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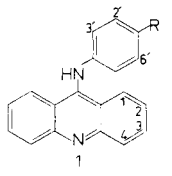
Potential Antitumor Agents. 23. 4′-(9-Acridinylamino)alkanesulfonanilide Congeners Bearing Hydrophilic Functionality

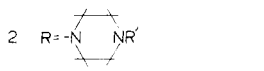
Bruce F. Cain,* Graham J. Atwell, and William A. Denny

New Zealand Cancer Society's Experimental Chemotherapy Laboratory, Auckland, New Zealand. *Received January 17, 1977*

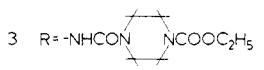
From structure–anti-L1210 relationships developed earlier for the 4′-(9-acridinylamino)alkanesulfonanilides it was predicted that congeners bearing both lipophilic 3-acridine substituents and compensatory hydrophilic function(s), together providing an overall molecular lipophilic–hydrophilic balance close to optimum, should have augmented antitumor properties. The acceptability of a variety of hydrophilic functions, and optimum positioning of these, has now been investigated. A variety of sterically demanding, hydrophilic functions may be acceptably appended to the acridine 4(5) position suggesting considerable site bulk tolerance. A variant with both a lipophilic 3-acridine substituent (3-iodo) and a hydrophilic 5-(2,3-dihydroxypropoxy) function is markedly more active than previous examples in the early treated, intraperitoneally (ip) dosed, ip implanted L1210 system, the assay system employed in the structure–activity analyses. However, this latter compound, on ip administration, failed to significantly inhibit subcutaneously implanted L1210 whereas earlier variants, under the same conditions, provided significant tumor inhibition. In this drug series the observed order of relative drug effectiveness alters with changing site of tumor implantation.

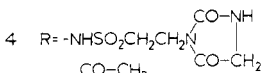
In our earlier analysis of the structure–antileukemic (L1210) relationships for the 4′-(9-acridinylamino)alkanesulfonanilides [1, R = $-NHSO_2(CH_2)_nCH_3$] it was

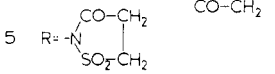


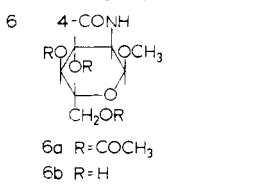


2a R′ = H
2b R′ = CH_3
2c R′ = $COCH_3$
2d R′ = SO_2CH_3
2e R′ = $CONH_2$









6a R = $COCH_3$
6b R = H

demonstrated that agent lipophilic–hydrophilic balance is a major factor in determining the level of attainable antitumor selectivity. For homologous members of the series there is a parabolic relationship between the logarithms of the observed maximum increases in life span in L1210 tests (log ILS_{max}) and acceptable measures of lipophilic–hydrophilic balance.[2] Further, analogues containing a hydrophobic 3-acridine substituent, which should be smaller than an isopropyl group but can acceptably be as large as an iodine atom, were shown to be more active than expected on the basis of their lipophilic character.[2] The prediction resulting from these studies was that if agents were prepared in which there was both a hydrophobic 3-acridine substituent and an acceptably positioned hydrophilic function, so that overall molecular lipophilic–hydrophilic balance was close to optimum, then such agents should have augmented antitumor properties.[2]

This communication describes an investigation of the acceptability of various hydrophilic structural units and optimum positioning of these.

Chemistry. Synthesis of the agent framework, represented by formula **1**, utilized acid-catalyzed coupling of a 9-chloroacridine component with the requisite R-substituted aromatic amine. The necessary aromatic amine unit for preparation of **9** resulted from Prévost benzoyloxylation (silver benzoate–I_2) of the aliphatic double bond of *N*-(2-propenyl)-4′-nitromethanesulfonanilide with following hydrolytic removal of the benzoyl groups and then nitro group reduction.

The 3-aminoacridine **11** resulted from reduction (Fe/H^+)[3] of the nitro precursor **10**, in turn prepared by the standard elaboration method.[1]

Reaction rates of 9-chloroacridine with usually encountered primary and secondary aromatic and aliphatic amines are respectively increased[4-6] and decreased[7] in acid media, permitting direct preparation of the dicationic analogues **12** and **14** by selective reaction of the requisite 9-chloroacridine with the primary aromatic amine function of 1-(4-aminophenyl)piperazine at low pH. Proof of the correct formulation of such products results from preparation of the identical acetylpiperazine analogue **16** either by application of the standard synthesis, utilizing 1-acetyl-4-(4-aminophenyl)piperazine as the side-chain

component, or by acetylation of the preformed acridine **14** as a terminal step.

The aromatic amine side-chain components have been prepared by reduction (H_2; Pd/C or Fe/H^+)[3] of the corresponding nitro compounds. Since all the amines produced on reduction bear electron-donor substituents, they are readily autoxidized and the products resulting from the catalytic absorption of the theoretical amount of hydrogen have accordingly been coupled directly with a 9-chloroacridine with minimum further manipulation.

Addition of thiolacetic acid to methyl acrylate provides methyl *S*-acetyl-3-mercaptopropionate[8] and following oxidation (Cl_2–H_2O) provided 2-methoxycarbonylethanesulfonyl chloride.[9-11] 2-Methoxycarbonyl-4′-nitroethanesulfonanilide, necessary for **34**, is readily prepared from this sulfonyl chloride. Saponification of the methyl ester in the latter sulfonanilide provided the corresponding acid necessary for elaboration of **33**. Treatment of the 3-(4-nitrophenylsulfamoyl)propionic acid intermediate with $SOCl_2$ provided a cyclic *N*-acylsulfonanilide[9,11] (cf. **32**) which, to suitable nucleophiles, acts as a cyclic anhydride and readily provided the corresponding amide (cf. **35**) and dimethylamide (cf. **36**) on treatment with the requisite amine. A similar sequence, starting from methyl 2-methylacrylate and thiolacetic acid, ultimately provided the methyl-branched isomer **37**.

Several alternate routes were developed to provide the 4-carboxamide congeners **40–49**. Treatment of 9(10*H*)-acridone-4-carboxylic acid with $SOCl_2$–DMF provided 9-chloroacridine-4-carbonyl chloride. In anhydrous, mildly basic media at low temperatures aliphatic amines react smoothly and selectively with the acid chloride moiety of this dichloro compound. The resulting 9-chloroacridine-4-carboxamides could then be coupled with a side-chain amine, under mild acid conditions, in the usual fashion. Alternatively, 9(10*H*)-acridone-4-carboxylic acid with tris(4-nitrophenyl) phosphite in pyridine solution provided 4-nitrophenyl 9(10*H*)-acridone-4-carboxylate. Treatment of this acridone with $SOCl_2$–DMF smoothly furnished the corresponding 9-chloroacridine which could be reacted with suitable side-chain amine components in acid media to provide analogues bearing a 4-(4-nitrophenyl ester) function (cf. **43**). Such 4-nitrophenyl esters react readily with primary aliphatic amines to also provide 4-carboxamide variants. Alternatively, at moderate temperatures and in basic media, 4-nitrophenyl 9-chloroacridine-4-carboxylate reacts readily with primary aliphatic amines providing 9-chloroacridine-4-carboxamides identical with those earlier prepared from the 9-chloroacridine-4-carbonyl chloride.

The 3-[9(10*H*)-acridon-4-yl]propionic acid precursor, necessary for preparation of **50**, was prepared by ring closure (polyphosphoric acid) of 3-[*N*-(2-carboxyphenyl)-2-aminophenyl]propionic acid. To prepare this latter acid by the Jourdan–Ullmann diphenylamine synthesis[12] requires 3-(2-aminophenyl)propionic acid as an intermediate. Catalytic reduction (H_2; Pd/C) of potassium 2-nitrocinnamate furnished the stable potassium salt of 3-(2-aminophenyl)propionic acid. Provided that the latter is employed as the aminocarboxylate ion in basic media, the normally facile ring closure of this compound to dihydrocarbostyril can be prevented. Thus in the basic conditions (K_2CO_3) of the Jourdan–Ullmann synthesis the salt reacted normally with potassium 2-chlorobenzoate to provide 3-[*N*-(2-carboxyphenyl)-2-aminophenyl]propionic acid. Mild treatment of the latter with polyphosphoric acid, and following treatment with alkali, provided 3-[9-(10*H*)-acridon-4-yl]propionic acid. As with the earlier discussed 9(10*H*)-4-carboxylic acid, reaction of 3-[9-(10*H*)-acridon-4-yl]propionic acid with $SOCl_2$–DMF, then excess NH_3 in anhydrous conditions, provided 3-(9-chloro-4-acridinyl)propionamide which reacted smoothly with the requisite side-chain amine to provide variant **50**.

A conventional Jourdan–Ullmann reaction utilizing 3-(3-aminophenyl)propionic acid and 2-chlorobenzoic acid provided 3-[*N*-(2-carboxyphenyl)-3-aminophenyl]propionic acid. Ring closure of the latter with H_2SO_4 afforded 3-[9(10*H*)-acridon-3-yl]propionic acid. As with previous acridones bearing carboxylic acid functions, treatment of this latter compound with $SOCl_2$–DMF produced the 9-chloroacridinecarbonyl chloride which, on selective reaction with ammonia, provided the corresponding 9-chloroacridinecarboxamide necessary for the preparation of **51**.

The acridone intermediates necessary for preparation of analogues **52–55** were prepared by etherification of the requisite hydroxyacridone. For example, reaction of 4-hydroxy-9(10*H*)-acridone and 2-bromoethanol (K_2CO_3, 2-butanone) provided 4-(2-hydroxyethoxy)-9(10*H*)-acridone. Masking of hydroxyl functions, in such acridones, by O-acetylation then following reaction with $SOCl_2$–DMF provided a substituted 9-chloroacridine which could be reacted with amine side-chain components in the usual manner. Partial loss of masking *O*-acetyl functions during the coupling reaction required either complete demasking or reacetylation before attempted product purification. The dihydroxyalkoxyacridones necessary for **53–55** were similarly prepared from the requisite hydroxyacridone and 2,2-dimethyl-4-iodomethyl-1,3-dioxolane, with following acid-catalyzed cleavage of the dioxolane protective function. Acetylation of the hydroxyl groups so liberated then provided protected derivatives which could be converted to 9-chloroacridines and these were then coupled with amine components as before. Conveniently, 2,2-dimethyl-4-(*p*-toluenesulfonyloxymethyl)-1,3-dioxolane could be employed directly in place of the corresponding 4-iodomethyl derivative if KI was included in the etherification reaction medium.

Results and Discussion

In earlier work lipophilic–hydrophilic balance of agents, as measured by R_m values from reversed phase chromatography, was shown to be a dominant factor influencing antileukemic activity.[1] A parabolic relationship between R_m values of the homologous 4′-(9-acridinylamino)alkanesulfonanilides [**1**, R = $-NHSO_2(CH_2)_nCH_3$] and the logarithms of the maximum increases in life span (log ILS_{max}) in L1210 tests was demonstrated.[1] Almost invariably an added drug substituent will alter lipophilic character and there will be a resultant change in biologic activity due to this alteration alone. Additionally, the added substituent may alter steric, electronic, and hydrophobic drug-site interactions as well as affecting rates of either metabolic or direct chemical activation and/or deactivation. To attempt to divorce the totalled contribution to biologic activity of the latter factors from that resulting from change in lipophilic–hydrophilic balance alone, the observed log ILS_{max} for a substituted derivative has been compared with that predicted by the parabolic reference curve at the R_m value of the derivative. That is, activity comparisons of agents have been made at the equivalent of equilipophilicity. As before,[1] the difference between the log ILS_{max} predicted from the reference curve and that actually observed (Δ log ILS) has been employed as a measure of the totalled substituent contributions to L1210 activity. The variations observed in L1210 screening data are such that Δ log ILS figures of less that 0.2 should

not be considered as significant.

Since an unsubstituted sulfonamide function is not a prerequisite for activity in this series,[2] hydrophilic functionality might acceptably be attached to the sulfonamide nitrogen. An example of such substitution (**9**) conferred little additional polar character (cf. R_m values of 8 and **9**) and the structural change involved provided an inactive analogue. 2'(6')-Substituents were earlier shown dystherapeutic, and steric inhibition of site binding was suggested.[2] Molecular models suggest that, because of intramolecular interactions, some component of the 1'-substituent of **9** will inevitably intrude into the space where 2'(6')-substituents normally reside. It appears that functions attached to the 1' position might require to be so structured that they intrude as little as possible into the space immediately adjacent to the 2' or 6' positions.

A 1' electron-donor substituent proved necessary for anti-L1210 activity but there did not appear to be increased activity associated with the most powerful electron-donor substituents employed [$-NH_2$, CH_3NH-, $(CH_3)_2N-$].[13] This seeming paradox likely results from the increasing ease of agent destruction, by nucleophilic displacement of the 9-anilino function, as the electron-donor character of the 1'-substituent increases.[14] The facile agent reaction with thiols (0.1 M 2-mercaptoethanol; pH 7.3; 37 °C) has been employed to quantitate the ease of agent destruction in vitro.[14] For example, the parent **8** under these assay conditions has a half-life ($T_{1/2}$) of 55 min. Presumably by steric effects acceptable 3'-substituents can stabilize agents (cf. **39**; $T_{1/2}$ = 106 min). Certain acridine substituents markedly stabilize agents; for example, an acridine 3-NH_2 lengthens $T_{1/2}$ by ninefold.[14] More powerful electron-donor 1'-substituents augment the lability of these agents (e.g., **1**, R = NH_2; $T_{1/2}$ = 12 min). If intrinsic antitumor selectivity was responsive to the donor properties of the 1' function, but masked by coincreasing agent lability, then inclusion of both 1' electron-donor and -stabilizing substituents in a single molecule might provide a more tumor-selective agent. While a CH_3O- group is an effective donor function an earlier example bearing this substituent (**1**, R = $-OCH_3$) proved L1210 inactive[13] whereas the slightly more hydrophilic 3-NO_2 derivative (**10**) proved weakly active and the yet more polar example **11**, containing a stabilizing 3-NH_2 group, has provided even greater activity. The small Δ log ILS value for the latter compounds suggests that the appended groups are conferring activity by making the compounds more hydrophilic and not by their own intrinsic contributions. From **11**, as example, it appears that hydrophilic functionality might acceptably be attached through an ether function to the 1' position. However, aromatic $-N(alkyl)_2$ substituents are more powerful electron donors than CH_3O- and might provide significant activity enhancement if suitable thiolysis stabilizing groups were also present. The desire to link from such substituents to hydrophilic functionality, and consideration of the steric requirements suggested above, prompted examination of the sterically constrained piperazine system (cf. **12–18**).

Those piperazines in which the outermost ring nitrogen is ionized at physiologic pH values (**12–15**) proved inactive, even when acridine pK_a was reduced by nitro group substitution (**12** and **13**), a device successfully restoring activity in dicationic variants earlier investigated.[15] Masking the basic character of the exterior piperazine nitrogen atom by acylation, as in **16–18**, restored tumor-inhibitory properties. Similarly, a range of alternative electron-donor substituents bearing hydrophilic functionality, which would be predicted not to intrude into the area adjacent to the 2'(6') positions, was prepared and screened (**19–38**). Most of these substituents (**16–38**) did not confer marked hydrophilic character. Further, intercomparison of these variants shows that many have high negative Δ log ILS values. If such results reflect drug-site interactions, and are not imposed by host derived factors, then substituent space distal to the 1' position appears proscribed and would require accurate mapping to be effectively utilized. A 1'-NHCONHR group has conferred highest activity, as evidenced by Δ log ILS figures (**20–22**), but again does not furnish adequate hydrophilic character. Unfortunately, congeners bearing the more hydrophilic 1'-$NHCONH_2$ group could not be maintained intact during laboratory manipulation and there was continual breakdown with the earlier prepared,[13] tumor active, 1'-NH_2 compound resulting. It is not known if congeners **20–24** are active per se or act by in vivo delivery of the 1'-NH_2 analogue.

Of the remaining nuclear positions of 1 available for attachment of hydrophilic functionality 1(8), 2(7), 2'(6'), and 3'(5') have been shown unacceptable[1,2] and it is proposed that the 3(6) positions should be reserved for lipophilic substituents capable of hydrophobic site bonding.[2] As shown by examples **40–55** hydrophilic functionality can be acceptably attached to the remaining acridine 4(5) positions.

The group of 4-carboxamide variants **40–49** contains several hydrophilic examples which display excellent antileukemic activity and, with the simpler amide analogues **40–42**, there is no marked loss in dose potency. The excellent activity of the derivatives containing either a bulky glucosaminide residue (**47** and **49**), or a variety of hydrophilic groups (**40–42**, **44**, **45**, **50**, **52–55**), suggests that there is considerable site bulk tolerance about the 4(5)-acridine position. With our DNA intercalation site model[1] such 4(5) functionality would reside in the relatively uncluttered major groove of the DNA.

Surprisingly, the glycinamide variant **45** has closely similar R_m value to the glucosaminide **47** and appears more hydrophilic than the dihydroxyamide **44**. This is contrary to predictions made by summation of π constants for the component polar functions and presumably results from the contiguity of these, with a consequent breakdown in π additivity.[17]

From the R_m value of variant **47** it was possible to predict[1] that the corresponding propanesulfonanilide (**49**) should have close to optimum lipophilic character and, as shown (Table I), this agent does provide excellent life extensions in L1210 tests although relatively massive doses are necessary.

Interposing a short alkyl segment between the 4-carboxamide function and the acridine ring, as in **50**, did not materially affect ip L1210 activity or dose potency. In contrast, the corresponding 3-isomer (**51**), which might match the alkyl segment to the proposed hydrophobic site area,[1,2] was significantly less dose potent and appeared less L1210 active than might be expected from R_m values.

Acridine 4(5)-hydroxy ether derivatives also proved acceptable; useful hydrophilic character was conferred and there was retention of dose potency and antileukemic efficacy (**52–55**). The availability of 3-iodo-5-methoxyacridone from earlier work[18] permitted the ready synthesis of the corresponding 3-iodo-5-dihydroxypropoxy analogue **55**. This agent provided a probe to examine our earlier conclusion that an agent containing both a hydrophobic 3-acridine substituent and an acceptably positioned, compensatory, hydrophilic unit would prove more active in ip L1210 tests.[1] The single entry in Table I for variant

Table I. Physicochemical and L1210 Screening Data for the 9-Anilinoacridines

No.	Type	R	Substituents	Mp, °C	Formula	Analyses[a]	R_m[b]	O.D.[c]	L1210 ILS, %[d] ip	L1210 ILS, %[d] sc	Δ log ILS
8	1	$-NHSO_2CH_3$		Parent for comparison purposes			0.00	45	107	28	
9	1	$-N(SO_2CH_3)CH_2CHOHCH_2OH$		290–291	$C_{23}H_{23}N_3O_4S\cdot HBr$	C, H, N, Br	−0.03	65	−[e]		>−0.68
10	1	$-OCH_3$	3-NO_2	290–292	$C_{20}H_{15}N_3O_3\cdot HCl$	C, H, N, Cl	+0.49	90	33		+0.08
11	1	$-OCH_3$	3-NH_2	333 dec	$C_{20}H_{17}N_3O\cdot HCl$	C, H, N, Cl	+0.31	15	66		+0.06
12	2a		3-NO_2	143–144	$C_{23}H_{21}N_5O_2\cdot 1.5H_2O$	C, H, N	−0.81	15	−		
13	2b		3-NO_2	301 dec	$C_{24}H_{23}N_5O_2\cdot 2HBr\cdot H_2O$	C, H, N, Br	−0.78	60	−		
14	2a			329 dec	$C_{23}H_{21}N_4\cdot 2HBr$	C, H, N, Br	−0.80	15	−		
15	2b			296–297	$C_{24}H_{24}N_4\cdot 2HBr$	C, H, N, Br[f]	−0.68	50	−		
16	2c			317 dec	$C_{25}H_{24}N_4O\cdot HCl$	C, H, N, Cl	+0.23	75	81		0.00
17	2d			328 dec	$C_{24}H_{24}N_4O_2S\cdot HCl$	C, H, N, S	+0.21	200	69		−0.09
18	2e			246 dec	$C_{24}H_{23}N_5O\cdot HCl$	C, H, N, Cl	−0.11	37	78		−0.20
19	1	$-NHNHCOOC_2H_5$		174–176	$C_{22}H_{20}N_4O_2\cdot HCl\cdot 1.5H_2O$	C, H, N, Cl	+0.41	25	67		+0.23
20	1	$-NHCONHCH_3$		268–270	$C_{21}H_{18}N_4O$	C, H, N	+0.25	60	112 (2)	−	+0.17
21	1	$-NHCONHCH_3$	3′-OCH_3	214–216	$C_{22}H_{20}N_4O_2\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl	+0.51	50	72		+0.46
22	3			241 dec	$C_{27}H_{18}N_5O_3\cdot HCl$	C, H, N, Cl	+0.59	50	29		+0.16
23	1	$-NHCONHNHCOOCH_3$		266–268	$C_{22}H_{19}N_5O_3\cdot HCl$	C, H, N, Cl	−0.04	>500[g]	67[h]		−0.25[h]
24	1	$-NHCOOCH_2CHOHCH_2OH$		245 dec	$C_{25}H_{21}N_3O_4\cdot HCl$	C, H, N, Cl	−0.06	25	63	−	−0.28
25	1	$-NHSO_2CH_2CH_2NHCOCH_3$		292–294	$C_{23}H_{22}N_4O_3S\cdot HCl$	C, H, N, Cl	−0.06	50	94	−	−0.11
26	1	$-NHSO_2CH_2CH_2NHCOOCH_3$		287 dec	$C_{23}H_{22}N_4O_4S\cdot HCl$	C, H, N, Cl	+0.24	>500[g]	67[h]		−0.06[h]
27	1	$-NHSO_2CH_2CH_2NHSO_2CH_3$		204–205	$C_{22}H_{22}N_4O_4S_2\cdot HCl$	C, H, N, S	−0.14	110	33		−0.57
28	1	$-NHSO_2CH_2CH_2NHCONH_2$		204–205	$C_{22}H_{21}N_5O_3S\cdot HCl\cdot H_2O$	C, H, N, Cl	−0.21	200	53	−	−0.34
29	4			297 dec	$C_{24}H_{21}N_7O_4S\cdot HCl$	C, H, N, Cl	−0.19	160	51	−	−0.37
30	1	$-NHSO_2CH_2CH_2N(CH_3)COCH_3$		294 dec	$C_{24}H_{24}N_4O_3S\cdot HCl\cdot H_2O$	C, H, N, Cl	+0.05	200	54		−0.33
31	1	$-NHSO_2(CH_2)_3NHCOCH_3$		265–266	$C_{24}H_{26}N_4O_3S\cdot HCl$	C, H, N, Cl	−0.01	>500[g]	26[h]		−0.67[h]
32	5			216 dec	$C_{22}H_{17}N_3O_3S\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl	+0.13	200	33		−0.49
33	1	$-NHSO_2CH_2CH_2COOH$		126 dec	$C_{22}H_{19}N_3O_4S\cdot HCl\cdot H_2O$	C, H, N, Cl	+0.10	350	−		>−0.63
34	1	$-NHSO_2CH_2CH_2COOCH_3$		287 dec	$C_{23}H_{21}N_3O_4S\cdot HCl$	C, H, N, Cl	+0.57	>500[g]		−	
35	1	$-NHSO_2CH_2CH_2CONH_2$		186 dec	$C_{22}H_{20}N_4O_3S\cdot HCl\cdot H_2O$	C, H, N, Cl	−0.34	>500[g]	67[h]		−0.18[h]
36	1	$-NHSO_2CH_2CH_2CON(CH_3)_2$		241 dec	$C_{24}H_{24}N_4O_3S\cdot HCl\cdot 2H_2O$	C, H, N, Cl	+0.17	150	63		−0.18
37	1	$-NHSO_2CH_2CH(CH_3)CONH_2$		122–124	$C_{23}H_{22}N_4O_3S\cdot HCl$	C, H, N, Cl	−0.11	50	−		>−0.69
38	1	$-NHSO_2CH_2CH_2OCH_3$		279 dec	$C_{22}H_{21}N_3O_3S\cdot HCl$	C, H, N, Cl	+0.21	280	79	25	−0.03
39	1	$-NHSO_2CH_3$	3′-OCH_3	For comparison purposes			+0.18	6.7	114 (2)	27	+0.09
40	1	$-NHSO_2CH_3$	3′-OCH_3,4-$CONH_2$	197 dec	$C_{22}H_{20}N_4O_4S\cdot HCl\cdot 1.5H_2O$	C, H, N, Cl	−0.27	25	140	36	+0.10
41	1	$-NHSO_2CH_3$	3′-OCH_3,4-$CONHCH_3$	87–88	$C_{23}H_{22}N_4O_4S\cdot HCl$	C, H, N, Cl	+0.06	8.3	166 (1)	59	+0.17
42	1	$-NHSO_2CH_3$	3′-OCH_3,4-$CON(CH_3)_2$	262–263	$C_{24}H_{24}N_4O_4S\cdot HCl\cdot 0.5H_2O$	C, H, N, Cl	+0.09	15	84	61	−0.11
43	1	$-NHSO_2CH_3$	3′-OCH_3,4-$COOC_6H_4$-*p*-NO_2	216–217	$C_{28}H_{22}N_4O_7S\cdot HCl\cdot 1.5H_2O$	C, H, N, Cl					
44	1	$-NHSO_2CH_3$	3′-OCH_3,4-$CONHCH_2$-$CHOHCH_2OH$	98–99	$C_{25}H_{26}N_4O_6S\cdot HCl\cdot 1.5H_2O$	C, H, N, Cl	−0.36	92	72		−0.13
45	1	$-NHSO_2CH_3$	3′-OCH_3,4-$CONHCH_2CONH_2$	224 dec	$C_{24}H_{23}N_5O_5S\cdot HCl$	C, H, N, Cl	−0.54	62	105 (2)	41	+0.30

46	6a	$-NHSO_2CH_3$	3'-OCH_3	207 dec	$C_{35}H_{38}N_5O_{12}S\cdot HCl$	C, H, N, Cl	+0.62	>400[g]	–	
47	6b	$-NHSO_2CH_3$	3'-OCH_3	338 dec	$C_{29}H_{32}N_4O_9S\cdot HCl$	C, H, N	−0.57	180	100	49	+0.33
48	6a	$-NHSO_2(CH_2)_2CH_3$	3'-OCH_3	213–214	$C_{37}H_{42}N_4O_{12}\cdot HCl$	C, H, N; Cl[f]					
49	6b	$-NHSO_2(CH_2)_2CH_3$	3'-OCH_3	181 dec	$C_{31}H_{36}N_4O_9S\cdot HCl$	C, H, N, Cl	−0.10	400	180	51	+0.17
50	1	$-NHSO_2CH_3$	3'-OCH_3,4-$CH_2CH_2CONH_2$	211–213	$C_{24}H_{24}N_4O_4S\cdot HBr$	C, H, N, Br	−0.17	15	94	30	−0.11
51	1	$-NHSO_2CH_3$	3'-OCH_3,3-$CH_2CH_2CONH_2$	209–211	$C_{24}H_{24}N_4O_4S\cdot HBr$	C, H, N, Br	−0.25	125	58		−0.30
52	1	$-NHSO_2CH_3$	3'-OCH_3,4-OCH_2CH_2OH	230–231	$C_{23}H_{22}N_3O_5S\cdot HCl\cdot 2H_2O$	C, H, N, Cl	+0.09	5	112 (2)	28	+0.01
53	1	$-NHSO_2CH_3$	3'-OCH_3,4-$OCH_2CHOHCH_2OH$	164–166	$C_{24}H_{25}N_3O_6S\cdot HCl$	C, H, N, Cl	−0.09	17	123	43	0.00
54	1	$-NHSO_2CH_3$	3'-CH_3,4-$OCH_2CHOHCH_2OH$	98–99	$C_{24}H_{27}N_3O_5S\cdot HCl$	C, H, N, Cl	−0.03	100	110 (2)	41	−0.04
55	1	$-NHSO_2CH_3$	3'-OCH_3,3-I,5-OCH_2-$CHOHCH_2OH$	208 dec	$C_{24}H_{24}IN_3O_6S\cdot HCl$	C, H, N, Cl	+0.06	62	(6)	–	

[a] Analyses for the indicated elements were within ±0.4% of the theoretical figures for the formula quoted. [b] R_m values from reversed phase partition chromatography; see ref 20. [c] Optimum dose in mg/kg/day for ip qd 1–5 treatment. [d] ILS = increase in life span = (T/C % − 100). Figures are quoted for L1210 assays employing an initial tumor burden of 10^5 cells implanted either ip or sc. Numbers of 50-day survivors, per group of six animals, are provided in parentheses. [e] –, significant life extension (> 25%) was not obtained. [f] Br: calcd, 30.15; found, 30.6. [g] Maximum dose employed; optimum dose may be higher. [h] Higher drug doses may provide higher figures. [i] Cl: calcd, 4.4; found, 3.95.

55 does not adequately portray the antileukemic efficacy of this agent in such tests. Numbers of 50-day survivors ("cures") have been observed in ip screening tests with this compound from the optimum dose (62 mg/kg) down to 24 mg/kg. The ratio of the optimum drug dose, in such tests, to that providing 40% increase in life span has been employed as a chemotherapeutic index.[19] Such indices derived for **55**, and the progenitor **39**, were respectively 33 and 6.9. In terms of the ip L1210 test system employed, these results do appear to justify our earlier SAR analyses and conclusions.

However, the close drug–tumor cell contact provided in such tests, in which there is early, limited period, ip dosage of ip implanted tumor, may provide an overly optimistic impression of the activity of any agent.[16] Drug concentration gradients resulting between the peritoneal cavity and those host tissues providing limiting toxicity will reflect the pharmacokinetic properties of the drug employed, as well as intrinsic resistance to metabolism, rates of excretion, and direct chemical destruction. If high concentration gradients become established then the ratio of the drug concentration at administration point to that reaching tissues providing dose limitation will be high. For a constant toxic load to limiting tissues a higher applied ip drug concentration will be permissible with a rapidly removed drug, relative to that which can be applied of a less rapidly removed, more readily equilibrating agent. The higher applied ip concentration of the more rapidly removed drug could then provide a relatively higher, local, tumor cell kill. The maximum life extension recorded with such a drug does not necessarily provide a realistic appraisal of the intrinsic antitumor selectivity of the agent.

Earlier it was shown that there is a lack of parallelism between the life extensions obtained when a range of drugs of this series were ip dosed to animals bearing either ip or subcutaneously (sc) implanted tumor.[16] In tests employing sc implanted L1210 and ip drug dosing **55** failed to provide significant life extension. In contrast, other drug congeners (Table I; ref 16), which are less effective than **55** against ip inoculated L1210, show convincing activity against the sc implanted tumor. Whether **55** is classed as "more active" than other drug congeners appears to depend on the site of tumor implantation employed in the test system.

For convenience, ip dosed, ip implanted tumor systems cannot be excelled as primary screening tools. However, to provide quantitative measures of antitumor effectiveness for either derivation of QSAR, or selection of clinical trial candidates, test systems mirroring the clinical situation, as regards location of tumor burden and drug administration route, might be better employed.[16]

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for these 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 makers' supplied stem corrected thermometer; melting points are as read. NMR spectra were obtained on a Varian A-60 spectrometer (Me_4Si). IR spectra (KBr) were recorded using a Beckmann 237 Infracord. UV spectra were recorded on a Shimadzu UV-200.

To monitor the progress of reactions, purification of products, etc., TLC on SiO_2 (Merck SiO_2, F_{254}) was used. For the products listed in Table I the most convenient solvents are the top phase of *n*-BuOH–HOAc–H_2O (5:1:4, v/v) and $CHCl_3$ containing 2–8% MeOH. The partition chromatographic methods used in measuring R_m values have been described earlier.[20]

***N*-(2-Propenyl)-4'-nitromethanesulfonanilide.** To a suspension of K_2CO_3 (0.054 M) in DMF (25 mL) was added

4′-nitromethanesulfonanilide (0.052 M) and allyl bromide (4.4 mL, 0.052 M) and the heterogeneous mixture heated on a steam bath for 1 h. On addition of 5% KOH–H_2O (50 mL) to the cooled mixture, and shaking, crude product crystallized. The solid was washed well with aqueous KOH and H_2O, dried, and crystallized once from EtOH and then from MeOH. Pure product was obtained as pale yellow crystals of mp 60–61 °C (76%). Anal. ($C_{10}H_{12}N_2O_4S$) C, H, N.

***N*-(2,3-Dibenzoyloxypropyl)-4′-nitromethanesulfonanilide.** To a solution of the aforementioned compound (0.011 M) in C_6H_6 (100 mL) was added silver benzoate (0.022 M) and traces of H_2O were removed by azeotropic distillation. I_2 (0.011 M) was added to the cooled, stirred mixture and, when dissolved, the mixture was heated on a steam bath for 6 h. Solids were removed and the resulting solution was evaporated to dryness. Multiple crystallizations from EtOH provided pure product as colorless needles of mp 121–122 °C (47%). Anal. ($C_{24}H_{22}N_2O_8S$) C, H, N.

***N*-(2,3-Dihydroxypropyl)-4′-nitromethanesulfonanilide.** The foregoing compound (5 mM) was dissolved in a solution of NaOH (0.75 g) in 85% MeOH–H_2O and the mixture boiled under reflux conditions for 1 h. After addition of HOAc (1.1 mL) volatiles were removed in vacuo and the residue was triturated with ice-cold 10% $KHCO_3$–H_2O. Product crystallized from boiling H_2O (50 mL) as colorless needles of mp 86–87 °C (61%). Anal. ($C_{10}H_{14}N_2O_6S$) C, H, N.

1-Acetyl-4-(4-nitrophenyl)piperazine was prepared by acylation of 1-(4-nitrophenyl)piperazine in pyridine solution with excess Ac_2O. Product crystallized from small volumes of EtOH or large volumes of H_2O as yellow needles of mp 150–151 °C (82%). Anal. ($C_{12}H_{15}N_3O_3$) C, H, N.

1-Methylsulfonyl-4-(4-nitrophenyl)piperazine. To an ice-water cooled, stirred solution of 1-(4-nitrophenyl)piperazine (0.02 M) in pyridine (15 mL) methanesulfonyl chloride (0.02 M) was added in dropwise fashion and the resulting solution allowed to stand at room temperature overnight. As much excess pyridine as possible was removed in vacuo and crude product precipitated with 2 N HCl. Crystallization from HOAc–H_2O provided pure product as yellow needles of mp 217–218 °C (76%). Anal. ($C_{11}H_{15}N_3O_4S$) C, H, N, S.

1-Carbamoyl-4-(4-nitrophenyl)piperazine. 1-(4-Nitrophenyl)piperazine (0.024 M) was dissolved in H_2O (50 mL) containing HOAc (0.048 M) by warming and the solution cooled to 5 °C and then KCNO (0.025 M) stirred in. After heating on a boiling water bath for 0.5 h HOAc (0.024 M) was added to the hot solution which was then cooled to 5 °C and a further quantity of KCNO added. The cycle of heating, acidification, cooling, etc., was repeated after addition of two further quantities of KCNO; TLC monitoring then showed reaction to be complete. After ice cooling crude product was collected. Recrystallization from boiling H_2O (500 mL) provided pure product as pale yellow needles of mp 157 °C dec (82%). Anal. ($C_{11}H_{14}N_3O{\cdot}H_2O$) C, H, N.

***N*^{1}-(4-Ethanamido-3-methoxyphenyl)-*N*^{3}-methylurea.** A solution of 2-methoxy-4-aminoacetanilide (0.075 M) in pyridine (100 mL) at 5 °C was treated with methyl isocyanate (0.92 M). After standing at room temperature for 2 days excess pyridine was removed in vacuo and the residue triturated with EtOH. Crystallization from EtOH provided pure product as colorless prisms of mp 221–223 °C (83%). Anal. ($C_{11}H_{15}N_3O_3$) C, H, N.

1-Ethoxycarbonyl-4-(4-nitrophenylcarbamoyl)piperazine. A solution of freshly purified 4-nitrophenyl isocyanate (0.015 M) in dry C_6H_6 (30 mL) was filtered into a solution of 1-ethoxycarbonylpiperazine (0.015 M) in C_6H_6 (5 mL). On gentle warming product started to crystallize and reaction was completed by 3 min of boiling. After cooling product was collected, washed well with C_6H_6, dried, and crystallized from EtOH–H_2O. Pure product was obtained as pale yellow plates of mp 193–194 °C (72%). Anal. ($C_{14}H_{18}N_4O_5$) C, H, N.

3-(4-Nitrophenylcarbamoyloxy)-1,2-propanediol. 4-Nitrophenyl isocyanate (9.8 mM) was added portionwise to a stirred solution of 2,2-dimethyl-4-hydroxymethyl-1,3-dioxolane (0.01 M) in dry pyridine (5 mL) at 10 °C. After 2 h at room temperature addition of H_2O (20 mL) precipitated an oil which slowly solidified on stirring. Two crystallizations from EtOH provided pure nitrophenylurethane as pale yellow prisms of mp 136–138 °C (81%). Anal. ($C_{13}H_{16}N_2O_6$) C, H, N.

Boiling the above acetal (5 mM) with 2 N HCl (150 mL) for 30 min, filtration of the hot solution, and thorough cooling provided crystalline diol. Recrystallization from boiling H_2O provided pure product as needles of mp 162–164 °C (92%). Anal. ($C_{10}H_{12}N_2O_6$) C, H, N.

2-Phthalimido-4′-nitroethanesulfonanilide. A solution of 4-nitroaniline (0.06 M) in pyridine (27.5 mL) was stirred in an ice–salt bath while 2-phthalimidoethanesulfonyl chloride (0.05 M) was added portionwise so that the temperature remained below −5 °C. When addition was complete the mixture was stirred in the cooling bath until all acid chloride had dissolved and then stored in a refrigerator for 12 h. The mixture was then heated on a steam bath until homogeneous and then for 1 h further and as much pyridine as possible was removed in vacuo. To the resulting brown gum, boiling MeOH (75 mL) was added and the mixture boiled and stirred until a smooth paste of crystalline product resulted. After thorough cooling the crystals were collected, washed with cold MeOH (2 × 20 mL) and much H_2O, and dried. Crystallization from DMF provided pure product as very pale yellow needles of mp 214–215 °C (68%). Anal. ($C_{16}H_{13}N_3O_6S$) C, H, N, S.

Similarly was prepared **3-phthalimido-4′-nitropropanesulfonanilide**: pale yellow needles from DMF–EtOH; mp 231–232 °C (69%). Anal. ($C_{17}H_{15}N_3O_6S$) C, H, N, S.

2-Amino-4′-nitroethanesulfonanilide. To a vigorously stirred suspension of the precursor phthalimido derivative (0.023 M) in boiling EtOH (75 mL) hydrazine hydrate (98%, 0.046 M) was added in one portion, a clear yellow solution resulting in ca. 3 min. Shortly afterwards 1,4-phthalazinedione started to crystallize from the solution. After 30 min further stirring and boiling, HOAc (10 mL) was added and EtOH removed in vacuo. The remaining solids were extracted with successive quantities of boiling 0.5 N HOAc (60 and then 2 × 30 mL) and the clarified extracts evaporated to dryness in vacuo to yield a thick colorless gum. After solution in the minimum quantity of boiling H_2O and cooling, excess NH_4OH (pH >10) was added. Excess NH_3 was then removed by heating the solution in a steam bath under vacuum with swirling until the yellow amine suddenly crystallized from solution. After thorough cooling the base was collected, washed well with water, and dried. Crystallization from EtOH–H_2O provided pure product as yellow needles of mp 225–226 °C (92%). Anal. ($C_8H_{11}N_3O_4S{\cdot}H_2O$) C, H, N, S.

Similarly was prepared **3-amino-4′-nitropropanesulfonanilide**: yellow needles from EtOH–H_2O of mp 230–231 °C (93%). Anal. ($C_9H_{13}N_3O_4S$) C, H, N, S.

2-Ethanamido-4′-nitroethanesulfonanilide. 2-Amino-4′-nitroethanesulfonanilide (0.01 M) was dissolved in H_2O (10 mL) containing NaOH (0.06 M) and the solution cooled to 5 °C and crushed ice (15 g) added. To the well-stirred solution Ac_2O (0.02 M) was added and after 0.5 h an additional quantity (0.02 M) was stirred in. After a further 0.5 h of stirring the precipitated crystals were collected and recrystallized from EtOH–H_2O, pure product being obtained as very pale yellow needles of mp 192–193 °C (86%). Anal. ($C_{10}H_{13}N_3O_5S$) C, H, N, S.

3-Ethanamido-4′-nitropropanesulfonanilide was similarly prepared and was obtained as pale yellow needles from EtOH–H_2O: mp 279–280 °C (83%). Anal. ($C_{11}H_{15}N_3O_5S$) C, H, N, S.

2-(Methoxycarbonylamino)-4′-nitroethanesulfonanilide. 2-Amino-4′-nitroethanesulfonanilide (0.01 M) was dissolved in H_2O (20 mL) containing NaOH (1.6 g) and the solution cooled to 5 °C. Methyl chloroformate (0.03 M) was added in dropwise fashion to the stirred cooled solution so that the temperature remained below 5 °C. When no second liquid phase remained the solution was acidified with HOAc and the precipitated crude product collected. Crystallization from 65% EtOH–H_2O provided pure product as colorless plates of mp 154–155 °C (97%). Anal. ($C_{10}H_{13}N_3O_6S$) C, H, N, S.

2-Methanesulfonamido-4′-nitroethanesulfonanilide. 2-Amino-4′-nitroethanesulfonanilide (0.01 M) was dissolved in DMF (25 mL) containing Et_3N (0.02 M). The solution was stirred and cooled to −5 °C and then methanesulfonyl chloride (0.011 M) added at that temperature. After overnight refrigeration volatiles were removed in vacuo and the crude product was dissolved in H_2O (12 mL) containing NaOH (1.2 g). After clarification of the solution product was recovered by acidification with HOAc. Recrystallization from HOAc–H_2O provided pure product as

colorless needles of mp 206–207 °C (71%). Anal. ($C_9H_{13}N_3O_6S_2$) C, H, N, S.

2-[2-(Ethoxycarbonylamino)ethanamido]-4'-nitroethanesulfonanilide. 2-(Ethoxycarbonylamino)ethanoic acid (0.02 M) and 2-amino-4'-nitroethanesulfonanilide (0.02 M) were suspended in pyridine (25 mL) and the mixture was cooled to below 5 °C. To the stirred mixture PCl_3 (0.0133 M) was slowly added so that the temperature remained below 5 °C. After stirring at this temperature for 1 h the clear solution was heated on a steam bath for 0.5 h and volatiles were removed in vacuo. Addition of sufficient cold 1 N HCl to neutralize remaining pyridine and trituration provided crude solid product. Crystallization from boiling H_2O (470 mL) provided pure product as very pale yellow needles of mp 149–150 °C (61%). Anal. ($C_{13}H_{18}N_4O_7S$) C, H, N.

2-(1,3-Diazacyclopentane-2,5-dion-1-yl)-4'-nitroethanesulfonanilide. A sample of the aforementioned compound (9.6 mM) was dissolved in 4 N KOH (20 mL) and the solution heated on a steam bath for 1 h. Acidification of the cooled solution with HCl precipitated product. After thorough washing with 10% $KHCO_3$–H_2O product was crystallized from EtOH–H_2O and pure compound was obtained as colorless needles of mp 210 °C dec (61%). Anal. ($C_{11}H_{12}N_4O_6S\cdot 2H_2O$) C, H, N.

2-Methanamido-4'-nitroethanesulfonanilide. To Ac_2O (5 mL) maintained at 0 °C 95% HCOOH (2.5 mL) was slowly added. The solution was heated to 50 °C for 15 min and cooled to 0 °C; then a cold solution of 2-amino-4'-nitroethanesulfonanilide (0.02 M) in HCOOH (6 mL) was added. After overnight refrigeration the solution was warmed to 50 °C for 0.5 h, volatiles were removed in vacuo at the same temperature, and crude product precipitated by addition of H_2O (50 mL) and thorough cooling. Recrystallization from a moderate volume of boiling H_2O provided pure product as colorless needles of mp 182–183 °C (68%). Anal. ($C_9H_{11}N_3O_5S$) C, H, N, S.

2-Methylamino-4'-nitroethanesulfonanilide. A sample of the preceding *N*-formyl derivative (0.019 M) was suspended in dry tetrahydrofuran (30 mL) and oven dried [110 °C (vacuum)] $NaBH_4$ (0.055 M) added. To the stirred, ice-cooled mixture $BF_3\cdot Et_2O$ (0.115 M) was added in dropwise fashion. When addition was complete the reaction mixture was heated under reflux conditions for 8 h and then volatiles were removed in vacuo. After cautious addition of H_2O the mixture was adjusted to pH 7.5–8.0 with NH_4OH and the precipitated yellow crystals were collected after thorough cooling. Solution in a twofold excess of 10% HOAc–H_2O and clarification removed a small quantity of insoluble nonbasic material and basification of the filtrate with NH_4OH, as before, returned essentially pure product as yellow needles of mp 196–197 °C (71%). Anal. ($C_9H_{13}N_3O_4S$) C, H, N.

2-(*N*-Methylethanamido)-4'-nitroethanesulfonanilide. A sample of the preceding compound (0.01 M) was dissolved in H_2O (10 mL) containing NaOH (0.075 M) and the solution cooled at −15 °C until a thick slurry of ice crystals resulted. To the well stirred slurry was added Ac_2O (0.029 M) and the mixture stirred for 0.5 h. A further quantity of Ac_2O (0.029 M) was then added and the heterogenous mixture stirred for 30 min longer. Recrystallization of the precipitated crystals from EtOH–H_2O provided pure product as colorless needles of mp 220–221 °C (84%). Anal. ($C_{11}H_{15}N_3O_5S$) C, H, N.

Methyl 3-(4-Nitrophenylsulfamoyl)propionate. 4-Nitroaniline (0.116 M) was dissolved in pyridine (50 mL) and the well-stirred solution maintained at 0–5 °C while 2-methoxycarbonylethanesulfonyl chloride[8-11] (0.11 M) was slowly added. The clear solution was refrigerated overnight and heated on a steam bath for 0.5 h and then excess pyridine was removed in vacuo. Addition of H_2O and sufficient 2 N HCl to neutralize remaining traces of pyridine precipitated crude product. Crystallization from MeOH–H_2O provided pure product as pale yellow needles of mp 139–140 °C (52%). Anal. ($C_{10}H_{12}N_2O_6S$) C, H, N.

3-(4-Nitrophenylsulfamoyl)propionic acid resulted when the aforementioned methyl ester (0.017 M) was dissolved by stirring in a solution of KOH (0.05 M) in H_2O (26 mL) and the solution stored at room temperature for 1 h. Precipitation with 12 N HCl provided crude product which was collected, washed well with H_2O, and then dissolved in 10% aqueous $KHCO_3$ (26 mL). Reprecipitation, from the clarified solution by acidification provided pure product. On crystallization from Me_2CO–H_2O this compound separated as colorless needles of mp 176–177 °C (91%). Anal. ($C_9H_{10}N_2O_6S$) C, H, N.

2-(4-Nitrophenyl)-1-thia-2-azacyclopentan-3-one 1,1-Dioxide. The preceding carboxylic acid (9.1 mM) was suspended in $SOCl_2$ (8 mL) and the mixture heated under reflux conditions until a clear solution resulted (2 h) and then for 0.5 h further. On evaporation product crystallized and a single crystallization from Me_2CO–H_2O provided pure product as colorless needles of mp 209–210 °C (93%). Anal. ($C_9H_8N_2O_5S$) C, H, N, S.

3-(4-Nitrophenylsulfamoyl)propionamide could be prepared by solution of the preceding compound in concentrated NH_4OH, reaction being complete when a homogeneous solution resulted, or by stirring of methyl 3-(4-nitrophenylsulfamoyl)propionate with excess concentrated NH_4OH for 12 h. In both cases, removal of volatiles in vacuo provided essentially pure amide. After removal of traces of coproduced 3-(4-nitrophenylsulfamoyl)propionic acid, by 10% $KHCO_3$–H_2O lavage, crystallization of the insoluble residue from EtOH–H_2O provided pure product as colorless needles of mp 171–172 °C. Anal. ($C_9H_{11}N_3O_5S$) C, H, N.

2-Methyl-3-(4-nitrophenylsulfamoyl)propionamide was similarly prepared by ammonolysis of the corresponding methyl ester and was obtained from EtOH–H_2O as pale yellow needles of mp 167–168 °C (87%). Anal. ($C_{10}H_{13}N_3O_5S$) C, H, N, S.

3-(4-Nitrophenylsulfamoyl)-*N*,*N*-dimethylpropionamide. 2-(4-Nitrophenyl)-1-thia-2-azacyclopentan-3-one 1,1-dioxide (0.0195 M) was suspended in aqueous dimethylamine (26% w/v; 40 mL) plus dioxane (10 mL) and the mixture stirred at room temperature until homogeneous and then for 12 h longer. The mixture was evaporated to dryness in vacuo, the residue was shaken well with 10% aqueous $KHCO_3$ (25 mL), and the crystals were collected. Acidification of the $KHCO_3$ solution returned 3-(4-nitrophenylsulfamoyl)propionic acid (3.2 g). Crystallization of the insoluble residue from EtOH–H_2O provided pure dimethylamide as colorless needles of mp 293–294 °C (38% conversion). Anal. ($C_{11}H_{15}N_3O_5S$) C, H, N, S.

2-Methoxy-4'-nitroethanesulfonanilide was prepared from 2-methoxyethanesulfonyl chloride and 4-nitroaniline in pyridine solution in the usual manner.[1,2] Pure product separated as pale yellow needles from EtOH–H_2O: mp 234–236 °C (46%). Anal. ($C_9H_{12}N_2O_5S$) C, H, N, S.

4-(2-Hydroxyethoxy)-9(10*H*)-acridone. 4-Hydroxy-9(10*H*)-acridone (0.02 M), KI (0.5 g), K_2CO_3 (0.07 M), and 2-bromoethanol (7.4 mL, 0.093 M) were suspended in 2-butanone (75 mL) and the heterogeneous mixture was stirred and boiled for 5 h. Inorganic salts were removed by filtration and washed thoroughly with boiling Me_2CO. Evaporation of solvents and shaking of the residue with 5% KOH–H_2O provided crude product. After crystallization once from EtOH–H_2O the compound was dissolved in 1:1 HOAc–H_2O, decolorizing charcoal added, and the solution clarified. Addition of H_2O to the boiling filtrate until turbid and then slow cooling provided highly crystalline product. One further crystallization from EtOH–H_2O provided pure product as yellow needles of mp 235–236 °C (82%). Anal. ($C_{15}H_{13}NO_3$) C, H, N.

4-(2-Acetyloxyethoxy)-9(10*H*)-acridone. The aforementioned hydroxy compound (0.01 M) was suspended in Ac_2O (10 mL) and pyridine (10 mL) and the whole mixture heated on a steam bath for 0.5 h. Removal of volatiles in vacuo and addition of H_2O provided crude material. Crystallization from HOAc–H_2O provided pure product as pale yellow needles of mp 113.5–114 °C (86%). Anal. ($C_{17}H_{15}NO_4\cdot H_2O$) C, H, N.

4-(2,3-Dihydroxypropoxy)-9(10*H*)-acridone. A suspension of 4-hydroxy-9(10*H*)-acridone (0.019 M), K_2CO_3 (0.05 M), KI (0.5 g), and 2,2-dimethyl-4-(*p*-toluenesulfonyloxymethyl)-1,3-dioxolane (0.028 M) in DMF (15 mL) was stirred and heated on a steam bath for 6 h. As much DMF as possible was removed in vacuo at steam bath temperature and then crude product removed by exhaustive extraction with boiling Me_2CO. To remove unreacted 4-hydroxyacridone the residue resulting on evaporation of the Me_2CO extracts was dissolved by warming with 1 N KOH solution in 85% MeOH–H_2O (50 mL), then MeOH removed in vacuo, and H_2O (25 mL) added. The precipitated material was collected, best by centrifugation, washed with 1 N KOH and H_2O, and then dried. TLC monitoring showed that invariably there was some cleavage of the dioxolane function during reaction and work-up.

Accordingly, the crude product was boiled with 80% HOAc–H_2O (10 mL) for 30 min to complete hydrolysis of the remaining dioxolane function and to thereby ensure a single major product. After removal of HOAc in vacuo, product was crystallized from MeOH–EtOAc and then from HOAc–H_2O. Pure product was obtained as colorless needles of mp 230–231 °C (61%). Anal. ($C_{16}H_{15}NO_4 \cdot H_2O$) C, H, N.

4-(2,3-Diacetyloxypropoxy)-9(10*H*)-acridone. The aforementioned product (0.0165 M) was suspended in a mixture of pyridine (16 mL) and Ac_2O (24 mL) and the whole mixture stirred and heated at 100 °C until a homogeneous solution resulted and then for 1 h further. After removal of volatiles in vacuo, product was dissolved in EtOAc and the solution washed successively with 2 N HCl, H_2O, 10% $KHCO_3$, and H_2O and then dried (Na_2SO_4). After evaporation to small volume ligroine was added to turbidity in the hot solution; on scratching and cooling product crystallized. Recrystallization from EtOAc–ligroine provided pure product as colorless needles of mp 126–127 °C (82%). Anal. ($C_{20}H_{19}NO_6$) C, H, N.

4-Hydroxy-6-iodo-9(10*H*)-acridone. A suspension of 4-methoxy-6-iodo-9(10*H*)-acridone (0.043 M) in constant boiling aqueous HBr (250 mL) was slowly distilled through an efficient fractionating column, the heating being so maintained that the temperature at the column head did not exceed 115 °C and H_2O as produced was continuously removed. After 16 h of heating H_2O (1 L) was added and the precipitated mixed acridones were collected. Hydroxyacridones were removed by boiling with successive quantities of 5% KOH–H_2O (100 mL) until the extracts were no longer colored. Acidification of the extracts while hot precipitated crude product in granular form. The crystalline potassium salt of the required compound was obtained by solution of crude product in the minimum volume of hot 5% KOH–H_2O and then portionwise addition of solid KCl to the hot solution until the red-orange potassium salt started to separate. Slow cooling provided a highly crystalline potassium salt. The collected salt was dissolved in hot H_2O containing a little KOH, the solution clarified, and product precipitated in the hot solution by addition of HOAc. A further crystallization from 2-ethoxyethanol–H_2O provided a TLC homogeneous product as orange-red needles of mp 343–346 °C (62%). Anal. ($C_{13}H_8INO_2$) C, H, N, I.

4-(2,3-Dihydroxypropoxy)-6-iodo-9(10*H*)-acridone. 4-Hydroxy-6-iodo-9(10*H*)-acridone (0.012 M), K_2CO_3 (0.032 M), KI (0.5 g), and 2,2-dimethyl-4-(*p*-toluenesulfonyloxymethyl)-1,3-dioxolane (0.018 M) in DMF (10 mL) were heated together on a steam bath for 2 h. Further sulfonic ester (9 mM) and K_2CO_3 (0.01 M) were then added and heating was continued for a further 2 h; TLC monitoring then demonstrated that all starting hydroxyacridone had reacted. As much DMF as possible was removed in vacuo and the residue extracted to completion with boiling Me_2CO. The semisolid mass resulting on removal of Me_2CO was dissolved in 1 N KOH in 85% MeOH–H_2O by warming and the solution was boiled for 10 min, HOAc (10 mL) was then added, and boiling continued for a further 10 min. After removal of volatiles in vacuo, crude product was extracted into EtOAc and the solution washed successively with 2 N HCl, H_2O, 10% $KHCO_3$, and then saturated brine, dried (Na_2SO_4), and evaporated. Crystallization from HOAc–H_2O and then from MeOH provided pure product as buff needles of mp 276–277 °C (71%). Anal. ($C_{16}H_{14}INO_4$) C, H, N, I.

4-(2,3-Diacetyloxypropoxy)-6-iodo-9(10*H*)-acridone. A sample of the forementioned compound (7.2 mM) was suspended in pyridine (12 mL) plus Ac_2O (16 mL) and the mixture stirred at 100 °C until a solution resulted and then for 1 h longer. After removal of volatiles in vacuo, product was removed in EtOAc and the resulting solution washed successively with 2 N HCl, H_2O, 10% $KHCO_3$, and then brine; the solution was dried (Na_2SO_4) and evaporated to 10 mL. Trituration and occasional refrigeration induced crystallization after several days. Further crystallization from EtOAc–ligroine provided pure compound as colorless prisms of mp 201–203 °C (83%). Anal. ($C_{20}H_{18}INO_6$) C, H, N, I.

4-Nitrophenyl 9(10*H*)-Acridone-4-carboxylate. Finely powdered 9(10*H*)-acridone-4-carboxylic acid (0.083 M) and 4-nitrophenol (0.16 M) were suspended in pyridine (200 mL) and the mixture was stirred vigorously at 60 °C while PCl_3 (4.4 mL, 0.053 M) was added in dropwise fashion. The heterogeneous mixture was then immediately heated in a steam bath, a clear solution resulting in minutes. Shortly afterward product started to crystallize and after 1 h of further heating the mixture was cooled thoroughly and product was collected, washed well with MeOH, H_2O, and then MeOH, and dried. The compound as obtained directly from the reaction mixture (87%) was homogeneous to TLC criterion. For analysis a sample was recrystallized from a large volume of DMF and was obtained as yellow needles of mp 280–281 °C. Anal. ($C_{20}H_{12}N_2O_5$) C, H, N.

4-Nitrophenyl 9-Chloroacridine-4-carboxylate. A sample of the preceding compound (5.6 mM) was heated with $SOCl_2$ (6 mL) containing DMF (0.02 mL) under reflux conditions until a clear solution resulted and then for 0.5 h longer. Volatiles were removed in vacuo, a little dry C_6H_6 added, and the mixture again evaporated. The residue was dissolved in EtOH-free dry $CHCl_3$ (100 mL), the whole mixture cooled in ice, and ice-cold 10% $KHCO_3$ (20 mL) added. The $CHCl_3$ layer was washed with $KHCO_3$ and dried (Na_2SO_4), C_6H_6 (15 mL) added, and the solution evaporated to 20 mL. On cooling pure product separated as very pale yellow needles of mp 194–196 °C (94%). Anal. ($C_{20}H_{11}ClN_2O_4$) C, H, N, Cl.

9-Chloroacridine-4-carboxamide. Method A. A suspension of 9(10*H*)-acridone-4-carboxylic acid (8.3 mM) was suspended in $SOCl_2$ (20 mL) containing DMF (0.02 mL) and the heterogeneous mixture heated under reflux conditions until a clear solution resulted and then for 0.5 h further. After removal of volatiles in vacuo a little dry C_6H_6 was added and this solvent then evaporated. The residue was dissolved in dry EtOH-free $CHCl_3$ (100 mL) and the cold (5 °C) solution added to dioxane (20 mL) which had been previously saturated with dry NH_3 at 5 °C. After stirring for 15 min H_2O (100 mL) was added, the $CHCl_3$ layer washed with 2 N NH_4OH and dried (Na_2SO_4), and 65% EtOH–H_2O (15 mL) added to the clarified solution. Distillation of $CHCl_3$ to incipient crystallization and following thorough cooling provided pure product (72%).

Method B. 4-Nitrophenyl 9-chloroacridine-4-carboxylate (5 mM) was dissolved in dry EtOH-free $CHCl_3$ (72 mL) and the solution cooled with stirring to 5 °C. Concentrated aqueous NH_4OH (10 mL) was added and the mixture stirred at room temperature for 4 h. Sufficient $CHCl_3$ was added to dissolve the crystalline product which had separated, the $CHCl_3$ solution washed with 2 N NH_4OH and dried (Na_2SO_4), and 65% EtOH–H_2O (25 mL) added to the dry, clarified solution. Distillation of $CHCl_3$ to incipient crystallization and thorough cooling provided pure product (78%).

Products from both routes had identical melting points of 228 °C dec, not depressed on admixture. TLC failed to resolve the two samples. Anal. ($C_{14}H_9ClN_2O$) C, H, N, Cl.

4-(*N*-Methylcarbamoyl)-9-chloroacridine and 4-(*N*,*N*-dimethylcarbamoyl)-9-chloroacridine were best prepared by application of method A using the requisite amine in place of NH_3. These reactive 9-chloro compounds were best immediately coupled with the requisite aromatic amine as detailed earlier.[1]

3′-Methoxy-4′-[4-(4-nitrophenoxycarbonyl)-9-acridinylamino]methanesulfonanilide (43). 4-Nitrophenyl 9-chloroacridine-4-carboxylate (0.011 M) was stirred with dry EtOH-free $CHCl_3$ (40 mL) at 5–10 °C and a cold solution of 4′-amino-3′-methoxymethanesulfonanilide (0.012 M) in a mixture of EtOH (100 mL), $CHCl_3$ (60 mL), and H_2O (4 mL) was added. The mixture was slowly warmed with stirring, product starting to crystallize as a temperature of ca. 50 °C was reached. Reaction was completed by boiling for 0.5 h, the mixture cooled thoroughly, and product collected. Recrystallization was by solution in 65% Me_2CO–H_2O and then addition of hot 10% aqueous NaCl to incipient crystallization and following cooling. Product (**43**, Table I) was obtained as brick-red crystals.

4′-(4-Carbamoyl-9-acridinylamino)-3′-methoxymethanesulfonanilide (40). To a stirred suspension of the aforementioned compound (1.3 mM) in DMF (15 mL) was added concentrated aqueous NH_4OH (2 mL), a homogeneous solution rapidly resulting. After 4 h at room temperature volatiles were removed in vacuo and, after addition of HOAc (3 mL) and thorough mixing, product was dissolved by addition of boiling H_2O (75 mL). Solid NaCl was added to the hot clarified solution to incipient crystallization and the mixture cooled thoroughly. The brick-red crystals were collected, well washed with saturated aqueous NaCl, and then dissolved in hot 0.05 N HOAc. 20% NaCl–H_2O was

added to the hot solution until crystallization initiated; slow cooling then provided pure product (**40**, Table I) as red needles (62%). This compound could not be distinguished by melting point, mixture melting point, or TLC from the product formed by mild acid-catalyzed coupling of 9-chloroacridine-4-carboxamide and 4'-amino-3'-methoxymethanesulfonanilide, using the standard methods detailed earlier.[1,2]

45. To a well-stirred suspension of **43** (0.17 mM) and glycinamide hydrochloride (0.25 mM) in DMF (7.5 mL) at room temperature was added Et_3N (0.059 mM), a homogeneous solution rapidly resulting. After 24 h at room temperature crude product was precipitated by addition of 10% aqueous $KHCO_3$ (10 mL) and H_2O (75 mL). The solid was collected, washed well with H_2O, and dissolved in hot 0.5 N HOAc. Addition of solid NH_4Cl to the hot clarified solution to 20% concentration and then slow cooling provided crystalline material. A further crystallization from 0.05 N HOAc–NH_4Cl provided pure product (**45**, Table I) as red needles (68%). **44** and **46** were similarly prepared employing 3-aminopropane-1,2-diol and *O*-triacetyl-α-methyl-D-glucosaminide hydrobromide,[21] respectively, in place of glycinamide hydrochloride and compensatory adjustment of the quantity of Et_3N used.

Protecting *O*-acetyl functions of **46** were removed (providing **47**) by treatment with NH_3–MeOH in the fashion normally employed in carbohydrate chemistry.

3-[*N*-(2-Carboxyphenyl)-2-aminophenyl]propionic Acid. 2-Nitrocinnamic acid (0.026 M) was suspended in H_2O (25 mL) at 60 °C and $KHCO_3$ (0.028 M) added as permitted by the resulting effervescence. When a clear solution resulted 10% Pd/C catalyst (0.5 g) was added and hydrogenation carried out at 45 psi of H_2 and 25 °C until the theoretical amount of H_2 had been absorbed. After removal of catalyst the solution was evaporated to dryness in vacuo, the potassium salt of 3-(2-aminophenyl)-propionic acid crystallizing in the latter stages. A sample probe of the crystalline salt on solution in the minimum quantity of H_2O and acidification immediately provided dihydrocarbostyril (83% yield), identical with an authentic sample by melting point, mixture melting point, and TLC.

To the remaining, thoroughly dried [P_2O_5 (vacuum)] potassium salt were added K_2CO_3 (0.015 M), potassium 2-chlorobenzoate (0.026 M), catalytic Cu (0.05 g), Cu_2O (0.05 g), and 2-ethoxyethanol (6 mL). The mixture was heated in an oil bath under reflux conditions for 2 h. H_2O (100 mL) was added to the cooled mixture and the whole mixture shaken until all salts had dissolved. Clarification and then acidification provided crude product which was collected and washed well with boiling H_2O. The crystals were dissolved in H_2O (100 mL) containing Na_2CO_3 (6 g), decolorizing charcoal (1 g) was added, and, after stirring for 15 min, the mixture was filtered and the product precipitated with acid, collected, and washed well with boiling H_2O. The crystals were dissolved in Me_2CO by boiling, an equal volume of hot EtOH was added, and the solution was distilled until crystallization initiated. A further crystallization from DMF–H_2O provided homogeneous product as yellow prisms of mp 214 °C dec (44%). Anal. ($C_{16}H_{15}NO_4$) C, H, N.

3-[9(10*H*)-Acridon-4-yl]propionic Acid. The aforementioned product (8.8 mM) was suspended in poly(phosphoric acid) (82% P_2O_5, 25 g) and the mixture heated on a steam bath with occasional swirling until a homogeneous solution resulted (1 h) and then for 0.5 h longer. Addition of warm H_2O (125 mL) precipitated a solid which was dissolved in 10% NaOH (15 mL), a small quantity of nonacidic material was removed by filtration, and product was recovered by acidification. Crystallization from DMF–H_2O provided pure product as yellow needles of mp 279 °C dec (89%). Anal. ($C_{16}H_{14}NO_3$) C, H, N.

3-[*N*-(2-Carboxyphenyl)-3-aminophenyl]propionic Acid. 3-(3-Aminophenyl)propionic acid (0.145 M), 2-chlorobenzoic acid (0.145 M), K_2CO_3 (0.29 M), catalytic Cu (0.3 g), and Cu_2O (0.3 g) were suspended in 2-ethoxyethanol (40 mL) and the mixture was heated in an oil bath under reflux conditions for 2 h. Sufficient boiling H_2O to dissolve all salts was added, the resulting solution clarified, and crude product precipitated by acidification. Crystallization from EtOH–H_2O and then from EtOAc–ligroine provided pure product as yellow needles of mp 168–170 °C (62%). Anal. ($C_{16}H_{15}NO_4$) C, H, N.

3-[9(10*H*)-Acridon-3-yl]propionic Acid. The preceding compound (0.07 M) was suspended in 98% H_2SO_4 (35 mL) and the mixture heated on a steam bath with occasional swirling for 4 h. Cooling and addition of ice precipitated crude product which was collected and well washed with boiling H_2O. Solution in H_2O (200 mL) containing Na_2CO_3 (10 g) by warming, following filtration and then acidification, returned product from which traces of nonacidic material had been removed. Multiple crystallizations from EtOAc were necessary to rid the 3-(acridon-3-yl)propionic acid of traces of the 3-(acridon-1-yl)propionic acid also produced in the ring-closure step. Pure product separated from EtOAc as yellow needles of mp 297 °C dec (68%). Anal. ($C_{16}H_{13}NO_3$) C, H, N.

Acridones bearing carboxylic acid functions were converted to the corresponding 9-chloroacridinecarbonyl chloride by treatment with $SOCl_2$–DMF as described for 9(10*H*)-acridone-4-carboxylic acid. As in transformations of the latter compound treatment with dry NH_3 at low temperature provided the relatively labile 9-chloroacridinecarboxamides which, after immediate purification, as before,[1,2] were promptly allowed to react with side-chain amines by our standard method.

Methods for generation, purification, and handling of 9-chloroacridines, as well as conditions for mild acid-catalyzed coupling of these to aromatic amines, and purification of the resulting 9-anilinoacridines have been adequately detailed earlier.[1,2,16]

Biological Testing. 10^5 L1210 cells were inoculated either intraperitoneally or subcutaneously, above the right axilla, into 18.5–22.5-g C_3H/DBA_2 F_1 hybrid mice. Ip drug treatment started 24 h later and continued for 5 days. An animal dose was contained in a volume of 0.2 mL. Dose levels were separated by 0.18 log dose intervals and there were six animals per dose level and one control group for every six test groups.

In sc L1210 assays a series of drug dilutions providing 0.18 log dose intervals has been screened on the one occasion, the median dose being the optimum observed in earlier ip L1210 assays. For acceptance of sc L1210 test results, with any single compound, the highest dose must provide evidence of drug toxicity, as shown by premature animal deaths, or lessened levels of life extension in comparison with those seen at lower doses. The lowest dose of the dilution series must be clearly suboptimal, as evidenced by shorter life extension than seen with higher doses, or clear average body weight gain. As necessary, screening was repeated with adjustment of doses until, in one batch of tests, a drug dilution series provided acceptable spanning of the optimum drug dose.

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Biologically Oriented Organic Sulfur Chemistry. 15. Organic Disulfides and Related Substances. 41. Inhibition of the Fungal Pathogen *Histoplasma capsulatum* by Some Organic Disulfides[1]

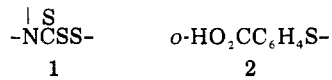
Lamar Field,* John A. R. Grimaldi, Jr., Wayne S. Hanley, Mark W. Holladay, Ramanathan Ravichandran, Lawrence J. Schaad, and C. Emory Tate

Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235. *Received December 28, 1976*

In an extension of promising inhibitory results in vitro against *Histoplasma capsulatum*, correlated earlier using substituent constants developed by regression analysis with 77 disulfides, one symmetrical and 14 unsymmetrical disulfides were prepared (3–17). About half were active in vitro against *H. capsulatum* (and one against *Candida albicans*). Groups that seemed most to lead to promising inhibition among the unsymmetrical disulfides were *o*-$HO_2CC_6H_4$, $(CH_2)_4SO_2Na$, $Me_2NC(S)$, *p*-ClC_6H_4, and perhaps *p*-$CH_3C_6H_4$; the first two also might be used to increase solubility. Earlier inhibitory promise of the morpholino group did not materialize. None of the group 3–17 was significantly active in vivo. The unsymmetrical disulfides were prepared by reaction of thiols with sulfenyl chlorides or with acyclic or cyclic thiolsulfonates. Two six-membered heterocyclic disulfides (5 and 6) were prepared by a novel cyclization, in which carbon disulfide reacted with an (*N*-alkylamino)ethyl Bunte salt, followed by ring closure; an explanation is suggested for formation of a thiazoline when the *N*-alkyl group is absent. One of the disulfides disproportionated with astonishing ease (31; 0.3–1 h at 25 °C).

On the basis of promising inhibitory activity in vitro,[2] we tested numerous classes of organic sulfur compounds against *Histoplasma capsulatum*,[3] the organism responsible for histoplasmosis in man. This paper reports exploration of some attractive structure–activity relationships that developed.

The trithiopercarbamate moiety (1) has been one of the most promising,[2,3d,f,g] particularly with the nitrogen atom



1 2

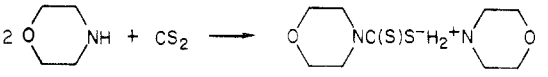
as part of a morpholine ring or bearing two methyl groups (minimum inhibitory concentration, MIC, 1–2.5 μg/mL).[3d,g] Since the *o*-carboxyphenylthio moiety (2) is a promising latentiating group for biologically active thiols,[4] its use to latentiate 1 deserved attention. Table I shows two target compounds selected, 3 and 4, along with other compounds tested as discussed below.

Incorporation of the trithio moiety 1 into a cyclic system, exemplified in trithiopercarbamates 5 and 6 (Table I), might lead to substances active via intramolecular latentiation. Since *p*-chlorobenzenethiol has been one of the most active of a number of thiols tested (MIC, 2.5–7.5 μg/mL),[3a,f] combination with *N,N*-dimethyl-1 in the known trithiopercarbamate 7[5] also was attractive (Table I).

During biological evaluation of 3–7, we developed substituent constants for inhibitory effects of disulfides in vitro by linear regression analysis using the Free–Wilson method.[3g] The single morpholino compound included had a favorable constant. To assess the promise of the morpholino group further, testing of 3 was complemented by that of 8–12 (Table I). A seventh morpholino disulfide sought proved unstable, in a chemically significant way described below.

Finally, five other disulfides of varied structure, predicted to be inhibitory from their substituent constants, were prepared and tested (13–17 in Table I). Table I lists all compounds tested (3–17), together with values calculated where possible for the minimum inhibitory concentrations (MIC).[3g]

Chemistry. Equation 1 shows the preparation of the dithio acid salt 18. As eq 2 shows, 18 was thioalkylated

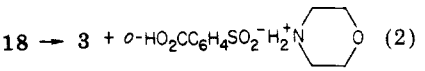


(1)

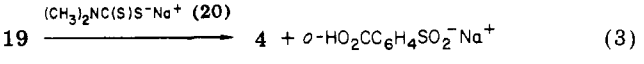
18

o-$HO_2CC_6H_4SO_2SC_6H_4$-*o*-CO_2H +

19



(2)



(3)

with the thiolsulfonate 19 to give the first compound of Table I, 3, in an extension of a reaction we have studied previously.[4a] A similar reaction led to 4 (eq 3). Purification of 3 and 4 was difficult because both disulfides dissolved so slowly in ethanol (the only promising solvent for recrystallization) that significant decomposition occurred when amounts of more than ca. 0.2 g were recrystallized (broadening of IR bands and melting point; cf. Experimental Section).

Since 2-(*n*-decylamino)ethanethiol had shown promising activity (MIC, 7.5–10 μg/mL),[3a] a thiolsulfonate counterpart of it, 21,[6] was substituted for the carboxyphenyl thiolsulfonate (19) in reactions like those of eq 2 and 3 with the two salts (18 and 20). These efforts were abandoned