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# QSAR Study on Some Pyridoacridine Ascididemin Analogues as Anti-tumor Agents

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**Abstract**—Pyridoacridine ascididemin analogues have been reported as anticancer agents for their interesting antitumor activity against human cancer cells. A quantitative structure–activity relationship (QSAR) analysis of ascididemin analogues was attempted using the physicochemical parameters and the electrotopological state atom (ETSA) indices. This study indicates that the electron withdrawing substituents with higher MR (molar refractivity) value at $R_1$ position favor the anti-tumor activity and the presence of NHR (R is hydrogen or alkyl group) at the $R_3$ position has contribution to the anti-tumor activity. ETSA indices have been incorporated as independent variable in the QSAR model with physicochemical parameters. It clearly suggests the importance of atoms 2, 3, 4, 5, 6 and 7 to the anti-tumor activity.
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## Introduction

Ascididemin is a unique polycyclic aromatic marine alkaloid drawing attention due to its significant anti-tumor activity. Ascididemin having pyrido[2,3,4-κl]-acridine skeleton is isolated from the ascidian *Didemnum* sp. by Kobayashi et al.[1] This is a conventional topoisomerase-II poison that is responsible for DNA cleavage. Therefore the synthesis or isolation of various analogues of ascididemin has been performed to allow their biological evaluation as potential anti-cancer agents.

Ascididemin has not a sole mode of action.[2] Relaxation assays of ascididemin have been performed using super coiled DNA, showed that it can stimulate double stranded cleavage of DNA by topoisomerase-II, but exerts only a very weak effect on topoisomerase-I[3] and it is also reported that it has a little effect on the catalytic activities of both the topoisomerase-I and -II.[4] Two structural features are important in respect to the mechanism of action of this type of compounds—a double N-bay region and iminoquinone heterocyclic ring. These structural features hypothesized two possible mechanism of action: (1) generation of reactive oxygen species facilitated by metal binding to the common phenanthroline bay region, and (2) production of reactive oxygen species by direct reduction of the iminoquinone moiety.[5] This alkaloid was found to strongly induce apoptosis in HL-60 and P388 leukemia cells probably by inducing a mediator of the apoptosis pathway.

In an attempt to identify the physicochemical and structural features required or responsible for anticancer activity and also to establish the pharmacophoric requirements of some pyridoacridine ascididemin derivatives reported by Delfourne et al.,[6] QSAR studies were undertaken using physicochemical parameters of substituents and electro topological state atom index (ETSA) of some common atoms as a part of our program of rational drug design.[7] Anticancer activity data of these compounds, general structure of which is shown in Figure 1 are listed in Table 1. $IC_{50}$ refers to the nano-molar concentration of the compounds required for 50% inhibition of the growth of human cancer cells. $IC_{50}$ values are transformed to $pIC_{50}$ (negative logarithm of $IC_{50}$) to get the linear relationship in the equation.

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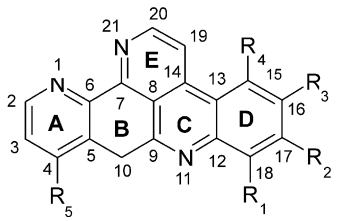


**Figure 1.** General structure of ascididemin analogues.

**Table 1.** Anti-tumor activity data of ascididemin analogues

| Cpd. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $IC_{50}$ (nM) | $pIC_{50}$ |
|---|---|---|---|---|---|---|---|
| **1** | H | H | H | H | H | 100 | −2.000 |
| **2** | H | Br | H | H | H | 120 | −2.079 |
| **3** | H | H | H | H | OH | 3200 | −3.505 |
| **4** | H | H | H | H | $OCH_3$ | 480 | −2.681 |
| **5** | $NO_2$ | H | H | H | H | 10 | −1.000 |
| **6** | $NH_2$ | H | H | H | H | 53 | −1.724 |
| **7** | H | H | Br | H | H | 80 | −1.903 |
| **8** | H | H | $NH_2$ | H | H | 21 | −1.322 |
| **9** | H | H | $NHCH_2CH_2Cl$ | H | H | 7 | −0.845 |
| **10** | H | H | $N(CH_2CH_2Cl)_2$ | H | H | 100 | −2.000 |
| **11** | H | H | $NHCH_2CH_2N(CH_3)_2$ | H | H | 60 | −1.778 |
| **12** | H | H | Cl | H | H | 270 | −2.431 |
| **13** | H | H | $CH_3$ | H | H | 60 | −1.778 |
| **14** | H | H | $OCH_3$ | H | H | 90 | −1.954 |
| **15** | H | H | $N(CH_3)_2$ | H | H | 37 | −1.568 |
| **16** | H | H | $NHCH_2Ph$ | H | H | 140 | −2.146 |
| **17** | H | H | $NH_2$ | Br | H | 140 | −2.146 |

## Results and Discussion

Multiple Linear Regression (MLR) analysis using physicochemical parameters and indicator variables showed the field effect of the substituents at $R_1$ position may be important for the activity as shown in eq 1:

$$\begin{aligned} pIC_{50} = &-1.9800(\pm0.1186) + 1.4780(\pm0.5745)\sigma_I R_1 \quad (1)\\ &- 1.1130(\pm0.2855)I_1 + 0.4573\\ &\times(\pm0.2186)I_2\\ n = 17;&\quad R = 0.8428;\quad \%EV = 71.03;\\ R^2_A = 0.6434;&\quad F_{(3,13)} = 10.623;\\ p < 0.0008;&\quad SEE = 0.3673\\ PRESS = 3.6956;&\quad SSY = 6.0536;\\ R^2_{CV} = 0.3895;&\quad S_{PRESS} = 0.5332;\\ P.S.E. = 0.4663.& \end{aligned}$$

where $n$ is the number of data points, $R$ is correlation coefficient. %EV, $R^2_A$, $F$, $p$, S.E.E. PRESS, SSY, $R^2_{CV}$, $S_{PRESS}$, P.S.E. are percentage of explained variance, adjusted $R^2$, ratio between the variances of observed and calculated activities, probability factor related to $F$-ratio, standard error of estimate, predicted residual sum of squares, total sum of squares, cross validated $R^2$, standard error of PRESS and uncertainty factor respectively.[9] The value within the parentheses is confidence intervals of corresponding parameters. $\sigma_I R_1$ is the field effect at $R_1$ position and it is quite clear that electron withdrawing substituents at $R_1$ position increases activity as evidenced by its positive regression coefficients. $I_1$ represents the presence of substitution at $R_5$ position. Negative coefficient of $I_1$ indicates that substitution at $R_5$ position may be detrimental to the activity. $I_2$ represents the presence of NHR (R, H or alkyl group) at $R_3$ position and presence of NHR at $R_3$ position may be advantageous to the activity as indicated by its positive coefficients. Eq 1 explains up to 71.03% of the variances in the activity data.

If the molar refractivity of the substituents at $R_1$ position ($MRR_1$) is taken as a variable instead of $\sigma_I R_1$ as they are highly auto-correlated, the resultant equation (eq 2) is also found good as indicated by its importance in explaining the variation of activity. The resultant equation explains up to 71.06% of the variation of activity.

$$\begin{aligned} pIC_{50} = &-2.1019(\pm0.1423) + 1.1269 \quad (2)\\ &\times(\pm0.4372)MRR_1 - 1.1072(\pm0.2856)I_1\\ &+ 0.4631(\pm0.2189)I_2\\ n = 17;&\quad R = 0.8430;\quad \%EV = 71.06;\\ R^2_A = 0.6439;&\quad F_{(3,13)} = 10.642;\\ p < 0.0008;&\quad S.E.E. = 0.3671;\\ PRESS = 3.6319;&\quad SSY = 6.0536;\\ R^2_{CV} = 0.4000;&\quad S_{PRESS} = 0.5286;\quad P.S.E = 0.4623. \end{aligned}$$

The statistical qualities of both equations (eqs 1 and 2) are almost same as suggested by the statistical parameters shown below the equations.

After deletion of outlier which may be acting through different mechanism of action, from eqs 1 and 2 yielded eqs 3 and 4, respectively, where the value of correlation coefficient ($R$) increases.

$$\begin{aligned} pIC_{50} = &-1.9800(\pm0.1036) + 1.4780(\pm0.5019)\sigma_I R_1 \quad (3)\\ &- 1.1130(\pm0.2495)I_1 + 0.6650(\pm0.2123)I_2\\ n = 16;&\quad DC = \mathbf{17};\quad R = 0.8912;\\ \%EV = 79.42;&\quad R^2_A = 0.7427;\quad F_{(3,12)} = 15.434;\\ p < 0.0002;&\quad S.E.E. = 0.3209;\\ PRESS = 2.9804;&\quad SSY = 6.0053;\\ R^2_{CV} = 0.5037;&\ S_{PRESS} = 0.4984;\quad P.S.E = 0.4301. \end{aligned}$$

$$\begin{aligned} pIC_{50} = &-2.1019(\pm0.1243) + 1.1269 \quad (4)\\ &\times(\pm0.3819)MRR1 - 1.1072(\pm0.2495)I_1\\ &+ 0.6708(\pm0.2124)I_2\\ n = 16;&\quad DC = \mathbf{17};\quad R = 0.8914;\\ \%EV = 79.46;&\quad R^2_A = 0.7432;\quad F_{(3,12)} = 15.470;\\ p < 0.0002;&\quad S.E.E. = 0.3206;\\ PRESS = 2.9167;&\quad SSY = 6.0053;\\ R^2_{CV} = 0.5143;&\quad S_{PRESS} = 0.4930;\quad P.S.E = 0.4270. \end{aligned}$$

where DC is deleted compound behaves as outlier.

Statistical qualities of eqs 3 and 4 increase as $R$, %EV, $R^2_A$, $F$ values increase and $p$, S.E.E., $S_{PRESS}$, P.S.E. values decrease. Both equations explain more than 79% of the variances. $F$-ratios of these two equations increase by around 5 units than eqs 1 and 2. Predictive powers ($R^2_{CV}$) of both equations (eqs 3 and 4) also increase as comparison to eqs 1 and 2.

Attempt was made to investigate whether the electro topological parameters could be incorporated in the Hansch equation as an independent parameter or not. From the correlation analysis, it is found that ETSA indices like $S_2$, $S_3$, $S_4$, $S_5$, $S_6$, and $S_7$ can be used separately along with either $\sigma_I R_1$ or $MRR_1$ and the indicator parameter $I_2$, where $S_2$, $S_3$, $S_4$, $S_5$, $S_6$, and $S_7$ are the ETSA indices of atom nos 2, 3, 4, 5, 6, 7 respectively. The values of these ETSA indices and $\sigma_I R_1$, $MRR_1$, $I_1$, $I_2$ are shown in Table 2 and the correlation matrix is presented in the Table 3. Incorporation of E-state indices ($S_2$–$S_7$) individually increases the correlation coefficient ($R$) and the resulted equations were of good statistical quality.

$$\begin{aligned} pIC_{50} = &-15.5341(\pm2.5065) + 2.3465 \\ &\times (\pm0.4522)\sigma_I R_1 + 7.4296(\pm1.3929)S2 \\ &+ 0.7046(\pm0.1868)I_2 \\ n = 16;&\ \ DC = \mathbf{17};\ \ R = 0.9152; \\ \%EV = 83.77;&\ \ R^2_A = 0.7971;\ \ F_{(3,12)} = 20.6400; \\ p < 0.0000;&\ \ S.E.E. = 0.2850; \\ PRESS = 2.4768;&\ \ SSY = 6.0053; \\ R^2_{CV} = 0.5876;&\ \ S_{PRESS} = 0.4543;\ \ P.S.E = 0.3934. \end{aligned} \quad (5)$$

$$\begin{aligned} pIC_{50} = &-10.5877(\pm1.6008) + 2.1190 \\ &\times (\pm0.4488)\sigma_I R_1 + 4.3721(\pm0.8305)S3 \\ &+ 0.6989(\pm0.1887)I_2 \\ n = 16;&\ \ DC = \mathbf{17};\ \ R = 0.9136; \\ \%EV = 83.47;&\ \ R^2_A = 0.7933;\ \ F_{(3,12)} = 20.1910; \\ p < 0.0000;&\ \ S.E.E. = 0.2877; \\ PRESS = 3.9849;&\ \ SSY = 6.0053; \\ R^2_{CV} = 0.3364;&\ \ S_{PRESS} = 0.5763;\ \ P.S.E = 0.4991. \end{aligned} \quad (6)$$

$$\begin{aligned} pIC_{50} = &-3.5298(\pm0.2670) + 1.5909(\pm0.4416)\sigma_I R_1 \\ &+ 0.7453(\pm0.1392)S4 + 0.6587(\pm0.1878)I_2 \\ n = 16;&\ \ DC = \mathbf{17};\ \ R = 0.9157; \\ \%EV = 83.85;&\ \ R^2_A = 0.7981;\ \ F_{(3,12)} = 20.7670; \\ p < 0.0000;&\ \ S.E.E. = 0.2843; \\ PRESS = 1.9657;&\ \ SSY = 6.0053; \\ R^2_{CV} = 0.6727;&\ \ S_{PRESS} = 0.4047;\ \ P.S.E = 0.3506. \end{aligned} \quad (7)$$

$$\begin{aligned} pIC_{50} = &-6.6875(\pm0.7981) + 2.4150(\pm0.4343)\sigma_I R_1 \\ &+ 4.0688(\pm0.7155)S5 + 0.6974(\pm0.1786)I_2 \\ n = 16;&\ \ DC = \mathbf{17};\ \ R = 0.9230; \\ \%EV = 85.19;&\ \ R^2_A = 0.8149;\ \ F_{(3,12)} = 23.0070; \\ p < 0.0000;&\ \ S.E.E. = 0.2722; \\ PRESS = 1.9845;&\ \ SSY = 6.0053; \\ R^2_{CV} = 0.6695;&\ \ S_{PRESS} = 0.4067;\ \ P.S.E = 0.3521. \end{aligned} \quad (8)$$

$$\begin{aligned} pIC_{50} = &-8.7980(\pm1.1629) + 2.7046(\pm0.4480)\sigma_I R_1 \\ &+ 7.0352(\pm1.2318)S6 + 0.7292(\pm0.1772)I_2 \\ n = 16;&\ \ DC = \mathbf{17};\ \ R = 0.9235; \\ \%EV = 85.28;&\ \ R^2_A = 0.8160;\ \ F_{(3,12)} = 23.1790; \\ p < 0.0000;&\ \ S.E.E. = 0.2714; \\ PRESS = 1.7176;&\ \ SSY = 6.0053; \\ R^2_{CV} = 0.7140;&\ \ S_{PRESS} = 0.3783;\ \ P.S.E = 0.3276. \end{aligned} \quad (9)$$

$$\begin{aligned} pIC_{50} = &-12.0476(\pm1.8711) + 3.8499 \\ &\times (\pm0.5883)\sigma_I R_1 + 10.6856(\pm2.0225)S7 \\ &+ 0.7879(\pm0.1863)I_2 \\ n = 16;&\ \ DC = \mathbf{17};\ \ R = 0.9140; \\ \%EV = 83.55;&\ \ R^2_A = 0.7944;\ \ F_{(3,12)} = 20.3130; \\ p < 0.0000;&\ \ S.E.E. = 0.2869; \\ PRESS = 1.9764;&\ \ SSY = 6.0053; \\ R^2_{CV} = 0.6709;&\ \ S_{PRESS} = 0.4058;\ \ P.S.E = 0.3514. \end{aligned} \quad (10)$$

Improvement of statistics in eqs 5–10 proves the importance of topological parameters along with physicochemical parameters. Eqs 5–10 explain more than 83% of the variances in the activity data. Cross-validated $R^2$ values of these equations increase to significant level. $F$-ratios also increase by 5–8 units in comparison to eqs 3 and 4, which in turn increase the statistical confidence.

Analysis of the eqs 5–10 reveals that ETSA indices like $S_2$, $S_3$, $S_4$, $S_5$, $S_6$, and $S_7$ have almost equal important contribution to the activity as the equations have similar statistical significance. From the autocorrelation study of E-state indices of common atoms it is revealed that E-state indices of atom nos 2, 3, 4, 5, 6, 7 are highly auto-correlated and cannot be used in a single equation. Hence an average of these ($S_{av}$) is considered as single best variable. As the ETSA indices are derived from the effects of electro negativity and topology of neighboring atoms, the value of $S_2$, $S_3$, $S_4$, $S_5$, $S_6$, and $S_7$ are interdependent at these positions (2, 3, 4, 5, 6 and 7).

**Table 2.** Physicochemical parameters and ETSA values of ascididemin analogues

| Compd | $\sigma_I R_1$ | $MRR_1$ | $I_1$ | $I_2$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | $S_6$ | $S_7$ | $S_{av}$[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 0.000 | 0.103 | 0.000 | 0.000 | 1.824 | 1.965 | 2.093 | 1.166 | 0.981 | 0.957 | 1.498 |
| **2** | 0.000 | 0.103 | 0.000 | 0.000 | 1.821 | 1.962 | 2.089 | 1.156 | 0.973 | 0.946 | 1.491 |
| **3** | 0.000 | 0.103 | 1.000 | 0.000 | 1.611 | 1.603 | 0.242 | 0.767 | 0.747 | 0.804 | 0.962 |
| **4** | 0.000 | 0.103 | 1.000 | 0.000 | 1.767 | 1.881 | 0.830 | 1.025 | 0.890 | 0.895 | 1.215 |
| **5** | 0.670 | 0.973 | 0.000 | 0.000 | 1.746 | 1.871 | 1.976 | 1.002 | 0.851 | 0.792 | 1.373 |
| **6** | 0.140 | 0.352 | 0.000 | 0.000 | 1.808 | 1.946 | 2.068 | 1.128 | 0.951 | 0.918 | 1.470 |
| **7** | 0.000 | 0.103 | 0.000 | 0.000 | 1.821 | 1.963 | 2.089 | 1.158 | 0.971 | 0.944 | 1.491 |
| **8** | 0.000 | 0.103 | 0.000 | 1.000 | 1.808 | 1.952 | 2.077 | 1.142 | 0.951 | 0.918 | 1.475 |
| **9** | 0.000 | 0.103 | 0.000 | 1.000 | 1.818 | 1.960 | 2.087 | 1.147 | 0.958 | 0.929 | 1.483 |
| **10** | 0.000 | 0.103 | 0.000 | 0.000 | 1.818 | 1.960 | 2.087 | 1.139 | 0.950 | 0.920 | 1.479 |
| **11** | 0.000 | 0.103 | 0.000 | 1.000 | 1.831 | 1.971 | 2.100 | 1.158 | 0.971 | 0.945 | 1.496 |
| **12** | 0.000 | 0.103 | 0.000 | 0.000 | 1.807 | 1.951 | 2.076 | 1.141 | 0.950 | 0.916 | 1.474 |
| **13** | 0.000 | 0.103 | 0.000 | 0.000 | 1.828 | 1.969 | 2.097 | 1.167 | 0.983 | 0.959 | 1.501 |
| **14** | 0.000 | 0.103 | 0.000 | 0.000 | 1.813 | 1.956 | 2.082 | 1.145 | 0.955 | 0.923 | 1.479 |
| **15** | 0.000 | 0.103 | 0.000 | 0.000 | 1.828 | 1.969 | 2.097 | 1.161 | 0.974 | 0.949 | 1.496 |
| **16** | 0.000 | 0.103 | 0.000 | 0.000 | 1.830 | 1.970 | 2.098 | 1.152 | 0.965 | 0.937 | 1.492 |
| **17** | 0.000 | 0.103 | 0.000 | 1.000 | 1.804 | 1.949 | 2.073 | 1.132 | 0.938 | 0.901 | 1.466 |

[a]$S_{av}$ is the average value of $S_2$, $S_3$, $S_4$, $S_5$, $S_6$ and $S_7$.

**Table 3.** Correlation matrix of the physicochemical parameters, ETSA indices and biological activity

| | $\sigma_I R_1$ | $MRR_1$ | $I_1$ | $I_2$ | $S_2$ | $S_3$ | $S_4$ | $S_5$ | $S_6$ | $S_7$ | $S_{av}$ | $pIC_{50}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $\sigma_I R_1$ | 1.00 | 1.00 | −0.12 | −0.15 | −0.24 | −0.15 | 0.06 | −0.27 | −0.37 | −0.66 | −0.09 | 0.40 |
| $MRR_1$ | | 1.00 | −0.12 | −0.16 | −0.24 | −0.15 | 0.07 | −0.26 | −0.36 | −0.65 | −0.08 | 0.40 |
| $I_1$ | | | 1.00 | −0.18 | −0.78 | −0.79 | −0.98 | −0.81 | −0.76 | −0.53 | −0.92 | −0.72 |
| $I_2$ | | | | 1.00 | 0.18 | 0.18 | 0.19 | 0.19 | 0.17 | 0.15 | 0.19 | 0.47 |
| $S_2$ | | | | | 1.00 | 0.99 | 0.88 | 0.99 | 0.98 | 0.86 | 0.96 | 0.58 |
| $S_3$ | | | | | | 1.00 | 0.90 | 0.99 | 0.96 | 0.80 | 0.96 | 0.63 |
| $S_4$ | | | | | | | 1.00 | 0.90 | 0.86 | 0.62 | 0.98 | 0.74 |
| $S_5$ | | | | | | | | 1.00 | 0.99 | 0.87 | 0.97 | 0.58 |
| $S_6$ | | | | | | | | | 1.00 | 0.93 | 0.94 | 0.50 |
| $S_7$ | | | | | | | | | | 1.00 | 0.76 | 0.22 |
| $S_{av}$ | | | | | | | | | | | 1.00 | 0.68 |
| $pIC_{50}$ | | | | | | | | | | | | 1.00 |

$$\begin{aligned} pIC_{50} = &-6.2048(\pm0.7072) + 1.9594 \\ &\times (\pm0.4191)\sigma_I R_1 + 2.8396(\pm0.4948)S_{av} \\ &+ 0.6740(\pm0.1780)I_2 \\ n = 16;&\quad DC = \mathbf{17};\quad R = 0.9240; \\ \%EV = 85.39;&\quad R^2_A = 0.8173;\quad F_{(3,12)} = 23.370; \\ p < 0.0000;&\quad S.E.E. = 0.2704; \\ PRESS = 1.6190;&\quad SSY = 6.0053; \\ R^2_{CV} = 0.7304;&\quad S_{PRESS} = 0.3673;\quad P.S.E = 0.3181. \end{aligned} \tag{11}$$

$$\begin{aligned} pIC_{50} = &-6.3363(\pm0.7115) + 1.4884 \\ &\times (\pm0.3191)MRR_1 + 2.8200 \\ &\times (\pm0.4953)S_{av} + 0.6814(\pm0.1785)I_2 \\ n = 16;&\quad DC = \mathbf{17};\quad R = 0.9239; \\ \%EV = 85.36;&\quad R^2_A = 0.8170;\quad F_{(3,12)} = 23.327; \\ p < 0.0000;&\quad S.E.E. = 0.2707; \\ PRESS = 1.6480;&\quad SSY = 6.0053; \\ R^2_{CV} = 0.7256;&\quad S_{PRESS} = 0.3706;\quad P.S.E = 0.3209. \end{aligned} \tag{12}$$

These two final equations (eqs 11 and 12) give important pieces of information at the molecular level to the structure activity relationship of this type of compounds. Eq 11 explains 85.39% of the variance of activity whereas eq 12 can explain 85.36% of the variance of activity. *F*-ratios of eqs 11 and 12 are of same confidence level as that of eqs 5–10. The values within the parentheses of all final equations (eqs 3, 4, 11 and 12) are of more than 95% confidence intervals as supported by the *t*-statistics and *p*-values shown in Table 4. The observed, calculated and residual values of the anticancer activity of these four final equations are given in Table 5. The predictive powers of these equations were confirmed by Leave-One-Out (LOO-) method[9] and the LOO-predicted values (pred) and predicted residuals (pres) are shown in Table 6. Cross-validated $R^2$ values of eqs 11 and 12 are significant (0.7304 and 0.7256), respectively. These equations clearly suggest that the presence of electron donating $R_1$ substituents have detrimental effects to the activity (as evidenced from positive coefficients of $\sigma_I R_1$) whereas the presences of bulkier $R_1$ substituents increase the activity (as evidenced from positive coefficients of $MRR_1$). Another important observation is that the presence of

**Table 4.** *t*- and *p*- values of eqs 3, 4, 11 and 12

| Eq | Intercept/parameters | *t*-value | *p*-value | Eq | Intercept/parameters | *t*-value | *p*-value |
|---|---|---|---|---|---|---|---|
| 3 | Intercept | −19.1149 | 0.0000 | 4 | Intercept | −16.9056 | 0.0000 |
| | $\sigma_I$_$R_1$ | 2.9447 | 0.0123 | | MR_$R_1$ | 2.9512 | 0.0121 |
| | $I_1$ | −4.4615 | 0.0008 | | $I_1$ | −4.4372 | 0.0008 |
| | $I_2$ | 3.1327 | 0.0086 | | $I_2$ | 3.1579 | 0.0083 |
| 11 | Intercept | −8.7736 | 0.0000 | 12 | Intercept | 8.9050 | 0.0000 |
| | $\sigma_I$_$R_1$ | 4.6753 | 0.0005 | | MR_$R_1$ | 4.6652 | 0.0005 |
| | $S_{av}$ | 5.7387 | 0.0001 | | $S_{av}$ | 5.6939 | 0.0001 |
| | $I_2$ | 3.7857 | 0.0026 | | $I_2$ | 3.8169 | 0.0025 |

**Table 5.** Observed, calculated and residual activities of eqs 3, 4, 11 and 12

| Compd | Obs. value | Eq 3 | | Eq 4 | | Eq 11 | | Eq 12 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Calc | Res. | Calc | Res. | Calc | Res. | Calc | Res. |
| **1** | −2.0000 | −1.9800 | −0.0200 | −1.9858 | −0.0147 | −1.9520 | −0.0480 | 1.9596 | −0.0404 |
| **2** | −2.0790 | −1.9800 | −0.0990 | −1.9858 | −0.0937 | −1.9704 | −0.1086 | 1.9779 | −0.1011 |
| **3** | −3.5050 | −3.0930 | −0.4120 | −3.0930 | −0.4120 | −3.4721 | −0.0329 | 3.4692 | −0.0358 |
| **4** | −2.6810 | −3.0930 | 0.4120 | −3.0930 | 0.4120 | −2.7556 | 0.0746 | 2.7576 | 0.0767 |
| **5** | −1.0000 | −0.9897 | −0.0103 | −1.0054 | 0.0033 | −0.9932 | −0.0068 | 1.0162 | 0.0162 |
| **6** | −1.7240 | −1.7731 | 0.0491 | −1.7052 | −0.0119 | −1.7567 | 0.0327 | 1.6674 | −0.0566 |
| **7** | −1.9030 | −1.9800 | 0.0770 | −1.9858 | 0.0823 | −1.9709 | 0.0679 | 1.9784 | 0.0754 |
| **8** | −1.3220 | −1.3150 | −0.0070 | −1.3150 | −0.0070 | −1.3432 | 0.0212 | 1.3430 | 0.0210 |
| **9** | −0.8450 | −1.3150 | 0.4700 | −1.3150 | 0.4700 | −1.3191 | 0.4741 | 1.3191 | 0.4741 |
| **10** | −2.0000 | −1.9800 | −0.0200 | −1.9858 | −0.0147 | −2.0050 | 0.0050 | 2.0122 | 0.0122 |
| **11** | −1.7780 | −1.3150 | −0.4630 | −1.3150 | −0.4630 | −1.2827 | −0.4953 | 1.2829 | −0.4951 |
| **12** | −2.4310 | −1.9800 | −0.4510 | −1.9858 | −0.4457 | −2.0206 | −0.4104 | 2.0277 | −0.4033 |
| **13** | −1.7780 | −1.9800 | 0.2020 | −1.9858 | 0.2073 | −1.9439 | 0.1659 | 1.9516 | 0.1736 |
| **14** | −1.9540 | −1.9800 | 0.0260 | −1.9858 | 0.0313 | −2.0050 | 0.0510 | 2.0122 | 0.0582 |
| **15** | −1.5680 | −1.9800 | 0.4120 | −1.9858 | 0.4173 | −1.9557 | 0.3877 | 1.9633 | 0.3953 |
| **16** | −2.1460 | −1.9800 | −0.1660 | −1.9858 | −0.1607 | −1.9681 | −0.1780 | 1.9756 | −0.1704 |

**Table 6.** LOO- predicted (Pred), predicted residual (Pres) values of eqs 3, 4, 11 and 12

| Compd | Obs value | Eq 3 | | Eq 4 | | Eq 11 | | Eq 12 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pred | Pres | Pred | Pres | Pred | Pres | Pred | Pres |
| **1** | −2.0000 | −1.9777 | −0.0223 | −1.9841 | −0.0158 | −1.9462 | −0.0538 | −1.9547 | −0.0453 |
| **2** | −2.0790 | −1.9685 | −0.1105 | −1.9748 | −0.1042 | −1.9579 | −0.1211 | −1.9661 | −0.1128 |
| **3** | −3.5050 | −2.6810 | −0.8240 | −2.6810 | −0.8240 | −3.3498 | −0.1552 | −3.3367 | −0.1683 |
| **4** | −2.6810 | −3.5050 | 0.8240 | −3.5050 | 0.8240 | −2.7775 | 0.0965 | −2.7803 | 0.0993 |
| **5** | −1.0000 | −0.7384 | −0.2616 | −1.0747 | 0.0747 | −0.8258 | −0.1742 | −1.2259 | 0.2259 |
| **6** | −1.7240 | −1.7786 | 0.0547 | −1.7026 | −0.0214 | −1.7604 | 0.0364 | −1.6593 | −0.0646 |
| **7** | −1.9030 | −1.9890 | 0.0860 | −1.9956 | 0.0926 | −1.9787 | 0.0757 | −1.9871 | 0.0841 |
| **8** | −1.3220 | −1.3115 | −0.0105 | −1.3115 | −0.0105 | −1.3539 | 0.0319 | −1.3536 | 0.0316 |
| **9** | −0.8450 | −1.5500 | 0.7050 | −1.5500 | 0.7050 | −1.5562 | 0.7112 | −1.5561 | 0.7111 |
| **10** | −2.0000 | −1.9777 | −0.0223 | −1.9841 | −0.0158 | −2.0055 | 0.0055 | −2.0136 | 0.0136 |
| **11** | −1.7780 | −1.0835 | −0.6945 | −1.0835 | −0.6945 | −1.0345 | −0.7435 | −1.0348 | −0.7432 |
| **12** | −2.4310 | −1.9276 | −0.5034 | −1.9333 | −0.4977 | −1.9771 | −0.4539 | −1.9845 | −0.4465 |
| **13** | −1.7780 | −2.0035 | 0.2255 | −2.0104 | 0.2324 | −1.9640 | 0.1860 | −1.9728 | 0.1948 |
| **14** | −1.9540 | −1.9830 | 0.0290 | −1.9896 | 0.0356 | −2.0105 | 0.0565 | −2.0186 | 0.0646 |
| **15** | −1.5680 | −2.0279 | 0.4599 | −2.0351 | 0.4671 | −2.0015 | 0.4335 | −2.0106 | 0.4426 |
| **16** | −2.1460 | −1.9607 | −0.1853 | −1.9669 | −0.1791 | −1.9475 | −0.1985 | −1.9556 | −0.1903 |

NHR (R is hydrogen or alkyl group) at $R_3$ position improves or increases the antitumor activity (as evidenced from positive coefficient of $I_2$). The equations also reveal that atoms 2–7 are important contributors to the activity as indicated by the presence of $S_{av}$ as a parameter in the two equations. The positive coefficients of $S_{av}$ in the equations indicate that higher value corresponds to the greater anti-tumor activity.

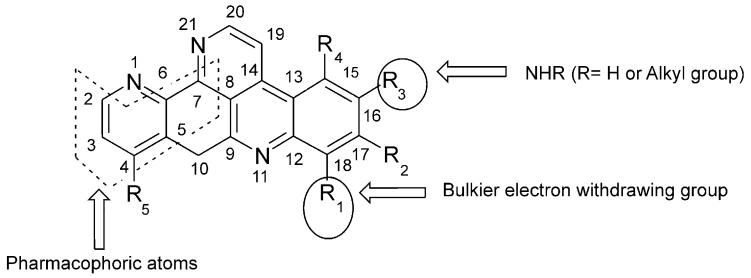


**Figure 2.** Pharmacophoric atoms and required physicochemical properties of substituents at $R_1$ and $R_3$ positions of asciddemin analogues for their anti-tumor activity.

## Conclusion

The QSAR study of pyrido[2,3,4-*kl*]acridine derivatives reveals some important information regarding the structural or substitutional requirement to the anti-tumor activity. Along with the physicochemical parameters ETSA indices have important contributions to the activity as both type of parameters appeared in the final QSAR equations. The pharmacophoric atoms and required physicochemical properties at $R_1$ and $R_3$ positions are shown in Figure 2. Here the QSAR analysis has been performed using the ring D analogues of asciddemin, however better and more statistically significant relations may be obtained, for which the compounds of this series with diverse substitution pattern are necessary.

## Experimental

### QSAR methodology

2-D QSAR study of some marine pyridoacridine asciddemin analogues was done for their in vitro anti-tumor activity against human cancer cells.

### Data set and parameters

The physicochemical parameters, like molar refractivity (MR), field effect ($\sigma_I$) and so on of the substituents were calculated by Molecular Modeling Pro. of Chem SW.[11] E-state indices of Kier and Hall, was employed in QSAR study to identify the required pharmacophore. The E- state indices[8] are derived from the value of electro negativity distributed over an atom according to its bonding degree of non-hydrogen atoms. The E-state index of an atom in a molecule is composed of an intrinsic state ($I_i$) and the perturbation effect ($\Delta_{ij}$), where the intrinsic state

$$I_i = \left[\left((2/N)^2\delta^v + 1\right)\delta\right] \quad (13)$$

$N$ = principle quantum number, $\delta^v$ = number of valence electron- number of hydrogen atom attached, $\delta$ = number of sigma electron-number of hydrogen atom attached and the perturbation effect

$$\Delta_{ij} = \sum \left(I_i - I_j\right)/r_{ij}^2 \quad (14)$$

in which $r_{ij}$ is the topological distance between the atoms, given as the number $i$ and $j$. The atoms of the molecules were numbered consecutively keeping the serial number of atoms same in all molecules and ETSA indices were calculated using program 'Mouse' developed in our laboratory. Besides these, some indicator parameters were also used in order to find out the role of the specific substituent at the specific position towards the biological activity.

### Correlation analysis[10]

Physicochemical parameters, ETSA indices and the biological activity data were subjected to correlation analysis and intercorrelated parameters were eliminated stepwise.

### Multiple regression analysis[10]

Multiple regression analysis was carried out by 'Multi regress' a program developed in our laboratory. The statistical quality of the regression equation were justified by parameters like correlation coefficient ($R$), percentage of explained variance (%EV), adjusted $R^2$ ($R^2A$), variance ratio ($F$), standard error of estimate (S.E.E) All the final equations have regression coefficients, intercepts and variance ratio ($F$) significant to more than 95% level. Use of more than one variable in the multivariate equation was justified by autocorrelation study. The predictive powers of the equation are validated by Leave-one-out (LOO-) cross validation method. Predicted residual sum of square (PRESS), total sum of squares (SSY), cross-validated $R^2$ ($R^2_{CV}$), standard error of PRESS ($S_{PRESS}$) and predictive standard error or uncertainty factor (P.S.E) for the final equations are considered for the validation of the models.

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