# $N^6$-Substituted *N*-Alkyladenosine-5′-uronamides: Bifunctional Ligands Having Recognition Groups for A1 and A2 Adenosine Receptors

R. A. Olsson,*† Shozo Kusachi,† Robert D. Thompson,† Dieter Ukena,‡ William Padgett,‡ and John W. Daly‡

*Suncoast Cardiovascular Research Laboratory, Departments of Internal Medicine and Biochemistry, University of South Florida College of Medicine, Tampa, Florida 33612, and Laboratory of Bioorganic Chemistry, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205.*
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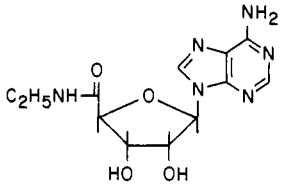
The coronary vasoactivity of *N*-ethyl-1′-deoxy-1′-(6-amino-9*H*-purin-9-yl)-β-D-ribofuranuronamide (NECA, 1) is over 2 orders of magnitude greater than that of adenosine, and the vasoactivity of certain $N^6$-substituted adenosines is as much as 1 order of magnitude greater. Such results suggest that a combination of appropriate modifications at $N^6$ and C-5′ might additively augment the agonist potency of adenosine. At low temperatures 1-deoxy-1-(6-chloro-9*H*-purin-9-yl)-2′,3′-*O*-isopropylidene-β-D-ribofuranosyl chloride (**5**), obtained in three steps from inosine, reacts with amines to yield uronamides. The subsequent reaction of such uronamides with amines at elevated temperatures displaces the purine 6-chloro group to yield, after deblocking, *N*-alkyl(or aryl)-$N^6$-alk(ar)yladenosine-5′-uronamides. At the coronary artery A2 receptor the potency of $N^6$-modified analogues of 1 is similar to that of the $N^6$-substituted adenosine, rather than equal to or greater than 1. As agonists in the A2 receptor-mediated stimulation of adenylate cyclase in plasma membranes of PC12 pheochromocytoma cells or human platelets, $N^6$-substituted analogues of 1 are intermediate between the high potency of 1 and the lower potency of the $N^6$-substituted adenosines. At the A1 receptor of rat brain the potency of an $N^6$-substituted analogue of 1 is often greater than that of the corresponding $N^6$-substituted adenosine. At all four receptors, replacing the ethyl group of *N*-ethyl-$N^6$-3-pentyladenosine-5′-uronamide by larger alkyl groups reduces potency; amides of secondary amines are inactive or have only marginal activity. Analogues of 1 containing a chiral center in the $N^6$ substituent retain the stereoselectivity characteristic of each of the four receptors. Thus, at either A1 or A2 adenosine receptors, adenosine analogues interact with both the $N^6$ and the C-5′ receptor regions. However, the effects of $N^6$ and C-5′ modifications on potency are less than additive, evidence that the interaction of a substituent with its receptor region influences the interaction of other substituents with their respective receptor regions.

Adenosine receptors that either inhibit (A1 or Ri) or stimulate (A2 or Ra) the activity of adenylate cyclase mediate many of the biological effects of adenosine. Frequently these receptors are identified pharmacologically by the rank order of potency within sets of adenosine analogues that include $N^6$-substituted adenosines and *N*-alkyladenosine-5′-uronamides. At A1 receptors the $N^6$-substituted adenosines are very potent, whereas at A2 receptors the adenosine-5′-uronamides are likewise very potent.[1,2] Thus, selectivity for one or the other receptor depends on the chemical attributes of, literally, the opposite ends of the adenosine molecule.

*N*-Ethyl-1′-deoxy-1′-(6-amino-9*H*-purin-9-yl)-β-D-ribofuranuronamide (NECA, 1), the prototypic A2 adenosine receptor agonist, is an exceedingly potent coronary vasodilator. Structure–activity correlations show that 1 and

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cyclopropyl and also the methoxy amidic congeners are the most potent of the adenosine-5′-uronamides as coronary vasodilators,[3,4] a result that supports the idea that the C-5′ region of the coronary receptor contains a subregion that interacts in a positive manner with two alkyl carbons or an isosteric methoxy group. The uronamides of larger primary amines have either lower activity or none at all. The uronamides of dialkylamines are inactive, evidence that vasoactivity may depend on the amide hydrogen, which could participate in hydrogen bonding with the receptor or, intramolecularly, with purine N-3. Alternatively, the C-5′ receptor region may not be wide enough to accommodate two alkyl substituents on the amide nitrogen.

Subsequent structure–activity studies confirm the extraordinary coronary vasoactivity of the adenosine-5′-uronamides 1 and **6**. The molar potency ratio (MPR) vs. adenosine of 1 is 150 and that of the cyclopropyl congener, **6**, is 94. In keeping with other evidence that the coronary artery adenosine receptor is of the A2 type, the prototypic A1 receptor agonists are much less potent than 1. For example, the coronary MPR of $N^6$-(1-phenylprop-2(*R*)-yl)adenosine is 4.3 and that of $N^6$-cyclohexyladenosine is 1.6.[5] Although the $N^6$-substituted adenosines are not nearly as potent as 1, several have substantially more coronary vasoactivity than adenosine. Additionally, well-defined structure–activity rules appear to account for the differences in coronary vasoactivity among the $N^6$-substituted adenosines, indicating that the coronary artery adenosine receptor contains an $N^6$ region of specialized structure.[6,7]

The present report describes a new synthesis and assays of the biological activities of $N^6$-modified *N*-alkyladenosine-5′-uronamides, adenosine analogues that combine, in the same molecule, the functional groups for recognition of both the $N^6$ and the C-5′ receptor regions. Since appropriate modifications of the adenosine molecule

† University of South Florida College of Medicine.
‡ National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.

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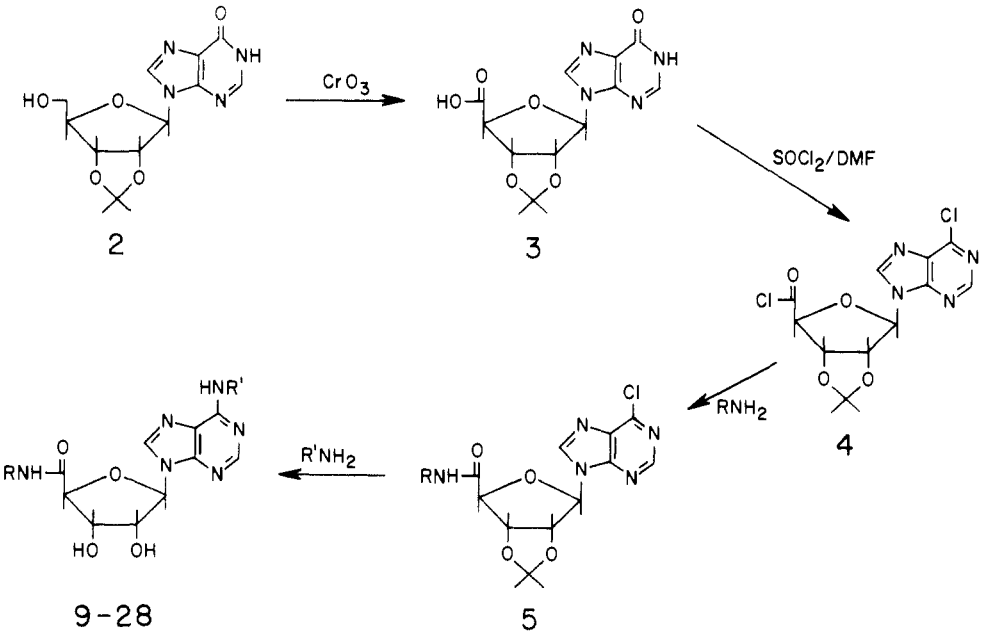
**Scheme I**




at either $N^6$ or at C-5′ can increase coronary vasoactivity, we reasoned that combining such modifications in the same molecule should result in an additive increase in coronary vasoactivity. However, initial assays of coronary vasoactivity rejected this hypothesis, showing instead that an $N^6$-substituted *N*-ethyladenosine-5′-uronamide has activity very nearly identical with that of the corresponding $N^6$-substituted adenosine. In view of this unexpected finding, we extended the investigation to two other examples of A2 adenosine receptors and also to the rat cerebral cortex A1 adenosine receptor.

**Chemistry.** Certain $N^6$-alkyl, -acyl, and -carbamoyl derivatives of the *N*-alkyladenosine-5′-uronamides are known.[8-10] The synthesis of the $N^6$-alkyl analogues entailed $N^1$ alkylation followed by Dimroth rearrangement in alkali. Such an approach is quite limited in scope; only methyl, benzyl, or allylic halides will alkylate $N^1$ of adenosine to an extent that is synthetically useful,[11] so many $N^6$-substituted adenosine-5′-uronamides are not likely to be accessible by this route. Moreover, the kinds of $N^6$ substituents that are accessible have negative or at best indifferent effects on biological activity.[6,7] Accordingly, we considered alternative routes to the amides of $N^6$-substituted adenosine-5′-uronic acids. The usual synthesis of adenosine-5′-ribofuranuronic acid, namely the oxidation of 2′,3′-*O*-isopropylideneadenosine with $KMnO_4$/KOH,[12] is not applicable to $N^6$-substituted adenosines because it ablates the $N^6$ substituent through oxidative cleavage. An alternative method of oxidation employing $KMnO_4$/acetic acid (see the Experimental Section) has several advantages over alkaline permanganate in the preparation of adenosine-5′-riburonic acid. These include a 2:1 rather than 4:1 ratio of $KMnO_4$ to nucleoside, a reaction time of 12 h rather than 3 days, and destruction of the $MnO_2$ precipitate by $H_2O_2$, which greatly simplifies the isolation of the product. However, $KMnO_4$/acetic acid, like $KMnO_4$/KOH, degrades $N^6$ substituents. In studies preliminary to those described below, the $KMnO_4$/acetic acid oxidation of 2′,3′-*O*-isopropylidene-$N^6$-2-phenethyladenosine resulted in extensive degradation to a tar that contained phenylacetic acid.

A new general synthesis of $N^6$-modified *N*-alkyladenosine-5′-uronamides is shown in Scheme I. The oxidation of 2′,3′-*O*-isopropylideneinosine (**2**) with $CrO_3$/acetic acid[13] affords the uronic acid **3** in ca. 50% yield. The reaction of **3** with $SOCl_2$/DMF results in a double chlorination to form the key intermediate in this synthesis, 1′-deoxy-1′-(6-chloro-9*H*-purin-9-yl)-2′,3′-*O*-isopropylidene-β-D-ribofuranosyl chloride (**4**). The reactivities of the two chloro groups of **4** differ so greatly that amines can displace them regioselectively. At low temperatures amines react exclusively with the C-5′ chloro group to form the 6-chloropurine-5′-ribouronamide **5**. Displacement of the purine 6-chloro group to form the *N*-alkyl-$N^6$-alkyladenosine-5′-uronamides requires higher temperatures and longer reaction times. The acid stability conferred on the glycosylic bond by the C-5′ carbonyl function[14] facilitates removal of the isopropylidene group; heating in 1 N HCl for 1 h at 60 °C deblocks these nucleosides without cleaving the glycosylic bond to yield **8–27**. Piperidine is an exception to such a course of events, displacing both of the chloro groups of **4** at low temperatures.

Table I lists the properties of the new analogues used in this study. We previously reported that when the alkyl carbon attached to $N^6$ of an adenosine is an asymmetric center, the *R* diastereomer will crystallize but the *S* diastereomer will not.[6] It is thus interesting that in the case of the uronamides this rule is reversed; analogues **15** and **17**, which are *S* diastereomers, crystallize readily, but their *R* diastereomers do not.

**Coronary Vasoactivity.** The assays of coronary vasoactivity used 39 dogs. During the control periods prior to the estimation of the $EC_{50}$ of adenosine, heart rate averaged (mean ± SEM) 97 ± 5 beats/min, mean arterial

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**Table I.** Characteristics of Novel *N*-Alkyl $N^6$-Substituted Adenosine-5′-ribofuranuronamides

| no. | formula (fw) | yield, % | mp, °C | $[\alpha]^{25}_D$, deg | UV: $\lambda_{max}$, nm ($\epsilon$) | purificn[a] | elements anal.[b] |
|---|---|---|---|---|---|---|---|
| **10** | $C_{14}H_{20}N_6O_4$ (336.35) | 42 | 176–177 | | 275 (19 100) | B (60) | C, H, N |
| 11 | $C_{17}H_{26}N_6O_4$ (378.44) | 11 | 176–177 | | 269 (18 100) | B (50–70) | C, H, N |
| 12 | $C_{18}H_{26}N_6O_4$ (382.38) | 47 | 133–135 | | 270 (18 800) | B (60–80) | C, H, N |
| 13 | $C_{21}H_{26}N_6O_4$ (426.48) | 47 | 157–158 | −104.5 | 270 (18 000) | B (50–60) | C, H, N |
| 14 | $C_{21}H_{26}N_6O_4$ (426.48) | 59 | 182–183 | +37.5 | 270 (17 600) | B (50–60) | C, H, N |
| 15 | $C_{22}H_{28}N_6O_4$ (440.51) | 33 | 161–163 | −81 | 271 (18 100) | B (50–60) | C, H, N |
| 16 | $C_{22}H_{28}N_6O_4$ (440.51) | 51 | 177–179 | +27 | 270 (19 200) | A | C, H, N |
| 17 | $C_{19}H_{22}N_6O_5$ (414.43) | 62 | 189–90 | | 287 (19 400) | A | C, H, N |
| 18 | $C_{23}H_{30}N_6O_7$ (502.53) | 29 | 154–155 | | 271 (15 700) | A | C, H, N |
| 19 | $C_{18}H_{26}N_6O_4$ (390.45) | 64 | 181–183 | | 269 (20 000) | A | C, H, N |
| 20 | $C_{18}H_{28}N_6O_4$ (392.46) | 41 | 191–193 | | 269 (18 300) | A | C, H, N |
| 21 | $C_{16}H_{24}N_6O_4$ (364.41) | 10 | 126–128 | | 269 (18 500) | B (60) | C, H, N |
| 22 | $C_{18}H_{26}N_6O_4$ (390.45) | 63 | 169–170 | | 269 (19 200) | A | C, H, N |
| 23 | $C_{20}H_{32}N_6O_4$ (420.52) | 66 | 211–212 | | 270 (17 900) | A | C, H, N |
| 24 | $C_{22}H_{28}N_6O_4$ (440.51) | 61 | 174–175 | | 269 (17 400) | B (60) | C, H, N |
| 25 | $C_{17}H_{26}N_6O_4$ (378.44) | 68 | 168–169 | | 269 (18 700) | A | C, H, N |
| 26 | $C_{19}H_{30}N_6O_4$ (406.49) | 58 | 148–150 | | 269 (19 000) | A | C, H, N |
| 27 | $C_{20}H_{32}N_6O_4$ (420.52) | 26 | 139–141 | | 270 (19 200) | B (70) | C, H, N |
| 28 | $C_{20}H_{30}N_6O_4$ (416.48) | 36 | 189–191 | | 281 (22 000) | A | C, H, N |

[a] Abbreviations: A, crystallization ($CH_3OH/H_2O$); B, low-pressure liquid chromatography, isocratic elution with $CH_3OH/H_2O$; (% $CH_3OH$); C, low-pressure liquid chromatography, elution with $CH_3OH/H_2O$ gradient (begin and end $CH_3OH$, %). [b] Elemental analyses agreed within ±0.4% of theoretical.

**Table II.** Coronary Vasoactivity of $N^6$-Substituted *N*-Alkyladenosine-5′-uronamides and Corresponding $N^6$-Substituted Adenosines

| no. | C-5′ subst | $N^6$ subst | MPR vs. adenosine: uronamide | MPR vs. adenosine: $N^6$-subst adenosine |
|---|---|---|---|---|
| | | A. Adenosine-5′-uronamides | | |
| 1 | $NHC_2H_5$ | H | 150 ± 35 (5)[a] | |
| 6 | NH-c-$C_3H_5$ | H | 94 ± 12 (5)[a] | |
| 7 | $NHCH_3$ | H | 11 | |
| 8 | $NH_2$ | H | 4.7 | |
| | | B. *N*-Ethyl $N^6$-Substituted Adenosine-5′-uronamides and Adenosines | | |
| 9 | $NHC_2H_5$ | $CH_3$ | 0.46 ± 0.11 (5) | 0.5[b] |
| 10 | $NHC_2H_5$ | $(CH_3)_2$ | 0 (3) | 0[c] |
| 11 | $NHC_2H_5$ | $CH(C_2H_5)_2$ | 3.3 ± 0.25 (5) | 4.0 ± 1.0 (5)[c] |
| 12 | $NHC_2H_5$ | c-$C_6H_{11}$ | 1.5 ± 0.24 (5) | 1.6 ± 0.2 (5)[a] |
| 13 | $NHC_2H_5$ | (*R*)-$C_6H_5CH_2CH(CH_3)$ | 4.3 ± 0.6 (5) | 4.3 ± 0.7 (5)[c] |
| 14 | $NHC_2H_5$ | (*S*)-$C_6H_5CH_2CH(CH_3)$ | 0.22 ± 0.04 (5) | 0.41 ± 0.06 (5)[c] |
| 15 | $NHC_2H_5$ | (*R*)-$C_6H_5CH_2CH(C_2H_5)$ | 3.8 ± 1.3 (4) | 9.0 ± 0.34 (5)[d] |
| 16 | $NHC_2H_5$ | (*S*)-$C_6H_5CH_2CH(C_2H_5)$ | 0.18 | 0.36 ± 0.02 (5)[d] |
| 17 | $NHC_2H_5$ | 4-$CH_3OC_6H_4$ | 2.0 | 3.5 |
| 18 | $NHC_2H_5$ | 3,4,5-$(CH_3O)_3C_6H_2C_2H_4$ | 3.0 | 5.2 |
| | | C. *N*-Alkyl-$N^6$-3-pentyladenosine-5′-uronamides | | |
| 19 | NH-c-$C_3H_5$ | $CH(C_2H_5)_2$ | 1.9 | |
| 20 | $NHCH(CH_3)_2$ | $CH(C_2H_5)_2$ | 0.23 | |
| 21 | $NHCH_3$ | $CH(C_2H_5)_2$ | 0.24 | |
| 22 | $NHCH_2CH{=}CH_2$ | $CH(C_2H_5)_2$ | 0.078 | |
| 23 | $NHCH(C_2H_5)_2$ | $CH(C_2H_5)_2$ | 0 | |
| 24 | $NHCH_2C_6H_5$ | $CH(C_2H_5)_2$ | 0 | |
| 25 | $N(CH_3)_2$ | $CH(C_2H_5)_2$ | 0 | |
| 26 | $N(C_2H_5)_2$ | $CH(C_2H_5)_2$ | 0 | |
| 27 | $N(CH_3)(C_4H_9)$ | $CH(C_2H_5)_2$ | 0 | |
| 28 | piperidyl[e] | piperidyl | 0 | |

[a] Reference 5. [b] Reference 15. [c] Reference 6. [d] Unpublished. [e] The corresponding $N^6$-substituted adenosine is inactive. Data are the means of duplicate assays or mean ± SEM of the number of assays listed in parentheses.

pressure 95 ± 4 mmHg, coronary flow rate 87 ± 6 mL/min per 100 g, arterial blood $P_{O_2}$ 134 ± 7 mmHg, $P_{CO_2}$ 39 ± 1 mmHg, and pH 7.38 ± 0.01. Coronary flow rate at the peak of a reactive hyperemia response to 30 s of coronary occulsion was 483 ± 43% of control and during the administration of a supramaximum dose of adenosine was 510 ± 44% of control. The $EC_{50}$ of adenosine averaged 1.1 ± 0.12 μM.

Table II summarizes the results of the assays of coronary vasoactivity and compares the potencies of the uronamides with those of the corresponding $N^6$- or C-5′-modified adenosines, when known. In accordance with earlier reports,[3,4] the potency ranking of the *N*-alkyladenosine-5′-uronamides not modified at $N^6$ was ethyl > cyclopropyl > methyl > unsubstituted, i.e. **1** > **6** > **7** > **8**. Analogues **7** and **8** are 33 and 100 times more potent than their respective 5′-(alkylamino)-5′-deoxy congeners,[15] illustrating the importance of the amide group for coronary vasoactivity.

The coronary vasoactivities of the $N^6$-substituted *N*-ethyluronamides **9**–**18** were very similar and in some cases identical with the potencies of the corresponding $N^6$-substituted adenosines. Such a result clearly rejects the hypothesis that the $N^6$ and C-5′ substituents will contribute additively to activity. Rather, the actual potencies of **9**–**18** were 2 orders of magnitude less than predicted by the hypothesis. Thus, the biological effects of the $N^6$ sub-

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**Table III.** Potency of *N*-Alkyl N$^6$-Substituted Adenosine-5′-uronamides and N$^6$-Substituted Adenosines at PC12 Cell A2 Adenosine Receptors

| no. | C-5′ subst | N$^6$ subst | adenylate cyclase stimulation $EC_{50}$,[a] nM: uronamide | adenylate cyclase stimulation $EC_{50}$,[a] nM: N$^6$-subst adenosine |
|---|---|---|---|---|
| | | A. Adenosine-5′-uronamides | | |
| 1 | $NHC_2H_5$ | H | 130 ± 10 | |
| 6 | NH-c-$C_3H_5$ | H | 110 ± 10 | |
| 7 | $NHCH_3$ | H | 720 ± 60 | |
| 8 | $NH_2$ | H | 1560 ± 80 | |
| | | B. *N*-Ethyl N$^6$-Substituted Adenosine-5′-uronamides and Adenosines | | |
| 9 | $NHC_2H_5$ | $CH_3$ | 6300 ± 1000 | 17300 ± 1200 |
| 10 | $NHC_2H_5$ | $(CH_3)_2$ | >30000 | >100000 |
| 11 | $NHC_2H_5$ | $CH(C_2H_5)_2$ | 820 ± 100 | 1200 ± 50 |
| 12 | $NHC_2H_5$ | c-$C_6H_{11}$ | 1400 ± 300 | 1830 ± 290 |
| 13 | $NHC_2H_5$ | (*R*)-$C_6H_5CH_2CH(CH_3)$ | 340 ± 30 | 980 ± 120 |
| 14 | $NHC_2H_5$ | (*S*)-$C_6H_5CH_2CH(CH_3)$ | 3300 ± 140 | 4200 ± 980 |
| 15 | $NHC_2H_5$ | (*R*)-$C_6H_5CH_2CH(C_2H_5)$ | 280 ± 20 | 820 ± 160 |
| 16 | $NHC_2H_5$ | (*S*)-$C_6H_5CH_2CH(C_2H_5)$ | 2200 ± 120 | 3600 ± 1400 |
| 17 | $NHC_2H_5$ | 4-$CH_3OC_6H_4$ | 1200 ± 30 | 1800 ± 140 |
| 18 | $NHC_2H_5$ | 3,4,5-$(CH_3O)_3C_6H_2C_2H_4$ | 420 ± 90 | 2200 ± 800 |
| | | C. *N*-Alkyl-*N*$^6$-3-pentyladenosine-5′-uronamides | | |
| 19 | NH-c-$C_3H_5$ | $CH(C_2H_5)_2$ | 540 ± 30 | |
| 20 | $NHCH(CH_3)_2$ | $CH(C_2H_5)_2$ | 5100 ± 300 | |
| 21 | $NHCH_3$ | $CH(C_2H_5)_2$ | 6400 ± 600 | |
| 22 | $NHCH_2CH{=}CH_2$ | $CH(C_2H_5)_2$ | 8900 ± 2900 | |
| 23 | $NHCH(C_2H_5)_2$ | $CH(C_2H_5)_2$ | >100000 | |
| 24 | $NHCH_2C_6H_5$ | $CH(C_2H_5)_2$ | >100000 | |
| 25 | $N(CH_3)_2$ | $CH(C_2H_5)_2$ | >100000 | |
| 26 | $N(C_2H_5)_2$ | $CH(C_2H_5)_2$ | >100000 | |
| 27 | $N(CH_3)(C_4H_9)$ | $CH(C_2H_5)_2$ | >100000 | |
| 28 | piperidyl | piperidyl | >100000 | |

[a] Data are mean ± SEM of three assays.

stituents, including the ability of **15–18** to recognize the stereoselectivity of the coronary receptor, are fully expressed, but those of the C-5 uronamide group are suppressed.

The activities of the *N*-alkyl-*N*$^6$-3-pentyladenosine-5′-uronamides **19–27** show that although the positive contributions of the amide substituent are suppressed, alterations of the amide nevertheless affect activity. The structure–activity rules that describe the potencies of the *N*-alkyladenosine-5′-uronamides[4] apply as well to their N$^6$-substituted congeners. The potency order ethyl > cyclopropyl > methyl in the ranking **11** > **19** > **21** parallels that in the ranking of **1** > **6** > **7**. Further, the adenosine-5′-uronamides of large primary amines or those of dialkylamines are inactive; such is likewise the case with the N$^6$-substituted analogues **22–27**.

**Stimulation of Adenylate Cyclase in PC12 Cell Membranes.** Adenosine analogues stimulate the activity of adenylate cyclase in membranes from PC12 cells through interaction with an A2 adenosine receptor: *N*-ethyladenosine-5′-uronamide (**1**) is more potent than 2-chloroadenosine, which in turn is more potent than N$^6$-substituted adenosines.[16] Table III summarizes studies of the effects of N$^6$-substituted adenosines and of the various adenosine-5′-uronamides on adenylate cyclase activity of PC12 cell plasma membranes. The potency ranking of the adenosine-5′-uronamides, **6** ≅ **1** > **7** > **8**, is consistent with that expected for an A2 receptor. The agonist potencies of the ethyluronamides **9–18** are intermediate between those of the N$^6$-substituted adenosines and that of **1**. Thus, the potency of an N$^6$-substituted uronamide is on the average about 2 times higher than that of the corresponding N$^6$-substituted adenosine but remains 2–50 times less potent than **1**. The N$^6$ region of the PC12 cell receptor shows the low stereoselectivity typical of A2 receptors; the *R*/*S* diastereomer potency ratios for the *N*$^6$-(1-phenylprop-2-yl)- and *N*$^6$-(1-phenylbut-2-yl)-adenosines average 4.4 whereas *R*/*S* potency ratios of the corresponding uronamides, **13**/**14** and **15**/**16**, average 8.8.

The N$^6$-monosubstituted *N*-ethyladenosine-5′-uronamides **9** and **11–18** are fully as efficacious in stimulating adenylate cyclase as **1** itself; the efficacies of these analogues relative to that of **1** range between 0.86 and 1.0. *N*-Ethyl-*N*$^6$-dimethyladenosine-5′-uronamide (**10**) has an efficacy of 0.53 at the highest concentration tested, 30 μM. Most of the N$^6$-substituted adenosines are also as efficacious at **1**, the relative efficacies ranging between 0.83 and 1.0. However, *N*$^6$-(4-methoxyphenyl)adenosine, which corresponds to **17**, has an efficacy of 0.70 and *N*$^6$,*N*$^6$-dimethyladenosine, which corresponds to **10**, has an efficacy of only 0.22 at the highest concentration tested, 100 μM.

The potency rank order of three uronamides of *N*$^6$-3-pentyladenosine, **19** ≅ **11** > **21**, parallels the ranking **6** ≅ **1** > **7** of the corresponding adenosine-5′-uronamides. The 2-propyl- and allylamides **20** and **22** are weak agonists whereas the amides of bulkier amines, **23–28**, are inactive. The efficacies relative to that of **1** of the *N*$^6$-3-pentyladenosine-5′-uronamides **19–22** are as follows: 0.78, 0.84, 1.0, and 0.59. The efficacies relative to **1** of uronamides **23–28**, which are derivatives of bulky primary amines of dialkylamines, are 0.20 or less at 100 μM, the highest concentration tested.

**Stimulation of Adenylate Cyclase of Human Platelet Membranes.** Adenosine analogues stimulate the activity of adenylate cyclase in membranes from human platelets through interaction with an A2 adenosine receptor: the uronamides are more potent than N$^6$-substituted adenosines.[17] Table IV summarizes estimates of the

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(17) Ukena, D.; Boehme, E.; Schwabe, U. *Naunyn Schmiedebergs Arch. Pharmacol.* **1984**, *327*, 36.

**Table IV.** Potency of $N^6$-Substituted *N*-Alkyladenosine-5′-uronamides and $N^6$-Substituted Adenosines at Human Platelet A2 Adenosine Receptors

| no. | C-5′ subst | $N^6$ subst | adenylate cyclase stimulation $EC_{50}$,[a] nM: uronamide | adenylate cyclase stimulation $EC_{50}$,[a] nM: $N^6$-subst adenosine |
|---|---|---|---|---|
| | | A. Adenosine-5′-uronamides | | |
| 1 | $NHC_2H_5$ | H | 300 ± 30 | |
| 6 | NH-c-$C_3H_5$ | H | 260[b] | |
| 7 | $NHCH_3$ | H | 1700[b] | |
| 8 | $NH_2$ | H | 5700[b] | |
| | | B. *N*-Ethyl $N^6$-Substituted Adenosine-5′-uronamides and Adenosines | | |
| 9 | $NHC_2H_5$ | $CH_3$ | 5900 ± 1100 | >100000 |
| 10 | $NHC_2H_5$ | $(CH_3)_2$ | >100000 | >100000 |
| 11 | $NHC_2H_5$ | $CH(C_2H_5)_2$ | 950 ± 80 | 4400 ± 1900 |
| 12 | $NHC_2H_5$ | c-$C_6H_{11}$ | 600 ± 70 | 1680 ± 360 |
| 13 | $NHC_2H_5$ | (*R*)-$C_6H_5CH_2CH(CH_3)$ | 470 ± 10 | 3100 ± 430 |
| 14 | $NHC_2H_5$ | (*S*)-$C_6H_5CH_2CH(CH_3)$ | 2200 ± 210 | 5800 ± 980 |
| 15 | $NHC_2H_5$ | (*R*)-$C_6H_5CH_2CH(C_2H_5)$ | 260 ± 140 | 750 ± 30 |
| 16 | $NNH_2H_5$ | (*S*)-$C_6H_5CH_2CH(C_2H_5)$ | 2370 ± 170 | 2900 ± 250 |
| 17 | $NHC_2H_5$ | 4-$CH_3OC_6H_4$ | 1550 ± 190 | 4500 ± 1100 |
| 18 | $NHC_2H_5$ | 3,4,5-$(CH_3O)_3C_6H_2C_2H_4$ | 680 ± 60 | 3400 ± 570 |

[a] Data are mean ± SEM of three assays. [b] From ref 17.

potency of $N^6$-substituted adenosines and of uronamides 1 and **9–18** at the A2 adenosine receptor of human platelet membranes. The potency of 1 is significantly lower at the platelet A2 receptor than at the PC12 cell A2 receptor. However, the potencies of $N^6$-substituted uronamides **9–18** are similar at the two receptors. $N^6$-substituted adenosines are on the average 5 times less potent than the corresponding $N^6$-substituted uronamides. However, with the exception of *N*-ethyl-$N^6$-(1-phenylbut-2(*R*)-yl)adenosine-5′-uronamide (**15**), which is equipotent with 1, the $N^6$-substituted uronamides are up to 20 times less potent than 1. The *R*/*S* diastereomer activity ratio for uronamides **13**/**14** is 4.7 and that of uronamides **15**/**16** is 9.1, in both instances nearly twice that of the corresponding $N^6$-substituted adenosines. Analogues **19–28** were not tested at the platelet A2 receptor.

As in the case of the PC12 cell adenylate cyclase, the *N*-ethyl $N^6$-substituted adenosine-5′-uronamides are fully as efficacious as 1, efficacy relative to 1 ranging between 0.91 and 1.0, at the platelet A2 receptor. The relative efficacy of the $N^6$,$N^6$-dimethyluronamide **9** is only 0.18 at 100 μM, the highest concentration tested. However, in contrast to the PC12 cell adenylate cyclase, none of the $N^6$-substituted adenosines are as efficacious as 1 at the platelet adenylate cyclase A2 receptor, relative efficacy ranging between 0.71 and 0.86. The efficacies of $N^6$-methyladenosine and $N^6$,$N^6$-dimethyladenosine are 0.46 and 0.06, respectively, at 100 μM, the highest concentration tested.

**Competition with [$^3$H]-$N^6$-Cyclohexyladenosine for Binding to A1 Receptors in Rat Cerebral Cortical Membranes.** Various adenosine analogues exhibit potency profiles for the antagonism of [$^3$H]-$N^6$-cyclohexyladenosine to rat cerebral cortical membranes that are consonant with those expected of an A1 receptor. $N^6$-Substituted analogues such as $N^6$-(1-phenylprop-2(*R*)-yl)adenosine (*R*-PIA) are very potent; 2-chloroadenosine and 1 are less but nearly equipotent and $N^6$-(1-phenylprop-2(*S*)-yl)adenosine is much less potent.[18] Table V summarizes the potencies vs. the binding of [$^3$H]-$N^6$-cyclohexyladenosine to rat cerebral cortical A1 adenosine receptors. Both 1 and **6** are, as expected,[19] rather potent agonists at this A1 receptor. The methylamide **7** and the unsubstituted amide **8** are 1 order of magnitude less potent.

The affinities for the brain A1 receptor of the $N^6$-substituted *N*-ethyluronamides **9–18** are as high or higher than those of the corresponding $N^6$-substituted adenosines. The increases in potency brought about by C-5′ modification are modest, only 2- to 3-fold. Analogues **13–16**, whose $N^6$ substituents contain a chiral center, show about the same degree of stereoselectivity as the adenosines. C-5′ substituents on the amide nitrogen larger than an ethyl or cyclopropyl markedly reduced potency. The rank order among three of the $N^6$-3-pentyluronamides, **11** > **19** > **21**, parallels that of these amides not substituted at N-6, namely **1** > **6** > **7**. Unlike A2 receptors, the brain A1 receptor exhibits a certain amount of tolerance of large amidic alkyl groups and of the amides of secondary amines. The activities of such analogues, **22–27**, are curtailed, in some instances profoundly, but activity is not altogether absent. Only *N*-piperidyl-$N^6$-(1-piperidyl)purine-5′-uronamide (**28**) is completely inactive, but the fact that the purine C-6 substituent is a tertiary amine could of itself account for such a result.[19]

**Summary.** Alterations of the adenosine molecule at either $N^6$ or at C-5′ markedly affect the activity of this nucleoside at A1 as well as at A2 adenosine receptors. An $N^6$ substituent usually enhances potency at A1 receptors but in most cases will reduce potency at an A2 receptor. There are, however, a few well-defined examples of an $N^6$ substituent increasing potency at an A2 receptor; e.g., $N^6$-(phenylbut-2(*R*)-yl)adenosine is 1 order of magnitude more potent than adenosine at the dog coronary artery A2 adenosine receptor. In contrast, the *N*-alkyladenosine-5′-uronamides, particularly the *N*-ethyl and *N*-cyclopropyl congeners, are the most potent A2 adenosine receptor agonists identified to date. At the dog coronary artery A2 adenosine receptor, for example, they are 2 orders of magnitude more potent than adenosine.[5] Such uronamides appear to retain high agonist activity at A1 receptors.

The present study of analogues that combine an $N^6$ substituent and a C-5′ uronamide group in the same molecule reveals marked differences in the way such combinations affect agonist potency at an example of an A1 receptor and three examples of A2 receptors. There are two approaches to the structure–activity analysis of bifunctional ligands such as the *N*-alkyl $N^6$-substituted adenosine-5′-uronamides. One may consider such ana-

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Table V. Potency of $N^6$-Substituted Adenosine-5′-uronamides and $N^6$-Substituted Adenosines at Rat Brain A1 Adenosine Receptor

| no. | C-5′ subst | $N^6$ subst | $IC_{50}$,[a] nM: uronamide | $IC_{50}$,[a] nM: $N^6$-subst adenosine |
|---|---|---|---|---|
| | | A. Adenosine-5′-uronamides | | |
| 1 | $NHC_2H_5$ | H | 10.2 ± 0.6 | |
| 6 | NH-c-$C_3H_5$ | H | 10.5 ± 0.7 | |
| 7 | $NHCH_3$ | H | 105 ± 25 | |
| 8 | $NH_2$ | H | 70 ± 3 | |
| | | B. *N*-Ethyl $N^6$-Substituted Adenosine-5′-uronamides and Adenosines | | |
| 9 | $NHC_2H_5$ | $CH_3$ | 68 ± 10 | 120 ± 22 |
| 10 | $NHC_2H_5$ | $(CH_3)_2$ | 9600 ± 2200 | 10000 |
| 11 | $NHC_2H_5$ | $CH(C_2H_5)_2$ | 1.2 ± 0.1 | 1.5 ± 0.3 |
| 12 | $NHC_2H_5$ | c-$C_6H_{11}$ | 0.86 ± 0.06 | 1.7 ± 0.3 |
| 13 | $NHC_2H_5$ | (*R*)-$C_6H_5CH_2CH(CH_3)$ | 1.1 ± 0.3 | 2.4 ± 0.1 |
| 14 | $NHC_2H_5$ | (*S*)-$C_6H_5CH_2CH(CH_3)$ | 31 ± 1 | 105 ± 15 |
| 15 | $NHC_2H_5$ | (*R*)-$C_6H_5CH_2CH(C_2H_5)$ | 0.62 ± 0.05 | 0.52 ± 0.04 |
| 16 | $NHC_2H_5$ | (*S*)-$C_6H_5CH_2CH(C_2H_5)$ | 33 ± 3 | 26 ± 6 |
| 17 | $NHC_2H_5$ | 4-$CH_3OC_6H_4$ | 1.0 ± 0.2 | 4.6 ± 1.6 |
| 18 | $NHC_2H_5$ | 3,4,5-$(CH_3)_3C_5H_2C_2H_4$ | 29 ± 4 | 54 ± 6 |
| | | C. *N*-Alkyl-$N^6$-3-Pentyladenosine-5′-uronamides | | |
| 19 | NH-c-$C_3H_5$ | $CH(C_2H_5)_2$ | 2.0 ± 0.1 | |
| 20 | $NHCH(CH_3)_2$ | $CH(C_2H_5)_2$ | 11 ± 3 | |
| 21 | $NHCH_3$ | $CH(C_2H_5)_2$ | 20 ± 2 | |
| 22 | $NHCH_2CH{=}CH_2$ | $CH(C_2H_5)_2$ | 17 ± 8 | |
| 23 | $NHCH(C_2H_5)_2$ | $CH(C_2H_5)_2$ | 1400 ± 110 | |
| 24 | $NHCH_2C_6H_5$ | $CH(C_2H_5)_2$ | 104 ± 3 | |
| 25 | $N(CH_3)_2$ | $CH(C_2H_5)_2$ | 13000 | |
| 26 | $N(C_2H_5)_2$ | $CH(C_2H_5)_2$ | 1700 ± 420 | |
| 27 | $N(CH_3)(C_4H_9)$ | $CH(C_2H_5)_2$ | 10% @ 10 μM[b] | |
| 28 | piperidyl | piperidyl | 0% @ 10 μM[b] | |

[a] Data are mean ± SEM of three assays. [b] Percentage displacement of [$^3$H]-$N^6$-cyclohexyladenosine at indicated concentration of uronamide.

logues as congeners of 1, thus assessing the impact of an $N^6$-substituent on the potency contributed by the amide group. Alternatively, one may assess the effect of introducing a C-5′ amide group on the potency of an $N^6$-substituted adenosine. Although the two approaches yield equivalent results, we have chosen to analyze our observations in terms of the influence of an amide group on the activity of an $N^6$-substituted adenosine.

At the A1 receptor, where the $N^6$ substituent is expected to be the primary determinant of potency, an ethyluronamide group often increases potency, though only 2- to 3-fold over that of the $N^6$-substituted adenosine. Large alkyl- or dialkylamide groups abolish the activity of uronamides at A2 receptors, but at the A1 receptor such analogues retain some activity. At the dog coronary artery A2 receptor the activity of an adenosine-5′-uronamide depends strongly on the nature of the amide substituent; it is clear from many studies that an ethyl substituent is optimum. An $N^6$ substituent exerts the same effect on an adenosine-5′-uronamide as on an adenosine but *completely* suppresses the potential of the amide function to contribute the vasoactivity. At the A2 adenosine receptors of PC12 cells and of human platelets, by contrast, an appropriate amide group can modestly increase the activity of certain $N^6$-substituted adenosines. At both the coronary and the PC12 cell A2 receptors a large alkyl- or dialkylamide eliminates activity.

## Experimental Section

Melting points were estimated on a Thomas-Hoover apparatus and are uncorrected. A Perkin-Elmer 241 MC spectropolarimeter served for estimates of $[\alpha]_D$. $^1$H NMR spectra of samples dissolved in $Me_2SO$-$d_6$ were recorded at 60 MHz, and resonances are reported as chemical shifts ($\delta$) from the $(CH_3)_4Si$ internal standard. MHW Laboratories, Tucson, AZ, performed the elemental analyses, which agreed within ±0.4% of calculated composition. Assays of purity by reversed-phase HPLC revealed that product accounted for >99% of the UV-absorbing material in samples assayed for biological activity. Earlier publications detail the methods used to assay the coronary vasoactivity of adenosine and its analogues[5,15] in an open-chest dog preparation. As in previous studies, an assay of the potency of adenosine preceded each analogue assay. Relative vasoactivity is expressed as a molar potency ratio (MPR), the quotient of the $EC_{50}$ of adenosine divided by that of the analogue. The assays of adenylate cyclase activity in plasma membranes of human platelets,[17] PC-12 pheochromocytoma cells,[16] and of inhibition of [$^3$H]-$N^6$-cyclohexyladenosine binding to rat cerebral cortical A1 receptors[18] have been described. Throughout this report experimental data are reported as mean ± SEM.

***N*-Ethyladenosine-5′-uronamide (1).** A solution of 50 g (163 mmol) of 2′,3′-*O*-isopropylideneadenosine in 750 mL of 80% acetic acid was magnetically stirred in an ice bath. When the contents of the flask reached 10 °C, 60 g (380 mmol) of solid $KMnO_4$ was added portionwise over 1–2 h, and the mixture was stirred overnight at room temperature. The addition of 30% $H_2O_2$ destroyed excess $KMnO_4$ and rendered the solution colorless. Diluting the reaction mixture with 1–2 vol of ice water precipitated product, which was filtered off, washed with water and a little ether, and then dried: yield 35 g (67%); mp 274–276 °C (lit.[12] mp 276 °C). Heating the product for 30 min at 70 °C in a mixture of 350 mL of water and 60 mL of concentrated HCl, cooling, and adjusting to pH 4 with 6 N NaOH precipitated product, which was filtered off and washed with cold water and acetone; yield 25 g (55%).

A suspension of adenosine-5′-uronic acid (18 g, 64 mmol) in 500 mL of absolute $C_2H_5OH$ was magnetically stirred and cooled to 5 °C in an ice bath. $SOCl_2$ (30 mL) was added dropwise at a rate that kept the temperature <10 °C. The mixture was stirred 18 h with exclusion of moisture, and then the HCl salt of the ethyl uronate was filtered off and washed with absolute $C_2H_5OH$ and dry $(C_2H_5)_2O$ to remove traces of $SOCl_2$. The precipitate was dissolved in water and adjusted to pH 8 with solid $NaHCO_3$. The precipated product was filtered and crystallized from $C_2H_5OH$ to yield, in two crops, 18 g (86%) of product, mp 207–210 °C (lit.[20] mp 207–209 °C).

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Ethyl adenosine-5′-uronate (5 g, 16.2 mmol) in a 50-mL flask was cooled in an ice bath and treated with 3 mL (46 mmol) of anhydrous $C_2H_5NH_2$. The thick slurry was stirred overnight, at which time the product had solidified. Product was suspended in dry $(C_2H_5O)_2O$, filtered, and crystallized from $C_2H_5OH$; yield 4 g (80%).

**2′,3′-*O*-Isopropylideneinosine-5′-uronic Acid (3).** A solution of 25 g (81.1 mmol) of 2′,3′-*O*-isopropylideneinosine (**2**) in 300 mL of glacial acetic acid was vigorously stirred in a 500-mL flask containing a large magnetic stirring bar. When cooling in an ice bath reduced the temperature to just above the freezing point of the acetic acid (ca. 17 °C), 8.9 g (89 mmol) of $CrO_3$ was added portionwise and the dark mixture was stirred at room temperature for 48 h. Product was filtered off and washed with acetic acid. Repeated crystallizations from boiling acetic acid freed the product of traces of Cr salts: yield 15.7 g (60%) of an amorphous white powder; mp 272–274 °C (lit.[13] mp 252 °C).

**2′,3′-*O*-Isopropylidene-*N*-ethyl-6-chloropurine-5′-uronamide (5).** A mixture of 6.5 g (20 mmol) of **3**, 3 mL (40 mmol) of $SOCl_2$, 1.5 mL (19.4 mmol) of *N,N*-dimethylformamide, and 250 mL of dry $CHCl_3$ was heated at reflux with the exclusion of moisture for 5–6 h. Vacuum evaporation of solvents yielded a syrup that was disolved in 80 mL of dry $CHCl_3$. This solution was added to an ice-cold solution of 14 mL of anhydrous $C_2H_5NH_2$ in 150 mL of dry $CHCl_3$. After 20 min at <10 °C this solution was extracted with 3 × 250 mL of dilute HCl, once with 250 mL of saturated $NaHCO_3$, and with 2 × 50 mL of water. Drying ($MgSO_4$) and evaporation gave a pale yellow syrup that was used directly in the next step.

***N*-Ethyl-*N*$^6$-cyclohexyladenosine-5′-uronamide (13).** A solution of **5** (7.36 g, 20 mmol), cyclohexylamine (2.1 g, 21.2 mmol), and $(C_2H_5)_3N$ (5.5 mL, 40 mmol) in 200 mL of absolute $C_2H_5OH$ was heated as reflux with exclusion of moisture. After 48 h the solvent was evaporated to leave a syrupy residue. The addition of dry $(C_2H_5)_2O$ precipitated $(C_2H_5)_3$·HCl, which was filtered off. The residue after evaporation was purified by LPLC on a 3 × 30 cm column of C-18 silica gel equilibrated with $CH_3OH/H_2O$ (3:2, v/v). The column was eluted with a linear gradient of $CH_3OH/H_2O$ generated by pumping $CH_3OH$ into $CH_3OH/H_2O$ (3:2, v/v). Fractions containing the product was pooled and evaporated, and the residue was heated in 1 N HCl for 1 h at 60 °C. Cooling and neutralization with $NaHCO_3$ precipitated a white solid that was purified by preparative reversed-phase LPLC employing a linear gradient formed from two solutions of $CH_3OH/H_2O$ (3:2, v/v; 4:1, v/v). Evaporation of appropriate fractions yielded 3.5 g (47%) of white powder.

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**Registry No.** 1, 35920-39-9; **2**, 2140-11-6; **3**, 28440-13-3; **5**, 103201-21-4; **6**, 50908-62-8; **7**, 35788-27-3; **8**, 35788-21-7; **9**, 72209-31-5; **10**, 103201-22-5; **11**, 103201-23-6; **12**, 103201-24-7; **13**, 103201-25-8; **14**, 103201-26-9; **15**, 103201-27-0; **16**, 103201-28-1; **17**, 103201-29-2; **18**, 103201-30-5; **19**, 103201-31-6; **20**, 103201-32-7; **21**, 103201-33-8; **22**, 103201-34-9; **23**, 103201-35-0; **24**, 103201-36-1; **25**, 103201-37-2; **26**, 103201-38-3; **27**, 103201-39-4; **28**, 103224-48-2; $C_2H_5NH_2$, 75-04-7; 2′,3′-*O*-isopropylideneadenosine, 362-75-4; adenosine-5′-uronic acid, 3415-09-6; ethyl adenosine-5′-uronate hydrochloride, 50663-70-2; ethyl adenosine-5′-uronate, 35803-57-7; cyclohexylamine, 108-91-8.

# Nonquaternary Cholinesterase Reactivators. 4. Dialkylaminoalkyl Thioesters of α-Keto Thiohydroximic Acids as Reactivators of Ethyl Methylphosphonyl- and 1,2,2-Trimethylpropyl Methylphosphonyl-acetylcholinesterase in Vitro

Clifford D. Bedford,* Michiko Miura, Jeffrey C. Bottaro, Robert A. Howd, and Harold W. Nolen III

*Organic Chemistry Program and Biomedical Research Laboratory, SRI International, Menlo Park, California 94025.*
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In the search for improved lipophilic centrally active acetylcholinesterase (AChE) antidotes, a series of α-keto thiohydroximates were prepared and evaluated for their ability to reactivate AChEs inhibited by ethyl *p*-nitrophenyl methylphosphonate (EPMP) and soman (GD). The compounds conformed to the general structure 4-$RC_6H_5C(O)C(NOH)S(CH_2)_nN^+R'R''\cdot X^-$ where R = H, $CH_3$, F, Br, Cl, $OCH_3$, CN; R′ = $CH_3$, $C_2H_5$, *i*-$C_3H_7$; R″ = H, $CH_3$; X = Cl, I; and *n* = 2, 3. In this series, varying R substituents on the aryl ring produced compounds with oxime p$K_a$ values from 6.8 to 8.0, optimum for an AChE reactivator. Increasing lipophilicity of the amine segment correlated with reactivator potency, as did electron-withdrawing groups on the aryl moiety, presumably due to increased binding to hydrophobic sites surrounding the AChE active site. The in vitro reactivation potency of the α-keto thiohydroximates approaches and even surpasses that of 2-PAM and toxogonin for GD-inhibited AChE. These initial findings point to additional structure–activity relationships to assist in the design of improved antidotal compounds.

Although the highly toxic nature of organophosphorus compounds has been known for many years,[1-10] there still exists serious limitations in the antidotal therapy available against poisoning of these compounds. Most toxic organophosphorus esters are irreversible inhibitors of acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7; AChE).[1,11-13] Conventional therapy against organo-

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