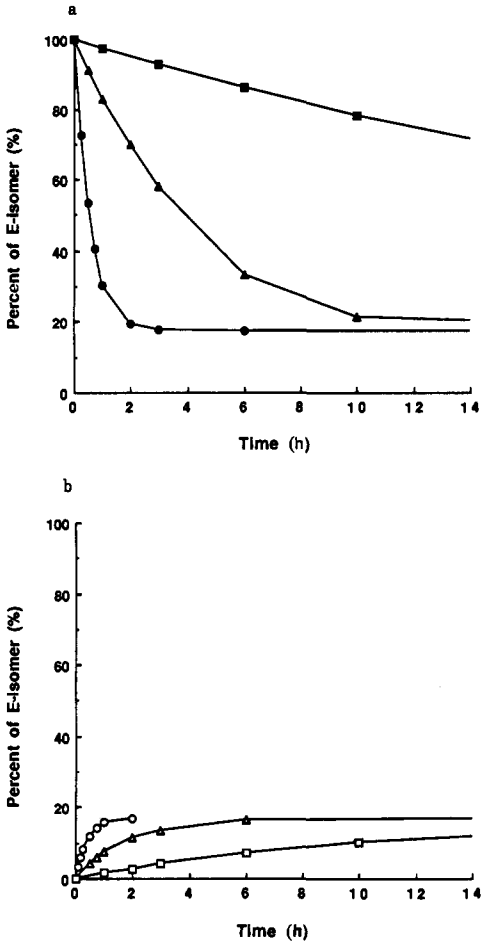

Figure 2. Time courses of the E–Z isomerization of (a) 1 (E-isomer) [(●) 0.1 mM; (▲) 1.0 mM; (■) 10.0 mM] and (b) 2 (Z-isomer) [(○) 0.1 mM; (△) 1.0 mM; (□) 10.0 mM] in DMSO. Experimental conditions: (1) solvent, DMSO; (2) container, colorless glass vial of 5 mL volume; (3) light source, fluorescent lamp, 100-W white light, type FLR110H. W/A/100, Toshiba Electric Co., Ltd., Tokyo; (4) illumination, 1000 lx (normal fluorescent lighting in usual laboratories); (5) temperature, room temperature; (5) determination, HPLC method. The values of the half-life ($t_{1/2}$) of 1 at 0.1, 1.0, and 10.0 mM were 0.5, 3.6, and 27 h, respectively.

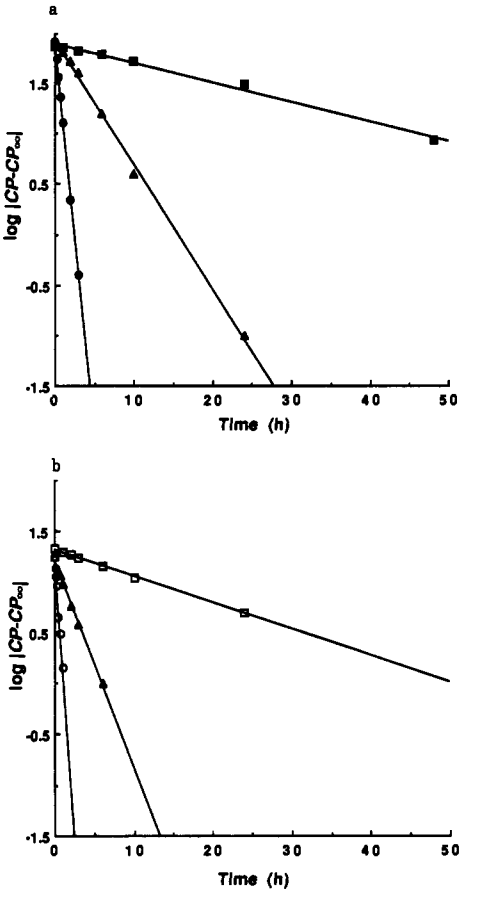

Figure 3. First-order plots of the E–Z isomerization of (a) 1 (E-isomer) [(●) 0.1 mM; (▲) 1.0 mM; (■) 10.0 mM) and (b) 2 (Z-isomer) [(○) 0.1 mM; (△) 1.0 mM; (□) 10.0 mM] in DMSO. $\log|CP - CP_\infty| = -(k_{obs}/2.303)t$ + const where CP and CP_∞ are the concentrations of compounds at time t and infinity where equilibrium mixture was obtained, respectively. Equilibrium mixture was usually obtained after 24–120 h. The values of pseudo-first-order rate constants for the isomerization (k_{obs}) of 1 at 0.1, 1.0, and 10.0 mM were 1.8, 0.28 and 0.045, respectively. Those of 2 at 0.1, 1.0, and 10.0 mM were 2.4, 0.47, and 0.060, respectively.

site (A_{2a}) is exclusively localized in the brain regions such as striatum, olfactory tubercle, and nucleus accumbens. The pharmacological profile of adenosine related agents in competing for the binding of [^{3}H]NECA (+ 50 nM N-cyclopentyladenosine) to striatal membranes has been found to be consistent with an interaction at high-affinity A_2 receptors (A_{2a}) in a variety of mammalian species.[9,10] We used this procedure for examining the so-called high-affinity A_2 receptors (A_{2a}) in the previous study. However recent studies suggest that [^{3}H]NECA binds not only to sites showing either A_2- or A_1-receptor characteristics, but also to sites that do not represent any known receptor.[11,12] Thus A_2 receptor binding was also performed with [^{3}H]-4-[2-[[6-amino-9-(N-ethyl-β-D-ribofuranuronamidosyl)-9H-purin-2-yl]amino]ethyl]benzenepropanoic acid (CGS 21680) in rat striatal membranes. CGS 21680 has been reported to be an A_{2a}-selective agonist.[13] All procedures in the binding assay were done in the dark in order to avoid photoisomerization. Compounds 1, 2, and 3 were dissolved in DMSO and the final concentration of DMSO in the assay was 0.9%.[14] These compounds were apparently soluble in our assay system. The results are listed in Table I.

Table I shows that the E-isomer (1) possesses high affinity at the A_{2a} receptor (K_i = 1.0 nM) and resulted in high A_2 selectivity (62-fold). On the other hand, the Z-isomer (2) eliminated or dramatically decreased affinity at the A_1 or A_{2a} receptor, respectively (K_i > 10 μM, K_i = 860 nM). The affinity and selectivity of the equilibrium mixture (3) (K_i = 390 ± 68 nM for A_1; K_i = 7.9 ± 0.055 nM for A2; K_i ratio of A_1/A_2 = 49) were almost the same as those of 1 in the previous study (K_i = 430 ± 150 nM for A_1; K_i = 7.8 nM for A_2; K_i ratio of A_1/A_2 = 55).[1] Thus the A_1 and A_2 affinity of 1 in the previous study was confirmed to be that of the equilibrium mixture (E–Z) which is still a significantly potent and selective adenosine A_2 antagonist. Further, the binding affinity of 1 determined with [^{3}H]CGS 21680 was consistent with the affinity determined with [^{3}H]NECA as ragioligand. Thus inhibition of NECA binding (+50 nM CPA) could be correlated with that of [^{3}H]CGS 21680 binding in 8-styrylxanthines and might be still useful for examining A_{2a} receptors.

Compound 1 (KF 17837) was previously proved to be a selective adenosine A_2 antagonist in vivo (oral administration).[1] As described above, photoisomerization of 1 easily occurred in DMSO solution. Thus we examined the possibility of the E–Z isomerization in the animal body and further examined whether this in vivo antagonism

Table I. A_1 and A_2 Adenosine Receptor Binding of 1,3-Dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthines

compd	K_i,[a] nM		K_i ratio
	A_1	A_2	A_1/A_2
1	62 ± 11	1.0 ± 0.057	62
2	>10000	860 ± 120	>12
3 (equilibrium mixture)	390 ± 68	7.9 ± 0.055	49
	430 ± 150[b]	7.8 ± 2.7[c]	55
	1500 ± 780[d]		190

[a] A_1 binding was carried out with N^6-[^{3}H]cyclohexyladenosine in rat forebrain membranes as described,[16] and A_2 binding was carried out with [^{3}H]CGS 21680 in rat striatal membranes by a modification of the method described by Bruns.[9] All procedures were done in the dark. Concentration–inhibition curves were carried out in duplicate with five or more concentrations of each test agent, and IC_{50} values were calculated from computerization of logit log curve. IC_{50} values were converted to K_i values as described.[17] The assays were carried out three or more times, and standard errors (SEM) are given in the table. Xanthines were dissolved in DMSO, and the final concentration of DMSo in the assay was 0.9%.[8] [b] A_1 binding was carried out with N^6-[^{3}H]cyclohexyladenosine in rat forebrain membranes under usual light as described.[8,16] [c] A_2 binding was carried out with N-[^{3}H]ethyladenosin-5′-uronamide in the presence of 50 nM cyclopentyladenosine in rat striatal membranes under usual light.[9] [d] A_1 binding was carried out with N^6-[^{3}H]cyclohexyladenosine in guinea pig forebrain membranes under usual light.[8]

might be realized by 1 or the equilibrium mixture 3. No photoisomerization of 1 was observed in 0.3% Tween 80 suspension that was used in oral administration, presumably due to its low water solubility (0.06 μg/mL). Plasma and brain concentrations of 1 and 2 were measured using HPLC 4 h after oral administration of 1 at a dose of 30 mg/kg in rats.[15] Plasma and brain concentrations of 1 were 0.065 μg/mL and 0.076 μg/g brain, respectively. None of the *Z*-isomer (2) was detected in plasma and brain. Although these concentrations of 1 did not show its good oral bioavailability, they are sufficient to fully antagonize adenosine receptors in the heart and the CNS. Many xanthines are known to have a short half-life (<1 h) in vivo, but compound 1 had a long half-life ($t_{1/2}$ = 5.17 h) that might be useful for in vivo experiments. Oral administration of 1 at a dose of 10 mg/kg inhibited NECA-induced decrease in blood pressure and CGS-21680-induced locomotor depression (intercerebroventricular injection). Detailed studies will be published elsewhere.

The most significant finding in this study is that the *E*-isomer 1 possesses 800-fold higher affinity at the A_2 receptor than its *Z*-isomer and may be a useful pharmacological probe in vivo for elucidating the physiological and pathophysiological roles of the A_2 receptor.[18]

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(4) Column: YMC-Pack ODS-A, 5 μm, 6 mm i.d. × 150 mm; mobile phase: CH_3CN–water = 7:3; flow rate: 1.0 mL/min; detection: ν = 246 nm.

(5) Column: YMC-Pack ODS-A, 10 μm, 30 mm i.d. × 500 mm; mobile phase: CH_3CN–water = 7:3; flow rate: 90 mL/min; detection: ν = 246 nm.

(6) 1: mp 165–166 °C (2-PrOH/H_2O); IR (KBr) 1692, 1657 cm^{-1}; MS *m/z* 412; ^{1}H NMR (DMSO-d_6) δ 7.60 (1 H, d, *J* = 15.8 Hz), 7.40 (1 H, d, *J* = 2.0 Hz), 7.28 (1 H, dd, *J* = 2.0, 8.4 Hz), 7.18 (1 H, d, *J* = 15.8 Hz), 6.99 (1 H, d, *J* = 8.4 Hz), 4.02 (3 H, s), 3.99 (2 H, t, *J* = 7.2 Hz), 3.90–3.80 (2 H, m), 3.85 (3 H, s), 3.80 (3 H, s), 1.85–1.50 (4 H, m), 1.00–0.86 (6 H, m). Anal. Calcd for $C_{22}H_{28}N_4O_4$: C, 64.06; H, 6.84; N, 13.58. Found: C, 64.06; H, 6.82; N, 13.80. **2**: mp 126.9–127.2 °C (CH_3CN/H_2O); IR (KBr) 1696, 1654, 1542, 1521 cm^{-1}; MS *m/z* 412; ^{1}H NMR (DMSO-d_6) δ 7.28 (1 H, d, *J* = 8.4 Hz), 7.20 (1 H, s), 6.94 (1 H, d, *J* = 12.7 Hz), 6.39 (1 H, d, *J* = 12.7 Hz), 3.93 (2 H, t, *J* = 7.4 Hz), 3.84 (2 H, t, *J* = 6.9 Hz), 3.77 (6 H, s), 1.75–1.50 (4 H, m), 0.86 (3 H, t, *J* = 7.4 Hz), 0.85 (3 H, t, *J* = 7.4 Hz). Anal. Calcd for $C_{22}H_{28}N_4O_4$: C, 64.06; H, 6.84; N, 13.58. Found: C, 64.12; H, 7.09; N, 13.54.

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(14) Compound 1 was stirred in the solvent (20 mL) at 20 °C. Then the supernatant was filtered, diluted with acetonitrile, and analyzed by HPLC. Although compound 1 is sparingly soluble to water (0.06 μg/mL), it is more soluble to 0.5% Tween 80 and DMSO (4.2 and 6600 μg/mL, respectively). Thus we believe that addition of DMSO to medium (0.9%) increased the solubility of compound 1.

(15) Compounds 1 and 2 in plasma and brain homogenates were extracted with CH_3CN. Supernatants were analyzed by HPLC. Column: YMC-Pack ODS AM-312, 5 μm, 6 mm i.d. × 150 mm; mobile phase: 1% acetic acid–CH_3CN = 25:75 (10 mM $NaClO_4$); flow rate: 1 mL/min; detection: ν = 360 nm. Retention times of 1 and 2 were 7.6 and 6.0 min, respectively.

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