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Design and synthesis of acridone-4-carboximide derivatives and their cytotoxic studies in cancer cells

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ABSTRACT

In search of developing potent cytotoxic molecules we have designed and synthesized novel scaffold by linking amines to acridone moiety. Novel acridone-4-carboximide derivatives were evaluated for their cytotoxic potentials against drug sensitive, resistant breast cancer cell lines (MCF7/wt, MCF7/mr and MCF7/dx) by using SRB assay. Moreover, Protein ligand interactions of acridone-4-carboxamide derivatives with P-glycoprotein was performed. Compound 1 and 2 have shown relatively potent cytotoxic activity among the derivatives against sensitive, resistant cell lines. Further, compound 8 has shown good cytoxicity against sensitive cells, but it has poor activity in resistant cells. Perhaps, these derivatives were substrate for MDR pumps P-gp and BCRP. The predicted dock score was in the range of -10.48 to -4.71 attributed for compound 1 and 5 respectively. Protein residues such as TYR953, LEU339 and TYR310 were the major contributors for the H-bonding with docked ligands. Compound 1 showed 2 H-bonds, one each with TYR953, and TYR310 with a bond distance of 2.33Å, and 1.81Å respectively. Hydrogen bonds with both the residues were formed with 2 carbonyl oxygen. Additionally, dominant hydrophobic interactions were formed each between a phenyl ring of acridone nucleus and PHE983, toluene nucleus and PHE72 an TYR953.

KEYWORDS: Acridone, SRB assay, Breast cancer, MCF7, Multidrug Resistance.

1. INTRODUCTION

Cancer is a disease characterized by a change in the controlled mechanisms that manage cell proliferation, differentiation and is continuing to be a major health problem in developing as well as undeveloped countries (Harris et al., 1993)[1]. Malignancy is caused by abnormalities in cells, which might be due to inherited genes or caused by outside exposure of the body to chemicals, radiation, or even infectious agents (Liotta et al., 1991; Mignatti et al., 1993)[2,3]. Multidrug resistance (MDR) is a phenomenon by which cancer cells evade the cytotoxic effects of chemotherapeutic agents. MDR is one of the major difficulties to the successful pharmacologic treatment of tumors (Simon et al., 1994)[4]. Several different mechanisms have been proposed to explain the development of an MDR phenotype in cancer cells, such as an alteration in the drug's specific target, the reduced uptake or increased efflux of a drug, the reduced capacity to enter apoptosis, an increase in the ability to repair DNA damage, a different compartmentalization, and an increased rate of detoxification of the drug (Gottesman et al., 2002)[5]. The over expression of efflux pumps that belong to the ATP-binding cassette family of transporters, such as the Pglycoprotein (P-gp) and the MDR-associated proteins (MRPs) causes tumor cells to become resistant to a variety of anticancer drugs (von Bossanyi et al., 1997)[6].

Several drugs have been investigated in the continuing quest to reverse P-gp mediated drug resistance in cancer and some have undergone clinical trials, but now none of them are in clinical use. Unfortunately, most of these drugs suffer clinically from their intrinsic toxicity or from undesired effects on the pharmacokinetics of the accompanying anticancer drugs. Therefore, identification of novel therapeutic strategies that will reverse resistance or sensitize resistant tumor cells to chemotherapeutics or immunotherapeutics remains one of the toughest challenges yet to overcome.

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Several planar molecules containing tricyclic structures with different side chains have been reported to possess useful cytotoxic and/or cytostatic potencies. These tricyclic systems include anthraquinone, acridine, and xanthene. A number of acridone alkaloids have been isolated from plants of the Rutaceae family. Acronycine possesses significant antitumor activity (Hughes et al., 1948; Svoboda et al., 1966)[7,8]. Glyfoline, another natural acridone alkaloid, was found to be the most potent compound for inhibition of cellular growth of human leukemia HL-60 cells in vitro (Chou et al., 1989)[9]. Several acridone derivatives have been shown to be a P-glycoprotein (P-gp)-mediated multidrug resistance (MDR) inhibitor (Hyfali et al., 1993; Walstab et al., 1999; Traunecker et al., 1999; Horton et al., 1997)[10-13].

Acridones have been studied as P-gp and to a lesser extent as MRP family protein inhibitors. An acridonecaboxamide derivative GF120918 has been identified as inhibitor of P-gp and ABCG2 (Wallstab et al., 1999)[11]. The potency of this molecule was evaluated by dose dependent sensitization of CHRC5, OVI/DXR and MCF7/Adr cells to the cytotoxicity of vincristine and doxorubicin respectively. Another imidazo acridone derivative (C1311) has a very good anticancer and anti- MDR activity against a number of human cancer cell lines (Wieslaw et al., 1990)[14]. In recent studies a series of acridone derivatives have been synthesized as reverters of drug resistance in cancer and developed an efficient pharmacophore model (Rajendra Prasad et al., 2008; Rajendra Prasad et al., 2011; Rajendra Prasad et al., 2013)[15-17]. In view of potency of acridones against resistant cells, in the present study we designed and synthesized acridone carboxamide derivatives and tested their cytotoxic properties against sensitive and resistant cancer cells.

2. MATERIALS AND METHODS

All chemicals and solvents were supplied by Sigma Aldrich, India and S.D.Fine Chemicals Limited, Mumbai. Reactions were monitored by TLC and compounds were purified by using column chromatography with silica gel Merck Grade 60 (230–400 mesh, 60; Merck, Germany). Melting points were recorded on a Tempirol hot-stage with microscope (AGA International, Ambala City, Haryana, India) and are uncorrected. Elemental analysis was performed and found values are within 0.4% of theoretical values unless otherwise noted. ^{1}H- and ^{13}C-NMR spectra were recorded in DMSO-d6 solution in a 5-mm tube on a Bruker drx 500 Fourier transform spectrometer (Bruker Bioscience, USA) and TMS was used as internal standard. Chemical shifts are expressed as δ (ppm) values. The spectrometer was internally locked to the deuterium frequency of the solvent. To obtain molecular weight information, acridone derivatives were analyzed by ESI-MS spectrometry. Collision-induced dissociation (CID) spectra were acquired in the positive ion mode on an MDS Sciex (Concord, Ont., Canada) API 4000 triple quadrupole mass spectrometry with a direct infusion of each acridone at a concentration of 10 µM in 50 % methanol, at a flow rate of 25 µL/ min. The instrument was operated with a spray voltage of 5.5kV, a declustering potential of 50 eV, a source temperature of 100^{0} C, a GSI value of 50 and the curtain gas set at 10. Ultra-pure nitrogen was used as both curtain gas and collision gas. MS/MS spectra of the protonated molecule of each drug were acquired and multiple reaction monitoring (MRM) transition for important fragments were monitored as the collision energy was ramped from 5-100 V (step size 0.5 V). The data for the fragment-ion curves represent an average of five consecutive experiments.

2.1. Chemistry

2.1.1. Preparation of 2, 2'-iminodibenzoic acid (C): Ullmann Condensation:

To a mixture of o- chloro benzoic acid (7.8 g, 0.05 mole), anthranilic acid (6.85 g, 0.05 moles) and copper powder (0.2 g) in 60 mL isoamylalcohol, dry potassium carbonate (4 g) was slowly added and the contents were allowed to reflux for 7-8 hours in an oil bath at 170 ^{0} C. The isoamylalcohol was removed by steam distillation and the mixture was poured into one liter of hot water and acidified with concentrated hydrochloric acid. The precipitate formed was filtered, washed with hot water and collected. The crude acid was dissolved in aqueous sodium hydroxide solution, boiled in the presence of activated charcoal and filtered. On acidification of the filtrate with concentrated hydrochloric acid, light yellowish precipitate was obtained which was washed with hot water and recrystallized from aqueous methanol to give light yellow solid (yield 8.8 g, 70 %, mp 187^{0}C)

2.1.2. Cyclisation of 2, 2'-iminodibenzoic acid to 9-Oxo-9, 10-dihydroacridine -4-carboxylic acid (D)

Five grams of 2, 2'-iminodibenzoic acid (C) was taken into a round bottom flask and 50 g of polyphosphoric acid was added to it. The reaction mixture was shaken well and heated on a water bath at 100^{o}c for 3 hours. Appearance of yellow colour indicated the completion of the reaction. Then, it was poured into one liter of hot water and made alkaline by liquor ammonia. The yellow precipitate that formed was filtered and collected. The sample 9-oxo-9, 10-dihydroacridine-

4-carboxylic acid (D) was recrystallized from acetic acid. Further, the purity of the compound was checked by TLC and the purified product was characterized by spectral methods.

Yield 70%; mp: 326-328^{0}C; ^{1}H NMR (DMSO-d_6) δ ppm: 12.68 (s, 1H, OH), 9.23 (s, 1H, NH), 7.29-8.48 (m, 7H, Ar-H); ^{13}C NMR (DMSO-d_6) δ: 178.4, 158.3, 139.5, 138.2, 133.5, 131.9, 129.5, 128.5, 125.4, 124.3, 123.4, 120.9 ; ESI-MS (m/z, %): 240.01 (100); Anal. Calculated for $C_{14}H_9NO_3$: C-70.29, H-3.79, N – 5.86; Found: C-69.96, H-3.47, N-5.63.

2.1.3. General method for synthesis of 9-oxo-N-(p-tolyl)-9, 10-dihydroacridine-4-carboxamide (1):

To the suspension of compound **D** (1 gm, 4.18 mmol) in 15 ml of dry toluene, thionyl chloride (0.42 ml, 5.81mmol) and freshly dried pyridine (0.46 ml, 5.81 mmol) were added. The reaction mixture was stirred at room temperature for 3-4 hrs and then combined with excess of p-toluidine (1.38 gm, 12.89 mmol) and triethylamine (1.72 ml, 12.37 mmol) and stirred for another 3 hrs. The reaction was monitored by TLC. The following day the solvent was removed under vacuum and water was added to the solid residue. The precipitate was filtered, washed with water and dried. The crude product was crystallized from n-butanol-DMF (19:1).

Yield: 70%, mp: 338-340^{0}C. ^{1}H NMR (DMSO-d_6) δ ppm: 2.33(3H, CH_3, s), 6.98-8.54 (m, 11H, Ar-H), 10.61 (s, 1H, CONH),11.81 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 175.7, 167.3, 139.5, 138.1, 133.5, 132.1, 129.9, 129.2, 129.2, 126.4, 121.8, 121.5, 121.5, 121.3, 120.4, 119.9, 118.8, 118.0, 21.3; ESI-MS (m/z, $[M+H]^+$ %): 329.2 (100); Anal. Calculated for $C_{21}H_{16}N_2O_2$: C-76.81, H-4.91, N – 8.53; Found: C-76.49, H-4.58, N-8.31.

2.1.4. Preparation of 4-[(4-phenylpiperazine-1-carbonyl] acridin-9(10H)-one (2):

Compound 2 was prepared from ACA (**D**) and 1-phenyl piperazine (0.98ml, 6.449 mmol) as yellow solid, using an approach similar to that described for compound 1.

Yield: 75 %, mp: 178-180 ^{0}C. ^{1}H NMR (DMSO-d_6) δ ppm: 3.10 (m, 2H, CH_2), 3.25 (m, 4H, CH_2), 3.90 (m, 2H, CH_2), 7.19-8.29 (m, 12H, Ar-H), 10.91 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 175.7, 168.9, 149.6, 139.3, 138.1, 133.3, 131.8, 129.6, 129.6, 127.5, 126.5, 121.9, 121.5, 121.4, 119.9, 118.8, 117.7, 114.3,53.3, 53.3, 50.0, 50.0; ESI-MS (m/z, $[M+H]^+$ %): 384.2 (100); Anal. Calculated for $C_{24}H_{21}N_3O_2$: C-75.18, H-5.52, N-10.96; Found: C-74.85, H-5.21, N-10.74.

2.1.5. Preparation of 4-[(4-ethylpiperazine-1- carbonyl] acridin-9(10H)-one (3)

Compound 3 was prepared from ACA (**D**) and 1-ethyl piperazine (0.81ml, 6.449 mmol) as pale yellow solid, using an approach similar to that described for compound 1.

Yield: 45 %, mp: 231-233^{0}C. ^{1}H NMR (DMSO-d_6) δ ppm**:** 1.00-1.11 (t, 3H, CH_3), 1.18-1.23 (m, 2H, CH_2), 2.72-2.89 (m, 4H, CH_2), 2.92-3.10 (m, 4H, CH_2), 7.31-8.46 (m, 7H, Ar-H), 10.87 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 177.7, 167.9, 138.7, 138.5, 133.3, 131.7, 127.9, 126.5, 121.5, 119.7, 118.8, 117.7, 56.5, 56.5,50.4, 50.4, 49.6,13.3 ;ESI-MS (m/z, $[M+H]^+$ %): 336.2 (100); Anal. Calculated for $C_{20}H_{21}N_3O_2$: C-71.62, H-6.31, N – 12.53; Found: C-71.29, H-5.99, N-12.32.

2.1.6. Preparation of 4-[(2-oxopyrrolidine-1-carbonyl) acridin-9(10H)-one (4)

Compound 4 was prepared from ACA (**D**) and 2-pyrrolidinone (0.49ml, 6.449 mmol) as yellow solid, using an approach similar to that described for compound 1.

Yield: 60 %, mp: 205-207 ^{0}C. ^{1}H NMR (DMSO-d_6) δ ppm: 2.06-2.09 (m, 2H, CH_2), 3.08-3.22 (t, 2H, CH_2), 3.98 (t, 2H, CH_2), 7.35-8.54 (m, 7H, Ar-H), 10.93 (s, 1H, NH),; ^{13}C NMR (DMSO-d_6) δ: 175.7, 172.4, 170.6, 139.6, 138.0, 133.3, 132.1, 129.9, 126.5,121.5, 120.4, 119.9, 118.7, 49.0, 32.0, 17.2; ESI-MS (m/z, $[M+H]^+$ %): 307.2 (100); Anal. Calculated for $C_{18}H_{14}N_2O_3$: C-70.58, H-4.61, N-9.15; Found: C-70.25, H-4.30, N-8.94.

2.1.7. Preparation of 4-(4-(furan-2-carbonyl) piperazine-1-carbonyl) acridin-9(10H)-one (5)

Compound 5 was prepared from ACA (**D**) and 1-(2-furoyl) piperazine (0.43ml, 3.22 mmol) as yellow solid, using an approach similar to that described for compound 1.

Yield: 55 %, mp: 180-182 ^{0}C. ^{1}H NMR (DMSO-d_6) δ ppm: 3.64 (m, 4H, CH_2), 3.85 (m, 4H, CH_2), 7.02-8.54 (m, 10H, Ar-H), 10.90 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 175.7, 168.9, 155.1, 147.0, 143.8, 139.3, 138.1, 133.3, 131.8, 127.5, 126.5, 121.5, 121.4, 119.9, 118.8, 117.9, 117.7, 111.7, 49.9,49.8; ESI-MS (m/z, $[M+H]^+$ %): 402.2 (100); Anal. Calculated for $C_{23}H_{19}N_3O_4$: C-68.82, H-4.77, N-10.47; Found: C-68.51, H-4.43, N-10.26.

2.1.8. Preparation of 1-[(9-oxo-9, 10-dihydroacridine-4-carbonyl) piperidine-2, 6-dione (6)

Compound 6 was prepared from ACA (**D**) and glutarimide (0.729ml, 6.449 mmol) using an approach similar to that described for compound 1.

Yield: 68 %, mp: 204-206 ^{0}C. ^{1}H NMR (DMSO-d_6) δ ppm: 2.41-2.43(m, 4H, CH_2), 3.06 (m, 2H, CH_2), 7.18-8.55 (m, 7H, Ar-H), 11.96 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 175.7, 174.7, 174.8, 168.0, 139.6, 138.1, 133.7, 132.1, 129.9, 126.3,121.5, 120.4, 119.9, 118.8, 31.8, 31.8, 16.9; ESI-MS (m/z, %): 334.1 (100); Anal. Calculated for $C_{19}H_{14}N_2O_4$: C-68.26, H-4.22, N-8.38; Found: C-67.93, H-3.90, N-8.16.

2.1.9. Preparation of 1-[(9-oxo-9, 10-dihydroacridin-4-carbonyl) piperidine-4-carboxylic acid (7)

Compound 7 was prepared from ACA (**D**) and Isonipecotic acid (0.83 gm, 6.44 mmol) using an approach similar to that described for compound 1.

Yield: 65 %, mp: 222-224 ^{0}C. ^{1}H NMR (DMSO-d_6) δ ppm: 1.61-1.86 (m, 4H, CH_2), 2.37 (m, 1H, CH), 3.29-3.39 (m, 4H, CH_2), 10.93 (s, 1H, NH), 11.1(s, 1H, OH); ^{13}C NMR (DMSO-d_6) δ: 176.7, 175.1, 172.5, 139.3, 138.1, 133.3, 131.8, 127.5, 126.5,121.5, 121.4, 119.9, 118.8, 117.7, 44.9, 44.9, 39.9, 28.7; ESI-MS (m/z, %): 351.1 (19); Anal. Calculated for $C_{20}H_{18}N_2O_4$: C-68.56, H-5.18, N-8.00; Found: C-68.22, H-4.87, N-7.78;

2.1.10. Preparation of 4-(4-(4-acetylphenyl) piperazine -1-carbonyl) acridin-9(10H)-one (8)

Compound 8 was prepared from ACA (**D**) and 4-piperazino acetophenone (0.576 gm, 3.22 mmol) as yellow solid, using an approach similar to that described for compound 1.

Yield: 68 %, mp: 172-175 ^{0}C. ^{1}H NMR (DMSO-d_6) δ ppm: 2.45(s, 3H, CH_3), 3.26 (m, 4H, CH_2), 3.51 (m, 4H, CH_2), 6.95-8.42 (m, 11H, Ar-H), 10.90 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ: 197.0, 175.7, 168.9, 139.3, 138.0, 135.7, 133.3, 131.8, 130.5, 129.7,129.7, 127.8,127.9, 127.5, 126.5, 121.3, 121.6, 119.7, 118.6, 117.3, 53.3, 50.0, 26.6; ESI-MS (m/z, $[M+H]^+$ %): 426.1(100); Anal. Calculated for $C_{26}H_{23}N_3O_3$: C-73.39, H-5.45, N-9.88; Found: C-73.05, H-5.11, N-9.66;

2.2. In-vitro cytotoxic studies by SRB assay:

The acridone derivatives were evaluated for cytotoxicity against different cancer cells by using the sulforhodamine B (SRB) (Keepers et al., 1991)[18]. In brief, cells were cultured in RPMI 1640 supplemented with 10 % fetal calf serum, and cultures were passed twice a week using trypsin EDTA to detach the cells from their culture flasks. The fast growing cells were harvested, counted, and plated at suitable concentrations in 96-well microplates. After incubation for 24 hours, the compounds were dissolved in the culture medium, added to the culture wells in triplicate and incubated further for 72 hours at 37^{0}C under 5% CO_2 atmosphere. The cultures were fixed with cold TCA and stained with 0.4 % SRB dissolved in 1 % acetic acid. After dissolving the bound stain with 150 µl of 10 mM unbuffered Tris base (Tris(hydroxymethyl)aminomethane) solution using gyratory shaker, absorbance was measured at 540 nm using a microplate reader (Tecan). The cytotoxicity was assessed by measuring the concentration required to inhibit protein synthesis by 50 % (i.e., IC_{50}) as compared. Each value represents the mean of triplicate experiments.

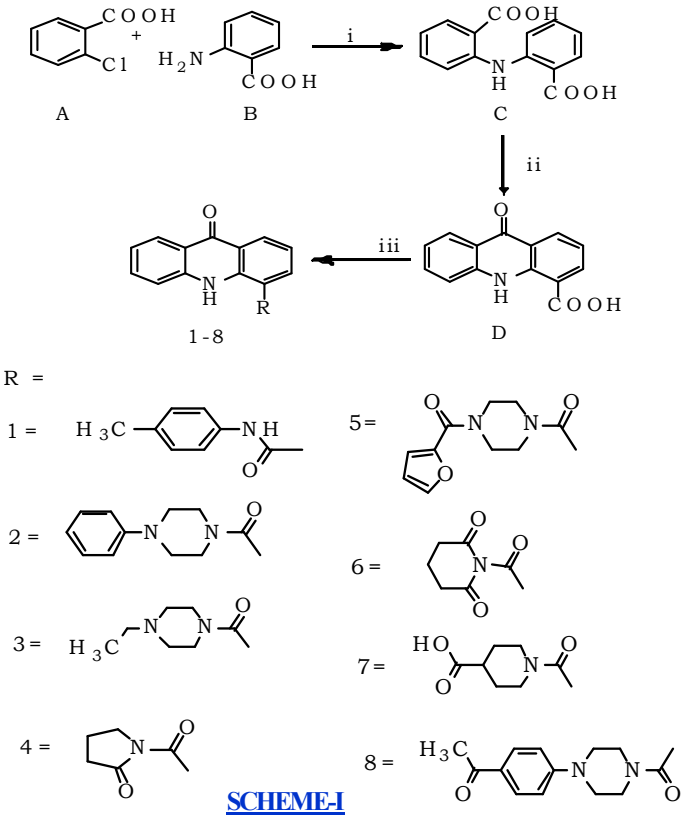
2.3. Molecular Docking Studies

Digital 3D structure of P-glycoprotein was retrieved from PDB website (PDB ID: 3G60) and structurally optimized using PRIME module of the Schrodinger Suite (Prime, 2013)[19]. Protein structure was optimized by removing non-protenous components such as waters, ions, and ligands, adding missing amoni acids, satisfying valency by adding hydrogen atoms, and energy was minimized using OPLS force field. Similarly, structures of all the listed molecules were drawn and optimized using Ligprep tool of Schrodinger Suit and used for molecular docking studies (Ligprep, 2013)[20]. Protein ligand interaction of the data set ligands with geometrically optimized p-gp were identified using Glide XP protocol of Schrodinger suite. A grid was generetaed on the protein and was used prior for docking (Babu et al., 2014)[21].

3. RESULTS AND DISCUSSION

3.1. Chemistry

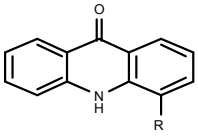
We first synthesized 2, 2'-iminodibenzoic acid (IA) on a preparative scale, using the method of Ulmann copper-catalysed condensation of anthranilic acid with ortho-chlorobenzoic acid. Cyclization to the acridone-4-carboxylic acid (ACA) (IB) was achieved by heating compound 1 with excess polyphosphoric acid at 100 ^{0}C (Gaidukevich et al., 1985; Rewcastle et al., 1985)[22,23]. The synthesis of ACA (IB) and its amides is shown in Scheme 1. *N*-Substituted acridone-4-carboxamides were synthesized by amide bond formation between an activated form of ACA and an appropriate amine. A convenient technique for carboxylic group activation was developed that allowed us to obtain high yields of the products. Following this methodology, we prepared an essentially complete set of acridone-4-carboxamides whose amide fragments were formed by the alkyl groups or aryl-, heteryl-ring systems bearing exocyclic groups at different ring positions. The structures of the compounds studied are presented in Table 1.

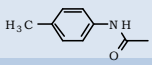
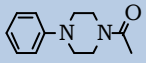
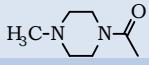
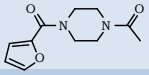
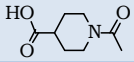
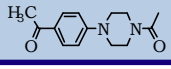


SCHEME-I

Reagents and conditions: (i) Cu, K_2CO_3, Isoamylalcohol, Reflux for 6-8hrs, 160^{0}C; (ii) PPA, 100^{0}C, 3hrs (iii) $SOCl_2$ / Dry Pyridine, Dry Toluene, amine, Et_3N, room temp, 7-8hrs

Table: 1 Physical characterization data of acridone-4-carboxamide derivatives



Compound code	R	Molecular Formula	Mol. Weight	Yield %	Melting point ^{o}C	TLC R_f Value
D	-COOH	$C_{14}H_9NO_3$	239.22	69	326-328	0.4
1		$C_{21}H_{16}N_2O_2$	328.36	72	338-340	0.5
2		$C_{24}H_{21}N_3O_2$	383.44	80	178-180	0.6
3		$C_{19}H_{19}N_3O_2$	321.37	36	130-132	0.7
4		$C_{18}H_{14}N_2O_3$	306.32	70	205-207	0.6
5		$C_{23}H_{19}N_3O_4$	401.41	45	180-182	0.5
6		$C_{19}H_{14}N_2O_4$	334.33	68	204-206	0.6
7		$C_{20}H_{18}N_2O_4$	350.37	65	222-224	0.8
8		$C_{26}H_{23}N_3O_3$	425.48	68	172-175	0.6

3.2. In vitro Cytotoxicity Sudies:

Synthesized acridone derivatives were evaluated for their *in-vitro* cytotoxic activity in comparison with standard drugs mitoxantrone, doxorubicin against different breast cancer cell lines MCF7-wt including its drug resistant cell lines MCF7/mr (BCRP expressed), MCF7/dx (Pgp expressed) by using sulforhodamine B (SRB) assay. The cytotoxic date is presented in Table 2 as IC_{50} concentrations in micromoles and the results shown that compound **1** and **2.**

Table: 2 *In vitro* cytotoxic activity of the compounds against sensitive and resistant human cancer cell lines in comparison with reference drugs doxorubicin (DX) and mitoxantrone (MR).

Compound	Cell lines[a]/IC_{50} (µM) ± SEM[b]		
	MCF-7/wt	MCF-7/mr	MCF-7/dx
1	6.5±1.8	7.4±2.1	8.3±2.5
2	4.5±1.6	5.1±1.8	6.5±2.1
4	12.2±2.5	22.2±2.6	26.5±2.4
6	23.0±3.9	31.0±2.1	47.8±2.8
7	10.5±2.3	22.8±1.9	33.1±2.5
8	6.1±2.0	10.5±1.6	8.5±1.9
DX	0.098	ND	3.7
MR	0.001	1.8	ND

[a]MCF7/wt, MCF7/mr (BCRP expressed), MCF7/dx (Pg-P expressed) which are breast cancer cell lines. [b]SEM - standard error of the mean

3.3. Molecular Docking Studies

The results in terms of dock score (XP GScore) was presented in the Table 3. Predicted dock scores were in range of -10.48 to -4.71 attributed for compound **1** and **5** respectively. Protein residues such as TYR953, LEU339 and TYR310 were the major contributors for the H-bonding with docked ligands. Compund **1** showed 2 H-bonds, one each with TYR953, and TYR310 with bond distance of 2.33Å, and 1.81Å respectively. Hydrogen bonds with both the residues were formed with 2 carbonyl oxygen (Figure 1). Additionally, dominant hydrophobic interactions were formed each between phenyl reing of acridone nucleus and PHE983, toluene nucleus and PHE72 an TYR953 (Figure 2).

Table 3: Protein ligand interactions of acridone-4-carboxamide derivatives with P-glycoprotein

Compound	Dock score	No. of Hydrogen Bonds	Interacted Protein Residues	H-bond Distance (Å)
1	-10.48992	2	TYR953, TYR310	2.33, 1.81
2	-6.200387	2	TYR953, LEU339	2.12, 1.46
3	-8.70411	1	TYR953	2.25
4	-5.01719	1	TYR953	2.17
5	-4.7177	--	--	--
6	-5.062077	2	TYR953, TYR310	2.13, 1.67
7	-4.9061	--	TYR953	1.98
8	-5.062077	2	TYR953, TYR310	2.13, 1.67

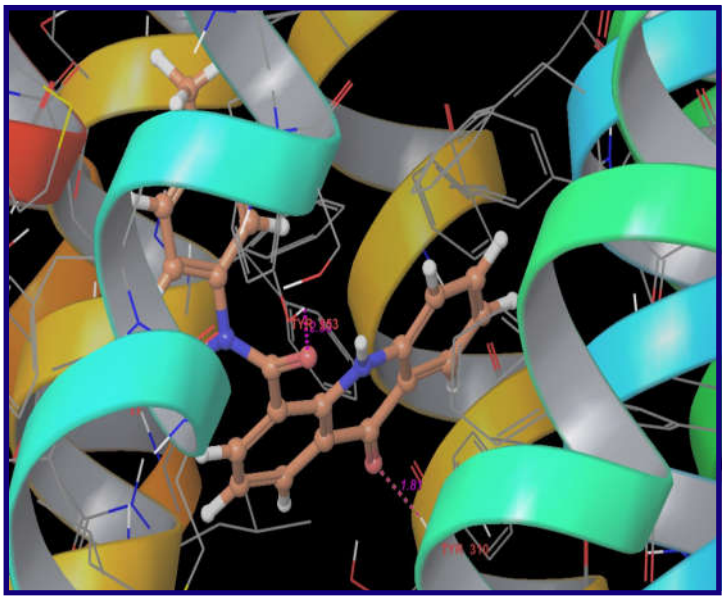


Figure 1: Protein-ligand binding interactions and conformations of compound 1

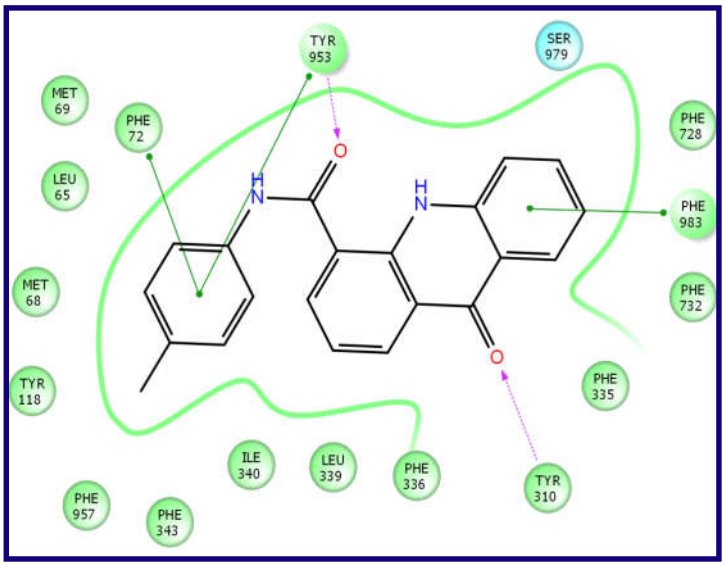


Figure 2: Hydrogen bonding and hydrophobic interactions of compound 1 with P-glycoprotein

Cytotoxicity studies have shown relatively potent cytotoxic activity among the derivatives against sensitive, resistant cell lines. Further, compound **8** has shown good cytoxicity against sensitive cells but it has lower activity against drug resistant cells. Compounds exhibited good to moderate cytotoxic properties against different sensitive and resistant cancer cells. Structure activity relationship analysis suggests the presence of larger hydrophobic group attached to the six membered cyclic nucleus at R1 position increases the cytotoxic activity against both drug sensitie and resistance cell lines.

4. CONCLUSION

In the present investigation, we have designed a novel scaffold by tagging acridone with different secondary amines through amide Bridge, with the aim to enhance the cytotoxicity. The molecules were evaluated for their cytotoxic activity against sensitive and resistant (P-gp and BCRP expressed) breast cancer cells and three types of colon cancer cells. Results indicate compound 1 and 2 shown good cytotoxic activity against MCF7/wt cells with IC_{50} of 6.5 and 4.5 µM respectively. The compound 2 also showed good activity against drug resistant cell lines MCF-7/mr and MCF-7/dx with IC_{50} of 5.1 µM and 6.5 µM respectively. These acridone analogues were shown good cytotoxicity in resistant cancer cells (P-gp and BCRP expressed), Perhaps these derivatives were substrate for MDR pumps P-gp and BCRP. Further, Compund 1 showed two H-bonds, one each with TYR953, and TYR310 with bond distance of 2.33Å, and 1.81Å respectively. Hydrogen bonds with both the residues were formed with two carbonyl oxygen. Additionally, dominant hydrophobic interactions were formed each between phenyl ring of acridone nucleus and PHE983, toluene nucleus and PHE72 and TYR953.

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Conflict of Interest

Authors declare no conflicts

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