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Bis(dimethylamino)-*s*-triazinyl Antiinflammatory Agents

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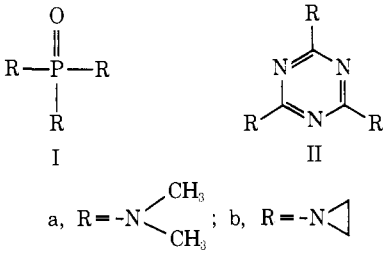
In 1964, Chang, Terry, and Borkovec[1] reported their remarkable discovery that dimethylamino analogs Ia and IIa of two cytotoxic aziridinyl compounds, tepa (Ib) and tretamine (IIb), retained the insect sterilizing properties of the latter but possessed greatly lowered toxicity to mammals. Because certain cytotoxic agents had been reported to have a beneficial effect on patients with rheumatoid arthritis,[2] we undertook the evaluation of Ia and IIa as antiinflammatory agents. Hexamethylphosphoramide (Ia) was completely without effect in a rat paw edema assay.[3] However, the significant reduction in paw volume produced by hexamethylmelamine (IIa) led us to synthesize the titled compounds in an effort to extend this activity to novel triazines.



I II

a, R = -N(CH3)2 ; b, R = -N(CH2)2

The new 6-alkyl- and 6-aryltriazines were obtained in fair to good yields by reacting 1,1,5,5-tetramethylbiguanide with the appropriate acid chlorides. Physical properties and pharmacological data for the compounds of most interest are presented in Table I. Antiinflammatory activity was evaluated using a kaolin-induced paw edema assay in rats.[3] The 48-hr LD_{50} was determined in mice after a single intraperitoneal injection.

During the course of this work several reports appeared in the patent literature describing the synthesis and antiinflammatory activity of 6-cycloalkyl- and 6-aryl-2,4-diamino-*s*-triazines.[4,5] At least one compound of this type, 6-phenyl-2,4-diamino-*s*-triazine, has been studied in human subjects and found to cause an acute rise in plasma corticosteroids without inducing other evidence of stress.[6] Because N-methylated congeners of drugs containing amino groups have sometimes exhibited decreased toxicity and/or modified biological activity,[7] we obtained and tested the analog of most interest in each of the reported diaminotriazine series (Table I, 6 and 7) for comparison with our tetramethyl derivatives.

As expected, the methylated aminotriazines **1–5** were less toxic than the corresponding primary amino derivatives **6** and **7**. The effect of methylation on antiinflammatory activity was variable but generally resulted in enhanced activity in the 6-cycloalkyltriazine series and unaltered or slightly reduced activity with 6-aryltriazines.

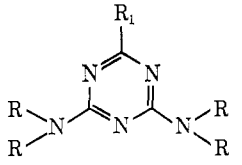
Compound **4**, the most effective analog in the present study, was also tested for its ability to inhibit inflammation using the granuloma pouch technique.[8] It provided 16, 46, and 100% inhibition of inflammation at dose levels of 7.5, 30, and 75 mg/kg, suggesting a potency of at least 2.5 times indomethacin in this assay.

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus equipped with a corrected thermometer. Analyses indicated by elemental symbols agree with calculated values within ±0.4%.

General Procedure for Preparation of Bis(dimethylamino)-*s*-triazines. A solution of the acid chloride (10.5 mmol) in 10 ml of acetone was added dropwise with cooling (ice–water bath) and vigorous stirring to a solution of 1,1,5,5-tetramethylbiguanide[9] in 10 ml of 5% aqueous sodium hydroxide. The resulting slurry was allowed to warm to room temperature with continued stirring over a period of 2 hr. The acetone was removed *in vacuo* and the product was isolated by filtration, washed with ice water, dried, and recrystallized from aqueous methanol. All of the triazines exhibited a moderately strong characteristic absorption at 795–810 cm^{-1} in the ir spectra (KBr). Yields and melting points of the triazines thus prepared are presented in Table I.

Table I. 2,4-Diamino-*s*-triazines



Compd no.	R_1	R	Mp, °C	Yield, %	Formula	Analyses	Anti-inflammatory activity[a]	LD_{50}, mg/kg
1	Cyclopropyl	CH_3	70–71	50	$C_{10}H_{17}N_5$	C, H, N	40	730
2	Cyclohexyl	CH_3	50–51	64	$C_{13}H_{23}N_5$	C, H, N	33	>1000
3	C_6H_5	CH_3	103–104	81	$C_{13}H_{17}N_5$	C, H, N	24	>1000
4	*p*-FC_6H_4	CH_3	121.5–122.5	83	$C_{13}H_{16}FN_5$	C, H, N	51	575
5	2-Furyl	CH_3	110–112	88	$C_{11}H_{15}N_5O$	C, H, N	27	575
6[b]	Cyclopropyl	H	294–297				22	333
7[c]	C_6H_5	H	226–228				33	545

[a] Except for **3** and **6**, compounds were tested at multiple dose levels between 12 and 150 mg/kg using seven animals per dose level. The figures given are per cent inhibition following a single po administration of the compound at 150 mg/kg. In our hands, indomethacin caused a 50% reduction in inflammatory response at 20–30 mg/kg in this rat paw edema assay.[3] [b] Lit.[1] 296–298°. [c] Purchased from Aldrich Chemical Co.

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Ethyl Adenosine-5′-carboxylate. A Potent Vasoactive Agent in the Dog

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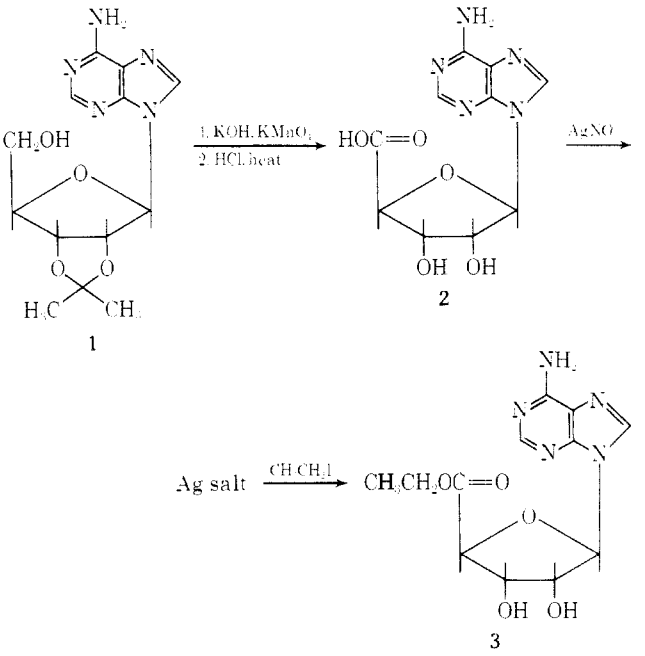
The activity of exogenous adenosine and adenosine nucleotides as coronary vasodilators is well documented.[1-3] The effect is of a short duration, however, probably because the compounds are rapidly metabolized and very quickly reach low, steady-state concentrations; adenosine itself is taken up rapidly by red blood cells and tissues[4] and converted to inosine[5] while phosphorylated derivatives do not cross biological membranes readily. Indeed, the hemodynamic effects are usually studied by administration *via* the coronary artery or by direct injection into the atrium. Berne, *et al.*,[6-9] argue that adenosine is an important physiological mediator of vasodilation. Although the exact role of adenosine has not been completely elucidated, there is little doubt that it is a potent, vasoactive substance.

As part of a program to prepare related compounds with similar activity but with a longer duration of action, ethyl adenosine-5′-carboxylate (3) was synthesized by the reaction sequence shown in Scheme I. The cardiovascular effects were tested in the dog preparation described by Schoepke, *et al.*,[10] in which blood pressure, heart rate, and coronary sinus Po_2 are continuously monitored. Changes in the last variable can be related to the coronary blood flow when oxygen extraction by the myocardium does not alter markedly. The *in vitro* effects on adenosine and adenylate deaminase were also assessed.

Scheme I. Synthesis of Ethyl Adenosine-5′-carboxylate



Results and Discussion

Typical cardiovascular data obtained are shown in Table I. As expected adenosine elicited marked responses of a short duration when given intravenously and essentially no effects when administered intraduodenally at doses as high as 30 mg/kg. On the other hand, 3 exhibited a marked, long-acting effect in the Po_2 test which, like intravenous adenosine, was initiated essentially instantaneously after intravenous administration of 0.10 mg/kg doses and lower; the intraduodenal activity, which became evident approximately 5 min after administration, was of particular significance since it indicated that 3 was well absorbed when given by this route and that it was not hydrolyzed rapidly to adenosine-5′-carboxylic acid. The latter was orders of magnitude less active in the preparation.

Table I. Effect on Cardiovascular Parameters in Anesthetized Dogs[a]

Compd	Injection route[c]	Dose, mg/kg	Po_2,[b] mm Change, %	Po_2,[b] mm Duration, min	Mean aortic pressure, mm Change, %	Mean aortic pressure, mm Duration, min	Heart rate, beats/min Change, %	Heart rate, beats/min Duration, min
Adenosine	iv	2	150	4	−50	2	−40	3
	id	15	3	15	0	0	−2	15
	id	30	8	20	0	0	0	0
3	iv	0.01	19	18	−1	9	5	5
	iv	0.05	104	32	−1	8	17	32
	iv	0.10	145	50	−5	26	17	50
	id	0.025	24	83	−3	60	13	65
	id	0.10	57	89	−2	60	2	36
	id	0.50	118	102	−25	88	34	75

[a] Peak changes are shown; the duration is the total time elapsed from the initiation of the change until return to pretest value. The range of the initial basal values of all the preparations was: Po_2, 15–25 mm; blood pressure, 90–120 mm; heart rate, 100–140 beats/min. The values cited are the averages of three measurements with a deviation of the order of 40%; iv dose responses were obtained in a given animal in three different preparations, while a total of six dogs were utilized to obtain the id data. [b] Po_2 = partial pressure of oxygen. [c] iv = intravenous; id = intraduodenal.