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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]

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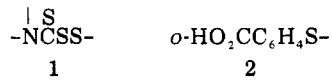
In an extension of promising inhibitory results in vitro against *Histoplasma capsulatum*, correlated earlier using substituent constants developed by regression analysis with 77 disulfides, one symmetrical and 14 unsymmetrical disulfides were prepared (3–17). About half were active in vitro against *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

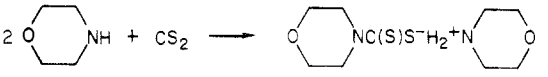
as part of a morpholine ring or bearing two methyl groups (minimum inhibitory concentration, MIC, 1–2.5 μg/mL).[3d,g] Since the *o*-carboxyphenylthio moiety (2) is a promising latentiating group for biologically active thiols,[4] its use to latentiate 1 deserved attention. Table I shows two target compounds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

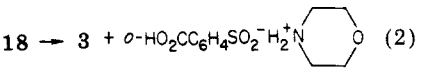


(1)

18

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

19



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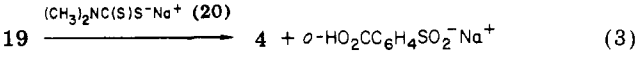
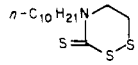
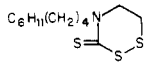


(3)

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).

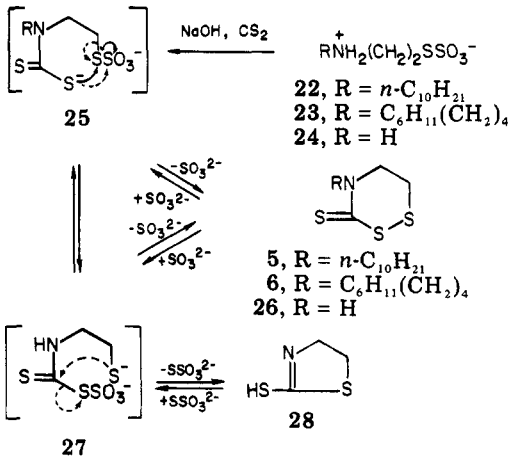
Since 2-(*n*-decylamino)ethanethiol had shown promising activity (MIC, 7.5–10 μg/mL),[3a] a thiolsulfonate counterpart of it, 21,[6] was substituted for the carboxyphenyl thiolsulfonate (19) in reactions like those of eq 2 and 3 with the two salts (18 and 20). These efforts were abandoned

Table I. Inhibitory Effects of Compounds on *H. capsulatum*

Compd	Structure	In vitro, MIC (μg/mL)[a] Found	Predicted by model for 2-groups[b]	Predicted by model for 4-groups[b]	In vivo, extension of mouse life, %[c] Dose, mg/kg[d]	Respective %[c]
3	$OC_4H_8NC(S)SSC_6H_4$-*o*-CO_2H[e]	>10[f]	3	3	50, 25, 13	−7, −5, −2[g]
4	$(CH_3)_2NC(S)SSC_6H_4$-*o*-CO_2H	<1[f]	6	7		
5		>10[f]	*h*	*h*		
6		10[f]	*h*	*h*		
7	$(CH_3)_2NC(S)SSC_6H_4$-*p*-Cl	5[f]	*h*	1	50, 25, 13	+11, +13, −14[g]
8	$OC_4H_8NC(S)SSC_2H_5$[e]	10[i]	3	3	25,[j] 13, 6	+5, +5, +5[k]
9	$OC_4H_8NC(S)SSC(CH_3)_3$[e]	>40[i]	2	2-3	40, 20, 10	+11, +5, −4[k]
10	$OC_4H_8NC(S)SSC_6H_5$[e]	40[i]	2	2	25,[j] 13, 6	+9, +11, +5[k]
11	$OC_4H_8NC(S)SS$-C_6H_4-*p*-CH_3	40[i]	1	1	50, 25, 13	+9, 0, +5[k]
12	$[ONC_4H_8C(S)S]_2$	40[i]	1	2	25,[j] 13, 6	+5, +5, +5[k]
13	*p*-$CH_3C_6H_4SS(CH_2)_4SO_2Na$	20[i]	3	2	50, 25, 13	−21, −6, +15[l]
14	*p*-$CH_3OC_6H_4CH_2SS(CH_2)_4SO_2Na$	20[i]	3	3	50, 25, 13	+9, −19, −2[l]
15	*p*-$CH_3C_6H_4SS(CH_2)_2NH_3Cl$	>20[i]	3	2-6	50, 25, 13	+9, 0, +4[l]
16	*p*-$CH_3OC_6H_4C(S)SSC_6H_4$-*p*-CH_3	20[i]	*h*	1	50, 25, 13	−2, +7, +2[l]
17	*p*-$CH_3C_6H_4SSCH_2CO_2H$[m]	20[i]	3	2-5	50, 25, 13	0, −6, +17[l]

[a] MIC = minimum inhibitory concentration by a standard method (see text). [b] Calculated as reported in ref 3g. [c] Extension in percent of lifetimes beyond controls (see text). [d] Given sc at 0 and 4 h postinfection on days 0, 1, and 2 postinfection for a total of six doses. [e] OC_4H_8N = c-$O(CH_2CH_2)_2N$-. [f] Amphotericin B, 0.125 μg/mL. [g] Amphotericin B given similarly at 50 mg/kg gave +70%. [h] Lack of constants for one or more groups precluded calculation. [i] Amphotericin B, 0.0625 μg/mL. [j] Toxic at higher doses. [k] Amphotericin B given similarly at 25 mg/kg gave +58%. [l] Amphotericin B given similarly at 50 mg/kg gave +108%. [m] An analytically pure sample could not be obtained; see text.

Scheme I

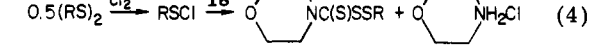


when TLC showed that the products contained both possible symmetrical disulfides (presumably from disproportionation), as well as the sulfinate salt produced from 21. Efforts to effect purification failed.

n-$C_{10}H_{21}NH(CH_2)_2SO_2S(CH_2)_2NH$-$n$-$C_{10}H_{21}\cdot 2HCl$
21

As Scheme I shows, heterocycles 5 and 6 of Table I were obtained from the Bunte salts 22[7] and 23;[8] yields exceeded 90%. Efforts to obtain an unsubstituted counterpart (26) from 24 by the same procedure led only to 2-thiazoline-2-thiol (28). Conversion of 24 to 28 with pyridine–aqueous sodium carbonate and carbon disulfide was reported earlier, although under more vigorous conditions.[9] It is interesting that the probable intermediate 25 produces 5 and 6 when R is a long chain but the thiazoline 28 when R is a hydrogen atom. The equilibria of Scheme I seem to explain this difference. Thus compounds 5, 6, and 26 may be the first products produced (kinetic control), as the solid arrows in 25 indicate. When R is a long-chain substituent, both 5 and 6 (being sparingly soluble) immediately precipitate so that the equilibria favor their formation. In the formation of 28, on the other hand, the kinetic product 26 presumably remains in solution until it either returns to 25 or goes directly to 27, which then cyclizes to 28 as shown by the dotted arrows (cf. ref 9). Conversion of 25 to 28 by displacement from carbon of SSO_3^{2-} seems unlikely. The thiazoline 28 did *not* precipitate from solution when 24 was subjected to the mild conditions used for 5 and 6 and had to be isolated by evaporation. Hence 28 may be a product of thermodynamic control, toward which the equilibria drift. An attempt to prepare 26 from 24 using carbon disulfide with triethylamine in ether yielded only starting material (95%). An effort to convert 5 (Table I) to the *N*-*n*-decyl analogue of 28 (as the thiono form) with Na_2SO_3 was unpromising, perhaps because of the low solubility of 5.

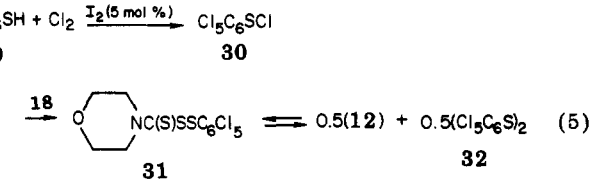
Of the other morpholino disulfides beside 3 in Table I, 8–11 were prepared essentially by the method of eq 4 and the disulfide 12 by oxidizing the thiolate salt 18 with



(4)

iodine. No problems were encountered except for the existence of 12 in three polymorphic forms, two of which had been observed but not recognized as polymorphs.

On the other hand, preparation of a final morpholino disulfide, 31, as shown in eq 5, led to several chemical features that deserve mention. Disulfide 31 was of interest



(5)

because the pentachlorophenyl group has a promising

substituent constant, although this group was used only once in the regression analysis (MIC = 3 for **31**, calculated by either method of Table I).[3g] Purification of the thiol **29** proved necessary to circumvent problems caused by impurities in commercial **29**. Attempted conversions of **29** to the sulfenyl chloride **30** with sulfuryl chloride, catalyzed by pyridine,[10] led mostly to the disulfide **32**. Reaction of **32** with sulfuryl chloride,[10,11] with or without pyridine or iodine catalysis, or with chlorine, left at least 35% of **32**. Chlorination of **29** to **30**, catalyzed by "a few crystals of iodine",[12] also gave mostly **32**. However, use of 5 mol % of iodine with **29** and chlorine led to **30** in quantitative yield (the long-term stability of **30** at 5 °C incidentally is worth noting). Conversion to **31** was best achieved by adding **30** and **18** concurrently to solvent at −70 °C and separating **31** from the coproducts **12** and **32** chromatographically. It is chemically significant that **31** probably disproportionates more rapidly to the symmetrical disulfides (**12** and **32**) than any unsymmetrical disulfide we have studied,[13] perhaps because the thiolate ions corresponding to both **12** and **32** are such good leaving groups. Thus disproportionation of **31** in benzene was significant at ~25 °C after only ~0.3 h and seemed to have reached an equilibrium point after ~1 h. Even solid **31** disproportionated significantly after a week at 5 °C. An attempt to evaluate **31** biologically thus did not seem worthwhile.

High rank of the groups p-$CH_3C_6H_4$, p-$CH_3OC_6H_4CH_2$, and $(CH_2)_4SO_2Na$ in the regression analysis prompted synthesis of the water-soluble combinations **13** and **14**.[3g] Equation 6 shows the synthesis, which was based on our earlier work.[14] Excess **33** was used because its facile

$$\mathrm{RSNa} + \overbrace{(\mathrm{CH_2})_4\mathrm{SO_2S}}^{\mathbf{33}} \xrightarrow{\Delta} \mathrm{RSS(CH_2)_4SO_2Na} \quad (6)$$

13, R = p-$CH_3C_6H_4$; **14**, R = p-$CH_3OC_6H_4CH_2$

$$0.5(\mathrm{RS})_2 + 0.5[\mathrm{S(CH_2)_4SO_2Na}]_2 \quad (7)$$

removal promised purification with minimum disproportionation. In disproportionation (eq 7), the half-life of **13** was found to be ~9 h at 25 °C in D_2O and that of **14** ~1.7 h at 68 °C in H_2O.

The potential of $(CH_2)_2NH_3^+$ and CH_2CO_2H as solubilizing groups for the moiety p-$CH_3C_6H_4SS$ led to **15** and **17** of Table I as target compounds; **16** was sought because of good constants for C(S) and p-$CH_3OC_6H_4$.[3g] We first tried to synthesize **15** and **17** using a method of Harpp and co-workers,[15] i.e., by attack of the appropriate thiol on *N*-(*p*-tolylthio)phthalimide. The products showed several TLC spots (including those of the symmetrical disulfides), however, and good separations for **15** and **17** could not be achieved. We next tried *p*-toluenesulfenyl chloride (**34**) with the appropriate thiol. This synthesis for **15** succeeded and, indeed, gave a better yield (70%) than our earlier one involving a thiolsulfonate (42%).[16] Disulfide **16** was obtained similarly from **34** and the dithio acid, but in the lower yield of 39%, probably because of side reactions and the considerable purification needed. Synthesis of **17** from **34** has been reported but with no detail.[17] Although NMR and IR spectra were satisfactory for **17** obtained by the reported method, and although TLC gave only one spot (different from either symmetrical disulfide), neither a good yield nor analysis could be obtained for **17**; the matter was not pursued since the biological properties of the sample tested were not notable (Table I). An effort to prepare 2-chloroethyl 2-(*n*-decylamino)ethyl disulfide hydrochloride, containing two promising groups,[3g] by reaction of 2-chloroethanesulfenyl chloride and 2-(*n*-decylamino)ethanethiol produced only bis[2-(*n*-decylamino)ethyl] disulfide.

Evidence that the unsymmetrical disulfides were not 1:1 mixtures of the two symmetrical ones was afforded by good agreement with the reported melting point for the known compounds **7**, **9**, **10**, **12**, and **15**. For the others, at least three of the following were applicable, depending on the circumstances: (1) use of well-established methods of preparation and purification; (2) proper elemental analysis (possible only for the unsymmetrical disulfide or in the improbability that a precise 1:1 ratio of the symmetrical ones would survive purification); (3) significant differences in solubility of the unsymmetrical disulfide from one or both symmetrical ones; (4) all previously unknown unsymmetrical disulfides showed only one spot in TLC; (5) sharp melting point (not characteristic of a mixture);[16] (6) study of disproportionation.

Biological Results and Structure–Activity Relations. Table I shows the results of evaluations of **3–17** against *H. capsulatum* in vitro and in vivo, together with values for amphotericin B as a standard drug for histoplasmosis. In vitro results were obtained using the standard agar dilution assay method;[18a] values of the amphotericin B standard did not vary more than fourfold from test to test (e.g., from 0.016 to 0.25 for the standard MIC reported of 0.0625 μg/mL).

Results calculated by the method involving 1/MIC and two groups (X–SS–X′) or four groups (X_1–X_2–SS–X_3–X_4) are shown where availability of substituent constants permitted calculation.[3g] Ranges, as illustrated with **9**, **15**, and **17**, can occur depending on choice of groups, e.g., whether in the four-group method for **15** one uses p-$C_6H_4CH_3$ + bond or 1,4-C_6H_4 + CH_3. The in vitro assays were done on a different strain (*H. capsulatum* 26) from that on which the regression analysis was based (*H. capsulatum* Darling, strain H-7) and presumably also reflect a number of other indefinable differences probable with assays being done by different groups. Hence present results can be used to correct the earlier constants only qualitatively. Relative effects of substituents to one another and to amphotericin B should be inferable from Table I, however. Table I shows that all compounds found active were predicted to be, although not all compounds predicted to be active proved so.

Making allowances for differences in amphotericin standards (cf. footnotes, Table I), one can conclude that disulfides that have significant activity in vitro are **4**, **6**, **7**, and **8**, and perhaps **13**, **14**, **16**, and **17**. The most promising groups for conferring both activity and solubility thus seem to be o-$HO_2CC_6H_4$ (cf. **4**) and $(CH_2)_4SO_2Na$ (cf. **13** and **14**). The most promising for conferring activity seem to be $Me_2NC(S)$ (cf. **4** and **7**), p-ClC_6H_4 (cf. **7**), and perhaps p-$CH_3C_6H_4$ (cf. **13**, **16**, and **17**). The morpholino group may show a little promise (cf. **8**), but much less than one disulfide in the regression analysis led us to hope (cf. **3**, **9–12**).[3g] Cyclization offers little apparent advantage (cf. **5** and **6**).

Even though about half of the disulfides show in vitro activity, use of such compounds may well be mainly as topical or agricultural fungicides, since none of the group **3–17** was significantly active in vivo. In vivo evaluations of Table I were done essentially as before by evaluating the effect of the drug in prolonging lifetimes of mice that had been irradiated with x rays and then infected once and drugged twice daily during 3 days.[18b] The reproducibility in this method depends on the virulence of the organism; hence, to be considered significantly active, a compound should show significant and reproducible extension of

survival over untreated controls and should compare favorably with amphotericin B as a positive control that has performed reasonably well in the series of tests. With 3–7 an initial assay resulted in no promise, but since the strain was less virulent than usual the two best candidates were rechecked later with the results shown in Table I.

Disulfide 7 also showed significant activity in vitro against *Candida albicans* (conventional disk-plate assay, 0.156 μg/mL; amphotericin B showed 0.125 μg/mL), but it showed no significant activity in *Candida*-infected mice (for procedures, see ref 18b).

Approximate toxicities for ip doses of typical compounds may be helpful rough guides, although they show merely whether two to three of three mice survived the lower dose but not the higher; these were ~0.1–0.3 g/kg for 3–5 and 7 and ~1 g/kg for 6.

Experimental Section

Melting points were determined using a Thomas-Hoover stirred-liquid apparatus and are corrected; for compounds 3 and 4, capillaries were inserted at 155 °C, and the rate of heating was 1–2°/min. NMR and/or IR spectra were consistent with structures assigned (instability of 31 precluded spectra). NMR spectra were done with a Joelco JNM-MH-100 spectrometer using Me_4Si as an internal standard [or, with 13 and 14 in D_2O, with $Me_3Si(CH_2)_3SO_3Na$]; IR spectra were obtained using Nujol mulls or KBr pellets with a Beckman Model IR10 or Perkin-Elmer 727 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Where analyses are indicated by symbols of the elements, results obtained were within ±0.4% of theoretical values. Moist extracts usually were dried using anhydrous $MgSO_4$, and solvent then was removed under reduced pressure using a rotary-flask evaporator. TLC was done using Brinkmann silica gel G, unless otherwise stated, with solvents specified.

Materials. Morpholinium 4-morpholinecarbodithioate (18) was prepared from 22.6 g (297 mmol) of CS_2 and 42.9 g (493 mmol) of morpholine by stirring for 0.5 h in 500 mL of cold Et_2O: yield of precipitate, 59.2 g (96%); sublimes without melting, as reported.[19] Bis(*N,N*-3-oxapentamethylenethiocarbamyl) disulfide (12) was prepared by adding 1 L of 0.1 N aqueous I_2–KI during ca. 1 h to 25.1 g (100 mmol) of the salt 18 in 1 L of H_2O. The 12 that precipitated (15.3 g, 95%), when recrystallized from benzene–ether in different preparations, gave 12 with three different melting points, each unchanged by further recrystallization. Taken simultaneously, these were 137–140, 139.5–142 [Anal. ($C_{10}H_{16}N_2O_2S_4$) C, H, N], and 142.5–145 °C, corresponding to polymorphic forms of 12 (lit. mp 136–137[20] and 145 °C.[21] *o*-Mercaptobenzoic acid (Aldrich Chemical Co.) was recrystallized (EtOH–H_2O).[22] Sodium *N,N*-dimethyldithiocarbamate dihydrate (20, Wateree Chemical Co.) was precipitated from Me_2CO with hexane or heptane as odorless fine white needles. *p*-Chlorophenyl *N,N*-dimethyltrithiopercarbamate (7) was obtained by treating 20 with *p*-chlorobenzenesulfenyl chloride essentially as reported,[5] but with CH_2Cl_2 as the reaction solvent, use of a small amount of H_2O to dissolve the 20, and purification of the product (7) by recrystallization (CCl_4, $CHCl_3$). The yield was 49%, mp 112–114 °C (lit.[5] 76%, mp 112–114 °C). Bunte salts 22[7] and 23[8] have been reported. Compound 24 was a commercial product, as were any not mentioned.

***o*-Carboxyphenyl *N,N*-3-Oxapentamethylenetrithiopercarbamate (3).** *o*-Carboxyphenyl *o*-carboxybenzenethiolsulfonate 19 (5.80 g)[4a] was taken up in EtOH (60 mL) and stirred briefly, and insoluble bis(*o*-carboxyphenyl) disulfide was removed (weight after an Et_2O wash and rapid drying, 0.37 g). The amount of 19 left in solution thus was 5.43 g (16.05 mmol). As soon as possible, 5.22 g (20.86 mmol, ~30% excess) of 18 was added as a slurry in 20 mL of EtOH. The mixture was stirred for 2.75 h; 5.10 g (101%) of white 3 separated. Washing with cold H_2O and cold EtOH and drying gave 3.76 g (74%) of 3 with mp 164–167 °C dec. This 3 was tested biologically. Recrystallization of 1.00 g in batches of ca. 25 mg from EtOH was accomplished with much rubbing, minimal heating (30–60 s total for each batch), and rapid vacuum filtration of each solution. Cooling, filtration, and drying led to 0.41 g of 3: mp 170–172 °C dec; TLC (EtOH) gave only one spot, R_f 0.56. Anal. ($C_{12}H_{13}NO_3S_3$) C, H, S.

***o*-Carboxyphenyl *N,N*-Dimethyltrithiopercarbamate (4).** As with 3, thiolsulfonate 19 (13.39 g)[4a] was stirred with EtOH (135 mL), and the disulfide was removed. To the 19 left in solution (12.54 g, 37.1 mmol), 5.31 g (37.1 mmol) of 20 was added in EtOH (20 mL) as soon as possible. Precipitation began in ca. 5 min. Stirring was continued ca. 3 h. Filtration gave white solid. Washing with H_2O and drying gave 7.03 g of 4 (69%): mp 166 °C (sinters), 167.5–169 °C dec. A 6.10-g portion of this 4 was finely powdered and swirled in 0.20-g batches with 10 mL of boiling EtOH for 30 s. In each instance, solid then was separated by rapid filtration using vacuum into a dry ice chilled flask. After the entire 6.10-g portion had been thus treated, the filtrate was warmed to room temperature and filtered, and 4 dried to yield 1.13 g of white solid, mp 168.5–169.5 °C dec (crop 1). The precipitate remaining from the first treatment with EtOH was similarly processed to give 0.64 g of white solid, mp 167.5–169 °C dec (crop 2). This procedure was repeated three more times, giving yield and melting point for each crop number as follows: 3, 0.26 g, mp 167.5–168.5 °C dec; 4, 0.36 g, mp 167.5–168 °C dec; 5, 0.16 g, mp 166–167 °C dec. The final undissolved solid (1.67 g, mp 165–166.5 °C dec) was considered to be a sixth crop. The first five crops, with 0.20 g of similarly prepared 4, were tested biologically [for the composite, mp 166.5–167 °C dec, mmp (with authentic 4) 166.5–167 °C dec]; IR spectrum consistent with previously prepared 4. An analytical sample of 4 was obtained from 1.00 g, prepared in like manner but recrystallized in 25-mg batches as for 3 to give 0.38 g: mp 171–172 °C dec; TLC (EtOH) gave only one spot, R_f 0.64. Anal. ($C_{10}H_{11}NO_2S_3$) C, H, S.

4-*n*-Decyl-5,6-dihydro-1,2,4-3(4*H*)-dithiazinethione (5). *S*-2-(*n*-Decylamino)ethanethiosulfuric acid (22, 2.04 g, 6.86 mmol) in 100 mL of H_2O was stirred during simultaneous addition (ca. 0.25 h) of CS_2 (0.41 mL, 6.86 mmol) and NaOH (15.09 mmol in 54 mL of H_2O). A precipitate of 5 quickly formed. After addition was complete, the mixture was stirred for 0.5 h and centrifuged. Solid was separated and dried to yield 1.84 g (92%) of 5, mp 61–63 °C. Recrystallization (hexane) left the melting point unchanged. TLC gave a single spot (CH_2Cl_2, R_f 0.77; 3:1 $CHCl_3$–hexane, R_f 0.53). Anal. [$C_{13}H_{25}NS_3$, mol wt calcd 291 (found 291, mass spectrum)] C, H, N, S.

4-(4-Cyclohexylbutyl)-5,6-dihydro-1,2,4-3(4*H*)-dithiazinethione (6). As with 5, *S*-2-(4-cyclohexylbutylamino)-ethanethiosulfuric acid (23, 10.20 g, 34.54 mmol) was stirred as a suspension in 200 mL of H_2O during simultaneous addition of CS_2 (2.08 mL, 34.54 mmol) and NaOH (76 mmol in 69 mL of H_2O). Heavy precipitation occurred. The mixture was stirred for 0.5 h and centrifuged: yield of 6, 9.43 g (94%); mp 81–84 °C. Recrystallization from hexane and CCl_4 gave 6 with a constant melting point of 86–87.5 °C. The 6 showed only one spot in TLC (CH_2Cl_2). Anal. ($C_{13}H_{23}NS_3$) C, H, N, S.

2-Thiazoline-2-thiol (28). The procedure used for preparing 5 was applied with *S*-2-aminoethanethiosulfuric acid (24; 2.08 g, 13.22 mmol), NaOH (29.08 mmol in 28 mL of H_2O), and CS_2 (0.8 mL, 13.22 mmol). The clear solution that resulted was evaporated after 4 h. Washing of the residue with H_2O and recrystallization led to 0.70 g (44%) of 28, mp 102.5–105 °C. Repetition of the reported procedure[9] gave 28 (identical IR spectra) in 32% yield: mp 102–104 °C; mmp 102.5–105 °C (lit.[9] mp 105–106 °C).

Ethyl (8), *tert*-Butyl (9), Phenyl (10), and *p*-Tolyl (11) *N,N*-3-Oxapentamethylenetrithiopercarbamate. Typically, 3.5 g (2.27 mL, 49 mmol) of Cl_2 was condensed using dry ice–acetone and then was allowed to volatilize (0.3 h) into a stirred solution of 50 mmol of diethyl, di-*tert*-butyl, diphenyl, or di-*p*-tolyl disulfide in a solvent (50 mL of hexane for 9, 50 mL of CH_2Cl_2 for 8 and 10, and 125 mL of CH_2Cl_2 for 11) below −20 °C (~22 °C for 9). The resultant red-orange solution of RSCl was added during ~0.5 h to a suspension of 25.1 g (100 mmol) of the salt 18 in 150–500 mL of CH_2Cl_2 at −30 ± 10 °C. The mixture was allowed to warm to ~22 °C with stirring during 2 h. Morpholine hydrochloride was separated by filtration, and the filtrate was washed with H_2O to neutrality. Crude solid obtained after drying and evaporation of solvent was recrystallized from Et_2O (10 and 11), Et_2O–pentane (8), or petroleum ether (9). Results were as follows: 9, 85%, mp 61.5–63.5 °C (lit.[23] mp 61 °C); 10, 45%, mp 58.5–60 °C (lit.[24] mp 59.5–61 °C); 8, 45%, mp 32–33 °C [Anal.

($C_7H_{13}NOS_3$) C, H, N]; **11**, 95%, mp 72–73.5 °C [Anal. ($C_{12}H_{15}NOS_3$) C, H, N]. Each of the compounds **8**–**11** gave a single spot by TLC.

Pentachlorophenyl *N*,*N*-3-Oxapentamethylenetrithiopercarbamate (31). Commercial pentachlorobenzenethiol (**29**) was purified[25] and then was converted to **pentachlorobenzenesulfenyl chloride (30)**, in a method based on a published one,[12] by bubbling Cl_2 (dried by passage through concentrated H_2SO_4) for 3 h at ~5 bubbles/s into a solution at the reflux temperature of 25.0 g (88.4 mmol) of **29** and 1.12 g (4.42 mmol, 5 mol %) of I_2 in 400 mL of CCl_4; a characteristic red-brown color developed after ~0.5 h. Too little I_2 led mostly to the disulfide **32** (mp 234–235 °C),[25] even with longer reaction times. Removal of solvent left 28.8 g (103%) of **30** as bright orange crystals, mp 100.5–103 °C (lit.[12] mp 103–104 °C). Such **30** could be stored at 5 °C for over 6 months (the melting point indicated little conversion to disulfide **32**). Any significant amount of **32** was readily recognizable by its insolubility in molten **30** and its high melting point. It could be removed by treating **30** with pentane and filtering. Rapid conversion of **30** to **32** occurs in pentane at ~25 °C (some precipitation of **32** within 0.3 h).

To prepare **31**, a solution of 1.63 g (5.14 mmol) of **30** [prepared by dissolution of crude **30** in pentane (140 mL) and filtration] was added concurrently with 1.16 g (4.63 mmol) of **18**, in ~1/16 portions of each, during ~1 h to 5 mL of pentane at ca. −70 °C. The mixture then was stirred 10 min and precipitate (2.24 g) was removed by rapid filtration; TLC (benzene; morpholine hydrochloride was insoluble) of this precipitate gave one spot with R_f 0.31 (**31**), along with two others having R_f 0.02 and 0.66 (**12** and **32**, respectively). Benzene was added to 1.50 g of this precipitate until further dissolution did not occur (~30 mL), and insoluble material was discarded. Chromatography of the solution during ~0.5 h on 30 g of light-shielded Woelm silica gel gave **32**, followed by **31**, which was collected at −78 °C in a light-shielded flask. Several fractions initially contained only **31** (TLC). However, evaporation led to 32 mg (2% yield if **31**) of solid, which had undergone disproportionation and gave three TLC spots corresponding to **12**, **31**, and **32**. In a similar trial, fractions that initially showed only **31** by TLC showed three spots after 1 h at 5 °C. Evaporation of a fresh portion, however, gave pale yellow **31**: mp 155–160 °C; R_f 0.31. When this **31** was dissolved in benzene, TLC at first showed only one spot for **31** but within 0.3 h at ~25 °C traces of **12** and **32** appeared; lack of further change after ~1 h suggested that equilibration to **12** and **32** was largely complete. That an equilibrium is involved was shown by three spots (within 10 min) for a solution saturated with **12** and **32** (R_f 0.02, 0.33, and 0.66). After 1 week at 5 °C, TLC showed that the solid **31** had disproportionated to **12**, **31**, and **32**. Anal. ($C_{11}H_8Cl_5NOS_3 \cdot 2H_2O$) H; C: calcd, 27.54; found, 27.97; Cl: calcd, 36.95; found, 36.46.

Other syntheses of **31** at 0 to −78 °C gave no better results (addition of **18** as a solid or in H_2O to **30** in pentane or CH_2Cl_2; addition of **30** in CH_2Cl_2 to an aqueous solution or suspension of **18** in CH_2Cl_2).

Sodium 4-(*p*-Tolyldithio)butanesulfinate Monohydrate (13). The methanolic NaOMe from 0.53 g (23.0 mmol) of Na and 15 mL of MeOH was added dropwise to a solution of 1,2-dithiane 1,1-dioxide **33** (7.00 g, 46.0 mmol)[26] and *p*-toluenethiol (2.86 g, 23.0 mmol) in 30 mL of MeOH during 2–3 min with good stirring. The mixture was stirred for 3 min more, and 800 mL of Me_2CO then was added to precipitate **13**. The **13** was separated by filtration, repeatedly washed with Me_2CO (to remove unreacted **33**), and was dried at ~2 mm overnight: yield of **13**, 5.60 g (77%); mp ~250 °C dec; TLC showed only one spot (R_f 0.54, MeOH); IR (KBr pellet) 3300, 2900, 1490, 1445, 1000, 960, 790, and 730 cm^{-1}; NMR (D_2O) δ 1.6 [m, 4 H, $SSCH_2(CH_2)_2CH_2SO_2Na$], 2.1 (s, 3 H, H_3C-Ph), 2.4–3.0 [m, 4 H, $SSCH_2(CH_2)_2CH_2SO_2Na$], 6.8–7.3 (d of d, 4 H, Ph). Anal. ($C_{11}H_{15}NaO_2S_3 \cdot H_2O$) C, H, H_2O; S: calcd, 30.38; found, 29.87. Anal. ($C_{11}H_{15}NaO_2S_3$) H, S; C: calcd, 44.30; found, 43.68.

TLC (MeOH) studies on a solution of 50 mg of **13** in 5 mL of MeOH at 65 °C showed that **13** began to disproportionate (three spots) in ~0.8 h and came to equilibrium in ~2 h (no further change). In estimation by NMR of the disproportionation of **13** (20 mg) in D_2O (0.9 mL) at 25 °C, the *p*-tolyl disulfide which precipitated had little effect on the signal; the change in integral of methyl relative to methylene protons, which signified precipitation of *p*-tolyl disulfide, indicated the half-life to be ~9 h; that the precipitate was *p*-tolyl disulfide was confirmed by IR, NMR, TLC, and mixture melting point.

Sodium 4-(*p*-Methoxybenzyldithio)butanesulfinate Hemihydrate (14). Much as with **13**, the methanolic NaOMe from 0.53 g (23.0 mmol) of Na and 15 mL of MeOH was added to **33** (7.00 g, 46.0 mmol) and *p*-methoxy-α-toluenethiol (3.55 g, 23.0 mmol) in 25 mL of MeOH during 10 min at ca. −75 °C with good stirring. After 30 min, a TLC spot corresponding to the thiol (or thiolate salt) disappeared, and a new spot for **14** became prominent. Me_2CO (1000 mL) then was added. **14** was removed, washed with Me_2CO, and dried at ~2 mm overnight: yield of **14**, 5.90 g (76%); mp ~255 °C; TLC showed only one spot (R_f 0.56, MeOH); IR (KBr pellet) 3400, 2950, 1600, 1500, 1460, 1300, 1250, 1180, 1100, 1030, 1010, 990, 960, 820, 740, and 720 cm^{-1}; NMR (D_2O) δ 1.5 [m, 4 H, $CH_2(CH_2)_2CH_2SO_2Na$], 2.1–2.5 [m, 4 H, $CH_2(CH_2)_2CH_2SO_2Na$], 3.7 (s, 3 H, H_3COPh), 3.8 (s, 2 H, *p*-$CH_3OC_6H_4CH_2SS$), 6.8–7.3 (d of d, 4 H, Ph). Anal. ($C_{12}H_{17}NaO_3S_3 \cdot 0.5H_2O$) C, H, S; H_2O: calcd, 2.67; found, 3.28. Anal. ($C_{12}H_{17}NaO_3S_3$) C, S; H: calcd, 5.21; found, 4.70.

Study of thermally induced disproportionation of **14** (five samples each of 200.0 mg in 10 mL of H_2O at 68 ± 0.5 °C) much as reported previously,[27] except by centrifuging, washing, drying, and then weighing the insoluble *p*-methoxybenzyl disulfide formed, permitted estimation of a half-life of ~1.7 h from the following values for percent disproportionation (at minutes given in parentheses): 17 (40); 48 (100); 87 (240); 88 (420); 90 (651); (we thank C. H. Lee for these results). The combined samples of precipitated *p*-methoxybenzyl disulfide, recrystallized from EtOH–H_2O, had mp 100–101 °C (lit.[28] 101 °C). Use of this method with **13** was unsuccessful because of emulsions.

2-(*p*-Tolyldithio)ethylamine Hydrochloride (15). *p*-Toluenesulfenyl chloride (**34**; 5.00 g, 31.6 mmol)[29] was added during 10 min to a solution of 3.59 g (31.6 mmol) of 2-aminoethanethiol hydrochloride in 75 mL of absolute EtOH at −10 °C. The mixture was stirred for 20 min more, and 0.1 g of precipitate was removed by filtration. Evaporation of the EtOH left a white solid which showed TLC spots for an unidentified impurity and for **15** (1:1 Me_2CO–EtOH). Removal of the impurity by repeated washing with CH_2Cl_2 left 5.21 g (70%) of **15**: mp 136–138 °C (lit.[16] mp 136–137 °C); **15** had an IR spectrum congruent with that of authentic **15**[16] and showed only one TLC spot (R_f 0.62, 1:1 Me_2CO–EtOH).

***p*-Tolyl *p*-Thioanisoyl Disulfide (16). *p*-Dithioanisic acid** was prepared, by a procedure based on one for *p*-dithiotoluic acid,[30] by adding 30.0 g (160.4 mmol) of *p*-bromoanisole in 30 mL of dry Et_2O to 3.9 g (160.4 mmol) of dried Mg shavings under N_2 in 40 mL of dry Et_2O (to initiate reaction an I_2 crystal, 5 drops of ethyl bromide, and gentle heat were necessary). When reaction began, addition of *p*-bromoanisole was continued at a rate (1 h) to maintain gentle reflux. The mixture was stirred 3 h more under reflux. Carbon disulfide (12.2 g, 160.4 mmol) in 25 mL of Et_2O then was added during ~1 h at −10 to −15 °C. Stirring was continued overnight at ~25 °C, and the solution then was poured into 100 g of ice and 300 mL of cold 6 N HCl. The red ether layer was separated, combined with three 25-mL Et_2O extracts of the aqueous layer, and extracted with 20-mL portions of 5% aqueous NaOH until an extract was light yellow. The aqueous solution of the sodium salt thus obtained was covered with ether, 6 N HCl was added until the aqueous solution was slightly acidic, and the Et_2O extract then was washed with H_2O. This process was carried out twice more. The ether extract then was dried and evaporated to give 15.87 g (54%) of *p*-dithioanisic acid as red oil; the acid seemed stable in Et_2O for about a month at ~5 °C. *p*-Toluenesulfenyl chloride (**34**; 6.3 g, 39.8 mmol)[29] in 50 mL of petroleum ether was added during 30 min to a solution of the *p*-dithioanisic acid (7.32 g, 39.8 mmol) in 30 mL of petroleum ether at −5 to −10 °C. The clear deep red solution was stirred for 15 min more and then was chilled to −50 °C. After 20 min, insoluble red semisolid was removed by filtration. This solid was dried overnight at 2 mm and then was rubbed well with five 10-mL portions of hexane. Evaporation of the hexane left crude **16** (mp ~82 °C), which was recrystallized five times from aqueous methanol to give 4.8 g (39%) of **16**: mp 97–99 °C (unchanged by further recrystallization); TLC gave only one spot using MeOH,

Me_2CO, $CHCl_3$, or CCl_4. Anal. ($C_{15}H_{14}OS_3$) C, H, S.

TLC (CCl_4) showed that with **16** (50 mg) in refluxing MeOH (10 mL), change from an original single spot for **16** to a total of three spots (**16** plus the two symmetrical disulfides) began to occur at ~19 h; **16** itself showed no change in TLC after ~5 months at 5 °C.

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Design, Synthesis, and Correlation Analysis of 7-Substituted 4-Hydroxyquinoline-3-carboxylic Acids as Inhibitors of Cellular Respiration

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Fifteen 7-substituted 4-hydroxyquinoline-3-carboxylic acids have been designed to minimize covariance between the physicochemical substituent parameters: π, MR, and σ_p. The molecules have been synthesized and evaluated for their ability to inhibit the respiration of Ehrlich ascites cells as a whole cell model and for their ability to inhibit malate dehydrogenase as an intracellular target enzyme model. Correlation analysis indicates that ascites cell inhibition is linearly related to π and that malate dehydrogenase inhibition is linearly related to MR.

Explorations of potential applications of quantitative structure–activity relationships (QSAR, correlation analysis) continue in our attempts to characterize and exploit metabolic and structural differences between normal and malignant tissue for purposes of chemotherapy. Alterations in the neoplastic cell membranes have been reported which result in changes in antigenic and transport properties.[1] Renewed and more detailed examinations of glycolysis and respiration pathways have been conducted.[2] Some cancer cells have exhibited abnormal levels or activities of lactate dehydrogenase,[3,4] malate dehydrogenase,[3–6] and other enzymes.[7,8] Selective inhibition of these enzymes in neoplastic tissue should increase the potential for chemotherapy of solid tumor systems as well as provide new candidates for inclusion in combination therapy. Selectivity, of course, is the key and is extremely critical