

# Synthesis and Biological Evaluation of Novel Triazolyl-Acridine Derivatives as Cytotoxic Agents

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Singh *et al.*: Synthesis of Novel Triazolyl-Acridine derivatives as Cytotoxic Agents

Novel triazolyl-acridine compounds were synthesized in 4 series of 9-(2-(substituted phenyl-1H-1,2,3-triazol-1-yl)ethoxy)acridine, 9-(3-(4-(2-substituted phenyl)-1H-1,2,3-triazol-1-yl)propoxy)acridine, N-(2-(4 substituted phenyl-1H-1,2,3-triazol-1-yl)ethyl)acridin-9-amine and N-(3-(4-(substituted phenyl)-1H-1,2,3-triazol-1-yl)propyl)acridin-9-amine using appropriate synthetic procedures and screened for cytotoxic activity. The structures of all synthesized compounds were confirmed by Fourier-transform infrared spectroscopy, proton nuclear magnetic resonance and mass spectroscopy and these compounds were assayed *in vitro* for cytotoxic activity against MCF-7 (human breast adenocarcinoma cell line) and HT-29 (human colon adenocarcinoma cell line) cells. Tested compounds showed better cytotoxic activities in terms of IC<sub>50</sub> value against MCF-7 and HT-29 cells. Methyl substituted compound MPP-9 exhibited excellent sensitivity with IC<sub>50</sub> value 1 and 2 μM, against MCF-7 and HT-29, respectively. Unsubstituted MPP-1 and chloro-substituted MPP-2 and MPP-5 also exhibited good IC<sub>50</sub> value ranges from 2-4 μM against both cell lines. These compounds were active at micro molar concentrations. Data study revealed that synthesized compounds are promising leads for future as cytotoxic agents.

**Key words:** Acridine, Triazol, Cytotoxic, Cell line

Without a doubt, no one deny the fact that cancer is an epidemic greater than one of the biggest medical challenges of the century<sup>[1]</sup>. There are many types of cancer e.g. breast cancer, colon cancer, lung cancer, prostate cancer, but all types of cancer have similarity of uncontrolled proliferation or uncontrolled symmetric cell division<sup>[2]</sup>. Technologies for diagnosis and treatment are highly advanced now a day, but the treatment still remains poor.

Literature survey revealed many heterocyclic organic compounds like acridines, triazoles, which are effective against cancer. Acridine is a tricycle organic compound with a nitrogen heterocycle (fig. 1) having molecular formulae C<sub>13</sub>H<sub>9</sub>N, which can also be named as 10-azaanthracene and dibenzo[b,e]pyridine<sup>[3]</sup>. Inhibitory activity on tumor cells and binding to DNA is the popular molecular strategy. DNA targeting is a recent trend in innovation of new anticancer agents. In 1998, a quantitative structure-activity relationship (QSAR) analysis of 9-anilinoacridines with respect to

antitumor activity and binding to DNA was reported by Gao *et al.*<sup>[4]</sup>. Intercalative binding of proflavine to bacterial nucleic acids is the site of action<sup>[5]</sup>, which led to the development of acridine derivatives for modern anticancer chemotherapy e.g. m-AMSA, although the actual site of action of such derivatives is now established at the level of DNA coiling enzymes (topoisomerase) rather than DNA itself, damage being caused by the stabilization of the enzyme-DNA cleavage complex<sup>[6,7]</sup>. Acridine derivatives are not only effective antitumor<sup>[8]</sup> but also effective anti-inflammatory<sup>[9]</sup>, antibacterial<sup>[10]</sup>, antimalarial<sup>[11]</sup> and antiviral agents<sup>[12]</sup>.

Triazoles are heterocyclic organic compounds containing five-membered ring with 3 nitrogen and

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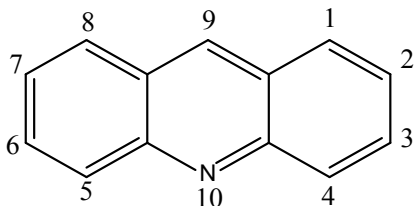
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**Fig. 1: Structure of acridine**

Two carbon atoms. Two isomeric forms of triazoles exist namely 1,2,3-triazole and 1,2,4-triazole<sup>[13]</sup>, with molecular formula  $C_2H_3N_3$ . 1,2,3-triazole is a basic aromatic heterocycle. The medicinal chemists have considered the synthesis of 1,2,3-triazole based heterocycles as the corner stone of medicinal chemistry due to their important biological activities like anticancer<sup>[14]</sup>, antitubercular<sup>[15]</sup>, antibacterial<sup>[16]</sup>, antiinflammatory<sup>[17]</sup>, antimicrobial<sup>[18]</sup> and antiviral<sup>[19]</sup>. The features possessed by the 1,2,3-triazoles make them pharmaceutically important molecules.

Thus, both acridines and 1,2,3-triazoles are important pharmacophores with anticancer activity. Both compounds are heterocyclic, small in size, with ease of synthesis and reported to possess extensively anticancer activity. Therefore in this investigation both structures were combined to synthesize novel triazolyl-acridine derivatives for evaluating anticancer activity against different cell lines.

## MATERIALS AND METHODS

Synthetic grade chemicals were used. Thin layer chromatography (TLC) on precoated TLC plates was used to monitor the synthesis and finally prepared compounds. Chloroform:methanol (9:1) was used as the mobile phase for TLC and iodine chamber to visualize spots. Open glass capillary method was used for melting point determination. Infrared (IR) spectra were recorded on Bruker's FT-IR spectrophotometer as KBr pellet and values are expressed in  $cm^{-1}$ .  $^1H$  nuclear magnetic resonance spectroscopy (NMR) spectral analysis of the synthesized compounds were recorded on a Bruker Avance II 400 MHz in deuterated chloroform ( $CDCl_3$ ) using tetramethylsilane as internal standard. Chemical shift values were recorded on  $\delta$ -scale. Syntheses of the triazolyl-acridine derivatives were performed according to the scheme presented in fig. 2.

### Synthesis of 9-chloroacridine and N-phenylanthranilic acid:

9-Chloroacridine was synthesized using modified Ullman-Goldberg reaction<sup>[20]</sup>. N-phenylanthranilic

acid was synthesized by mixing equal quantities of (0.038 mol) of o-chlorobenzoic acid and aniline with 0.12 g of copper powder in 40 ml isoamyl alcohol to which 6 g of dry potassium carbonate was added slowly and the final mixture was refluxed for 6 to 8 h. After reflux, isoamyl alcohol was removed by distillation, the mixture poured into 500 ml of hot water and filtered. The filtrate was acidified with concentrated hydrochloric acid. Yellow precipitate formed, filtered, washed with hot water and collected.

### Synthesis of 9-chloroacridine by cyclization of n-phenylanthranilic acid:

Five grams (0.023 mol) of N-phenylanthranilic acid was mixed with 16 ml (27 g, 0.176 moles) of phosphorus oxychloride taken in a 500 ml round bottom flask fitted with a water-cooled condenser and heated about 15 min at 85-90°, then temperature is raised to 135-140°, where it was maintained for 2 h. Excess phosphorus oxychloride was removed by simple distillation. After cooling, the residue was poured into a well-stirred mixture of 20 ml of concentrated ammonia solution, 50 g of ice and 20 ml of chloroform in a separating funnel and allowed to stand for 30-40 min. The chloroform layer was separated and evaporated, greenish gray powder obtained was 9-chloroacridine.

### Addition of alkanediol to 9-chloroacridine<sup>[21]</sup>:

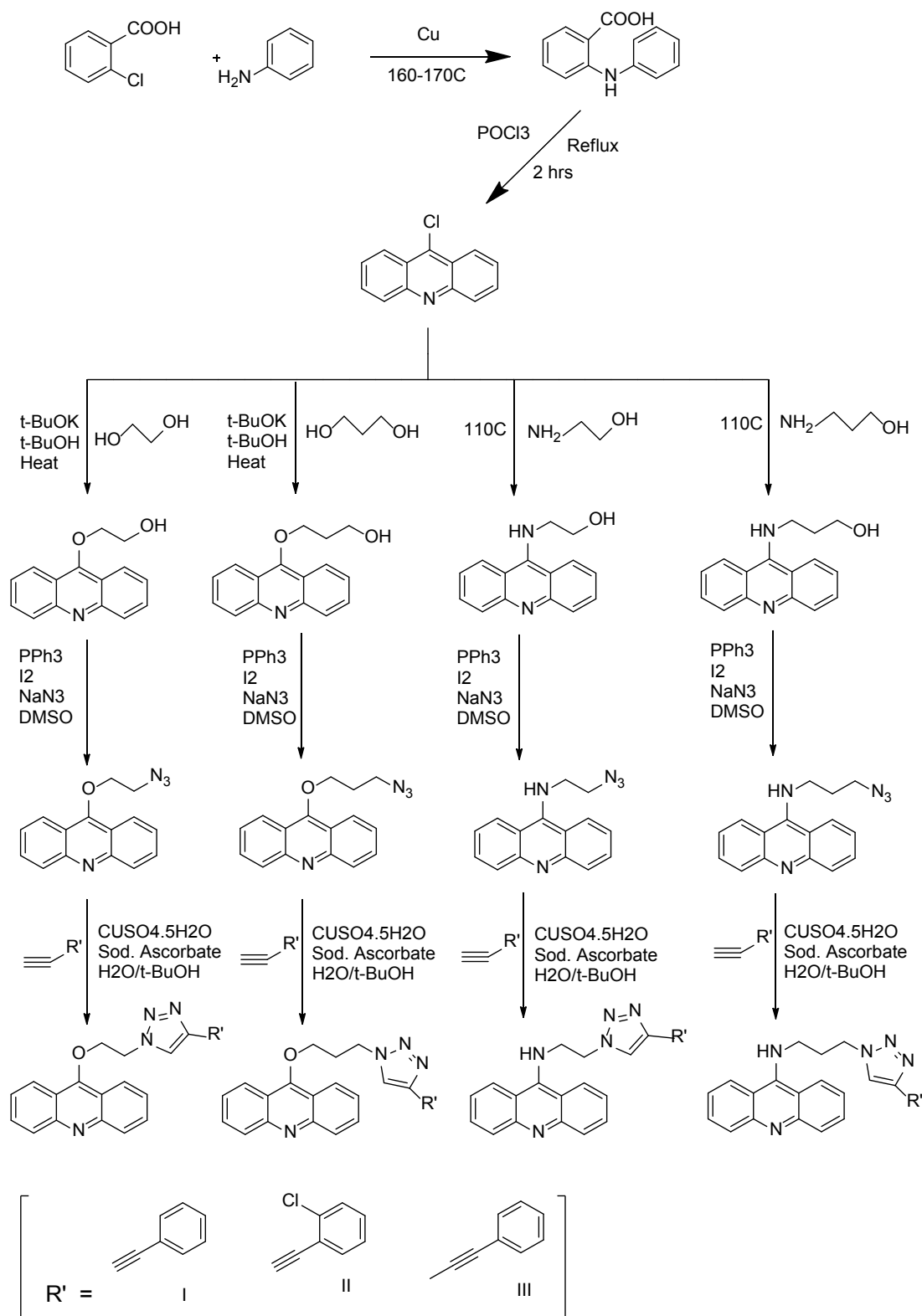
Dissolved 0.2 g (1.0 mmol) of 9-chloroacridine in 2.0 ml of ethylene glycol, under inert atmosphere, a 1.0 M solution of potassium t-butoxide in t-butyl alcohol (1.5 ml, 1.5 mmol, 1.5 equiv) was added, stirred at 80° for 18 h, then quenched with saturated  $NaHCO_3$ . The mixture was extracted with  $CH_2Cl_2$ , dried over anhydrous  $MgSO_4$ , concentrated in vacuum, and purified by recrystallization from  $CHCl_3$  to yield 9-acridinyl alkanediol as the product.

### Conversion of 9-chloroacridine to amino alcohols:

9-chloroacridine (1.0 g, 5.1 mmol) and alkanolamine (5 equiv) was heated at 110° for 6 h, under inert atmosphere and then cooled. NaOH 1 N was added and extracted with  $CHCl_3$ . The organic layers were washed with brine, dried over anhydrous  $Na_2SO_4$  and evaporated under reduced pressure to yield 9-acridinyl alkanolamine as the product.

### Conversion of alcohols into azides<sup>[22]</sup>:

Firstly, 3 mmol of 9-acridinyl alkanediol or 9-acridinyl alkanolamine, 3.6 mmol of triphenylphosphine and



**Fig. 2: Synthetic scheme to prepare target compounds MPP-1 to MPP-12**

3.6 mmol of iodine was triturated in a mortar and pestle for 10 min, when exothermic reaction took place, paste like consistency appeared. Separately, 12 mmol of sodium azide was dissolved in DMSO, the solution was mixed with acridine paste and stirred for 30 min on a magnetic stirrer. Upon completion of the reaction, ice-

cooled solution of 50 ml of sodium thiosulphate was added and extracted with 30 ml of diethyl ether 3 times. The combined organic layer was washed with brine (50 ml). On evaporation a crude product obtained, which was purified by column chromatography using 5 % ethyl acetate in hexane.

**Conversion of azides in to triazoles<sup>[23]</sup>:**

Three mmol of alkyne and, 3 mmol of azide were suspended in 12 ml of a 1:1 water/tert-butanol mixture. Freshly prepared 1 M sodium ascorbate solution was added, followed by 0.03 mmol of copper (II) sulfate pentahydrate in 100 ml of water. The heterogeneous mixture was stirred vigorously overnight. When TLC analysis indicated complete consumption of the reactants, the reaction mixture was diluted with 50 ml of water and cooled in ice, and the white precipitate was collected as filtrate. After being washed with cold water (225 ml), the precipitate was dried under vacuum to obtain pure triazole compounds, MPP-1 to MPP-12.

MPP-1, 9-(2-(4-phenyl-1H-1,2,3-triazol-1-yl)ethoxy)acridine, yield: 85 %, melting point (MP): 223°,  $R_f$ : 0.45; FT-IR (KBr):  $\text{cm}^{-1}$  3051 C-H str. (triazole), 1463 C-H str. (methylene), 1651 C=N str. (Ar), 1592 N=N str., 1524 C=C str. (Ar), 1308 C-N str., 1254 C-O str. (ether), C-Cl str. (Ar);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$ : 8.14 (d, 2H,  $J=9.44$ , CH), 7.99 (d, 2H,  $J=8.2$ , CH), 7.82 (t, 2H,  $J=4.96, 4.2$ , CH), 7.79 (d, 2H,  $J=6.92$ , CH), 7.63 (t, 2H,  $J=4.08, 3.68$ , CH), 7.59 (s, 1H, CH-triazole), 7.53 (t, 2H,  $J=2.8, 2.96$ , CH-Ar), 7.45 (t, 1H,  $J=2.48, 4.72$ , CH-Ar), 4.50 (t, 2H,  $J=6.56, 7.32$ , CH), 4.16 (t, 2H,  $J=5.24, 5.08$ , CH) ppm; MS (m/z): 367 (M+1).

MPP-2, 9-(2-(4-(2-chlorophenyl)-1H-1,2,3-triazol-1-yl)ethoxy)acridine, yield: 83 %, MP: 242°,  $R_f$ : 0.68; FT-IR (KBr):  $\text{cm}^{-1}$  3048 C-H str. (triazole), 1466 C-H str. (methylene), 1656 C=N str. (Ar), 1541 N=N str., 1510 C=C str. (Ar), 1313 C-N str., 1278 C-O str. (ether), 836 C-Cl str. (Ar);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  8.12 (d, 2H,  $J=10.28$ , CH), 7.94 (d, 2H,  $J=6.8$ , CH), 7.79 (t, 2H,  $J=5.08, 6.84$ , CH-Ar), 7.74 (d, 1H,  $J=8.76$ , CH), 7.64 (t, 2H,  $J=5.24, 3.6$ , CH), 7.59 (s, 1H, CH-triazole), 7.57 (d, 1H,  $J=7.84, 2.96$ , CH-Ar), 7.39 (t, 1H,  $J=4.0, 4.2$ , CH-Ar), 7.35 (t, 1H,  $J=2.88, 6.44$ , CH-Ar), 4.49 (t, 2H,  $J=2.08, 7.52$ , CH), 4.16 (t, 2H,  $J=7.24, 2.56$ , CH) ppm. MS (m/z): 401 (M+1).

MPP-3, 9-(2-(4-(o-tolyl)-1H-1,2,3-triazol-1-yl)ethoxy)acridine, yield: 82 %, MP: 212°,  $R_f$ : 0.58; FT-IR (KBr):  $\text{cm}^{-1}$  3052 C-H str. (triazole), 1455 C-H str. (methylene), 1436 C-H str. (methyl), 1651 C=N str., 1524 N=N str., 1308 C-N str., 1254 C-O str. (ether).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  8.14 (d, 2H,  $J=6.8$ , CH), 7.98 (d, 2H,  $J=6.84$ , CH), 7.83 (t, 2H,  $J=6.44, 1.16$ , CH-Ar), 7.63 (t, 2H,  $J=3.6, 5.32$ , CH), 7.60 (s, 1H, Ar-CH), 7.56 (d, 1H,  $J=9.64$ , CH-Ar), 7.33-7.27 (m, 3H, CH-Ar), 4.51

(t, 2H,  $J=4.88, 4.64$ , CH), 4.15 (t, 2H,  $J=3.6, 5.2$ , CH), 2.58 (s, 3H,  $\text{CH}_3$ ) ppm. MS (m/z): 381 (M+1).

MPP-4, 9-(3-(4-phenyl-1H-1,2,3-triazol-1-yl)propoxy)acridine, yield: 90 %, MP: 242°,  $R_f$ : 0.46; FT-IR (KBr):  $\text{cm}^{-1}$  3054 C-H str. (triazole), 1452 C-H str. (methylene), 1654 C=N str., 1541 N=N str., 1511 C=C str. (Ar), 1307 C-N str., 1259 C-O str. (ether);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  8.13 (d, 2H,  $J=9.28$ , CH), 7.97 (d, 2H,  $J=8.84$ , CH), 7.82 (t, 2H,  $J=1.84, 6.08$ , CH-Ar), 7.79 (d, 2H,  $J=8.44$ , CH), 7.63 (t, 2H,  $J=4.64, 6.0$ , CH), 7.59 (s, 1H, Ar-CH), 7.53 (t, 2H,  $J=4.6, 5.32$ , CH-Ar), 7.42 (t, 1H,  $J=4.12, 4.6$ , CH-Ar), 4.46 (t, 2H,  $J=1.6, 5.44$ , CH), 4.06 (t, 2H,  $J=5.08, 2.0$ , CH), 2.21-2.17 (p, 2H, CH) ppm. MS (m/z): 381 (M+1)

MPP-5, 9-(3-(4-(2-chlorophenyl)-1H-1,2,3-triazol-1-yl)propoxy)acridine, yield: 81 %, MP: 221°,  $R_f$ : 0.43; FT-IR (KBr):  $\text{cm}^{-1}$  3072 C-H str. (triazole), 1436 C-H str. (methylene), 1658 C=N str., 1545 N=N str., 1504 C=C str. (Ar), 1309 C-N str., 1256 C-O str. (ether), 836 C-Cl str. (Ar);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  8.14 (d, 2H,  $J=6.12$ , CH), 7.99 (d, 2H,  $J=5.12$ , CH), 7.82 (t, 2H,  $J=3.4, 2.88$ , CH-Ar), 7.76 (d, 1H,  $J=8.0$  CH), 7.63 (t, 2H,  $J=2.8, 3.6$ , CH), 7.59 (s, 1H, Ar-CH), 7.55 (d, 1H,  $J=7.36$ , CH-Ar), 7.39 (t, 1H,  $J=8.28, 3.36$ , CH-Ar), 7.35 (t, 1H,  $J=5.08, 3.08$ , CH-Ar), 4.48 (t, 2H,  $J=7.32, 3.44$ , CH), 4.07 (t, 2H,  $J=2.84, 4$ , CH) 2.21-2.17 (p, 2H, CH) ppm. MS (m/z): 415 (M+1).

MPP-6, 9-(3-(4-(o-tolyl)-1H-1,2,3-triazol-1-yl)propoxy)acridine: yield, 79 %, MP: 215°C,  $R_f$ : 0.61; FT-IR (KBr):  $\text{cm}^{-1}$  3073 C-H str. (triazole), 1468 C-H str. (methylene), 1438 C-H str. (methyl), 1652 C=N str., 1546 N=N str., 1507 C=C str. (Ar), 1313 C-N str., 1255 C-O str. (ether);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  8.14 (d, 2H,  $J=6.8$ , CH), 7.98 (d, 2H,  $J=9.84$ , CH), 7.81 (t, 2H,  $J=2.36, 3.6$ , CH-Ar), 7.62 (t, 2H,  $J=7.08, 2.48$ , CH), 7.59 (s, 1H, Ar-CH), 7.56 (d, 1H,  $J=10.04$ , CH), 7.35 (t, 1H,  $J=2.88, 6.44$ , CH-Ar), 7.30-7.26 (m, 2H, CH-Ar), 4.48 (t, 2H,  $J=4.2, 5.16$ , CH), 4.09 (t, 2H,  $J=6.4, 3.96$ , CH), 2.59 (s, 3H,  $\text{CH}_3$ ), 2.21-2.17 (p, 2H, CH) ppm. MS (m/z): 395 (M+1).

MPP-7, N-(2-(4-phenyl-1H-1,2,3-triazol-1-yl)ethyl)acridin-9-amine: yield, 82 %, MP: 232°,  $R_f$ : 0.49; FT-IR (KBr):  $\text{cm}^{-1}$  3339 N-H str. (secondary amine), 3075 C-H str. (triazole), 1468 C-H str. (methylene), 1655 C=N str., 1545 N=N str., 1510 C=C str. (Ar), 1311 C-N str.;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  8.18 (d, 2H,  $J=7.44$ , CH), 7.98 (d, 2H,  $J=6.8$ , CH), 7.81 (d, 2H,  $J=8.64$ , CH), 7.78 (t, 2H,  $J=2.56, 4.44$ , CH-Ar), 7.62 (t, 2H,  $J=5.24, 5.08$ , CH), 7.59 (s, 1H, Ar-CH), 7.52 (t, 2H,  $J=3.24$ ,

7.2, CH-Ar), 7.42 (t, 1H,  $J=5.36, 3.36$ , CH-Ar), 5.61 (t, 2H,  $J=4.2, 4.8$ , CH), 4.01 (s, 1H, Ar-NH), 3.56 (t, 2H,  $J=4.56, 6.48$ , CH) ppm. MS (m/z): 366 (M+1).

MPP-8, N-(2-(4-(2-chlorophenyl)-1H-1,2,3-triazol-1-yl) ethyl)acridin-9-amine, yield: 85 %, MP: 236°,  $R_f$ : 0.54; FT-IR (KBr):  $\text{cm}^{-1}$  3334-N-H str. (secondary amine), 3061-C-H str. (triazole), 1452-C-H str. (methylene), 1650-C=N str., 1545-N=N str., 1505-C=C str. (Ar), 1310 C-N str., 832C-Cl str.;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  8.19 (d, 2H,  $J=8.24$ , CH), 7.95 (d, 2H,  $J=7.52$ , CH), 7.78 (t, 2H,  $J=1.32, 5.32$ , CH-Ar), 7.74 (d, 1H,  $J=8.76$ , CH), 7.61 (t, 2H,  $J=1.64, 3.92$ , CH), 7.59 (s, 1H, Ar-CH), 7.55 (d, 1H,  $J=9.32$ , CH-Ar), 7.40-7.35 (m, 2H, CH-Ar), 5.60 (t, 2H,  $J=3.88, 3.36$ , CH), 4.00 (s, 1H, NH), 3.56 (t, 2H,  $J=2.12, 4.92$ , CH) ppm. MS (m/z): 400 (M+1).

MPP-9, N-(2-(4-(o-tolyl)-1H-1,2,3-triazol-1-yl)ethyl)acridin-9-amine: yield, 86 %, MP: 227°,  $R_f$ : 0.62; FT-IR (KBr):  $\text{cm}^{-1}$  3379-N-H str. (secondary amine), 3069-C-H str. (triazole), 1475-C-H str. (methylene), 1435-C-H str. (methyl), 1655-C=N str., 1590-N=N str., 1538-C=C str. (Ar), 1308-C-N str.;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  8.20 (d, 2H,  $J=11.12$ , CH), 7.96 (d, 2H,  $J=7.32$ , CH), 7.78 (t, 2H,  $J=1.36, 4.72$ , CH-Ar), 7.62 (t, 2H,  $J=3.04, 5.24$ , CH), 7.59 (s, 1H, Ar-CH), 7.57 (d, 1H,  $J=10.28$ , CH-Ar), 7.33-7.28 (m, 3H, CH-Ar), 5.59 (t, 2H,  $J=3.36, 4.96$ , CH), 4.01 (s, 1H, NH), 3.56 (t, 2H,  $J=3.24, 3.2$ , CH), 2.59 (s, 3H,  $\text{CH}_3$ ) ppm. MS (m/z): 380 (M+1).

MPP-10, N-(3-(4-phenyl-1H-1,2,3-triazol-1-yl)propyl)acridin-9-amine, yield: 89 %, MP: 217°,  $R_f$ : 0.67; FT-IR (KBr):  $\text{cm}^{-1}$  3365-N-H str. (secondary amine), 3078-C-H str. (triazole), 1457-C-H str. (methylene), 1656-C=N str., 1597-N=N str., 1535-C=C str. (Ar), 1302-C-N str.;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  8.20 (d, 2H,  $J=10.48$ , CH), 7.98 (d, 2H,  $J=9.36$ , CH), 7.82-7.77 (m, 4H, CH-Ar), 7.62 (t, 2H,  $J=1.52, 4.64$ , CH), 7.59 (s, 1H, Ar-CH), 7.52 (d, 2H,  $J=3.68$ , CH-Ar), 7.43 (t, 1H,  $J=5.24, 5.08$ , CH-Ar), 4.01 (s, 1H, NH), 3.37 (t, 2H,  $J=0.52, 6.96$ , CH), 2.58-2.54 (p, 2H, CH) ppm. MS (m/z): 480 (M+1).

MPP-11, N-(3-(4-(2-chlorophenyl)-1H-1,2,3-triazol-1-yl)propyl)acridin-9-amine, yield: 83%, MP: 242°,  $R_f$ : 0.62; FT-IR (KBr):  $\text{cm}^{-1}$  3374-N-H str. (secondary amine), 3031-C-H str. (triazole), 1449-C-H str. (methylene), 1647-C=N str., 1513-C=C str. (Ar), 1338-C-N str., 794-C-Cl str.;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  8.14 (d, 2H,  $J=7.48$ , CH), 7.97 (d, 2H,  $J=7.24$ , CH), 7.79 (t, 2H,  $J=1.92, 3.96$ , CH), 7.73 (d, 1H,  $J=6.52$ , CH), 7.62 (t, 2H,  $J=2.88, 5.64$ , CH), 7.59 (s, 1H, Ar-

CH), 7.57 (d, 1H,  $J=7.0$ , CH-Ar), 7.41 (t, 1H,  $J=6.64, 2.92$ , CH-Ar), 7.36 (t, 1H,  $J=4.56, 4.32$ , CH-Ar), 4.48 (t, 2H,  $J=4.24, 4.72$ , CH-Ar), 4.01 (s, 1H, NH), 3.36 (t, 2H,  $J=3.36, 3.96$ , CH), 2.57-2.53 (p, 2H, CH) ppm. MS (m/z): 414 (M+1).

MPP-12, N-(3-(4-(o-tolyl)-1H-1,2,3-triazol-1-yl)propyl)acridin-9-amine, yield: 88 %, MP: 252°,  $R_f$ : 0.57; FT-IR (KBr):  $\text{cm}^{-1}$  3332-N-H str. (secondary amine), 3082-C-H str. (triazole), 1463-C-H str. (methylene), 1401-C-H str. (methyl), 1683-C=N str., 1579-N=N str., 1544-C=C str. (Ar), 1266-C-N str.;  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  8.20 (d, 2H,  $J=5.68$ , CH), 7.98 (d, 2H,  $J=4.84$ , CH), 7.79 (t, 2H,  $J=4.32, 3.92$ , CH), 7.64 (t, 2H,  $J=3.48, 6.2$ , CH), 7.59 (s, 1H, Ar-CH), 7.58 (d, 1H,  $J=8.32$ , CH-Ar), 7.33-7.28 (m, 3H, CH-Ar), 4.48 (t, 2H,  $J=5.0, 3.32$ , CH), 4.01 (s, 1H, NH), 3.36 (t, 2H,  $J=5.36, 1.4$ , CH), 2.59 (s, 3H,  $\text{CH}_3$ ), 2.57-2.53 (p, 2H, CH) ppm. MS (m/z): 394 (M+1).

### Biological evaluation:

All newly synthesized triazolyl-acridines were assayed *in vitro* for cytotoxic activity against MCF-7 (human breast adenocarcinoma cell line) and HT-29 (human colon adenocarcinoma cell line) cells, which were maintained at 37° in a humidified atmosphere (90 %) containing 5 %  $\text{CO}_2$ <sup>[24]</sup>.

### MTT Assay:

All the synthesized triazolyl-acridine compounds were dissolved in DMSO and serially diluted with complete medium to get a range of test concentrations. DMSO concentration was kept <0.1 % in all the samples. HT-29 colon carcinoma and MCF7 cells breast adenocarcinoma maintained in appropriate conditions were seeded in 96 well plates and treated with different concentrations of the test samples and incubated at 37°, 5 %  $\text{CO}_2$  for 96 h. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) reagent was added to the wells and incubated for 4 h; the dark blue formazan product formed by the cells was dissolved in DMSO under a safety cabinet and read at 550 nm in triplicate. Percent inhibitions were calculated and plotted with the concentrations used to calculate the inhibitory concentration ( $\text{IC}_{50}$ ) values<sup>[25]</sup>. The results of MTT assay summarized in Tables 1 and 2.

## RESULTS AND DISCUSSION

Structures of novel synthesized triazolyl-acridine compounds, MPP-1 to MPP-12 were confirmed by FT-IR,  $^1\text{HNMR}$  and mass spectroscopy data as well



**TABLE 1: CYTOTOXIC ACTIVITY OF SYNTHESIZED COMPOUNDS AGAINST MCF-7 CELL LINE**

Compound code	Concentration ( $\mu\text{M}$ )					IC <sub>50</sub> value ( $\mu\text{M}$ )
	10	1	0.1	0.01	0.001	
MPP-1	74.3±0.23	42.55±0.00	23.66±0.04	14.29±0.08	10.31±0.01	2
MPP-2	55.48±0.71	43.26±0.82	29.21±1.07	7.54±0.23	1.22±0.09	2
MPP-3	68.36±1.58	50.1±2.55	13.24±0.45	1.26±0.14	2.3±0.00	>10
MPP-4	56.64±1.59	33.64±0.22	18.54±1.49	12.35±0.92	3.69±0.27	>10
MPP-5	56.64±0.86	33.21±0.59	21.54±0.38	2.38±0.91	1.25±0.53	4
MPP-6	58.95±0.17	45.56±0.24	12.54±0.21	3.36±0.09	2.05±0.09	10
MPP-7	41.26±0.47	32.35±1.16	23.64±0.39	10.11±0.73	2.37±0.38	>10
MPP-8	38.65±0.80	28.66±0.33	12.64±0.13	10.56±0.22	2.3±0.21	>10
MPP-9	68.1±0.27	50.2±0.69	32.15±0.98	13.65±0.44	10.1±0.46	1
MPP-10	32.15±0.09	16.54±0.24	10.2±0.09	2.5±0.12	1.1±0.25	>10
MPP-11	31.21±0.09	23.5±0.30	12.3±0.39	6.27±0.59	2.13±0.10	>10
MPP-12	39.21±0.19	25.32±0.59	13.5±0.20	8.24±0.00	1.34±0.08	>10

Results expressed as mean±SD of % inhibition, IC<sub>50</sub> values calculated from MTT assay results

**TABLE 2: CYTOTOXIC ACTIVITY OF SYNTHESIZED COMPOUNDS AGAINST HT-29 CELL LINE**

Compound code	Concentrations ( $\mu\text{M}$ )					IC <sub>50</sub> value ( $\mu\text{M}$ )
	10	1	0.1	0.01	0.001	
MPP-1	72.3±0.13	44.22±0.74	32.7±0.06	15.74±0.63	11.2±0.12	2
MPP-2	58.17±0.18	44.6±0.12	32.91±1.26	10.8±0.01	2.47±0.29	2
MPP-3	32.87±0.55	15.66±0.21	12.74±0.81	2.47±0.21	1.45±0.28	>10
MPP-4	52.7±0.47	32.84±0.46	22.85±0.33	18.19±0.72	2.47±0.32	>10
MPP-5	53.44±0.23	37.6±0.35	22.5±0.17	7.24±0.18	3.54±0.45	4
MPP-6	54.25±0.42	22.61±0.2	18.4±0.18	1.2±0.07	1.1±0.12	10
MPP-7	45.26±0.01	25.63±0.78	18.54±0.37	12.31±0.35	1.25±0.16	>10
MPP-8	38.65±0.32	28.66±0.28	12.64±0.10	10.56±0.33	2.3±0.16	>10
MPP-9	55.3±0.63	44.21±0.24	32.26±0.52	15.6±0.36	2.36±0.21	2
MPP-10	45.26±0.44	32.15±0.41	21.26±0.62	6.35±0.42	1.25±0.32	>10
MPP-11	42.3±0.12	22.21±0.36	12.42±0.36	6.12±0.41	2.31±0.01	>10
MPP-12	32.3±0.37	20.1±0.56	11.21±0.89	4.91±0.71	1.22±0.56	>10

Results expressed as mean±SD of % inhibition, IC<sub>50</sub> values calculated from MTT assay results

as their distinct  $R_f$  values in TLC analysis. FT-IR spectroscopic data clearly confirmed the formation of target compounds, by showing stretching of triazole's C-H bond at 3000  $\text{cm}^{-1}$ , methylene's C-H bond about 1465  $\text{cm}^{-1}$ , aryl alkyl ether's C-O between 1200-1275  $\text{cm}^{-1}$  (MPP-1 to MPP-6 compounds), secondary amine's N-H bond between 3310-3350  $\text{cm}^{-1}$  (MPP-7 to MPP-12 compounds) and disappearance of azide's peaks found between 2120-2160  $\text{cm}^{-1}$ . Similarly proton NMR signal characterized the target compounds by  $\delta$ -value of, CH at triazole ring at 7.59 ppm,  $\text{CH}_2$  ranges 2.1-5.5 ppm and NH at 4.01 ppm (compound MPP-7 to compounds MPP-12).

All synthesized triazolyl-acridine compounds (MPP-1 to MPP-12) were evaluated *in vitro* for cytotoxicity against MCF-7 and HT-29. IC<sub>50</sub> values were calculated by MTT assay, using five different concentrations of all triazolyl-acridine compounds. MCF-7 cell line was found highly sensitive against methyl substituted compound (MPP-9) with an IC<sub>50</sub> of 1  $\mu\text{M}$ , unsubstituted compound (MPP-1) and chlorine substituted

compounds (MPP-2, MPP-5) exhibited IC<sub>50</sub> of 2, 4  $\mu\text{M}$ , respectively. HT-29 cell line was found sensitive with IC<sub>50</sub> of 2  $\mu\text{M}$  against MPP-1, MPP-2, MPP-9 and MPP-5 with IC<sub>50</sub> of 4  $\mu\text{M}$ . The data clearly showed inhibition of growth of MCF-7 and HT-29 cells, which decreased with increasing doses administered. At the final dose, there was only minimal amount of cells survived.

Present study concluded that novel synthesized triazolyl-acridine compounds inhibited the growth of cells tested in a dose-dependent manner. MPP-1, MPP-2 and MPP-9 demonstrated greater activity while MPP-5 was moderately active in inhibiting the growth of both cell line tested. These can still be considered good candidates for the development of anticancer drugs since these demonstrated activities against cancer cells. These compounds also can be used as drug leads for the development of better candidate drugs or these can be used in combination with other antineoplastic drugs to complement their therapeutic effects. However, more *in vitro* and *in vivo* mechanistic studies are required to understand the full potential of these compounds.

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Nil.

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