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**THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF A HIGHLY SELECTIVE  
ADENOSINE A<sub>2a</sub> RECEPTOR AGONIST**

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**Abstract:** Three novel nucleosides **1**, **2**, and **3** were prepared that contained side chains at the 2-position of adenosine. Compound **1** was shown to be the most selective A<sub>2a</sub> receptor agonist reported to date having an A<sub>1</sub>/A<sub>2</sub> ratio of 2400. In addition, compound **1** was shown to reduce blood pressure in rats and dogs with only minimal effects on heart rate.

Adenosine effects changes in cAMP production in smooth muscle and cardiac tissue through its binding to G protein-coupled adenosine receptors.<sup>1-8</sup> The binding of adenosine agonists to A<sub>1</sub> and A<sub>2a</sub> adenosine receptors cause inhibition or stimulation of adenylyl cyclase, respectively. Stimulation of A<sub>1</sub> receptors is thought to mediate the dromo, chrono and inotropic effects of adenosine in cardiac tissue, while stimulation of the A<sub>2a</sub> receptor is believed to mediate its vasodilatory effects.<sup>3</sup> In the present study we will discuss the synthesis of a series of new A<sub>2a</sub> receptor agonists (**1**, **2**, and **3**; Figure 1) and their *in vitro* activities. In addition, the *in vivo* cardiovascular effects of compound **1** in rats and dogs will be presented.

### **Chemistry**

Compounds **1**, **2** and **3** were prepared by the two methods shown in Schemes 1 and 2. Compound **1** was prepared by displacement of the 2-chloro group of compound **6** using R-amphetamine (Scheme 1). This reaction requires relatively high temperatures (130-140

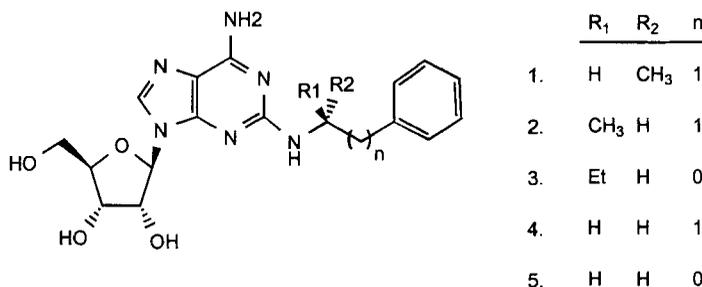


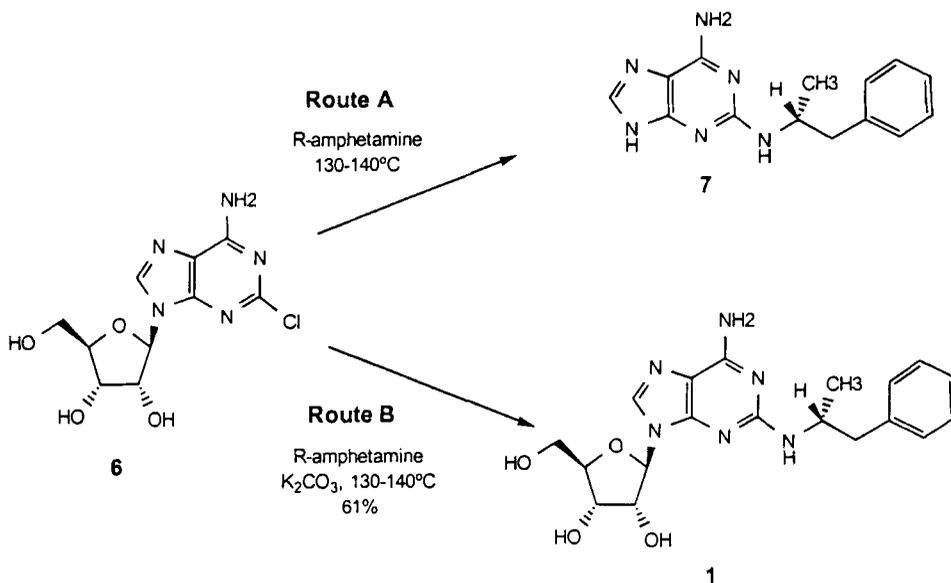
Figure 1

°C). The displacement also evolves hydrochloric acid, which is trapped as the amine salt, and at these elevated reaction temperatures causes a significant amount of aglycone **7** to form (Scheme 1, Route A). To inhibit aglycon formation, finely ground potassium carbonate was added to the reaction mixture whereby compound **1** was obtained and only a trace amount of aglycon **7** was detected (Scheme 2, Route B). The side chain S-isomer of **1** (compound **2**) and the related isomeric compound **3** were obtained according to Scheme 2 starting with compound **6**, for which compounds the vicinal diols were first protected as an acetonide (compound **8**) using 2,2-dimethoxypropane and to p-toluenesulfonic acid in dimethylformamide. The acetonide **8** was then treated with D-amphetamine or (R)-1-phenylpropylamine to give compounds **9** and **10**, respectively. Removal of the acetonide group of compound **9** was accomplished with 1M hydrochloric acid to give compound **2** in good yield. Aqueous trifluoroacetic acid was used for the analogous transformation of **10** to **3**.

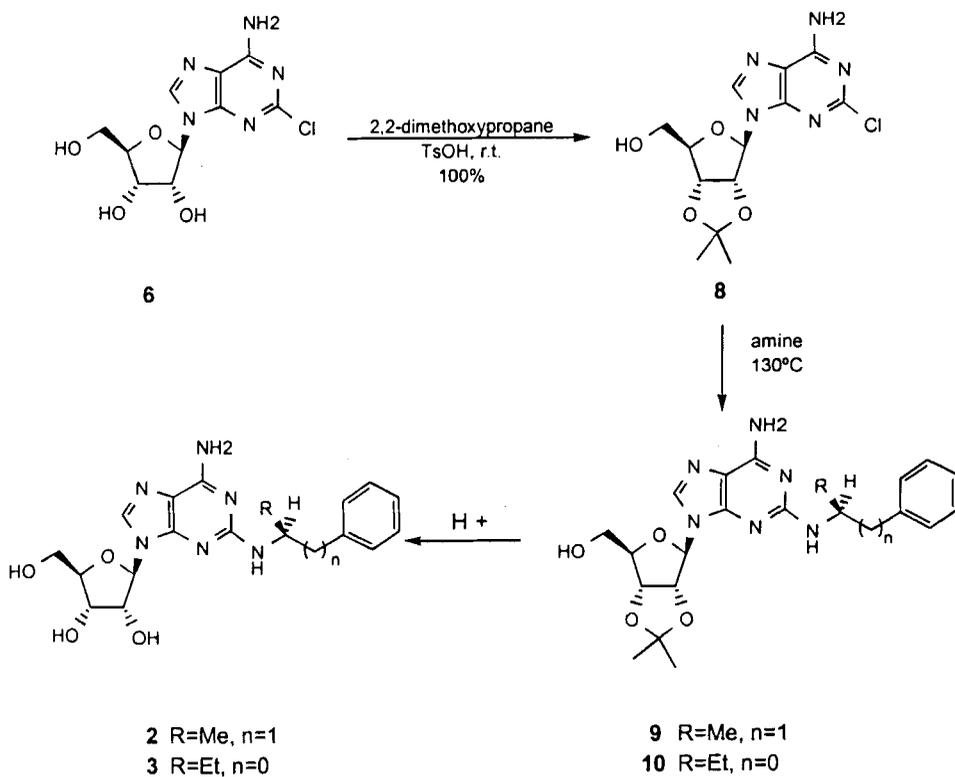
## Results

**In vitro receptor data:** Compounds **1**, **2** and **3** were evaluated for their ability to displace [<sup>3</sup>H]N<sub>6</sub>-cyclohexyladenosine from A<sub>1</sub> receptors and [<sup>3</sup>H]NECA from A<sub>2a</sub> receptors using rat brain membrane preparations.<sup>9,10</sup> Shown in Table 1 are the binding affinities expressed as K<sub>i</sub> values and the selectivity ratio for the A<sub>1</sub> and A<sub>2a</sub> receptors. Compound **1** was shown to be highly selective as an agonist for the A<sub>2a</sub> receptor with a selectivity ratio of 2400.

**In vitro heart rate effects:** The direct effect of compound **1** on heart rate was



Scheme 1



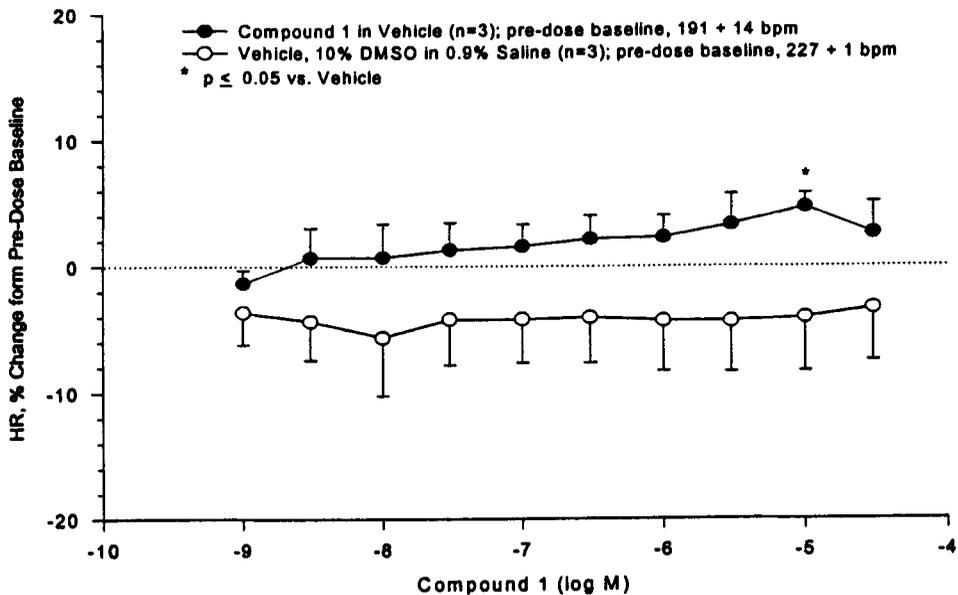
Scheme 2

Table 1

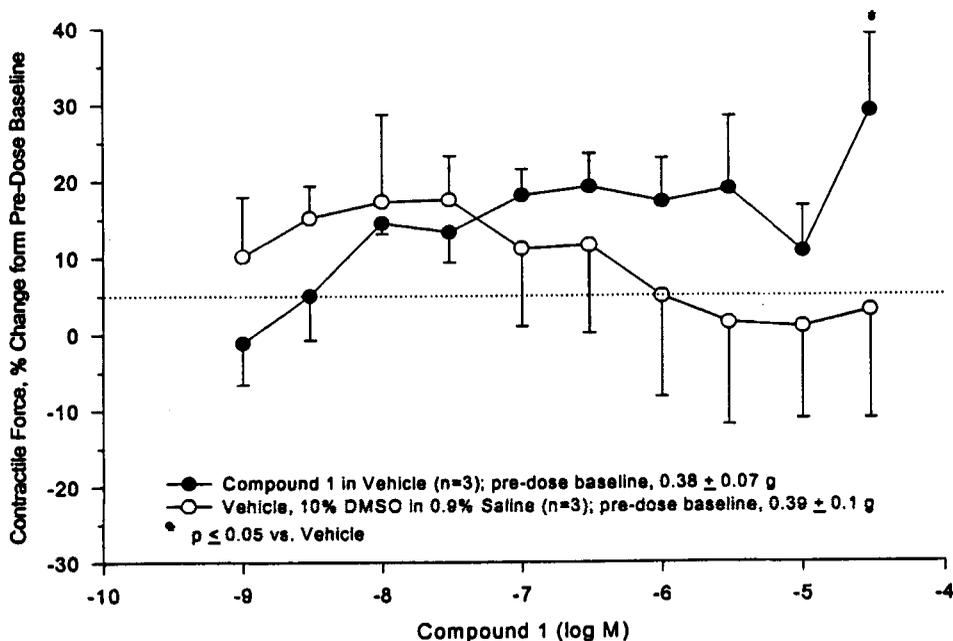
<u>Compound</u>	<u>A<sub>1</sub> Receptor Ki (nM)</u>	<u>A<sub>2a</sub> Receptor Ki (nM)</u>	<u>A<sub>1</sub>/A<sub>2a</sub> Selectivity</u>
1	8,400	3.5	2400
2	5,800	158	37
3	>10	>10	nd
4	977	68	14 <sup>11</sup>
5	10,471	5,888	1.8 <sup>11</sup>

determined *in vitro* using spontaneously beating isolated guinea pig atrial pairs. Pre-dose baseline values for heart rate and contractile force for the treatment group was not significantly different from those for the vehicle group ( $p > 0.05$ , unpaired t-test). When compared to the corresponding vehicle effect, HR was slightly, but significantly, increased ( $p \leq 0.05$ , two-way repeated measures ANOVA) in the presence of only one concentration, 10  $\mu\text{M}$ , of compound 1 (Figure 2). As shown in Figure 3, contractile force was significantly increased ( $p \leq 0.05$ , two-way repeated measures ANOVA) in the presence of only one concentration, 30  $\mu\text{M}$ , of compound 1.

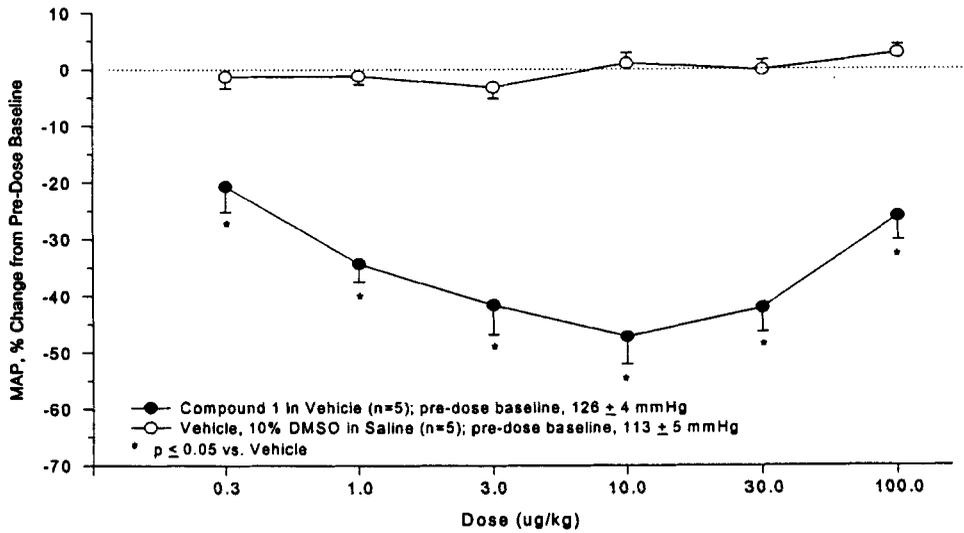
***In vivo* mean arterial pressure and heart rate effects in anesthetized normotensive and spontaneously hypertensive rats:** The effect of compound 1 on mean arterial pressure (MAP) and heart rate (HR) was evaluated *in vivo* in anesthetized normotensive and spontaneously hypertensive (SHR) rats. In the normotensive rats, baseline MAP and HR values prior to the first dose were not significantly different between the vehicle and treatment groups ( $p > 0.05$ , unpaired t-test). Increasing doses of compound 1 administered intravenously decreased MAP 21%, 34%, 42%, 47%, 42%, and 26%, respectively (Figure 4). All of these decreases were significantly different ( $p \leq 0.05$ , two-way, repeated measures ANOVA) from the corresponding changes following vehicle administration. Forty minutes after the fourth dose and 45 min after the fifth dose of compound 1, MAP, although at a new stable plateau, had not completely recovered to the



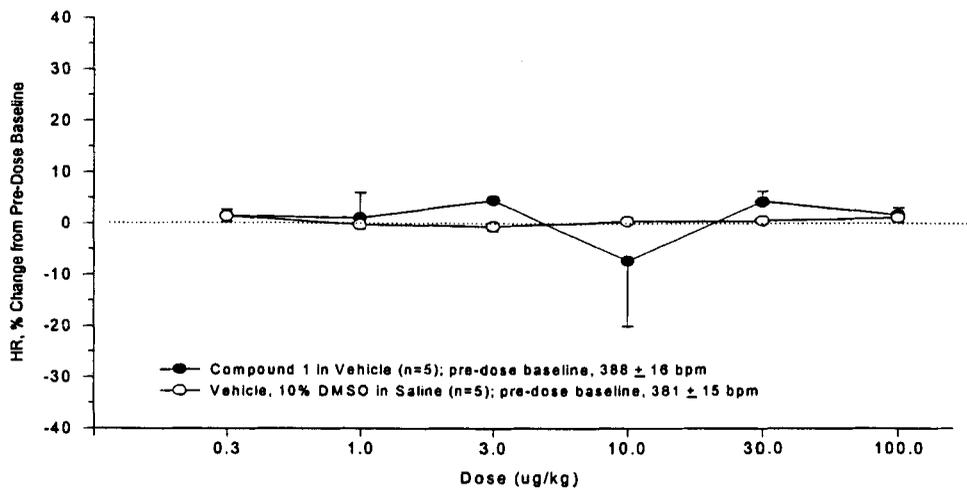
**Figure 2**  
 Effect of Compound 1 on Heart Rate (HR) in Isolated Guinea Pig Atrial Pairs



**Figure 3**  
 Effect of Compound 1 on Contractile Force in Isolated Guinea Pig Atrial Pairs



**Figure 4**  
Effect of Intravenous Compound 1 on Mean Arterial Pressure (MAP) in Anesthetized Normotensive Rats



**Figure 5**  
Effect of Intravenous Compound 1 on Heart Rate (HR) in Anesthetized Normotensive Rats

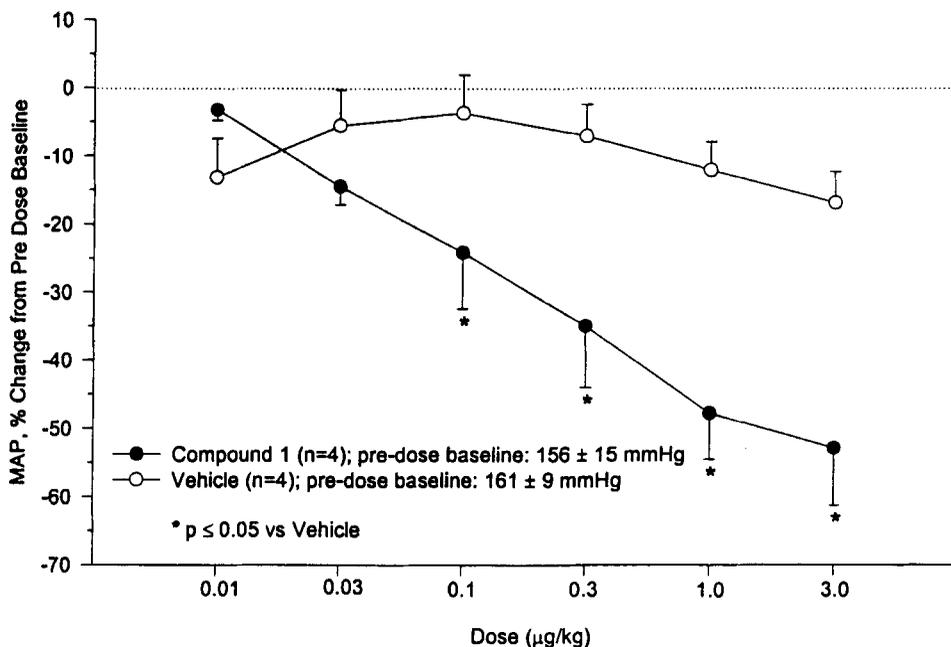


Figure 6

**Effect of Intravenous Compound 1 on Mean Arterial Pressure (MAP) in Anesthetized Spontaneously Hypertensive Rats**

respective pre-dose baseline. When compared to the corresponding changes following vehicle administration, compound 1 had very little, if any, effect on HR (Figure 5;  $p > 0.05$ , two-way, repeated measures ANOVA).

In the SHR, prior to the first dose, baseline MAP and HR values were nearly identical for the vehicle and treatment groups. When escalating doses of compound 1 were administered intravenously, MAP decreased 3%, 13%, 23%, 34%, 47% and 52%, respectively (Figure 6). The 23%, 34%, 47% and 52% decreases were significantly different than the corresponding changes following vehicle administration. Recovery of MAP was incomplete between the third, fourth, and fifth doses of compound 1. The first three doses of compound 1 had little or no effect on HR but the three highest doses increased HR 8%, 14% and 23%, respectively (Figure 7). Recovery of HR to pre-dose levels was incomplete between the third, fourth, and fifth doses of compound 1.

***In vivo* mean arterial pressure and heart rate effects in the anesthetized and conscious beagle dog:** The effect of compound 1 on mean arterial pressure (MAP) and

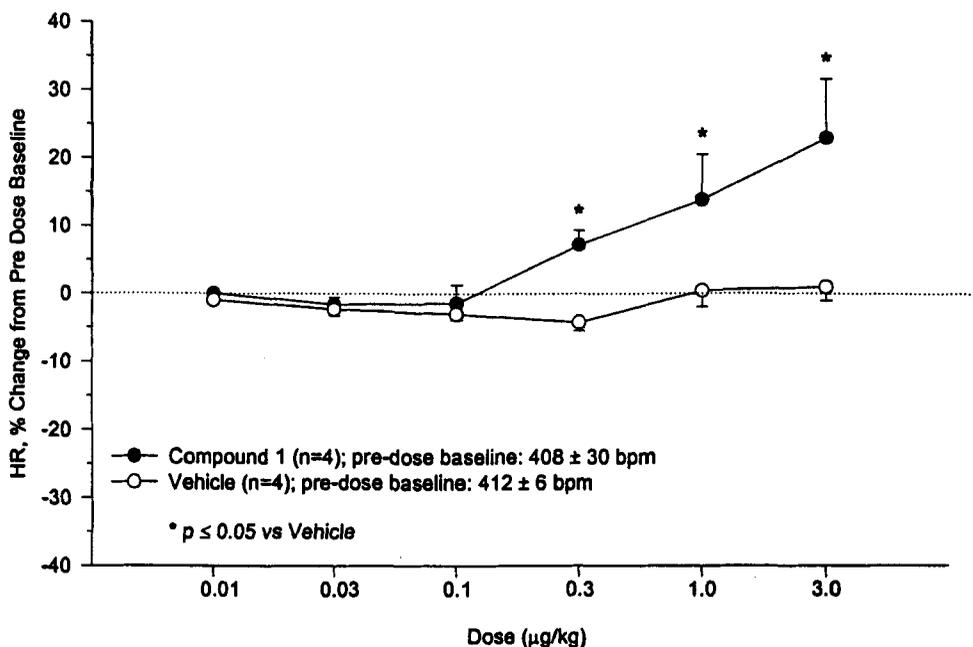


Figure 7

**Effect of Intravenous Compound 1 on Heart Rate (HR) in Anesthetized Spontaneously Hypertensive Rats**

heart rate (HR) was evaluated in anesthetized and conscious normotensive beagle dogs as follows:

**Anesthetized dogs; 0.1 mg/kg, iv:** Two anesthetized dogs received *iv* vehicle and 5 min later 0.1 mg/kg of compound 1. As shown in Figure 8, two min after compound 1 was administered MAP decreased 36%, recovered slightly and remained about 31% below pre-dose baseline for the remainder of the experiment. Heart rate responded in a reciprocal fashion (Figure 8). Two min after compound 1 was administered HR increased 20% and remained 15 to 23% above pre-dose baseline for the remainder of the experiment.

**Anesthetized dog; 3 x 0.1 mg/kg, iv:** A third dog received three, 0.1 mg/kg doses of compound 1 at 20 min intervals. As shown in Figure 9, the first 0.1 mg/kg dose decreased MAP 57% below pre-dose baseline 2 min after dosing. MAP recovered

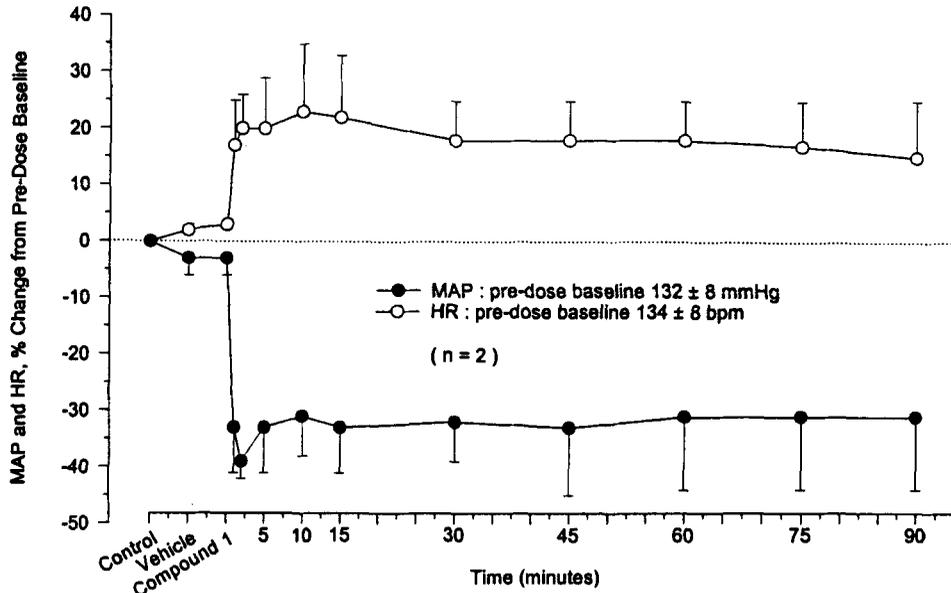


Figure 8

**Effect of Intravenous Compound 1 (0.1 mg/kg) on Mean Arterial Pressure and Heart Rate in Anesthetized Dogs**

slightly during the next 15 min, decreased slightly after the second dose of compound 1 and then remained 43 to 45% less than baseline during the remainder of the experiment. Compound 1 increased HR 15% above baseline 2 min after dosing (Figure 9). It declined to 8% above baseline at 10 min and 6-8% for the remainder of the study.

Conscious dog; 0.1 mg/kg, iv: One dog was dosed *iv* with 2 ml of vehicle (50% DMSO/50 % 0.9% sodium chloride) and the next day with vehicle containing 0.1 mg/kg of compound 1. As shown in Figure 10, MAP decreased 62% below pre-dose baseline the first hour after administration of compound 1 while it increased only 3% after vehicle administration. It remained 59-65% below baseline for the next 3 hours, waned to 28-37% below baseline during the fifth through seventh hours and returned to vehicle control values 8 hours after dosing. HR responded in a reciprocal fashion (Figure 10). It increased 89% above pre-dose baseline the first hour after compound 1 was administered and 22% after vehicle administration. HR increased to 96 to 110% above baseline during the next 3 hours after compound 1 was administered, waned to 40-58% above baseline

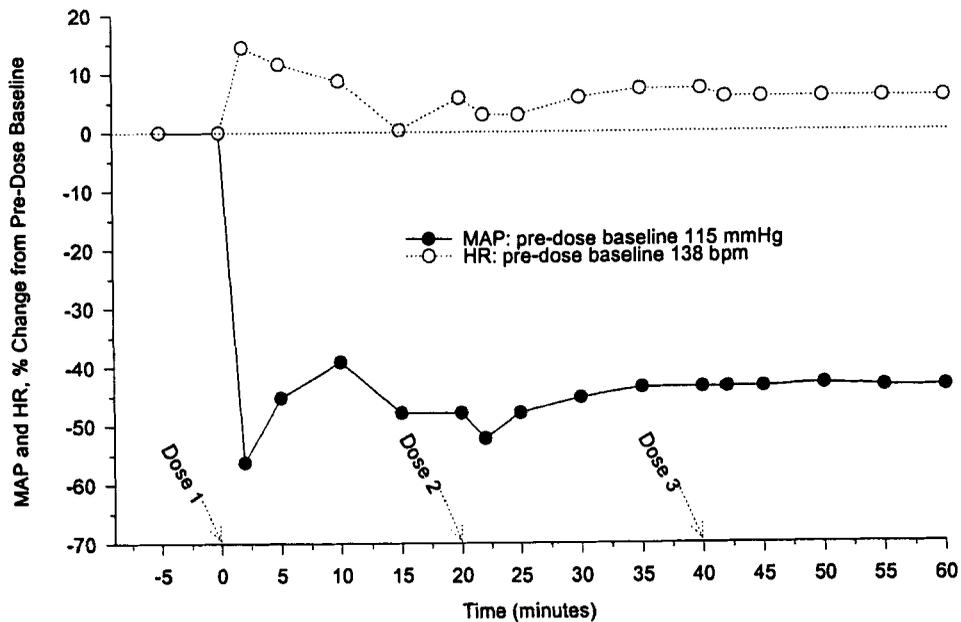


Figure 9

Effect of Intravenous Compound 1 (0.1 mg/kg x 3) on Mean Arterial Pressure and Heart Rate in Anesthetized Dog

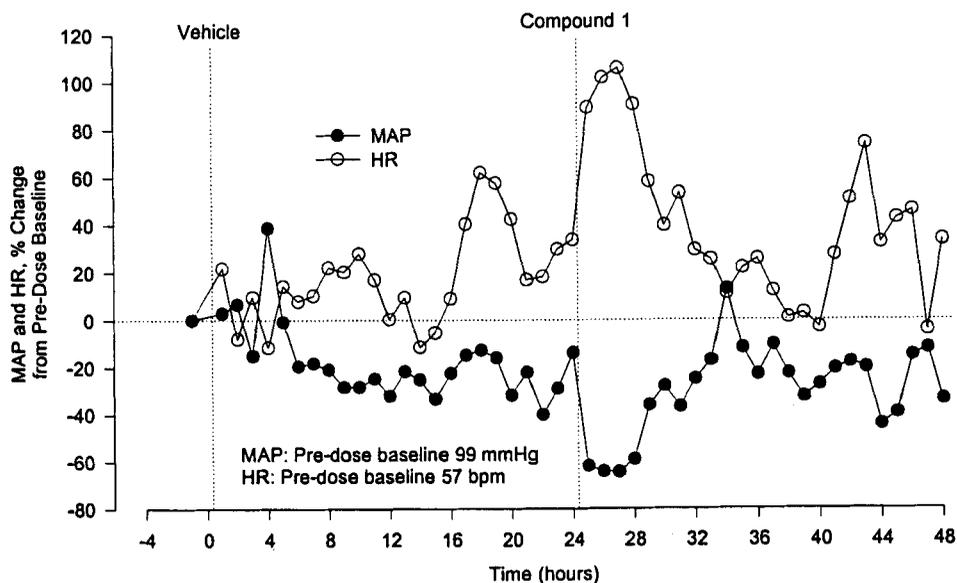


Figure 10

Effect of Intravenous Compound 1, 0.1 mg/kg, on Mean Arterial Pressure and Heart Rate in a Conscious, Normotensive Beagle

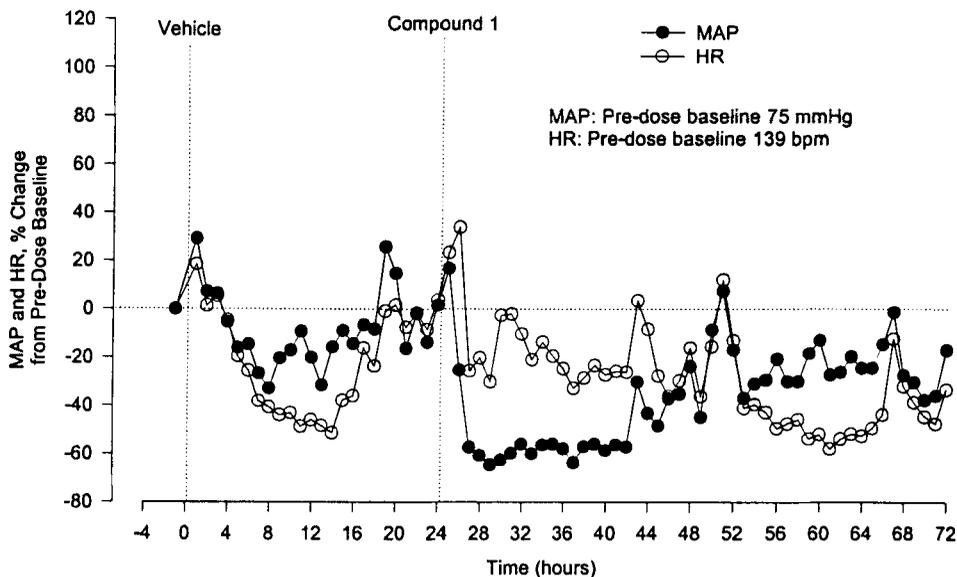


Figure 11

**Effect of Oral Compound 1, 1.0 mg/kg, on Mean Arterial Pressure and Heart Rate in a Conscious, Normotensive Beagle**

for the fifth through the seventh hours and returned to vehicle control values 8 hours after dosing.

Conscious dog; 1.0 mg/kg, oral: Another dog was dosed with an empty gelatin capsule and the next day with a capsule containing 1 mg/kg of compound 1. As shown in Figure 11, the third hour MAP decreased 57% below pre-dose baseline after compound 1 was administered and increased only 6% above baseline after vehicle administration. During the next 21 hours MAP averaged 53% less than baseline following compound 1 and 11% less after vehicle. The twentyfifth through the fortyeighth hour after dosing, MAP averaged 23% below baseline following compound 1 and 8% below after vehicle. HR (Figure 11) increased 34% above pre-dose baseline the second hour following compound 1; it increased only 1% after vehicle. Over the next 22 hours HR averaged 20% less than baseline following compound 1 and 24% less after vehicle. The 25th through 48th hour after dosing HR averaged 39% less than baseline following compound 1 and 21% less after vehicle.

## Discussion

Compound **1** was shown to be highly selective as an agonist for the  $A_{2a}$  receptor with a selectivity ratio of 2400. Thus, **1** is the most selective  $A_{2a}$  agonist reported to date with respect to  $A_1$  affinity. Compound **2** is a diastereomer of compound **1** where the methyl group of the amphetamine side-chain is changed from the R-configuration to the S-configuration. Compound **2** had dramatically reduced selectivity for the  $A_{2a}$  receptor, being only two-fold more selective than the non-chiral normethyl derivative **4**.<sup>11</sup> The chiral (R)-1-phenylpropyl analog **3** was devoid of activity for either  $A_1$  or  $A_{2a}$  receptors. This was not totally unexpected, since compound **5** is known not to possess high affinity for  $A_1$  or  $A_{2a}$  receptors.<sup>11</sup> Francis, et al. have shown that a number of similar analogs possess selectivity ratios ranging from 1-530 and based on this body of data it appears that the (R)-amphetamine side chain at the 2-position of adenosine is the optimal substituent for binding at the  $A_{2a}$  receptor.<sup>11</sup> The adenosine  $A_{2a}$  receptor appears to be very sensitive to chiral substitution at the  $\alpha$ -position from the nitrogen on the 2-substituent. Francis, et al. have shown when chiral substituents were placed in the  $\beta$ -position, minimal selectivity was achieved.<sup>11</sup>

Ideally, a selective  $A_{2a}$  agonist would be an effective antihypertensive agent, possessing vasodilatory, hypotensive actions, without the negative cardiac effects associated with  $A_1$  receptor activation. Indeed, a putative selective  $A_{2a}$  adenosine receptor agonist has been shown to cause a dose-dependent reduction in systolic blood pressure with minimal reflex tachycardia in conscious spontaneously hypertensive rats.<sup>12</sup> Alternatively, the hypotensive effect of  $A_{2a}$  agonists may be accompanied by a marked, sustained reflex tachycardia.<sup>3,13</sup> Compound **1**, a proposed selective adenosine  $A_{2a}$  receptor agonist, did not decrease HR or contractile force of isolated guinea pig atria at any of the doses studied; in fact, there was a small increase in HR (less than 5% following 10  $\mu$ M) and an increase in contractile force (less than 30% following 30  $\mu$ M). The observation that compound **1** did not decrease HR and contractile force, as would be expected with an  $A_1$  receptor agonist, suggests that it has little, if any, functional  $A_1$  agonist activity in isolated guinea pig atria and is in agreement with the receptor binding data showing that it has minimal  $A_1$  receptor affinity.

In anesthetized normotensive rats, *iv* administration of 0.3-10  $\mu$ g/kg of compound **1** produced dose-dependent decreases in blood pressure with little effect on HR.

Following administration of 30 and 100 ug/kg, the hypotensive effect, although significantly different from the corresponding vehicle effect, was not dose-related. The reason for this attenuated hypotensive effect is not clear, but may be due to the observation that after the preceding dose, MAP, although at a stable plateau, did not completely return to the pre-dose baseline. In the anesthetized spontaneously hypertensive rat, six doses of compound 1 produced dose-dependent decreases in MAP while only the top three doses produced modest increases in heart rate. Compound 1 is a potent hypotensive agent in the anesthetized dog as well, with heart rate effects being similar to those observed in the anesthetized spontaneously hypertensive rat. In the conscious dog dosed intravenously with 0.1 mg/kg of compound 1, there was a hypotensive effect of 7 hr duration but a tachycardic effect greater than that in the anesthetized dog was observed. In the conscious dog dosed with a ten-fold larger dose of compound 1, 1.0 mg/kg, there was a profound depressor effect that lasted more than 24 hr but HR exceeded baseline value at only the first and second hr after dosing. When these values were compared to the corresponding vehicle values there was a real increase at only the second hour.

In summary, it appears that compound 1 can lower blood pressure in conscious and anesthetized animals without resulting in serious tachycardia. Although an increase in HR is presumably a reflex-mediated response due to a fall in blood pressure, other mechanisms, including modulation of platelet, renal and central neural function, may contribute to the homeostatic responses consequent to blood pressure lowering.<sup>14</sup> When considered together, these results indicate that the pharmacologic profile of compound 1 is consistent with that for a selective adenosine A<sub>2a</sub> receptor agonist.

## Experimental

**(R)-2-[(Phenylisopropyl)amino]adenosine (1):** 2-Chloroadenosine (6, 2.0 g, 6.6 mmol) K<sub>2</sub>CO<sub>3</sub> (finely ground, 0.5 g, 3.5 mmol) were heated to 130-140 °C in (R)-amphetamine (6.0 g, 44.1 mmol) for 8 h in a sealed tube. The reaction was cooled to room temperature and the mixture was dissolved in 10% NaOH and extracted with Et<sub>2</sub>O. The ether layer was extracted with aq NaOH (2x25 mL) and the combined aqueous layers were filtered and concentrated *in vacuo*. The residue was extracted with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (4:1) and

concentrated *in vacuo*. The product was purified by flash chromatography using CH<sub>2</sub>Cl<sub>2</sub>/MeOH (17:3) to give **1** (1.62 g, 61%), mp 96 °C (effervescence); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub> + D<sub>2</sub>O) δ 7.89 (s, 1H), 7.24 (d, 4H, J=4.4 Hz), 7.16 (m, 1H), 5.72 (d, 1H, J=6.25 Hz), 4.57 (m, 1H), 4.10 (m, 2H), 3.88 (m, 1H), 3.61 (m, 1H), 3.45 (m, 1H), 2.95 (m, 1H), 2.51 (m, 1H), 1.05 (d, 3H, J=6.4Hz); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) δ 158.6, 155.6, 151.5, 139.8, 136.3, 129.2, 128.0, 113.5, 87.0, 72.9, 70.6, 61.7, 47.9, 42.1, 20.1; [α]<sub>D</sub><sup>20</sup> = -35.8° (c=1.00, 0.1% HCl); MS (CI/CH<sub>4</sub>) m/z 401 (M<sup>+</sup>).

**(S)-2-[Phenylisopropyl]amino]adenine Dihydrochloride (2):** A stirred solution of compound **8** (0.64 g, 1.9 mmol) in (S)-amphetamine (4.5 g) was heated to 130 °C for 5 h under N<sub>2</sub>. The reaction was purified by flash chromatography (CHCl<sub>3</sub>-3% to 5% to 10% MeOH) followed by radial chromatography four times (CHCl<sub>3</sub>-4% to 6% to 8% to 10% MeOH) to provide **9** (0.56 g, 68%).

Compound **9** (0.46 g, 1.05 mmol) was heated to 45 °C with 1M HCl (40 mL) for 15 min. The reaction was cooled and poured into sat NaHCO<sub>3</sub> (300 mL). The mixture was extracted with CHCl<sub>3</sub> (3x150 mL), dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo* to provide a solid (0.4 g). This material was then treated with ethereal HCl. The resulting solid was filtered and dried under high vacuum over P<sub>2</sub>O<sub>5</sub>. Recrystallization from 10% MeOH/Et<sub>2</sub>O provided **2** (0.18 g, 40%), mp 155 °C (dec); <sup>1</sup>H-NMR (D<sub>2</sub>O) δ 8.17 (s, 1H), 7.25 (d, 4H), 7.16 (m, 1H), 5.86 (d, 1H), 4.74 (t, 1H), 4.39 (t, 1H), 4.31 (q, 1H), 4.15 (q, 1H), 3.87 (m, 1H), 3.75 (m, 1H), 2.92 (m, 1H), 2.78 (m, 1H), 1.25 (d, 3H); [α]<sub>D</sub><sup>20</sup> = +8.00° (c=0.67, H<sub>2</sub>O); MS (CI/CH<sub>4</sub>) m/z 401 (M<sup>+</sup>).

**(R)-2-[(1-Phenylpropyl)amino]adenosine (3):** Compound **8** (3.4 g, 9.95 mmol) was combined with (R)-1-phenylpropylamine (8.62 g) and heated at reflux for 18 h. Excess amine was removed by distillation. After cooling, the material was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>-3% MeOH). The appropriate fractions were combined, concentrated and triturate with Et<sub>2</sub>O to provide **10** (1.38 g, 32%).

Compound **10** (0.43g, 1.0mmol) was dissolved in TFA (20 mL) and H<sub>2</sub>O (2 mL). After 15 min, the solution was concentrated *in vacuo* and the aqueous layer was saturated with Na<sub>2</sub>CO<sub>3</sub>. The organic layer was separated, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (CH<sub>2</sub>Cl<sub>2</sub>-5% to 10% to 15% MeOH). The appropriate fractions were combined and concentrated and the

product was crystallized from Me<sub>2</sub>CO to give **3** (72 mg, 18 %); <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>) δ 7.87 (s, 1H), 7.38 (d, 1H), 7.27 (t, 2H), 7.16 (t, 1H), 6.60-6.73 (m, 3H, D<sub>2</sub>O exch), 5.69 (d, 1H), 5.31 (d, 1H), 5.02 and 5.12 (br s and d, 2H), 4.83 (q, 1H), 4.52 (dd, 1H), 4.17 (dd, 1H), 3.88 (dd, 1H), 3.64-3.37 (m, 1H), 3.47-3.59 (m, 1H), 2.08 (s, 3H), 1.63 (m, 2H), 0.96 (t, 3H); IR (KBr) 3423-3290 cm<sup>-1</sup>; MS (CI/CH<sub>4</sub>) m/z 401 (M<sup>+</sup>).

**Spontaneously beating isolated guinea pig atrial pairs:** Male Hartley guinea pigs (304-438 g) were anesthetized with phenobarbital, 50 mg/kg i.p. The atria were rapidly removed and mounted vertically, as a pair, under a resting tension of 1 g in Krebs' Ringer bicarbonate solution of the following composition (mM): NaCl, 188.2; KCl, 4.6; CaCl<sub>2</sub>·2H<sub>2</sub>O, 2.5; KH<sub>2</sub>PO<sub>4</sub>, 1.2; MgSO<sub>4</sub>, 1.2; dextrose, 10.0; and NaHCO<sub>3</sub>, 24.8. The Krebs' Ringer bicarbonate solution was maintained at 37°C and continually bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub> to maintain a pH of 7.4. Heart rate (HR) and contractile force were measured with a force displacement transducer and recorded on a Modular Instruments M-5000 Signal Processing Center. The atria were allowed to equilibrate for 60 min prior to making experimental observations. Each atrial pair was incubated with increasing concentrations (1 nM–30 μM) of compound **1** (treatment group) or a corresponding volume of vehicle (10% DMSO in 0.9% saline; vehicle group). Each concentration or volume remained in contact with the tissue for 5 min before the next addition. Absolute HR and contractile force values were converted to percent change from baseline.

**Mean arterial pressure and heart rate in anesthetized normotensive and spontaneously hypertensive (SHR) rats:** Male, normotensive Sprague-Dawley rats (394-644 g) and spontaneously hypertensive rats (380-460 g) were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and atropine (0.4-0.5 mg/kg) was administered i.p. to reduce pulmonary secretions. The trachea was cannulated and the animals were allowed to breathe spontaneously. A femoral vein was cannulated and used for the administration of sodium pentobarbital (as needed to maintain anesthesia) and test compound. A femoral artery was cannulated and was connected to either a Cobe CDX III (normotensive rat studies) or a Statham model P-23-id (SHR studies) pressure transducer for the measurement of pulsatile or MAP. HR was recorded from the blood pressure signal using a Gould (normotensive rat studies) or Western Graphtec (SHR studies) biotachometer. All signals were recorded on a Gould 4000 Brush Recorder (normotensive rat studies) or a Western Graphtec Linearcorder (SHR studies). For each

study, two groups of rats were tested: a vehicle group (n=5) which received vehicle only (0.2 ml of 10% DMSO in 0.9% saline) administered *iv* over 30 sec and a treatment group (n=5) which received ascending doses of compound 1 (0.3, 1.0, 3.0, 10, 30, and 100 ug/kg for the normotensive rat studies and 0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 ug/kg for the SHR studies) in 0.2 ml of vehicle administered *iv* over 30 sec. For the normotensive rat studies the doses were administered at least 20 min apart to allow MAP or HR to return to baseline or to reach a new stable baseline. For the SHR studies, the six vehicle and MDL doses were administered 20, 20, 60, 120, 120, and 120 min apart, respectively. Absolute MAP and HR values were converted to % change from baseline.

**Mean arterial pressure and heart rate in anesthetized and conscious normotensive beagle dogs:**

Experiments were performed in three anesthetized beagles, one male and two females, weighing 8.0 to 13.5 kg that served as controls in other experiments. Compound 1 was administered at the end of the initial experiment. In some cases the initial experiment lasted more than 8 hours. Anesthesia was induced (32.5 mg/kg) and maintained (4.5 mg/kg/hr) with *iv* sodium pentobarbital. The animals were intubated and respired with room air using a respirator. A femoral artery and both femoral veins were cannulated to monitor arterial blood pressure, administer test drugs and infuse anesthetic, respectively. Mean arterial pressure (MAP) was recorded using a Statham pressure transducer. Heart rate (HR) was recorded using a Graphtec biotachometer. All signals were recorded on a Western Graphtec recorder. Compound 1 was dissolved in 1 ml of 42.5 % DMSO/57.5 % 0.9 % sodium chloride (vehicle) and administered over 20-30 seconds followed by a 2 ml flush of 0.9 % sodium chloride. Absolute MAP and HR values were converted to % change from pre-dose baseline values. Experiments were performed in two conscious male beagles weighing 14.6 and 15.8 kg. MAP was measured using surgically-implanted Data Sciences blood pressure transmitters. The blood pressure cannula was located in a femoral artery. At least one week was allowed for recovery from surgery before initiating an experiment. MAP was recorded as 5 min averages, HR as 30 min averages. The dogs were acclimated to a metabolism cage and the following schedule: clean and feed at 09:00 hr, dose at 15:00 hr. The dogs were fed standard dog chow; water was available *ad lib*. Absolute MAP and HR values were converted to % change from pre-dose baseline values.

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