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DMFDMA catalyzed synthesis of 2-((Dimethylamino)methylene)-3,4-dihydro-9-arylacridin-1(2*H*)-ones and their derivatives: *in-vitro* antifungal, antibacterial and antioxidant evaluations

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Abstract: A series of 3,4-dihydro-9-arylacridin-1(2*H*)-ones was synthesized and enaminone function was introduced at the C-2 position using DMFDMA catalyst which in turn successfully converted into pyrazole, isoxazol, 1-phenyl-1*H*-pyrazole by treating it with reagents such as hydrazine, hydroxylamine and phenylhydrazine. These newly synthesized compounds were evaluated for their antibacterial activity against a series of Gram-Positive bacteria including Staphylococcus *aureus*, Bacillus *cereus*, Staphylococcus *aureus* MLS16 and Gram-Negative bacteria including Klebsiella *planticol*, Escherichia *coli* and Pseudomonas *aeruginosa* and also against fungal strains including Candida *albicans*, Candida *parapsilosis*, Candida *glabrata*, Candida *aaseri*, Aspergillus *niger* and Issatchenkia *hanoiensis*. The compounds **3a** and **6a** exhibited considerable antifungal activity (MIC value 0.007 and 0.006 μM) against Candida *albicans* and Aspergillus *niger* respectively. The compound **4a** showed excellent antibacterial activity towards Escherichia *Coli* (MIC = 0.003 μM) and the compound **5a** found to show prominent DPPH radical scavenging activity with EC_{50} value 16.85±1.5μg mL^{-1}.

Keywords: DMFDMA; Enaminones; antifungal; antibacterial; antioxidant evaluations.

1 Introduction

N, N-Dimethylformamide dimethyl acetal (DMFDMA) also known as 1, 1-dimethoxy-N, N-dimethylmethylamine or 1, 1-dimethoxytrimethylamine, as its name indicates, is a derivative of formamide [1] acts as methylating and formylating agent. Formylated products obtained by treating compounds with active methylene group and DMFDMA have proved to be very useful intermediates in the synthesis of heterocycles [1-4]. Enaminone derivatives have been proven to be valuable synthon for the synthesis of a wide variety of biologically active heterocycles [4]. On the other hand, the functionalized acridines are often used to design and synthesize the compounds with various pharmacological activities such as antibacterial [5], antifungal [5], anti-inflammatory [6], anticancer [7], anti-cytotoxic [8] and anti-malarial activities [9]. Though the synthesis of enaminone and its derivatives from simple ketones using DMFDMA are available in the literature, only a very few reports are available on the enaminones from active methylene of the various heterocycles [1-4 and 10-14]. This prompted us to attempt the present work in which we have used the active methylene group of 3,4-dihydro-9-arylacridin-1(2*H*)-ones to synthesize the corresponding enaminone which in turn used as a synthon to obtain the corresponding pyrazole, isoxazole and 1-aryl-1*H*-pyrazole using hydrazine, hydroxylamine, phenylhydrazine respectively.

Ethical approval: The conducted research is not related to either human or animal use.

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2 Materials and Methods

Melting points (mp) reported in this work are recorded in Elchem microprocessor based DT apparatus in open capillary tubes and are uncorrected. ^{1}H NMR and ^{13}C NMR are recorded in Bruker 400 MHz NMR spectrometer with TMS as an internal reference. The chemical shift values are reported in parts per million (δ, ppm) from internal standard TMS. High-Resolution Mass spectra were recorded using Bruker *MaXis* HR-MS (ESI-Q-TOF-MS) instrument. All the reagents were purchased from Aldrich and used as received. Excess solvents were removed under vacuum. Organic extracts were dried with anhydrous Na_2SO_4. Silica gel $60F_{254}$aluminum sheets were used in analytical thin-layer chromatography. Visualization of spots on TLC plates was effected by UV illumination, exposure to iodine vapour and heating the plates dipped in a $KMnO_4$ stain. In column chromatography, the silica gel with 230-400 mesh size was used for the purification.

2.1 General procedure for the synthesis of *3,4-dihydro-9-arylacridin-1(2H)-ones*.

The 2-amino benzophenone **1a** (0.1 g, 0.0005 M) was treated with 1,3-cyclohexanedione (0.06 g, 0.0005 M) in presence of *ortho*-phosphoric acid (catalyst) in ethanol and refluxed for 12 hrs. After the completion of the reaction the crude was poured into ice cold water and the formed precipitate was filtered and dried to afford the product **2a**. The obtained product was used without further purification. And the procedure was repeated with **1b-c** to get **2b-c**.

2.1.1 *3,4-dihydro-9-phenylacridin-1(2H)-one* (2a)

Yellow solid, (yield 86%), m.p: 156-157ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.00 (d, *J* = 9.00 Hz, 1H), 7.69 (dd, *J* = 9.00 Hz, 2.20 Hz, 1H), 7.53-7.49 (m, 4H), 7.41 (d, *J* = 2.20 Hz, 1H), 7.15 (dd, *J* = 7.00 Hz, 2.20 Hz, 2H), 3.36 (t, *J* = 6.40 Hz, 2H), 2.70 (t, *J* = 6.40 Hz, 2H), 2.28-2.20 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 197.7, 162.5, 150.5, 147.0, 136.8, 132.6, 132.4, 130.1, 128.3, 128.3, 128.0, 127.9, 126.7, 124.4, 40.6, 34.5, 21.2.

2.1.2 *7-chloro-3,4-dihydro-9-phenylacridin-1(2H)-one* (2b)

Yellow solid, (yield 80%), m.p: 185-187 ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.97 (d, *J* = 8.80 Hz, 1H), 7.68 (dd, *J* = 8.80 Hz, 2.40 Hz, 1H), 7.53-7.47 (m, 3H), 7.41 (d, *J* = 2.40 Hz, 1H), 7.15 (dd, *J* = 7.20 Hz, 2.40 Hz, 2H), 3.35 (t, *J* = 6.20 Hz, 2H), 2.70 (t, *J* = 6.20 Hz, 2H), 2.27-2.21 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 197.7, 162.5, 150.5, 147.0, 136.8, 132.6, 132.4, 130.2, 128.3, 128.3, 127.9, 126.7, 124.4, 40.6, 34.5, 21.2.

2.1.3 *7-chloro-9-(2-chlorophenyl)-3,4-dihydroacridin-1(2H)-one* (2c)

Yellow solid, (yield 78%), m.p: 180-181ºC ; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.02 (d, *J* = 9.00 Hz, 1H), 7.71 (dd, *J* = 9.00 Hz, 1.40 Hz, 1H), 7.55 (d, *J* = 7.60 Hz, 1H), 7.46-7.42 (m,2H), 7.30 (s, 1H), 7.10 (d, *J* = 6.80 Hz, 1H), 3.37 (t, *J* = 6.00 Hz, 2H), 2.80-2.65 (m, 2H), 2.24 (t, *J* = 6.00 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 197.4, 162.5, 147.4, 147.0, 135.9, 133.1, 132.8, 132.1, 130.7, 130.7, 130.4, 125.9, 125.5, 40.1, 34.4, 21.2.

2.2 General procedure for synthesis of (E)-2-((dimethylamino)methylene)-3,4-dihydro-9-arylacridin-1(2*H*)-ones (3a-c)

A mixture of 3,4-dihydro-9-phenylacridin-1(2H)-one (0.1g, 0.0005 M) **2a-c** with DMFDMA (0.1 mL, 0.0005 M) in toluene (5 mL) and refluxed for 8-10 hrs to afford the product **3a**. The mixture was then evaporated under reduced pressure to afford the solid, which was filtered, washed with hexane and then dried. The similar procedure was repeated with **2b-c** to get **3b-c**. The obtained product used for further analysis without any purification process.

2.2.1 *(E)-2-((dimethylamino)methylene)-3,4-dihydro-9-phenylacridin-1(2H)-one* (3a)

Colourless solid (yield 86%), m.p: 162-163ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.98 (d, *J* = 8.80 Hz, 1H), 7.62 (dd, *J* = 8.80 Hz, 2.10 Hz, 2H), 7.50-7.45 (m, 4H), 7.42 (d, *J* = 2.10 Hz, 1H), 7.22 (d, *J* = 7.60 Hz, 2H), 3.22 (t, *J* = 6.40 Hz, 2H), 3.10 (s, 6H), 3.02 (t, *J* = 6.40 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 185.2, 162.0, 151.1, 148.3, 146.1, 137.9, 131.7, 131.2, 130.0, 129.0, 128.5, 128.3, 128.1, 127.9, 127.4, 126.9, 126.4, 104.7, 43.5, 34.6, 23.2. HRMS-ESI (m/z) calcd for $C_{22}H_{20}N_2O$ $[M+H]^+$ = 329.1654, found = 329.1660.

2.2.2 *(E)-7-chloro-2-((dimethylamino)methylene)-3,4-dihydro-9-phenylacridin-1(2H)-one* (3b)

Colourless solid, (yield 85%), m.p: 172-174ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.98 (d, *J* = 9.20 Hz, 1H), 7.62 (dd, *J* = 9.20 Hz, 2.40 Hz, 2H), 7.50-7.46 (m, 3H), 7.44 (dd, *J* = 12.80 Hz, 2.40 Hz, 1H), 7.22 (dd, *J* = 7.80 Hz, 1.80 Hz, 2H), 3.22 (t, *J* = 6.40 Hz, 2H), 3.10 (s, 6H), 3.02 (t, *J* = 6.40 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 185.2, 162.0, 151.1, 148.3, 146.1, 137.9, 131.7, 131.2, 130.0, 129.0, 128.5, 128.3, 128.1, 127.9, 127.4, 126.9, 126.4, 104.7, 43.5, 34.6, 23.2. HRMS- ESI (m/z) calcd for $C_{22}H_{19}ClN_2O$ $[M+H]^+$ = 362.1654, found = 362.1654.

2.2.3 *(E)-7-chloro-2-((dimethylamino)methylene)-9-(2-chlorophenyl)-3,4 dihydroacridin-1(2H)-one* (3c)

Light brown solid, (yield 84%), m.p.: 158-159ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.00 (d, *J* = 9.00 Hz, 1H), 7.64 (dd, *J* = 9.00 Hz, 2.40 Hz, 2H), 7.53 (dd, *J* = 7.10 Hz, 1.80 Hz, 1H), 7.42-7.36 (m, 2H), 7.27 (d, *J* = 2.40 Hz, 1H), 7.15 (dd, *J* = 7.10 Hz, 1.80 Hz, 1H), 3.24 (t, *J* = 6.40 Hz, 2H), 3.10 (s, 6H), 3.03 (t, *J* = 6.40 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 184.7, 161.9, 151.3, 146.4, 145.0, 137.1, 132.4, 132.1, 131.4, 130.2, 129.6, 129.2, 128.9, 127.0, 126.5, 125.6, 104.2, 43.6, 34.5, 23.1. HRMS- ESI (m/z) calcd for $C_{22}H_{18}Cl_2N_2O$ $[M+H]^+$ = 397.0874, found = 397.0871.

2.3 General procedure for the synthesis of 4,5-dihydro-11-aryl-2*H*-pyrazolo[3,4-a] acridines (4a-c)

A mixture of compound **3a** (0.1 g, 0.0003 M), hydrazine hydrate (0.05 mL, 0.0101 M) and 1-2 drops of acetic acid (catalyst) in ethanol (10 mL) was refluxed at 80 °C for 6-8 h. The mixture was then extracted with ethyl acetate, dried over sodium sulphate and concentrated under reduced pressure to afford the crude product **4a**. The obtained crude product was purified by silica gel column chromatography using ethylacetate/hexane (4:6) eluent. To check there reproducibility the reaction and to get **4b-c**, the procedure was repeated with **3b-c**.

2.3.1 *4,5-dihydro-11-phenyl-2H-pyrazolo[3,4-a]acridine* (4a)

Colourless solid, (yield 76%), m.p: 166-168ºC;^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.40 (d, *J* = 8.00 Hz, 1H), 7.65 (dd, *J* = 5.40 Hz, 1.80 Hz, 4H), 7.41 (d, *J* = 3.00 Hz, 1H), 7.39 (d, *J* = 3.00 Hz, 1H), 7.37 (d, *J* = 5.40 Hz, 2H), 7.33 (d, *J* = 8.00 Hz, 1H), 3.40 (t, *J* = 6.80 Hz, 2H), 2.98 (t, *J* = 6.80 Hz, 2H), 1.90 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 158.1, 148.60, 147.1, 142.7, 135.9, 130.2, 129.3, 128.7, 128.5, 127.3, 127.0, 126.7, 117.4, 115.1, 33.7, 17.6; HRMS- ESI (m/z) calcd for $C_{20}H_{15}N_3$ $[M+H]^+$ = 298.1344, found = 298.1338.

2.3.2 *9-chloro-4,5-dihydro-11-phenyl-1H-pyrazolo[3,4-a] acridine*(4b)

Colourless solid, (yield 79%), m.p: 155-157ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.00 (d, *J* = 8.40 Hz, 1H), 7.69 (dd, *J* = 8.40 Hz, 1.20 Hz, 1H), 7.62-7.59 (m, 2H), 7.58 (t, *J* = 2.20 Hz, 1H), 7.54 (dt, *J* = 1.20 Hz, 7.50 Hz, 1H), 7.39 (s, 1H), 7.35 (dd, *J* = 7.50 Hz, 1.60 Hz, 1H), 7.18 (d, *J* = 2.20 Hz, 1H), 3.38 (t, *J* = 3.00 Hz, 2H), 3.00 (t, *J* = 3.00 Hz, 2H), 2.16 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 159.0, 144.7, 138.9, 137.0, 136.7, 135.9, 132.4, 130.3, 130.1, 130.0, 129.7, 129.6, 129.1, 128.0, 127.9, 124.7, 119.3, 118.9, 34.4, 29.7. HRMS- ESI (m/z) calcd for $C_{20}H_{14}ClN_3$ $[M+H]^+$ = 332.0955, found = 332.1001.

2.3.3 *9-chloro-11-(2-chlorophenyl)-4,5-dihydro-1H-pyrazolo[3,4-a]acridine* (4c)

Brown solid, (yield 59%), m.p: 164-166ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.01 (d, *J* = 6.90 Hz, 1H), 7.71 (d, *J* = 6.90 Hz, 1H), 7.61-7.57 (m, 2H), 7.53 (t, *J* = 6.90 Hz, 1H), 7.39 (s, 1H), 7.36 (dd, *J* = 6.00 Hz, 1.80 Hz, 1H), 7.19 (d, *J* = 1.80 Hz, 1H), 3.38 (t, *J* = 3.40 Hz, 2H), 3.00 (t, *J* = 3.40 Hz, 2H), 2.00 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 159.3, 144.8, 136.2, 135.1, 133.8, 132.6, 131.0, 130.7, 130.5, 130.1, 128.0, 127.4, 124.2, 120.2, 34.4, 18.6. HRMS- ESI (m/z) calcd for $C_{20}H_{13}Cl_2N_3$ $[M+H]^+$ = 366.0565, found = 366.0565.

2.4 General procedure for the synthesis of 4,5-dihydro-11-phenylisoxazolo[5,4-a] acridines (5a-c)

A mixture of compound **3a** (0.1 g, 0.0003 M) in ethanol (10 mL), hydroxyl amine hydrochloride (0.02 g, 0.0003 M) was refluxed at 80°C for 6-8 h. After the completion of the reaction, the reaction mixture was poured into ice water; the obtained crude was filtered, dried to afford the product **5a**. The obtained product was purified by silica gel column chromatography using ethylacetate/hexane (4:6) eluent. Similar procedure was adopted with **3b-c** to get **5b-c**.

2.4.1 *4,5-dihydro-11-phenylisoxazolo[5,4-a]ccridine* (5a)

Colourless solid, (yield 86%), m.p: 160-161ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.11 (s, 1H), 8.05 (d, *J* = 8.20 Hz, 1H), 7.64 (t, *J* = 8.20 Hz, 1H), 7.54 (t, *J* = 2.40 Hz, 3H), 7.48 (d, *J* = 7.90 Hz, 1H), 7.41 (t, *J* = 7.90 Hz, 1H), 7.34 (dd, *J* = 6.60 Hz, 2.40 Hz, 2H), 3.44 (t, *J* = 7.20 Hz, 2H), 2.95 (t, *J* = 7.60 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 158.1, 148.6, 147.1, 142.7, 135.9, 130.2, 129.3, 128.7, 128.5, 127.3, 127.0, 126.7, 117.4, 115.1, 33.7, 17.6. HRMS- ESI (m/z) calcd for $C_{20}H_{14}N_2O$ $[M+H]^+$ = 299.1184, found = 299.1191.

2.4.2 *9-chloro-4,5-dihydro-11-phenylisoxazolo[3,4-a] Acridine* (5b)

Yellow solid, (yield 69%), m.p: 152-154ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.12 (s, 1H), 7.98 (d, *J* = 8.80 Hz, 1H), 7.66 (dd, *J* = 5.20 Hz, 2.20 Hz, 1H), 7.63 (d, *J* = 2.00 Hz, 1H), 7.62 (dd, *J* = 8.80 Hz, 2.00 Hz, 1H), 7.56 (dd, *J* = 4.90 Hz, 2.50 Hz, 2H), 7.32 (dd, *J* = 4.90 Hz, 2.50 Hz, 2H), 3.43 (t, *J* = 7.20 Hz, 2H), 2.96 (t, *J* = 7.20 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 163.8, 158.4, 148.6, 146.2, 145.5, 141.7, 135.2, 132.6, 132.2, 131.3, 130.9, 130.3, 129.4, 129.2, 129.2, 128.9, 128.7, 128.5, 128.2, 127.6, 125.7, 125.3, 122.5, 117.9, 115.9, 33.7, 17.5. HRMS- ESI (m/z) calcd for $C_{20}H_{13}ClN_2O$ $[M+H]^+$ = 332.0950, found = 332.0950.

2.4.3 *9-chloro-11-(2-chlorophenyl)-4,5-dihydroisoxazolo[5,4-a]Acridine* (5c)

Yellow solid, (yield 49%), m.p: 168-170ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.12 (s, 1H), 7.98 (d, *J* = 8.80 Hz, 1H), 7.62 (d, *J* = 8.80 Hz, 2.40 Hz, 1H), 7.56 (dd, *J* = 9.00 Hz, 2.00 Hz, 2H), 7.43 (d, *J* = 2.40 Hz, 1H), 7.30 (dd, *J* = 9.00 Hz, 2.40 Hz, 2H), 3.41 (t, *J* = 7.30 Hz, 2H), 2.97 (t, *J* = 7.30 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 160.8, 152.2, 147.9, 147.4, 146.3, 144.6, 136.6, 129.9, 129.8, 129.6, 129.6, 129.5, 128.6, 128.5, 128.4, 128.2, 128.0, 127.7, 127.5, 126.7, 126.4, 126.2, 126.1, 125.9, 125.7, 33.9, 29.7. HRMS- ESI (m/z) calcd for $C_{20}H_{12}Cl_2N_2O$ $[M+H]^+$ = 367.0405, found = 367.0403.

2.5 General procedure for the synthesis of *4,5-dihydro-1,11-diaryl-1*H*-pyrazolo[3,4-a] acridine* (6a-c)

A mixture of compound **3a** (0.1 g, 0.0003 M), phenyl hydrazine (0.03 g, 0.0003 M) in glacial acetic acid (5 mL), was refluxed at 110°C for 12 h. The reaction mixture was then extracted with DCM, dried over sodium sulphate and concentrated under reduced pressure to afford the crude product **6a**. The obtained crude product was purified by silica gel column chromatography using ethylacetate/hexane (4:6) eluent. To check the reproducibility similar procedure was adopted with **3b-c** to get **6b-c**.

2.5.1 *4,5-dihydro-1,11-diphenyl-1*H*-pyrazolo[3,4-a] ccridine* (6a)

Yellow solid, (yield 66%), m.p: 182-183ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.09 (d, *J* = 7.00 Hz, 1H), 7.71 (t, *J* = 7.00 Hz, 2H), 7.54 (d, *J* = 7.60 Hz, 2H), 7.46 (d, *J* = 7.00 Hz, 2H), 7.42 (dd, *J* = 6.00 Hz, 2.00 Hz, 2H), 7.36 (d, *J* = 2.00 Hz, 1H), 7.34 (t, *J* = 2.40 Hz, 2H), 7.31 (d, *J* = 7.60 Hz, 1H), 7.28-7.27 (m, 1H), 7.25 (d, *J* = 2.40 Hz, 1H), 3.28 (t, *J* = 6.60 Hz, 2H), 2.98 (t, *J* = 6.60 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 152.2, 147.9, 147.4, 146.3, 144.6, 136.6, 129.9, 129.6, 129.6, 129.5, 128.5, 128.4, 128.2, 127.7, 127.7, 126.7, 126.4, 126.1, 126.0, 125.7, 33.9, 29.7. HRMS- ESI (m/z) calcd for $C_{26}H_{19}N_3$ $[M+H]^+$ = 374.1675, found = 374.1655.

2.5.2 *9-chloro-4,5-dihydro-1,11-diphenyl-1*H*-pyrazolo[3,4-a]acridine* (6b)

Yellow solid, (yield 58%), m.p: 175-177ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.10 (d, *J* = 8.60 Hz, 1H), 7.71 (t, *J* = 8.60 Hz, 2H), 7.54 (d, *J* = 7.60 Hz, 2H), 7.47 (d, *J* = 7.10 Hz, 2H), 7.36 (dd, *J* = 7.60 Hz, 2.40 Hz, 2H), 7.35 (d, *J* = 1.80 Hz, 1H), 7.31 (d, *J* = 7.10 Hz, 2H), 7.28 (t, *J* = 1.80 Hz, 1H), 7.27 (d, *J* = 5.60 Hz, 1H), 3.29 (t, *J* = 6.20 Hz, 2H), 2.99 (t, *J* = 6.20 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 160.9, 152.9, 147.9, 147.4, 146.3, 144.6, 136.6, 129.9, 129.8, 129.6, 129.6, 128.6, 128..5, 128.4, 128.2, 128.0, 127.6, 126.6, 126.4, 126.2, 126.1, 126.0, 125.7, 33.9, 29.7. HRMS- ESI (m/z) calcd for $C_{26}H_{18}ClN_3$ $[M+H]^+$ = 408.1268, found = 408.1267.

2.5.3 *9-chloro-11-(2-chlorophenyl)-4,5-dihydro-1-phenyl-1*H*-pyrazolo[3,4-a]acridine* (6c)

Brown solid, (yield 59%), m.p: 187-189ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.10 (d, *J* = 8.60 Hz, 1H), 7.71 (t, *J* = 8.60 Hz, 2H), 7.54 (d, *J* = 7.60 Hz, 2H), 7.47 (d, *J* = 7.00 Hz, 2H), 7.42 (d, *J* = 1.40 Hz, 2H), 7.36 (dd, *J* = 7.60 Hz, 2.40 Hz, 2H), 7.31 (d, *J* = 7.00 Hz, 1H), 7.28 (t, *J* = 1.40 Hz, 1H), 3.29 (t, *J* = 6.20 Hz, 2H), 2.99 (t, *J* = 6.20 Hz, 2H).^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 160.9, 152.2, 147.9, 147.4, 146.3, 144.6, 136.6, 129.9, 129.8, 129.6, 129.6, 129.5, 128.6, 129.5, 128.4, 128.2, 128.0,

127.7, 127.6, 126.7, 126.4, 126.2, 126.1, 126.0, 125.7, 33.9, 29.7. HRMS- ESI (m/z) calcd for $C_{26}H_{17}Cl_2N_3$ $[M+H]^+$ = 442.0881, found = 442.0881.

2.6 Spectral Characterization

All these newly synthesized compounds **3a-c** to **6a-c** were characterized using ^{1}H-NMR, ^{13}C-NMR, DEPT-135, H, H-COSY, C, H-COSY, HR-MS (ESI) and the data included in the experimental section. Consequently, all the newly derived target molecules **3a-c** to **6a-c** subjected to the *in-vitro* anti-fungal, anti-bacterial and antioxidant evaluations and their results were discussed under biological evaluations.

2.6.1 Spectral Characterization of Compound 3c

The compound **3c** has been characterized by ^{1}H-NMR, ^{13}C-NMR, DEPT-135, H, H-COSY, C, H-COSY, HR-MS (ESI) spectral data. The ^{1}H-NMR spectrum of compound **3c** exhibited the following chemical shifts, d 8.00 d [*J* = 9.00 Hz, one proton], 7.64 dd [*J* = 9.00 Hz, 2.40 Hz, two proton], 7.53 dd [*J* = 7.10 Hz, 1.80 Hz, one proton], 7.42-7.36 m [two protons], 7.27 d [*J* = 2.10 Hz, one proton], 7.15 dd [*J* = 7.10 Hz, 2.10 Hz, one proton], 3.24 t [*J* = 6.40 Hz, two protons], 3.10 s [six protons], 3.03 t [*J* = 6.40 Hz, two protons]. The examination of H, H-COSY spectrum of the molecule shows that the singlet at d 3.10 ppm which is integrating for six protons due to the two methyl groups of the molecule at C-18 and C-19 and it is appeared as a single peak due to symmetry. The remaining two methylene protons have appeared at d 3.24 and 3.03 ppm which are integrating for two protons of each at C-14 and C-15. The signals at d 8.00 d [*J* = 9.00 Hz, one proton], 7.64 dd [*J* = 9.00 Hz, 2.40 Hz, two protons] and 7.27 d [*J* = 2.40 Hz, one proton] are appearing as coupling partners, which is assigned to C-8, C-7 and C12 respectively. The signals at d 7.53 dd [*J* = 7.10 Hz, 1.80 Hz, one proton] and 7.16 dd [*J* = 7.20 Hz, 1.60 Hz, one proton] ppm are assigned to C-10 and C-13 respectively and are found to couple with the multiplet in the range of 7.42-7.36 ppm and hence the multiplet assigned to the protons at C-11 and C-12. The ^{13}C-NMR spectrum exhibited the following chemical shift values: d 23.1, 34.5, 43.6, 104.2, 125.6, 126.5, 127.0, 128.9, 129.2, 130.2, 131.4, 132.1, 132.4, 137.1, 145.6, 146.4, 151.3, 161.9, 184.7 ppm. The up-field signal at d 23.1 ppm has been assigned as a methylene carbon at C-15 and one more signal at d 34.5 ppm represents at C-14. And the remaining two methyl groups at C-18 and C-19 appear as a singlet at d 43.6 ppm is due to symmetry. The extreme downfield signal at d 84.7 ppm is indicates the carbonyl group at C-22. The signal at d 151.3 ppm has been assigned to the carbon at C-17 due to the deshielding effect of adjacent functional groups. The C-7 and C-8 carbons were identified as d 130.2 and 132.4 ppm respectively. The aliphatic CH_2 carbons at (C-14 and C-15) carbons were distinguished using the DEPT-135 spectrum of the compound. Remaining aromatic carbon attached hydrogen appears from the aromatic region from d 151.4 to 125.6 ppm. Similarly, the *ortho*, *meta* and *para* carbons of phenyl group at C-4 were identified as d 129.2, 126.5 and 125.6 ppm respectively. The signals at d 104.2, 127.0, 132.1, 137.2, 145.0, 146.4, 161.9 and 184.7 ppm were due to the non-proton bearing carbons at C-20, C-6, C-3, C-23, C-16, C-2, C-22 and *ipso* positions. The formation of compound **3c** also been supported by the observation of (ESI-HRMS) m/z value at 397.0871 in the mass spectrum.

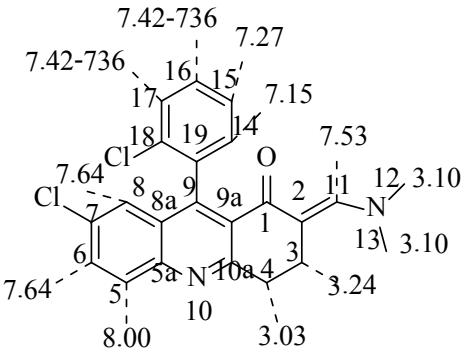


Figure 1: Summary of Proton d Values of **3c.**

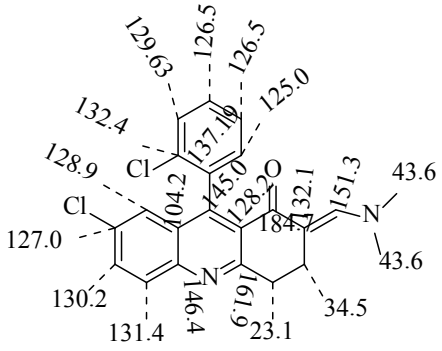


Figure 2: Summary of Carbon d Values of **3c.**

2.6.2 Spectral Characterization of Compound 4a

The compound **4a** has been characterized by ^{1}H-NMR, ^{13}C-NMR, DEPT-135, H, H- COSY, C, H-COSY, HR-MS (ESI) spectral data. The ^{1}H-NMR spectrum of compound **4a**

exhibited the following chemical shifts, d 8.40 d [J = 8.00 Hz, one proton], 7.64 dd [J = 5.40 Hz, 1.80 Hz, four protons], 7.41 d [J = 3.00 Hz, one proton], 7.39 d [J = 3.00 Hz, one proton], 7.37 d [J = 5.40 Hz, two protons], 7.33 d [J = 8.00 Hz, one proton], 3.40 t [J = 6.80 Hz, two protons], 2.98 t [J = 6.80 Hz, two protons], 1.90 s [one proton]. The examination of H, H-COSY spectrum of the molecule shows that the two triplets at d 3.40 and 2.98 ppm which is integrating for two protons of each are assigned to C-14 and C-15. The signal at d 8.40 ppm which integrates for one proton is assigned to C-8. The signal at d 7.64 [J = 5.40 Hz, 1.80 Hz, four protons] is assigned to C-7, C-6, C-5 and C-9 respectively. The signal at d 7.41 d [J = 3.00 Hz, one proton], 7.39 d [J = 3.00 Hz, one proton] are appearing as a coupling partner, which is assigned to C-10 and C-11 respectively. And a signal at d 7.33 d [J = 8.00 Hz, one proton] is assigned to C-13. The ^{13}C-NMR spectrum exhibited the following chemical shift values: d 17.6, 33.7, 115.4, 117.2, 126.7, 127.0, 127.3, 128.7, 128.0, 128.8, 130.2, 135.9, 142.7, 147.1, 148.6, 158.1 ppm. The up-field signal at d 17.6 ppm has been assigned as methylene carbon at C-15 and one more signal at d 34.5 ppm represents at C-14. The extreme downfield signal at d 158.1 ppm is assigned to C-18. The proton bearing carbons are identified using HSQC spectrum. The signal at d 126.0 ppm has been assigned to the carbon at C-16 due to the shielding effect of adjacent functional groups. The C-7 and C-8 carbons were identified as d 129.8 and 128.7 ppm respectively. The aliphatic CH_2 carbons at (C-14 and C-15) carbons were distinguished using the DEPT-135 spectrum of the compound. Remaining aromatic carbon attached hydrogen appears from the aromatic region from d 129.8 to 18.7 ppm. Similarly, the *ortho*, *meta* and *para* carbons of phenyl group at C-4 were identified as d 129.3, 129.1 and 126.5 ppm respectively. The signals at d 117.9, 127.0, 127.3, 130.2, 135.9, 142.7, 147.1, 158.1 ppm were due to the non-proton bearing carbons at C-21, C-17, C-20, C-3, C-19, C-18, C-2 and *ipso* positions. The formation of compound **4a** also been supported by the observation of (ESI-HRMS) m/z value at 298.1338 in the mass spectrum.

2.6.3 Spectral Characterization of Compound 5a

The compound **5a** has been characterized by ^{1}H-NMR, ^{13}C-NMR, DEPT-135, H, H- COSY, C, H-COSY, HR-MS (ESI) spectral data. The ^{1}H-NMR spectrum of compound **5a** exhibited the following chemical shifts, d 8.11 s [one proton], 8.05 d [J = 8.20 Hz, one proton], 7.64 t [J = 8.20 Hz, one proton], 7.54 t [J = 2.40 Hz, three protons], 7.48 d [J = 7.90 Hz, one proton], 7.41 d [J = 7.90 Hz, one proton], 7.34 dd [J = 6.60 Hz, 2.40 Hz, two protons], 3.44 t [J = 7.20 Hz, two

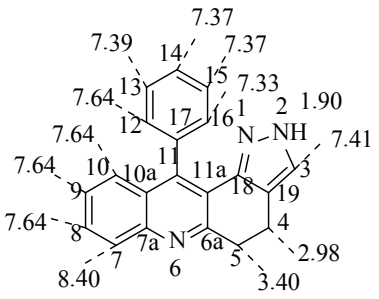


Figure 3: Summary of Proton d Values **4a.**

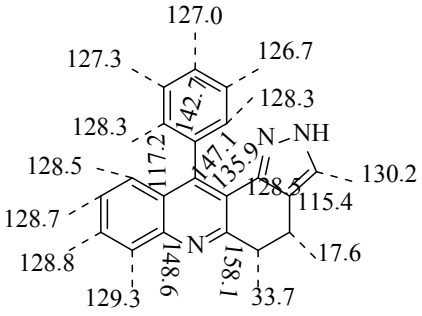


Figure 4: Summary of Carbon d Values **4a.**

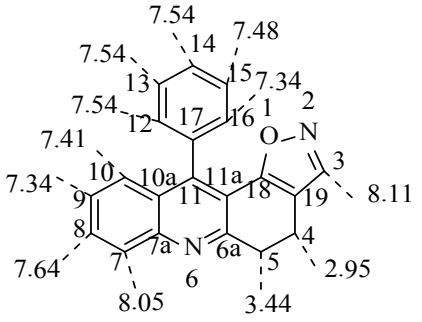


Figure 5: Summary of Proton d Values **5a.**

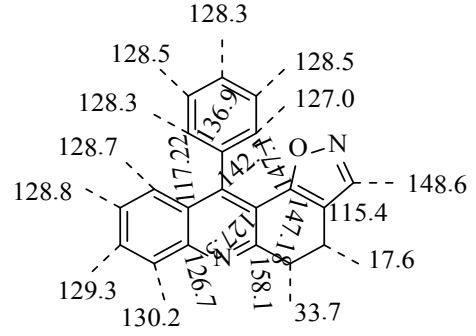


Figure 6: Summary of Carbon d Values **5a.**

protons], 2.95 t [*J* = 7.60 Hz, two protons]. The examination of H, H-COSY spectrum of the molecule shows that the two triplets at d 3.44 and d 2.95 ppm which is integrating for two protons of each are indicated at C-14 and C-15. The signal at d 8.05d [*J* = 8.20 Hz, one proton] and 7.64 t [*J* = 8.20 Hz, one proton] are appearing as a coupling partner, which is assigned to C-8 and C-7 respectively. The signal at d 8.11 ppm is assigned to C-16. The signals at d 7.54 t [*J* = 2.40 Hz, three protons] and 7.34 dd [*J* = 6.60 Hz, 2.40 Hz, two protons] with a coupling constant of [*J* = 6.00 Hz, 2.40 Hz, two protons]. And the signals at d 7.48 d [*J* = 7.90 Hz, one proton] and 7.41 d [*J* = 7.90 Hz, one proton] are appearing as a coupling partners is assigned for C-10 and C-9 respectively. The ^{13}C-NMR spectrum exhibited the following chemical shift values: d 17.6, 33.7, 115.1, 117.1, 126.7, 127.0, 127.3, 128.5, 128.7, 129.3, 130.2, 135.9, 142.7, 147.1, 148.6, 158.1 ppm. The up-field signal at d 33.7 ppm has been assigned as a methylene carbon at C-14 and one more signal at d 17.6 ppm represents at C-15. The extreme downfield signal at d 158.1 ppm is assigned to C-2. The signal at d 148.6 ppm has been assigned to the carbon at C-16 due to shielding effect of adjacent functional groups. The C-7 and C-8 carbons were identified as d 130.3 and 130.9 ppm respectively. The aliphatic CH_2 carbons at (C-14 and C-15) carbons were distinguished using DEPT-135 spectrum of the compound. Remaining aromatic carbon attached hydrogen appears from aromatic region from d 148.6 to 125.7 ppm. Similarly, the *ortho*, *meta* and *para* carbons of phenyl group at C-4 were identified as d 129.7, 129.2 and 128.5 ppm respectively. The formation of compound **5a** also been supported by the observation of (ESI-HRMS) m/z value at 299.1191 in the mass spectrum.

2.6.4 Spectral Characterization of Compound 6a

The compound **6a** has been characterized by ^{1}H-NMR, ^{13}C-NMR, DEPT-135, H, H-COSY, C, H-COSY, HR-MS (ESI) spectral data. The ^{1}H-NMR spectrum of compound **6a** exhibited the following chemical shifts, d 8.09 d [*J* = 7.00 Hz, one proton], 7.71 t [*J* = 7.00 Hz, two protons], 7.54 d [*J* = 7.60 Hz, two protons], 7.46 d [*J* = 7.00 Hz, two protons], 7.42 dd [*J* = 6.00 Hz, 2.00 Hz, two protons], 7.36 d [*J* = 2.00 Hz, one proton], 7.34 t [*J* = 2.40 Hz, two protons], 7.31 d [*J* = 7.60 Hz, one proton], 7.28-7.27 m [one proton], 7.25 d [*J* = 2.40 Hz, one proton], 3.28 t [*J* = 6.60 Hz, two protons], 2.98 t [*J* = 6.60 Hz, two protons]. The examination of H, H-COSY spectrum of the molecule shows that the two triplets at d 3.28 and 2.98 ppm which is integrating for two protons of each are due to C-14 and C-15. The signals at d 8.09 d [*J* = 7.00 Hz, one proton], 7.71 t [*J* = 7.00 Hz, two protons] and 7.46 d [*J* =

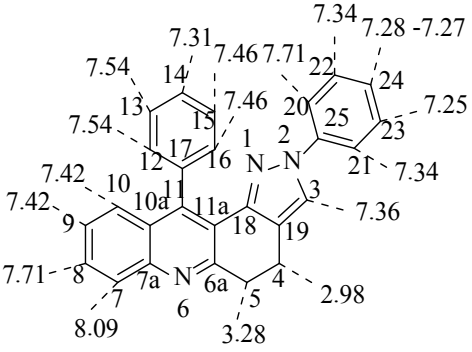


Figure7: Summary of Proton d Values of **6a.**

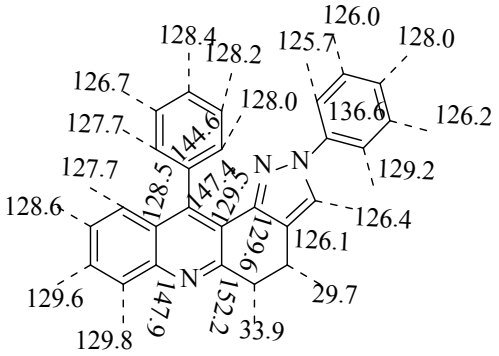


Figure 8: Summary of Carbon d Values of **6a.**

7.00 Hz, two protons] are appearing as a coupling partners is assigned to C-8, C-7, C-9, C-5 and C10 respectively. The signals at d 7.54 d [*J* = 7.60 Hz, two protons] and 7.31 d [*J* = 7.60 Hz, one proton] are appearing as a coupling partners is assigned to C-12, C-13 and C-11 respectively. Similarly, the signals at d 7.34 t [*J* = 2.40 Hz, two protons] and 7.25 d [*J* = 2.40 Hz, one proton] is assigned for phenyl substituent at C-17. The ^{13}C-NMR spectrum exhibited the following chemical shift values: d 29.7, 33.9, 125.7, 126.0, 126.1, 126.2, 126.4, 126.7, 127.6, 127.7, 128.0, 128.2, 128.4, 128.5, 128.6, 129.5, 129.6, 129.8, 129.9, 136.6, 144.6, 146.3, 147.4, 147.9, 152.2 ppm. The up-field signal at d 33.9 ppm has been assigned as a methylene carbon at C-14 and one more signal at d 29.7 ppm represents at C-15. The extreme downfield signal at d 152.2 ppm is assigned to C-2. The signal at d 129.8 ppm has been assigned to the carbon at C-16 due to shielding effect of adjacent functional groups. The C-7 and C-8 carbons were identified as d 129.6 and 128.6 ppm respectively. The aliphatic CH_2 carbons at (C-14 and C-15) carbons were distinguished using the DEPT-135 spectrum of the compound. Remaining aromatic carbon attached hydrogen appears from the aromatic region from d 152.2 to 146.6 ppm. Similarly, the *ortho*, *meta* and *para* carbons

of phenyl group at C-4 were identified as d 127.7, 126.7 and 125.9 ppm respectively. The signals at d 126.1, 129.9, 136.6, 144.6, 146.3, 147.4, 147.9, 152.2 ppm were due to the non-proton bearing carbons at C-18, C-3, C-4, C-17, C-19, C-20,C-2 and *ipso* positions. The formation of compound **6a** also been supported by the observation of (ESI-HRMS) m/z value at 374.1655 in mass the spectrum.

2.7 *In-vitro* antibacterial studies by micro dilution method

The indicator strains used were Staphylococcus *aureus* MTCC 737, Bacillus *Cereus* MTCC 430, Staphylococcus *aureus* MLS16, Klebsiella *planticola* MTCC 2277, Escherichia *Coli* MTCC 1687 and Pseudomonas *aeruginosa* MTCC 424. The test bacterial strains were grown in nutrient broth medium and inoculums prepared were stored at 4°C for further use. The dilutions of the bacterial inoculums were cultured on a nutrient agar medium to check for the presence of contamination. The bacterial suspension was adjusted with sterile saline to a final concentration of approximately 10^7 CFU/mL in a final volume of 100 µL per well in 96 well plate. The bacterial pathogens were inoculated in Muller-Hinton broth along with different concentrations of compounds including 0.488, 0.244, 0.122, 0.061, 0.030, 0.015, 0.007, 0.003, 0.001 and 0.001 µM. *Neomycin* (Sigma) was used as positive control and Muller-Hinton culture media alone was used as negative control. The bacterial pathogens were incubated along with compounds for 24 h. After incubating for 24 h, 40 µL of INT dye solution was added to each well. The INT dye solution was prepared by dissolving 0.02% of 20 mg INT in 100 mL of 40% DMF. Further the samples were incubated for 2 h. Then the samples were spectrometrically read at 450 nm using TRIAD multi-mode reader which measures the reduction of INT dye by bacterial pathogens. The percentage of bacterial growth inhibition was calculated using the formula:

$$\% \text{ of bacterial growth inhibition} = (1 - Ac/Ao) \times 100.$$

Where Ac represents intensity of the wells with compound concentration C; Ao represents intensity of the negative control without compound concentrations. Further, minimum inhibitory concentration, MIC (µM) values representing the lowest concentration of the compound which exhibited significant decrease in bacterial viability (>95%) was determined. All the experiments were performed in triplicate and the results were expressed as the average of three independent experiments. The results were given as **Table-3** along with the minimum inhibitory concentrations. The compounds exhibited a broad spectrum of antibacterial activity against the tested Gram-positive bacterial pathogens.

2.8 *In-vitro* antioxidant activity by DPPH free radical scavenging method

The antioxidant activity of the compounds was assayed by measuring scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. In this experiment, different concentrations (10, 20, 40, 80, and 200 µg mL^{-1}) of each compound were prepared in methanol. Then, working solutions were mixed with 100 µL 160 µM methanol solution of DPPH. The reaction mixture was thoroughly mixed for 2 min. The samples were then incubated for 30 min in the dark at 35°C. After incubating for 30 min, the absorbance of the samples was measured spectroscopically at 517 nm using a TRIAD multimode reader. In this experiment, ascorbic acid was used as positive control and methanol solution as a negative control. The radical scavenging potential is expressed as an EC_{50} value, representing the compound concentration at which 50% of the DPPH radicals was scavenged. All tests were performed in triplicate, and values are presented as means. The ability of the compound to scavenge the formation of DPPH free radicals was measured in a concentration-dependent manner and the DPPH scavenging activity calculated in terms of the effective concentration.

2.9 *In-vitro* antifungal studies by broth dilution method

The antifungal activity of the compounds was determined by broth dilution method using *p*-iodonitrotetrazolium (INT) dye reduction method in 96 well plates. Different test fungal pathogens used in the study include Candida *albicans* MTCC 227 (MTCC-Microbial Type Culture Collection), Candida *parapsilosis* MTCC 6510, Candida *glabrata* MTCC 3984, Candida *aaseri* MTCC 1907, Aspergillus *niger* MTCC 1344 and Issatchenkia *hanoiensis* MTCC 4755. The test fungal strains (10^7 CFU/mL) were inoculated in 100 µL of potato dextrose broth and the test cultures were stored at 4°C for further experiments. The purity of the test cultures was verified by culturing on nutrient agar medium. In a 96-well plate, fungal test cultures were inoculated with the final concentration of approximately 10^7 CFU/mL in the final volume of 100 µL per well. To each fungal suspension, compound was

added at different concentrations of 0.720, 0.360, 0.180, 0.090, 0.045, 0.011, 0.005, 0.002 and 0.001 μM. In positive control experiments, miconazole (Sigma) was added to each fungal test culture, while potato dextrose broth medium alone was used as negative control. The fungal pathogens were incubated along with test compound for 24 h at 30°C. Aspergillus strain was incubated at 25°C for 72 h. After incubation periods, 40 mL INT test reagent containing 0.02% 20 mg INT in 100 mL 40% dimethylformamide (DMF) was added to each well. The samples were further incubated for 2 h at 37°C. Reduction of INT dye was monitored spectrometrically by measuring samples at 450 nm using a TRIAD multimode reader. The percentage of fungal pathogen growth inhibition was calculated using the formula:

% of fungal pathogen growth inhibition = (1–Ac/Ao) × 100.

Where A_C is the INT absorbance of wells with compound at concentration C and A_0 represents the INT absorbance of the negative control without compound. The minimum inhibitory concentration (MIC, μM) of the samples represents the lowest concentration of the compound with >95% decrease in fungal growth. All experiments were performed in triplicates, and the results are expressed as the average of three independent experiments.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Chemistry

In the present work, a series of 3,4-dihydro-9-arylacridin-1(2*H*)-one based enaminones and their derivatives were synthesized. In the first step different benzophenone derivatives (**1a-c**) were converted into their corresponding 3,4-dihydro-9-arylacridin-1(2H)-ones (**2a-c**) by treating them with 1, 3-cyclohexanedione (**Scheme**) in ethanol in the presence of *ortho*-phosphoric acid as a catalyst. The obtained products **2a-c** were further treated with DMFDMA catalyst to form corresponding enaminones (**3a-c**) under the conventional heating method, in which different solvents such as DMF, xylene, toluene, acetonitrile and dioxane were examined for the effective conversion of **2a-c** to **3a-c (Scheme)**. The yield was observed to be better with toluene and hence, toluene was considered as a suitable solvent for this reaction (**Table-1**). All the newly formed enaminones **3a-c** were characterized using

Table 1: Optimization for the synthesis of 3,4-dihydro-9-arylacridin-1(2*H*)-ones based enaminones.

S. No	Solvents	T (°C)	Time (h)	Yield (%)
1	DMF	90	14	18
2	DMF	100	12	38
3	DMF	>110	8	51
4	Xylene	120	6	66
5	Toluene	110	6	85
6	Acetonitrile	90	48	12
7	Dioxane	100	36	11

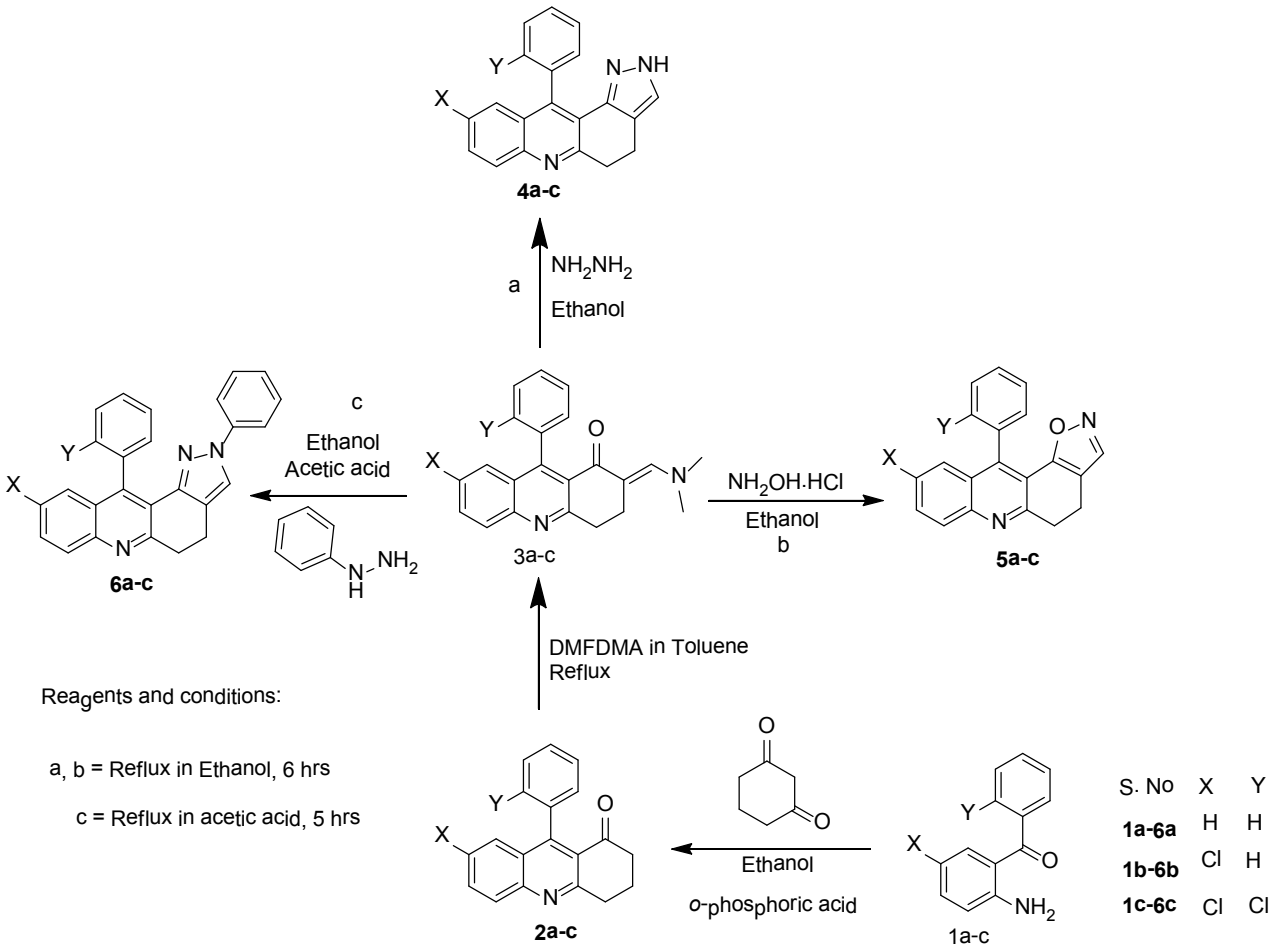
^{1}H-NMR, ^{13}C-NMR, DEPT-135, H, H-COSY, HSQC, HR-MS (ESI) and the detailed data included in the experimental part.

The above enaminone derivatives (**3a-c**) afford various cyclized derivatives of 3,4-dihydro-9-arylacridin-1(2*H*)-ones such as **4a-c**, **5a-c,** and **6a-c** (**Scheme**) while it is treated with reagents such as hydrazine, hydroxylamine hydrochloride and phenylhydrazine. All these newly synthesized compounds were characterized using ^{1}H-NMR, ^{13}C-NMR, DEPT-135, H, H-COSY, HSQC, HR-MS (ESI) and the data included in the experimental section. Consequently, all the newly derived compounds **3a-c**, **4a-c**, **5a-c** and **6a-c** were subjected to the antifungal, anti-bacterial and anti-oxidant evaluations and their results are discussed under biological evaluation.

3.2 Biological evaluations

3.2.1 *In-vitro* antifungal activity

The antifungal activity of the compounds is presented in terms of MIC in **Table-2**. These minimum inhibitory concentration values demonstrate that the compounds exhibited good to moderate antifungal activity against the tested fungal pathogens. Among the compounds, **3a** and **6a** showed the highest antifungal activity with MIC value 0.007 and 0.006 μM against Candida *albicans* and Aspergillus *niger* respectively. In the case of Candida *parapsilosis*, promising antifungal activity was observed for compounds **3a**, **5a** and **6a** with MIC values such as 0.028, 0.031 and 0.023 μM respectively. In the case of Aspergillus *niger*, compound **6a** showed good antifungal activity as compared with positive control miconazole with MIC value of 0.006 μM. In the case of Candida *glabrata*, compound **4a** exhibited good antifungal activity with MIC value 0.031 μM. The other compounds such as **4b**, **5b-c** and **6b-c** failed to exhibit



Scheme 1: Synthesis of enaminone of 3,4-dihydro-9-arylacridin-1(2*H*)-ones and their derivatives.

the activity against the all the fungal pathogens tested and hence not included in **Table-2**. The compound which showed lower antifungal activity is due to substitution on benzene ring, whereas compound **3a, 4a** and **6a** has no such substitution and hence exhibiting better activity.

The results demonstrate that the compounds possess good antifungal activity against the fungal pathogens. Among all the compounds, the compounds **3a** and **6a** exhibited considerable fungal activity (MIC value as 0.89 μM) against Candida *albicans* MTCC 227 and Aspergillus *niger* MTCC 1344 respectively. Whereas other compounds, the anti-fungal activity is lower than **3a** and **6a** due to substitution on the aryl ring but in **3a** and **6a** there is no substitution on it.

3.2.2 *In-vitro* antibacterial activity

The compounds exhibited promising broad-spectrum antibacterial activity against the tested Gram-positive and Gram-negative bacteria pathogens. The antibacterial activity of the compounds is presented in terms of minimum inhibitory concentration in **Table-3**. In case of S. *aureus* strain, the compounds **5a** and **6a** exhibited the highest antibacterial activity with minimum MIC value (0.015 and 0.012 μM) compared with the positive control. In the case of *K. planticola* strain, compounds **4a** exhibited the highest antibacterial activity with minimum MIC value (0.007 μM) compared with the positive control. The compounds **4a** and **5a** showed promising antibacterial activity in the case of test strain Bacillus *cereus* with MIC value 0.007 μM. Similarly, in the case of Staphylococcus *aureus* compounds **3b** and **6a** exhibited good antibacterial activity with minimum MIC values (0.006 and 0.012 μM) in comparison with neomycin standard. Furthermore, it was observed that compound **4a** showed excellent antibacterial activity compared with neomycin standard against Escherichia *coli* bacterial pathogen with MIC value (0.003 μM). In the case of Pseudomonas *aeruginosa*, compared with

Table 2: Antifungal activity of compounds by broth dilution method.

Compounds	Minimum Inhibitory Concentration, MIC (μM)					
	Candida *albicans* MTCC 227	Candida *parapsilosis* MTCC 6510	Candida *aaseri* MTCC 1907	Candida *glabrata* MTCC 3984	Issatchenkia *hanoiensis* MTCC 4755	Aspergillus *niger* MTCC 1344
3a	0.007	0.028	0.228	0.057	0.057	0.057
3b	-	0.051	-	-	-	0.051
3c	-	0.094	0.378	0.094	0.094	0.047
4a	-	0.063	0.252	0.031	-	-
4c	-	-	-	0.113	0.113	-
5a	-	0.031	0.251	0.062	0.251	-
6a	-	0.023	-	0.050	-	0.006

- : No Activity; Miconazole 0.022 † Standard.

Table 3: Antibacterial activity of compounds by micro dilution method.

Compounds	Minimum Inhibitory Concentration, MIC (μM)					
	Gram Positive Bacteria			Gram Negative Bacteria		
	Staphylococcus *aureus* MTCC 737	Bacillus *cereus* MTCC 430	Staphylococcus *aureus* MLS16	Klebsiella *planticola* MTCC 2277	Escherichia *coli* MTCC 1687	Pseudomonas *aeruginosa* MTCC 424
3a	-	-	-	-	0.007	0.007
3b	-	-	0.006	-	-	-
4a	0.031	0.007	0.252	0.007	0.003	0.007
4c	-	-	0.056	0.056	-	-
5a	0.015	0.007	0.125	-	0.015	0.031
6a	0.012	-	0.012	0.050	0.025	0.100
6c	0.021	-	0.042	-	-	-

– : No Activity; Nm-Neomycin (0.030) † Standard.

standard neomycin (MIC 0.030 μM), compound **3a** and **4a** exhibited the highest antibacterial activity with MIC of 0.007 and 0.007 μM. This data clearly suggests that the compounds exhibit promising broad-spectrum antibacterial activity against test bacterial pathogens. The compound **4a** showed excellent antibacterial activity towards Escherichia *coli* MTCC 1687 (MIC value 0.003 μM). The other compounds such as **3c**, **4b**, **5b-c** and **6b** failed to exhibit the activity against the all the bacterial pathogens tested and hence are not included in **Table-3**. The compound which showed lower antibacterial activity is due to substitution on the aryl ring, whereas in compounds **3a, 4a** and **6a** has no such substitution and hence exhibiting better activity.

The data clearly suggested that the compounds exhibit promising broad-spectrum of antibacterial activity against test bacterial pathogens. The compound **4a** showed excellent antibacterial activity towards Escherichia *Coli* MTCC 1687 (MIC value 0.003 μM). Whereas other compounds, the anti-bacterial activity is low due to substitution on benzene ring but in compound **4a** there is no substitution on it.

3.2.3 *In-vitro* antioxidant activity

The results of free radical-scavenging capacity of the compounds by DPPH methods are shown in **Table-4**. All of the compounds except **3c**, **4b** and **5b-c** demonstrated inhibitory activity against the DPPH free radicals. The order of free radical-scavenging capacity of the compounds was **5a**>**4a**>**3a**>**6b**>**6a**>**4c** = **3b**>**6c**. The compounds **4a** and **5a** showed the highest free radical-scavenging capacity as compared to standard ascorbic acid with EC_{50} values 18.9 ± 1.9 and 16.85 ±1.5μgmL^{-1} respectively.

Table 4: *In-vitro* antioxidant activity by DPPH free radical scavenging method.

Compounds	EC_{50} (concentration of 50% scavenging of DPPH free radicals) [μgmL^{-1}]
3a	19.58 ± 1.1
3b	33.5 ± 1.7
4a	18.9 ± 1.9
4c	33.5 ± 1.7
5a	16.85 ±1.5
6a	24.8 ± 1.3
6b	20.74 ± 1.6
6c	41.7 ± 2.1
Ascorbic acid†	18.22 ± 1.25

4 Conclusion

The 3,4-dihydro-9-arylacridin-1(2*H*)-one based enaminones was successfully synthesized by exploring the synthetic potential of DMFDMA which in turn converted into number of biologically potent heterocycles. In addition all the newly synthesized compounds were subjected to the *in-vitro* antifungal, antibacterial and antioxidant evaluations to find their efficacy and the potent lead molecules were found.

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