



ORIGINAL ARTICLE

1st Cancer Update

Synthesis and anticancer study of 9-aminoacridine derivatives

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9-Aminoacridine;
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Abstract Acridine and its derivatives, well known as DNA intercalates lead to cell cycle arrest and apoptosis. 9-Aminoacridine derivatives were synthesized, characterized and evaluated against lung cancer (*A-549*) cell line and cervical cancer (*HeLa*) cell line by MTT assay. Compound **9** exhibited potent anticancer activity with CTC_{50} (13.75 & 18.75 $\mu\text{g/ml}$) for cervical cancer cell (*HeLa*) line and lung cancer cell (*A-549*) line respectively. *In vitro* short term cytotoxicity evaluation of compound **9** was carried out by Dalton's Lymphoma Ascites (DLA) with percentage growth inhibition CTC_{50} (337.5 $\mu\text{g/ml}$). Compound **7** also exhibited good anticancer activity with CTC_{50} (31.25 & 36.25 $\mu\text{g/ml}$) for cervical cancer cell (*HeLa*) line and lung cancer cell (*A-549*) line respectively. Further *in vivo* study of newly synthesized 9-aminoacridine derivative can give a ray of light in the field of anticancer drugs.

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1. Introduction

Acridine and its derivatives, well known as DNA intercalates, have been widely studied from a variety of viewpoints, such as synthesis, physicochemical properties structural requirements and biological activities (Gooch and Beal, 2004). Acridine was first developed as dyes and during the early 20th century and its pharmacological properties were evaluated. Limited numbers of anticancer drugs are available in the market. It can be concluded that there is an urgent need to search for new anticancer agents (Sondhi et al., 2010; Howell et al., 2009; Settimo et al., 1998; Denny et al., 1982). During the last 20–30 years a large number of derivatives belonging to the general class of aniline acridine have been prepared and evaluated extensively as anti-inflammatory (Sondhi et al., 2005), anti-malarial (Kumar et al., 2009), antimicrobial (Auparakkitanon et al., 2003) and anticancer agents (Tomar et al., 2010; Petrikaite et al., 2007).

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The great majority of antitumor agents in the present clinical use are thought to exert their cytotoxic action by interfering with DNA metabolism, some binding non-covalently and reversibly to DNA and exerting their action either by inhibition of nucleic acid synthesis or by inhibition of DNA breakage and repair phenomenon (Roninson et al., 1984; Baguley, 1982). The mechanism of action of acridine moiety in the chemotherapy revealed, interference of acridine with the activity of topoisomerase and telomerase enzymes and other cellular targets such as cyclin dependent kinase. An effect of acridine derivatives on DNA synthesis raised the hypothesis that acridine moieties' secondary effect on biochemical pathways, including protein and lipid metabolism exists, which suggests acridine derivatives could be considered multi targets drugs (Zahir et al., 1996; Helene, 1988; Wang, 1996).

Inspired by the wide range of useful activities possessed by acridine derivatives and in continuation of our efforts in search of potential anticancer agents, we synthesized 9-anilinoacridine derivatives. The newly synthesized compounds evaluated for anticancer activities against two cancer cell lines i.e. human small cell lung cancer (*A-549*) cell line and human endothelial cervical cancer (*HeLa*) cell line by MTT assay (Fig. 1). The most potent compound among two tested compounds was evaluated for *in vitro* short term anticancer study by *Dalton's Lymphoma Ascites (DLA)*. The results of these studies are reported in this paper.

2. Experiment

All chemicals used were of reagent grade and purified as per need of the reaction. Progress of the reaction was monitored by TLC using chloroform: methanol (10:1) system. 3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA, EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai and Dimethyl Sulfoxide (DMSO) and Propanol from E. Merck Ltd., Mumbai, India.

2.1. Chemistry

9-Chloro-2-(un)substituted acridine derivatives synthesized by modified Ullman-Goldberg reaction (Scheme 1). Modified Ullmann-Goldberg involves reaction of *o*-chlorobenzoic acid with (substituted/un-substituted) aniline in the presence of

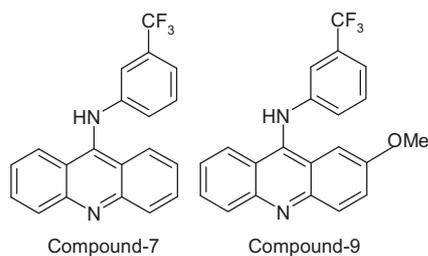
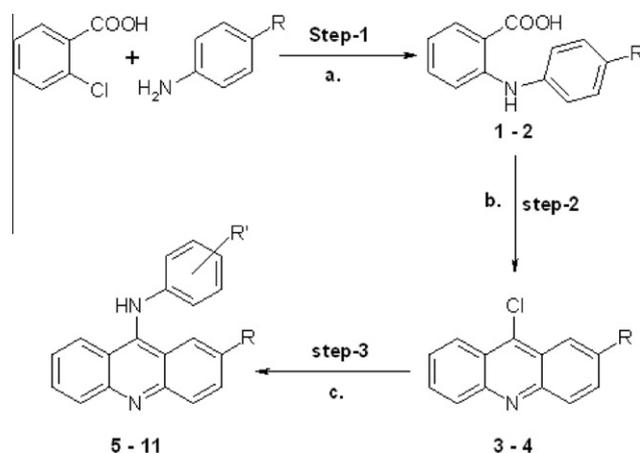


Figure 1 Structure of 9-aminoacridine derivatives which tested against cancer cell lines.



Scheme 1 (a) Sodium acetate, Cu, CuO and DMF, heating at 160–170 °C for 2 h. (b) Cyclization by freshly distilled POCl₃ under nitrogen atmosphere. (c) Phenol, heating at 100 °C, with primary aromatic amine. Where R = H for un-substituted acridine derivative and R = OCH₃ for substituted acridine derivatives. When R = H then R' = 4'-CH₃ 2'-COOH, 3'-CF₃, when R = OCH₃ then R' = 4'-Cl 3'-CF₃ 3'-CH₃.

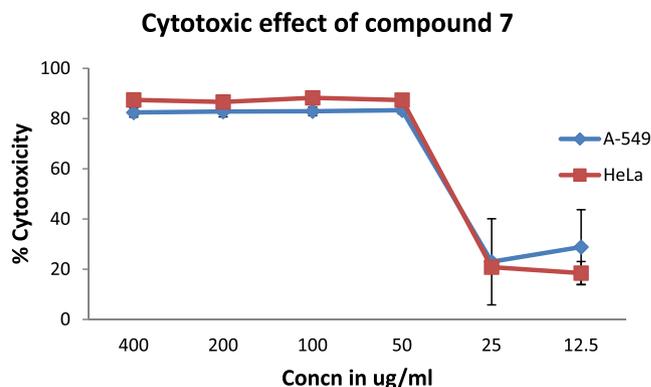


Figure 2 Cytotoxic effect of compound 7 on *A-549* and *HeLa* cell lines.

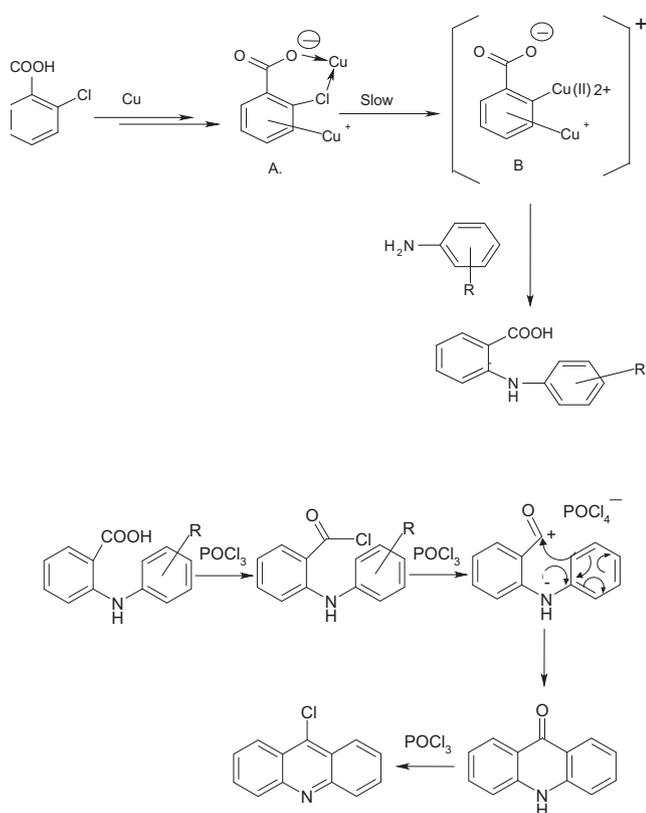
copper (acts as catalyst) and copper oxide (acts as co-catalyst) in DMF as solvent to give intermediate product. Cyclization of intermediate product with freshly distilled phosphorus oxychloride under nitrogen atmosphere gives 9-chloro (substituted/un-substituted) acridines.

9-Chloro-2-(un)substituted acridine (0.001 mol) was dissolved in phenol at 100 °C for 1 h under a nitrogen atmosphere and allowed to react with (substituted/un-substituted) primary amine (0.001 mol) under reflux to give the final product. Here, phenol reacted with 9-chloro-2-(un)substituted acridine to give phenoxyacridine which readily reacted with primary aromatic amine to give the final products (5-11).

The mechanism of reaction described below in the text. The newly synthesized compounds purified by re-crystallization with methanol/ethyl acetate. The structure of newly synthesized compounds was confirmed by proton NMR and IR spectroscopy.

2.2. Mechanism of reactions

- I. N-phenyl(substituted/un-substituted)anthranilic acid:
 II. 9-Chloro(substituted/un-substituted)acridine:



2.3. Characterization

Melting points (mp) were determined using Veego microprocessor based programmable melting point apparatus, in open capillaries and are uncorrected. $^1\text{H-NMR}$ spectra were recorded on BRUKER AVANCE II 400 NMR Spectrophotometer. All experiments were carried out in $\text{d}_6\text{-DMSO}$, CDCl_3 as solvent and ^1H chemical shift of the solvent was used as secondary reference and referred to the TMS signal from the usual relationship; the values of chemical shift (δ) are given in ppm. IR spectra were taken using PERKIN ELMER spectrophotometer over the range of $450\text{--}4000\text{ cm}^{-1}$ in KBr tablets.

2.4. Synthesis

2.4.1. General procedure

9-Chloro (un)substituted acridine (0.001 mol) dissolved in phenol at $100\text{ }^\circ\text{C}$ for 1 h under a nitrogen atmosphere and allowed to react with (substituted/un-substituted) primary amine (0.001 mol) under reflux for another two hrs. The reaction

was monitored by TLC using chloroform: methanol as solvent. After completion of the reaction, cooling down to room temperature, the residue was dissolved in ethanol (5 ml) and then poured into diethyl ether. The deposit was collected by filtration on Buckner funnel and washed with diethyl ether. The residue obtained, dried at $70\text{--}80\text{ }^\circ\text{C}$ in oven for 10–15 min and re-crystallized with methanol/ethyl acetate. The precipitate and washed with ethyl acetate.

2.4.2. Synthesis of 9-substituted acridine derivative

2.4.2.1. *Synthesis of N-(phenyl)anthranilic acid (1)*. The conditions were same as employed for step-1 of Scheme 1. Aniline 7.4 ml (0.08 mol) was reacted with o-chlorobenzoic acid 6.3 gm (0.04 mol) to give a yellow colored product. Yield – 74%, R_f – 0.68, m.p. $182\text{ }^\circ\text{C}$ (lit. $182\text{--}183\text{ }^\circ\text{C}$).

2.4.2.2. *Synthesis of 9-chloroacridine (3)*. The conditions were same as employed for step-2 of Scheme 1. Five grams (0.023 mol) of N-phenylanthranilic acid was reacted with 16 ml (0.176 mol) of phosphorus oxychloride to give a green colored product. Yield – 85%, R_f – 0.83, m.p. $117\text{ }^\circ\text{C}$ (lit. $117\text{--}118\text{ }^\circ\text{C}$).

^1H , NMR (400 MHz, CDCl_3): 8.34–8.32 (d, 2H aromatic), 8.2–8.1 (d, 2H aromatic), 7.77–7.73 (t, 2H aromatic), 7.58–7.54 (m, 2H aromatic).

2.4.2.3. *9-[(4'-Methyl)phenylamino]acridine (5)*. The conditions were same as employed for step-3 of Scheme 1 (General procedure A). 0.137 gm (0.001 mol) of 2-aminobenzoic acid was reacted with 0.213 gm (0.001 mol) of 9-chloroacridine to give a yellow colored product. Yield – 90%, R_f – 0.72, m.p. $282\text{ }^\circ\text{C}$.

I.R. (KBr) cm^{-1} ν_{max} : 3464, 3085, 3029, 2899, 1633, 1583, 1521, 1473, 1373, 1275, 1159, 1113, 1034.

^1H , NMR (400 MHz, $\text{d}_6\text{-DMSO}$) in ppm: 14.92 (s, 1H, NH^+), 11.2 (s, 1H, NH), 8.29–8.26 (d, 2H, aromatic), 8.22–8.20 (d, 2H, aromatic), 7.71–7.76 (t, 2H, aromatic), 7.23–7.12 (m, 6H, aromatic), 2.32 (s, 3H, $-\text{CH}_3$).

2.4.2.4. *9-[(2'-Carboxy)phenylamino]acridine hydrochloride (6)*. The conditions were same as employed for step-3 of Scheme 1 (General procedure A). 0.107 gm (0.001 mol) of p-toluidine was reacted with 0.213 gm (0.001 mol) of 9-chloroacridine to give a greenish yellow powder. Yield – 86%, R_f – 0.78, m.p. $262\text{ }^\circ\text{C}$.

I.R. (KBr) cm^{-1} ν_{max} : 2951, 1721, 1632, 1584, 1549, 1522, 1369, 1216, 1177, 1157, 1132, 1073, 1035.

^1H , NMR (400 MHz, $\text{d}_6\text{-DMSO}$) in ppm: 15.53 (s, 1H, NH^+), 11.7 (s, 1H, NH), 8.44–8.42 (d, 2H, aromatic), 8.20–8.16 (m, 3H, aromatic), 7.97–7.93 (t, 2H, aromatic) 7.57–7.53 (m, 1H, aromatic) 7.49–7.40 (m, 3H, aromatic), 7.26–7.24 (d, 1H, aromatic).

2.4.2.5. *9-[(3'-Trifluoromethyl)phenylamino]acridine hydrochloride (7)*. The conditions were as employed for step-3 of Scheme 1 (General procedure A), 0.124 ml (0.001 mol) of 3-amino benzotrifluoride was reacted with 0.213 gm (0.001 mol) of 9-chloroacridine to give an orange colored product. Yield – 88%, R_f – 0.90, m.p. $276\text{--}278\text{ }^\circ\text{C}$.

I.R. (KBr) cm^{-1} ν_{max} : 3318, 3023, 2881, 2740, 1638, 1581, 1163, 1114, 748, 698, 655, 655.

^1H , NMR (400 MHz, CDCl_3): 14.92 (s, 1H, NH^+), 11.67 (s, 1H, NH), 8.24–8.22 (d, 4H, aromatic), 7.64–7.62 (d, 1H, aromatic), 7.55–7.49 (q, 3H, aromatic), 7.39–7.34 (t, 2H, aromatic), 7.13–7.09 (t, 2H, aromatic).

2.4.3. Synthesis of 2,9 substituted acridine derivative

2.4.3.1. Synthesis of *N*-(4-methoxyphenyl)anthranilic acid (**2**).

The conditions were same as employed for step-3 of Scheme 1 (General procedure A). Anisidine 9.8 gm (0.08 mol) was reacted with *o*-chlorobenzoic acid 6.3 gm (0.04 mol) to give a yellow colored product. Yield – 73%, R_f – 0.51, m.p. 185 °C (lit. 186–187 °C).

2.4.3.2. Synthesis of 2-methoxy-9-chloroacridine (**4**). The conditions were same as employed for step-3 of Scheme 1 (General procedure A). 5.6 g (0.023 mol) of *N*-(2-methoxy phenyl)anthranilic acid was reacted with 16 ml (0.176 mol) of phosphorus oxychloride to give a green colored product. Yield – 82%, R_f – 0.64, m.p. 150–152 °C (lit. 152–153 °C).

^1H , NMR (400 MHz, d_6 -DMSO): 9.01–8.98 (dd, 2H, aromatic), 8.62–8.60 (d, 1H, aromatic), 8.16–8.12 (t, 1H, aromatic), 7.97–7.93 (t, 1H, aromatic), 7.88–7.85 (dd, 1H, aromatic), 7.72–7.71 (s, 1H, aromatic), 4.1 (s, 3H, $-\text{CH}_3$).

2.4.3.3. 2-Methoxy-9-[(4'-chloro)phenylamino]-acridine hydrochloride (**8**). The conditions were same as employed for step-3 of Scheme 1 (General procedure A). 0.127 gm (0.001 mol) of *p*-chloroaniline was reacted with 0.243 gm (0.001 mol) of 2-methoxy-9-chloroacridine to give yellow colored product. Yield – 87%, R_f – 0.79, m.p. 292 °C.

I.R. (KBr) cm^{-1} ν_{max} : 3428, 2899, 2748, 1629, 1581, 1547, 1513, 1442, 1231, 1159, 1091, 1019, 756, 683, 503.

^1H , NMR (400 MHz, d_6 -DMSO): 15.27 (s, 1H, NH^+), 11.09 (s, 1H, NH), 8.32–8.30 (d, 2H, aromatic), 8.14–8.12 (d, 1H, aromatic), 7.82 (d, 1H, aromatic), 7.77 (s, 1H, aromatic), 7.58–7.54 (d, 1H, aromatic), 7.42–7.30 (m, 5H, aromatic), 3.8 (s, 3H, $-\text{OCH}_3$).

2.4.3.4. 2-Methoxy-9-[(3'-trifluoromethyl)phenylamino]-acridine hydrochloride (**9**). The conditions were same as employed for step-3 of Scheme 1 (General procedure A). 0.124 ml (0.001 mol) of 3-aminobenzotrifluoride was reacted with 0.243 gm (0.001 mol) of 2-methoxy-9-chloroacridine to give yellow powder. Yield – 79%, R_f – 0.85, m.p. 260 °C.

I.R. (KBr) cm^{-1} ν_{max} : 3378, 3122, 1612, 1582, 1566, 1518, 1442, 1364, 1262, 1124, 1082, 988, 974.

^1H , NMR (400 MHz, CDCl_3): 14.9 (s, 1H, NH^+), 11.18 (s, 1H, NH), 8.19–8.17 (d, 1H, aromatic), 8.01–7.99 (d, 1H, aromatic), 7.85–7.82 (t, 2H, aromatic), 7.56–7.52 (t, 3H, aromatic), 7.46–7.44 (d, 1H, aromatic), 7.32–7.31 (d, 1H, aromatic), 7.26–7.15 (t, 1H, aromatic), 6.95–6.92 (d, 1H, 1H, aromatic), 3.8 (s, 3H, $-\text{OCH}_3$).

2.4.3.5. 2-Methoxy-9-[(4'-methyl)phenylamino]-acridine hydrochloride (**10**). The conditions were same as employed for step-3 of Scheme 1 (General procedure A). 0.107 gm (0.001 mol) of *p*-toluidine was reacted with 0.243 gm (0.001 mol) of 2-methoxy-9-chloro acridine to give a yellow solid powder. Yield – 85%, R_f – 0.82, m.p. 278–280 °C.

I.R. (KBr) cm^{-1} ν_{max} : 3398, 3017, 2924, 2834, 1598, 1573, 1536, 1431, 1308, 1228, 1189, 1012, 985, 946.

^1H , NMR (400 MHz, d_6 -DMSO): 14.76 (s, 1H, NH^+), 11.16 (s, 1H, NH), 8.15–8.13 (d, 3H, aromatic), 7.86–7.82 (t, 1H, aromatic), 7.67–7.66 (d, 1H, aromatic), 7.58–7.55 (d, 1H, aromatic), 7.34–7.25 (m, 5H, aromatic), 3.73–3.71 (s, 3H, $-\text{OCH}_3$), 2.56 (s, 3H, $-\text{CH}_3$).

3. Anticancer evaluation

3.1. Cell lines and culture medium

A-549 (human, small cell lung carcinoma) and *HeLa* (human, epithelial cervical cancer) cell cultures were procured from National Center for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 $\mu\text{g}/\text{ml}$) and amphotericin B (5 $\mu\text{g}/\text{ml}$) in a humidified atmosphere of 5% CO_2 at 37 °C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtiter plates (Tarsons India Pvt. Ltd., Kolkata, India).

3.2. Preparation of test solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

3.3. Cell viability by MTT Assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using DMEM medium containing 10% FBS. To each well of the 96-well microtiter plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once with medium and 100 μl of different test concentrations of test drugs was added on to the partial monolayer in microtiter plates. The plates were then incubated at 37 °C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 μl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37 °C in 5% CO_2 atmosphere. The supernatant was removed and 100 μl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a micro plate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line (Fig. 2 and Fig. 3).

%Growth Inhibition

$$= 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \right) \times 100$$

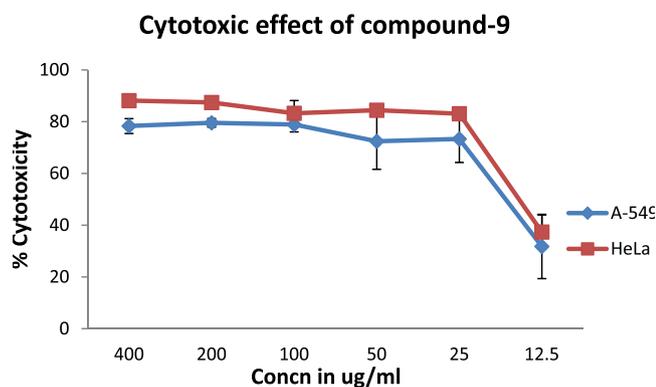


Figure 3 Cytotoxic effect of compound 5a on A-549 and HeLa cell lines.

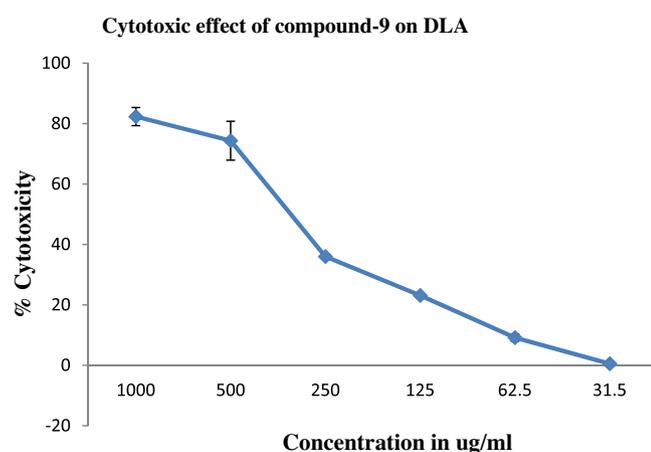


Figure 4 Cytotoxic effect of compound-9 on DLA.

3.4. In vitro short term cytotoxicity study on DLA

3.4.1. Cell lines and propagation

Dalton's Lymphoma Ascites (DLA) cells were a gift sample from the JSSCP, Ooty, India and were propagated and maintained in peritoneal cavity of Swiss albino mice as per standard procedures (Dagli et al., 1992; Harb, 1991).

3.4.2. Preparation of test solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with MEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

3.4.3. In vitro short term cytotoxicity studies

DLA cells were cultured in the peritoneal cavity of mice by injecting intraperitoneally a suspension of DLA cells (1.0×10^5 cells/ml). The cells were withdrawn from the peritoneal cavity of the mice between 15 and 20 days with the help of a sterile syringe. The cells were washed with HBSS and centrifuged for 10–15 min at 1200 rpm. The procedure was repeated thrice. The cells were then suspended in known

Table 1 Cytotoxic properties of test drugs on A-549 and HeLa cell lines.

Sr. No.	Test drug No.	CTC ₅₀ in µg/ml against (A-549) cell line ^a	CTC ₅₀ in µg/ml against (HeLa) cell line ^b
1	7	36.25	31.25
2	9	18.75	13.75

^a Dose in µg/ml required to produce 50% inhibition of A-549 cancer cells ($n = 2, p < 0.01$).

^b Dose in µg/ml required to produce 50% inhibition of HeLa cancer cells ($n = 2, p < 0.01$).

quantity of HBSS and the cell count was adjusted to 2×10^6 cells/ml. The diluted cell suspension was distributed into micro centrifuge tubes (0.1 ml containing 2×10^6 cells). The cells were exposed to drug dilutions and incubated at 37 °C for 3 h. After 3 h, dye exclusion test was carried out i.e. equal quantities of drug treated cells and trypan blue (0.4%) were mixed and left for a minute. It was then loaded in a hemocytometer and viable and non-viable counts were recorded within 2 min.

The percentage growth inhibition was calculated and CTC₅₀ value is generated from the dose-response curves for each cell line (Fig. 4).

%Growth Inhibition

$$= 100 - \left(\frac{\text{Total Cells} - \text{Dead Cells}}{\text{Total Cells}} \right) \times 100$$

3.5. Observations

Refer Tables 1 and 2.

3.6. Graphical representation of cytotoxic effect of drugs

Figs. 2 and 3 show cytotoxic effect of test drugs on A-549 and HeLa cells after 72 h of drug treatment. The cell viability was determined by MTT assay ($n = 2, p < 0.01$).

Fig. 4 shows cytotoxic effects of test drugs on DLA cells after 3 h of drug treatment and cell viability was determined by trypan blue dye exclusion technique.

4. Results and discussion

9-Aminoacridine derivatives have been known for their potential anti-cancer, antimicrobial, and anti-inflammatory activities. We synthesized and characterized new 9-aminoacridine derivatives. Newly synthesized compounds were evaluated for *in vitro* anticancer activity against cervical cancer cell line and lung cancer cell line.

N-(substituted/un-substituted) phenyl anthranilic acid was a starting intermediate for the synthesis of 9-aminoacridine derivatives. N-phenylanthranilic acid was prepared by modified Ullmann-Goldberg reaction. Sodium acetate used as base instead of K₂CO₃. Also copper oxide is used as co-catalyst. If K₂CO₃ is used as base, then there is a possibility of double substitution on amino group. So mixture of N-phenylanthranilic acid and N,N-diphenylanthranilic acid will be obtained. Sodium acetate does not give such products. Simple Ullmann-

Table 2 Cytotoxic properties of short listed test drug on *DLA* cells.

Sr. No.	Test drug No.	Test conc. in $\mu\text{g/ml}$	% Cytotoxicity	CTC ₅₀ in $\mu\text{g/ml}$
1	9	1000	82.33	337.50
		500	74.34	
		250	36.01	
		125	23.13	
		62.5	9.17	
		31.25	0.52	

Goldberg reaction, involves K_2CO_3 and copper as catalyst and after completion of the reaction, mixture was allowed to stand overnight. Hence reaction time was about 15–18 h. In modified Ullmann-Goldberg procedure total reaction was 2–3 h, no need to stand overnight. N-phenyl anthranilic acid was obtained as yellow powder. 9-Chloro (substituted/un-substituted) acridine was obtained by reacting N-phenylanthranilic acid with freshly distilled phosphorous oxychloride under nitrogen atmosphere.

The purity of synthesized compounds was checked by performing TLC. The structure of synthesized compounds was determined by ^1H NMR and IR spectroscopy.

In NMR, a peak appeared at about 15 ppm representing the NH^+ proton, $-\text{NH}$ peak appeared at about 12–13 ppm, and aromatic protons gave their signals at 6–9 ppm, whereas OCH_3 and CH_3 gave their peaks at 2–5 ppm. In IR spectroscopy, the structure was confirmed by functional group identification. Secondary amine gave its peak value at 3400 cm^{-1} . Aromatic keto group gave their peaks at about 1710 cm^{-1} . Benzene ring gave its signal in range of $1400\text{--}1600\text{ cm}^{-1}$. Aliphatic C–H signals appeared at $2800\text{--}3000\text{ cm}^{-1}$ whereas aromatic C–H peaks appeared at $3000\text{--}3100\text{ cm}^{-1}$. Carboxylic acid gave a broad peak in the region of 2951 cm^{-1} and overlaps other peaks in this region like aliphatic and aromatic C–H peaks. C–N peaks appeared at $1200\text{--}1300\text{ cm}^{-1}$. C–Cl peaks appeared at about $540\text{--}785\text{ cm}^{-1}$ whereas C–F peaks appeared at about $1000\text{--}1400\text{ cm}^{-1}$.

The majority of drugs used for the treatment of cancer today are cytotoxic (cell killing) drugs that work by interfering in some way with the operation of cell's DNA. 9-Aminoacridine based drugs have shown good anticancer properties through different pathways [Guo et al., 2009]. *In vitro* anticancer activities of newly synthesized compounds were determined by using MTT assay [Francis and Rita, 1986]. Two compounds were selected for their anti-cancer evaluation against lung cancer (*A-549*) and cervical cancer (*HeLa*) cell lines. Compounds **7** and **9** were selected for anticancer evaluation against lung cancer cell line and cervical cancer cell line. Compounds **7** and **9** gave anticancer activity against lung cancer cell line with CTC₅₀ ($36.25\text{ }\mu\text{g/ml}$ & $18.75\text{ }\mu\text{g/ml}$) respectively, whereas against cervical cancer cell line, gave anticancer activity with CTC₅₀ ($31.25\text{ }\mu\text{g/ml}$ & $13.75\text{ }\mu\text{g/ml}$) respectively as described in Table 1. Compound **9** has shown potent anticancer activities against both the cell lines and so it was selected for *in vitro* short term cytotoxicity evaluation by Dalton's Lymphoma Ascites (DLA) with percentage growth inhibition value CTC₅₀ $337.5\text{ }\mu\text{g/ml}$ as mentioned in Table 2.

The structure activity relationship revealed that:

1. Substitution by methoxy group on acridine ring at position '2' and bulkier CF_3 group at position '3' on phenyl group attached to acridine ring gave potent activity against lung cancer cell line (*A-549*) and cervical cancer cell line (*HeLa*) than un-substituted 9-aminoacridine derivative.
2. Substitution of electron releasing group on acridine ring at position-2 enhances the anticancer activity.

These newly synthesized derivatives can be further evaluated for *in vivo* anticancer activities and more new compounds can be designed.

5. Conclusion

In summary, 9-aminoacridine derivatives were synthesized and characterized by thin layer chromatography, ^1H NMR and IR techniques. Anticancer evaluation was done for selected two compounds among the total synthesized compounds against lung cancer (*A-549*) cell line and cervical cancer (*HeLa*) cell line by using MTT assay. The new acridine derivatives found to possess potent to good anticancer activity.

In vivo anticancer evaluation studies can also be carried out for newly synthesized acridine derivatives in future. Structural modification may lead to the synthesis of more acridine derivatives and can be evaluated for their anticancer activities *in vitro* as well as *in vivo*.

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