Chemotherapy is often the treatment of choice for many types of cancer and the search for new chemotherapeutic agents still plays a major role in the fight against cancer. A reasonable approach in this area deals with use of compounds interacting with DNA and/or inhibiting enzymes critical for cell survival and replication. Amsacrine is one such compound, a well-known antiproliferative agent used to treat some types of cancers including acute adult leukaemia\(^1\). The poisoning of topo II activity inhibits the relegation process and causes lethal double-strand breaks in DNA, leading to cell cycle arrest and apoptosis. The intercalative property was referred to the planar aromatic system of the acridine moiety\(^2\).

In the same context, acridines have gained strong ground for various biological activities like antimicrobial\(^3\), antioxidant\(^4\), antitumor\(^5-8\), antimalarial\(^9\), antiinflammatory\(^10\), analgesic\(^11\), antileishmanial\(^12\), antinociceptive\(^13\), acetylcholinesterase inhibitory\(^14\) and antiherpes\(^15\). Amsacrine is the best known compound of 9-anilinoacridines series. It was one of the first DNA-intercalating agents to be considered as a topoisomerase II inhibitor. The intercalation process is the strongest type of reversible binding to the double helical DNA in compounds with sufficiently large coplanar aromatic chromophore. Several detailed SAR studies of acridine-based DNA-intercalating agents suggest that the mode of binding is important and the chromophore intercalate with the DNA base pairs. The chemical modification of acridines such as the introduction of different substitutions or heterocyclic rings were allowed expansion of research on the structure activity relationship to afford new insight into molecular interactions at the receptor level\(^16\). In fact, it is well-established that slight structural modification on 9-anilinoacridines may bring various pharmacological effects. Similarly oxazine derivatives also have various biological activities\(^17-20\) like antimicrobial, antitumor.

In this paper \textit{in vitro} and \textit{in vivo} antitumor activity against Daltons lymphoma ascites (DLA) cell lines were described. In continuation of our previous research work\(^21\) on searching new potent cytotoxic agents, 9-anilinoacridine analogues bearing the oxazine residue on anilino rings were synthesized for antitumor evaluation. Acridine derivatives possessed a diverse range of pharmacological activities\(^22\). Hence the main objective of this study was to determine the antibacterial and antitumor activities of oxazine substituted 9-anilino acridine derivatives. The results revealed that the newly synthesized derivatives exhibited significant antitumor activities.

\textbf{Synthesis, Characterization and Antitumour Activity of Some Novel Oxazine Substituted 9-Anilinoacridines and their 3D-QSAR Studies}

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Kalirajan \textit{et al.}: Oxazine bearing 9-anilinoacridines and Antitumour activity

A series of oxazine substituted 9-anilinoacridines were synthesized, characterized, and evaluated for antitumor activity against Daltons lymphoma ascites cells using \textit{in vitro} and \textit{in vivo} methods. Results indicated that these conjugates exhibited significant antitumour activity on Daltons lymphoma ascites cells. Among these agents, compounds 4b, 4c, 4e and 4j were the most cytotoxic with CTC\(_{50}\) value of 96.5-190 µg/ml (0.125-0.352 µM). 3D QSAR study was performed using PHASE module of Schrodinger suite.

**Key words:** Acridine, Oxazine, Synthesis, Antitumour, 3D-QSAR
Materials and Methods

Melting points were obtained on Veego VMP-1 apparatus in open capillary tubes and are uncorrected. The reactions were monitored by thin-layer chromatography (TLC) on silica gel thin-layer plates. Compounds were analysed for C, H, N and analytical results obtained for these elements were within ±0.5 % of the calculated values for the formula shown. All reagents were of commercially quality or were purified before use. Organic solvents were of analytical grade or were purified by standard procedures. IR spectra were obtained using a Perkin Elmer FT-IR spectrometer spectrum two model. 1H nuclear magnetic resonance (NMR) and 13C NMR were recorded on Bruker Avance III 500 MHz spectrometer. Chemical shifts are in parts per million (ppm). Mass spectra of the final compounds were recorded on a Jeol GC-Mate mass spectrometer.

General procedure for synthesis of chalcones (3a-k):

The chalcones were synthesized by using the general Claisen-Schmidt condensation as reported[23]. 1-[4-(Acridin-9-ylamino) phenyl]ethanone (2) was synthesized by the reaction of 4-aminoacetophenone was refluxed with 9-chloroacridine as reported[23].

(E)-1-(4-(acridin-9-ylamino)phenyl)-3-(4-methoxy phenyl)prop-2-en-1-one (3b):

Yellow powder, % yield: 58; MP: 179-181°, IR (υ, cm\(^{-1}\)): 3273 (NH), 3100-3000 (ArCH); MS: m/z 434.52 (M\(^+\)), 1624 (α, β-unsaturated C=O), 1606 and 1518 (ArC=C), 7.90 and 7.56 (2H, s, CH=CH), 11.21 (1H, s, NH); 1H NMR (DMSO-d6, δ ppm): 6.65-8.02 (16H, m, ArCH), 7.90 and 7.56 (2H, s, CH=CH), 11.21 (1H, s, NH); 13C NMR (DMSO-d6, δ ppm): 189 (C=O), 145.3, 120.4 (CH=CH), 153.5, 150.8, 148.4, 148.5, 143.2, 141.2, 136.3, 136.1, 132.4, 131.7, 130.8, 129.6, 130.5, 128.6, 127.2, 127.1, 127.2, 121.6, 119.5, 116.3. Anal. calcd. for C\(_{28}\)H\(_{19}\)ClN\(_2\)O: C, 77.32; H, 4.42; N, 6.45; found: C, 77.25; H, 4.29; N, 6.62.

(E)-1-(4-(acridin-9-ylamino)phenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (3c):

Yellow powder, % yield: 54; MP: 228-230°, IR (υ, cm\(^{-1}\)): 3044 (NH), 3100-3000 (ArCH), 1626 (υ, α, β-unsaturated C=O), 1577 and 1498 (ArC=C), 748 (ArCH); MS: m/z 460.58 (M\(^+\)), 1H NMR (DMSO-d6, δ ppm): 6.65-8.02 (16H, m, ArCH), 7.90 and 7.56 (2H, s, CH=CH), 11.21 (1H, s, NH); 13C NMR (ppm): 181 (C=O), 53.47 (OCH\(_3\)), 54.32 (OCH\(_3\)), 104.8-158.8 (aromatic carbons). Anal. calcd. for C\(_{29}\)H\(_{22}\)N\(_2\)O\(_3\): C, 78.29; H, 5.33; N, 6.13; found: C, 78.25; H, 5.36; N, 6.12.

(E)-1-(4-(acridin-9-ylamino)phenyl)-3-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (3d):

Yellow powder, % yield: 73; MP: 210-212°, IR (υ, cm\(^{-1}\)): 3230 (NH), 3100-3000 (ArCH); MS: m/z 450.34 (M\(^+\)), 1H NMR (DMSO-d6, δ ppm): 6.65-8.02 (16H, m, ArH), 7.90 and 7.56 (2H, s, CH=CH), 11.21 (1H, s, NH); 13C NMR (ppm): 181 (C=O), 53.47 (OCH\(_3\)), 54.32 (OCH\(_3\)), 104.8-169.3 (aromatic carbons). Anal. calcd. for C\(_{26}\)H\(_{24}\)N\(_2\)O\(_3\): C, 77.42; H, 5.85; N, 6.33; found: C, 77.46; H, 5.92; N, 6.37.
6.64 (15H, m, ArCH), 10.74 (1H, s, NH), 7.58 and 7.91 (2H, ss, CH=CH); 1^3C NMR (ppm): 180 (C=O), 53.17 (OCH3), 105.7-167.5 (aromatic carbons). Anal. calcd. for C26H22N2O: C, 79.97; H, 4.64; N, 7.17; found: C, 79.88; H, 4.53; N, 7.25.

(E)-1-(4-(acridin-9-ylamino)phenyl)-3-(pyridin-2-yl)prop-2-en-1-one (3f):

Yellow powder, % yield: 48; MP: 174-177°; IR (υ, cm^-1): 3028 (NH), 3100-3000 (ArCH), 1627 (α, β-unsaturated C=O), 1579 and 1498 (Ar=C=C), 744 (Ar=CH); MS: m/z 402.15 (M^+); 1^3C NMR (ppm): 181 (C=O), 110.3-157.3 (aromatic carbons). Anal. calcd. for C27H18N2O: C, 81.49; H, 5.33; N, 8.23; found: C, 81.53; H, 5.37; N, 8.13.

(E)-1-(4-(acridin-9-ylamino)phenyl)pent-2-en-1-one (3j):

Yellow powder, % yield: 54; MP: 195-200°; IR (υ, cm^-1): 3044 (NH), 3100-3000 (Ar st CH), 1626 (α, β-unsaturated C=O), 1582 and 1496 (Ar=C=C), 1280 (CN), 748 (Ar=CH); MS: m/z 352.43 (M^+); 1^3C NMR (ppm): 181 (C=O), 114.2-153.6 (aromatic carbons). Anal. calcd. for C25H20N2O: C, 76.25; H, 5.33; N, 6.13; found: C, 74.29; H, 5.33; N, 6.13.

(E,2E,4E)-1-(4-(acridin-9-ylamino)phenyl)hexa-2,4,13,19-oxazin-4-yl)phenol (4a):

A mixture of chalcone 3a-k (0.02 mol), urea (0.02 mol) were dissolved in sodium hydroxide in ethanol (10 ml), stirred for about for 2-3 h on a magnetic stirrer. This mixture was poured into 400 ml of cold water with continuous stirring for 1 h. This was kept in refrigerator for 24 h. The precipitate obtained was filtered, washed and recrystallized using petroleum ether:benzene (5:5). The reaction was monitored by TLC using methanol:water (5:3).

4-(6-(4-(acridin-9-ylamino)phenyl)-2-amino-2H-1,3-oxazin-4-yl)phenol (4a):

Yellow powder, % yield: 54; MP: 108-111°, IR (υ, cm^-1): 3389 (NH2), 3282 (NH), 1586 and 1437 (Ar=C=C), 1176 (Ar=C=N), 3223 (Ar-CH=O), 819 (Ar=CH); MS: m/z 458. 51 (M^+); 1^3C NMR (ppm): 5.62 (s, 1H, OH), 6.85-7.94 (16H, m, ArCH), 7.46-7.48 (2H, d, CH), 7.95 (1H, s, NH), 6. 12 (2H, s, NH); 1^3C NMR (ppm): 179.7 169.6, 163.7, 150.2, 148.3, 148.5, 145.3, 120.5, 142.5, 141.2, 136.2, 136.1, 132.5,
N-(4-(2-amino-4-(4-methoxyphenyl)-2H-1,3-oxazin-6-yl)phenyl)acridin-9-amine (4b):
Yellow powder, % yield: 72; MP: 142-145°, IR (υ, cm⁻¹): 3014 (NH), 2961 (Ar st CH), 1588 and 1468 (ArC=C), 1177 (ArC=N), 1295 (C-O), 820 (ArCH); MS: m/z 472.54 (M⁺); ¹H NMR (DMSO-d₆, δ ppm): 3.36, (3H, d, OCH₃), 1.61-2.15 (2H, m, ArCH), 7.46-7.48 (2H, m, CH), 11.25 (1H, s, NH), 6.12 (2H, s, NH₂); ¹³C NMR (ppm): 167.9, 158.4, 158.1, 149.1, 148.9, 145.1, 144.7, 141.2, 137.3, 136.5, 133.5, 132.1, 130.2, 130.9, 129.6, 128.5, 127.3, 127.2, 126.8, 121.2, 120.5, 119.1, 116.3, 116.1, 114.6, 112.4 (aromatic carbons), 54.63 (OCH₃). Anal. calc. for C₃₇H₂₆N₄O₂: C, 76.27; H, 5.14; N, 11.87; found: C, 76.32; H, 5.17; N, 11.91.

N-(4-(2-amino-4-(3,4-dimethoxyphenyl)-2H-1,3-oxazin-6-yl)phenyl)acridin-9-amine (4c):
Yellow powder, % yield: 67; MP: 162-165°, IR (υ, cm⁻¹): 3352 (NH), 3005 (Ar st CH), 1600 and 1583 (ArC=C), 1642 (ArC=N), 1263 (CO), 744 (ArCH); MS: m/z 502.52 (M⁺); ¹H NMR (DMSO-d₆, δ ppm): 3.80, 3.86 (6H, d, OCH₃), 6.85-7.97 (16H, s, ArCH), 7.48 (1H, s, NH), 6.12 (2H, s, NH₂); ¹³C NMR (ppm): 185.96, 167.1, 153.66, 150.71, 148.9, 145.1, 142.5, 141.87, 137.3, 136.5, 133.6, 132.2, 130.8, 130.9, 129.6, 128.5, 127.3, 127.92, 125.55, 123.21, 120.5, 119.9, 112.7, 111.54, 110.49, (aromatic carbons), 55.69 (OCH₃), 55.53 (OCH₃). Anal. calc. for C₃₅H₂₄N₄O₂: C, 74.18; H, 5.16; N, 11.12; found: C, 74.23; H, 5.12; N, 11.18.

N-(4-(2-amino-4-(4-methoxyphenyl)-2H-1,3-oxazin-6-yl)phenyl)acridin-9-amine (4d):
Yellow powder, % yield: 57; MP: 169-172°, IR (υ, cm⁻¹): 3332 (NH), 2993 (Ar st CH), 1589 and 1563 (ArC=C), 3227 (Ar-OH), 1653 (ArC=N), 1178 (C-O), 750 (ArC=H); MS: m/z 488.47 (M⁺); ¹H NMR (DMSO-d₆, δ ppm): 3.36, (3H, d, OCH₃), 5.34 (s, 1H, OH), 7.27-7.71 (18H, m, ArH), 8.28 (1H, s, NH), 6.27 (2H, s, NH₂); ¹³C NMR (ppm): 40.03 (OCH₃), 163.9, 161.7, 151.3, 149.4, 147.1, 145.3, 141.5, 140.3, 138.5, 136.2, 133.1, 132.5, 130.8, 130.1, 129.3, 128.2, 127.3, 127.1, 126.3, 121.6, 120.2, 119.2, 117.3, 116.8, 114.6, 112.4 (aromatic carbons). Anal. calc. For C₃₀H₂₄N₄O₂: C, 73.68; H, 4.87; N, 11.36; found: C, 73.68; H, 4.87; N, 11.36.

N-(4-(2-amino-4-(furan-2-yl)-2H-1,3-oxazin-6-yl)phenyl)acridin-9-amine (4f):
Brown powder, % yield: 55; MP: 200-203°; IR (υ, cm⁻¹): 3349 (NH), 3224 (NH), 1586 and 1473 (ArC=C), 1636 (ArC=N), 1259 (CO), 748 (ArCH); MS: m/z 443.47 (M⁺); ¹H NMR (DMSO-d₆, δ ppm): 6.06-8.49 (16H, m, ArCH), 8.40-8.43 (2H, m, CH), 5.45 (1H, s, NH), 4.54 (2H, s, NH₂); ¹³C NMR (ppm): 168.4, 167.3, 153.7, 150.5, 147.3, 145.2, 142.1, 141.4, 138.6, 136.5, 134.3, 132.7, 131.3, 130.1, 129.5, 128.8, 127.4, 127.2, 126.2, 121.1, 120.7, 119.2, 116.7, 116.4, 114.2, 112.7, 109.3 (aromatic carbons). Anal. calc. for C₃₀H₂₂N₄O; C, 75.87; H, 4.74; N, 15.79; found: C, 75.87; H, 4.74; N, 15.79.

N-(4-(2-amino-4-(pyridin-3-yl)-2H-1,3-oxazin-6-yl)phenyl)acridin-9-amine (4g):
Brown powder, % yield: 53; MP: 135-137°, IR (υ, cm⁻¹): 3347 (NH), 3221 (NH), 1587 and 1478 (ArC=C), 1632 (ArC=N), 1278 (CO), 827 (ArCH); MS: m/z 443.47 (M⁺); ¹H NMR (DMSO-d₆, δ ppm): 6.12-8.47 (16H, m, ArCH), 8.38-8.42 (2H, m, CH), 5.75 (1H, s, NH), 4.76 (2H, s, NH₂); ¹³C NMR (ppm): 171.5, 167.4, 155.3, 151.3, 147.1, 145.2, 142.5, 141.5, 138.3, 137.5, 134.2, 132.5, 131.7, 130.4, 129.9, 128.5, 127.6, 127.3, 126.7, 121.5, 120.3, 119.6, 117.4, 116.7, 114.5, 112.2, 109.6, 107.4 (aromatic carbons). Anal. calc. for C₃₀H₂₁N₄O; C, 75.81; H, 4.75; N, 15.78; found: C, 75.83; H, 4.74; N, 15.75.
N-(4-(2-aminobenzylidene)-2H-1,3-oxazine-6yl)phenyl)acridin-9-amine (4i):

Yellow powder, % yield: 51; MP: 173-176 °C, IR (υ, cm⁻¹): 3242 (NH), 2930, 2868 (CH), 1662 (C=O), 1549 (C=C), 1461 (C-H). MS: m/z 662.50 (M⁺); 1H NMR (DMSO-d6, δ ppm): 6.75-7.85 (10H, m, ArH), 7.36 (2H, s, NH), 6.18 (2H, s, NH); 13C NMR (ppm): 175.9, 168.4, 157.1, 151.3, 145.2, 143.2, 140.7, 138.7, 136.5, 135.5, 134.2, 131.3, 130.7, 129.3, 128.2, 127.2, 126.5, 124.5, 121.3, 119.6, 115.2, 112.8 (aromatic carbons), 57.6 (CH₃). Anal. calc. for C₂₅H₂₂N₄O: C, 76.77; H, 5.48; N, 13.78; found: C, 76.77; H, 5.48; N, 13.75.

Pharmacological evaluation:

All the oxazine substituted 9-anilinoacridine derivatives 4a-k were screened for antibacterial activity and short term in vitro antitumour activity against DLA cells. All the synthesized final compounds 4a-k exhibited significant cytotoxic activities. The compounds 4b and 4h were further screened for in vivo antitumour activity against DLA cells.

Short-term study for in vitro antitumour activity[26]:

Short term in vitro antitumour activity of the compounds was assayed by determining the percent viability of DLA cells using trypan blue dye exclusion technique. DLA cells were cultured in the peritoneal cavity of healthy albino mice weighing 25-30 g by injecting a suspension of DLA cells (1×10⁶ cells/ml) intraperitoneally. The cells were aspirated aseptically from the peritoneal cavity of the mice on d 15. The cells were washed with Hank’s balanced salt solution (HBSS) and centrifuged for 10-15 min at 1500 rpm in the cooling centrifuge. The pellet was re-suspended with HBSS and the process was repeated three times. Finally the cells were suspended in a known quantity of HBSS and the cell count was adjusted to 2×10⁶ cells/ml. 0.1 ml of the diluted cell suspension was distributed into Eppendorf tubes and exposed 0.1 ml each of the different concentration of the drug in phosphate buffer saline and incubated at 37 °C, 5 % CO₂ for 3 h. After 3 h, trypan blue dye exclusion test was performed to determine percent viability. For testing viability using dye exclusion method, the pooled cells from wells of each concentration were mixed with 0.4 % trypan blue in a ratio of 1:1 and the number of stained, non-stained and total number of cells were counted using haemocytometer. The percent inhibition and CTC₅₀ values were calculated.

In vivo antitumour activity:

In vivo antitumour activities of selected compounds were carried out using DLA tumor model in mice[26]. Male Swiss albino mice were divided into 7 groups of 6 animals each. Except normal group (group 1) all the animals were injected intraperitoneally (i.p.) with 1×10⁶ DLA cells. Group-1 and group 2 animals received vehicle (0.5 % CMC, 10 ml/kg, p.o) and served as a normal and control, respectively. Group-3 animals received 5-fluorouracil (10 mg/
kg, i.p) and treated as standard group. Group 4 and 5 animals received compound 4b at a dose of 10 and 20 mg/kg, p.o., respectively. Group 6 and 7 animals received compound 4h at a dose of 10 and 20 mg/kg, p.o., respectively. The treatment was started 24 h after tumour inoculation and continued for a period of 24 d. Through body weight analysis in each group, mean survival time (MST) and increase in the life span was calculated. The treatment protocols received approval from the Institutional Animal Ethics Committee.

RESULTS AND DISCUSSION

The reaction sequences leading to the various oxazine substituted 9-anilinoacridines were outlined in the fig. 1. This synthetic pathway was based on the preparation of oxazine substituted 9-anilino acridines 4a-k\(^{[25]}\) from 9-chloroacridine 1. 1-(4-(acridine-9-ylamino)phenylethanone 2\(^{[23]}\) was prepared from compound 1, which was refluxed with p-aminoacetophenone. The various chalcone substituted 9-anilinoacridines 3a-k\(^{[21]}\) were prepared by the reaction of 2 with various aldehydes and these chalcone derivatives were allowed to cyclized with urea afford the corresponding oxazine substituted 9-anilinoacridines 4a-k. Synthesis, characterization and evaluation of biological activities of novel oxazine substituted 9-anilinoacridines are described in this paper. The synthesized compounds were purified by column chromatography. The final yield of the derivatives was in the range of 48-73 %. The compounds obtained were stable in the solid as well as in the solution state. The new compounds were completely characterized by IR, \(^1\)H NMR, \(^{13}\)C NMR, mass spectral data and elemental analysis. The IR spectra of compounds 4a-k showed intense bands in the region 1200-1300 cm\(^{-1}\) due to carbonyl stretching and broad bands in the region 3200-3400 cm\(^{-1}\) due to NH stretching. The \(^1\)H NMR spectra also support the structure of the compounds 4a-k. The NH proton appeared at 7.9-8.1 and NH\(_2\) proton at 5.9-6.3. The mass spectra of all compounds 4a-k showed molecular ion peaks confirming their molecular weight.

![Fig. 1: Reaction sequences for oxazine substituted 9-anilinoacridines](image)

a. 2-BuOH+4-aminoacetophenone, refluxed at 130-140\(^\circ\) for 3 h on an oil bath, b. EtOH+10 % NaOH+R-CHO, stirred at room temperature for 8 h and c. cyclization with urea, stirred for 2-3 h.
The pharmacological properties of the compounds greatly depended on the number and the chemical nature of the substituents. The synthesized final compounds 4a-k were subjected to short term study for \textit{in vitro} antitumor activity against DLA cells. The compounds 4b, 4c, 4e-4j exerted significant antitumor activity against DLA cells at the concentration of 96.5-190 µg/ml (0.125-0.352 µM, Table 1).

The compounds 4b and 4h were screened further to evaluate \textit{in vivo} antitumor activity against DLA cells. The \textit{in vivo} study was carried out for 24 d. Body weight gradually increased for many groups. The body weight analysis, MST and % increase in life span at the dose of 10 and 20 mg/kg in Swiss albino mice inoculated with DLA cells ($1 \times 10^6$) were calculated (Table 2).

The 3D-QSAR model was generated by PHASE module of Schrodinger suite 2012. The predictive ability was analysed for the training set as well as the test set molecules. The features represented by the model with hydrogen bond donor, electron withdrawing group and hydrophobic/non-polar group (fig. 2). Blue colour region represented the favourable position for substitution and the red colour region represented the non-favourable position for substitution of groups.

In conclusion, acridine family includes a wide range of tricyclic molecules with various biological properties. Considered as potential antitumour agents since the 1980s, numerous acridine derivatives have been synthesised and successfully assessed for their antitumour activity. On this basis, authors recently demonstrated that diverse compounds of the oxazine substituted 9-anilinoacridine series exerted potent antitumour activities. It was revealed that these

### TABLE 1: SHORT TERM \textit{IN VITRO} ANTICANCER ACTIVITY AGAINST DLA CELLS

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>CTC\textsubscript{50} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>385</td>
</tr>
<tr>
<td>4b</td>
<td>107</td>
</tr>
<tr>
<td>4c</td>
<td>190</td>
</tr>
<tr>
<td>4d</td>
<td>250</td>
</tr>
<tr>
<td>4e</td>
<td>118</td>
</tr>
<tr>
<td>4f</td>
<td>116</td>
</tr>
<tr>
<td>4g</td>
<td>105</td>
</tr>
<tr>
<td>4h</td>
<td>96.5</td>
</tr>
<tr>
<td>4i</td>
<td>140</td>
</tr>
<tr>
<td>4j</td>
<td>115</td>
</tr>
<tr>
<td>4k</td>
<td>365</td>
</tr>
</tbody>
</table>

DLA- Dalton’s lymphoma ascites cells, CTC\textsubscript{50} concentration required to reduce viability by 50%.

### TABLE 2: \textit{IN VIVO} ANTICANCER ACTIVITY AGAINST DLA CELLS

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Compound</th>
<th>MST (In Days)</th>
<th>% ILS</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (Control)</td>
<td>10</td>
<td>CMC (0.05 %)</td>
<td>13±2.7</td>
<td>--</td>
</tr>
<tr>
<td>3 (Standard)</td>
<td>10</td>
<td>5-Fluorouracil</td>
<td>23.3±0.8</td>
<td>79.46</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>5b</td>
<td>17.33±1.6</td>
<td>33.30</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>5b</td>
<td>23.33±1.0</td>
<td>79.46</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>5h</td>
<td>19.33±1.0</td>
<td>48.69</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>5h</td>
<td>23.33±1.1</td>
<td>79.46</td>
</tr>
</tbody>
</table>

MST values are mean±SD, n=6, MST: mean survival time, % ILS: percent increase in life span.

Fig. 2: 3D QSAR model based on compounds 4b and 4h

3D QSAR model for compounds 4b and 4h illustrates (A) hydrogen bond donor feature, (B) electron withdrawing group and (C) hydrophobic feature. The blue colour region represents favourable position for substitution and the red colour region represents non-favourable position for substitution.
agents exhibited significant cytotoxicity against DLA cell growth. Results observed in the present study clearly demonstrated that some derivatives of the oxazine substituted 9-anilinoacridine family could exert interesting antitumour activity. The compounds 4a-k showed significant antitumour activity and have the potential to be developed as useful drugs after further refinement. These derivatives certainly provide impetus to design future antitumour agents with greater therapeutic potential.

Acknowledgements:

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Conflict of interest:

There is no conflict of interest among authors.

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