Table I. Pharmacological Results

compd	guinea pig[a] tracheal test ED_{50}[b] (SE)	intrinsic act.[c]	atrial test ED_{25}[d] (SE)	intrinsic act.[c]	anesthetized dogs[a] inhibn of bronchoconstriction, equipotent dose at ED_{50}[e]	increase in heart rate, equipotent dose at ED_{25}[e]
l-isoproterenol					1.00[g] (0.65-1.52)	1.00[h] (0.29-1.95)
procaterol	$1.10 (\pm 0.22) \times 10^{-8}$	0.98	$5.70 (\pm 1.63) \times 10^{-9}$	0.60		
terbutaline	$1.04 (\pm 0.19) \times 10^{-6}$	0.70	$2.64 (\pm 1.13) \times 10^{-6}$	0.73		
4	$2.25 (\pm 0.26) \times 10^{-7}$	0.50	*f*	0.09	41.7 (23.5-83.0)	87.1 (13.8-283.8)
9	$1.22 (\pm 0.28) \times 10^{-6}$	0.60	$5.66 (\pm 2.10) \times 10^{-7}$	0.27		

[a] $n = 5$. [b] Molar concentration for 50% of the maximum response to each compound. [c] *l*-Isoproterenol = 1. [d] Molar concentration for 25% of the maximum response to each compound. [e] Relative to *l*-isoproterenol = 1.00; 95% confidence limits in parentheses. [f] Maximal activity was obtained at 1×10^{-5} mol/L. [g] Mean ED_{50} value, obtained at a dose of 0.054 μg/kg. [h] Mean ED_{25} value, obtained at 0.021 μg/kg.

concentrated sulfuric acid and 10 mL of water was added dropwise a solution of 0.645 g (9.35 mmol) of sodium nitrite in 2 mL of water at below 5 °C. Stirring was continued for a further 10 min and 15 g of ice–water was added to the solution. Then the solution was heated at 80–90 °C for 2 h. The reaction mixture was cooled, and the precipitate was collected and washed with water. The crystalline sulfate obtained was dissolved in aqueous KOH solution with stirring and cooling in ice–water, and the resulting solution was adjusted to pH 8.5 with concentrated hydrochloric acid. The precipitate was collected, washed with water, and suspended in MeOH. The suspension was acidified to pH 1 with concentrated hydrochloric acid and evaporated. The residue was washed with cold MeOH and recrystallized from water to give 1.34 g (48%) of **4** as the hydrochloride 0.25-hydrate: mp 279–281 °C dec. NMR (Me_2SO-d_6–D_2O) δ 7.04 and 6.80 (1 H, d, J = 2.2 Hz, aromatic CH) and 5.69 [1 H, br d, J = 4 Hz, >C*H*OH]. Anal. ($C_{16}H_{23.5}N_2O_{3.25}Cl$) C, H, N.

3,4-Dihydro-5-[1-hydroxy-2-(isopropylamino)butyl]-7-hydroxycarbostyril (9). A solution of 0.48 g (1.45 mmol) of **4** in 50 mL of water was reduced in the presence of 0.1 g of Palladium black in a Paar hydrogenator at 70 °C under a hydrogen atmosphere of 6 kg/cm² for 12 h. The catalyst was removed and the aqueous solution was evaporated. The residue was recrystallized from water to give 0.23 g (46%) of **9** as the hydrochloride monohydrate, mp 177–180 °C. Anal. ($C_{16}H_{27}N_2O_4Cl$) C, H, N.

Pharmacology. Methods. A. Guinea Pig Tracheal Test. This test was performed as described previously,[1] without the expression of ED_{50} values. ED_{50} values were determined from dose–response curves as the values for 50% of the maximum response to each compound. The concentration–response curve for compound **4** on tracheal strips was shifted in a parallel fashion to the right in the presence of propranolol as follows (molar concentration of propranolol, ED_{50} value and maximum response to **4** with *l*-isoproterenol): 1×10^{-8}, 2.3×10^{-6}, 0.58; 3×10^{-8}, 4.8×10^{-6}, 0.42; 1×10^{-7}, 1.1×10^{-5}, 0.27.

B. Guinea Pig Atrial Test. This test was carried out as described previously,[1] using the cumulative method of drug administration described by Van Rossum.[13] The effects of the test compounds were expressed as percentages of the maximum response to *l*-isoproterenol. ED_{25} values were obtained from dose–response curves as the value for 25% of the maximum response to each compound.

C. In Vivo Assay Using Anesthetized Dogs. This test was performed as described previously.[2]

(13) J. M. Van Rossum, *Arch. Int. Pharmacodyn. Ther.*, **143**, 299 (1963).

Toxicity Quantitative Structure–Activity Relationships of Colchicines

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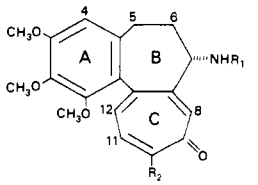
A method for extracting LD_{50} values from antitumor test data is described. A quantitative structure–activity relationship (QSAR) for 7- and 10-substituted colchicines is presented. This correlation equation closely parallels that which had been derived earlier for potency. This result indicates that attempts to modify 7- and 10-substituted colchicines in order to decrease toxicity will likely produce a simultaneous decrease in potency. Ring A modified colchicines do not obey the potency and toxicity correlations. 4-Substituted colchicines appear promising in terms of decreased toxicity, greater ILS, and a broader therapeutic range.

Colchicine (**1**), a potent mitotic inhibitor, has long been known to have antitumor properties.[1] As an agent against human tumors, however, its performance has been disappointing.[2] Major factors which preclude the use of colchicine as a clinical agent are severe toxicity and an extremely narrow therapeutic range. While P388-tumored mice treated with colchicine at the optimal dose (0.50



1 (colchicine), $R_1 = COCH_3$; $R_2 = CH_3O$

(1) Eigsti, O. J.; Dustin, P. "Colchicines in Agriculture, Medicine, Biology and Chemistry"; Iowa State College Press: Ames, Iowa, 1955; Chapter 1.
(2) Dowling, M. D.; Krakoff, I. H.; Karnofsky, D. A. "Chemotherapy of Cancer"; Cole, W. H., Ed.; Lea and Febiger: Philadelphia, 1970; Chapter 1.

Table I. Physicochemical and Toxicity Data of Colchicines

no.	substituents R_1	substituents R_2	log P	I	log $(1/LD_{50})$ obsd	log $(1/LD_{50})$ pred (eq 3)	$\lvert\Delta$ log $(1/LD_{50})\rvert$
1	CH_3CO	CH_3O	1.03	1.0	5.46	5.59	0.13
2[a]	CH_3CO	CH_3O	0.93	1.0	5.82	5.58	0.24
3	H	CH_3O	1.10	0.0	3.71	3.88	0.17
4	CH_3	CH_3O	1.53	0.0	3.63	3.85	0.22
5[b]		CH_3O	2.07	0.0	4.21	3.69	0.52
6	$ClCH_2CO$	CH_3O	1.71	1.0	5.54	5.53	0.01
7	FCH_2CO	CH_3O	1.19	1.0	5.69	5.60	0.09
8	HCO	CH_3O	1.02	1.0	5.16	5.59	0.43
9	C_6H_5CO	CH_3O	2.94	1.0	4.24	4.85	0.61
10	CH_3CO	$CH_3OCOCH(NH_2)(CH_2)_4NH$	−2.10	1.0	3.00	3.01	0.01
11	$ClCH_2CO$	CH_3S	2.34	1.0	5.65	5.27	0.38
12	$HOCH_2CO$	CH_3S	1.50	1.0	5.66	5.57	0.09
13	C_2H_5OCO	CH_3S	3.12	1.0	4.19	4.68	0.49
14	α-L-arabinosyl	CH_3S	1.40	0.0	3.98	3.87	0.11
15	β-D-glucosyl	CH_3S	1.25	0.0	3.71	3.88	0.17
16[c]	β-D-glucosyl, $(CH_3CO)_4$	CH_3S	4.61	0.0	3.81	1.02	2.78
17	CH_3CO	CH_3S	1.66	1.0	5.62	5.54	0.08
18	CH_3CO	$C_6H_5CH_2S$	3.21	1.0	4.22	4.60	0.38
19	$Cl(CH_2)_2NHCO$	CH_3S	3.53	1.0	4.93	4.26	0.67
20	H	CH_3S	1.73	0.0	3.75	3.81	0.06
21	CH_3CO	$(C_2H_5)_2N$	2.56	1.0	4.64	5.14	0.50
22	CF_3CO	CH_3O	2.36	1.0	5.73	5.26	0.47
23	p-$NO_2C_6H_4CO$	CH_3O	2.96	1.0	4.41	4.83	0.42
24[c]	CH_3CO	$C_6H_5CH_2NH$	2.14	1.0	4.28	5.37	1.09
25	CH_3CO	CH_3NH	0.58	1.0	5.72	5.50	0.22
26	CF_3CO	CH_3S	2.99	1.0	5.51	4.80	0.71

[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] Not included in eq 3.

mg/kg) routinely show an ILS of ca. 85%, this dose usually produces a severe weight loss.[3] The LD_{50} in mice is about 1 mg/kg. As little as 7 mg has proven fatal to humans.[4]

Many analogues of colchicine have been synthesized with a view to reducing toxicity, while retaining the antitumor properties of the parent. Most modifications of colchicine have been made in the 7 and/or 10 positions. In a previous study,[5] eq 1 was formulated for a number of 7- and 10-

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

$$n = 24; r = 0.932; s = 0.412; \log P_0 = 1.17$$

substituted colchicines acting against P388 lymphocytic leukemia in mice.

C in eq 1 is the concentration (moles per kilogram) which produces a T/C of 140 (ILS = 40). The indicator variable I represents the presence of an acyl group on the nitrogen atom at position 7. The general conclusions of that study were that there are rather narrow limits on manipulation of 7- and 10-substituents in terms of enhancement of potency. The value of log P_0 sets a limit on the potency which can be obtained by manipulation of the lipophilic/hydrophilic balance. An amide nitrogen at position 7 favors potency. A further conclusion of that investigation was that strong electron-withdrawing groups at position 10 are deleterious.

A complete QSAR description of colchicine must inevitably address the problem of toxicity. Taken together, potency and toxicity correlations should reveal whether there is any room for manipulation of substituents to retain relative potency while decreasing toxicity. In this paper a toxicity QSAR of 7- and 10-substituted colchicines is presented.

In the course of this investigation, a method for obtaining the LD_{50} of drugs from tumor-bearing mice through toxicity day survival data was established and tested.

Since it appeared from the original study that ring A modified colchicines did not obey eq 1, three additional 4-substituted colchicines were synthesized and tested, and the results are presented here.

Biological and Chemical Data. Under protocols[6] established by the National Cancer Institute (NCI) for testing in P388 leukemia, mice are tumored intraperitoneally (ip) and treatment begins the following day. Untreated controls normally die within 9–13 days. Deaths which occur prior to the 5th day after tumor implantation are attributed to drug toxicity. Accordingly, the day 5 survival observation is referred to as "toxicity day". Toxicity day observations are retained as part of the biological record for all drugs tested in the NCI screening program. Similar procedures have been followed for all antitumor testing, although the toxicity day may be different for different tumors. Since the determination of toxicity of drugs in tumored animals is a byproduct of all NCI testing, it seemed reasonable to attempt to make use of these data.

Toxicity data were retrieved for 26 compounds which had been administered to mice bearing P388 leukemia on a chronic regimen (QD 1–9). Thus, each compound had been administered once daily for 4 days before deaths were noted. LD_{50} values were calculated by the probit method,[7]

(3) Drug Evaluation Branch, Division of Cancer Treatment, National Cancer Institute.
(4) Woodbury, D. M.; Fingl, E. "The Pharmacological Basis of Therapeutics", 5th ed.; Goodman, L. S.; Gilman, A., Eds.; Macmillan: New York, 1975; Chapter 17.
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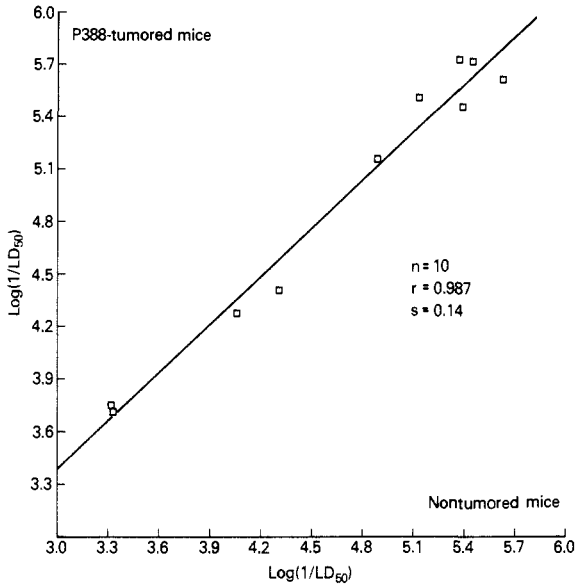

Figure 1. Plot of eq 2 showing the relationship of log $(1/LD_{50})$ derived from nontumored and P388-tumored mice.

Table II. Comparison of the Toxicity Day LD_{50} in Nontumored and P388-Tumored Mice

	LD_{50}, mg/kg	
no.	nontumored	tumored
1	1.6	1.4
8	5.0	2.7
14	251	102
17	1.0	1.0
20	177	69
22	1.9	0.8
23	25	20
24	41	25.1
25	1.4	0.8
26	3.5	1.4

and the log $(1/LD_{50})$ values are given in Table I. These same compounds had been used in the potency correlation (eq 1).

The precision of this method may be illustrated as follows. The LD_{50} of colchicine (**1**) calculated from tumored animal data was 1.37. The upper and lower 95% confidence intervals were 1.58 and 1.22, respectively. Similar confidence intervals were obtained for other members of the series.

Day 5 toxicities of nontumored animals (four ip injections/dose/mouse) were measured for 10 of the compounds in the series (**1**, **8**, **14**, **17**, **20**, and **22–26**), and the results compared with the toxicities in Table I. The results of this comparison are given in Table II and eq 2. Figure 1 is

$$\log (1/LD_{50})_{\text{tumored}} = 0.631\ (\pm 0.583) + 0.918\ (\pm 0.122) \log (1/LD_{50})_{\text{nontumored}} \quad (2)$$

$$n = 10;\ r = 0.974;\ s = 0.140;\ F_{1,9} = 298.9;\ p < 0.001$$

a plot of eq 2, showing the linear relationship between the two methods for LD_{50} determination.

As one would expect, the LD_{50} for tumored mice is somewhat less than that for healthy, nontumored animals. The relationship is well-defined and should provide a useful tool in the design of anticancer agents. Without scheduling additional biological assays, requiring the expenditure of both time and scarce test compound, an in vivo test system not only can establish the presence of antitumor activity but also can provide an approximate LD_{50}, allowing a therapeutic ratio to be calculated. The latter quantity is a very useful measure to have in appraising a series of antitumor analogues.

The method of calculation of physicochemical parameters of the compounds in Table I has been described.[5]

Results and Discussion

Equation 3 was developed from the data in Table I.

$$\log (1/LD_{50}) = 3.54\ (\pm 0.41) + 0.58\ (\pm 0.22) \log P - 0.24\ (\pm 0.08) (\log P)^2 + 1.71\ (\pm 0.43)\ I \quad (3)$$

$$n = 24;\ r = 0.904;\ s = 0.401;\ \log P = 1.19\ (0.88\text{–}1.53)$$

$$\log (1/LD_{50}) = 3.65\ (\pm 0.57) + 0.44\ (\pm 0.29) \log P - 0.14\ (\pm 0.08) (\log P)^2 + 1.24\ (\pm 0.52)\ I \quad (3a)$$

$$n = 26;\ r = 0.790;\ s = 0.564$$

Equation 3a contains all of the compounds, whereas compounds **16** and **24** were omitted in the development of eq 3. Compound **16** did not fit the activity equation (eq 1) and it was substantially underpredicted by eq 3. It is probably a prodrug of **15** and this is supported by the observation that the LD_{50} values of **15** and **16** are very close. It is not clear why compound **24** is overpredicted. This is a relatively insoluble compound and that may be a contributing factor. There was virtually no autocorrelation between the parameters log P and I (arc cos 0.02 = 88.8°).[8] The development of eq 3 is given in Table III.

Table III. Development of Equation 3

intercept	log P	$(\log P)^2$	I	r	s	$F_{1,X}$	p	eq
3.83			1.23	0.625	0.698	14.07	<0.005	A
4.06		0.10	1.51	0.728	0.627	6.28[a]	<0.25	B
3.54	0.58	0.24	1.71	0.904	0.401	31.36[b]	<0.001	3

[a] This F value was obtained in comparison with eq A. [b] This F value was obtained in comparison with eq B.

Table IV. Comparison of Observed and Predicted Potency and Toxicity of 4-Substituted Colchicines

	log $(1/C)$			log $(1/LD_{50})$		
4-substituent	obsd	pred[a]	$\lvert\Delta \log (1/C)\rvert$	obsd	pred[b]	$\lvert\Delta \log (1/LD_{50})\rvert$
CHO (**27**)	5.92	6.49	0.57	4.14	5.44	1.30
CN (**28**)	4.46	6.53	2.07	3.80	5.47	1.67
CH_2OH (**29**)	4.58	6.27	1.69	3.40	5.25	1.85

[a] Predicted by eq 1. [b] Predicted by eq 3.

Equation 3 differs very little from eq 1. It indicates that the toxicity of these 7- and 10-substituted colchicines is strongly influenced by the presence of an amide nitrogen at position 7. There is a parabolic dependence on the partition coefficient with a log P close to that of eq 1 (1.19 vs. 1.17). A comparison of eq 1 and 3 leads to the conclusion that for 7- and 10-substituted colchicines toxicity and potency are inseparable. There is no clear way to manipulate substituents to decrease toxicity without decreasing potency.

In the previous study, there was some indication that ring A modified colchicines might behave differently from 7- and 10-modified analogues. This was based on the substantial overprediction of the potency of compound **2**, the 3-demethyl derivative. In the course of this investigation, three ring A substituted analogues were synthesized by literature methods and tested. These were the 4-formyl (**27**), 4-cyano (**28**), and 4-(hydroxymethyl) (**29**) derivatives.[9] The partition coefficients of these compounds were calculated as follows:

$$(1)\ \log P_{27} = \log P_1 + \pi_{CHO} = 1.03 - 0.65 = 0.38$$

$$(2)\ \log P_{28} = \log P_1 + \pi_{CN} = 1.03 - 0.57 = 0.46$$

$$(3)\ \log P_{29} = \log P_1 + \pi_{CH_2OH} = 1.03 - 1.03 = 0.0$$

The potency and toxicity of each of these three analogues was calculated by eq 1 and 3 and compared with observed values. The results are given in Table IV.

It is of considerable interest that all of the 4-substituted colchicine analogues (**27–29**) are overpredicted in terms of potency. Compounds **28** and **29** are overpredicted by almost 2 log units. All three derivatives were predicted to be considerably more toxic than they were observed to be. Therefore, eq 1 and 3 do not predict the potency or toxicity of 4-substituted colchicines. Ring A modification, especially at the 4 position, appears to result in colchicine derivatives that differ fundamentally from those which are modified at the 7 or 10 positions.

Table V presents the biological data of 4-formylcolchicine (**27**). In terms of maximum ILS produced, this compound appears to surpass the parent. It is considerably less toxic and has a much broader therapeutic range.

Table V. Comparison of 4-Formylcolchicine (27) and Colchicine (1) against in Vivo P388 Murine Leukemia[a]

	% ILS			
	4-formylcolchicine		colchicine	
dose, mg/kg	trial 1	trial 2	trial 1	trial 2
12.5		2		
6.25	105	105		
3.12	94	80		
1.56	60	75		
1.0			toxic	toxic
0.78	54	49		
0.5			78	89
0.39	51	49		
0.25			46	58
0.12			33	40
0.06			27	24

[a] The standard protocol[6] used by the National Cancer Institute for the P388 test system was followed. Hydroxypropylcellulose was used to suspend 4-formylcolchicine in saline; colchicine was dissolved in water. Drugs were administered ip on days 1-9 (nine injections) after tumor implantation. Median survival times of the control groups in trials 1 and 2 were 11.2 and 10.7 days, respectively.

It seems clear, as Hansch[10] has observed, that anticancer agents will yield satisfactory QSAR and that guidelines can be provided for the design of new agents. In this and the previous study, our analysis has shown that further modifications of positions 7 and 10 of colchicine are not likely to be profitable. Having established some important limitations on the design of colchicine analogues, a promising synthetic lead has been uncovered, i.e., modification of the 4 position of ring A. In addition, a strategy for obtaining toxicity from screening data has been described. While LD_{50} values thus obtained are not intended for translation into higher animals or man, they should provide a readily accessible and valuable parameter to the medicinal chemist in the search for improved anticancer agents. Work is in progress which is designed to further explore ring A modified colchicines.

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Preparation and Antibacterial Activity of Δ^1-Thienamycin

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Δ^1-Thienamycin (**2**), a double-bond isomer of thienamycin, was prepared by isomerizing *N*-[[(*p*-nitrobenzyl)oxy]carbonyl]thienamycin *p*-nitrobenzyl ester (**5b**) with DBU in Me_2SO followed by hydrogenolysis of the protecting groups. When evaluated in a disc-diffusion antibacterial assay, Δ^1-thienamycin was found to be essentially devoid of activity. The lack of antibacterial activity was ascribed to a chemically less reactive β-lactam amide bond than that found in thienamycin.

The recently discovered carbapen-2-em family of antibiotics, which includes thienamycin,[1] the epithienamycins,[2] the olivanic acids,[3] and PS-5,[4] exhibits unusual and highly desirable antibacterial properties. Of these, thienamycin

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