Potential Antitumor Agents. 37. Organophosphorus Derivatives of 9-Anilinoacridine

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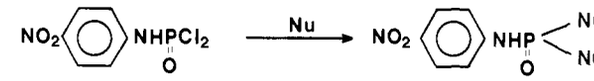
A series of 9-anilinoacridine derivatives substituted in the anilino ring with a variety of phosphoramide and related substitutents has been prepared, and the antitumor activity has been evaluated both in vivo and in vitro against the L1210 or P-388 mouse leukemia systems. The DNA-binding properties were measured using the ethidium displacement method, and the structural requirements for strong binding were found to differ from those for antileukemic activity. For high biological activity a marked preference for oxygen-containing substituents on the phosphorus atom was noted, while for high DNA binding a requirement for nitrogen-containing or cyclized substituents was observed. The most active congeners, as assayed in both in vitro and in vivo systems, were comparable in activity to the clinically utilized anilinoacridine derivative *N*-[4′-(9-acridinylamino)-3′-methoxyphenyl]methanesulfonamide (*m*-AMSA, amsacrine).

In earlier work from this laboratory with the 9-anilinoacridines, the marked antitumor effect of the 1′-methanesulfonamide derivative (compound 1, Table I) was revealed.[1] A search for more potent congeners culminated in the development of the clinical agent 4′-(9-acridinylamino)methanesulfon-*m*-anisidine (*m*-AMSA, 2).[2,3] As a further extension of this study, we decided to investigate the effect of replacing the sulfonamide group with a range of phosphorus-containing substituents. We have previously demonstrated that both DNA binding and agent lipophilic–hydrophilic balance are major factors in determining the level of antitumor activity,[4] and it was expected that the wide structural variation available in the phosphorus series would provide many opportunities for optimizing these parameters.

Chemistry. The formation of the organophosphorus anilinoacridines involved mild acid-catalyzed coupling of 9-chloroacridine with the requisite aromatic amine component in methanol. The latter compounds were obtained by catalytic hydrogenation (Pd/C) of the analogous nitro- or benzylurethane derivatives and were normally coupled directly without further isolation. The nitro aromatic species were generally prepared by published procedures, or by nucleophilic attack on *N*-(4-nitrophenyl)phosphoramidic dichloride (3) (Scheme I).

Benzyl *N*-(4-aminophenyl)carbamate (**4a**) was obtained by reduction (Fe/H$^+$) of the analogous nitro precursor and was used in place of 4-nitroaniline for the majority of the phosphorylation reactions because of the greater reactivity of its amino group (Scheme II). The phosphoryl substrates were all prepared by known literature procedures[5] (see Experimental Section), and coupled with amine **4a** in pyridine.

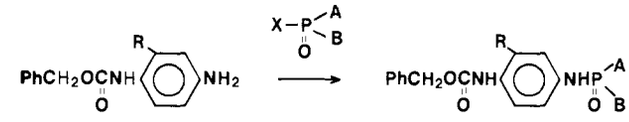
Scheme I[a]



3

[a] Nu = nucleophile, e.g., alkoxy, alkylamino, or dialkylamino.

Scheme II[a]



4a, R = H
b, R = OCH_3

[a] X = Cl or Br; A, B = alkyl, alkoxy, or alkylamino.

Results

The antitumor and DNA binding results for all of the phosphorus-containing 9-anilinoacridines are summarized in Table I, together with data from related examples of some sulfur- and carbon-containing congeners.

The dimethylphosphinamide derivative **5** was synthesized first as the compound bearing the closest resemblance to the active antitumor agent 1. However, although the DNA binding is superior to that of 1, **5** shows only weak cytotoxic activity. Analogues containing nitrogen and oxygen substituents were then prepared.

The tetramethyl triamide **7** shows a slight improvement in in vivo activity compared to **5**, while the very water-soluble dimethyl derivative **6** is less active. The high DNA binding but low activity shown by compounds **5** and **6** is to be noted, as it clearly demonstrates that factors other than a high DNA affinity play a major part in determining the antitumor activity of this series of compounds. Inclusion of the cyclic portion of the antitumor agent cyclophosphamide gave compound **8**, which displayed greater in vitro activity than the earlier congeners, despite showing a lower in vivo activity. The trend toward greater in vitro activity was continued when a second oxygen atom was included in the ring, and, in fact, the cyclic phosphoramidates **9** and **10** both display significant in vivo activity as well.

On moving from the cyclic compounds **9** and **10** to the acyclic diester **11**, further improvement results, and the ILS_{max} for the P-388 system is comparable with that of *m*-AMSA (**2**). The dose potency of **11** is lower than that of *m*-AMSA but higher than that of AMSA (compound 1). The in vitro activity data for **11** are actually superior to that of both *m*-AMSA and AMSA.

The diethyl diester **12** is much less active than its dimethyl analogue **11**, and this is possibly due either to its greater lipophilic character or to an unfavorable steric interaction involving the 1′-substituent and its putative target. The higher DNA binding of **12** compared to that

† Deceased January 1981.

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(4) Part 36: W. A. Denny, B. F. Cain, G. J. Atwell, C. Hansch, A. Panthananickal, and A. Leo, *J. Med. Chem.*, **25**, 276 (1982).
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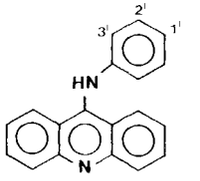
Table I



no.	substituents	mp, °C	formula	anal.[a]	log K[b]	ID_{50},[c] nM L1210	ID_{50},[c] nM P-388	opt dose,[d] mg/kg (P-388)	ILS_{max}[e] (P-388)
1	1'-$NHSO_2CH_3$		*f*		6.15	35	40	150	93
2	1'-$NHSO_2CH_3$, 3'-OCH_3		*g*		5.57	35	30	13.3	78
5	1'-$NHPO(CH_3)_2$	190-191	$C_{21}H_{20}N_3OP$	*h*	6.81	1300	1000	65	18
6	1'-$NHPO(NHCH_3)_2$	198 dec	$C_{21}H_{22}N_5OP \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl	6.84	2500	2000	50	11
7	1'-$NHPO[N(CH_3)_2]_2$	146-147	$C_{23}H_{26}N_3OP \cdot 0.25H_2O$	C, H, N	6.08	1500	1300	50	29
8		219 dec	$C_{22}H_{21}N_4O_2P \cdot 0.25H_2O$	C, H, N	6.58	800	450	50	9
9		225 dec	$C_{22}H_{20}N_3O_3P \cdot HCl$	C, H, N, Cl	6.78	110	50	30	47
10		250 dec	$C_{21}H_{18}N_3O_3P \cdot HCl$	C, N, Cl; H[i]	6.52	180	110	30	54
11	1'-$NHPO(OCH_3)_2$	189 dec	$C_{21}H_{20}N_3O_3P$	C, H, N	6.26	15	10	33	74
12	1'-$NHPO(OC_2H_5)_2$	210 dec	$C_{23}H_{24}N_3O_3P \cdot HCl$	C, H, N, Cl	6.47	230	200	110	28
13	1'-$NHPO(CH_3)OCH_3$	215 dec	$C_{21}H_{20}N_3OP \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl	6.34	100	70	38	49
14	1'-$NHPO_2(OCH_3)^-Na^+$	220 dec	$C_{20}H_{17}N_3NaO_3P \cdot 1.5H_2O$	C, H, N	5.37	2000	4000	nt	
15	1'-$NHPO_2NH_2^-Na^+$	217 dec	$C_{18}H_{16}NaN_4O_2P \cdot 0.5H_2O$	C, H, N	4.83	1800	2800	nt	
16	2'-$NHPO(OCH_3)_2$	215 dec	$C_{21}H_{20}N_3O_3P \cdot HCl$	C, H, N, Cl	5.66	1800	1400	250	34
17	2'-$NHSO_2CH_3$		*f*		5.73	110	90	66	53
18	1'-$CH_2PO(OCH_3)_2$	225 dec	$C_{22}H_{21}N_2O_3P \cdot HCl$	C, H, N, Cl	5.67	670	1400	125	2
19	1'-$CH_2SO_2CH_3$	270 dec	$C_{21}H_{18}N_2O_2S \cdot HCl$	C, H, N, Cl	5.76	250	270	330	49
20	1'-$CONHPO(OCH_3)_2$	224 dec	$C_{22}H_{20}N_3O_4P \cdot HCl$	C, H, N, Cl	5.56	>7900	4800	190	12
21	1'-$NHPO(OCH_3)_2$, 3'-OCH_3	135 dec	$C_{22}H_{22}N_3O_4P$	C, H, N	5.64	70	36	8.9	67
22	1'-$NHCO_2CH_3$		*f*		6.36	60	26	30	98
23	1'-$NHCOCH_3$		*f*		6.30	100	54	30	57
24	1'-$NHCONHCH_3$		*j*		6.24	100	86	30	53

[a] Analyses for the indicated elements were within ±0.4% of the calculated values for the formula provided. [b] Association constant for binding to poly(dA-dT), determined as previously described.[14] [c] Concentration of drug inhibiting growth of cell cultures by 50% over 3 days.[13] [d] Optimal dose given by intraperitoneal injection of drug solution in 0.1 mL of 30% aqueous ethanol on days 1, 5, and 9 after ip injection of 10^6 P-388 tumor cells. [e] Percentage increase in life at optimal dosage. Control animals died on average 11 days after tumor inoculation. Values of less than 20% are statistically nonsignificant. No long-term survivors were recorded. [f] Reference 1. [g] Reference 2. [h] Structure confirmed by ^1H NMR: δ 1.55 (d, 6 H, J = 14 Hz, P-CH_3). [i] H: calcd, 5.5; found, 4.5. [j] Reference 6.

of 11 tends to argue against the latter possibility, although it does not rule out the possibility that there is some form of interaction that preferentially favors a methyl ester group over the ethyl analogue. Thus, compound **13**, which only contains a single methyl ester group, is actually more biologically active than **12** even though the presence of a methyl substituent was previously shown to produce a compound of low activity (compound **5**).

To further investigate drug–target site interactions, we prepared and tested the methyl phosphoramidate **14**. Very poor in vitro antitumor activity was observed, together with low DNA binding, which is presumably caused by anionic repulsions between the charged phosphoramidate and DNA phosphate oxygens. Similar results were obtained with the charged diamide **15**.

With the 1′-$NHPO(OCH_3)_2$ side chain having been established as the best phosphoramide substituent studied, the effect of position was next investigated. The 2′-substituted isomer **16** was prepared but found to be much less active than **11**. Thus, there appears to be an orientational requirement associated with the activity of the $NHPO(OCH_3)_2$ group that is more severe than that for the methanesulfonamide group, as shown by a comparison between compounds **1** and **17**.

A loss in activity was found on replacing the amide NH group with a CH_2 group (compound **18**); yet, interestingly, the sulfur-containing congener **19** still shows appreciable activity. Insertion of a carbonyl group between the aromatic ring and the amide nitrogen was also investigated (compound **20**), but again only low antitumor activity was observed. Thus, the amide NH group was confirmed as being the best link group.

Finally, having established that the 1′-$NHPO(OCH_3)_2$ substituent gave the best results in terms of antileukemic activity, we next included the 3′-OCH_3 group of *m*-AMSA (**2**) to see if the resulting compound (**21**) would show the same increase in dose potency that occurs between **1** and **2**. The increase in dose potency was actually observed, and compound **21** was slightly more dose potent that *m*-AMSA.

Discussion

In the results obtained for the above phosphorus-containing 9-anilinoacridines, several factors appear important. As with the sulfur-containing derivatives, greatest activity is seen with amide substituents at the 1′-position on the anilino ring. For high antitumor activity, a strong preference for oxygen-containing substituents was noted in the phosphorus series, with nitrogen- and alkyl-containing substituents being less favorable. Interestingly, this result is similar to that observed in the related carboxamide series where the methoxy-substituted carbamate confers greater activity than the methyl or methylamino analogues (compounds **22–24**, cf. L1210 data in ref 6). Extrapolating to the sulfonamide series, we predict that if the 1′-$NHSO_2OCH_3$ substituent were to be chemically stable, it would have to produce compounds of even greater activity than AMSA or *m*-AMSA (**1** and **2**).

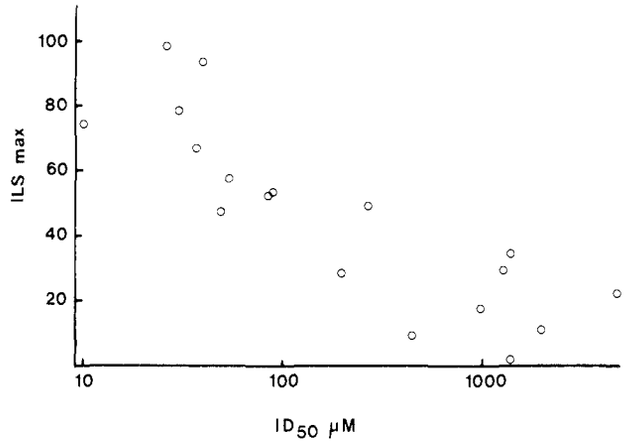


Figure 1. Correlation of in vivo and in vitro P-388 antitumor activity for compounds in Table I.

One feature of the results is the significant correlation between in vitro and in vivo biological activity (Figure 1). The correlation coefficients for regressions of ILS_{max} vs. log ID_{50} for P-388 and L1210 cell cultures are 0.85 and 0.83, respectively. The correlations are somewhat better than that obtained previously for a wider series of 9-anilinoacridine derivatives[13] and illustrate the usefulness of the cell culture method for rapid assessment of the antileukemic potential of newly developed agents.

Experimental Section

Where analyses are indicated only by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Analyses were performed by Dr. A. D. Campbell, Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal melting point apparatus with the maker's supplied stem-corrected thermometer; melting points are as read.

To monitor the progress of reactions, purified products, etc., we used TLC on SiO_2 (Merck SiO_2, F_{254}). The most convenient solvents were the top phase of *n*-BuOH–HOAc–H_2O (5:1:4, v/v) and $CHCl_3$ containing 10% MeOH.

Each of the intermediate compounds has been numbered so as to indicate which of the acridine derivatives in Table I is derived from it.

Standard Coupling Procedure. A solution of 9-chloroacridine (5 mmol) in 50 mL of methanol containing 1 drop of concentrated NH_3 (aq) was combined with a methanolic solution of the substituted aniline component (5.25 mmol), and two drops of concentrated HCl were added to initiate the reaction, which was evidenced by the appearance of a deep-red color. (The amine components were normally obtained in solution, without further isolation, by hydrogenation of the appropriate nitro- or benzylurethane precursors.) After 5–10 min, the solution was concentrated to a small volume and allowed to stand as crystallization commenced. After being diluted with ethyl acetate to ensure complete crystallization, the mixture was filtered and the dark-red hydrochloride salt was washed well with dry acetone. The product was purified by recrystallization from MeOH–EtOAc.

Conversion of the hydrochloride salt to the free base was achieved by the addition of 1.1 equiv of $KHCO_3$ (aq) to an aqueous methanolic solution of the salt. Removal of the methanol gave the free base, which was recrystallized from either aqueous methanol or anhydrous solvents, such as ethyl acetate or benzene.

***N*-(4-Nitrophenyl)phosphoramidic Dichloride (3).**[7] A mixture of 4-nitroaniline (75 g, 0.51 mol) and freshly distilled $POCl_3$ (150 mL) was heated to reflux on an oil bath and main-

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tained at that temperature until HCl evolution ceased (2–3 h). The homogeneous solution was allowed to cool, and the dichloride (3) that crystallized out on standing was filtered off, washed with petroleum ether, and dried: yield 130 g (94%).

Benzyl *N*-(4-Aminophenyl)carbamate (4a). A mixture of 4-nitroaniline (20 g, 0.15 mol) and MgO (5 g) in 150 mL of anhydrous acetone was treated with 30 mL of commercial benzyl chloroformate, and the resulting slurry was stirred vigorously at room temperature with occasional warming to dissolve the precipitate. After 12 h, DMF (25 mL) was added, and the mixture was heated to dissolve the product and filtered. Removal of the acetone and crystallization from aqueous DMF gave 34.4 g (87%) of the nitrourethane, mp 155–156 °C. Anal. ($C_{14}H_{12}N_2O_4$) C, H, N.

The nitrourethane was dissolved in 100 mL of DMF, 30 mL of water, and 10 mL of concentrated HCl, and to the hot solution was added, in portions, Fe powder (20 g) at a rate so as to maintain gentle reflux. After a further 30 min at reflux, the mixture was treated with 30 mL of concentrated NH_3 (aq) and filtered through Celite, and the solvent was removed in vacuo. The residue was extracted with aqueous methanesulfonic acid, and the solution was clarified with charcoal–Celite and neutralized with NH_3 (aq) to precipitate the product (**4a**): yield 33 g (92%); mp 102–103 °C [EtOH (aq)]. Anal. ($C_{14}H_{14}N_2O_2$) C, H, N.

The aminophenylurethane **4a** was also obtained by selective acid hydrolysis of the acetyl group from the product obtained by reaction of benzyl chloroformate with 4-aminoacetanilide, mp 200–201 °C (acetone). Anal. ($C_{16}H_{16}N_2O_3$) C, H, N.

Benzyl *N*-(3-Methoxy-4-aminophenyl)carbamate (4b). 2-Methoxy-4-nitroaniline was treated with benzyl chloroformate as above to give benzyl *N*-(2-methoxy-4-nitrophenyl)carbamate in 94% yield, mp 130–131 °C. Anal ($C_{15}H_{14}N_2O_5$) C, H, N. Reduction of the nitro compound with Fe/H^+ as before gave the desired amine **4b**: yield 97%; mp 77–78 °C (toluene–petroleum ether). Anal. ($C_{15}H_{16}N_2O_3$) C, H, N.

General Procedure for Phosphorylations. A solution of amine **4a** (2.42 g, 10 mmol) in pyridine (10 mL) was treated with 1.5–2.0 equiv of the phosphoric halide at 0 °C, and the mixture was allowed to warm slowly to room temperature. After an additional 4–12 h, the reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was washed successively with water, dilute methanesulfonic acid, water, dilute $KHCO_3$ solution, and brine and then dried over Na_2SO_4. Removal of the solvent, followed by recrystallization from either aqueous DMF or acetone, gave the phosphorylated product.

The phosphoric halides were all prepared by known literature procedures,[5] 2-chloro-2-oxo-1,3,2-dioxaphospholane was obtained by oxidation of 2-chloro-1,3,2-dioxaphospholane with molecular oxygen,[8] 2-chloro-2-oxo-1,3,2-dioxaphosphorinane was similarly prepared from 2-chloro-1,3,2-dioxaphosphorinane,[8] 2-chloro-2-oxo-1,3,2-oxazaphosphorinane was prepared from $POCl_3$ and 3-hydroxy-1-propylamine,[9] reaction of dimethyl methylphosphonate with 1 equiv of PCl_5 gave methyl methylphosphonochloridate,[5] dimethyl phosphorobromidate was prepared from trimethyl phosphite and bromine, and dimethylphosphinic chloride was purchased from Alfa Ventron Co.

***N*-(4-Nitrophenyl)dimethylphosphinamide (5a).** Dimethylphosphinic chloride (2 g, 17.7 mmol) in dry dimethoxyethane (5 mL) was slowly added to a solution of 4-nitroaniline (1.50 g, 11 mmol) in dry pyridine (5 mL) at 0 °C. The mixture was allowed to warm slowly to room temperature and, after an additional 4 h, water was added and the product was collected and washed well with water. Recrystallization from aqueous ethanol removed unreacted 4-nitroaniline and gave 0.78 g (34%) of the dimethylphosphinamide **5a**, mp 235 °C. Anal ($C_8H_{11}N_2O_3P$) C, H, N.

Because of the low yields associated with the above phosphorylation of 4-nitroaniline, all subsequent phosphorylation reactions involve amines **4a** or **4b**.

***N*,*N*,*N*',*N*'-Tetramethyl-*N*''-(4-nitrophenyl)phosphoric Triamide (6a).** A stirred suspension of a mixture of the dichloride **3** (10.2 g, 0.04 mol) and of finely ground dimethylamine hydrochloride (10 g, 0.12 mol) in 150 mL of dry CH_2Cl_2 was treated dropwise with an excess of Et_3N (30 mL) at 0 °C. The mixture was allowed to warm slowly to room temperature; after 30 min, dilute HCl was added, and the aqueous layer was washed with aqueous methanesulfonic acid, water, K_2CO_3 solution, and brine and then dried over $MgSO_4$. Removal of the solvent and recrystallization from aqueous methanol (to remove 4-nitroaniline) gave 3.84 g (35%) of **6a**, mp 214 °C. Anal. ($C_{10}H_{17}N_4O_3P$) C, H, N.

***N*,*N*'-Dimethyl-*N*''-(4-nitrophenyl)phosphoric Triamide (7a).** A solution of dichloride **3** (6.37 g, 0.025 mol) in 200 mL of dry CH_2Cl_2 and 25 ml of dry pyridine was treated with a slow stream of methylamine gas at 0 °C until precipitation of CH_3NH_3Cl ceased. The mixture was filtered to remove the precipitate, and the solvent was removed under vacuum. The residue was extracted with acetone, and the solution was filtered, diluted with EtOAc, and concentrated to give 0.9 g (15%) of **7a**, mp 193–194 °C. Anal. ($C_8H_{13}N_4O_3P$) C, H, N.

2-[4-(Benzyloxycarboxamido)phenyl]-2-oxo-1,3,2-oxazaphosphorinane (8a). Reaction of 2-chloro-2-oxo-1,3,2-oxazaphosphorinane with amine **4a** gave the cyclic phosphordiamidate **8a**: yield 26%; mp 227 °C [DMF (aq)]. Anal. ($C_{17}H_{20}N_3O_4P$) C, H; N: calcd, 11.6; found, 12.5.

2-[4-(Benzyloxycarboxamido)phenyl]-2-oxo-1,3,2-dioxaphosphorinane (9a). Reaction of 2-chloro-2-oxo-1,3,2-dioxaphosphorinane with amine **4a** gave the cyclic phosphoramidate **9a**: yield 38%; mp 209 °C [DMF (aq)]. Anal. ($C_{17}H_{19}N_2O_5P \cdot 0.4H_2O$) C, H, N.

2-[4-(Benzyloxycarboxamido)phenyl]-2-oxo-1,3,2-dioxaphospholane (10a). Treatment of amine **4a** with 2-chloro-2-oxo-1,3,2-dioxaphospholane gave the cyclic phosphoramidate **10a**: yield 33%; mp 162 °C (acetone–petroleum ether). Anal. ($C_{16}H_{17}N_2O_5P$) C, H, N.

Dimethyl *N*-(4-Nitrophenyl)phosphoramidate (11a). Solid dichloride **3** (12.75 g, 0.05 mol) was added in portions to a cooled solution of sodium methoxide (3.80 g of Na, 0.165 mol, 3.3 equiv) in dry methanol (50 mL), and after being stirred for an additional 5 min at low temperature, the mixture was diluted with 250 mL of ice-cold water and rapidly filtered. Treatment of the filtrate with dilute hydrochloric acid gave a precipitate of the diester, which was collected, washed well with water, and dried: yield 8.70 g (71%); mp 163–164 °C [MeOH (aq)]. Anal. ($C_8H_{11}O_5P$) C, H, N.

Diethyl *N*-(4-Nitrophenyl)phosphoramidate (12a). A suspension of the dichloride **3** (6.7 g, 0.026 mol) in 50 mL of absolute EtOH was treated with a solution of NaOEt in EtOH until the solution remained basic. After neutralization with dilute HCl, the solvent was removed under vacuum, and the product was recrystallized from aqueous methanol to remove a trace of 4-nitroaniline: yield 5.5 g (77%); mp 139–140 °C. Anal. ($C_{10}H_{15}N_2O_5P$) C, H, N.

Methyl *N*-[4-(Benzyloxycarboxamido)phenyl]methylphosphonamidate (13a). Reaction of amine **4a** with methyl methylphosphonochloridate gave the phosphonamidate **13a**: yield 21%; mp 185 °C; NMR (Me_2SO-d_6) δ 1.57 (d, 3 H, J = 16 Hz, PCH_3), 2.50 (m, 1 H, NH), 3.65 (d, 3 H, J = 12 Hz, $POCH_3$), 5.13 (s, 2 H, CH_2Ph), 6.10 (m, 1 H, NH), 6.73–7.30 (m, 4 H, aromatic H), 7.35 (s, 5 H, Ph).

Sodium Methyl *N*-(4-Nitrophenyl)phosphoramidate (14a). Solid dichloride **3** (7.7 g, 0.03 mol) was slowly added to a stirred solution of potassium acetate (7.5 g) in 15 mL of anhydrous methanol, and after the addition was complete, the mixture was warmed on a water bath for 15 min. After dilution with EtOAc and filtration to remove the inorganic precipitate, the solvent was removed under vacuum. The residue was extracted into Na_2CO_3 solution and filtered, and the water was removed under vacuum. Recrystallization from MeOH–$CHCl_3$ gave 1 g of the sodium salt (13%): mp 287 °C dec. Anal. ($C_7H_8N_2O_5P \cdot 0.5H_2O$) C, H, N.

Sodium *N*-(4-Nitrophenyl)phosphorodiamidate (15a). To a cooled (<10 °C) solution of 15 mL of concentrated NH_3 (aq) was slowly added solid dichloride **3** (5 g, 0.02 mol), and after filtration to remove an insoluble fraction, the solution was carefully acidified with concentrated HCl. The precipitate was collected, washed with water, and dissolved in aqueous Na_2CO_3. Evaporation of the solvent and recrystallization from MeOH–EtOAc gave 1.20 g (25%) of the sodium salt, mp >300 °C dec. Anal. ($C_6H_7N_3NaO_4P \cdot 2.5H_2O$) C, H, N.

Dimethyl *N*-(3-Nitrophenyl)phosphoramidate (16a). *N*-(3-Nitrophenyl)phosphorodichloridate was prepared from 3-nitroaniline and $POCl_3$ by the same procedure as outlined for the

dichloride 3. Reaction with sodium methoxide in methanol as before gave the dimethyl ester, mp 144 °C (MeOH). Anal. ($C_8H_{11}N_2O_5P$) C, H, N.

Dimethyl (4-Aminobenzyl)phosphonate (18a). Reaction of freshly distilled benzyl bromide with trimethyl phosphite gave dimethyl benzylphosphonate, which was treated with HNO_3/H_2SO_4 to give the known 4-nitro compound.[10] Hydrogenation (Pd/C) gave the 4-amino derivative (18a), mp 103 °C (benzene–petroleum ether). Anal. ($C_9H_{14}NO_3P$) C, H, N.

Methyl 4-Nitrobenzyl Sulfone (19a). Reaction of benzyl bromide with aqueous sodium sulfite gave sodium benzylsulfonate, which was converted to the sulfonyl chloride with PCl_5 and reduced to the sulfinite with Zn powder. Alkylation with dimethyl sulfate then gave benzyl methyl sulfone, which was converted to the 4-nitro derivative with HNO_3/H_2SO_4.[11]

Dimethyl *N*-(4-Nitrobenzoyl)phosphoramidate (20a). Reaction of 4-nitrobenzamide with PCl_5, followed by treatment with sodium methoxide in methanol, gave a 65% yield of compound **20a**, mp 154–155 °C (lit.[12] mp 153–154 °C).

Dimethyl *N*-[4-(Benzyloxycarboxamido)-3-methoxyphenyl]phosphoramidate (21a). Reaction of benzyl *N*-(4-amino-2-methoxyphenyl)carbamate (**4b**) (10 g, 3.6 mmol) with an excess (2 equiv) of freshly prepared dimethyl phosphorobromidate in pyridine at 0 °C gave, after normal workup, an oil, which was purified by chromatography on silica (CH_2Cl_2-MeOH, 25:1): yield 5.68 g (41%); mp 115 °C (acetone–petroleum ether). Anal. ($C_{17}H_{21}N_2O_6P$) C, H, N, P.

Antitumor Testing. F1 hybrid (DBA/2J × C57B/J) mice (19–21 g) of either sex were inoculated with 10^6 P-388 murine leukemia cells on day 0. Drugs, normally as solutions but at the highest doses as suspensions (sonicated) in 30% aqueous ethanol, were administered intraperitoneally in a volume of 0.1 mL on days 1, 5, and 9. Average survival times were measured for each group of six mice, and the percentage increase life span was calculated with respect to the control animals (20–30 mice). Control animals survived 11.0 days on average. Drug doses ranged from a toxic level downwards at 0.67-fold intervals. No long-term survivors were recorded.

Cultures of P-388 leukemia cells and L1210 leukemia cells were used to test drug cytotoxicity over a 3-day incubation time. Conditions for cell culture and drug addition were identical with those previously described.[13]

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Imidodisulfamides. 2.[1] Substituted 1,2,3,4-Tetrahydroisoquinolinylsulfonic Imides as Antagonists of Slow-Reacting Substance of Anaphylaxis

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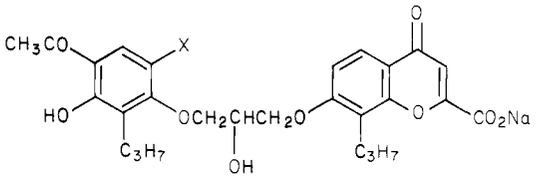
As part of a study of the influence of structural modifications of *N',N''*-bis(aralkyl)imidodisulfamides on their ability to selectively antagonize SRS-A activity, a few conformationally constrained structures were examined. Among these derivatives having a conformationally restricted alkylene side chain, substituted 1,2,3,4-tetrahydroisoquinolinylsulfonic imides produced optimum SRS-A antagonist activity and selectivity. These compounds were tested for antagonism of partially purified SRS-A induced contractions of isolated guinea pig ileum. In this series of tetrahydroisoquinolines, the effect of aromatic ring substitution, as well as substitution and variation of the size of the heterocyclic ring on SRS-A antagonist activity and selectivity, was studied.

The important roles of SRS-A and the leukotrienes in inducing bronchospasm in human allergic asthma and in anaphylactic shock in animals are well established.[2,3] The search for a potent and selective SRS-A antagonist as an agent for the therapy or prophylaxis of human asthma has intensified recently, especially after the chemical structures of SRS-A and the leukotrienes were elucidated.[4,5] A potent and specific SRS-A antagonist is the chromonecarboxylic acid 1 (FPL-55712).[6] This is of considerable value as a pharmacological tool for the identification of the SRS-A site(s) of action. Recently, the 6-iodo derivative of 1, i.e., 2 has been disclosed to be a more potent SRS-A antagonist than 1 with longer duration of action and antihistaminic properties.[7] Other structurally unrelated compounds with anti-SRS-A activity have also been described recently.[8]



1 (FPL-55712), X = H
2, X = I

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