Quantitative Structure–Activity Relationship Analysis of Combretastatins: A Class of Novel Antimitotic Agents

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Received October 24, 1990; accepted January 22, 1991

Combretastatins and their synthetic analogues, having structural features resembling that of colchicine, also have similar modes of action. In this report we have correlated the cytotoxicity of combretastatins against the murine leukemic cell line L1210 with physicochemical parameters such as the summation of the Hansch–Fujita π constant, which was used as an index of lipophilicity of the substituent groups on ring A ($\Sigma\pi_a$) and ring B ($\Sigma\pi_b$), the vector summation of the group dipole moments of ring A ($\Sigma\mu_a$) and ring B ($\Sigma\mu_b$), the nature of the linker chain between ring A and ring B ($B_{t\text{-}L}$), indicator parameters $(NOH)_a$ and $(NOH)_b$, which represent the number of hydroxyl groups on ring A and ring B, respectively, and the summation of π values of the substituents on the linker ($\Sigma\pi_L$). Cytotoxicity correlated well with ($\Sigma\pi_b$), $(NOH)_a$, ($B_{t\text{-}L}$), and ($\Sigma\mu_b$), and the dependency on ($\Sigma\pi_b$) was found to be parabolic.

KEY WORDS: *Combretum caffrum;* combretastatins; tubulin inhibitor; quantitative structure–activity relationship (QSAR); hydrophobic interaction; dipolar interactions; antimitotic agents; cytotoxicity.

INTRODUCTION

Combretastatins belong to the class of antimitotic agents which exert their antineoplastic effect by inhibiting mitosis and microtubule assembly, hence resulting in an accumulation of cells in the metaphase. The structural resemblance of combretastatin to colchicine and its stimulatory effect on tubulin-dependent GTP hydrolysis (1) indicates that combretastatin binds to tubulin at the same site as that of colchicine. This binding site is shared by other compounds that are structurally related to colchicine, such as maytansine, steganacin, taxols, podophyllotoxin, and cornigerin (1,2), but not the vinca alkaloids (vincristine, vinblastine), which interact with tubulin at a different site. Combretastatins have been reported to bind to tubulin quite firmly and rapidly, and unlike for colchicine, the binding is not temperature dependent (1).

Several naturally occurring combretastatins and their derivatives have been isolated or synthesized (Fig. 3). One such compound is combretastatin A-4 (NSC-817373) (Fig. 1) extracted from the bark of a South American tree *Combretum caffrum* (Combretaceae) (2,3). Both combretastatin A-4 and another analogue, combretastatin A-2 (Fig. 2), showed excellent cytotoxicity against murine P388, L1210 lymphocytic leukemia cells, and human cell lines such as LoVo, HT-29, Colo-205, DLD-1, and HCT-15 colon carcinoma cell lines among many others tested (4,5).

Most congeneres have two substituted benzene rings joined by a two-carbon spacer (usually ethylene) and exhibiting predominantly a *cis* configuration, which is usually more active than the *trans* isomer (13).

Several hypotheses have been proposed (especially for the colchicines) about the location of the binding site on one of the tubulin subunits. Assuming involvement of only the α chain (6,7) or the β chain (8,9,10) of tubulin, individually, one cannot account for all the experimental findings. Lin *et al.* (1) proposed a model for the binding site of colchicine on tubulin that spans both subunits and took into account their substantial sequence homology (11,12). They proposed that a subsite exists for colchicine binding on each tubulin subunit at homologous locations on the two peptide chains. Because of their structural similarity to colchicine, combretastatins are thought to fit this binding site.

From the mechanism of action of these agents and the structure–activity analysis of the combretastatins (5,13), it appears that apart from the hydrophobicity of the functional groups on the A and the B rings and the substituent group dipole moments on the benzene rings, other parameters may also determine the biological activity of these compounds.

In this paper, we analyzed the correlation between the cytotoxicity of the compounds against the L1210 cell line and some of these physicochemical parameters. A good correlation was obtained between their cytotoxicity and the summation of the hydrophobicity values of the functional groups on ring B of combretastatins ($\Sigma\pi_b$) (14), vector summation of the group dipole moments of ring B ($\Sigma\mu_b$), the bond type (single or double) of the spacer between the two phenyl rings ($B_{t\text{-}L}$), and an indicator variable $(NOH)_a$, which designates the number of free hydroxy groups on ring A.

METHODS

The biological activities of the compounds listed in Table IV were adapted from a paper by Lin *et al.* (13) for the QSAR analysis. All the compounds that were analyzed have a bridge length of two carbons between the two phenyl groups. The ones having a variable bridge length between the two phenyl groups were not taken into consideration. Moreover, the latter compounds exhibited decreased potency with increased chain length. The compounds in which the two phenyl rings are fused into a phenanthrene-like ring structure were also left out because of the difference in their basic structure as compared to the rest of the compounds.

In Lin and co-workers' report (13) L1210 murine leukemia cells were used to determine the effects of these compounds on cell proliferation. The cells were grown in spinner culture at a density of 105 cells/ml in RPMI 1640 medium having 5% fetal calf serum with penicillin and streptomycin added. The cells were counted with a Coulter counter (15).

$\Sigma\pi_a$, $\Sigma\pi_b$, $\Sigma\mu_a$, and $\Sigma\mu_b$ were calculated by us and are shown in Table V along with other parameters and the bio-

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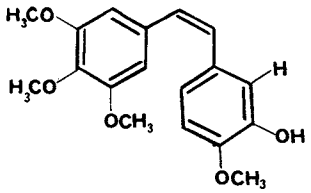

Fig. 1. The structure of combretastatin A-4.

logical activity. $\Sigma\pi_a$ and $\Sigma\pi_b$ are the summation of the π values of all the individual functional groups (16,17) on ring A and ring B, respectively. $\Sigma\mu_a$ and $\Sigma\mu_b$ are the vector summation of the group dipole moment (18–20) of the functional groups on ring A and ring B, respectively. The indicator variable, $B_{t\text{-}L}$, accounts for the nature of the two carbon unit bridge. A value of 1 was assigned if the bridge was unsaturated and 0 if it was saturated.

The other indicator variables, $(NOH)_a$ and $(NOH)_b$, represent the number of hydroxyl groups on ring A and ring B, respectively. $(NOH)_a$ is a parameter that combines lipophilicity as well as polar interaction and hydrogen bonding of the ring A in the correlation equation. $\Sigma\pi_L$ represents the summation of the π values of the substituent group on the linker.

The cytotoxicity, IC_{50} (molar concentration), was transformed to $\log(1/IC_{50})$.

Stepwise regression of the data was accomplished by a BMDP statistical software package (21).

RESULTS AND DISCUSSION

From the SAR analysis of the combretastatins (13) it is evident that the *cis* isomer is comparatively more active than the *trans* isomer. The bridge length between the two benzene rings seems to be critical. The compounds with an ethyl or an ethylene bridge were more active than those with a longer bridge. It has been hypothesized that the A and the B rings of combretastatins have two separate binding sites on α and β chains of tubulin (1), respectively. So if the two rings are far apart, the affinity for the binding site decreases, resulting in a reduced cytotoxicity of the compounds.

Combretastatins usually have the R configuration at position 1_a (22). The racemic compounds are relatively less active than the R forms, suggesting that the R configuration at position 1_a (Table IV) is of functional importance.

The data set was treated as a whole and also in subsets. The whole data set comprised of combretastatins having free vicinal methoxy and/or hydroxy groups and also those in which the vicinal groups are fused to yield a methylenedioxy

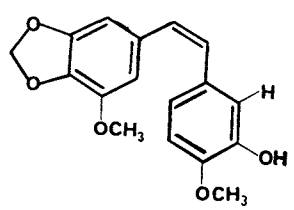

Fig. 2. The structure of combretastatin A-2.

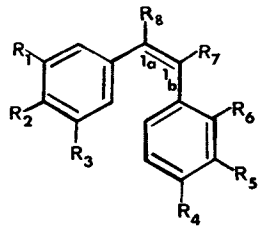

Fig. 3. The general structure of combretastatins and the synthetic analogues.

bridge (benzodioxole ring system) on the A ring. The subset comprised of only those compounds which have free vicinal functional groups on ring A. This was done to determine any possible effects of the compounds with a dioxole ring on the correlation analysis of the compounds lacking that moiety.

Stepwise regression was also separately performed on both the data sets, by leaving out first the *trans* isomers and then both the *trans* isomers and the racemic forms of the compounds to determine whether the geometric isomers and the racemic forms affected the correlation analysis. Only the best equations from these analyses are cited in Table III. It is interesting to note that the inclusion of the *trans* isomers or the racemic forms of the compounds does not affect the overall correlations. QSAR analysis of the geometric isomers could not be performed separately due to very few pairs of data points available.

Table I. Equations Correlating Cytotoxicity with Physicochemical Parameters for All the Compounds in Table IV

(1) $\log(1/IC_{50}) = -1.29(\Sigma\mu_b) - 0.53$
$n = 30, r = 0.45, s = 1.00, F(1,28) = 7.18$

(2) $\log(1/IC_{50}) = -1.11(\Sigma\mu_b) + 0.79(B_{t\text{-}L}) - 0.33$
$n = 30, r = 0.58, s = 0.94, F(1,27) = 5.10$

(3) $\log(1/IC_{50}) = -1.04(\Sigma\mu_b) + 0.96(B_{t\text{-}L}) + 1.12(\Sigma\pi_a) - 0.04$
$n = 30, r = 0.61, s = 0.92, F(1,26) = 1.97$

(4) $\log(1/IC_{50}) = -1.44(\Sigma\mu_b) + 0.95(B_{t\text{-}L}) + 1.47(\Sigma\pi_a) - 0.49(\Sigma\pi_b)^2 - 0.76$
$n = 30, r = 0.66, s = 0.89, F(1,25) = 2.77$

(5) $\log(1/IC_{50}) = -1.31[\pm0.68]\,(\Sigma\mu_b) + 1.05[\pm0.49]\,(B_{t\text{-}L}) + 0.98[\pm1.11]\,(\Sigma\pi_a) - 3.23[\pm1.08]\,(\Sigma\pi_b)^2 - 4.49[\pm1.63]\,(\Sigma\pi_b) - 1.82$
$n = 30, r = 0.87, s = 0.59, F(1,24) = 31.65$
$(\Sigma\pi_b)_0 = -0.69[-1.03, -0.49]$

(5a) $\log(1/IC_{50}) = -1.31[\pm0.69]\,(\Sigma\mu_b) + 1.04[0.49]\,(B_{t\text{-}L}) - 0.62[\pm0.72]\,(NOH)_a - 3.23[\pm1.64]\,(\Sigma\pi_b)^2 - 4.49[\pm1.64]\,(\Sigma\pi_b) - 1.86$
$n = 30, r = 0.87, r^2 = 0.76, s = 0.59, F(1,24) = 31.42$
$(\Sigma\pi_b)_0 = -0.69[-1.03, -0.49]$

(6) $\log(1/IC_{50}) = -1.22(\Sigma\mu_b) + 1.02(B_{t\text{-}L}) + 1.21(\Sigma\pi_a) - 3.10(\Sigma\pi_b)^2 - 4.33(\Sigma\pi_b) - 0.28(\Sigma\mu_a) - 2.19$
$n = 30, r = 0.88, s = 0.59, F(1,23) = 1.47$

(7) $\log(1/IC_{50}) = -1.32(\Sigma\mu_b) + 0.89(B_{t\text{-}L}) + 1.34(\Sigma\pi_a) - 3.34(\Sigma\pi_b)^2 - 4.61(\Sigma\pi_b) - 0.39(\Sigma\mu_a) + 0.25(\Sigma\pi_L) - 2.58$
$n = 30, r = 0.89, s = 0.57, F(1,22) = 2.50$

(8) $\log(1/IC_{50}) = -1.27(\Sigma\mu_b) + 0.86(B_{t\text{-}L}) + 1.24(\Sigma\pi_a) - 3.32(\Sigma\pi_b)^2 - 4.89(\Sigma\pi_b) - 0.36(\Sigma\mu_a) + 0.23(\Sigma\pi_L) - 0.22(NOH)_b - 2.38$
$n = 30, r = 0.89, s = 0.58, F(1,21) = 0.58$

Table II. Equations Correlating Cytotoxicity with Physicochemical Parameters for All the Compounds in Table IV, Without the Benzodioxole Ring System

(9) $\log(1/IC_{50}) = -1.35(\Sigma\mu_b) - 0.56$
$n = 25, r = 0.50, s = 0.98, F(1,23) = 7.74$

(10) $\log(1/IC_{50}) = -1.20(\Sigma\mu_b) + 0.82(B_{t\text{-}L}) - 0.45$
$n = 25, r = 0.62, s = 0.91, F(1,22) = 4.54$

(11) $\log(1/IC_{50}) = -1.12(\Sigma\mu_b) + 1.09(B_{t\text{-}L}) - 0.98(NOH)_a - 0.15$
$n = 25, r = 0.69, s = 0.86, F(1,21) = 3.73$

(12) $\log(1/IC_{50}) = -1.38(\Sigma\mu_b) + 1.08(B_{t\text{-}L}) - 1.15(NOH)_a - 0.39(\Sigma\pi_b)^2 - 0.61$
$n = 25, r = 0.71, s = 0.85, F(1,20) = 1.43$

(13) $\log(1/IC_{50}) = -1.29[\pm0.59]\ (\Sigma\mu_b) + 1.39[\pm0.48]\ (B_{t\text{-}L}) - 0.89[\pm0.64]\ (NOH)_a - 3.27[\pm1.05]\ (\Sigma\pi_b)^2 - 4.63[\pm1.56]\ (\Sigma\pi_b) - 1.84$
$n = 25, r = 0.91, r^2 = 0.83, s = 0.50, F(1,19) = 37.82$
$(\Sigma\pi_b)_0 = -0.71[-0.96, -0.54]$

(14) $\log(1/IC_{50}) = -1.41(\Sigma\mu_b) + 1.29(B_{t\text{-}L}) - 0.95(NOH)_a - 3.54(\Sigma\pi_b)^2 - 4.93(\Sigma\pi_b) + 0.23(\Sigma\pi_L) - 2.09$
$n = 25, r = 0.93, s = 0.48, F(1,18) = 2.93$

(15) $\log(1/IC_{50}) = -1.40(\Sigma\mu_b) + 1.27(B_{t\text{-}L}) - 0.96(NOH)_a - 3.52(\Sigma\pi_b)^2 - 4.91(\Sigma\pi_b) + 0.23(\Sigma\pi_L) - 0.04(\Sigma\mu_a) - 2.14$
$n = 25, r = 0.93, s = 0.49, F(1,17) = 0.03$

(16) $\log(1/IC_{50}) = -1.40(\Sigma\mu_b) + 1.27(B_{t\text{-}L}) - 0.96(NOH)_a - 3.52(\Sigma\pi_b)^2 - 4.91(\Sigma\pi_b) + 0.23(\Sigma\pi_L) - 0.04(\Sigma\mu_a) - 0.002(NOH)_b - 2.14$
$n = 25, r = 0.93, s = 0.51, F(1,16) = 0.00$

Table III. Equations Correlating Cytotoxicity with Physicochemical Parameters for Both Sets of Data Excluding Geometric Isomers and Racemic Forms[a]

(1) $\log(1/IC_{50}) = -0.71[\pm0.72]\ (NOH)_a - 4.30[\pm1.72]\ (\Sigma\pi_b) - 3.08[\pm1.18]\ (\Sigma\pi_b)^2 - 1.25[0.61]\ (\Sigma\mu_b) + 1.48[\pm0.54]\ (B_{t\text{-}L}) - 1.64$
$n = 20, r = 0.92, s = 0.50, (\Sigma\pi_b)_0 = -0.70[-1.01, -0.51]$

(2) $\log(1/IC_{50}) = -0.73[\pm0.68]\ (NOH)_a - 4.36[\pm1.61]\ (\Sigma\pi_b) - 3.13[\pm1.08]\ (\Sigma\pi_b)^2 - 1.27[\pm0.58]\ (\Sigma\mu_b) + 1.47[\pm0.51]\ (B_{t\text{-}L}) - 1.69$
$n = 23, r = 0.91, s = 0.49, (\Sigma\pi_b)_0 = -0.69[-0.96, -0.52]$

(3) $\log(1/IC_{50}) = -0.53[\pm0.78]\ (NOH)_a - 4.41[\pm1.76]\ (\Sigma\pi_b) - 3.23[\pm1.19]\ (\Sigma\pi_b)^2 - 1.28[\pm0.69]\ (\Sigma\mu_b) + 1.23[\pm0.53]\ (B_{t\text{-}L}) - 1.76$
$n = 24, r = 0.89, s = 0.57\ (\Sigma\pi_b)_0 = -0.68[-1.04, -0.47]$

(4) $\log(1/IC_{50}) = -0.54[\pm0.75]\ (NOH)_a - 4.48[\pm1.63]\ (\Sigma\pi_b) - 3.29[\pm1.08]\ (\Sigma\pi_b)^2 - 1.31[\pm0.64]\ (\Sigma\mu_b) + 1.22[\pm0.49]\ (B_{t\text{-}L}) - 1.82$
$n = 27, r = 0.89, s = 0.56, (\Sigma\pi_b)_0 = -0.68[-0.99, -0.49]$

[a] Equation (1) represents correlation of cytotoxicity to that of physicochemical parameters of the *cis* compounds only excluding the dioxole ring and without the *trans* and racemic form. Equation (2) represents correlation of cytotoxicity to that of physicochemical parameters of the *cis* and the racemic compounds excluding the dioxole ring and without the *trans* form. Equation (3) represents correlation of cytotoxicity to that of physicochemical parameters of compounds with the dioxole ring but excluding the *trans* and the racemic form. Equation (4) represents correlation of cytotoxicity to that of physicochemical parameters of compounds with the dioxole ring and the racemic form but excluding the *trans* form.

Table IV. The Substituent Groups of the Combretastatin Analogs[a]

No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	B	C
1	−OMe	−OMe	−OMe	−OMe	−OH	−H	−H	−H	d	*cis*
2	−OMe	−OMe	−OMe	−OMe	−OH	−OH	−H	−H	d	*cis*
3	−OH	−OMe	−OMe	−OMe	−OH	−H	−H	−H	d	*cis*
4	−OH	−OMe	−OMe	−OMe	−OH	−H	−H	−H	s	*cis*
5	−OH	−OMe	−OMe	−OMe	−OMe	−H	−H	−H	d	*cis*
6	−OMe	−OMe	−OMe	−OMe	−OH	−H	−H	−OH	s	*cis*
7	−OMe	−OMe	−OMe	−OMe	−OH	−OH	−H	−H	s	*cis*
8	−OMe	−H	−OMe	−OMe	−OH	−H	−H	−H	s	*cis*
9	−OMe	−H	−OMe	−OH	−OH	−H	−H	−H	s	*cis*
10	−OMe	−OMe	−OMe	−OH	−OH	−H	−H	−H	s	*cis*
11	−OMe	−H	−OMe	−OH	−H	−H	−H	−H	s	*cis*
12	−OMe	−OMe	−OMe	−OH	−H	−H	−H	−H	s	*cis*
13	−OMe	−OMe	−OMe	−OMe	−OH	−H	−H	−H	d	*trans*
14	−OMe	−OMe	−OMe	−OMe	−OCOMe	−H	−H	−H	d	*cis*
15	−OMe	−OMe	−OMe	−OMe	−OCOMe	−H	−H	−H	s	*cis*
16	−OMe	−OMe	−OMe	−OMe	−OCOMe	−OCOMe	−H	−H	d	*cis*
17	−OMe	−OMe	−OMe	−OMe	−OMe	−H	−H	−H	d	*cis*
18	−OH	−OMe	−OMe	−OMe	−OMe	−H	−H	−H	d	*trans*
19	−OMe	−OMe	−OMe	−OMe	−OH	−H	−H	−H	s	*cis*
20	−OMe	−OMe	−OMe	−OMe	−OH	−H	−H	−OH	s	*RS*
21	−OMe	−OMe	−OMe	−OMe	−OH	−H	−H	−OCOMe	s	*RS*
22	−OMe	−OMe	−OMe	−OMe	−OH	−H	−OH	−H	s	*RS*
23	−OMe	−OMe	−OMe	−OMe	−OH	−H	=O	−H	s	*cis*
24	−OMe	−OMe	−OMe	−OMe	−OMe	−H	−H	−H	s	*cis*
25	−OMe	−OMe	−OMe	−OMe	−OMe	−OMe	−H	−H	s	*cis*
26	=O−CH−O=	−OMe	−OMe	−OH	−H	−H	−H	−H	d	*cis*
27	=O−CH−O=	−OMe	−OMe	−OH	−OH	−H	−H	−H	d	*cis*
28	=O−CH−O=	−OMe	−OMe	−OH	−H	−H	−H	−H	d	*trans*
29	=O−CH−O=	−OMe	−OMe	−OH	−OH	−H	−H	−H	s	*cis*
30	=O−CH−O=	−OMe	−OMe	−OMe	−OMe	−H	−H	−H	s	*cis*

[a] R_1–R_8 represent the substituents at positions 1 through 8. B indicates the type of bond of the linker. d represents double bond and s represents single bond. C indicates the configuration. R and S represent rectus and sinister, respectively.

Table V. The Physico Chemical Parameters and Cytotoxicity of Combretastatin and Its Analogs

No.	$\Sigma\pi_a$[a]	$\Sigma\pi_b$[a]	$(NOH)_a$	$(NOH)_b$	$\Sigma\pi_L$[a]	$(B_{t\text{-}L})$	$\Sigma\mu_a$[b]	$\Sigma\mu_b$[b]	IC_{50} (mol/L)[c]
1	−0.06	−0.69	0	1	0	1	−2.60	−2.91	7.0×10^{-6}
2	−0.06	−1.36	0	2	0	1	−2.60	−3.06	6.0×10^{-4}
3	−0.71	−0.69	1	1	0	1	−2.77	−2.91	4.0×10^{-5}
4	−0.71	−0.69	1	1	0	0	−2.77	−2.91	3.0×10^{-3}
5	−0.71	−0.04	1	0	0	1	−2.77	−2.62	2.0×10^{-3}
6	−0.06	−0.69	0	1	−1.16	0	−2.60	−2.91	5.0×10^{-5}
7	−0.06	−1.36	0	2	0	0	−2.60	−3.06	2.0×10^{-3}
8	−0.04	−0.69	0	1	0	0	−1.27	−2.91	1.0×10^{-3}
9	−0.04	−1.34	0	2	0	0	−1.27	−3.20	1.0×10^{-3}
10	−0.06	−1.34	0	2	0	0	−2.60	−3.20	3.0×10^{-3}
11	−0.04	−0.67	0	1	0	0	−1.27	−1.57	4.0×10^{-3}
12	−0.06	−0.67	0	1	0	0	−2.60	−1.57	5.0×10^{-2}
13	−0.06	−0.69	0	1	0	1	−2.60	−2.91	2.0×10^{-5}
14	−0.06	−0.66	0	0	0	1	−2.60	−3.04	7.0×10^{-6}
15	−0.06	−0.66	0	0	0	0	−2.60	−3.04	1.0×10^{-4}
16	−0.06	−1.30	0	0	0	1	−2.60	−3.27	6.0×10^{-5}
17	−0.06	−0.04	0	0	0	1	−2.60	−2.62	3.0×10^{-4}
18	−0.71	−0.04	1	0	0	1	−2.77	−2.62	3.0×10^{-2}
19	−0.06	−0.69	0	1	0	0	−2.60	−2.91	2.0×10^{-4}
20	−0.06	−0.69	0	1	−1.16	0	−2.60	−2.91	9.0×10^{-4}
21	−0.06	−0.69	0	1	−0.91	0	−2.60	−2.91	2.0×10^{-4}
22	−0.06	−0.69	0	1	−1.16	0	−2.60	−2.91	9.0×10^{-4}
23	−0.06	−0.69	0	1	−3.87	0	−2.60	−2.91	3.0×10^{-3}
24	−0.06	−0.04	0	0	0	0	−2.60	−2.62	2.0×10^{-2}
25	−0.04	−0.04	0	0	0	0	−1.27	−2.62	3.0×10^{-2}
26	−0.07	−0.69	0	1	0	1	−1.92	−2.91	1.0×10^{-4}
27	−0.07	−1.36	0	2	0	1	−1.92	−3.06	4.0×10^{-3}
28	−0.07	−0.69	0	1	0	1	−1.92	−2.91	4.0×10^{-4}
29	−0.07	−1.36	0	2	0	0	−1.92	−3.06	8.0×10^{-3}
30	−0.07	−0.06	0	0	0	0	−1.92	−2.60	4.0×10^{-2}

[a] The Hansch–Fujita π constant calculated by the additive principle of Lien *et al.* (16,17).

[b] $\Sigma\mu$, vector summation of the group dipole moments of the functional groups on the two rings. Dipole moment for 1,3-dioxalane obtained from the literature (20).

[c] IC_{50} values adapted from Lin *et al.* (13) and expressed as moles per liter.

Upon stepwise regression of the whole data set we obtained Eqs. (1)–(8) for the correlation of $\log(1/IC_{50})$ with $\Sigma\pi_a$, $\Sigma\pi_b$, $\Sigma\mu_a$, $\Sigma\mu_b$, $B_{t\text{-}L}$, $(NOH)_a$, $(NOH)_b$, and $\Sigma\pi_L$ (Table I). Equations (9)–(16) (Table II) were generated upon regression of the subset. Close examination of the equations in the tables shows that Eqs. (5) and (13) in both the tables are the best equations. It is important to note that occasionally $(NOH)_a$ can be replaced by $\Sigma\pi_a$ in the equation. So we tried to correlate $\Sigma\pi_a$ with $(NOH)_a$, and they showed an excellent correlation [$\Sigma\pi_a = -0.65\,(NOH)_a - 0.06$ $n = 30$, $r = 0.99$, $s = 0.008$]. Thus substitution of $(NOH)_a$ by $\Sigma\pi_a$ does not alter the equation [Eq. (5a); Table I].

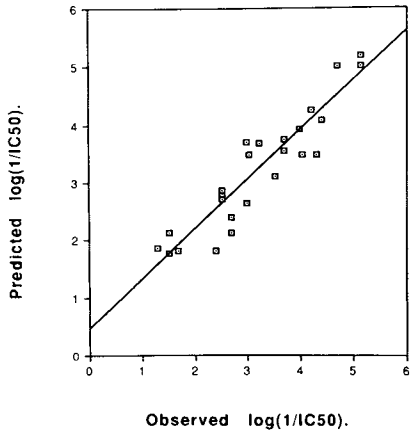

Fig. 4. Plot of predicted $\log(1/IC_{50})$ against observed $\log(1/IC_{50})$ from Eq. (5a) in Table I.

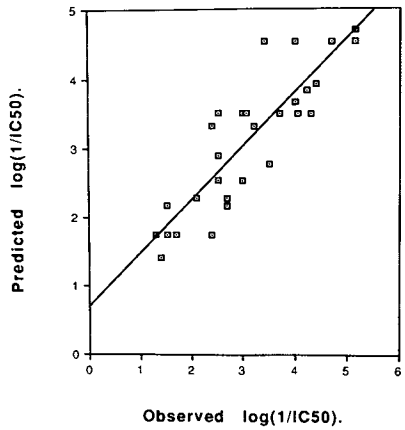

Fig. 5. Plot of predicted $\log(1/IC_{50})$ against observed $\log(1/IC_{50})$ from Eq. (13) in Table II.

The best equation correlates the cytotoxicity [log(1/IC_{50})] with $\Sigma\pi_b$, $(\Sigma\pi_b)^2$, $(NOH)_a$, $B_{t\text{-}L}$, and $\Sigma\mu_b$. The rest of the parameters are not significant, as the addition of these.

Parameters [$\Sigma\pi_a$, $\Sigma\mu_a$, $(NOH)_b$, $\Sigma\pi_L$] to the equation did not improve the F statistics, possibly due to the narrow ranges of these values. The best equation in Table I has a correlation coefficient of 0.87, with a standard deviation of 0.59. So 76% ($r^2 = 0.76$) of the variance in the data can be explained by this equation (Fig. 4).

The best equation in Table II has a correlation coefficient of 0.91, with a standard deviation of 0.50. So 84% ($r^2 = 0.84$) of the variance in the data set can be accounted for by this equation (Fig. 5).

The regression analysis of $\log(1/IC_{50})$ against π_b showed a vertical clustering, which means that a few different values for the $\log(1/IC_{50})$ were reflected by three discrete π_b values as the independent variable. Although this did not affect the overall analysis in this case, it could be a potential source of interference if insufficient data points were included.

It appears that cytotoxicity has a negative dependency on both $\Sigma\pi_b$ and $(\Sigma\pi_b)^2$. It has to be taken into consideration that the $\Sigma\pi_b$ values of all the compounds are negative, which makes the contribution from $\Sigma\pi_b$ positive and that from $(\Sigma\pi_b)^2$ negative toward the biological activity. It is also seen that some parameters such as $\Sigma\pi_b$, $(NOH)_a$, $\Sigma\mu_b$, and $B_{t\text{-}L}$ contribute significantly toward the cytotoxicity of these compounds.

The parabolic dependency on $\Sigma\pi_b$ is expected because the compounds have to cross the biological membranes to exert their effect. It is known that compounds that are too hydrophilic cannot easily penetrate the biological membrane. Although the hydrophobic compounds are capable of penetrating the biological membrane, they cannot partition into the adjoining water compartment (cytoplasm) easily and hence cannot gain access to their site of action. So a balance has to be achieved whereby penetration is facilitated and the compound is capable of partitioning across both the membrane barriers and the cytoplasmic compartment. The optimum $(\Sigma\pi_b)_o$ value for the compounds tested is found to lie in the range of 0.69 to 0.71.

As $\Sigma\pi_a$ values are effectively the same for almost all the compounds tested, and as the length of the linker is also the same in all the cases, the contribution from these two moieties toward the total lipophilicity of the compound is almost constant. The lipophilicity of the compound is therefore dependent primarily on the lipophilicity of the substituents on ring B as represented by $\Sigma\pi_b$.

The bond type ($B_{t\text{-}L}$) has a positive contribution toward $\log(1/IC_{50})$. Therefore, unsaturation of the linker contributes positively toward cytotoxicity. Saturation of the linker ensures free rotation of the two phenyl rings, probably distorting the rigid structure, leading to reduced binding of the compounds toward the active site on tubulin.

The vector summation of the dipole moments of the functional groups on ring B ($\Sigma\mu_b$) has a negative contribution to cytotoxicity. An increase in the dipole moment is reflected in the reduced cytotoxicity of these compounds. The increased dipole moment might pose a problem for these species to cross the membrane and also in binding to the receptor on tubulin. This observation leads to the suggestion that hydrophobic interaction rather than a polar interaction plays a major role in binding of these compounds to the receptors.

ACKNOWLEDGMENTS

This work has been supported in part by the Hong-Yen and Lin Run Charitable Foundation and by BRSG Grant S07RR05792 awarded to the School of Pharmacy of the University of Southern California.

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