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Research Article

# Microwave Irradated Synthesis, Characterization and Evaluation for their Antibacterial and Larvicidal Activities of some Novel Chalcone and Isoxazole Substituted 9-Anilino Acridines

**Abstract**

**Introduction:** Chalcone, isoxazole and acridines have diverse biological activities. A series of novel chalcone and isoxazole substituted 9-anilinoacridines were synthesized for their antibacterial, larvicidal, activities.

**Methods:** A series of novel chalcone and isoxazole substituted 9-anilinoacridines (3a-h and 4a-h) were synthesized from 9-chloroacridine by microwave irradiation method. The antibacterial evaluation was performed by cup-plate method and screened for their larvicidal activity by larval bioassay method.

**Result:** The compounds 3d, 3e, 3f, 3h, 4d, 4f have significant antibacterial activity against Gram +ve bacteria like *Staphylococcus aureus*, *Bacillus megaterium*, and Gram –ve *Escherichia coli, Klebsiella pneumoniae* at 25µg/ml. Compounds 3c, 3f, 4a, 4f have significant larvicidal activity against culex and anopheles species at $LC_{50}$value of 17-36ppm.

**Conclusion:** Many of the compounds have significant antibacterial and larvicidal activities, which are used for further refinement.

## Introduction

The design of noble chemical entities like Acridine, chalcone and isoxazole derivatives could lead to availability of better drugs for the treatment of various diseases. In the same context, acridines have gained strong ground for various biological activities like antimicrobial [1], antioxidant [2], anticancer [3-7], antimalarial [8,9], anti-inflammatory [10], analgesic [11], antileishmanial [12], antinociceptive [13], acetyl cholinesterase inhibitors [14] and antiherpes [15] etc. Acriflavine and Ethacridine are the known antibacterial agents with acridine moiety. Amsacrine is the best known compound of 9-anilinoacridines series. It was one of the first DNA-intercalating agents to be considered as a Topoisomerase II inhibitor. Several detailed SAR studies of acridine-based DNA-intercalating agents suggest that the mode of binding is important and the chromophore intercalate with the DNA base pairs. The chemical modification of acridines such as the introduction of different substitutions were allowed expansion of research on the structure activity relationship to afford new insight into molecular interactions at the receptor level [16]. In fact, it is well established that slight structural modification on 9-anilinoacridines may bring various pharmacological effects. Similarly isoxazoles are well known to be an important class of compounds with a wide range of biological activities [17-19] like antimicrobial, anticancer etc. Antibacterial activity against various Gram +ve and Gram –ve bacteria and larvicidal activity against culex and anopheles species are described. As a part of our ongoing research on searching new potent antimicrobial and larvicidal agents, we have synthesized 9-anilinoacridine analogues bearing the isoxazole residue on anilino rings for antimicrobial and larvicidal evaluation. The results revealed that the newly synthesized derivatives exhibited significant biological activities.

## Materials and Methods

Melting points were obtained on Veego VMP-1 apparatus in open capillary tubes and are uncorrected. The reactions were monitored by TLC on silica gel thin layer plates. Compounds were analyzed for C, H, N and analytical results obtained for these elements were within ±0.5% of the calculated values for the formula shown. IR spectra were obtained using a Perkin Elmer spectrum-2 FT-IR spectrometer. $^{1}$HNMR and $^{13}$C NMR were recorded on Bruker A VIII 500 MHz Spectrometer. Chemical shifts are in parts per million (ppm). Mass spectra of the final compounds were recorded on a JEOL GC mate Mass Spectrometer. Microwave irradiation was carried out in cata scientific microwave synthesis system 2400 MHZ, catalyst systems, India.

### Synthesis and characterisation of the compounds

The compounds were synthesized by microwave irradiation (MW) methods and schematized in scheme 1. The yield and reaction time were summarized (Table 1).

*Synthesis of 1-[4-(Acridin-9-ylamino) phenyl]ethanone (2) by MW method*

In a 250mL flask, a mixture of 4.06 g (0.03 Mole) of 4-aminoacetophenone, 5.4528 g (0.0256 Mole) of 9-chloroacridine and 20 mL of 2-butanol were taken and subjected to microwave irradiation for 3 min at 65% intensity (455 W) under reflex condenser. After completion of reaction, the reaction mixture was allowed to cool to room temperature and it was poured into 150 ml of ice water. A precipitate formed was filtered by suction, washed with water and dried crystallized from ethanol. The yield was 71%.

*General procedure for synthesis of Chalcones (3a-h) by MW method [20]*

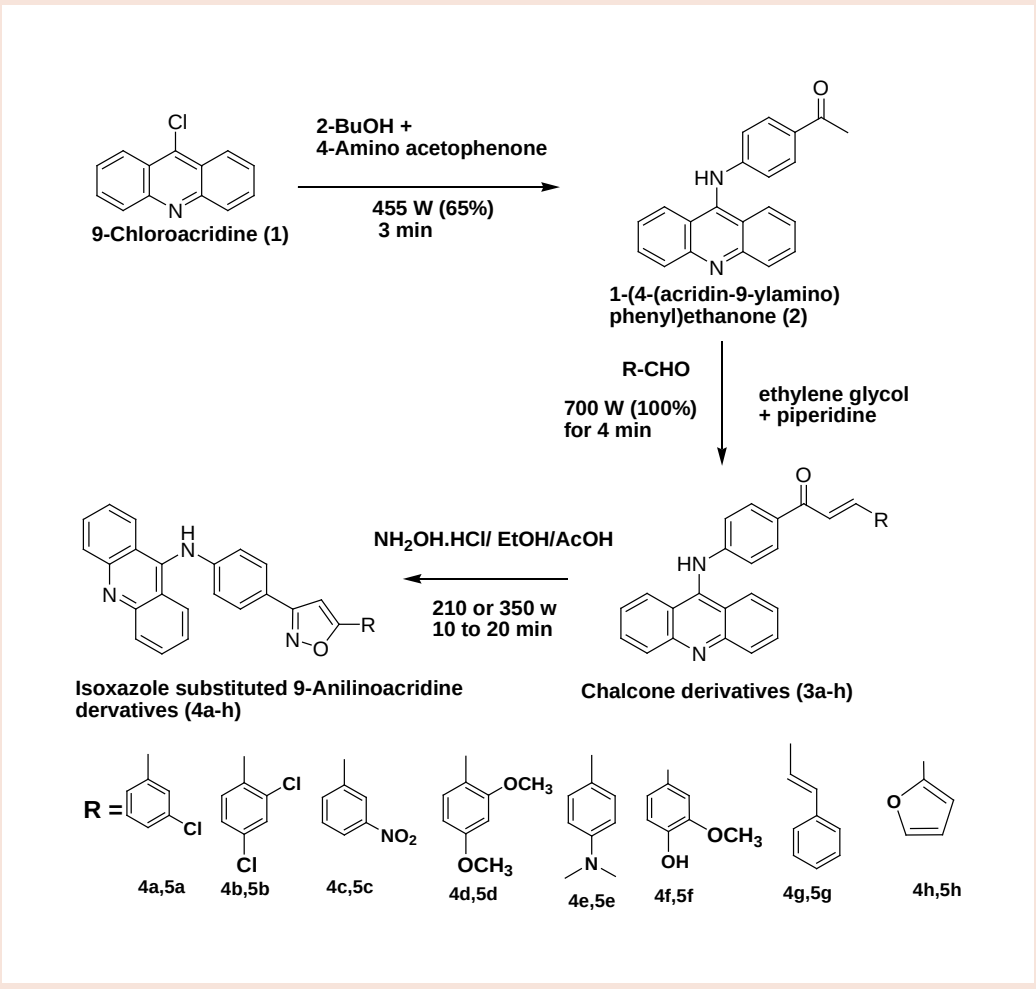
In a 25 ml flask 0.0032 mole of corresponding aldehyde, 5 ml of ethylene glycol, 0.5 ml of piperidine and 0.9984 g (0.0032 Mole) of 9-anilinoacridine were taken and subjected to microwave irradiation for 5 min at 100% intensity (700W). Irradiation was carried out in successive 30 sec periods to avoid over heating of the solvent and the reaction mixture. After completion of the reaction, the reaction mixture was cooled and poured into 100 ml of water. The precipitated solid was filtered, washed with water, dried and rcrystallizede from ethanol.

*E)-1-(4-(acridin-9-ylamino) phenyl)-3-(3-chlorophenyl)prop-2-en-1-one (3a)*

This compound was obtained as a yellow powder; m.p.: 196-198 °C; Anal. Calc. for $C_{28}H_{19}ClN_2O$: C, 77.32; H, 4.42; N, 6.45; Found: C, 77.25; H, 4.29; N, 6.62; IR (υ, $cm^{-1}$): 3302 (N-H), 3100-3000 (Ar C-H), 1624 (α,β-unsat. C=O), 1606 & 1518 (Ar C=C), 1267 (C-N), 748 (Ar C-H); ms: m/z 434.52 ($M^+$); $^1$HNMR (in ppm): 6.65- 8.02 (m, ArH), 7.90 and 7.56(s, α,β-unsaturated carbonyl ), 11.21(s, NH); $^{13}$CNMR (in ppm) :189 (C=O), 153.5,150.8, 143.2, 141.2, 136.3, 136.1, 132.4, 131.7, 130.8, 129.6, 130.5, 128.6, 127.2, 127.1, 127.2, 121.6, 119.5, 116.3.

*(E)-1-(4-(acridin-9-ylamino) phenyl)-3-(2,4-dichlorophenyl)prop-2-en-1-one (3b)*

This compound was obtained as a yellow powder; m.p.: 195-197 °C; Anal. Calc. for $C_{28}H_{18}Cl_2N_2O$: C, 71.68; H, 3.73; N, 5.90; Found:




**Scheme 1:**



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**Table 1:** Reaction conditions and yield of compounds by Micro wave irradiation method.

| Compound | Microwave irradiation method | | | |
|---|---|---|---|---|
| | % Intensity | Power in watts | Reaction time in min | Yield in % |
| **3a** | 100 | 700 | 4 | 67.83 |
| **3b** | 100 | 700 | 4 | 69.48 |
| **3c** | 100 | 700 | 4 | 60.52 |
| **3d** | 100 | 700 | 4 | 62.47 |
| **3e** | 100 | 700 | 4 | 62.36 |
| **3f** | 100 | 700 | 4 | 63.20 |
| **3g** | 100 | 700 | 4 | 55.49 |
| **3h** | 100 | 700 | 4 | 53.73 |
| **4a** | 30 | 210 | 15 | 72.61 |
| **4b** | 50 | 350 | 10 | 71.28 |
| **4c** | 20 | 210 | 15 | 68.19 |
| **4d** | 20 | 350 | 20 | 63.69 |
| **4e** | 50 | 350 | 15 | 62.76 |
| **4f** | 50 | 350 | 20 | 67.50 |
| **4g** | 50 | 350 | 15 | 65.43 |
| **4h** | 50 | 350 | 15 | 68.35 |

C, C, 71.73; H, 3.77; N, 5.92; IR (υ, $cm^{-1}$): 3269 (N-H), 3063-3000 (Ar C-H), 1649 (α,β-unsat. C=O), 1607 & 1512 (Ar C=C), 1231 (C-N), 761 (Ar C-H); ms: m/z 468.16 ($M^+$); $^1$HNMR (in ppm): 6.65- 8.02 (m, ArH), 7.90 and 7.56(s, α,β-unsaturated CH), 10.28(s, NH); $^{13}$CNMR (in ppm) :184.4(C=O), 157.3, 151.3, 142.1, 141.5, 136.7, 136.4, 132.1, 131.4, 130.3, 130.5, 129.2, 128.2, 127.6, 127.4, 127.2, 122.3, 118.3, 176.3, 114.8 (aromatic carbons).

*(E)-1-(4-(acridin-9-ylamino) phenyl)-3-(3-nitro phenyl)prop-2-en-1-one (3c)*

This compound was obtained as a yellow powder; m.p.: 188-190 °C; Anal. Calc. for $C_{28}H_{19}N_3O_3$: C, 75.47; H, 4.30; N, 9.45; Found: C, 75.26; H, 4.52; N, 9.51; IR (υ, $cm^{-1}$): 3034 (N-H), 3100-3000 (Ar C-H), 1626 (α,β-unsat. C=O), 1577 & 1498 (Ar C=C), 1529 & 1348 ($NO_2$), 1280 (C-N), 748 (Ar C-H); ms: m/z 445.37 ($M^+$); $^1$HNMR (in ppm): 6.65- 8.02 (m, ArH), 7.90 and 7.56(s, α,β-unsaturated CH), 10.34( 1H, s, NH); $^{13}$CNMR (in ppm) :189 (C=O), 149.7,149.3, 142.6, 140.5, 136.7, 136.12, 132.8, 131.5, 130.5, 130.1, 129.4, 128.5, 127.3, 127.2, 127.1, 122.6, 120.5, 114.8 (aromatic carbons).

*(E)-1-(4-(acridin-9-ylamino) phenyl)-3-(2,4-dimethoxy phenyl) prop-2-en-1-one (3d)*

This compound was obtained as a yellow powder; m.p.: 228-230 °C; Anal. Calc. for $C_{30}H_{24}N_2O_3$: C, 78.29; H, 5.33; N, 6.13; Found: C, 78.25; H, 5.36; N, 6.12; IR (υ, cm-1): 3044 (N-H), 3100-3000 (Ar st C-H), 1626 (α,β-unsaturated C=O), 1577 & 1498 (Ar C=C), 748 (Ar C-H); ms: m/z 460.58 ($M^+$); $^1$HNMR (in ppm): 6.65- 8.02 (m, ArH), 7.90 and 7.56(s, α,β-unsaturated CH), 11.21(s, NH), 3.23 (s, $OCH_3$). $^{13}$CNMR (in ppm):181 (C=O), 158.6, 151.7, 143.9, 142.1, 137.1, 136.7, 132.1, 131.3, 130.6, 129.3, 129.1, 128.7, 127.6, 127.5, 127.2, 121.4, 119.1, 117.1 (aromatic carbons), 53.49 ($OCH_3$), 54.31 ($OCH_3$).

*(E)-1-(4-(acridin-9-ylamino)phenyl)-3-(4-(dimethylamino) phenyl)prop-2-en-1-one(3e)*

This compound was obtained as Orange crystals; m.p.:165-167°C. Anal. Calcd. for $C_{30}H_{25}N_3O$: C, 81.26; H, 5.65; N, 9.43; Found: C, 81.09; H, 5.75; N, 9.30; IR (υ, $cm^{-}$): 3313 (N-H), 3099-2999 (Ar C-H), 1654 (α,β-unsaturated C=O), 1604 & 1500 (Ar- C=C), 1261 (C-N), 750 (Ar C-H); MS (m/z):444.20 ($m^+$+1); $^1$H NMR (ppm): 2.83 (s, $CH_3$), 7.54 (s, CH=CH), 8.02 to 6.62 (m, Ar-H), 11.03 (s, NH), 2.83 (s, $CH_3$). $^{13}$CNMR (in ppm):183.2(C=O), 158.6, 151.8, 144.5, 142.1, 137.4, 136.5, 132.7, 131.1, 130.3, 129.3, 129.5, 128.6, 127.1, 127.0, 127.2, 121.4, 119.1, 104.8, (aromatic carbons) 56.49 ($CH_3$), 55.37 ($CH_3$).

*(E)-1-(4-(acridin-9-ylamino) phenyl)-3-(4-hydroxy-3-methoxy phenyl)prop-2-en-1-one* **(3f)**

This compound was obtained as a yellow powder; m.p.: 210-212°C; Anal. Calc. for $C_{29}H_{26}N_2O_3$: C, 77.42; H, 5.85; N, 6.33; Found: C, 77.46; H, 5.92; N, 6.37; IR (υ, cm-1): 3304 (N-H), 3100-3000 (Ar st C-H), 1626 (α,β-unsat. C=O), 1587 & 1568 (Ar C=C), 3327 (Ar-OH), 748 (Ar C-H); ms: m/z 450.34 ($M^+$); $^1$HNMR (in ppm): 6.65-8.02 (m, ArH), 5.02(s, OH), 7.90 and 7.56(s, α,β-unsaturated CH), 3.45(s, $OCH_3$) 11.21(s, NH); $^{13}$CNMR (in ppm) :176 (C=O), 169.3, 152.8, 144.7, 142.7, 137.5, 136.8, 132.1, 131.2, 130.4, 129.2, 128.1, 128.0, 127.4, 127.3, 127.1, 121.5, 119.5, 110.2, (aromatic carbons), 42.03($OCH_3$).

*(2E,4E)-1-(4-(acridin-9-ylamino)phenyl)-5-phenylpenta-2,4-dien-1-one* **(3g)**

This compound was obtained as a yellow powder; m.p.:218-220 °C; Anal. Calcd. for $C_{30}H_{22}N_2O$: C, 84.50; H, 5.18; N, 6.58; Found: C, 84.72; H, 5.03; N, 6.42; IR (υ, $cm^{-1}$): 3234 (N-H), 3100-3000 (Ar C-H), 1633 (α,β-unsaturated C=O), 1600 & 1514 (Ar C=C), 1254 (C-N), 761 (Ar C-H); MS (m/z):427.1 ($m^+$+1), $^1$H NMR (ppm): 8.05 to 6.66 (m, 16H, Ar-H), 11.10 (s, NH), 7.23 and 7.77 (s, α,β-unsaturated CH); $^{13}$CNMR (in ppm) :184.12 (C=O), 176.3,155.2, 145.6, 142.5, 137.4, 136.7, 132.3, 131.3, 130.9, 129.8, 128.9, 128.6, 127.5, 127.3, 127.2, 122.4, 119.5, 112.2 (aromatic carbons).

**(***E)-1-(4-(acridin-9-ylamino)phenyl)-3-(furan-2-yl)prop-2-en-1-one* **(3h)**



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This compound was obtained as a yellow powder; m.p.:179-181°C; Anal. Calcd. for $C_{26}H_{18}N_2O_2$: C, 79.97; H, 4.64; N, 7.17; Found: C, 79.88; H, 4.53; N, 7.25; IR (υ, $cm^{-1}$): 3300 (N-H), 3057-3034 (Ar C-H), 1651 (α,β-unsaturated C=O), 1606 & 1512 (Ar C=C), 1230 (C-N), 1176 (C-O), 759 (Ar C-H); MS (m/z): 391.14 ($M^+$+1); $^1$H NMR (ppm): 8.04 to 6.64 (m, Ar-H), 10.74 (s, NH), 7.58 and 7.91(s, α,β-unsaturated CH); $^{13}$CNMR (in ppm) :182.46 (C=O), 162.3, 152.5, 143.7, 142.7, 138.3, 137.2, 133.6, 132.5, 131.8, 130.6, 130.5, 129.2, 128.2, 127.8, 127.5, 122.5, 119.5, 112.2 (aromatic carbons).

*General procedure for Synthesis of isoxazole substituted 9-anilinoacridines* **(4a-h)** *by MW method* [20]

In a 25ml beaker, 2.5g (0.02mol) anhydrous sodium acetate was dissolved in hot glacial acetic acid (10ml) and it was added to a solution of 0.81g (0.01mol) hydroxyl amine hydrochloride in absolute ethanol (10ml). A solution of 0.01mol of the corresponding chalcones 3a-h in 10ml of absolute ethanol was added and the above solution was taken in microwave oven flask and subjected to microwave irradiation at 350 Watt (50% intensity) for 5-10 mins. The reaction was monitored by TLC (Pet ether: Ethyl acetate 3:2). After completion of the reaction excess solvent was concentrated in vacuo at reduced pressure, residual solution obtained was cooled to room temperature, poured into ice cold water, neutralized with sodium hydroxide and washed well with water to remove unreacted hydroxyl amine hydrochloride and excess of sodium hydroxide. Dried crude product yield was noted and recrystallized from ethanol.

*N-(4-(5-(3-chlorophenyl) isoxazol-3-yl)phenyl)acridin-9-amine* (4a)

This compound is obtained as a Yellowish green powder; m.p.: 185-186°C; Anal. calcd. for $C_{28}H_{18}ClN_3O$: C, 75.08; H, 4.05; Cl, 7.92; N, 9.38; Found: C, 75.18; H, 4.05; N, 9.28; IR (υ, $cm^{-1}$): 3338 (N-H), 3048 (Ar st C-H), 1606 & 1473 (Ar C=C), 1157 (Ar C=N), 1230 (C-O), 744 (Ar C-H), 711 (C-Cl); MS: m/z 447.92 ($M^+$); $H^1$NMR(in ppm): 6.45-8.74 (m, ArH), 9.35(s, CH of isoxazole), 11.30(s, 9-NH); $C^{13}$NMR (in ppm) : 98.4 (C of isoxazole), 169.6, 163.5, 150.8, 148.4, 148.5, 145.3, 120.4, 143.2, 141.2, 136.3, 136.1, 132.4, 131.7, 130.8, 129.6, 130.5, 128.6, 127.2, 127.1, 127.2, 121.6, 119.5, 116.3,116.2 (Ar-Carbons).

*N-(4-(5-(2,4-dichlorophenyl) isoxazol-3-yl)phenyl)acridin-9-amine* (4b)

This compound is obtained as a Yellowish green powder; m.p.: 228-230°C; Anal. calcd. for $C_{28}H_{18}Cl_2N_3O$: C, 69.68; H, 3.65; Cl, 14.92; N, 8.38; Found: C, 69.75; H, 14.82; N, 8.43; IR (υ, $cm^{-1}$): 3343 (N-H), 3042 (Ar st C-H), 1608 & 1468 (Ar C=C), 1161 (Ar C=N), 1242 (C-O), 741 (Ar C-H), 715 (C-Cl); MS: m/z 482.52 ($M^+$); $H^1$NMR(in ppm): 6.38-8.65 (m, ArH), 9.32(s, CH of isoxazole), 10.38(s, NH); $C^{13}$NMR (in ppm) :97.8 (C of isoxazole), 169.2, 164.2, 150.8, 149.7, 148.3, 145.7, 143.2, 141.2, 136.3, 136.1, 132.4, 131.7, 130.3, 129.2, 130.2, 128.3, 127.4, 127.3, 127.2, 121.1, 120.8, 119.6, 116.1,116.4 (Ar-Carbons).

*N-(4-(5-(3-nitrophenyl) isoxazol-3-yl) phenyl) acridin-9-amine* (4c)

This compound is obtained as a yellow powder; m.p.: 175-177 °C; Anal. calcd. for $C_{28}H_{18}N_4O_3$: C, 73.35; H, 3.96; N, 12.22; O, 10.47. Found: C, 73.15; H, 3.56; N, 12.12; IR (υ, $cm^{-1}$): 3340 (N-H), 3057 (Ar st C-H), 1608 & 1473 (Ar C=C), 1519 (Ar-$NO_2$), 1159 (Ar C=N), 1228 (C-O), 742 (Ar C-H); MS: m/z 458.47 ($M^+$); $H^1$NMR ( in ppm): 6.85-8.91 (m, ArH), 9.97(s, CH of isoxazole), 11.11(s, 9-NH); $C^{13}$NMR (in ppm):98.4 (C of isoxazole), 169.6, 164.3, 150.4, 149.5, 148.2, 145.5, 143.7, 141.6, 136.7, 136.9, 132.7, 131.3, 130.5, 129.7, 130.3, 128.9, 127.5, 127.7, 127.1, 121.6, 120.3, 119.5, 116.3,116.7 (Ar-Carbons).

*N-(4-(5-(2,4-dimethoxyphenyl)isoxazol-3-yl)phenyl)acridin-9-amine* (4d)

This compound is obtained as a green powder; m.p.: 155-157°C; Anal. calcd. for $C_{30}H_{23}N_2O_3$: C, 76.15; H, 4.92; N, 8.82; Found: C, 76.22; H, 4.83; N, 8.75; IR (υ, $cm^{-1}$): 3340 (N-H), 3030 (Ar st C-H), 1604 & 1473 (Ar C=C), 1157 (Ar C=N), 1219 (C-O), 746 (Ar C-H); MS: m/z 474.32 ($M^+$+1); $H^1$NMR ( in ppm): 6.72-8.64 (m, ArH), 9.83(s, CH of isoxazole), 10.45(s, 9-NH), 3.23 (s, $OCH_3$); $C^{13}$NMR (in ppm):98.4 (C of isoxazole), 57.34 & 56.87 (2 $OCH_3$), 169.1, 163.8, 150.5, 149.2, 148.7, 145.6, 143.1, 141.5, 136.4, 136.6, 132.3, 131.5, 130.7, 129.8, 130.7, 128.3, 127.4, 127.2, 127.5, 121.5, 120.4, 119.3, 116.4,116.2(aromatic carbons).

*N-(4-(5-(4-(dimethylamino) phenyl) isoxazol-3-yl)phenyl)acridin-9-amine* (4e)

This compound is obtained as a red powder; m.p.: 256-258°C; Anal. calcd. for $C_{30}H_{24}N_4O$: C, 78.71; H, 5.32; N, 12.25; Found: C, 78.63; H, 5.28; N, 12.31; IR (υ, $cm^{-1}$): 3340 (N-H), 3026 (Ar st C-H), 1600 & 1473 (Ar C=C), 1159 (Ar C=N), 1253 (C-O), 744 (Ar C-H); MS: m/z 457.52 ($M^+$+1); $H^1$NMR ( in ppm): 6.34-8.34 (m, ArH), 9.75(s, CH of isoxazole), 10.72(s, NH), 3.27 (s, $OCH_3$); $C^{13}$NMR (in ppm):98.4 (C of isoxazole), 56.34 & 56.87 (2 $CH_3$), 169.5, 164.3, 151.2, 149.7, 148.9, 145.8, 143.5, 141.2, 136.5, 136.4, 132.5, 131.7, 130.4, 129.5, 130.4, 128.5, 127.7, 127.4, 127.5, 121.7, 120.4, 119.3, 116.7,116.6 ( aromatic carbons).

*5-(3-(4-(acridin-9-ylamino) phenyl) isoxazol-5-yl)-2-methoxyphenol* (4f)

This compound is obtained as a yellow powder; m.p.: 203-205°C; Anal. calcd. for $C_{29}H_{21}N_3O_3$: C, 75.75; H, 4.62; N, 9.22; Found: C, 75.68; H, 4.57; N, 9.24; IR (υ, $cm^{-1}$): 3342 (N-H), 2991 (Ar st C-H), 1589 & 1473 (Ar C=C), 1157 (Ar C=N), 1253 (C-O), 744 (Ar C-H); MS: m/z 459.52 ($M^+$); $H^1$NMR ( in ppm): 6.23-8.12 (m, ArH), 9.68(s, CH of isoxazole), 10.86(s, NH), 5.02(s, OH), 3.14 (s, $OCH_3$); $C^{13}$NMR (in ppm):97.2 (C of isoxazole), 58.37 ($OCH_3$), 168.4, 164.5, 150.9, 149.8, 148.7, 145.5, 144.1, 141.5, 136.3, 136.4, 132.6, 131.3, 130.7, 129.9, 130.3, 128.6, 127.3, 127.4, 127.6, 121.3, 120.4, 119.5, 116.4,116.5 (aromatic carbons).

*N-(4-(5-styrylisoxazol-3-yl) phenyl) acridin-9-amine* (4g)

This compound is obtained as a red powder; m.p.: 162-164°C; Anal. calcd. for $C_{30}H_{21}N_3O_3$: C, 81.76; H, 4.82; N, 9.52; Found: C, 81.78; H, 4.73; N, 9.54; IR (υ, $cm^{-1}$): 3340 (N-H), 3024 (Ar st C-H), 1600 & 1473 (Ar C=C), 1157 (Ar C=N), 1257 (C-O), 746 (Ar C-H); MS: m/z 439.52 ($M^+$); $H^1$NMR ( in ppm): 6.42-8.18 (m, ArH), 9.73(s, CH of isoxazole), 10.86(s, NH); $C^{13}$NMR (in ppm):98.2 (C of isoxazole), 68.3, 65.8(CH=CH), 68.3, 65.8(CH=CH), 164.6, 155.1, 151.1, 150.8,

148.4, 145.6, 144.1, 142.1, 136.3, 136.5, 132.4, 132.6, 130.5, 129.7, 130.3, 128.2, 126.7, 127.5, 127.7, 121.4, 120.5, 120.4, 116.6,116.3 ( aromatic carbons).

*N-(4-(5-(furan-2-yl) isoxazol-3-yl) phenyl) acridin-9-amine* (4h)

This compound is obtained as a yellow powder; m.p.: 198-200°C; Anal. calcd. for $C_{26}H_{17}N_3O_2$: C, 77.43; H, 4.24; N, 10.45; Found: C, 77.45; H, 4.27; N, 10.47; IR (υ, $cm^{-1}$): 3340 (N-H), 3030 (Ar st C-H), 1600 & 1473 (Ar C=C), 1157 (Ar C=N), 1232 (C-O), 746 (Ar C-H); MS: m/z 403.43 ($M^+$); $H^1$NMR (in ppm): 6.23-7.87 (m, ArH), 9.63(s, CH of isoxazole), 10.78(s, NH); $C^{13}$NMR (in ppm):98.2 (C of isoxazole), 68.3, 65.8(CH=CH), 159.3, 162.4, 151.4, 149.8, 148.9, 145.8, 144.6, 141.5, 136.7, 136.6, 134.6, 131.5, 130.7, 129.8, 130.3, 128.2, 127.6, 127.7, 127.5, 121.7, 120.5, 119.7, 116.5, 116.7 ( aromatic carbons).

## Pharmacology

Acridine derivatives possess a diverse range of pharmacological activities. Hence all the acridinyl chalcone derivatives, 3a-h and isoxazole substituted acridines 4a-h were screened for antibacterial and larvicidal activities. Many of the synthesized final compounds 3a-h, 4a-h have significant biological activities.

## Anti bacterial activity

All the chalcone and isoxazole substituted 9-anilino acridine derivatives (3a-h, 4a-h), were screened for their *in-vitro* antibacterial activity against various bacteria like *Staphylococcus aureus*, *Bacillus megaterium*, *Escherichia coli, Klebsiella pneumoniae,* by cup-plate method. Many of the synthesized compounds have significant antibacterial activity.

*In-vitro* antibacterial assay by Cup Plate Method [21]

Sterile nutrient agar plates were prepared, by pouring into Petri dishes in aseptic conditions. 0.1 ml of each standardized test organism culture was spread on the plates. The test compounds as well as the standard drug solutions and DMSO solvent control were placed in the cavity separately. The compounds were tested at the concentrations of 25, 50 and 100µg/ml against two Gram-positive and two Gram-negative bacteria. Then the plates were kept for 1h to allow the diffusion of solution into the medium. All the bacterial plates were incubated at 37 °C for 24 hrs. The zone of inhibition was measured in mm.

Larvicidal activity by Larval Bioassay Experiment [22]

The synthesized compounds 3a-h and 4a-h were also screened for their larvicidal activity by larval bioassay method as per the standard procedure recommended by WHO. The final 3rd instar or early 4th instar larva were collected according to larval size and degree of chitinization of respiratory siphon in 25 ml of water in 50 ml beakers. They were left for 15-20 min in these beakers for acclimatizing to experimental condition; at this stage unhealthy/ parasitized/damaged larvae were rejected. 25 larvas were collected with pasture pipette, placed on a filter paper for removal of excess water and they were placed in to 250 ml test solutions two replicates and control also kept. The beakers were covered with muslin cloth to avoid the entry of any other foreign material or organisms. The larval mortality was recorded in the test concentrations of each beaker after 24 h by counting the dead, moribund and alive larva. When the control mortality lies between 0- 10% then only the corrected mortality was calculated by using Abbot's formula.

## Results and Discussion

Our synthetic pathway (Scheme 1), the 9-chloro acridine 1, was refluxed with p-amino acetophenone to yield 1-(4-(acridine-9-ylamino) phenyl ethanone 2. The various chalcone substituted 9-anilino acridines 3a-h were prepared by the reaction of 2 with various aldehydes and these chalcone derivatives was allowed to cyclized with hydroxylamine hydro chloride afford the corresponding isoxazole substituted 9-anilino acridines 4a-h [20]. Synthesis, characterization and evaluation of biological activities of novel chalcone and isoxazole substituted 9-anilino acridines are described in this paper. The synthesized compounds were purified by column chromatography. The final yield of the derivatives was in the range of 57–78%. The new compounds were completely characterized by IR, $^1$HNMR, $^{13}$CNMR, Mass spectral data and elemental analysis. The IR spectra of compounds 4a-h showed intense bands in the region 1200– 1300 $cm^{-1}$ due to carbonyl stretching and broad bands in the region 3300–3400 $cm^{-1}$ due to NH stretching. The $^1$H NMR spectra also support the structure of the compounds 4a-h. The NH proton appeared at 10 - 11 and CH proton of isoxazole at 9-9.5. The mass spectra of all compounds 4a-h showed molecular ion peaks confirming their molecular weight. Results are summarized in Tables 1-3 and schematized in scheme 1. The chalcone and isoxazole substituted 9-anilino acridines have pharmacological properties of the compounds greatly depended on the number and the chemical nature of the substituents.

From Table 1, the reaction time was reduced from hours in conventional methods to minutes and the yield was increased in the microwave irradiation method. The synthesized compounds were characterized and confirmed by physical characters and spectral value etc.

For *in-vitro* antibacterial assay, all the synthesized compounds 3a-h and 4a-h have significant activity against Gram positive bacteria like *Staphylococcus aureus* and *Bacillus megaterium* and Gram negative bacteria like *Klebsiella pneumoniae* and *Escherichia coli* at concentration about 100 and 50µg/ml. But among those compounds 3e, 3f, 3h, 4d and 4f were showed significant activity even at the concentration of 25µg/ml against *Staphylococcus aureus* and the compounds 3g, 3f, 3h and 4f were showed significant activity even at the concentration of 25µg/ml against *Bacillus megaterium.* The compounds 3b, 3e and 4b were showed significant activity even at the concentration of 25µg/ml against *Escherichia coli.* The results were summarized (Table 2).

For larval bio assay, the test concentration of the synthesized compounds 3a-h and 4a-h have significant larvicidal activity against *Culex quinquefasciatus* and *Anopheles stephensi*. Among compounds 3a-c, 3f, 3g, 4a-d and 4f showed good larvicidal activity with $LC_{50}$ value of 22.91- 64.8 ppm on filarial vector *Culex quinquefasciatus* larvas. The compounds 3a-h, 4a-c, 4f and 4h have good larvicidal activity with $LC_{50}$ value of 22.42- 64.81 ppm on the malarial vector *Anopheles*

**Table 2:** Antibacterial activity of synthesized compounds 3a-h and 4a-h by cup and plate method.

| Compound | Zone of inhibition in mm | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Concentration (µg/ml) | | | | | | | | | | | | |
| | | *S.aureus (G+ve)* | | | *B.megaterium (G+ve)* | | | *K. pneumonia (G-ve)* | | | *E. coli (G-ve)* | | |
| | Ct | 25 | 50 | 100 | 25 | 50 | 100 | 25 | 50 | 100 | 25 | 50 | 100 |
| **3a** | - | - | 10 | 15 | - | 13 | 16 | - | 10 | 17 | - | 10 | 16 |
| **3b** | - | - | 12 | 18 | - | 12 | 17 | - | 16 | 19 | 10 | 15 | 20 |
| **3c** | - | 7 | 16 | 19 | - | 11 | 16 | - | 13 | 16 | - | 11 | 16 |
| **3d** | - | 7 | 16 | 20 | - | 10 | 15 | - | 10 | 13 | - | 10 | 14 |
| **3e** | - | 12 | 19 | 21 | - | 12 | 17 | - | 15 | 18 | 9 | 12 | 19 |
| **3f** | - | 11 | 16 | 20 | 10 | 14 | 19 | - | 10 | 15 | - | 11 | 16 |
| **3g** | - | 7 | 12 | 16 | 10 | 19 | 22 | - | 11 | 16 | - | 11 | 17 |
| **3h** | - | 11 | 19 | 22 | 10 | 14 | 20 | 8 | 14 | 19 | - | 16 | 19 |
| **4a** | - | - | 13 | 15 | - | 12 | 16 | - | 10 | 14 | - | 10 | 14 |
| **4b** | - | - | 12 | 16 | - | 13 | 17 | - | 14 | 19 | 10 | 15 | 20 |
| **4c** | - | 8 | 14 | 18 | - | 10 | 14 | - | 13 | 16 | - | 10 | 13 |
| **4d** | - | 11 | 18 | 20 | - | 9 | 15 | - | 10 | 14 | - | 10 | 14 |
| **4e** | - | - | 9 | 15 | - | 12 | 16 | - | 11 | 15 | - | 11 | 15 |
| **4f** | - | 9 | 17 | 21 | 8 | 11 | 16 | - | 9 | 12 | - | 10 | 13 |
| **4g** | - | - | 10 | 16 | - | 10 | 18 | - | 9 | 15 | - | 12 | 17 |
| **4h** | - | - | 12 | 16 | - | 14 | 16 | - | 12 | 16 | - | 13 | 18 |
| **Standard Gentamycin ( 25 µg/ml)** | 14 | | | | 16 | | | 21 | | | 20 | | |

**Table 3:** Larvicidal activity by Larval Bioassay Method.

| S.No | Compound | Culex species (ppm) | | Anopheles species (ppm) | |
|---|---|---|---|---|---|
| | | $LC_{50}$ | $LC_{95}$ | $LC_{50}$ | $LC_{95}$ |
| **1** | **3a** | 43.39 | 96.51 | 57.49 | >100 |
| **2** | **3b** | 53.32 | >100 | 63.42 | >100 |
| **3** | **3c** | 36.85 | 96.68 | 30.47 | 74.81 |
| **4** | **3d** | 81.42 | >100 | 22.45 | 75.29 |
| **5** | **3e** | 74.20 | >100 | 31.15 | 83.55 |
| **6** | **3f** | 17.11 | 50.43 | 23.87 | 70.67 |
| **7** | **3g** | 52.87 | >100 | 24.69 | 85.84 |
| **8** | **3h** | 92.23 | >100 | 55.59 | 95.58 |
| **9** | **4a** | 28.19 | >100 | 57.01 | >100 |
| **10** | **4b** | 48.43 | >100 | 63.51 | >100 |
| **11** | **4c** | 62.37 | >100 | 51.71 | 83.09 |
| **12** | **4d** | 64.81 | >100 | >100 | >100 |
| **13** | **4e** | >100 | >100 | >100 | >100 |
| **14** | **4f** | 22.91 | 72.02 | 22.42 | 72.58 |
| **15** | **4g** | >100 | >100 | 86.55 | >100 |
| **16** | **4h** | 71.42 | >100 | 64.81 | >100 |

$LC_{50}$, concentration which killed 50% of the larvas. $LC_{95}$, concentration which killed 95% of the larvas.

*stephensi* larvas. Among these compounds, the more polar OH group containing compound 3f showed more potent activity against both larvas. The results were summarized (Table 3).

## Conclusion

A series of novel chalcones and isoxazole bearing acridine moiety have been synthesized for various biological activities. On this basis, authors recently demonstrated that diverse compounds of the chalcone and isoxazole substituted 9-anilino acridine series exerted potent antibacterial and larvicidal activities. Results obtained in the present study clearly demonstrated that some derivatives of the chalcone and isoxazole substituted 9- anilino acridine family could exert interesting antibacterial and larvicidal activities and are likely to be useful as drugs after further refinement. These derivatives will encourage helping to design future antimicrobial and larvicidal agents with therapeutic potentials.

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