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ORIGINAL ARTICLE

1st Cancer Update

Synthesis and biological evaluation of new 9-aminoacridine-4-carboxamide derivatives as anticancer agents

Puneet Kumar *, Rajesh Kumar, Deo Nandan Prasad

Department of Pharmaceutical Chemistry, Shivalik College of Pharmacy, Nangal, Punjab 40126, India

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KEYWORDS

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Lung cancer;
Cervical cancer;
MTT assay;
Topoisomerase

Abstract The design of molecules that recognize the specific sequence of the DNA double helix or those that can stabilize DNA topoisomerase cleavable complex to stop the progression of DNA process, may be very useful in cancer chemotherapy. In the field of antituor DNA-intercalating agents, 9-aminoacridine-4-carboxamide derivatives play an important role due to their anti-proliferative properties. In the present study, 9-aminoacridine-4-carboxamide derivatives were designed, synthesized, characterized and evaluated against lung cancer (*A-549*) cell line and cervical cancer (*HeLa*) cell line *in vitro* by MTT assay. Compounds **5a**, **5b** and **5e** were selected for anticancer evaluation against the lung cancer cell line and cervical cancer cell line. Compound **5b** showed the maximum activity against the cervical cancer (*HeLa*) cell line with CTC_{50} (47.50 μg/ml) and compound **5e** showed the maximum activity against the lung cancer (*A-549*) cell line with CTC_{50} (100 μg/ml) among the tested compounds. The present study opens new vista for DNA intercalating anticancer compounds and their further *in vivo* investigation.

* Corresponding author. Address: Promed Exports Pvt. Ltd., Nalagarh, Himachal Pradesh 174101, India. Mobile: +91 98158 46831; fax: +91 1795 220527.
E-mail address: puneetsoni1984@gmail.com (P. Kumar).

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1. Introduction

The limited understanding of the mechanism involved in the development of drug resistance poses a major obstacle in the design and development of anticancer agents. This has a serious impact on the successful treatment of hematological malignancies. Understanding the phenomenon of multidrug resistance in tumor cells may help in the design of compounds that could be selectively cytotoxic for the resistant cells (Roninson et al., 1984). 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 (Baguley, 1982). In case of this nature, the DNA minor grove appears to be a potential site for the binding of a variety of agents and where antitumor activity is initiated. The binding energy is being contributed by ionic interactions, ion-dipole, ion-induced dipole interaction and specific hydrogen bonding either to thymidine 2-keto oxygen acceptor or guanine 2-amino donors (Baguley, 1982). The design of molecules that recognize specific sequence of the DNA double helix or those that can stabilize DNA topoisomerase cleavable complex to stop the progression of DNA processes, may be useful in cancer chemotherapy (Bodley and Liu, 1988; Zahir et al., 1996; Helene, 1988).

The first acridine based therapeutic agent specifically designed for cancer treatment was developed during 1970's. Acridine has been clinically utilized as a single agent or in combination with other anti neoplastic drugs in the treatment of acute non-lymphocytic, lymphocytic and acute myeloid leukemia. Acridine derivatives possessing fungicidal, anti-parasitic, antimicrobial, antitumor, anti-inflammatory, analgesics etc. activities are well documented in literature (Sondhi et al., 2005; Kumar et al., 2009; Auparakkitanon et al., 2003; Tomar et al., 2010; Petrikaite et al., 2007).

The 9-aminoacridine-4-carboxamide is a structure of interest to medicinal chemists and appears in many biologically active compounds mostly with anticancer application. In the field of antitumor DNA-intercalating agents, 9-aminoacridine-4-carboxamide derivatives play an important role due to their antiproliferative properties. Several cancer chemotherapeutics such as DACA have been developed as anticancer agents (Ma et al., 2008; Atwell et al., 1984). The first goal of this study was to synthesize a new 9-aminoacridine-4-carboxamide derivative by modified Ullmann–Goldberg reaction. Synthesis starting from the reaction of *o*-chlorobenzoic acid with anthranilic acid in the presence of copper (acts as catalyst) and copper oxide (acts as co-catalyst) to give compound (**1**). Cyclization of (**1**) was done with concentrated sulfuric acid to give 9-oxoacridan-4-carboxylic acid (**2**). Some sulfonated impurities may be present in compound (**2**), was purified by dissolving in ethanol and then acidified with glacial acetic acid to give product (**2**) after thorough cooling. Chlorination of (**2**) was done with freshly distilled thionyl chloride to give 9-chloroacridine-4-carbonyl chloride (**3**). The residual traces of thionyl chloride were removed by extracting it with dry dichloromethane. Compound (**3**) was reacted with isopropylamine in dry and cooled dichloromethane to give *n*-(isopropyl)-9-chloro acridine-4-carboxamide (**4**). This compound (**4**) was dissolved in phenol and allowed to react with substituted aromatic amine to give final products (**5a–5g**) under nitrogen atmosphere. The synthesized compounds were purified by extracting the products with ethanol and diethyl ether and recrystallized with methanol/ethylacetate solution. Another goal of this study was to investigate the cell growth inhibitory activity of selected newly synthesized derivatives on human small cell lung cancer (*A-549*) cell line and human endothelial cervical cancer (*HeLa*) cell line *in vitro* by MTT assay.

2. Experimental

All chemicals used were of reagent grade and purified as per the 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, dimethyl sulfoxide (DMSO) and propanol from E. Merck Ltd., Mumbai, India were obtained Scheme 1.

2.1. Characterization

Melting points (mp) were determined using Veego microprocessor based programmable melting point apparatus, in open capillaries and are uncorrected. ^{1}H NMR spectra were recorded on a BRUKER AVANCE II 400 NMR spectrophotometer. All experiments were carried out in d_6-DMSO, $CDCl_3$ as solvent and ^{1}H chemical shift of the solvent was used as a secondary reference and referred to the TMS signal from the usual relationship; the values of the chemical shift (δ) are given in ppm. IR spectra were taken using a PERKIN ELMER spectrophotometer over the range of 450–4000 cm^{-1} in KBr tablets. Molecular mass of the compounds was determined by mass spectroscopy using a Micromass, Q-Tof micro (Water make) spectrophotometer.

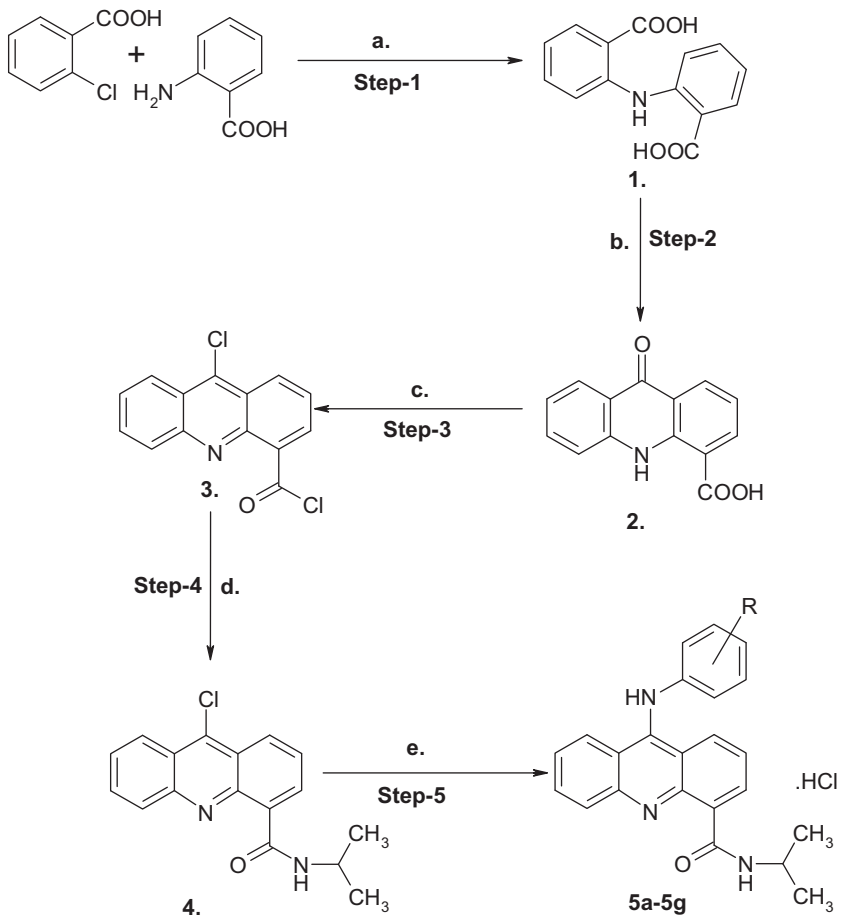
2.2. Synthesis

2.2.1. Synthesis of N-(2-carboxyphenyl)anthranilic acid (1)

2.2.1.1. Modified UllMann–Goldberg reaction. A mixture of *o*-chlorobenzoic acid 3.7 g (0.0233 mol), anthranilic acid 6.4 g (0.046 mol), anhydrous sodium acetate 3.0 g, copper powder 0.15 g, copper oxide 0.075 g, DMF 25 ml was refluxed for 4 h at 140–150 °C. The mixture was allowed to stand at room temperature for 10–15 min and the reaction mixture was poured into 200 ml of water. Green color precipitate was formed, the pH of the solution checked and if necessary, made acidic with conc. HCl. The precipitate was filtered and washed with hot water thoroughly. The collected precipitate was dissolved in aqueous sodium hydroxide solution and heated with activated charcoal and filtered. and acidified with conc. HCl. An yellow colored precipitate was formed and filtered and again washed with hot water, dried in an oven for 15–25 min at 70–80 °C. Yield 71%, mp 294–296 °C.

2.2.2. Synthesis of 9-oxoacridan-4-carboxylic acid (2)

A mixture of *N*-(2-carboxyphenyl)anthranilic acid (0.008 mol) and concentrated sulfuric acid (6 ml) was heated on a water bath for 4 h and cooled, then poured into ice cooled water. The precipitated solid was collected and washed well with hot water. This was dissolved in dilute aqueous sodium hydroxide solution and following filtration was diluted with equal volume of EtOH and then acidified with glacial acetic acid (this left any sulfonated impurities in the solution). The acridone acid that slowly crystallized from hot solution was collected after thorough cooling (about 4-8 h stored in refrigerator) and washed with dilute aqueous EtOH and dried, providing a pure product. Yield 78%, mp >300 °C.



Where R= 3'-CF_3, 4'-COOH, 4'-OCH_3, 4'-COOH, 3'-Cl, 4'-Cl, 4'-CH_3,

Scheme. 1 (a) Sodium acetate, Cu, CuO and DMF, heating at 140–150 °C for 4 h. (b) Cyclization by H_2SO_4 on water bath. (c) $SOCl_2$, two drops of DMF (d) Dry CH_2Cl_2, isopropylamine, 0–5 °C. (e) Phenol heating at 100 °C with primary aromatic amine.

*2.2.3. Synthesis of 9-chloroacridine-4-carbonyl chloride (**3**)*

A suspension of 9-oxoacridan-4-carboxylic acid 2 g in freshly distilled $SOCl_2$ (6 ml) containing DMF (two drops) was heated gently under reflux with stirring until homogeneous and then was refluxed for a further 45 min. The solution was evaporated to dryness in vacuo below 40 °C, and residual traces of $SOCl_2$ were removed by the addition of dry CH_2Cl_2 and complete re-evaporation of all solvent to give the crude product as yellow powder. Product obtained can be used without further purification in next step. Yield 96%.

*2.2.4. Synthesis of N-(isopropyl)-9-chloroacridine-4-carboxamide (**4**)*

An ice cold solution of isopropyl amine 1.61 ml (0.0187 mol) in dichloromethane (10 ml) was added within 5 min to a stirring ice cold suspension of (0.002 mol) of 9-chloroacridine-4-carbonylchloride in dichloromethane (10 ml) at 0–5 °C. The resulting solution was stirred at room temperature for another 2 h, washed twice with (40 ml) 10% sodium carbonate and then with (40 ml) brine, and finally dried over anhydrous sodium sulfate. After removal of the solvent in vacuo, the product was obtained as oil, which slowly solidified. This was extracted with hot dry benzene and pet. ether (1:5) and filtered quickly. Crystalline material rapidly separated and addition of further pet. ether completed the precipitation of the product. The yellow solid was collected, and washed with pet. ether and dried to give *N*-(isopropyl)-9-chloroacridine-4-carboxamide as the product. Yield 90%, mp 140 °C. IR (KBr) cm^{-1} ν_{max}: 3182, 3045, 2964, 1653, 1616, 1551, 1454, 1160, 1022, 747. ^1H NMR (400 MHz, $CDCl_3$): 11.66–1165(d, 1H, NH–C═O), 9.05–9.04(d, 1H, Ar), 8.59–8.57(d, 1H, Ar), 8.44–8.42(d, 1H, Ar), 8.11–8.09(d, 1H, Ar), 7.90–7.86(t, 1H, Ar), 7.77–7.75(t, 1H, Ar), 7.69–7.67(t, 1H, Ar), 4.47–4.42(m, 1H, –CH), 1.48–1.25(d, 6H, 2-CH_3).

*2.2.5. Synthesis of 4, 9-substituted acridine derivatives (**5a**–**5g**)*

2.2.5.1. General procedure. *N*-(Isopropyl)-9-chloroacridine-4-carboxamide (0.001 mol) was dissolved in phenol at 100 °C for 1 h under a nitrogen atmosphere and allowed to react with aromatic amine (0.001 mol) under reflux for another 2 h. The reaction was monitored by TLC. 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 was dried at 70–80 °C in an oven for 10–15 min. Recrystallized with methanol/ethylacetate. The precipitate was filtered and washed with ethyl acetate. Dried for 10–15 min.

*2.2.5.2. N-(Isopropyl)-9-[(3′-triflouromethyl)phenylamino) acridine-4-carboxamide hydrochloride (**5a**).* 0.124 ml (0.001 mol) of 3-aminobenzotriflouride was reacted with 0.3 g (0.001 mol) of *N*-(isopropyl)-9-chloroacridine-4-carboxamide to give a yellow product. Yield 88%, mp 208–214 °C. IR (KBr) cm^{-1} ν_{max}: 3402, 3222, 3051, 2973, 2929, 1619, 1566, 1446, 1167, 1131, 833, 748, 696, 650. NMR (400 MHz, d_6-DMSO): 15.39 (s, 1H, NH^+), 12.68 (s, 1H, NH), 9.35–9.33 (d, 1H, NH–C═O), 9.13–9.06 (d, 2H, Ar), 8.03–8.01 (d, 1H, Ar), 7.8–7.7 (m, 3H, Ar), 7.6–7.5 (m, 3H, Ar), 7.3–7.2 (m, 2H, Ar), 4.42–4.37 (m, 1H, –CH), 1.46–1.44 (d, 6H, 2-CH_3). M/S *m*/*e*: 426, 425, 424, 360.

*2.2.5.3. N-(Isopropyl)-9-[(4′-carboxy)phenylamino]-acridine-4-carboxamide hydrochlorde (**5b**).* 0.137 g (0.001 mol) of *p*-amino benzoic acid was reacted with 0.3 g (0.001 mol) of *N*-(1,1-isopropyl)-9-chloroacridine-4-carboxamide to give orange powder. Yield 76%, mp 276–278 °C. IR (KBr) cm^{-1} ν_{max}: 2925, 1709, 1620, 1579, 1518, 1429, 1354, 1271, 1166, 1105, 1007, 859, 755, 693. NMR (400 MHz, d_6-DMSO): 15.50 (s, 1H, NH^+), 12–57(s, 1H, NH), 9.07–9.06 (d, 1H, NH–C═O), 8.96–8.94 (d, 1H, Ar), 8.85–8.83 (d, 1H, Ar), 8.73–8.71 (d, 1H, Ar), 8.50–8.47 (d, 1H, Ar), 8.06–8.04 (d, 2H, Ar), 7.98–7.94(t, 1H, Ar), 7.91–7.89 (t,1H, Ar), 7.55–7.49 (q, 3H, Ar), 7.37–7.25(t, 1H, Ar), 3.68–3.40 (m, 1H, CH), 1.38–1.36 (d, 6H, 2-CH_3). M/S *m*/*e*: 401, 400.

2.2.5.4. N-(Isopropyl)-9-[(4′-methoxy)phenylamino]acridine-4-carboxamide (5c). 0.107 g (0.001 mol) of *p*-anisidine was reacted with 0.3 g (0.001 mol) of *N*-(isopropyl)-9-chloroacridine-4-carboxamide to give an orange color product. This product obtained as a pure form as it was treated with dilute sodium hydroxide. Yield 81%, mp 172–174 °C. IR (KBr) cm^{-1} ν_{max}: 3354, 3217, 3050, 2970, 2929, 1618, 1555, 1436, 1243, 1171, 1027, 912. NMR (400 MHz, $CDCl_3$): 12.34 (s, 1H, NH), 9.18–9.12 (t, 2H, Ar), 9.00–8.98 (d, 1H, NH–C═0), 8.16–8.14 (d, 1H, Ar), 7.78–7.75 (t, 1H, Ar), 7.62–7.60 (t, 1H, Ar), 7.73–7.26 (t, 2H, Ar), 7.24–7.16 (m, 2H, Ar), 6.84–6.82 (d, 2H, Ar), 4.37–4.35 (m, 1H, –CH), 3.78 (s, 3H, OCH_3), 1.44–1.439 (d, 6H, 2-CH_3).

*2.2.5.5. N-(Isopropyl)-9-[(2′-carboxyl)phenylamino]-acridine-4-carboxamide hydrochloride (**5d**).* 0.137 g (0.001 mol) of *o*-aminobenzoic acid was reacted with 0.3 g (0.001 mol) of *N*-(isopropyl)-9-chloroacridine-4-carboxamide to give a yellow product. Yield 83%, mp 226–229 °C. IR (KBr) cm^{-1} ν_{max}: 3449, 3251, 3041, 2971, 1710, 1623, 1567, 1442, 1362, 1205, 1079. NMR (400 MHz, d_6-DMSO): 15.79 (s, 1H, NH^+), 12.22 (s, 1H, NH), 9.5–9.4 (d, 1H, NH), 9.17–9.15 (d, 1H, Ar), 8.5–8.49 (d, 1H, Ar), 8.23–8.21 (t, 2H, Ar), 8.01–7.92 (m, 2H, Ar), 7.57–7.53 (t, 2H, Ar), 7.48–7.43 (m, 2H, Ar), 7.29–7.27 (d, 1H, Ar), 4.43–4.44 (m, 1H, –CH), 1.42–1.40 (d, 6H, 2-CH_3).

*2.2.5.6. N-(Isopropyl)-9-[(3′-chloro)phenylamino]-acridine-4-carboxamide hydrochloride (**5e**).* 0.11 ml (0.001 mol) of *m*-chloroaniline was reacted with 0.3 g (0.001 mol) of *N*-(isopropyl)-9-chloroacridine-4-carboxamide to give a yellow product. Yield 85%, mp 194–196 °C IR (KBr) cm^{-1} ν_{max}: 3396, 3227, 3052, 2971, 2873, 1619, 1573, 1475, 1439, 1324, 1166, 992, 748, 679, 628. NMR (400 MHz, d_6-DMSO): 15.31 (s, 1H, NH+), 12.5 (s, 1H, NH), 9.20–9.18 (d, 2H, Ar), 9.10–9.09 (d, 1H, NH–C═0), 8.15–8.13 (d, 1H, Ar), 7.87–7.87 (t, 1H, Ar), 7.85–7.83 (d, 1H, Ar), 7.37–7.33 (t, 2H, Ar), 7.30–7.20 (m, 3H, Ar), 7.20–7.18 (d, 1H, Ar), 4.41–4.36 (m, 1H, CH), 1.46–1.45 (d, 6H, 2-CH_3).

*2.2.5.7. N-(Isopropyl)-9-[(4'-chloro)phenylamino]-acridine-4-carboxamide hydrochloride (**5f**).* 0.11 ml (0.001 mol) of *p*-chloroaniline was reacted with 0.3 g (0.001 mol) of *N*-(isopropyl)-9-chloroacridine-4-carboxamide to give a yellow product. Yield 83%, mp 198–202 °C. IR (KBr) cm^{-1} ν_{max}: 3349, 3225, 3033, 2871, 2930, 1620, 1554, 1524, 1432, 1169, 1087, 1010, 912, 827, 757, 712. NMR (400 MHz, d_6-DMSO): 15.31 (s,1H, NH^+), 12.51 (s, 1H, NH), 9.10–9.08 (d, 1H, NH–C═0), 8.97–8.95 (d, 1H, Ar), 8.85–8.83(d, 1H, Ar), 8.46–8.44 (d, 1H, Ar), 7.95–7.91 (t, 1H, Ar), 7.87–7.85 (d, 1H, Ar), 7.49–7.37 (m, 6H, Ar), 4.40–4.39 (m, 1H, CH), 1.38–1.36 (d, 6H, 2-CH_3).

*2.2.5.8. N-(Isopropyl)-9-[(4'-methyl)phenylamino]-acridine-4-carboxamide hydrochloride (**5g**).* 0.107 g (0.001 mol) *p*-toluidine was reacted with 0.3 g (0.001 mol) of *N*-(isopropyl)-9-chloroacridine-4-carboxamide to givea yellow product. Yield 70%, mp 230–232 °C. NMR (400 MHz, $CDCl_3$): 14.72 (s, 1H, NH+), 12.46 (s, 1H, NH), 9.23–9.21 (d, 1H, NH–C═O), 9.03–9.02 (d, 1H, Ar), 8.94–8.92 (d, 1H, Ar), 8.15–8.12 (d, 1H, Ar), 7.79–7.75 (t, 1H, Ar), 7.63–7.61 (d, 1H, Ar), 7.30–7.25 (m, 3H, Ar), 7.21–7.19 (d, 1H, Ar), 7.16–7.13 (t, 2H, Ar), 4.39–4.34 (m, 1H, CH), 2.39–2.34 (s, 3H, –CH_3), 1.46–1.42 (d, 6H, 2-CH_3).

2.3. Anticancer evaluation

2.3.1. Cell lines and culture medium

A-549 (human, small cell lung carcinoma) and *HeLa* (human, epithelial cervical cancer) cell cultures were procured from the National Centre 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 µg/ml) and amphotericin B (5 µg/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 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

2.3.2. Preparation of test solutions

For cytotoxicity studies, each weighed test drug was separately dissolved in distilled DMSO and the 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 twofold dilutions were prepared from this for carrying out cytotoxic studies.

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

Sr. No	Test drug	CTC_{50} in μg/ml against (*A-549*) cell line[a]	CTC_{50} in μg/ml against (*HeLa*) cell line[b]
1	**5a**	325.00	700.00
2	**5b**	370.00	47.50
3	**5e**	100	130.00

[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 ($n = 2$, $p < 0.01$).

2.3.3. 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 microtitre 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 the medium and 100 μl of different test concentrations of the test drugs were added on to the partial monolayer in the microtitre 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 the 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 (see Table 1).

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

3. Results and discussion

9-Aminoacridine-4-carboxamide derivatives have been known for its potential anti-cancer, MDR inhibitor and anti-inflammatory activities. We designed, synthesized and characterized new 9-aminoacridine-4-carboxamide derivatives.

Five steps were involved in the synthesis of *N*-(isopropyl)-9 amino-4 carboxamide derivative (**5a**–**5g**). The lead compound, *N*-(isopropyl)-9-chloroacridine-4-carboxamide (**4**) is a very new compound and no previous such data are available for its anticancer activities. The synthesized compounds were purified by extracting the products with ethanol and diethyl ether followed by re-crystallized with methanol/ethyl acetate solution. NMR, IR and mass data reveal the structure of molecules of newly synthesized acridine derivative. A peak appeared at about 15 ppm representing the NH^+ proton, –NH peak appeared at about 12–13 ppm, –NH–C═O peak appeared at about 9–12 ppm and aromatic proton gave their signal in 6–9 ppm, Whereas OCH_3 and CH_3 gave their peak at 2–5 ppm. In IR, the structure was confirmed by functional group identification. Secondary amine gave its peak value at 3400 cm^{-1}. In carboxamide a peak appears at 3400 and 3200 cm^{-1}, first peak represents the secondary amino group whereas a peak at about 3200 is due to –OH peaks generated due to intramolecular bonding of hydrogen attached to ring nitrogen and oxygen atom of carbonyl group. Amide group of 9-chloroacridine gave their peak in at about 1653 cm^{-1} but amide group of 9-aminoacridine derivative gave their peaks at about 1620 cm^{-1}. C–Cl peaks appeared at about 540–785 cm^{-1} whereas C–F peaks appeared at about 1000–1400 cm^{-1}. Mass spectra were recorded only for two compounds and they reveal the molecular mass of the compounds.

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. As with any pharmaceutical, new anticancer drugs are developed in three step processes i.e., initial discoveries, molecular modification of known compound and development into a useful pharmaceutical. 9-Aminoacridine based drugs shows good anticancer properties through different pathways (Guo et al., 2009). DACA is an acridine-4-carboxamide derivative which shows good anticancer activities against various cancer cell lines (Adams et al., 2000). Inspired from the anticancer activities of DACA, we design a new acridine derivative for anticancer evaluation based upon molecular modification. *In vitro* anticancer activities of newly synthesized compounds were determined by using MTT assay (Francis and Rita, 1986). Lung cancer is mainly caused by smoking and radiation whereas cervical cancer is mainly caused by human papillomavirus which is a sexually transmitted disease. Three compounds were selected for their anti-cancer evaluation against lung cancer (*A-549*) and cervical cancer (*HeLa*) cell line. Anticancer activity was determined by using test drug treated group and control group. The tested compounds gave different anticancer activity based upon the position of functional group on aromatic ring attached to the acridine ring. Compounds **5a**, **5b** and **5e** were selected for anticancer evaluation against lung cancer cell line and cervical cancer cell line to see the effect of electron withdrawing group and size of electron withdrawing group at position 3′ and 4′ on aromatic ring attached with acridine ring. The newly synthesized compound **5a** contain ($-CF_3$) group whereas **5e** contains only (–Cl) group at position 3′ on aromatic ring attached to acridine ring. The group ($-CF_3$) is a much bulkier group than (–Cl) and shows less anticancer activity against both the cell lines i.e. *A-549* and *HeLa* cell lines. The third compound selected for anticancer evaluation is **5b** that contains (–COOH) group at para position on the aromatic ring shows excellent activity against *HeLa* cell lines. Compound **5e** shows maximum activity against lung cancer (*A-549*) cell line with CTC_{50} (100 μg/ml). Compounds **5a**

and **5b** also show good activity against lung cancer cell line with CTC_{50} (325 and 370 μg/ml), respectively. Compound **5b** shows maximum activity against cervical cancer (*HeLa*) cell line with CTC_{50} (47.50 μg/ml). Compounds **5a** and **5e** also show good activity with CTC_{50} (700 μg/ml and 130 μg/ml) against cervical cancer, respectively.

Based upon the abovementioned evaluation data against both cancer cell lines, Structure activity relationship reveals the following points:

1. 3′-substitution by electronegative atom (Cl) gave the maximum activity against lung cancer cell line than substitution at 3′ position by a bulkier (CF_3) group.

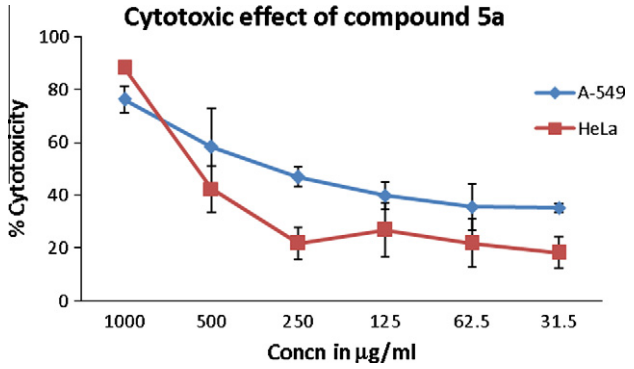


Figure 1 Cytotoxic effect of compound **5a** on *A-549* and *HeLa* cell lines.

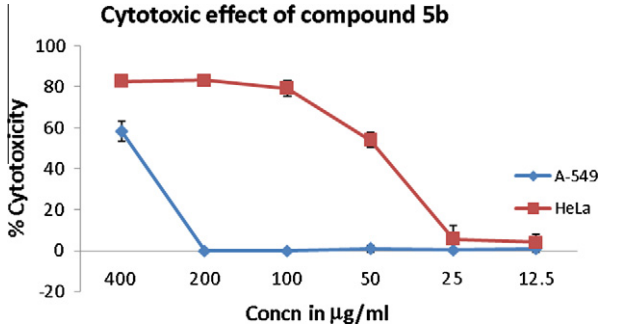


Figure 2 Cytotoxic effect of compound **5b** on *A-549* and *HeLa* cell lines.

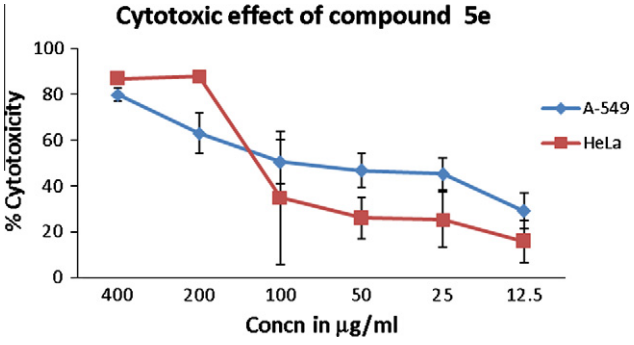


Figure 3 Cytotoxic effect of compound **5e** on *A-549* and *HeLa* cell lines.

2. Whereas substitution at 4′ by (–COOH) group gave maximum activity against cervical cancer cell line than 3′-substitution by electronegative atoms (Cl, CF_3) on aromatic ring attached to acridine ring against *HeLa* cell lines.
3. By considering the evaluated results of anticancer activities it can be assumed that substitution at 4′-position by another electron withdrawing group may increase the anticancer activity against both the cell lines.

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

3.1. Graphical representation of cytotoxic effect of drugs

Figs. 1–3 show cytotoxic effect of test drugs on *A-549* and *HeLa* cells after 72 h of drug treatment. Cell viability was determined by MTT assay ($n = 2$, $p < 0.01$).

4. Conclusion

In summary, 9-aminoacridine-4carboxamide derivatives have been designed, synthesized and characterized. Anticancer evaluation was done for selected three compounds among the total synthesized compounds against lung cancer (*A-549*) cell line and cervical cancer (*HeLa*) cell line. Tested compounds shown good activities against both the cell lines. In future, *in vivo* anticancer evaluation of these drugs can also be carried out for further study.

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