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ORIGINAL ARTICLE

Synthesis, characterization and antitumor activity of 2-methyl-9-substituted acridines

Rajesh Kumar [a,*], Ankita Sharma [a], Sarita Sharma [b], Om Silakari [c], Mandeep Singh [a], Manmeet Kaur [a]

[a] *Pharmaceutical Chemistry Division, Shivalik College of Pharmacy, Nangal, Punjab 140126, India*
[b] *Pharmaceutical Chemistry Division, Global College of Pharmacy, Khanpur Khuhi, Anandpur Sahib 140117, India*
[c] *Molecular Modeling Lab (MML), Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, Punjab 147002, India*

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KEYWORDS

2-Methylacridine;
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Intercalation

Abstract In the field of antitumor DNA-intercalating agents, 9-anilinoacridines play an important role due to their antiproliferative properties. Several cancer chemotherapeutics such as amascrine and nitracrine have been developed as anticancer agents. In the present study, several 2-methyl-9 substituted (**AS 0–8**) acridines were synthesized by nucleophilic substitution of 2-methyl-9-chloro-acridine (**AS**) with aromatic amines. The structures of novel compounds were determined using spectroscopic methods. Three compounds were evaluated for antiproliferative activity against *A-549* (Human, small cell lung carcinoma) and *MCF-7* (Human, breast cancer) cell lines using the MTT assay. Compound **AS-2** showed higher *in vitro* cytotoxic activity against *A-549* and *MCF-7* cancer cell lines with CTC_{50} 187.5 and 212.5 μg/ml respectively. The cancer cell cytotoxicity of acridines against *A-549* cell line was found to be more active than *MCF-7* cell line.

1. Introduction

Cancer is a popular generic term for malignant neoplasms. Malignant tumor is the most serious threat to human health in the world. The word cancer comes from Latin for crab probably because of the way a cancer adheres to any part that it seizes upon in an obstinate manner like the crab. It is a popular generic term whereas the actual medical term for cancer is neoplasia which comes from Greek meaning new formation (Parkin et al., 2005). Malignant neoplasm refers to the fact that the new growth has virulent or adverse properties that may be displayed in the body. Through expression of these properties, it can cause destruction of major organs, and in some cases, life threatening disturbances in body function (Alberg et al., 2005; Cohen et al., 2005). It is very well documented that an anticancer drug for a complete treatment of cancer is yet to be discovered. The current failure of cancer treatment could be attributed to the development of multidrug resistance (MDR) (Dimanche-Boitrel et al., 1993). This phenomenon is of considerable economic importance and often has grave consequences for health. It also serves as a major challenge to the pharmaceutical

* Corresponding author. Tel.: +91 09815844041; fax: +91 01887 221276.
E-mail address: rajeshduvedi@gmail.com (R. Kumar).
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industry because the development of resistance ensures that effective drugs become limited in their usefulness. The repeated use of chemotherapeutics leads the organism to become ineffective due to the onset of resistance or tolerance by the target cells or organism. The above setbacks have posed a major problem for the effective treatment of cancer as well as infectious diseases.

Acridine derivatives constitute a class of compounds that are used for commercial means for more than a century. Its derivatives are one of the more studied chemotherapeutic compounds, widely used as antimalarial, antiviral, antibacterial, antitumor, antiprotozoal, and antitubercular agents (Gamage et al., 1994; Valdes 2011; Goodwell et al., 2006; Wainwright 2001; Guddneppanavar et al., 2006; Giorgia et al., 2007; Aly and Abadi, 2004) and their antitumor activity with DNA-intercalative properties have been studied (Michael et al., 2004; Bacherikov et al., 2005). Ehrlich and Benda in 1912 proposed the use of acridine dyes as antibacterial agents and in 1917 it was clinically used as antibacterial. Acridine has been used as anti-infective agent for many years and now a vast majority of patients are dedicated to its use as antitumor agent. Quinacrine hydrochloride represents a good example of this statement, as it was launched in 1932 for the treatment of malaria and is now in clinical trial for the treatment of Creutzfeldt–Jakob disease, prostate cancer and advanced renal cell carcinoma (Belmont and Dorange, 2008). Amascrine also known as *m*-AMSA, obtained by Denny's group was the first synthetic drug that was approved for clinical use in 1976 for the treatment of leukemia. Nitracrine dihydrochloride hydrate also known as Ledakrin, developed by Ledochowski's group, is another oncolytic drug that was launched in the 1980s and approved for the clinical use.

Amascrine is the best known compound of 9-aminoacridine series (Su et al., 1995; Kohn et al., 1994). It was one of the first DNA intercalating agents to be considered as topoisomerase II inhibitor. It forms a ternary complex in which acridine is intercalated into DNA and the aniline side chain interacts with the enzyme. The stabilization of topoisomerase II-DNA cleavage complex by amascrine inhibits enzyme mediated DNA relegation (Denny, 2002). Among various derivatives of amascrine, asulacrine (CI-921) displayed interesting properties and went through phase I/II clinical trials. Both amascrine and asulacrine undergo reversible oxidation forming chemically reactive quinone-diimine which excreted by conjugation with thiols such as glutathione (Demeunynck et al., 2001). The new analogs were prepared to prevent their oxidation in which the substituents on aniline ring are in *meta* position to each other and became unable to form the quinone-diimine intermediate. In 2003 they prepared *m*-hydroxymethyl substituted derivatives having AHMA as lead compound (Chang et al., 2003). The CH_2OH function of AHMA was replaced by methyl group at *ortho*, *meta* and *para*-positions to NH_2 group to form AOT, AMT and APT compounds with the introduction of dimethylaminoethylcarboxamido and methyl group at C4 and C5 of acridine ring. These compounds show better *in vivo* cytotoxicity than AHMA. Further compounds AOA, AMA and APA with the methoxy group in place of methyl group at *ortho*, *meta*, *para*-positions to NH_2 have been prepared. Among them AOA exhibited a higher cytotoxicity than AMA and APA. Overall order of toxicity was AMAs > AMTs > AOAs > AOTs > APTs > APAs (Denny et al., 1982; Su et al., 1995; Belmont et al., 2007). A further AHMA-alkylcarbamate derivative that possesses a carbamate group has been prepared with a significant anticancer effect and lesser toxicity than amascrine (Su et al., 1999). The significant clinical use of several of these compounds is limited by problems such as side effects, drug resistance and poor bioavailability, which have encouraged further modifications to these compounds. Thus, with the purpose to discover better antiproliferative agents, a new series of 2-methylacridine derivatives were prepared.

2. Experimental

All the chemicals used for the synthetic work were purchased from Central Drug House Pvt. Ltd. (CDH), New Delhi and Hi-Media Laboratories Pvt. Ltd. Mumbai. Melting points were determined by using Veego microprocessor based programmable melting point apparatus in open capillaries and are uncorrected. The progress of reaction was monitored by TLC on silica gel G plates using Chloroform:Methanol (8:2) solvent system. Infra red (IR) spectra were recorded in KBr pellets on Shimadzu IR-Affinity Fourier Transformation spectrophotometer (ν max in cm^{-1}). ^{1}H-NMR spectra were recorded on BRUKER AVANCE II 400 NMR spectrometer using $CDCl_3$ and DMSO-d_6 as solvents and TMS as internal standard (chemical shift values expressed in δ ppm), Elemental analyses were carried out on a Perkin–Elmer 2400 CHN elemental analyzer.

2.1. Synthesis

2.1.1. Synthesis of 2-(p-tolylamino) benzoic acid

A mixture of *o*-chlorobenzoic acid (0.04 mol), *p*-toluidine (0.08 mol), sodium acetate (4.1 g), copper powder (0.2 g), copper oxide (0.1 g) and DMF (25 ml) was refluxed for 2 h at 160–170 °C. The mixture was then cooled and poured into water and acidified with concentrated hydrochloric acid. The precipitates so obtained were filtered and washed with hot water. Recrystallized product (light yellow crystals) was recovered from ethanol using charcoal. Yield: 74% (4.6 g), m.p.: 184 °C.

2.1.2. Synthesis of 2-methyl-9-chloroacridine

A mixture of 2-(*p*-tolylamino) benzoic acid (0.022 mol) and freshly distilled phosphorous oxychloride (0.176 mol) was slowly heated for about 15 min at 85–90 °C on a water bath and when the boiling subsided it was heated on a heating mantle for 2 h at 140–150 °C. The excess of oxychloride was removed by distillation. The residue after cooling was poured into a well-stirred mixture of cooled concentrated ammonia and chloroform (1:1). The mixture was stirred for 30 min. When no more undissolved solid remained, the chloroform layer was separated and extracts were dried over calcium chloride, filtered and finally the solvent was evaporated. A greenish powder was obtained as product.

2-Methyl-9-chloroacridine (AS)

Yield 86%; m.p. 146 °C; IR υ (cm^{-1}): 3026.31 (ArC-H), 2831.50 (C-H), 1600.92 and 1473.62 (ArC = C), 1340.53 (C-N), 775.3 and 756.10 (=C-H), 563.21 (C-Cl); ^{1}H-NMR ($CDCl_3$): δ 2.49 (s, 3H, CH_3), 7.49–7.54 (m, 2H, ArH), 7.66–7.70 (m, 1H, ArH), 8.01 (s, 1H, ArH), 8.06–8.08 (d, 1H, J = 8.88 Hz, ArH), 8.15–8.18 (d, 1H, J = 8.76 Hz, ArH), 8.26–8.28 (d, 1H, J = 8.64 Hz, ArH).

2.1.3. Synthesis of 2-methyl-9-substituted acridines

The substituted aromatic amines (0.011 mol) were dissolved in 50 ml of methanol and refluxed with 2-methyl-9-chloroacridine **AS** (1.14 g, 0.005 mol) for different hrs at 50–60 °C. TLC using chloroform: methanol as solvent (8:2) was used for the confirmation of the completion of the reaction and visualized in an iodine chamber. The reaction mixture was cooled and poured into diethyl ether. The hydrochloric salts were precipitated, filtered and recrystallized with ethanol/ether to give red, yellow or orange color.

2-Methyl-9-(phenylamino) acridine hydrochloride (AS-0)

Yield 88%; m.p. 278–279 °C; IR υ (cm^{-1}): 3566.64 (N–H), 3049.36 (ArC–H), 2831.60 (C–H), 1581.63 and 1517.98 (ArC═C), 1338.60 (C–N), 775.38 and 750.31 (═C–H); ^{1}H-NMR (DMSO-d_6): δ 2.40 (s, 3H, –CH_3), 7.12–8.11 (m, 12H, ArH), 11.2 (s, 1H, NH), 15.2 (s, 1H, HCl). Anal. Calcd for $C_{20}H_{17}ClN_2$: C, 74.88; H, 5.34; N, 8.73. Found: C, 74.50; H, 5.21; N, 8.67.

2-Methyl-9-[(4′-methoxy)phenylamino]acridine hydrochloride (AS-1)

Yield 82%; m.p. 270–271 °C; IR υ (cm^{-1}): 3500.30 (N–H), 3003.30 (ArC–H), 2831.3 (C–H), 1589.34 and 1508.33 (ArC═C), 1340.53 (C–N), 1354.53 (C–O), 775.38 and 759.95 (═C–H); ^{1}H-NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 7.02–7.04 (t, 2H, *J* = 6.96 Hz, ArH), 7.27–7.32 (m, 3H, ArH), 7.73–7.75 (dd, 1H, *J* = 8.20 Hz, ArH), 7.82–7.86 (t, 1H, *J* = 8.12 Hz, ArH), 8.05–8.07 (t, 1H, *J* = 4.76 Hz, ArH), 8.11–8.15 (t, 3H, *J* = 9.52 Hz, ArH), 11.29 (br s, 1H, –NH), 14.60 (s, 1H, HCl). Anal. Calcd for $C_{21}H_{19}ClN_2O$: C, 71.89; H, 5.46; N, 7.98. Found: C, 71.64; H, 5.38; N, 7.80.

*2-Methyl-9-[(3′-chloro)phenylamino]acridine hydrochloride (**AS-2**)*

Yield 82%; m.p. 238 °C; IR υ (cm^{-1}): 3566.38 (N–H), 3064.89 (ArC–H), 2829.57 (C–H), 1595.13 and 1512.19 (ArC═C), 1365.50 (C–N), 775.38 and 754.17 (═C–H), 553.57 (C–Cl); ^{1}H-NMR (DMSO-d_6): δ 2.43 (s, 3H, CH_3), 6.48–6.55 (m, 2H, ArH), 6.94–6.98 (t, 1H, *J* = 1.96 Hz, ArH), 7.31–7.48 (m, 1H, ArH), 7.75–7.77 (d, 1H, *J* = 8.72 Hz, ArH), 7.86–7.89 (t, 1H, *J* = 7.48 Hz, ArH), 8.16–8.25 (m, 5H, ArH), 11.2 (s, 1H, –NH), 15.1 (s, 1H, HCl). Anal. Calcd for $C_{20}H_{16}Cl_2N_2$: C, 67.62; H, 4.54; N, 7.89. Found: C, 67.38; H, 4.47; N, 7.69.

*2-Methyl-9-[(4′-chloro)phenylamino]acridine hydrochloride (**AS-3**)*

Yield 71%; m.p. 275–276 °C; IR υ (cm^{-1}): 3523.95 (N–H), 3101.54 (ArC–H), 2829.57 (C–H), 1578.13 and 1548.84 (ArC═C), 1278.81 (C–N), 775.38 and 750.31 (═C–H), 549.71 (C–Cl); ^{1}H-NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), 7.41–7.47 (m, 1H, ArH), 7.56–7.67 (m, 4H, ArH), 7.84–7.86 (d, 1H, *J* = 8.92 Hz, ArH), 7.93–7.97 (t, 1H, *J* = 7.40 Hz, ArH), 8.14–8.25 (m, 4H, ArH), 11.36 (s, 1H, NH), 15.05 (s, 1H, HCl). Anal. Calcd for $C_{20}H_{16}Cl_2N_2$: C, 67.62; H, 4.54; N, 7.89. Found: C, 67.36; H, 4.50; N, 7.72.

*2-Methyl-9-[(4′-methyl)phenylamino]acridine hydrochloride (**AS-4**)*

Yield 88%; m.p. 279–280 °C; IR υ (cm^{-1}): 3523.95 (N–H), 3101.54 (ArC–H), 2738.92 (C–H), 1583.58 and 1508.33 (ArC═C), 1236.37 (C–N), 762.32 and 758.02 (═C–H); ^{1}H-NMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 7.23–7.25 (d, 2H, *J* = 8.24 Hz, ArH), 7.29–7.33 (t, 3H, *J* = 8.16 Hz, ArH), 7.76–7.79 (dd, 1H, *J* = 8.72 Hz, ArH), 7.85–7.89 (t, 1H, *J* = 7.44 Hz, ArH), 7.98–8.04 (t, 2H, *J* = 8.72 Hz, ArH), 8.06–8.09 (t, 1H, *J* = 4.68 Hz, ArH), 8.12–8.14 (d, 1H, *J* = 9.08 Hz, ArH), 11.23 (s, 1H, –NH), 14.23 (s, 1H, HCl). Anal. Calcd for $C_{21}H_{19}ClN_2$: C, 75.33; H, 5.72; N, 8.37. Found: C, 75.28; H, 5.60; N, 8.24.

*2-Methyl-9-[(3′-triflouromethyl)phenylamino]acridine hydrochloride (**AS-5**)*

Yield 79%; m.p. 274–275 °C; IR υ (cm^{-1}): 3423.95 (N–H), 3101.54 (ArC–H), 2830.31 (C–H), 1595.13 and 1508.08 (ArC═C), 1338.60 (C–F), 1242.00 (C-N), 775.38 and 756.10 (═C–H); ^{1}H-NMR (DMSO-d_6): δ 2.54 (s, 3H, CH_3), 7.35–7.41 (m, 3H, ArH), 7.46–7.48 (d, 2H, *J* = 8.72 Hz, ArH), 7.81–7.83 (t, 1H, *J* = 7.84 Hz, ArH), 7.90–7.94 (t, 1H, *J* = 7.68 Hz, ArH), 8.09–8.16 (m, 3H, ArH), 8.21 (s, 1H, ArH), 11.31 (s, 1H, –NH), 14.80 (s, 1H, HCl). Anal. Calcd for $C_{21}H_{16}ClF_3N_2$: C, 64.87; H, 4.15; N, 7.20. Found: C, 64.68; H, 4.05; N, 7.16.

*2-Methyl-9-[(4′-triflouromethyl)phenylamino]acridine hydrochloride (**AS-6**)*

Yield 85%; m.p. 240–241 °C, IR υ (cm^{-1}): 3315.63 (N–H), 3161.33 (ArC–H), 2829.57 (C–H), 1583.56 and 1510.26 (ArC═C), 1356.60 (C–F), 1276.88 (C-N), 775.38 and 754.17 (═C–H); ^{1}H-NMR (DMSO-d_6): δ 2.54 (s, 3H, CH_3), 7.43–7.50 (m, 3H, ArH), 7.69–7.71 (d, 2H, *J* = 8.44 Hz, ArH), 7.84–7.87 (dd, 1H, *J* = 8.74 Hz, ArH), 7.94–7.98 (t, 1H, *J* = 7.36 Hz, ArH), 8.19–8.23 (m, 4H, ArH), 11.47 (s, 1H, -NH), 15.23 (s, 1H, HCl). Anal. Calcd for $C_{21}H_{16}ClF_3N_2$: C, 64.87; H, 4.15; N, 7.20. Found: C, 64.70; H, 4.05; N, 7.10.

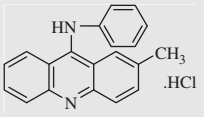
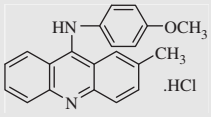
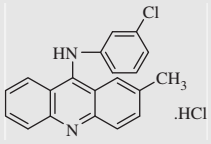
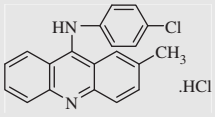
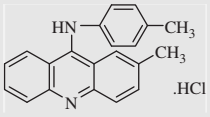
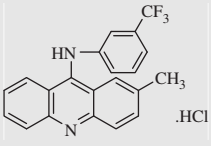
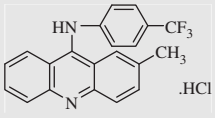
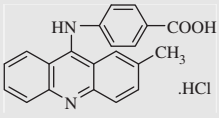
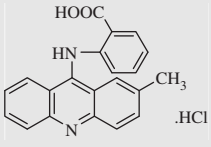
*2-Methyl-9-[(4′-carboxy)phenylamino]acridine hydrochloride (**AS-7**)*

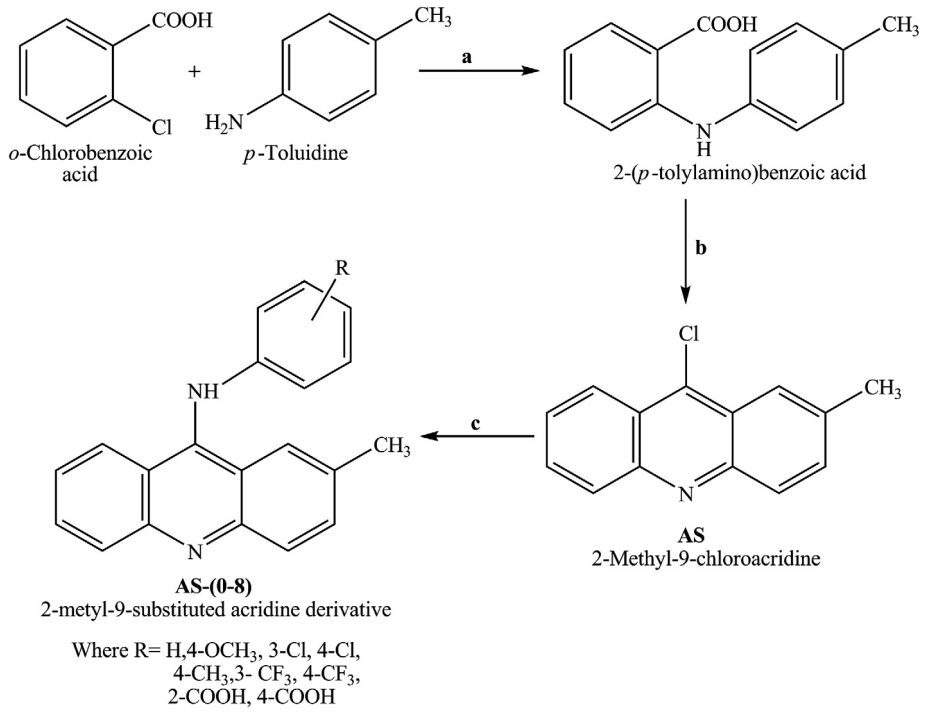
Yield 80%; m.p. 225 °C (dec.); IR υ (cm^{-1}): 3523.95 (N–H), 2951.09 (COOH), 3101.54 (ArC–H), 2829.57 (C–H), 1714.65 (C═O), 1550.77 and 1508.33 (ArC═C), 1255.66 (C–N), 775.38 and 754.17 (═C–H); ^{1}H-NMR (DMSO-d_6): δ 2.57 (s, 3H, CH_3), 6.58–6.60 (d, 2H, *J* = 8.60 Hz, ArH), 7.18 (s, 1H, ArH), 7.62 (s, 2H, ArH), 7.68–7.70 (d, 1H, *J* = 8.60 Hz, ArH), 7.80–7.82 (t, 1H, *J* = 7.56 Hz, ArH), 7.87–7.93 (m, 1H, ArH), 8.03–8.05 (d, 1H, *J* = 8.56 Hz, ArH), 8.16–8.21 (m, 2H, ArH), 11.26 (s, 1H, –NH), 15.03 (br s, 1H, HCl). Anal. Calcd for $C_{21}H_{17}ClN_2O_2$: C, 69.14; H, 4.70; N, 7.68. Found: C, 69.05; H, 4.64; N, 7.58.

*2-Methyl-9-[(2′-carboxy)phenylamino]acridine hydrochloride (**AS-8**)*

Yield 78%; m.p. 220 °C (dec.); IR υ (cm^{-1}): 3523.95 (N–H), 2951.09 (COOH), 3101.54 (ArC–H), 2831.50 (C–H), 1716.65 (C═O), 1585.85 and 1541.12 (ArC═C), 1236.37 (C–N), 773.24 and 752.24 (═C–H); ^{1}H-NMR (DMSO-d_6): δ 2.58 (s, 3H, CH_3), 6.53–6.57 (t, 1H, *J* = 7.96 Hz, ArH), 6.71–6.73 (d, 1H, *J* = 8.28 Hz, ArH), 7.16–7.23 (m, 2H, ArH), 7.38–7.52 (m, 2H, ArH), 7.76–7.83 (m, 2H, ArH), 7.91–7.98 (m, 1H, ArH), 8.11–8.18 (m, 1H, ArH), 8.32–8.39 (t, 1H, *J* = 8.04 Hz, ArH), 11.37 (s, 1H, –NH), 11.60 (br, 1H, –COOH), 15.57 (br s, 1H, HCl). Anal. Calcd for

Table 1 Physical data of the synthesized compounds.

Product code	Structure	M.P. (°C)	Time (hrs)	Color
AS-0		278–279	8	Yellowish green
AS-1		270–271	8	Dark yellow
AS-2		238	8	Greenish yellow
AS-3		275–276	5	Yellow
AS-4		279–280	8	Yellow
AS-5		274–275	6	Yellow
AS-6		240–241	5	Yellowish green
AS-7		225 (dec.)	7	Orange
AS-8		220 (dec.)	9	Dark orange



Scheme 1 **a** Sodium acetate, Cu Powder, CuO and DMF heating at 160–170 °C for 2 h. **b** Cyclization by freshly distilled $POCl_3$. **c** Methanol, heating at 50–60 °C with aromatic amine for different hrs.

$C_{21}H_{17}ClN_2O_2$: C, 69.14; H, 4.70; N, 7.68. Found: C, 69.08; H, 4.65; N, 7.60.

2.2. Cytotoxicity evaluation

2.2.1. Regents and chemicals

3-(4,5-Dimethyl thiazol-2-yl)-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.

2.2.2. Cell lines and culture medium

A-549 (Human, small cell lung carcinoma) and *MCF-7* (Human, Breast 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).

Table 2 *In vitro* antitumor activity of acridine derivatives.

Cell line	Compound	Concentration in µg/ml*					CTC_{50} in µg/ml
		1000	500	250	125	62.5	
A-549	AS-1	74.07 ± 0.04	57.29 ± 0.06	49.23 ± 0.07	14.16 ± 0.13	0.15 ± 0.17	300
	AS-2	81.04 ± 0.03	72.33 ± 0.04	67.97 ± 0.05	32.89 ± 0.10	16.35 ± 0.12	187.5
	AS-5	78.64 ± 0.03	61.65 ± 0.05	49.23 ± 0.07	17.86 ± 0.13	9.80 ± 0.13	262.5
MCF-7	AS-1	72.76 ± 0.04	63.83 ± 0.05	51.41 ± 0.07	14.16 ± 0.13	0.00 ± 0.16	237.5
	AS-2	83.87 ± 0.02	76.90 ± 0.03	55.55 ± 0.06	37.25 ± 0.09	10.25 ± 0.13	212.5
	AS-5	73.85 ± 0.04	59.91 ± 0.06	44.44 ± 0.08	13.50 ± 0.13	4.13 ± 0.14	337.5
		Concentration in µg/ml					
		10	5	2.5	1.25	0.625	
A-549	Taxol	78.95 ± 0.15	66.50 ± 0.08	58.75 ± 0.09	42.76 ± 0.04	19.67 ± 0.03	1.65
MCF-7	Taxol	71.25 ± 0.14	63.45 ± 0.06	49.15 ± 0.05	33.45 ± 0.04	19.50 ± 0.05	2.75

* Results are expressed as mean percent of MTT absorbance (ratio of absorbance in test compound treated and control cells). Data points represent means of three independent experiments ± SEM of $n = 2$, $p < 0.01$.

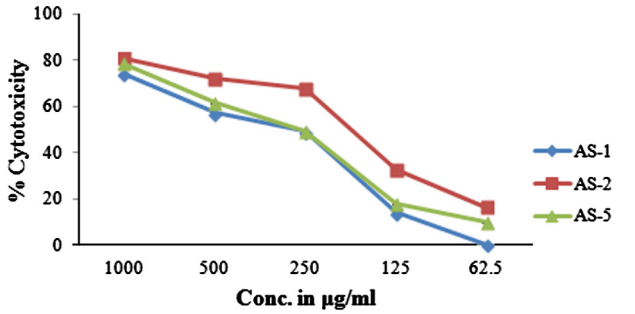


Figure 1 Cytotoxic assay of test samples by MTT against *A-549* cell line after 72 h of drug treatment.

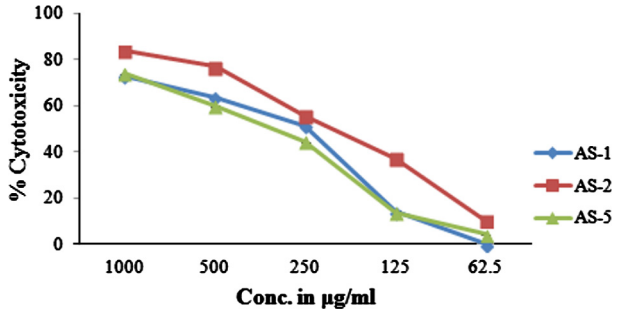


Figure 2 Cytotoxic assay of test samples by MTT against *MCF-7* cell line after 72 h of drug treatment.

2.2.3. 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 two fold dilutions were prepared from this for carrying out cytotoxic studies.

2.2.4. Cell viability by MTT assay

Cell viability following exposure to synthetic compounds was estimated by using the MTT reduction assay (Mosmann 1983; Francis and Rita, 1986; Antonini et al., 2006). 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 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 medium and 100 µl of different test concentrations of test drugs were added onto the partial monolayer in 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, 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and the concentration of the test drug needed to inhibit cell growth by 50% (CTC_{50}) values was generated from the dose–response curves for each cell line (Cookson et al., 2005; Yim et al., 2005). Each experiment was done in triplicate and results are expressed as means ± SEM for each determination. The following formula was used for the calculation of the percentage of cell viability (CV): CV (%) = (A of the individual test group/A of the control group) × 100.

3. Results and discussion

3.1. Chemistry

2-Methyl-9-substituted acridines (**AS 0–8**) were synthesized and given in Table 1. *p*-toluidine was a starting intermediate for the synthesis of 9-substituted acridines. Sodium acetate was used as a base where as Cu metal (catalyst) and CuO are used as co-catalysts to give 2-(*p*-tolylamino) benzoic acid. The product was treated with $POCl_3$ which gives 2-methyl-9-chloroacridine, **AS** (Boyer et al., 1999). **AS** was dissolved in methanol using different aromatic amines and refluxed for different hrs. at 50–60 °C to give corresponding 9-substituted derivatives of 2-methyl acridines as shown in Scheme 1 (Hagan et al., 1997). ^{1}H-NMR and IR data reveal the structure of molecules of newly synthesized acridine derivatives. In IR, the structure was confirmed by functional group identification. Secondary amine gave its peak at 3570–3400 cm^{-1}. C═O peak of the carboxylic group appeared at 1716–1714 cm^{-1} along with a broad peak of –COOH at 3000–2800 cm^{-1}. C–F peaks appeared at about 1350–1300 cm^{-1}. Whereas C–Cl peaks appeared at about 750–700 cm^{-1}. C–O peak of ether is at 1345 cm^{-1}. In ^{1}H-NMR, the structure was confirmed by the no. of proton, splitting of signals, and value of chemical shift in ppm. Peaks appearing at about 11.2–11.4 ppm represent NH proton, COOH peak appeared at about 11.6 ppm whereas $–OCH_3$ and $–CH_3$ gave their peaks at 2–5 ppm.

3.2. Cytotoxicity

The majority of drugs used for the treatment of cancer today are cytotoxic drugs that work by interfering in some way with the operation of cell's DNA. 9-anilinoacridine based drugs show good anticancer activity e.g. amascrine, nitracrine, DACA etc. Inspired from the anticancer activities of amascrine, we design new 9-anilinoacridines for anticancer evaluation based upon molecular modification. Antitumor activity was done on two cancer cell lines (*A-549* and *MCF-7*) by MTT assay. Three compounds **(AS-1, AS-2** and **AS-5)** were selected on the basis of the results of previous work for an evaluation of antiproliferative activity on both cell lines (Kumar et al., 2013). Compound **AS-2** showed good activity against *A-549* and *MCF-7* cancer cell lines with CTC_{50} 187.5 and 212.5 µg/ml respectively as shown in Table 2 and Figs. 1 and 2. All the evaluated compounds showed more activity against *A-549* cell line as compared to *MCF-7* cell line. Other compounds **AS-1** and **AS-5** showed a significant activity against (*A-549*) cancer cell lines with CTC_{50} 300 and 262.5 µg/ml. Also **AS-1** and **AS-5** showed a significant activity against (*MCF-7*) cancer cell lines with CTC_{50} 237.5 and 337.5 µg/ml respectively.

4. Conclusion

New acridine derivatives were prepared and evaluated for their antiproliferative activity. Various structural modifications were done on acridine ring as well as on aromatic ring attached to 9-position of acridine ring. A series of derivatives was prepared by substituting various electron withdrawing as well as electron releasing groups on the aromatic ring attached to 9-position of acridine ring. The new acridine derivatives are found to possess significant antiproliferative activity *in vitro*. An electron withdrawing group on *m*-position of aromatic amine was found to be more active than electron releasing group. Also substitution of Cl group on *m*-position of 9-position of acridine ring was found to be more active than CF_3 group on the same position. Structural modifications may lead to synthesize more acridine derivatives and can be evaluated for their anticancer, antimalarial, anti-inflammatory activities *in vitro* as well as *in vivo*.

Declaration of interest

The authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.arabjc.2012.12.035.

References

Alberg, A.J., Brock, M.V., Stuart, J.M., 2005. Epidemiology of lung cancer: looking to the future. J. Clin. Oncol. 23, 3175–3185.

Aly, E.L., Abadi, A.H., 2004. Synthesis and antitubercular acivity of 6-chloro(unsubsituted)-2-methoxy-9-subsituted acridines derivatives. Arch. Pharm. Res. 27, 713–719.

Antonini, I., Santoni, G., Lucciarni, R., Amantini, C., Sparpani, S., Magnano, A., 2006. Synthesis & biological evaluation of new assymetrical bisintercalators as potent antitumour drugs. J. Med. Chem. 49, 7198–7207.

Bacherikov, V.A., Chang, J.Y., Lin, Y.W., 2005. Synthesis and antitumour activity of 5-(9-acridinylamino) anisidine derivative. Biorg. Med. Chem. 13, 6513–6520.

Belmont, P., Bosson, J., Godet, T., Tiano, M., 2007. Acridine and acridone derivatives, anticancer properties and synthetic methods: where are we now? Anticancer Agents Med. Chem. 7, 139–169.

Belmont, P., Dorange, I., 2008. Acridine/acridone: a simple scaffold with a wide range of application in oncology. Exp. Opin. Ther. Patents 18, 1211–1222.

Boyer, G., Lormier, T., Galy, J.P., Llamas-Saiz, A.L., Foces-Foces, C., Fierros, M., Elguero, J., Virgili, A., 1999. X-ray crystallography at 170K of racemic 2,2′-dimethoxy-9,9′-biacridine and 1H NMR study of 2,2′-diacetoxy-9,9′-biacridine. Molecules 4, 104–121.

Chang, J.Y., Lin, C.F., Pan, W.Y., Bacherikov, V., Chou, T.C., Chen, C.H., Dong, H., Cheng, S.Y., Tasi, T.J., Lin, Y.W., Chen, K.T., Chen, L.T., Su, T.L., 2003. New analogs of AHMA as potential antitumor agents: synthesis and biological activity. Bioorg. Med. Chem. 11, 4959–4969.

Cohen, A.J., Ross, A.H., Ostro, B., 2005. The global burden of disease due to outdoor air pollution. J. Toxicol. Environ. Health 68, 1301–1307.

Cookson, J.C., Heald, R.A., Stevens, M.F.G., 2005. Antitumour polycyclic acridines. 17. Synthesis and pharmaceutical profile of pentacyclic acridinium salts designed to destabilize telomeric integrity. J. Med. Chem. 48, 7198–7207.

Demeunynck, M., 2004. Antitumour acridines. Exp. Opin. Ther. Pat. 14, 55–70.

Demeunynck, M., Charmantray, F., Martelli, A., 2001. Interest of acridine derivatives in the anticancer chemotherapy. Curr. Pharm. Des. 7, 1703–1724.

Denny, W.A., 2002. Acridine derivatives as chemotherapeutic agents. Curr. Med. Chem. 9, 1655–1665.

Denny, W.A., Chain, B.F., Atwell, G.J., Hansch, C., Leo, A., 1982. Potential antitumour agents. 36. Quantitative relationships between experimental antitumour activity, toxicity and structure for general class of 9-anilinoacridine agents. J. Med. Chem. 25, 276–313.

Dimanche-Boitrel, M.T., Garido, C., Chauffert, B., 1993. Kinetic resistance to anticancer agents. Cytotechnology 12, 347–356.

Francis, D., Rita, L., 1986. Rapid colorimetric assay for cell growth and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J. Immunol. Methods 89, 271–277.

Gamage, S.A., Tepsiel, N., Wilairat, P., Wajcik, S.J., Figgit, D.P., Ralph, R.K., Denny, W.A., 1994. Synthesis and *in vitro* evaluation of 9-anilino-3,6-diaminoacridines active against a multidrug resistant strain of the malaria parasite *Plasmodium falciparum*. J. Med. Chem. 37, 1486–1494.

Giorgia, C.D., Shimi, K., Boyer, G., Delmas, F., Galy, J.P., 2007. Synthesis and antileishmanial activity of 6-mono-subsituted and 3,6-disubsituted acridines obtained by arylation of proflavine. Eur. J. Med. Chem. 42, 1277–1284.

Goodwell, J.R., Basagoiti, F.P., Forsley, B.M., Shi, P.Y., Fergudon, D.M., 2006. Identification of compounds with anti-west nile virus activity. J. Med. Chem. 49, 2127–2137.

Guddneppanavar, R., Saluta, G., Kucera, G.L., Bierbach, U., 2006. Synthesis, biological activity and DNA damage profile of platinium-threading intercalator conjugates designed to target adenine. J. Med. Chem. 49, 3204–3214.

Hagan, D.J., Gimenez-Arnau, E., Schwalbe, C.H., Stevens, M.F.G., 1997. Antitumour polycyclic acridines. Part 1. Synthesis of 7H-pyrido-and 8H-quino-[4,3,2-*kl*]acridines by Graebe-Ulmann thermolysis of 9-(1,2,3-triazol-1-yl)acridines: application of differential scanning calorimetry to predict optimum cyclisation conditions. J. Chem. Soc. Perkin. Trans. 1, 2739–2746.

Kohn, K.W., Orr, A., Connor, P.M., 1994. Synthesis and DNA sequence selectivity of series of mono- & difunctional 9-aminoacridine nitrogen mustards. J. Med. Chem. 37, 67–72.

Kumar, P., Kumar, R., Prasad, D.N., 2013. Synthesis and biological evaluation of new 9-aminoacridine-4-carboxamide derivatives as anticancer agents. Arabian J. Chem. 6, 59–65.

Kumar, P., Kumar, R., Prasad, D.N., 2013. Synthesis and anticancer study of 9-aminoacridine derivatives. Arabian J. Chem. 6, 79–85.

Michael, S., Gaslonde, T., Tillequin, F., 2004. Benzo acronycine derivatives: a novel class of antitumour agents. Eur. J. Med. Chem. 39, 695–755.

Mosmann, T., 1983. Rapid colorimeteric assay for cellular growth & survival: application to proliferation & cytotoxicity assay. J. Immunol. Methods 65, 55–63.

Parkin, D.M., Bray, F., Ferlay, J., Pisani, P., 2005. Global cancer statistics 2002. CA Cancer J. Clin. 55 (2), 74–108.

Su, T.L., Chen, C.H., Huang, L.F., Chen, C.H., Basu, M.K., Zhang, X.G., Chou, T.C., 1999. Synthesis and structure-activity relationships of potential anticancer agents: alkylcarbamates of 3-(9-acridinylamino)-5-hydroxymethylaniline. J. Med. Chem. 43, 4741–4748.

Su, T.L., Chou, T.C., Kim, J.Y., Huang, J.T., Ciszeuska, G., Ren, W.Y., Otter, G.M., Sirotnak, F.M., Watanabe, K.A., 1995. 9-Substituted acridine derivatives with long half-life and potent antitumour activity: synthesis and structure activity relationships. J. Med. Chem. 38, 3226–3235.

Valdes, A.F.C., 2011. Acridine & acridones: old & new structure with antimalarial activity. Open Med. Chem. J. 5, 11–20.

Wainwright, M., 2001. Acridine-a neglected antibacterial chromophore. J. Antimicrob. Chemo. 47, 1–13.

Yim, S.K., Yun, C.H., Ahn, T., Jung, H.C., Pan, J.G., 2005. A continuous spectrophotometric assay for NADPH-cytochrome p450 reductase activity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. J. Biochem. Mol. Biol. 38 (3), 366–369.