Table VIII. Inhibition of Muscarinic Responses of Guinea Pig Ileal Longitudinal Smooth Muscle by 1,4-Dihydropyridines

no.	ID_{50}, M[a]	act. (III = 1.0)	torsion angle,[b] deg
I	9.0×10^{-10}	6.0	16
II	5.0×10^{-5}	0.0001	28
III (nifedipine)	5.1×10^{-9}	1.0	20
IV	3.0×10^{-7}	0.017	30

[a] Measured against tonic (slow) response to the muscarinic agonist, *cis*-2-methyl-4-[(dimethylamino)methyl]-1,3-dioxolane methiodide (CD), according to our previously described method.[12] [b] Average of the two torsion angles (C_2-C_3-C_4-C_5, C_3-C_4-C_5-C_6, Table VI) describing the distortion from planarity of C_4 of the 1,4-dihydropyridine ring.

pig ileal longitudinal smooth muscle,[2,12] are given in Table VIII. The order of activity, I > III > IV > II, demonstrates, as noted previously,[2,7,8] the highly detrimental effects of 4-substitution in the phenyl ring. The high activity of the pentafluoro derivative (I), which contains a 4-F substituent, may be due to the activity-enhancing effects of the two ortho and two meta substituents, which are sufficient to overcome any detrimental effects of 4-substitution. Structural studies are continuing to test this point and to extend the structure–activity correlation. 2-Substituted compounds are generally somewhat more active than 3-substituted compounds[2,7,8] and consistent with this the 3-cyano derivative (IV) is less active than the 2-nitro derivative (III).

Because of the limited number of structures determined and the presumed importance of electronic, hydrophobic, and other physical chemical factors, any correlation between biological activity and solid-state structure can be, at best, of limited scope. However, there is some indication that activity does vary according to the planarity of the 1,4-dihydropyridine ring, as measured by torsion angles about C4 (Table VIII), activity increasing with increasing ring planarity.

Acknowledgment. This work was supported by NIH Grants HL 16003 and RO 545417. We appreciate the continued interest and support of our colleagues A. B. Triggle, J. A. Triggle, D. B. Shefter, and D. M. Shefter.

Supplementary Material Available: Crystallographic data for molecules I–IV (Table I); fractional atomic coordinates for I with estimated standard deviations $\times 10^{-4}$ (Table II); fractional atomic coordinates and estimated standard deviations $\times 10^{-4}$ for compounds II–IV (Table III); bond distances (Å) for compounds I–IV (Table IV); bond angles (degrees) for compounds I–IV (Table V); torsion angles for compounds I–V (Table VI); and intermolecular hydrogen bond data for compounds I–IV (Table VII) (7 pages). Ordering information is given on any current masthead page.

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Lipophilic and Hydrophilic Esters of 4-Acetyl-2-(2-hydroxyethyl)-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one as Antihypertensive Agents

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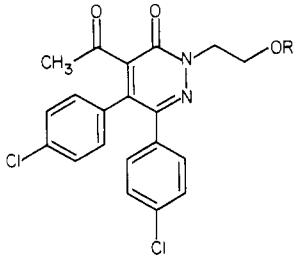
In an attempt to enhance the antihypertensive activity of 4-acetyl-2-(2-hydroxyethyl)-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one, 1, a series of lipophilic and hydrophilic esters was synthesized. These derivatives possessed increased lipid and aqueous solubility, respectively. The esters, in general, cause a larger blood-pressure drop than 1 when tested at high doses in the spontaneously hypertensive rat (SHR) model. At lower doses the antihypertensive activity is the same as with 1.

As described in the accompanying paper,[1] 4-acetyl-2-(2-hydroxyethyl)-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one (1) is active in the spontaneously hypertensive rat (SHR) and the deoxycorticosteroid (DOCA) rat models of hypertension. Evaluation of this compound established that, while a statistically significant reduction in blood pressure does occur following a dose of 3 mg/kg in these models, reduction of blood pressure to less than approximately 140 mmHg does not occur even at doses of 100 mg/kg. Indeed, a plot of the dose–response relationship for this compound plateaus at less than 50 mg/kg. It was also observed that 1 has limited solubility in both water and lipophilic solvents. In order to determine if the efficacy of this compound could be improved, a series of esters of the 2-hydroxy function was synthesized. The initial series which we will describe is a series of alkyl and aryl esters which were designed to have higher solubility in lipid solvents. The second series that we will discuss are amino acid esters of 1 with increased water solubility. The synthesis of amino acid derivatives as potential prodrugs has been reported previously.[2,3]



1, R = H
2, R = $COCH_3$

(1) R. Buchman, J. A. Scozzie, Z. S. Ariyan, R. D. Heilman, D. J. Rippin, L. J. Powers, and R. J. Matthews, *J. Med. Chem.*, under articles section in this issue.

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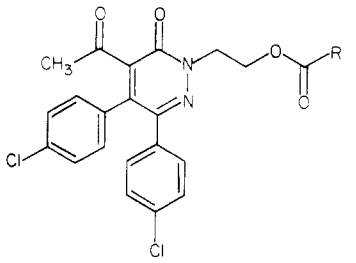
Table I. Esters of 2-(2-Hydroxyethyl)pyridazinones



no.	R	mp, °C	solvent	% yield	synth method	formula
2	CH_3	99-102		63	A	$C_{22}H_{18}Cl_2N_2O_4$
3	$C(CH_3)_3$	45-49		49	A	$C_{25}H_{24}Cl_2N_2O_4$
4	CH_2CH_3	82-86	C_6H_{12}	48	A	$C_{23}H_{20}Cl_2N_2O_4$
5	$CH(CH_3)_2$			37	A	$C_{24}H_{22}N_2Cl_2O_4$
6	$(CH_2)_{16}CH_3$	43-46		24	A	$C_{38}H_{50}Cl_2N_2O_4$
7	C_6H_5	98-100		45	A	$C_{27}H_{20}Cl_2N_2O_4$
8	$CH_2C_6H_5$	134-135	CH_2Cl_2-C_6H_{14}	40	A	$C_{28}H_{22}Cl_2N_2O_4$
9	CH_2NH-Cbz	84.5-86	MeCN	69	C	$C_{30}H_{25}Cl_2N_2O_6$
10	CH(NH-Cbz)$C(CH_2)_3NHC(NH)NHNO_2$	85-87		21	B	$C_{34}H_{33}Cl_2N_7O_8$
11	$CH(NH\text{-Cbz})CH_2C_6H_5$	59-60		42	B	$C_{37}H_{31}Cl_2N_3O_6$
12	$(CH_2)_2NH$-Cbz	49-52		33	D	$C_{31}H_{27}N_3Cl_2O_6$
13	$CH(NH\text{-Cbz})CH(CH_3)_2$	112-113	MeCN-Et_2O	36	B	$C_{33}H_{31}N_3Cl_2O_6$
14	CH_2NH-*t*-Boc	60-63		54	D	$C_{27}H_{27}N_3Cl_2O_6$
15	$CH(^+NH_3Br^-)(CH_2)_4NH_3^+Br^-$	125 s[a]	FD[b]		B, E	$C_{26}H_{28}Cl_2N_4O_4 \cdot 2HBr$
16	$CH_2NH_3^+Br^-$	110 s[a]	FD		E	$C_{22}H_{19}Cl_2N_3O_4 \cdot HBr$
17	$CH_2NH_3^+Cl^-$	127-130	FD	66	F	$C_{22}H_{19}Cl_2N_3O_4 \cdot HCl$
18	$CH(^+NH_3Cl^-)(CH_2)_4NH_3^+Cl^-$	140-144	FD	40	B, E	$C_{26}H_{28}Cl_2N_4O_4 \cdot 2HCl$
19	$CH(^+NH_3Cl^-)CH_2C_6H_5$	123-126	FD	52	E	$C_{29}H_{25}Cl_2N_3O_4 \cdot HCl$
20	$CH(^+NH_3Cl^-)CH(CH_3)_2$	124-128	FD	31	E	$C_{25}H_{25}Cl_2N_3O_4 \cdot HCl$[c]
21	$(CH_2)_2NH_3^+Cl^-$	138-140	FD	75	E	$C_{23}H_{21}Cl_2N_3O_4 \cdot HCl$
22	$CH(^+NH_3Cl^-)(CH_2)_2SCH_3$	102-105	FD	43	D, G	$C_{25}H_{25}Cl_2N_3O_4S$

[a] s = softens. [b] FD = freeze-dried. [c] Anal. H: calcd, 5.1; found, 4.5. Cl: calcd, 19.1; found, 19.0.

Chemistry. The aliphatic and aromatic esters were synthesized from 1 and the appropriate acid chloride (Table I). Amino acid esters were obtained by coupling a protected amino acid and 1 using standard methods of peptide synthesis. Dicyclohexylcarbodiimide (DCC)[4] or the mixed anhydride method[5] using isobutyl chloroformate or pivaloyl chloride was employed.

The Cbz groups in *N*-(carbobenzyloxy)glutamine, *N*-(carbobenzyloxy)glutamic acid γ-benzyl ester, and *N*-(carbobenzyloxy)nitroarginine proved to be very resistant to cleavage. Hydrogenation in acetic acid using 10% palladium on carbon as a catalyst did not remove the Cbz groups from these amino acid derivatives. The use of 70% hydrogen fluoride in pyridine[6] also failed to cleave the protecting groups of the glutamic acid ester. The *N*-*t*-Boc group of 14 was removed by treatment with hydrogen chloride in dioxane. The *N*-Cbz group of **9**, **11**, **12**, and **13** was removed by treatment with hydrogen bromide in acetic acid. The amino acid esters were found to be very water soluble, having an aqueous solubility of >200 mg/mL relative to 1, suggesting a 10^5 increase in aqueous solubility as the result of this derivatization.

Biological Activity. The activity of the lipophilic and hydrophilic esters of 1 is tabulated in Table II. The greatest differences in antihypertensive activity are seen at the higher dose levels where some of the esters cause a greater reduction in blood pressure. As illustrated in Figure 1 for **2**, the peak effect on blood pressure is at least 8 h postdose. This is true for 1, the lipophilic esters, and the hydrophilic esters.

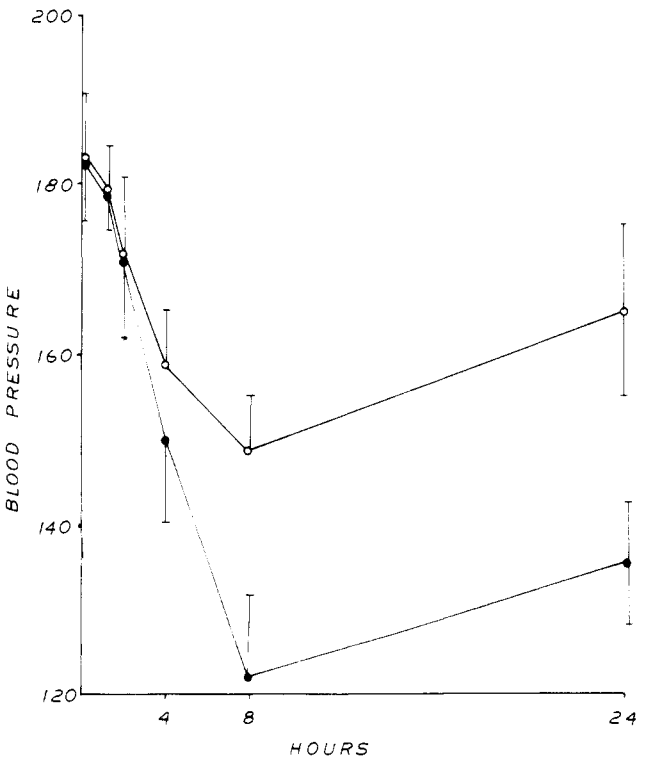


Figure 1. Effect on blood pressure after administration of compound 2: (○) 30 mg/kg; (●) 100 mg/kg. Points represent the average blood pressure of five animals. Vertical bars indicate the standard deviation ($n = 5$). For clarity, the bars are shown on only one side of each point for the 0, 1, 2, 4, and 8 h readings.

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Table II. Antihypertensive Activity. SHR Model

compd[a]	po dose, mg/kg	av blood pressure,[a] mmHg predose	4 h PD 1	24 h PD 1	4 h PD 2	24 h PD 2
1	100	218	173[b]	185	174[b]	193
	30	213	200	192	164[b]	206
2	100	182	150[b]	132[b]	122[b]	143[b]
	30	183	159[b]	165	140[b]	152[b]
	10	183	162[b]	181	149	176
3	30	205	200	202	158[b]	205
4	30	181	133[b]	136[b]	129[b]	168
6	30	181	168	162[b]	142[b]	137[b]
7	30	173	116[b]	139[b]	136	130[b]
8	30	174	165	154	146[b]	127[b]
15	50	231	179[b]	173[b]		
	17	189	165	162	143[b]	175
16	13	182	177	154[b]	145[b]	170
17	100	178	138[b]	131[b]	124[b]	166
	12	199	164[b]	161[b]	153[b]	164[b]
	4	184	162[b]	189	161	175
18	15 ia[c]	199	183	159[b]	171	
	15 sc[d]	181	169[b]	171	166	185
21	13	215	210	215	195[b]	209
α-Me-Dopa	100	178	161[b]	174	155[b]	166
guanethidine	50	187	154[b]	166[b]	138[b]	186[b]

[a] The following compounds (dose mg/kg) did not give a significant lowering of blood pressure at 4 or 24 h postdose (PD): 5 (30 mg/kg), 9–13 (100 mg/kg), 18 (15 mg/kg), 19 (15 mg/kg), 20 (14 mg/kg), and 22 (15 mg/kg). Compound 14 was not screened due to insufficient sample. [b] Indicates that level of significance $p = <0.05$ by the Student "t" test. [c] ia = intra-arterial. [d] sc = subcutaneous.

While the glycine ester **17** and the acetate ester **2** both cause slightly greater reductions in blood pressure after 4 h than does **1**, none of the compounds reduce the blood pressure to normal. After 9 h (Figure 2), **1** is more potent than **2** at low doses. The dose–response relationship for **2** is linear and shows a reduction of blood pressure to near normal at the highest dose tested (100 mg/kg). The reductions in blood pressure caused by administering higher doses of **1** are not as great as would be expected from a projection of a linear dose–response relationship for the lower doses. This is consistent with a limited bioavailability of **1** at higher doses.

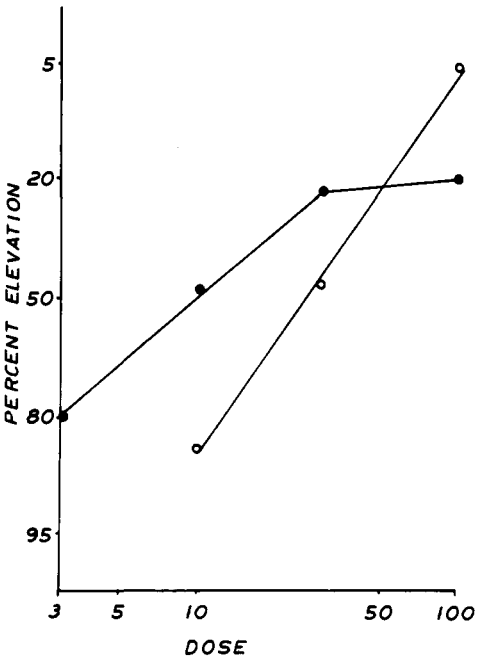

Figure 2. Dose–response relationships for compounds **1** (●) and **2** (○). Blood pressure readings are 9 h postdose in the SHR model.

Experimental Section

Elemental analyses were performed by the Central Analytical Department of Diamond Shamrock or by Galbraith Laboratories, Knoxville, Tenn. All analyses are within ±0.4% of calculated values, except as indicated in Table I. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Structural assignments are supported by IR, NMR, and, where necessary, mass spectra. Homogeneity of all compounds was evaluated prior to submission of the compounds for biological evaluation by silica gel TLC. Each method utilized to form the amino acid esters is illustrated using the glycine analogue (**17**) as an example.

Method A. The 2-(2-hydroxyethyl)pyridazinone (1 equiv), Et_3N (1.1 equiv), and acyl chloride (or anhydride) (1.1 equiv) were allowed to react in dry THF at 20 °C for 48 h. The reaction mixture was then filtered and the THF removed in vacuo. The residue was partitioned between EtOAc and 5% $NaHCO_3$, and the organic layer was separated and evaporated onto dry column silica gel. The residue from this evaporation was placed on a silica gel dry column and eluted with EtOAc/cyclohexane (1:3). The UV-absorbing region was separated and extracted with EtOAc, and the solvent was evaporated to give the product.

Method B. *N*-(Carbobenzyloxy)glycine (10.0 g, 0.0478 mol) and 200 mL of freshly distilled pyridine were cooled in an ice bath under an argon atmosphere, which was maintained during the experiment. To this cooled solution was added 10.0 g (0.0484 mol) of DCC in 25 mL of freshly distilled pyridine. A dropwise addition of 20.0 g (0.0496 mol) of **1** in 100 mL of freshly distilled pyridine was initiated, maintaining a temperature of <5 °C. Upon completion of this addition, the reaction was stirred for 10 min and the ice bath removed. After the reaction was stirred for 15 h at 20 °C, 1.0 g (0.0048 mol) of *N*-(carbobenzyloxy)glycine and 1.0 g (0.0048 mol) of DCC were added, and the reaction was stirred for 2 h at 20 °C. The reaction mixture was filtered to remove the precipitated dicyclohexylurea (DCU), and the precipitate was washed with pyridine and xylene. The filtrate was cooled in the refrigerator for 1.5 h and refiltered, and the filtrate was concentrated under reduced pressure. The residual light green oil was chromatographed on a dry silica gel column (1 m × 70 mm flat diameter) using 50:50 EtOAc/$CHCl_3$ as eluent. The UV-absorbing (R_f 0.65) portion of the column was extracted and filtered, and the filtrate was dried (Na_2SO_4). Concentration under reduced pressure afforded a crude light green oil that was crystallized from MeCN and petroleum ether to afford white crystals of **9**.

Method C. $SOCl_2$ (2.62 g, 0.022 mol), DMF (1.46 g, 0.020 mol), and 75 mL of $CHCl_3$ were stirred at 20 °C for 1.0 h, then *N*-(carbobenzyloxy)glycine (4.18 g, 0.020 mol) and EtOAc (75 mL) were added, and stirring was continued for 4 h at 20 °C. **1** (4.03

g, 0.010 mol) was then added and the reaction was stirred at 20 °C for 45 h. The reaction mixture was washed with 10% NaOH solution and the organic layer dried (Na_2SO_4). After filtration, the solution was concentrated under reduced pressure and the residue placed on a dry silica gel column. Elution with 90:10 $CHCl_3$/EtOAc afforded the desired compound **9** (R_f 0.35) as a white powder: yield 1.15 g (23%).

Method D. To a stirring mixture of *N*-(*tert*-butoxycarbonyl)glycine (7.01 g, 0.04 mol), Et_3N (4.05 g, 0.04 mol), and 250 mL of dry THF in an ice bath under an argon atmosphere was added pivaloyl chloride (4.9 mL, 4.81 g, 0.04 mol) at one time. After the mixture was stirred for 0.3 h, Et_3N (2.02 g, 0.02 mol) was added followed by the dropwise addition of 1 (8.07 g, 0.02 mol) in 100 mL of dry THF, maintaining the reaction temperature at <5 °C. The ice bath was removed and the reaction stirred at 20 °C for 15 h. Since TLC [Quantum MQ6F plates, EtOAc/$CHCl_3$ (1:1), UV visualization] indicated that 1 was still present, the reaction mixture was cooled in an ice bath and 7.01 g (0.04 mol) of *N*-(*tert*-butoxycarbonyl)glycine and 7.08 g (0.06 mol) of Et_3N were added. Pivaloyl chloride (4.80 g, 0.04 mol) was then added dropwise, maintaining the reaction temperature at <5 °C. After this addition, the ice bath was removed and the reaction was stirred at 20 °C for 18 h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure. The residue was taken up in EtOAc and washed sequentially with 10% NaOH solution, H_2O, and saturated NaCl solution. The organic layer was concentrated under reduced pressure and the residue chromatographed on dry silica gel column (1.3 m × 50 mm flat diameter) using EtOAc/$CHCl_3$ (1:1) as developer. The compound at R_f 0.60 was collected and rewashed with 10% NaOH solution to remove residual pivaloyl chloride. The organic layer was concentrated under high vacuum, where it foamed and crystallized affording 6.04 g of yellow solid, **14**.

Method E. Compound **9** (20.0 g, 0.34 mol) and 50 mL of glacial HOAc were stirred at 20 °C under an argon atmosphere. To this solution was added 50 mL of 4 N HBr/HOAc and the reaction was stirred for 1.25 h. The yellow reaction mixture was poured slowly into anhydrous Et_2O. The precipitate was washed with anhydrous Et_2O and the solvent decanted. The residue was dissolved in water, frozen, and lyophilized to produce a fluffy, off-white solid **16**: yield 15.21 g. To a solution of 13.13 g (0.024 mol) of **16** in 200 mL of H_2O was added 50 mL of 10% NaOH solution. The solution was extracted with EtOAc, and the combined organic layers were washed with a saturated NaCl solution and dried (Na_2SO_4). After filtration, the filtrate was concentrated to afford a yellow oil, which was dissolved in anhydrous Et_2O and poured slowly into an Et_2O–HCl solution (25 mL of 6 M HCl/dioxane to 600 mL of anhydrous Et_2O). The precipitate was washed with anhydrous Et_2O, dissolved in H_2O, frozen, and lyophilized. After 90 h, a light yellow powder (7.85 g) of the desired HCl salt, **17**, was harvested as a monohydrate.

Method F. Compound **9** (1.0 g, 1.68 mmol), 10% Pd/C (300 mg), and 50 mL of glacial HOAc were mixed and placed on a Parr hydrogenator. The mixture was shaken under 15 psi of H_2 for 16 h at 20 °C and then filtered through a Celite filter pad. The filtrate was made basic with a 10% NaOH solution and extracted with EtOAc. The organic phase was washed with saturated NaCl solution, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The red residue was dissolved in Et_2O and then slowly poured into Et_2O–HCl (35 mL of 6 M HCl/dioxane in 250 mL of anhydrous Et_2O). The precipitate was washed three times with 600-mL portions of anhydrous Et_2O. Yield of the desired salt, **17**, was 0.33 g.

Method G. A solution of 1.50 g (0.00268 mol) of **14**, 25 mL of dioxane, and 25 mL of 6 M HCl/dioxane was stirred at 20 °C for 2.75 h and then concentrated using high vacuum. The residue was poured into anhydrous Et_2O and the precipitate was washed two times with anhydrous Et_2O (2 × 600 mL). The solid residue was dissolved in H_2O, frozen, and lyophilized. After 45 h, the lyophilization afforded a white fluffy powder (1.16 g) of the desired salt, **17**, as the trihemihydrate.

Antihypertensive Activity. The antihypertensive activity of the compounds was determined by Pharmakon Laboratories, Scranton, PA, under the direction of Richard J. Matthews. The experimental procedure for these determinations is reported in the accompanying paper.[1]

Some Short-Chain N^6-Substituted Adenosine Analogues with Antitumor Properties[1]

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The compounds N^6-allyl-, N^6-isopropyl-, N^6-propargyl-, and N^6-(2-methylallyl)adenosine were prepared by reacting 6-chloropurine riboside with an excess of the corresponding amines in ethanol, in the presence of two acid acceptors resulting in virtually quantitative yields. The compounds showed biological activity in a number of in vitro and in vivo tumor cell systems. Very good increases in life spans of mice bearing mammary carcinoma were obtained by treatment with the N^6-allyl, N^6-isopropyl, and N^6-propargyl analogues, respectively. In rats, the N^6-allyl analogue slowed the rate of transplantable mammary tumor growth by one-fourth. The short-chain adenosine analogues are more active in the treatment of animal carcinomas than in the leukemia or sarcoma tumor cell systems.

A number of N^6-substituted adenosine analogues containing five or more carbon atoms in the substituent chain[2-4] including one with a nitrogen mustard moiety[5] were synthesized and examined in recent years, and a significant proportion of these were found to possess antitumor activity in a variety of in vitro systems. Several of the compounds also had moderate activity against murine L-1210 leukemia in vivo, with an increase in life span of 41–50% over controls. An interesting finding was that these compounds had no cytotoxic activity against a leukocyte cell line in vitro which had originated from normal cells, while against certain cultures of tumor cell

(1) Presented in part before the Division of Medicinal Chemistry of the American Chemical Society–Japan Chemical Society Congress in April, 1979. See M. H. Fleysher, R. J. Bernacki, and E. Mihich, "Abstracts of Papers", ACS/CSJ Chemical Congress, Honolulu, HI, Apr 1-6, 1979, American Chemical Society: Washington, D.C., 1979, Abstr MEDI 29.

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