of predictions of true unknowns.

Conclusions

A data set consisting of 157 aromatic amines has been studied using computer-assisted structure–activity methods. The 157 compounds were divided into subsets according to tumor sites, routes of administration, and activity. Differences in active site did not affect the results to a large extent. However, different pattern-recognition methods showed markedly different classification and predictive abilities.

In spite of the dissimilarity of the data sets' positive and negative compound distribution, there were some molecular structure descriptors that consistently were chosen as important. Prominent among these important descriptors were those coding size and shape information, e.g., number of rings and principal moments. These descriptors must be representing common factors important in the different subset studies. Final or conclusive meanings that attach to these descriptors relevant to structure–activity relationships would have to be determined by biological experiments.

These results are specific to the data sets used. They will be general to the extent that this data set mimics the universe of aromatic amino compounds. If this set of 157 compounds is a good representation of all aromatic amines, then the results should generalize. If the data set is not representative of the universal set, then the results are applicable only to the immediate compounds.

The results show that similar results can be obtained by using data of a mixed nature, that is, compounds which were tested using various protocols. It is not necessary to limit a study to one site, one route of administration, etc., in order to proceed. Of course, the results will be somewhat dependent on such factors, but mixed data sets can be used in computer-assisted SAR studies.

Quantitative Structure–Activity Relationships of Colchicines against P388 Leukemia in Mice

Frank R. Quinn* and John A. Beisler

Laboratory of Medicinal Chemistry and Biology, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205. Received January 7, 1980

A quantitative structure–activity relationship (QSAR) was derived for colchicine and 14 analogues acting against P388 lymphocytic leukemia in mice. Twelve additional compounds were synthesized to reinforce and confirm the correlation. The final correlation indicates that there is a parabolic dependence of antitumor potency on the partition coefficient with log P_0 = 1.17. When an amino nitrogen is present on the B ring, increased potency is favored by acylation of that nitrogen. The most potent compound of the series was the 7-fluoroacetamido analogue. Strong electron-withdrawing groups substituted at the 10 position of the tropolone ring destroy activity. Electron-releasing groups at position 10 improve potency slightly but have a limited effect.

Colchicine (1) is a potent mitotic inhibitor which occurs
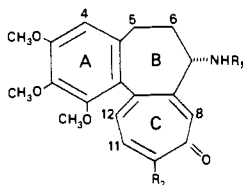
1 (colchicine), R_1 = CH_3CO; R_2 = CH_3O

naturally in the autumn crocus, *Colchicum autumnale L.* The antitumor property of colchicine has been recognized for over 3 millennia.[1] The antitumor effect has recently been shown by Kram and Schmitt[2] to be due to a binding of colchicine around a cysteine residue on the tubulin polypeptide chain. They conclude that this binding results from an interaction of the aromatic rings of colchicine with hydrophobic domains of tubulin.

The colchicines continue to find limited and sporadic use in the treatment of neoplasms.[3] The lack of interest in the colchicines among clinicians apparently arises from

the clear superiority of the vinca alkaloids in the management of advanced lymphomas and Hodgkin's disease.[4] The extreme toxicity of colchicine is another factor which works against its acceptance in the treatment of human cancer.[5]

Many attempts have been made to discover more effective and less toxic analogues of colchicine. This search has not been without some success. For example, deacetyl-*N*-methylcolchicine (colcemid, 4) has proven to be less toxic than colchicine and has been used in the treatment of chronic granulocytic leukemia.[6]

In the search for improved colchicines, a number of useful but sometimes contradictory qualitative structure–activity postulates have been developed. These are scattered in the literature and we have summarized them here. In their original monograph, Eigsti and Dustin[1] listed the following then known requirements for the antimitotic activity of colchicine derivatives: (1) at least one methoxy group on the A ring; (2) the amino group on the B ring should be acylated; (3) ring C should be seven membered;

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(1) Eigsti, O. J.; Dustin, P. "Colchicine in Agriculture, Medicine, Biology and Chemistry"; Iowa State College Press: Ames, Iowa, 1955; Chapter 1.

(2) Kram, R.; Schmitt, H. *J. Supramol. Struct.* **1978**, *Suppl. 2*, 328.

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Table I. Physicochemical and Antitumor Data of Colchicines against P388 Leukemia

| | substituents | | | | log (1/C) | | |
no.	R_1	R_2	log P	I	obsd	pred, eq 2	$\|\Delta$ log $(1/C)\|$
1	CH_3CO	CH_3O	1.03	1.0	6.46	6.67	0.21
2[a]	CH_3CO	CH_3O	0.93	1.0	5.82	6.66	0.84
3	H	CH_3O	1.10	0.0	4.69	4.52	0.17
4	CH_3	CH_3O	1.53	0.0	4.66	4.48	0.18
5[b]		CH_3O	2.07	0.0	4.03	4.28	0.25
6	$ClCH_2CO$	CH_3O	1.71	1.0	6.78	6.59	0.19
7	FCH_2CO	CH_3O	1.19	1.0	7.13	6.68	0.45
8	HCO	CH_3O	1.02	1.0	6.63	6.67	0.04
9	C_6H_5CO	CH_3O	2.94	1.0	5.76	5.74	0.02
10	CH_3CO	$CH_3OCOCH(NH_2)(CH_2)_4NH$	−2.10	1.0	3.61	3.54	0.07
11[c]	CH_3	CH_3S	2.22	0.0		4.19	
12	$ClCH_2CO$	CH_3S	2.34	1.0	6.74	6.27	0.47
13	$HOCH_2CO$	CH_3S	1.50	1.0	6.94	6.64	0.30
14	C_2H_5OCO	CH_3S	3.12	1.0	6.24	5.54	0.70
15	α-L-arabinosyl	CH_3S	1.40	0.0	4.39	4.52	0.12
16	β-D-glucosyl	CH_3S	1.25	0.0	4.39	4.52	0.13
17[d]	β-D-glucosyl, $(CH_3CO)_4$	CH_3S	4.61	0.0	3.81	0.98	2.83
18	CH_3CO	CH_3S	1.66	1.0	6.77	6.61	0.16
19	CH_3CO	$C_6H_5CH_2S$	3.21	1.0	4.76	5.43	0.67
20	$Cl(CH_2)_2NHCO$	CH_3S	3.53	1.0	4.93	5.01	0.08
21	H	CH_3S	1.73	0.0	4.57	4.43	0.14
22	CH_3CO	$(C_2H_5)_2N$	2.56	1.0	5.43	6.10	0.67
23	CF_3CO	CH_3O	2.36	1.0	6.36	6.25	0.11
24	$p\text{-}NO_2C_6H_4CO$	CH_3O	2.96	1.0	5.29	5.72	0.43
25[c]	CH_3CO	CH_3SO	−0.53	1.0		5.82	
26[c]	CH_3CO	CH_3SO_2	−0.58	1.0		5.76	
27[c]	CH_3CO	$C_6H_5CH_2SO_2$	0.97	1.0		6.67	
28	CH_3CO	$C_6H_5CH_2NH$	2.14	1.0		6.40	
29	CH_3CO	CH_3NH	0.58	1.0	6.52	6.57	0.05
30	CF_3CO	CH_3S	2.99	1.0	6.23	5.69	0.54

[a] 3-*O*-Demethyl. [b] In this compound, the C_7 carbon is substituted with a dimethylamino group having the same stereochemical configuration as colchicine. We thank Dr. A. Brossi for providing compound 5. [c] Inactive. [d] Not included in eq 2.

(4) there should be a methoxy, alkylamino, or alkylthio group on ring C. However, Schindler[7] showed that the acylamino group at position 7 was dispensable and the resulting analogue was approximately 10 times more active than colchicine in inhibiting the in vitro growth of a transplantable murine mast cell tumor. Schindler[8] also found that the carbonyl of ring C must be present.

In contrast to the postulate of Eigsti and Dustin,[1] that a seven-membered C ring is required, Leiter et al.[9] found in vivo tumor-inhibiting activity in sarcoma 37 for "colchinol" derivatives. In these congeners, the seven-membered C ring is replaced by a six-membered benzene ring and the carbonyl is replaced by a hydroxy or alkoxy group.

In a comprehensive study, Lettré determined the smallest concentration of a number of colchicine derivatives which produced inhibition of mitosis in cultured chicken heart fibroblasts.[10,11] His data suggest that a number of derivatives are more potent than colchicine and could conceivably be less toxic.

In order to determine whether the optimum antitumor colchicine has yet to be found, we have attempted to apply quantitative structure–activity techniques (QSAR) to in vivo data which has been accumulated by the National Cancer Institute (NCI). A search of the NCI chemical data base revealed that more than 100 analogues of colchicine had been subjected to biological testing in a variety of tumor systems. About 15 analogues showed sufficient activity in the P388 mouse leukemia test system for inclusion in this study. This paper presents the results of this study and the correlations which were achieved.

Methods. The compounds in this study were evaluated against lymphocytic leukemia P388 according to protocols established by the Division of Cancer Treatment, National Cancer Institute.[12] The mice were tumored intraperitoneally (ip), and the drugs were administered ip on days 1–9 with the QD 1–9 schedule (nine injections). A compound is considered active when it produces a percentage increase in survival time (% ILS) equal to or greater than 20%. However, in choosing a standard biological response (BR) for QSAR studies which deal with in vivo cancer systems, our experience has been that it is preferable to select a % ILS somewhat above the threshold of activity but not so high that moderately active but interesting analogues are excluded. In the present study, dose–response curves were plotted for each analogue; a % ILS of 40 was selected as the standard BR, and C is the concentration (moles/kilogram) which produces this response.

The octanol/water partition coefficients of the compounds in this study are given in Table I. The partition coefficients of 1, 3, 15, 16, 22, 24, and 28 were experimentally determined at pH 7.4.[13,14] The remaining log P values were calculated using π and/or fragment values.[13]

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(13) Hansch, D.; Leo, A. J. "Substituent Constants for Correlation Analysis in Chemistry"; Wiley Interscience: New York, 1979.
(14) The partition coefficients of 3, 15, 16, 22, 24, and 28 were measured at Pomona College, Contract N01-CM-67062.

The method of calculation is given in the Experimental Section.

Quantitative Structure–Activity Relationships. Equation 1 was developed using data from Table I for

$$\log (1/C) = 4.06\ (\pm 0.48) + 0.73\ (\pm 0.22) \log P -$$
$$0.27\ (\pm 0.08)\ (\log P)^2 + 2.15\ (\pm 0.54)\ I \quad (1)$$

$$n = 15;\ r = 0.954;\ s = 0.385;\ \log P_0 = 1.32\ (1.00\text{--}1.75)$$

$$\log (1/C) = 4.13\ (\pm 0.62) + 0.67\ (\pm 0.28) \log P -$$
$$0.19\ (\pm 0.07)\ (\log P)^2 + 1.77\ (\pm 0.59)\ I \quad (1a)$$

$$n = 16;\ r = 0.927;\ s = 0.499$$

compounds 1–4, 6–10, 12–16 and 20, all of which were selected from a survey of colchicine analogues previously tested by the NCI. Compound 17, the glucosylamino tetraacetate derivative, was omitted in the development of eq 1. This extremely lipophilic compound is probably a prodrug of 16 and would be expected to hydrolyze rapidly to the latter in the presence of esterases. Equation 1a contains all of the compounds.

In eq 1, $I = 1$ for the presence of an acyl group at the R_1 position and $I = 0$ for all other substituents. The autocorrelation between $\log P$ and the indicator variable I was 0.02, which indicates that those parameters are virtually orthogonal (arc cos 0.02 = 88.8°).

Equation 1 suggested that for this series of congeners in which principal structural variations were made at positions 7 and 10 there is a significant dependence on $\log P$. The optimal $\log P$ had a value of 1.32. There is an even greater dependence on the presence of the indicator variable I, suggesting that it is highly desirable to have an electron-withdrawing group adjacent to the N at position 7. In this series, the acyl carbonyl group serves that function and, as a consequence, renders the amino group less basic.

Chemistry. Equation 1 suggested the synthesis of 12 additional compounds (18, 19, and 21–30) to test, support, and expand the correlation. Compounds 18, 19, 22, and 25–29 were synthesized to probe electronic effects in the tropolone ring system which were not addressed by the original set of compounds used in the regression. Compounds 18, 19, 21, 22, and 29 were synthesized according to literature procedures[15-18] (see Experimental Section). Amides 23 and 24 were prepared from the tartrate of N-deacetylcolchicine[19] (3) using standard methods. Based on a reported method[20] for the conversion of methyl sulfides to sulfoxides or sulfones, compounds 25 and 26 were made by selective oxidations of thiocolchicine (18) with $NaIO_4$ or m-chloroperoxybenzoic acid, respectively. Sulfoxide 25 shows a strong absorption in the infrared at 1050 cm^{-1} which can be ascribed to the S=O stretching vibration.[21] Compound 26 exhibits bands at 1311 and 1136 cm^{-1} which are within the ranges characteristic of sulfones.[22] Within expectations, compound 27, which is a

Table II. Development of Equation 2

inter-cept	$\log P$	$(\log P)^2$	I	r	s	$F_{1,x}{}^a$
4.45			1.57	0.656	0.812	16.62
4.75		−0.12	1.91	0.762	0.715	7.60
4.11	0.70	−0.30	2.16	0.932	0.412	43.22

$^a F_{1,23;\alpha=0.005} = 11.37;\ F_{1,22;\alpha=0.01} = 7.95;\ F_{1,21;\alpha=0.001} = 14.59.$

benzyl sulfone, absorbs at frequencies of 1314 and 1142 cm^{-1}. Condensation of colchicine (1) with benzylamine provided 28. Compound 30 was prepared by trifluoroacetylation of N-deacetylthiocolchicine (21) in methylene chloride solution.

Structural assignments for all new compounds synthesized during the course of this study were verified by NMR and mass spectra. Compounds 21–30, for which mass spectra were measured, exhibited prominent M^+ ions, as well as peaks at M – 28. On electron impact, tropolones, including colchicine analogues, characteristically expel carbon monoxide from the molecular ion to give an abundant M – 28 ion.[23] Indeed, the M – 28 peaks in the spectra of 21 and 30 were the base peaks.

Results and Discussion

The 12 additional compounds (18, 19, and 21–30), together with compound 5 (see Table I, footnote b), were tested against P388 leukemia under the same conditions as the 15 original analogues. Compounds 5, 18, 19, 21–24, and 29–30 were active and were included in eq 2, together with the 15 original compounds from eq 1.

$$\log (1/C) = 4.11\ (\pm 0.42) + 0.70\ (\pm 0.22) \log P -$$
$$0.30\ (\pm 0.08)\ (\log P)^2 + 2.16\ (\pm 0.44)\ I \quad (2)$$

$$n = 24;\ r = 0.932;\ s = 0.412;\ \log P_0 = 1.17\ (0.91\text{--}1.45)$$

$$\log (1/C) = 4.22\ (\pm 0.56) + 0.58\ (\pm 0.28) \log P -$$
$$0.20\ (\pm 0.08)\ (\log P)^2 + 1.72\ (\pm 0.51)\ I \quad (2a)$$

$$n = 25;\ r = 0.883;\ s = 0.546$$

$$\log (1/C) = 4.22\ (\pm 0.26) + 0.72\ (\pm 0.13) \log P -$$
$$0.32\ (\pm 0.05)\ (\log P)^2 + 2.28\ (\pm 0.27)\ I \quad (2b)$$

$$n = 19;\ r = 0.982;\ s = 0.238$$

Table II gives the development of eq 2. Equation 2 is similar to eq 1 except for $\log P_0$, which is slightly lower (1.17 vs. 1.32). Compound 17 was omitted in the development of eq 2 for reasons cited in the development of eq 1. Equation 2a contains all of the compounds.

Equation 2 provides a reasonable fit of inherently variable murine in vivo antitumor data. If compounds with residuals >0.5 log unit are omitted (2, 14, 19, 22, and 30), the statistics are improved even further (eq 2b). In our view, eq 2 is to be preferred because lack of fit of the data associated with a particular compound may provide valuable insight.

Equation 2 was developed from data for compounds whose structural features are not too far removed from those of colchicine itself. With the exception of compound 2, substitutions occur only in the 7 and 10 positions. For these congeners, the correlation indicates that the most important parameter is I, denoting the presence of an amide at position 7. An inspection of the data [log (1/C)]

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in Table I will confirm the importance of this parameter. Those compounds lacking the amide group (3, 4, 5, 15, 16, and 21) are among the least potent members of this congeneric series.

For the analogues included in this study the correlation revealed, in addition, a parabolic dependence on log P and $(\log P)^2$ with an optimal value (log P_0) of 1.17. It is interesting to note that when we attempted a correlation of Lettré's in vitro data[10,11] for eight 10-(alkylamino) derivatives eq 3 was obtained.

$$\log (1/C) = 6.07 \ (\pm 1.28) + 0.44 \ (\pm 0.29) \ \Sigma E_{\mathrm{s}} +$$
$$2.10 \ (\pm 1.18) \log P - 0.68 \ (\pm 0.27) \ (\log P)^2 \ (3)$$

$$n = 8; \ r = 0.985; \ s = 0.272$$

In eq 3, ΣE_{s} is the sum of Taft steric parameter[13] values for alkyl substituents on the amino nitrogen. While the statistics of eq 3 are satisfying, the number of data points are less than one would like. For that reason, eq 3 is cited for purposes of comparison only. Nevertheless, it is interesting to observe that eq 3 shows a parabolic dependence on log P similar to eq 2 with a log P_0 (1.54) not too far from that of eq 2 (1.17).

Compound 2, the only A-ring-modified compound in this study, was the most poorly fit and was overpredicted by 0.8 log unit. It would be of considerable interest to determine whether other A-ring-modified colchicines show a similar lack of fit.

Compound 14, a carbamate, is underpredicted by 0.70 log unit for reasons that are not entirely clear. It is possible that this compound is acting as a carbamylating or alkylating agent. Compounds 19 and 22 are overpredicted by about 0.7 log unit. Both have fairly bulky groups at position 10, and, while the compounds in the present study did not permit an investigation of the steric effect at this position, Lettré's data (eq 3) suggest that it may be important.

Of the more active compounds in this study, the fluoroacetamide derivative (7) has both the optimal log P (1.19) and the required amide linkage, which is augmented in electron-withdrawing capability by the fluoro substituent. The fluoroacetamide is about 5 times more potent than the parent (1), which has a slightly suboptimal log P (1.03). The chloroacetamide analogue (6) has a partition coefficient about 0.5 log unit above log P_0. It is somewhat less potent than the fluoroacetamide but is still more potent than colchicine, perhaps due to the electron-withdrawing effect of the chloro substituent.

Lettré reported mitotic inhibition data for a set of 7-haloacyl derivatives of colchicine.[10,11] The activity of these congeners can be ranked as follows: F > Cl ≈ colchicine > Br > I. Since our in vivo data for two of these derivatives (6 and 7) showed the same trend, it seemed possible that electron-withdrawing substituents on the acyl carbon atom of the 7-acetamido group might be manipulated to improve potency. Compound 23, the trifluoroacetamide derivative, was synthesized to test this hypothesis. This derivative showed about the same potency as colchicine. Some of this lack of increased potency may be attributed to the fact that 23 has a log P about 1.2 log units above log P_0. Nevertheless, we were surprised to find that the presence of CF_3, one of the most powerful electron-withdrawing substituents known, did not produce even a modest increase in potency. It appears that the effect of electron-withdrawing groups on the amino nitrogen is limited.

The 7-benzamido derivative (9) is about 5 times less potent than colchicine. This is probably due to a partition coefficient 2.5 times log P_0. The p-nitrobenzamido derivative (24) was synthesized to see whether the electronic effect of the nitro group on the acyl carbon through resonance would compensate for the nonoptimal partition coeff. The partition coefficient of 24 is approximately the same as that of 9. However, the potency of the p-nitrobenzamido analogue was slightly less than that of 9. Thus, the presence of the strongly electron-withdrawing para-nitro group produced no beneficial effects.

Thiocolchicine (18), one of the 12 additional compounds, is slightly (2 times) more potent than colchicine. This may be due to the fact that CH_3S would be expected to decrease the electron density of the tropolone ring somewhat less than CH_3O. The sulfoxide (25) and the sulfones (26 and 27) were designed to further explore the effect of electron-withdrawing groups on the tropolone ring. The methylsulfinyl and methylsulfonyl groups yielded compounds with virtually identical partition coefficients, -0.53 and -0.58, respectively. If the Swain–Lupton field constant ($\mathcal{F}$) is taken as a measure of the electron-withdrawing potential of these groups, the values are quite close.[13] For CH_3SO, $\mathcal{F}$ = 0.52 vs. 0.60 for CH_3SO_2. The suboptimal hydrophobicity of these two compounds would be expected to adversely affect the antitumor activity and, indeed, both proved to be inactive. It was not possible, however, to ascribe this lack of activity to hydrophobic or electronic effects or both. Compound 27, the benzyl sulfone, was designed to separate these effects, since it has a partition coefficient (0.97) close to the optimal value. The benzyl sulfone was completely inactive in P388 leukemia, suggesting that electron-withdrawing substituents on the tropolone ring have a deleterious effect.

The 10-(benzylthio) compound (19) showed a modest potency in spite of a partition coefficient (3.21) which was about 3 times log P_0. The 10-(benzylamino) bioisostere (28) was synthesized to determine whether this activity could be improved by substituting the more electron-releasing nitrogen atom for the sulfur of 19. Also, compound 28 had the advantage of a somewhat more favorable log P (2.14). However, the benzylamino compound (28) offered no improvement over 19. In fact, the activity of 28 was only marginal (% ILS ≈ 25).

Azacolchicine (29) forms a series with colchicine (1) and thiocolchicine (18) in which the 10 position is in turn substituted by CH_3O, CH_3S, and CH_3NH. Azacolchicine and colchicine have approximately the same potency and both are slightly less potent than thiocolchicine. The suboptimal log P of 29 (0.58) is apparently evenly balanced by the beneficial effect of the electron-releasing amino group. When one compares azacolchicine (29) with the 1-(diethylamino) (22) and 10-(benzylamino) (28) derivatives, the potency decreases with increasing lipophilicity. This suggests that the beneficial effect of electron-releasing substituents on the tropolone ring is limited and is overshadowed by the overall hydrophobicity of the molecule.

Compound 30, the trifluoroacetyl analogue of thiocolchicine, was synthesized to see whether the combined effect of the 10-thio substituent and the 7-(trifluoroacetyl) group would improve the potency of (trifluoroacetyl)-colchicine (23). Since 30 had about the same potency as 23, it appears that the combination of substituents is not sufficient to overcome the overall unfavorable hydrophobicity of this analogue (log P = 2.99).

In conclusion, our correlation of the antileukemic potency of a number of colchicines modified in the 7 and 10 positions indicates that colchicine analogues have an optimal partition coefficient of about 1.17. When an amino group is present at position 7, potency is increased when the nitrogen is acylated. Potency is further enhanced by

the monosubstitution of a strong electron-attracting group such as F on the acyl carbon, provided the partition coefficient is close to log P_0. Electron-withdrawing groups at the 10 position tend to be deleterious, and eq 2 cannot be used to predict activity when such groups are present. Thiocolchicine (18) appears to be slightly more potent than colchicine.

Studies are in progress on a correlation of the structural parameters of colchicine analogues with acute toxicity in mice. In the event that potency and toxicity correlations can be viewed together, one may better judge whether further structural modifications can produce an improvement. If toxicity and potency correlations are exactly parallel, this is strong evidence that an increase in potency will be accompanied by a corresponding increase in toxicity. In addition, we are in the process of synthesizing and testing colchicine analogues in which substituents on the A ring have been modified. If is of interest to determine if A-ring-modified colchicines also fit eq 2 and if they will yield a toxicity correlation along with the compounds of the present study.

Experimental Section

(A) Chemical Methods. Mass spectra were obtained by direct probe insertion with a DuPont 21-492 spectrometer using a 75-eV ionizing voltage. Proton NMR spectra were recorded in deuteriochloroform solution with a Varian HA-100D spectrometer. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane, which was used as an internal standard. Infrared spectra were obtained in chloroform solution with a Perkin-Elmer Model 621 spectrophotometer. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed by the Section on Microanalytical Services and Instrumentation, NIAMDD, NIH, and by Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are reported only by the element symbols, results were within ±0.4% of the theoretical values.

Thiocolchicine (18), benzylthiocolchicine (19) and N-deacetylthiocolchicine (21) were prepared according to literature procedures.[15-17] The diethylamino compound (22) and the methylamino compound (29) were obtained using the method of Hartwell et al.[18] The melting points of compounds 18, 19, 21, 22, and 29 were in good agreement with published values, and NMR spectra were consistent with structural assignments. Colchicine (1) was purchased from S. B. Penick & Company, Lyndhurst, NJ.

N-(5,6,7,9-Tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl)-(S)-2,2,2-trifluoroacetamide (23). The tartrate of N-deacetylcholchicine (3) was prepared by reacting diazomethane with trimethylcolchicinic acid according to the method of Raffauf.[19] The free base was obtained by CHCl₃ extraction (3 × 15 mL) of a 0.5 N NaOH solution (40 mL) containing 710 mg (1.4 mmol) of the tartrate salt. After the extracts were dried and evaporated, the resulting oil was dissolved in 5 mL of dry CH₂Cl₂. While being cooled with an ice bath, the solution was treated with trifluoroacetic anhydride (0.5 mL) by dropwise addition over 2 min. The ensuing deep-red solution, protected with a CaSO₄ drying tube, was stirred for 45 min at ice temperature, followed by 90 min at room temperature. The reaction solution was pipetted into 0.5 N NaOH solution (40 mL) and extracted (3 × 20 mL) with CHCl₃. Evaporation of the dried extracts gave a syrup, which was layered with Et₂O and allowed to crystallize overnight. Yellow-orange crystals (370 mg, 59%) were obtained, which melted at 202–203 °C after recrystallization from ethyl acetate–cyclohexane: lit.[24] mp 203–205 °C; IR 1721, 1618, 1589, 1563, 1489, 1324, 1255, 1180, 1096 cm⁻¹; NMR δ 3.67, 3.91, 3.95 (3 s, 3 H each, aromatic OCH₃), 4.03 (s, 3 H, tropolone OCH₃), 6.55 (s, 1 H, C₄ H), 6.93 (d, $J = 10.5$ Hz, 1 H, C₁₁ H), 7.40 (d, $J = 10.5$ Hz, 1 H, C₁₂ H), 7.56 (s, 1 H, C₈ H); MS, m/e 453 (M⁺). Anal. (C₂₂H₂₂F₃NO₆, 453.4) C, H, N, F.

4-Nitro-N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl)-(S)-benzamide (24). The free base was liberated from the tartrate of 3 (508 mg, 1.0 mmol) as described above. The oily base was taken up in dry pyridine (1 mL) and treated with p-nitrobenzoyl chloride (223 mg, 1.2 mmol) while cooling the reaction solution with an ice bath. While being protected from moisture with a CaSO₄ tube, the solution was stirred at ice temperature for 30 min and then at room temperature for 60 min. The voluminous precipitate which separated from solution was dissolved in CHCl₃ (5 mL) and the stirring was continued for another 60 min. The reaction solution was poured into 1 N HCl (50 mL) and extracted with CHCl₃ (3 × 15 mL). The combined extracts were shaken with 3% NaHCO₃ solution (25 mL) and then dried and evaporated to give a yellow gum. The gum was chromatographed on a 2.5 × 18.5 cm silica column. Following elution with EtOAc (200 mL), the column was eluted with 300 mL of EtOAc containing 3% absolute EtOH, which gave 24 in the middle fractions as a yellow glass. Trituration of the glass with Et₂O gave 330 mg (65%) of an analytically pure, noncrystalline powder: IR 1664, 1613, 1600, 1589, 1528, 1487, 1348, 1253, 1094 cm⁻¹; NMR δ 3.78, 3.91, 3.97 (3 s, 3 H each, aromatic OCH₃), 4.01 (s, 3 H, tropolone OCH₃), 6.59 (s, 1 H, C₄ H), 6.98 (d, $J = 11$ Hz, 1 H, C₁₁ H), 7.46 (d, $J = 11$ Hz, 1 H, C₁₂ H), 7.73 (s, 1 H, C₈ H), 7.97 (s, 4 H, nitrobenzoyl protons); MS, m/e 506 (M⁺). Anal. (C₂₇H₂₆N₂O₈, 506.5) C, H, N.

N-[5,6,7,9-Tetrahydro-1,2,3-trimethoxy-9-oxo-10-(methylsulfinyl)benzo[a]heptalen-7-yl]-(S)-acetamide (25). Thiocolchicine (18; 1.66 g, 4 mmol) in MeCN (20 mL) solution was diluted with water (20 mL). While stirring and cooling with an ice bath, the solution was treated with sodium periodate (4.28 g, 20 mmol) in water (20 mL) and MeCN (20 mL) solution over 5 min in several small parts. The reaction solution was stirred for 1 h at 0 °C and then for 20 h at room temperature, then poured into water (400 mL), and extracted with CHCl₃ (4 × 60 mL). The combined extracts were dried and evaporated to give a residual gum from which 0.84 g (49%) of yellow needles separated from a EtOAc–Et₂O solution. Recrystallization from EtOAc gave the analytical sample: mp 261–262 °C; IR 1678, 1613, 1560, 1486, 1347, 1322, 1093, 1050 cm⁻¹; NMR δ 1.98 (s, 3 H, CH₃CON), 2.93 (s, 3 H, CH₃SO), 3.69, 3.92, 3.95 (3 s, 3 H each, aromatic OCH₃), 6.57 (s, 1 H, C₄ H), 7.41 (s, 1 H, C₈ H), 7.56 (d, $J = 10$ Hz, 1 H, C₁₁ H), 7.98 (d, $J = 10$ Hz, 1 H, C₁₂ H); MS, m/e 431 (M⁺). Anal. (C₂₂H₂₅NO₆S, 431.5) C, H, N, S.

N-[5,6,7,9-Tetrahydro-1,2,3-trimethoxy-9-oxo-10-(methylsulfonyl)benzo[a]heptalen-7-yl]-(S)-acetamide (26). A solution of thiocolchicine (18; 2.08 g, 5 mmol) in CHCl₃ (100 mL) was cooled with an ice bath and treated with m-chloroperoxybenzoic acid (1.90 g, 10 mmol). The reaction solution was stirred at 0 °C for 1 h and then stored in a 5 °C refrigerator for 21 h. After the reaction solution was diluted with CHCl₃ (100 mL), it was shaken with 3% NaHCO₃ solution (2 × 75 mL) and water (100 mL). After the CHCl₃ solution was dried, solvent was removed under vacuum and the resulting yellow foam was triturated several times with Et₂O to give a quantitative yield of the sulfone as an amorphous yellow-orange powder, which was analytically pure: IR 1678, 1622, 1580, 1488, 1321, 1311, 1136, 1094 cm⁻¹; NMR δ 2.02 (s, 3 H, CH₃CON), 3.39 (s, 3 H, CH₃SO₂), 3.71, 3.91, 3.94 (3 s, 3 H each, aromatic OCH₃), 6.54 (s, 1 H, C₄H), 7.40 (d, $J = 9$ Hz, 1 H, C₁₁ H), 7.58 (s, 1 H, C₈ H), 8.31 (d, $J = 9$ Hz, 1 H, C₁₂ H); MS, m/e 447 (M⁺). Anal. (C₂₂H₂₅NO₇S, 447.5) C, H, N, S.

N-[5,6,7,9-Tetrahydro-1,2,3-trimethoxy-9-oxo-10-[(phenylmethyl)sulfonyl]benzo[a]heptalen-7-yl]-(S)-acetamide (27). To a solution of 19 (983 mg, 2 mmol) in 40 mL of CHCl₃, which was stirred and cooled with ice, was added m-chloroperbenzoic acid (759 mg, 4.4 mmol). The reaction solution was stirred at 0 °C for 45 min and then at room temperature for 5 h. The solution was diluted with CHCl₃ (100 mL) and washed sequentially with 3% NaHCO₃ solution (2 × 50 mL) and water (50 mL). On evaporation of the dried CHCl₃ solution a brown gum was obtained, which was chromatographed on a 3 × 19 cm silica column. Elution with EtOAc (1250 mL) gave benzyl sulfone 27 (980 mg, 82%) as an amorphous yellow-orange powder after trituration with Et₂O: IR 1671, 1613, 1573, 1480, 1340, 1314, 1142, 1113, 1087 cm⁻¹; NMR δ 2.06 (s, 1 H, CH₃CON), 3.64, 3.88, 3.90 (3 s, 3 H each, aromatic OCH₃), 4.86 (s, 2 H, CH₂SO₂), 6.52 (s, 1 H, C₄ H), 7.19 (d, $J = 10$ Hz, 1 H, C₁₁ H), 7.33 (s, 5 H, C₆H₅), 7.60 (s, 1 H, C₈

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(24) Capraro, H. G.; Brossi, A. *Helv. Chim. Acta* **1979**, *62*, 965. We thank Dr. Brossi for providing us with a copy of the manuscript prior to publication.

H), 7.91 (d, J = 10 Hz, 1 H, C_{12} H); MS, m/e 523 (M⁺). Anal. ($C_{28}H_{29}NO_7S$, 523.6) C, H, N, S.

N-[5,6,7,9-Tetrahydro-1,2,3-trimethoxy-9-oxo-10-[(phenylmethyl)amino]benzo[a]heptalen-7-yl]-(S)-acetamide (28). A solution of colchicine (1; 1.60 g, 4 mmol) in absolute EtOH (40 mL) was treated with benzylamine (4 mL) and heated in a pressure bottle at 116 °C for 20 h. Volatile materials were removed under vacuum with a rotary evaporator. The residue was taken up in 75 mL of absolute EtOH and evaporated once again. Last traces of volatiles were removed with a mechanical pump, resulting in a gum from which 1.79 g (94%) of yellow prisms were obtained by crystallization from EtOAc–cyclohexane: mp 166–168 °C; IR 1672, 1601, 1583, 1500, 1462, 1428, 1404, 1349, 1322, 1142, 1096 cm⁻¹; NMR δ 1.97 (s, 1 H, CH_3CON), 3.61, 3.87, 3.92 (3 s, 3 H each, aromatic OCH_3), 4.60 (d, J = 6 Hz, 2 H, benzylic protons), 6.52 (s, 1 H, C_4 H), 6.63 (d, J = 12 Hz, 1 H, C_{11} H), 7.34 (s, 5 H, C_6H_5), 7.39 (d, J = 12 Hz, 1 H, C_{12} H), 7.65 (s, 1 H, C_8 H), 8.35 (d, J = 6 Hz, 1 H, BzNH); MS, m/e 474 (M⁺). Anal. ($C_{28}H_{30}N_2O_5$, 474.5) C, H, N.

N-[5,6,7,9-Tetrahydro-1,2,3-trimethoxy-9-oxo-10-(methylthio)-9-oxobenzo[a]heptalen-7-yl]-(S)-2,2,2-trifluoroacetamide (30). A stirred solution of N-deacetylthiocolchicine (21; 1.12 g, 3 mmol) in dry CH_2Cl_2 (15 mL) was cooled with an ice bath and treated with trifluoroacetic anhydride (0.9 mL). The reaction solution, while protected with a $CaSO_4$ drying tube, was stirred for 45 min at ice temperature and then for 60 min at room temperature. The resulting deep-red solution was poured into 0.5 N NaOH solution (80 mL) and extracted (3 × 50 mL) with $CHCl_3$. The dried extracts when evaporated produced a yellow solid, which when crystallized from EtOAc-cyclohexane gave 1.36 g (96%) of small yellow needles, mp 191–192 °C. Recrystallization from the same solvents raised the melting point by one degree and provided the analytical sample: IR 1721, 1608, 1485, 1322, 1095, 1019 cm⁻¹; NMR δ 2.45 (s, 3 H, CH_3S), 3.68, 3.91, 3.96 (3 s, 3 H each, aromatic OCH_3), 6.56 (s, 1 H, C_4 H), 7.12 (d, J = 10.5 Hz, 1 H, C_{11} H), 7.37 (d, J = 10.5 Hz, 1 H, C_{12} H), 7.51 (s, 1 H, C_8 H); MS, m/e 469 (M⁺). Anal. ($C_{22}H_{22}F_3NO_5S$, 469.5) C, H, N, S.

(B) Calculation of Partition Coefficients.

(1) $\Delta\pi$ for replacement of CH_3O by OH^{25} = −0.10

$$\log P_{[2]} = \log P_{[1]} - 0.10 = 1.03 - 0.10 = 0.93$$

(2) $\log P_{[4]} = \log P_{[3]} - f_{NH_2}^{1R} + f_{NH}^{1R} + f_{CH_3} + F_b =$
$$1.10 + 1.35 - 1.69 + 0.89 - 0.12 = 1.53$$

(3) $\log P_{[5]} = \log P_{[3]} - f_{NH_2}^{1R} + f_N^{1R} + 2f_{CH_3} + 2F_{bYN} =$
$$1.10 + 1.35 - 1.76 + 1.78 - 0.40 = 2.07$$

(4) $\log P_{[6]} = \log P_{[1]} - \log P_{CH_3CONH_2} + \log P_{ClCH_2CONH_2} =$
$$1.03 + 1.21 - 0.53 = 1.71$$

(5) $\log P_{[7]} = \log P_{[1]} - \log P_{CH_3CONH_2} + \log P_{FCH_2CONH_2} =$
$$1.03 + 1.21 - 1.05 = 1.19$$

(6) $\log P_{[8]} = \log P_{[1]} - \pi_{CH_3CONH} + \pi_{HCONH} =$
$$1.03 + 0.97 - 0.98 = 1.02$$

(7) $\log P_{[9]} = \log P_{[1]} - f_{NHCO} - f_{CH_3} - f^\phi_{CONH} + f_{C_6H_5} =$
$$1.03 + 2.71 - 0.89 - 1.81 + 1.90 = 2.94$$

(8) $\log P_{[10]} = \log P_{[1]} - \pi_{CH_3O} - f_H + f_{NH} + 4f_{CH_2} + f_{CH} +$
$f_{NH_2} + f_{COOH} + 7F_b + F_{P-1}^{NH_2 \to COOH} + F_{gBr} + F_Z^{26} =$
$$1.03 + 0.02 - 0.23 - 1.03 + 2.64 + 0.43 - 1.54 - 1.11 - 0.84 +$$
$$1.11 - 0.22 - 2.36 = -2.10$$

(9) $\log P_{[11]} = \log P_{[2]} - \pi_{CH_3O} + \pi_{CH_3S} = 1.59 + 0.02 + 0.61 =$
$$2.22$$

(10) $\log P_{[12]} = \log P_{[1]} - \log P_{CH_3CONH_2} + \log P_{ClCH_2CONH_2} -$
$\pi_{CH_3O} + \pi_{CH_3S} = 1.03 + 1.21 - 0.53 + 0.02 + 0.61 = 2.34$

(11) $\log P_{[13]} = \log P_{[1]} - \pi_{CH_3O} + \pi_{CH_3S} - f_H + f_{OH} +$
$F_{P-1}^{NHCO \to OH} = 1.03 + 0.02 + 0.61 - 0.23 - 1.64 + 1.83 = 1.62$

(12) $\log P_{[14]} = \log P_{[1]} - f_{CONH} + f_{OCONH} + \pi_{CH_3} - \pi_{CH_3O} +$
$\pi_{CH_3S} = 1.03 + 2.71 - 1.79 + 0.54 + 0.02 + 0.61 = 3.12$

(13) $\log P_{[17]} = \log P_{[16]} + 4\Delta\pi_{OH \to OCOCH_3}^{27} =$
$$1.25 + 4(0.84) = 4.61$$

(14) $\log P_{[18]} = \log P_{[1]} - \pi_{CH_3O} + \pi_{CH_3S} =$
$$1.03 + 0.02 + 0.61 = 1.66$$

(15) $\log P_{[19]} = \log P_{[1]} - \pi_{CH_3O} + \pi_{CH_3S} + f_{C_6H_5} - f_H + F_b =$
$$1.03 + 0.02 + 0.61 + 1.9 - 0.23 - 0.12 = 3.21$$

(16) $\log P_{[20]} =$

$\log P_{[18]} - f_{CONH} + f_{NHCONH} + 2f_{CH_2} + f_{Cl} + F_{P-2}^{N \to Cl} + 2F_b =$
$$1.66 + 2.71 - 2.18 + 1.32 + 0.06 + 0.02 - 0.24 = 3.53$$

(17) $\log P_{[21]} = \log P_{[3]} - \pi_{CH_3O} + \pi_{CH_3S} =$
$$1.10 + 0.02 + 0.61 = 1.73$$

(18) $\log P_{[23]} = \log P_{[1]} - \pi_{CH_3CONH} + \pi_{CF_3CONH} =$
$$1.03 + 0.97 + 0.08 = 2.08$$

(19) $\log P_{[25]} = \log P_{[2]} - \pi_{CH_3O} + \pi_{SOCH_3} =$
$$1.03 + 0.02 - 1.58 = -0.53$$

(20) $\log P_{[26]} = \log P_{[2]} - \pi_{CH_3O} + \pi_{SO_2CH_3} =$
$$1.03 + 0.02 - 1.63 = -0.58$$

(21) $\log P_{[27]} = \log P_{[2]} - \pi_{CH_3O} + \pi_{SO_2CH_2C_6H_5} =$
$$1.03 + 0.02 + 0.27 = 1.32$$

(22) $\log P_{[28]} = \log P_{[1]} - \pi_{CH_3O} + \log P_{C_6H_5CH_2NH_2} =$
$$1.03 + 0.02 + 1.09 = 2.14$$

(23) $\log P_{[29]} = \log P_{[1]} - \pi_{CH_3O} + \pi_{CH_3NH} =$
$$1.03 + 0.02 - 0.47 = 0.58$$

(24) $\log P_{[30]} = \log P_{[23]} - \pi_{CH_3O} + \pi_{CH_3S} =$
$$2.36 + 0.02 + 0.61 = 2.99$$

(25) This $\Delta\pi$ is derived from 1,2,3-trimethoxybenzene and is only valid for similar systems.

(26) F_Z is a zwitterion factor: A. Leo, personal communication.

(27) The net change in π for esterification of the OH groups was calculated from the log P values for ethanol and ethyl acetate minus a proximity effect of 0.08 per OH.