(27) Interestingly, Murdock (see ref 10) found that the bis(methyl disulfide) analogue of acetylaranotin had a higher anti-RNA polymerase activity than acetylaranotin itself, whereas the bis(methylthio) analogue was considerably less active.
(28) P. T. Beurskens, *Acta Crystallogr.*, 17, 462 (1964).
(29) Th. E. M. van den Hark, Thesis, University of Nijmegen, 1976.
(30) The X-Ray System, Technical Report TR-192 of the Computer Science Center, University of Maryland, College Park, Md., June 1972.

Comparative Analysis of the Cytotoxicity of Substituted [Phenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) Chelates. 2. Parabolic Correlations and Their Implications for Selective Toxicity[1]

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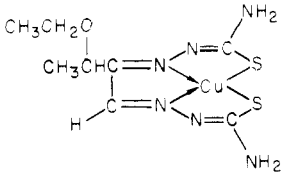
The synthesis of an extended series of para-substituted [phenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) chelates is reported. Subsequent biological evaluation and regression analysis have been performed, correlating pI_{50} with extrathermodynamic substituent parameters. Parabolic correlations with π have resulted which predict optimum lipophilic character of the para substituent with respect to Ehrlich ascites cytotoxicity ($\pi_0 = -2.13$) and with respect to ascites vs. liver slice cytotoxicity ($\pi_0 = -1.31$). Results indicated clearly that the chelate most toxic to the tumor cell model may not be the most selective.

Considerable interest in recent years has been shown in the development of metal chelates and chelating agents as potential antineoplastic agents. Aside from complexes of platinum,[2] some work has focused upon chelates of copper(II) such as Cu(II) KTS [[2-keto-3-ethoxybutyraldehyde bis(thiosemicarbazone)]copper(II) chelate; [kethoxal bis(thiosemicarbazone)]copper(II) chelate]] (1) and its analogues.[3]



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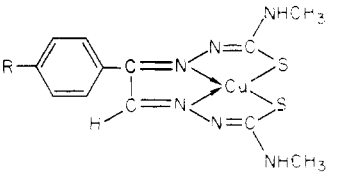
The mechanism of action of Cu(II) KTS as an antitumor agent has been the subject of intense study and some controversy. Several workers[4] have promulgated the view that the copper chelate is a lipophilicity-potentiated form of Cu^{2+}, better able to transport the latter species into the tumor cell, whereupon dissociation of the chelate and copper(II)-mediated cytotoxic effects upon enzymes involved in DNA synthesis occurs. An adjunct to this hypothesis has been advanced[5] more recently in the suggestion that while the proposed "shuttle" of copper chelate may well occur, the *primary* effect of Cu(II) KTS on tumor cells is that of interfering with their energy transport system; that is, inhibition of DNA synthesis might very well be observed as a secondary effect resulting from a lack of ATP. Unpublished investigations have indicated that Cu(II) KTS may have the ability to uncouple oxidative phosphorylation in isolated rat liver mitochondria at concentrations below those required for solutions of copper(II) ions alone.[5b] It is also known that Cu(II) KTS inhibits the respiration of both Ehrlich ascites cells and rat liver slices. These observations could be accounted for by the recent findings of Petering,[5] who demonstrated the ability of Cu(II) KTS to undergo a sluggish reduction to Cu(I) by thiols such as coenzyme A and lipoic acid. Such processes might be expected to interfere with various mitochondrial functions (i.e., pyruvate dehydrogenase complex, α-ketoglutarate dehydrogenase, etc.), ultimately having an effect on observed cellular respiration.

Based upon these considerations, our laboratory recently reported a preliminary study,[6] in which a series of substituted [phenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) chelates, **2**, was synthesized as more easily



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accessible and systematically variable analogues of Cu(II) KTS. The study, whose purpose was to investigate via quantitative structure–activity relationship (QSAR) techniques the design of potential antineoplastic chelates which would exhibit selective toxicity for a tumor cell model vs. a normal cell model, yielded some significant insights into the structural requirements needed to realize the desired goal. These are summarized in eq 1–3. The

$$pI_{50}\ (\text{liver}) = 0.14\ (\pm 0.09)\,E_s + 2.26\ (\pm 0.10) \quad (1)$$
$$n = 8;\ s = 0.12;\ r = 0.85$$

$$pI_{50}\ (\text{ascites}) = -0.69\ (\pm 0.33)\,\pi - 1.17\ (\pm 0.74)\,\sigma_p + 5.08\ (\pm 0.29) \quad (2)$$
$$n = 8;\ s = 0.28;\ r = 0.95$$

$$pI_{50}\ (\text{ascites}) - pI_{50}\ (\text{liver}) = -0.54\ (\pm 0.36)\,\pi - 1.03\ (\pm 0.81)\,\sigma_p + 2.42\ (\pm 0.31) \quad (3)$$
$$n = 8;\ s = 0.30;\ r = 0.92$$

negative coefficients associated with π and with σ_p in eq 3 suggested that the desired selective cytotoxicity against Ehrlich ascites tumor cells with minimal cytotoxicity to rat liver slice could be achieved by introducing substituents into **2** which would enhance water solubility while stabilizing the chelate against premature dissociation by electron donation through the conjugated ring system. However, it was reasonable to assume that continued increases in hydrophilic character would eventually lead to a drop in activity, thus establishing an optimum for this parameter. Based upon this projection, further analogues

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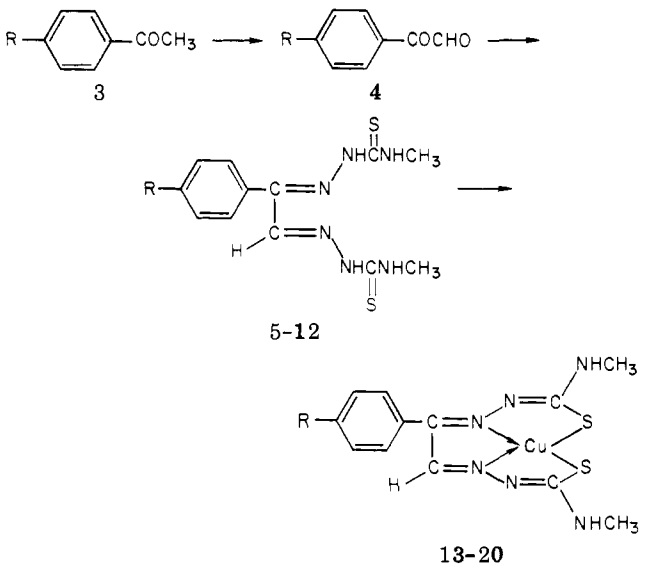
Scheme I



were designed with the primary objective of delineating the potential parabolic relationship in π which is frequently observed in whole cell or in vivo biological test systems[7] when compounds experience multiple partitionings en route to their intracellular sites of action.

This report, accordingly, relates the synthesis, testing, and revised correlation analysis of an additional eight congeners of **2** where a number of the new substituents have been selected to increase the range in π character.

Chemistry. The chelates were prepared according to the methods outlined in Scheme I, starting with the appropriately substituted acetophenones, **3**. Several of the acetophenones which were not readily available were synthesized as follows. *p*-Acetylphenoxyacetic acid (**3**, R = OCH_2CO_2H) was prepared from *p*-hydroxyacetophenone by treatment with bromoacetic acid in base.[21] *p*-Acetylbenzamide (**3**, R = $CONH_2$) was synthesized from *p*-cyanoacetophenone by reaction with hydrogen peroxide in base. Treatment of thioanisole with acetic–trifluoroacetic anhydride afforded *p*-mercaptomethylacetophenone (**3**, R = SCH_3).[22] Oxidation of *p*-mercaptomethylacetophenone via hydrogen peroxide in glacial acetic acid gave *p*-methylsulfonylacetophenone (**3**, R = SO_2CH_3).[23] Conversion of *p*-aminoacetophenone to *p*-acetamidoacetophenone (**3**, R = $NHCOCH_3$)[20] was accomplished by treatment with acetic anhydride in pyridine.

Selenium dioxide oxidation[8] of the acetophenones **3** in refluxing aqueous dioxane gave the desired arylglyoxals **4** in moderate to good yields. Conversion of **4** to the corresponding bis(4-methyl-3-thiosemicarbazones) **5–12** proceeded smoothly in the presence of excess 4-methyl-3-thiosemicarbazide and an acid catalyst with one exception. The reaction with phenylglyoxal-4-sulfonic acid (**4**, R = SO_3H) afforded low yields of a material which exhibited ultraviolet and infrared spectral characteristics of the desired product but gave an elemental analysis that did not conform to the anticipated molecular formula. The elemental analysis did reflect the presence of an additional molecule of thiosemicarbazide, presumably as the sulfonate salt. This was confirmed by proton NMR and by Dowex 50 ion exchange which effectively removed the thiosemicarbazide. Treatment of **5–12** with stoichiometric amounts of cupric acetate in hot methanolic solution followed by very slow cooling generally afforded the chelates **13–20**. The chelates could be readily identified by the characteristic hypsochromic shift in ultraviolet absorption maxima from 337 nm for **5–12** to 307 nm for **13–20**. This shift was accompanied by the appearance of a new band in the visible region at 500 nm.

It is noteworthy to mention that when the carboxylic acid analogues **16** and **17** were prepared utilizing cupric acetate monohydrate as the source of Cu(II), the strong carbonyl stretch band of the carboxylic acid moiety in the respective bis(thiosemicarbazones) (1695 and 1670 cm^{-1} for **8**; 1740 cm^{-1} for **9**) disappeared and a new peak at 1630 cm^{-1} appeared. Concomitant with this was the loss of bicarbonate solubility in the corresponding products **16** and **17**. These data are consistent with a complexation of the carboxylic acid moieties to Cu(II) in an as yet uncharacterized manner, a process which has precedent in the behavior of acetylsalicylic acid when similarly treated with cupric acetate.[9] Samples of the desired copper(II) chelates **16** and **17** not having this degree of carboxyl group complexation were successfully prepared, however, by substituting cupric sulfate as the source of Cu(II).

Also of interest is the observation that the *p*-acetamido analogue **20**, despite an apparently normal progress of reactions, was found to exhibit in its infrared spectrum a split amide I carbonyl stretching vibration [1660 (s), 1682 cm^{-1} (m)] and subsequently failed to yield virtually any respiratory inhibition of either of the two biological test systems. This latter finding is especially significant since eq 1–3 predict appreciable activity for this congener, particularly in the Ehrlich ascites test system. These observations lead us to speculate that the structure of the *p*-acetamido copper chelate analogue **20** may not be directly comparable to the remainder of the congeneric series.

Although many of the chelates proved too lipophilic for accurate determination of partition coefficients, the apparent log *P* of the sulfonate **13** (−0.55 ± 0.02), the carboxylate **16** (0.28 ± 0.03), the oxyacetate **17** (−0.30 ± 0.04), the methylsulfonyl **18** (2.66 ± 0.07), and the hydroxy **28** (3.29 ± 0.08) congeners has been measured. All of the partition coefficient determinations[10,11] were conducted using pH 7.4 phosphate (Ringer) as the aqueous phase and buffer-saturated octanol as the organic phase and thus represent apparent partition coefficients (uncorrected for ionization) which relate directly to the biological test medium used. Of the five chelates, the hydroxy, **28**, was assumed to be least affected by ionization and was used to estimate the log *P* for the parent, unsubstituted chelate, **21**, as 3.96 [log $P_{OH} - \pi_{OH}$ = 3.29 − (−0.67)]. Thus, the π values for the four substituents, **13** and **16–18**, in Table III are derived from the estimated log *P* of **21** and the measured log *P* (app) for those congeners.

To provide an indication of the influence of chelation on log *P*, two thiosemicarbazone log *P* values were also determined. The log *P* (app) of the carboxylate, **8**, is −0.93 (±0.0003). Thus, chelate formation here results in an increase in log *P* of 1.21 (from −0.93 for **8** to 0.28 for **16**). The log *P* (app) of the hydroxy thiosemicarbazone is 2.38 (±0.02). Chelate formation here results in an increase in log *P* of 0.91 (from 2.38 to 3.29 for **28**). These values indicate, then, that chelate formation increases the log *P* of the ligand by approximately one log unit while creating a lipid-soluble form of the metal.

Pertinent chemical and spectral properties of all new compounds prepared are summarized in Table I.

Biological Results. Compounds **13–20** were evaluated for their ability to inhibit the respiration of Ehrlich ascites cell suspensions as a tumor cell model and the results compared to the inhibition of rat liver slice respiration as a normal cell model. The rationale behind the choice of these biological test systems has been discussed previ-

Table I. Physical Properties

no.	R	% yield	mp, °C dec	λ_{max} (95% EtOH), nm (ϵ)	formula	analyses[a]
			4-R-phenylglyoxal bis(4-methyl-3-thiosemicarbazone)			
5	$-SO_3^-$	49	215-220	337 (23 000)	$C_{12}H_{15}N_6O_3S_3 \cdot H_2O$	C, H; N[b]
6	-CN	11	205-208	336 (17 300)	$C_{13}H_{15}N_7S_2$	C, H, N
7	$-SCH_3$	47	225-227	345 (22 100)	$C_{13}H_{18}N_6S_3$	C, H, N
8	$-CO_2H$	64	242-243	340 (23 700)	$C_{13}H_{16}N_6O_2S_2 \cdot 0.5H_2O$	C, H, N
9	$-OCH_2CO_2H$	79	200-203	341 (26 700)	$C_{14}H_{18}N_6O_3S_3$	C, H, N, S
10	$-SO_2CH_3$	59	238-239	343 (19 900)	$C_{13}H_{18}N_6O_2S_3$	C, H, N, S
11	$-CONH_2$	50	247-249	341 (19 300)	$C_{13}H_{17}N_7OS_2 \cdot H_2O$	C, H, N
12	$-NHCOCH_3$	52	232-233	348 (41 000)[c]	$C_{14}H_{19}N_7OS_2 \cdot H_2O$	C, H; N[d]
			[4-R-phenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) chelate			
13	$-SO_3^-$	87	277-279	310 (19 700), 495 (3000)	$C_{12}H_{13}N_6O_3S_3Cu \cdot H_2O$	H, N, S, Cu; C[e]
14	-CN	56	255-256	322 (25 600), 463 (5300)	$C_{13}H_{13}N_7S_2Cu$	C, H, N
15	$-SCH_3$	72	225-227	312 (25 000), 540 (5200)	$C_{13}H_{16}N_6S_3Cu$	C, H, N
16	$-CO_2H$	97	252-253	315 (16 200), 501 (2700)	$C_{13}H_{14}N_6O_2S_2Cu$	C, H, N, S
17	$-OCH_2CO_2H$	≃100	210-213	308 (20 200), 502 (4600)	$C_{14}H_{16}N_6O_3S_2Cu$	C, H, N, S
18	$-SO_2CH_3$	70	251	319 (29 600), 512 (5600)	$C_{13}H_{16}N_6O_3S_3Cu$	H, N; C[f]
19	$-CONH_2$	50	248-250	318 (27 300), 507 (4800)	$C_{13}H_{15}N_7OS_2Cu$	C, H; N[g]
20	$-NHCOCH_3$	82	272-274	312 (31 000), 499 (7000)[c]	$C_{14}H_{17}N_7OS_2Cu$	H, N, S; C[h]

[a] Within 0.4% of theory unless otherwise noted. [b] N: calcd, 20.67; found, 19.65. [c] Taken in CH_3OH. [d] N: calcd, 25.57; found, 26.29. [e] C: calcd, 30.79; found, 30.23. [f] C: calcd, 34.85; found, 35.40. [g] N: calcd, 22.75; found, 21.88. [h] C: calcd, 39.39; found, 40.69.

Table II. Observed and Predicted Respiratory Inhibition

		liver			ascites			pI_{50} (ascites) − pI_{50} (liver)	
no.	R	I_{50}[a]	pI_{50} obsd	pI_{50}[b] pred	I_{50}[c]	pI_{50} obsd	pI_{50}[d] pred	obsd	pred[e]
13	$-SO_3^-$	0.51 (0.35)	3.29	3.41	1.03 (0.47)	4.99	4.85	1.70	1.35
14	-CN	4.36 (1.56)	2.36	2.70	2.83 (0.66)	4.55	4.52	2.19	1.96
15	$-SCH_3$	10.70 (1.84)	1.97	2.49	1.51 (0.67)	4.82	4.75	2.85	2.46
16	$-COO^-$	0.25 (0.16)	3.60	3.26	0.25 (0.13)	5.60	5.33	2.00	1.89
17	$-OCH_2COO^-$	0.64 (0.40)	3.19	3.36	1.59 (0.31)	4.80	5.16	1.61	2.07
18	$-SO_2CH_3$	6.49 (1.69)	2.19[f]	2.89	0.37 (0.00)	5.43[f]	4.69	3.24[f]	1.97
19	$-CONH_2$	0.90 (0.65)	3.05	2.86	1.16 (0.24)	4.94	5.12	1.89	2.21
20	$-NHCOCH_3$	inactive	[f]	2.77	inactive	[f]	5.56	[f]	2.82
21	-H	1.56 (0.54)	2.81	2.60	0.32 (0.24)	5.49	5.09	2.69	2.27
22	-Br	2.00 (0.85)	2.70	2.44	8.92 (1.60)	4.05	4.31	1.35	1.87
23	-Cl	1.84 (0.75)	2.74	2.47	4.23 (1.60)	4.37	4.40	1.63	1.96
24	$-OCH_3$	3.14 (0.66)	2.50	2.60	0.48 (0.24)	5.32	5.43	2.82	2.78
25	$-CH_3$	2.87 (1.55)	2.54	2.50	3.00 (0.81)	4.52	4.99	1.98	2.29
26	$-NO_2$	3.67 (1.26)	2.44	2.65	4.83 (1.38)	4.32	4.26	1.88	1.83
27	$-C_6H_5$	5.90 (2.05)	2.23	2.24	11.80 (3.79)	3.93	3.71	1.70	1.48
28	-OH	1.36 (0.58)	2.87	2.72	0.09 (0.01)	6.03	5.81	3.16	3.00

[a] I_{50} values are in moles × 10^3; numbers in parentheses are standard deviations. [b] Predicted by eq 5. [c] I_{50} values are in moles per milligram of ascites cells × 10^5; numbers in parentheses are standard deviations. [d] Predicted by eq 9. [e] Predicted by eq 12. [f] These data points were not used in the derivation of eq 5, 9, and 12.

Table III. Substituent Constants Used in the Correlation Analyses

no.	R	π[a]	σ_p[a]	σ_p^+[b]
13	$-SO_3^-$	−4.51	0.09	0.09
14	-CN	−0.57	0.66	0.66
15	$-SCH_3$	0.61	0.00	−0.60
16	$-CO_2^-$	−3.68	0.00	−0.02
17	$-OCH_2CO_2$	−4.26	−0.06	−0.78[c]
18	$-SO_2CH_3$	−1.30	0.72	0.72
19	$-CONH_2$	−1.49	0.36	0.36
20	$-NHCOCH_3$	−0.97	−0.09	−0.60
21	-H	0.00	0.00	0.00
22	-Br	0.86	0.23	0.15
23	-Cl	0.71	0.23	0.11
24	$-OCH_3$	−0.02	−0.27	−0.78
25	$-CH_3$	0.56	−0.17	−0.31
26	$-NO_2$	−0.28	0.78	0.79
27	$-C_6H_5$	1.96	−0.01	−0.18
28	-OH	−0.67	−0.37	−0.92

[a] π values of compounds 13 and 16-18 are calculated from measured log P values as described in the chemistry section of the text; the remainder of the π values and all of the σ_p values are from C. Hansch et al., *J. Med. Chem.*, 16, 1207 (1973). [b] From ref 17. [c] Estimated as similar to compound 24.

Table IV. Cross-Correlation Matrix (r^2) between Substituent Constants Used in the Development of Equations 5, 9, and 12

	π	σ_p	σ_p^+
π	1.00	0.00	0.00
σ_p		1.00	0.84
σ_p^+			1.00

ously.[6] The respiratory rates were followed potentiometrically by oxygen electrode, and the percent inhibition of oxygen uptake was correlated with chelate concentration by the method of "least squares" to obtain values for 50% inhibition (I_{50}). The observed and predicted biological activities of all analogues of **2** prepared to date are summarized in Table II.

Analyses and Discussion. The biological data for the eight new congeners were combined with that previously reported[6] allowing correlation analyses to be conducted on the entire set, compounds 13-28. In the development of meaningful regression equations all potentially relevant combinations of the parameters, σ_p, σ_p^+, σ_p^-, MR, π, F, and R, were explored. The values for the substituent constants utilized in the reported equations are summarized in Table

III. Table IV is the correlation matrix for these substituent parameters.

The best correlations with liver slice respiratory inhibition are given in eq 4 and 5. In these equations, pI_{50}

$$pI_{50}\ (\text{liver}) = -0.17\ (\pm 0.09)\ \pi + 2.56\ (\pm 0.19) \quad (4)$$

$$n = 15;\ s = 0.31;\ r = 0.74$$

$$pI_{50}\ (\text{liver}) = -0.18\ (\pm 0.08)\ \pi + 2.60\ (\pm 0.16) \quad (5)$$

$$n = 14;\ s = 0.26;\ r = 0.82$$

is the negative logarithm of the molar concentration of chelate giving 50% inhibition, π is the Hansch hydrophobic substituent constant,[12] the numbers in parentheses are the 95% confidence intervals for the coefficients, n is the number of data points included in the regression, s is the standard deviation, and r is the correlation coefficient. It is apparent that the increase in number of data points has improved the definition of physicochemical influences on liver slice respiratory inhibition. The expanded range of π has essentially abolished the earlier[6] covariance between π and steric bulk as modeled by E_s or by MR. Equation 5 is the correlation with the methylsulfonyl analogue, 18, deleted. This derivative, which was almost inactive, was observed to be considerably less water soluble than the carboxamide congener, 19, despite apparently similar π values.

Addition of electronic or squared terms to eq 5 gave no improvement whatsoever. The small negative coefficient associated with π suggests that hydrophobic character does influence transport into the liver cell but also that a large variation in π character can be tolerated with relatively small effects on activity. Thus as the hydrophilicity of the congeners increases there is a small but definite increase in liver cell toxicity.

Equations 6–9 were extracted from correlations of

$$pI_{50}\ (\text{ascites}) = -0.14\ (\pm 0.16)\ \pi - 0.59\ (\pm 0.89)\ \sigma_p + 4.85\ (\pm 0.36) \quad (6)$$

$$n = 15;\ s = 0.54;\ r = 0.56$$

$$pI_{50}\ (\text{ascites}) = -0.41\ (\pm 0.29)\ \pi - 0.09\ (\pm 0.08)\ \pi^2 + 4.91\ (\pm 0.32) \quad (7)$$

$$n = 15;\ s = 0.48;\ r = 0.68$$

$$pI_{50}\ (\text{ascites}) = -0.53\ (\pm 0.21)\ \pi - 0.12\ (\pm 0.06)\ \pi^2 - 1.02\ (\pm 0.59)\ \sigma_p + 5.11\ (\pm 0.25) \quad (8)$$

$$n = 15;\ s = 0.33;\ r = 0.87$$

$$pI_{50}\ (\text{ascites}) = -0.48\ (\pm 0.19)\ \pi - 0.11\ (\pm 0.05)\ \pi^2 - 1.22\ (\pm 0.56)\ \sigma_p + 5.09\ (\pm 0.22) \quad (9)$$

$$n = 14;\ s = 0.28;\ r = 0.91$$

Ehrlich ascites respiratory inhibition data. Equation 6 is given to allow comparison with eq 2 from the first stage of this work.[6] The parabolic relationship, eq 7, is now the most significant two-parameter equation and is an improvement over the linear relationship in π alone ($F_{1,12} = 5.78$; $F_{1,12;\alpha=0.05} = 4.75$). Addition of an electronic term, Hammett σ_p, yields eq 8 which is an improvement over eq 7 ($F_{1,11} = 14.41$; $F_{1,11;\alpha=0.005} = 12.22$) and demonstrates that "ideal" lipophilic character has indeed been successfully bracketed by the extended series of chelates. Here again, deletion of the deviant methylsulfonyl congener, 18, sharpens the correlation somewhat (eq 9). Although eq 9 is based upon slightly less than five data points per independent variable, considerable significance can be attached to it since the two parameters, π and σ_p, are totally orthogonal (Table IV, $r^2 = 0.00$) with a range of over one log unit each.[13] It is obvious that eq 6, as well as eq 2, no longer provides an adequate explanation of the physicochemical influences on inhibition of ascites respiration. Equation 9 suggests that partitioning across the tumor cel membrane can be a limiting factor affecting biological activity. This equation can be employed to predict the optimum lipophilic character which should be possessed by a chelate substituent in order to maximize its potency against the tumor cells. Taking the partial derivative of pI_{50} with respect to π, holding σ_p constant, and setting this expression equal to zero allow one to solve the resulting relationship for this optimum π or π_0,[7] affording a value of −2.13 with a 95% confidence interval of −2.84 to −1.74. Equation 9 then allows one to estimate that the maximum inhibition of ascites respiration would be exerted by chelates which are stabilized by electron-donating substituents with π values in the range of −2.0. This does not necessarily ensure maximum selectivity against the tumor cell model vs. the liver cell model, however. One must consider eq 5 which suggests that a substituent having a π value of −2.0 may also be significantly toxic to liver.

As a final step in the correlation analysis then, the two biological test systems are compared quantitatively by correlating the differences in pI_{50}. This procedure, as described previously,[6] is similar in principle to the method used by Taft to derive the σ^* polar substituent parameter[14] and has been employed in biological correlation analyses.[15,16] Equation 10, a parabolic relationship, is the best

$$pI_{50}\ (\text{ascites}) - pI_{50}\ (\text{liver}) = -0.29\ (\pm 0.32)\ \pi - 0.10\ (\pm 0.09)\ \pi^2 + 2.37\ (\pm 0.36) \quad (10)$$

$$n = 15;\ s = 0.53;\ r = 0.59$$

$$pI_{50}\ (\text{ascites}) - pI_{50}\ (\text{liver}) = -0.35\ (\pm 0.30)\ \pi - 0.12\ (\pm 0.08)\ \pi^2 - 0.46\ (\pm 0.54)\ \sigma_p^+ + 2.39\ (\pm 0.33) \quad (11)$$

$$n = 15;\ s = 0.48;\ r = 0.71$$

$$pI_{50}\ (\text{ascites}) - pI_{50}\ (\text{liver}) = -0.26\ (\pm 0.24)\ \pi - 0.10\ (\pm 0.07)\ \pi^2 - 0.65\ (\pm 0.44)\ \sigma_p^+ + 2.27\ (\pm 0.27) \quad (12)$$

$$n = 14;\ s = 0.37;\ r = 0.81$$

two-parameter equation and is a significant improvement over the single-parameter linear relationship in π ($F_{1,12} = 6.29$; $F_{1,12;\alpha=0.05} = 4.75$). Addition of the electronic resonance donating parameter,[17] σ_p^+, gives a significant improvement in correlation ($F_{1,11} = 3.77$; $F_{1,11;\alpha=0.10} = 3.22$) and, as before, deletion of the methylsulfonyl data point affords further improvement (eq 12). Utilizing eq 12 to derive an optimum π value affords an estimate of $\pi_0 = -1.31$ (−1.92 to −0.24). These relationships provide a quantitative description of the structural requirements for maximum selectivity of chelate respiratory inhibitors. A substituent providing electron donation by resonance, large negative σ_p^+, and possessing a π value in the range of −1.3 should be ideal. For monosubstituted congeners, the hydroxy, 28, comes about as close as possible to these requirements.

Conclusions

The work reported here and previously[6] in this series has attempted to demonstrate how modern QSAR techniques might be utilized in a heuristic sense toward the

rational design of potential antineoplastic agents. An initial small set of chelates was synthesized and evaluated on a comparative basis in order to assess relative respiratory inhibition potencies toward a normal cell model vs. a tumor cell model. The congeners initially selected were such that accurate substituent parameter data were readily available and that the chelates could easily be synthesized from commercially available starting materials already possessing the desired substituents as part of their structures. Computer-assisted multiparameter regression analysis of the results of the initial biological tests indicated, however, that the latter dictate of synthetic ease had led to a group of chelates which were far too lipophilic to exhibit maximal in vitro activity and which were characterized by high covariance between lipophilic and steric parameters. Additional chelates were therefore designed with the aim of extending the range of substituent space, especially into more hydrophilic regions. The choice of substituents was largely made on the basis of "scatter-point" plots of various cross sections of substituent space (π vs. MR; π vs. σ_p; σ_p vs. MR), since the more systemic data banks of orthogonalized substituent parameters[18] and such selection aids as cluster analysis[19] were not yet available. The results of the revised correlation analysis reveal clearly that, in terms of the parabolic dependence of chelate transport into Ehrlich ascites cells, the early linear correlation, eq 2, represented the "right" or more lipophilic leg of a parabola. Furthermore, the π_0 estimate of −2.13 from eq 9 can serve to estimate the requirements for passive transport into the Ehrlich ascites tumor cell. Adding the π_0 to the calculated log P apparent for the unsubstituted chelate gives an ideal log P apparent of 1.83 (−2.13 + 3.96) which indicates that the ascites cell membrane is, in fact, relatively lipophilic.

Finally, the revised comparative analyses, eq 12, demonstrate the value of employing multiple biological test systems when attempting to develop agents exhibiting selective action. While a π_0 of −2.13 (or log P_0 of 1.83) may provide an agent which is the most potent against the tumor cell model, a π_0 of −1.31 (or log P_0 of 2.65) would provide the greatest selectivity and minimize liver cell cytotoxicity.

The results presented here have again emphasized the importance of judicious congener selection in the initial design phases of a project. Even more significantly, these investigations have established the value of utilizing multiple biological test systems and quantitative comparative analysis to monitor selectivity as well as potency.

Experimental Section

All melting points were taken on a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by Midwest Microlab, Ltd., Indianapolis, Ind., and were within 0.4% of theoretical values unless otherwise noted. IR, NMR, and UV data were consistent with the assigned structures. UV spectra were recorded on a Beckman Acta V spectrophotometer. IR spectra were recorded on a Beckman IR-33 or Beckman 4230 spectrophotometer as neat oils, Nujol mulls, or KBr pellets. NMR spectra were determined on a Hitachi Perkin-Elmer R-24 high-resolution NMR spectrometer as 20% solutions in $CDCl_3$, acetone-d_6, or Me_2SO-d_6 with Me_4Si as an internal standard. 4-Methyl-3-thiosemicarbazide was obtained from the Aldrich Chemical Co. The following substituted acetophenones **3** were obtained commercially: sodium *p*-acetylbenzenesulfonate and *p*-hydroxyacetophenone (Aldrich Chemical Co.), *p*-cyanoacetophenone (Trans-World Chemical Co.), and *p*-aminoacetophenone (K&K/ICN Laboratories). Representative synthetic procedures are presented below for the compounds described in Table I.

***p*-Acetamidoacetophenone (3, R = $-NHCOCH_3$).** To a stirred mixture of 5 mL of Ac_2O and 1.0 mL of pyridine was added 1.35 g (0.01 mol) of *p*-aminoacetophenone. The solid went into solution immediately, a mildly exothermic reaction ensuing. Within 10 min, a voluminous white precipitate of the reaction product was deposited. After stirring at room temperature overnight, the product was collected by suction, washed well with cold water, and air-dried. The crude yield was 1.77 g (85%). Recrystallization from dilute alcohol gave material of mp 170–172.5 °C (lit.[20] mp 171 °C).

***p*-Acetylphenoxyacetic Acid (3, R = $-OCH_2CO_2H$).** A mixture of 5.45 g (0.04 mol) of *p*-hydroxyacetophenone, 11.12 g (0.08 mol) of bromoacetic acid, and 21.0 g (0.15 mol) of anhydrous K_2CO_3 was refluxed for 24–48 h in acetone with stirring. A voluminous precipitate was observed to form upon mixing of all the components. Thereafter, the cooled reaction mixture was poured into 150 mL of ice–water, stirred well, and acidified to pH 2 (hydrion paper) via HCl addition. The tan solid which precipitated was collected via suction and recrystallized from hot water (Norit A) to afford the pure product as white needles; yield 5.05 g (64.7%); mp 174–177 °C (lit.[21] mp 172.5–174.5 °C).

***p*-Acetylbenzamide (3, R = $-CONH_2$).** To a solution of 10.0 g (0.069 mol) of *p*-cyanoacetophenone in 100 mL of 1.0 N NaOH was added with stirring at room temperature 40 mL of 30% H_2O_2 solution in a dropwise manner over 30 min. The deep red color of the initial solution faded to a pale yellow-green color and moderate effervescence due to O_2 evolution was noted. The reaction mixture was then stirred at 50–60 °C for an additional 3 h to ensure completion of reaction, after which it was quenched by pouring onto cracked ice. The white solid, which precipitated immediately, was collected via suction, air-dried, and recrystallized from aqueous alcohol: yield 7.5 g (67%); mp 191–194 °C. Anal. ($C_9H_9NO_2$) C, H, N.

***p*-Mercaptomethylacetophenone (3, R = $-SCH_3$)** was prepared in 68.5% yield according to the acylation of thioanisole via acetic-trifluoroacetic anhydride as described by Charbonneau and Smith:[22] bp 97–102 °C (0.10 Torr); mp 78–80 °C (from 95% ethanol) [lit.[22] bp 125 °C (0.05 Torr); mp 80.5–81.5 °C].

***p*-Methylsulfonylacetophenone (3, R = $-SO_2CH_3$)** was prepared from *p*-methylmercaptoacetophenone in 80.5% yield via the 30% hydrogen peroxide oxidation in refluxing glacial acetic acid described by Gregory.[23] Recrystallization of the crude product from 95% ethanol afforded snow-white needles of mp 127–130 °C (lit.[23] mp 127–128.5 °C).

***p*-Methylmercaptophenylglyoxal (4, R = SCH_3).** To a stirred solution of 1.1 g (0.01 mol) of SeO_2 in 15 mL of warm *p*-dioxane and 1.0 mL of water was added a solution of 1.66 g (0.01 mol) of *p*-mercaptomethylacetophenone in 15 mL of dioxane. After refluxing the reaction mixture for 24 h, the precipitated selenium was filtered off via suction from the cooled mixture: yield 0.70 g (90%). The solvent was then removed from the clear yellow filtrate via rotary evaporator to afford the crude glyoxal as a yellow-green oil [IR (neat) 1720 (–CHO), 1685 cm^{-1} [Ar-C(=O)–]]. The crude oil was digested in 100–200 mL of boiling water with stirring, treated with Norit A, gravity filtered, and allowed to cool slowly to room temperature. Gleaming silver-hued flakes of the glyoxal hydrate were deposited: yield 1.0 g (50%); mp 94–97 °C. Anal. ($C_9H_8SO_2 \cdot H_2O$) C, H, S.

***p*-Methylmercaptophenylglyoxal Bis(4-methyl-3-thiosemicarbazone) (7).** A solution of 2.0 g (0.01 mol) of *p*-methylmercaptophenylglyoxal hydrate in 30 mL of 95% ethanol was added dropwise over 30 min to a stirred, refluxing solution of 2.63 g (0.025 mol) of 4-methyl-3-thiosemicarbazide in a mixture composed of 35 mL of 95% ethanol, 25 mL of distilled, deionized water, and 2 mL of concentrated HCl. After a further 45 min of reflux, the reaction mixture was cooled to room temperature, and the bright yellow, crystalline precipitate was collected via suction, washed well on the filter with ice–water, and air-dried: yield 1.65 g (46.6%); mp 225–227 °C dec; UV (95% EtOH) λ_{max} 345 nm (ϵ 22150). The product was routinely protected from light as it showed a tendency to turn orange upon such exposure. Anal. ($C_{13}N_{18}N_6S_3$) C, H, N.

[*p*-Methylmercaptophenylglyoxal Bis(4-methyl-3-thiosemicarbazone)]copper(II) Chelate (15). To a refluxing solution of 0.50 g (0.0014 mol) of 7 in 500 mL of methanol was added with stirring over a 20-min interval a solution of 0.30 g (0.0015 mol) of cupric acetate monohydrate in 100 mL of deionized, distilled water via dropwise addition. After a further 10 min of reflux, the dark red reaction mixture was allowed to

cool slowly to room temperature with stirring. After overnight refrigeration, 0.42 g (72%) of a maroon-colored amorphous solid was collected: mp 225–226 °C dec; UV (95% EtOH) λ_{max} 312.5 nm (ϵ 24 950), 504 (5200). The analytical sample was stirred overnight with 1% (v/v) HCl, collected via suction, and washed at the filter with cold portions of deionized, distilled water and methanol. Anal. ($C_{13}H_{16}N_6S_3Cu$) C, H, N.

Biological Testing. A. Liver Slice. The procedure employed in obtaining rat liver slices of appropriate size has been described in some detail in a previous publication.[6] Methods for monitoring the effect of the several chelates on the respiration rate of the liver slices have similarly been discussed.

B. Ascites. The procedure employed in maintaining and harvesting the Ehrlich ascites tumor cells from Swiss white mice has been described in a previous publication.[6] Methods for monitoring the inhibitory effect of the copper chelates on the respiration rates of the ascites cell suspension have also been described earlier.

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Notes

Drugs Derived from Cannabinoids. 7.[1] Tachycardia and Analgesia Structure–Activity Relationships in Δ^9-Tetrahydrocannabinol and Some Synthetic Analogues

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Δ^9-Tetrahydrocannabinol (Δ^9-THC) and eight other synthetic analogues were found to induce a dose-related increase in heart rate in the conscious Wistar rat. In a comparison of tachycardia with analgesic activity (mouse hot-plate and antiwrithing tests) it was found that the water-soluble ester derivatives of **2a**, 1-hydroxy-3-(3-methyl-2-octyl)-6,6,9-trimethyl-7,8,9,10-tetrahydro-6*H*-dibenzo[*b*,*d*]pyran (DMHP), had the least potency for tachycardia and the greatest potency for analgesia. These findings suggest that these compounds may have promise as therapeutic agents.

Compounds structurally related to Δ^9-tetrahydrocannabinol (Δ^9-THC), the major active constituent of marihuana (cannabis sativa), are known to have analgesic properties.[2,3] In man, however, one of the most consistently observed physiological effects of these agents is tachycardia,[4,5] which would tend to reduce their potential usefulness as therapeutic agents. Heretofore, tachycardia activity has been difficult to assess because in the usual laboratory animal preparations cannabinoids induce a decrease in heart rate.[5] Recently, however, we found that in the conscious rat Δ^9-THC and other cannabinoids led to tachycardia similar to that seen in man[6,7] and further that the potency for this effect was generally less in the phenolic ester derivatives of Δ^9-THC than in the parent compound.[7-9]

Many of these compounds had been previously tested for antinociceptive activity in mice after oral administration and a number of them found to be more potent than codeine, propoxyphene (Darvon), and a narcotic analgesic, anileridine;[3a] in addition, several of the more

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