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# Comparative QSAR Studies on Substituted Bis-(acridines) and Bis-(phenazines)-Carboxamides: A New Class of Anticancer Agents

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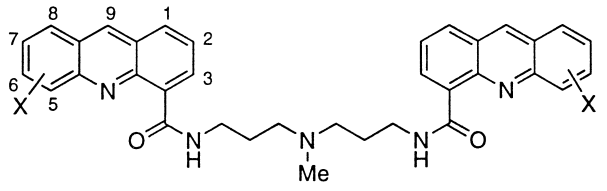
**Abstract**—Quantitative structure–activity relationships have been formulated for two sets of DNA binding topoisomerase agents (bis-acridines and bis-phenazines) acting on murine P388 leukemia cells, murine Lewis lung carcinoma ($LL_C$) cells and human Jurkat leukemia wild-type ($JL_C$) cells. For the acridines, all three QSARs (1–3) show only a (small negative) hydrophobic effect. In sharp contrast, the phenazines in all three studies (4–6) show a strong hydrophobic effect, with the optimum ClogP being near 7.3 for all examples. This suggests that, despite the structural similarity of the compounds, different modes of enzyme and/or DNA binding may be involved. 

## Introduction

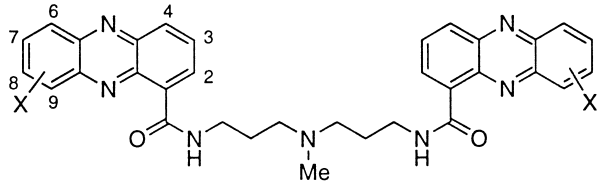
Early studies of structure–activity relationships (SAR) among cytotoxic DNA intercalating agents that act as topoisomerase (topo) inhibitors suggested a positive correlation between cytotoxic potency and the strength of reversible DNA binding.[1–4] This led to the evaluation of various dimeric compounds designed as potential bis-intercalating agents,[5–7] because bis-intercalation can theoretically greatly increase DNA binding. However, while many such compounds did show a bis-intercalative binding mode, their DNA binding strength (and cytotoxicities) generally did not improve as much as expected,[6,7] and the approach was abandoned.

This field was revived recently with the discovery of several new series of dimeric compounds comprised of lipophilic, neutral intercalators (naphthalimides,[8] imidazoacridinones,[9] acridines,[10] and phenazines[11]) joined by cationic linkers. These compounds do generally show significantly enhanced potencies in cell culture over the respective monomers[12] and in many cases broad-spectrum in vivo activity as well.

While many examples of these dimeric analogues have been made and tested in cell culture, no quantitative structure–activity relationship (QSAR) studies have been reported. Recently, Gamage et al.[10] reported SAR for a set of substituted bis[(acridine-4-carboxamides) propyl]methylamines (**I**). These are dimeric analogues of the acridinecarboxamide derivative *N*-[(2-dimethylamino)ethyl]acridine-4-carboxamide (DACA), a lipophilic mono-intercalator which is now in Phase II clinical trials[13] on the basis of its dual inhibition of both topo I and topo II,[14,15] and activity in resistant cell lines[16,17] and in experimental solid tumor models.[13,18] Subsequently Spicer et al.[11] reported SAR on related bis[(phenazine-1-carboxamide)propyl] methylamines (**II**), derived from the previously-reported[19] highly active monomers. These sets of compounds, with a fixed linker chain and variations only in the chromophore substituents, provided good data sets for comparative QSAR analysis, and we report this here.



(**I**)



(**II**)

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## Results and Discussion

Eqs (1)–(3) were developed for the growth inhibitory properties of bis(acridine-4-carboxamides), measured as $IC_{50}$ values (the concentration of drug to reduce cell numbers to 50% of control cultures). These provide an overview of the importance of substituent steric and hydrophobic effects in analogues of **I**.

(i) $IC_{50}$ values (molar) of substituted bis[(acridine-4-carboxamide)propyl]methylamines (**I**) in murine P388 leukemia cells (Table 1)[10]

$$\text{Log1/C} = -0.15(\pm0.14)\text{ClogP} + 4.00(\pm1.03)B5_5 - 1.06(\pm 0.26)(B5_5)^2 + 0.26(\pm0.24)L_6 - 0.23(\pm0.18)B5_7 + 4.69(\pm1.43)$$

$$n = 36 \quad r^2 = 0.800 \quad s = 0.313 \quad q^2 = 0.706 \qquad (1)$$

$B5_0 = 1.88$ (1.78–1.97)

Outliers: 6-$CF_3$, 3-Me, 5-$C_2H_5$, 6-$NMe_2$

(ii) $IC_{50}$ values (molar) of substituted bis[(acridine-4-carboxamide)propyl]methylamines (**I**) in murine Lewis lung carcinoma ($LL_c$) cells (Table 1)[10]

$$\text{Log1/C} = -0.24(\pm0.18)\text{ClogP} + 4.91(\pm1.33)B5_5 - 1.31(\pm0.34)(B5_5)^2 - 0.34(\pm0.22)B5_7 + 6.11(\pm1.64)$$

$$n = 38 \quad r^2 = 0.764 \quad s = 0.426 \quad q^2 = 0.690 \qquad (2)$$

$B5_0 = 1.87$ (1.78–1.97)

Outliers: 3-Cl, 3-Me, 5-$C_2H_5$

(iii) $IC_{50}$ values (molar) of substituted bis[(acridine-4-carboxamide)propyl]methylamines (**I**) in human Jurkat leukemia wild-type ($JL_c$) cells (Table 1)[10]

**Table 1.** Cytotoxicities of compound **I** against P388, $LL_C$ and $JL_C$ cells[10]

| | | | | | | P388 cells Log1/C | | | $LL_C$ cells Log1/C | | | $JL_C$ cells Log1/C | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | X | ClogP | $B5_5$ | $L_6$ | $B5_7$ | Obsd[a] | Calcd eq (1) | Δ | Obsd[a] | Calcd eq (2) | Δ | Obsd[a] | Calcd eq (3) | Δ |
| 1 | H | 6.74 | 1.00 | 2.06 | 1.00 | 6.89 | 6.93 | −0.04 | 7.52 | 7.72 | −0.20 | 6.96 | 7.16 | −0.20 |
| 2 | 1-Me | 7.34 | 1.00 | 2.06 | 1.00 | 6.77 | 6.84 | −0.07 | 7.85 | 7.57 | 0.28 | 7.39 | 7.07 | 0.32 |
| 3 | 1-Cl | 7.47 | 1.00 | 2.06 | 1.00 | 6.40 | 6.82 | −0.42 | 7.38 | 7.54 | −0.16 | 6.90 | 7.05 | −0.16 |
| 4 | 2-Me | 7.74 | 1.00 | 2.06 | 1.00 | 6.66 | 6.78 | −0.12 | 7.17 | 7.48 | −0.30 | 6.59 | 7.01 | −0.42 |
| 5 | 2-Cl | 8.30 | 1.00 | 2.06 | 1.00 | 6.95 | 6.69 | 0.26 | 8.23 | 7.34 | 0.89 | 7.34 | 6.94 | 0.41 |
| 6 | 3-Me | 7.34 | 1.00 | 2.06 | 1.00 | 5.46[b] | 6.84 | −1.38 | 5.62[b] | 7.57 | −1.95 | 5.89[b] | 7.07 | −1.18 |
| 7 | 3-Cl | 7.47 | 1.00 | 2.06 | 1.00 | — | — | — | 5.53[b] | 7.54 | −2.02 | 6.05[b] | 7.05 | −1.01 |
| 8 | 5-Me | 7.74 | 2.04 | 2.06 | 1.00 | 7.64 | 7.57 | 0.06 | 8.75 | 8.45 | 0.30 | 7.96 | 7.87 | 0.09 |
| 9 | 5-$C_2H_5$ | 8.80 | 3.17 | 2.06 | 1.00 | 6.77[b] | 5.68 | 1.09 | 7.57[b] | 6.04 | 1.53 | 6.95[b] | 5.94 | 1.01 |
| 10 | 5-$CHMe_2$ | 9.60 | 3.17 | 2.06 | 1.00 | 5.56 | 5.56 | 0.00 | 5.98 | 5.85 | 0.13 | 5.68 | 5.82 | −0.14 |
| 11 | 5-$C_6H_5$ | 10.52 | 3.11 | 2.06 | 1.00 | 5.91 | 5.58 | 0.33 | 5.97 | 5.82 | 0.14 | 5.60 | 5.86 | −0.26 |
| 12 | 5-OMe | 7.29 | 3.07 | 2.06 | 1.00 | 6.37 | 6.17 | 0.20 | 6.77 | 6.74 | 0.03 | 6.46 | 6.43 | 0.04 |
| 13 | 5-F | 7.07 | 1.35 | 2.06 | 1.00 | 7.80 | 7.40 | 0.40 | 7.46 | 8.28 | −0.82 | 7.31 | 7.66 | −0.35 |
| 14 | 5-Cl | 8.21 | 1.80 | 2.06 | 1.00 | 7.34 | 7.52 | −0.19 | 8.10 | 8.36 | −0.26 | 7.48 | 7.82 | −0.34 |
| 15 | 5-Br | 8.51 | 1.95 | 2.06 | 1.00 | 7.82 | 7.48 | 0.34 | 8.22 | 8.29 | −0.06 | 7.62 | 7.78 | −0.16 |
| 16 | 5-$CF_3$ | 8.64 | 2.61 | 2.06 | 1.00 | 6.62 | 6.90 | −0.28 | 7.41 | 7.56 | −0.15 | 6.94 | 7.20 | −0.26 |
| 17 | 5-$NMe_2$ | 7.90 | 3.08 | 2.06 | 1.00 | 5.67 | 6.05 | −0.38 | 6.17 | 6.56 | −0.38 | 6.75 | 6.31 | 0.43 |
| 18 | 6-Me | 7.74 | 1.00 | 2.87 | 1.00 | 6.46 | 6.98 | −0.52 | 7.25 | 7.48 | −0.22 | 6.75 | 7.01 | −0.26 |
| 19 | 6-OMe | 7.29 | 1.00 | 3.98 | 1.00 | 7.12 | 7.34 | −0.22 | 7.70 | 7.59 | 0.11 | 7.29 | 7.08 | 0.22 |
| 20 | 6-F | 7.07 | 1.00 | 2.65 | 1.00 | 7.62 | 7.03 | 0.59 | 7.70 | 7.64 | 0.06 | 7.28 | 7.11 | 0.18 |
| 21 | 6-Cl | 8.21 | 1.00 | 3.52 | 1.00 | 7.18 | 7.08 | 0.10 | 7.89 | 7.36 | 0.53 | 7.29 | 6.94 | 0.35 |
| 22 | 6-Br | 8.51 | 1.00 | 3.82 | 1.00 | 7.31 | 7.11 | 0.20 | 7.75 | 7.29 | 0.46 | 7.24 | 6.90 | 0.34 |
| 23 | 6-$CF_3$ | 8.64 | 1.00 | 3.30 | 1.00 | 5.02[b] | 6.96 | −1.94 | 6.80 | 7.25 | −0.46 | 6.45 | 6.88 | −0.43 |
| 24 | 6-$NMe_2$ | 7.90 | 1.00 | 3.53 | 1.00 | 6.12[b] | 7.13 | −1.01 | 6.50 | 7.44 | −0.94 | 6.65 | 6.99 | −0.34 |
| 25 | 7-Me | 7.74 | 1.00 | 2.06 | 2.04 | 6.57 | 6.54 | 0.03 | 7.14 | 7.12 | 0.02 | 6.56 | 6.59 | −0.03 |
| 26 | 7-$C_2H_5$ | 8.80 | 1.00 | 2.06 | 3.17 | 5.97 | 6.12 | −0.16 | 6.12 | 6.48 | −0.36 | 5.79 | 5.99 | −0.20 |
| 27 | 7-$CHMe_2$ | 9.59 | 1.00 | 2.06 | 3.17 | 5.84 | 6.00 | −0.17 | 5.68 | 6.28 | −0.60 | 5.68 | 5.87 | −0.19 |
| 28 | 7-$CMe_3$ | 10.39 | 1.00 | 2.06 | 3.17 | 5.75 | 5.89 | −0.13 | 5.85 | 6.08 | −0.23 | 5.97 | 5.76 | 0.21 |
| 29 | 7-$C_6H_5$ | 10.52 | 1.00 | 2.06 | 3.11 | 6.15 | 5.88 | 0.27 | 6.22 | 6.07 | 0.14 | 5.99 | 5.76 | 0.23 |
| 30 | 7-OMe | 7.29 | 1.00 | 2.06 | 3.07 | 6.88 | 6.37 | 0.51 | 7.70 | 6.88 | 0.82 | 6.83[b] | 6.24 | 0.59 |
| 31 | 7-F | 7.07 | 1.00 | 2.06 | 1.35 | 6.85 | 6.79 | 0.06 | 7.50 | 7.52 | −0.02 | 7.02 | 6.97 | 0.05 |
| 32 | 7-Cl | 8.21 | 1.00 | 2.06 | 1.80 | 6.77 | 6.52 | 0.25 | 7.34 | 7.09 | 0.25 | 6.87 | 6.62 | 0.25 |
| 33 | 7-Br | 8.51 | 1.00 | 2.06 | 1.95 | 6.65 | 6.44 | 0.20 | 7.46 | 6.96 | 0.50 | 6.65 | 6.52 | 0.13 |
| 34 | 7-$NMe_2$ | 7.90 | 1.00 | 2.06 | 3.08 | 6.01 | 6.28 | −0.27 | 6.74 | 6.72 | 0.02 | 6.12 | 6.15 | −0.03 |
| 35 | 8-Me | 7.74 | 1.00 | 2.06 | 1.00 | 6.75 | 6.78 | −0.03 | 7.80 | 7.48 | 0.32 | 7.15 | 7.01 | 0.14 |
| 36 | 8-Cl | 8.21 | 1.00 | 2.06 | 1.00 | 6.13 | 6.71 | −0.58 | 6.91 | 7.36 | −0.45 | 6.62 | 6.94 | −0.32 |
| 37 | 5,7-di-Me | 8.74 | 2.04 | 2.06 | 2.04 | 6.68 | 7.19 | −0.51 | 7.70 | 7.85 | −0.15 | 6.98 | 7.31 | −0.33 |
| 38 | 5,8-di-Me | 8.74 | 2.04 | 2.06 | 1.00 | 7.62 | 7.43 | 0.19 | 8.50 | 8.20 | 0.29 | 7.96 | 7.73 | 0.23 |
| 39 | 1,5-di-Me | 8.34 | 2.04 | 2.06 | 1.00 | 7.68 | 7.49 | 0.19 | 8.85 | 8.30 | 0.55 | 8.41 | 7.78 | 0.63 |
| 40 | 5-Me, 8-Cl | 9.21 | 2.04 | 2.06 | 1.00 | 7.39 | 7.35 | 0.03 | 8.06 | 8.08 | −0.03 | 7.70 | 7.66 | 0.04 |
| 41 | 1-Cl, 5-Me | 8.47 | 2.04 | 2.06 | 1.00 | 7.32 | 7.47 | −0.15 | 8.22 | 8.27 | −0.05 | 7.92 | 7.77 | 0.16 |

[a]Data taken from ref 10.
[b]Data points not included in deriving the respective eqs.

$$\text{Log1/C} = -0.14(\pm 0.14)\text{ClogP} + 4.19(\pm 0.92)\text{B5}_5 - 1.11(\pm 0.23)(\text{B5}_5)^2 - 0.40(\pm 0.17)\text{B5}_7 + 5.44(\pm 1.16)$$

$$n = 37 \quad r^2 = 0.842 \quad s = 0.293 \quad q^2 = 0.789 \qquad (3)$$

$\text{B5}_0 = 1.89$ (1.82–1.97)

Outliers: 3-Cl, 3-Me, 5-$C_2H_5$, 7-OMe

In the above equations, C is the concentration of drug (mol/litre) required to reduce cell number to 50% of control cultures in leukemia cells, $n$ is the number of data points, $r^2$ is the square of correlation coefficient, $q^2$ is the measure of quality of fit and $s$ is the standard deviation. ClogP is calculated octanol/water partition coefficient,[20] and B5 and L are Verloop's sterimol parameters. B5 defines the maximum width of substituent and L is the length of the substituent moiety. Es is Taft's classical steric parameter. All these parameters have been discussed previously, along with their applications.[21] The parameters were auto-loaded from our CQSAR database and the QSAR analysis was executed with the C-QSAR program. The parameters used in deriving QSARs were selected using permutation in CQSAR program, it automatically picks the best parameter to use. This program includes all the commonly used substituent parameters,[22] and its utility in correlation analysis has been discussed previously.[23,24]

Eqs (1)–(3), for activities of the compounds in three different cell lines are reasonably similar overall; this reflects the original observation[10] that the log1/C in the different cell lines were highly collinear. Looking at individual parameters, a common feature of the equations is that there is no positive role for hydrophobic properties (and in fact the negative ClogP term has marginal significance). This is not uncommon; we have often noted a lack of positive hydrophobic interaction of ligands interacting with DNA.[25,26] All three equations contain a steric parameter that is initially positive but then turns negative at a B5 value of about 1.9 for substituents in the 5-position. Gamage et al.[10] also noted that small 5-position substituents increase potency. They correlated derivatives with 5-substituents with MR and suggested that larger groups at this position decreased potency. However, we are rather surprised at the uniform optimum size ($\text{B5}_0$) for substituents in this location. Groups in the 7-position all show small negative steric effects, as also suggested in the original study. Eq (1) is slightly different from the other two in that a negative steric term of marginal importance for 6-substituents is also present (note confidence limits). There are four unexplained outliers associated with QSAR 1, but steric effects are the most difficult to parameterise and B5 can only be expected to give an approximate answer. Although $r^2$ is not high, the overall agreement among the equations is good.

The synthesis, evaluation and limited SAR study of related bis[(phenazine-1-carboxamide)propyl] methylamines (**II**) has recently been reported.[11] Because these compounds differ from those of class I by only by an additional ring nitrogen (the ring numbering is different), and were evaluated in the same biological screens, it was of interest to carry out comparative QSAR on these, and Eqs (4)–(6) were derived.

(iv) $IC_{50}$ values (molar) of substituted bis[(phenazine-1-carboxamide) propyl]methyl-amine (**II**) in Murine P388 leukemia cells (Table 2)[11]

$$\text{Log1/C} = 6.26(\pm 5.11)\text{ClogP} - 0.42(\pm 0.37)(\text{ClogP})^2 + 0.51(\pm 0.35)\text{L}_8 - 1.25(\pm 0.47)\text{Es}_9 - 17.85\ (\pm 17.73)$$

$$n = 17 \quad r^2 = 0.838 \quad s = 0.356 \quad q^2 = 0.762 \qquad (4)$$

$\text{logP}_0 = 7.37$ (6.94–10.25)

**Table 2.** Cytotoxicities of compound **II** against P388, $LL_C$ and $JL_C$ cells[11]

| | | | | | P388 cells Log1/C | | | $LL_c$ cells Log1/C | | | $JL_c$ cells Log1/C | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | X | ClogP | $L_8$ | $Es_9$ | Obsd[a] | Calcd eq (4) | Δ | Obsd[a] | Calcd eq (5) | Δ | Obsd[a] | Calcd eq (6) | Δ |
| 1 | H | 5.87 | 2.06 | 0.00 | 6.28[b] | 5.30 | 0.99 | 6.97[b] | 5.76 | 1.22 | 6.76[b] | 5.90 | 0.86 |
| 2 | 2-Cl | 5.68 | 2.06 | 0.00 | 4.92 | 5.04 | −0.12 | 5.08 | 5.28 | −0.20 | 5.62 | 5.65 | −0.03 |
| 3 | 3-Me | 6.87 | 2.06 | 0.00 | 6.33 | 6.15 | 0.18 | 7.32 | 7.27 | 0.05 | 6.68 | 6.70 | −0.02 |
| 4 | 3-Cl | 7.34 | 2.06 | 0.00 | 6.69 | 6.25 | 0.44 | 7.92 | 7.43 | 0.49 | 7.13 | 6.81 | 0.33 |
| 5 | 4-Me | 6.87 | 2.06 | 0.00 | 6.43 | 6.15 | 0.28 | 7.59 | 7.27 | 0.32 | 6.99 | 6.70 | 0.29 |
| 6 | 6-Me | 6.87 | 2.06 | 0.00 | 6.23 | 6.15 | 0.09 | 7.34 | 7.27 | 0.07 | 6.76 | 6.70 | 0.06 |
| 7 | 6-Cl | 7.31 | 2.06 | 0.00 | 5.11[b] | 6.25 | −1.14 | 6.82 | 7.43 | −0.61 | 6.68 | 6.81 | −0.13 |
| 8 | 7-Me | 6.87 | 2.06 | 0.00 | 6.21 | 6.15 | 0.06 | 7.14 | 7.27 | −0.12 | 6.55 | 6.70 | −0.15 |
| 9 | 7-Cl | 7.31 | 2.06 | 0.00 | 5.53 | 6.25 | −0.72 | 7.08 | 7.43 | −0.35 | 6.60 | 6.81 | −0.20 |
| 10 | 7-OMe | 6.60 | 2.06 | 0.00 | 6.22 | 6.00 | 0.22 | 7.19 | 7.01 | 0.19 | 6.71 | 6.56 | 0.15 |
| 11 | 8-Me | 6.87 | 2.87 | 0.00 | 6.16 | 6.56 | −0.40 | 6.97 | 7.27 | −0.30 | 6.83 | 7.05 | −0.22 |
| 12 | 8-Cl | 7.31 | 3.52 | 0.00 | 7.01 | 6.99 | 0.02 | 7.36 | 7.43 | −0.07 | 7.38 | 7.43 | −0.06 |
| 13 | 8-OMe | 6.60 | 3.98 | 0.00 | 7.13 | 6.97 | 0.15 | 7.62 | 7.01 | 0.61 | 7.52 | 7.39 | 0.14 |
| 14 | 9-Me | 6.87 | 2.06 | −1.24 | 7.82 | 7.69 | 0.13 | 8.80 | 8.61 | 0.19 | 8.24 | 8.04 | 0.21 |
| 15 | 9-Cl | 6.87 | 2.06 | −0.97 | 7.41 | 7.36 | 0.05 | 8.06 | 8.31 | −0.26 | 7.85 | 7.75 | 0.11 |
| 16 | 9-F | 6.17 | 2.06 | −0.55 | 6.53 | 6.33 | 0.20 | 6.92 | 6.98 | −0.06 | 6.75 | 6.82 | −0.07 |
| 17 | 9-OMe | 6.60 | 2.06 | −0.55 | 6.09 | 6.68 | −0.60 | 6.80[b] | 7.61 | −0.81 | 6.91 | 7.15 | −0.25 |
| 18 | 6-9-di-Me | 7.87 | 2.06 | −1.24 | 7.59 | 7.70 | −0.11 | 8.28 | 8.54 | −0.25 | 7.68 | 8.05 | −0.37 |
| 19 | 6-Cl, 9-Me | 8.31 | 2.06 | −1.24 | 7.54 | 7.43 | 0.11 | 8.28 | 7.99 | 0.29 | 8.01 | 7.80 | 0.21 |

[a]Data taken from ref 11.
[b]Data points not included in deriving the respective eqs.

Outliers: H, 6-Cl

(v) $IC_{50}$ values (molar) of substituted bis[(phenazine-1-carboxamide)propyl]methyl-amine (**II**) in murine lewis lung carcinoma ($LL_c$) cells (Table 2)[11]

$$\text{Log1/C} = 11.62(\pm 5.01)\text{ClogP} - 0.79(\pm 0.37)(\text{ClogP})^2 - 1.08(\pm 0.45)\text{Es}_9 - 35.12(\pm 17.71)$$

$$n = 17 \quad r^2 = 0.845 \quad s = 0.357 \quad q^2 = 0.617 \qquad (5)$$

$\text{logP}_0 = 7.32$ (7.09–7.77)

Outliers: H, 9-OMe

(vi) $IC_{50}$ values (molar) of substituted bis[(phenazine-1-carboxamide)propyl]methyl-amine (**II**) in human jurkat leukemia wild-type ($JL_c$) cells (Table 2)[11]

$$\text{Log1/C} = 5.86\ (\pm 3.21)\text{ClogP} - 0.40(\pm 0.23)(\text{ClogP})^2 + 0.43(\pm 0.22)\ L_8 - 1.08(\pm 0.29)\text{Es}_9 - 15.69(\pm 11.16)$$

$$n = 18 \quad r^2 = 0.903 \quad s = 0.226 \quad q^2 = 0.837 \qquad (6)$$

$\text{logP}_0 = 7.38$ (7.09–8.11)

Outlier: H

These equations show that the additional nitrogen atom in the ring system produces very significant changes. As for the bis(acridine-4-carboxamides), the three equations for the different cell lines are broadly similar, and similar parameters occur (ClogP, $Es_5$ rather than $B5_5$ and $L_6$). However, ClogP now becomes the parameter of overwhelming importance, with close agreement in the three examples on an optimum value ($\text{ClogP}_0 = 7.3$–$7.4$). The different apparent steric effects of the phenazine 9-substituents (equivalent to acridine 5-substituents) is most likely due to a smaller dataset in the phenazine case (no large substituents were studied). However, the striking dependence of log1/C on hydrophobicity in the QSAR between these structurally similar series is impressive.

It cannot be due to direct steric difference between ring CH or N. The difference in both calculated (ClogP) and measured (MlogP) overall hydrophobicity between the two parent molecules is also small. For **I** (X = H), MlogP = 3.40, ClogP = 3.41; for **II** (X = H), MlogP = 2.84, ClogP = 2.89. However, although we have not been able to uncover any substituent electronic effects from the QSAR studies, the electronic effects of the additional N would be significant, in two ways. Firstly it would increase charge transfer interactions between the chromophore and the receptor (likely DNA). Secondly, it markedly affects the $pK_a$ of the nitrogen adjacent to the carboxamide. For the monomeric compounds, the conjugate acid of unsubstituted acridine-4-carboxamide has a measured $pK_a$ of 3.54, while that of the phenazine-1-carboxamide is only 0.8.[28] While it is unlikely that either chromophore will be ionized in bulk solution at physiological pH, it is possible that the acridine (but not the phenazine) chromophore could become charged if bound in the DNA microenvironment. Whether the adjacent nitrogen is charged or not has a major effect on the conformation of the carboxamide, since it can adopt two different H-bond configurations; carboxamide O H-bonded to ring $NH^+$,[29] or carboxamide NH H-bonded to ring N.[29,30]

Overall, these QSAR studies indicate quite different dependencies of cytotoxicity on structure between the acridine and phenazine series. While limited previous studies[31] of other "aza-acridinecarboxamides" have suggested these are less interesting than phenazines, further work in this area would be interesting in terms of comparative QSAR studies.

The problem of outliers is a difficult one. For the acridines of set I the 3-Me is consistently misfit and the 3-Cl is out of line in two of the three QSAR. This could be due to a perturbation of the *ortho* amide moiety. The 5-ethyl group is also misfit in all three QSAR. Possibly this is not well accounted for by the $B5_5$ term that is far from a perfect steric parameter, but it is the best that we have. In the phenazines of set II the parent compound is an outlier in every instance. It is always more active than predicted. The steric parameter takes care of the large substituents, but not H. It will never be possible to design a set of steric parameters that will be anywhere near perfection for ligand–receptor interactions. Even knowing the geometry of the active site we are not yet able to predict how flexible it will be in accepting a ligand. Allosteric problems are even more difficult to deduce.

The formulation of a new QSAR is always interesting, however, until one establishes lateral relationships with other QSAR the result must be accepted with caution. We have been attempting to illustrate the importance of such an approach.[24–27] In the present study although $r^2$ is not particularly high, except for QSAR 6, the agreement between the three QSAR for each set is excellent. That is for QSAR 1–3 the hydrophobic terms are at best marginal. The steric effect of 5-substituents dominates the QSAR. The optimum steric effect ($B5_0$) for the three equations ranges from 1.87 to 1.89 for the three different types of cells. For QSAR 4–6 the optimal logP ($\text{logP}_0$) ranges from 7.32 to 7.38! Having this kind of agreement from 3 different test systems speaks well for the quality of the experimetal work and leaves no doubt about two different types of receptor interactions.

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