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SYNTHESIS AND ANTI-MICROBIAL ACTIVITIES OF SOME NOVEL SCHIFF BASES OF 5-SUBSTITUTED ISATIN DERIVATIVES

C.R. Prakash[1*], S. Raja[2], T. Panneer Selvam[1], G. Saravanan[3], V. Karthick and P. Dinesh Kumar

[*1]Department of Pharmaceutical Chemistry, D.C.R.M. Pharmacy College, Inkollu-523167 (A.P), India
[2] Bharat Institute of Technology (Pharmacy), Ibrahimpatnam, Hyderabad, Andhrapredesh, 501 510, India
[3]Medicinal Chemistry Research Laboratory, Bapatla College of Pharmacy, Bapatla-522101 (A.P), India
E.Mail: crp2020@gmail.com

ABSTRACT

In the present study, a series of novel Schiff bases of 5-substituted isatin were synthesized by condensation of imesatin with different aromatic aldehydes. The imesatins were synthesized by reaction of 5-substituted isatin with p-phenylenediamine. The chemical structures of the synthesized compounds were confirmed by means of IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectroscopy and Elemental analysis. These compounds were screened for antibacterial (Staphylococcus aureus ATCC 9144, Staphylococcus epidermidis ATCC 155, Micrococcus luteus ATCC 4698, Bacillus cereus ATCC 11778, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 2853 and Klebsiella pneumoniae ATCC 11298) and antifungal (Aspergillus niger ATCC 9029 and Aspergillus fumigatus ATCC 46645) activities by paper disc diffusion technique. The minimum inhibitory concentrations (MICs) of the compounds were also determined by agar streak dilution method. Among the synthesized compounds, 3-(4-(4-nitrobenzylideneamino)-phenylimino)indoline-2-one **5e** was found to be the most potent antimicrobial activity with MICs of 9.4, 10.4, 11.8, 9.8, 12.3, 12.0, 10.8, 12.3 and 14.7 μg.mL^{-1} against above mentioned respective strains. Compounds were found to exhibit more antibacterial than antifungal activity.

Key words: Isatin; Schiff base; Antibacterial; Antifungal.

INTRODUCTION

The synthesis of a newer class of anti-bacterial and anti-fungal agents is in need of time, especially against drug-resistant bacteria and fungi, such as gram-positive and gram-negative strains, which are responsible for a number of serious infections in the acute and chronic care units in hospitals. The search of new antimicrobial agents with reduced toxicity and lower side effects is of continuous process. One of the most frequently encountered heterocyclic in medicinal chemistry is isatin and its derivatives have gained unique importance due to the broad spectrum of pharmacological activities which are reflected by their use as antimicrobial[1-6], anticonvulsant[7,8] analgesic[9,10], antiinflammatory[10], anticancer[11,12], antitubercular[13], antiviral[14-16] and anti-HIV[17] activities. Schiff bases are used as substrates in the preparation of a number of industrial and biologically active compounds via ring closure, cycloaddition and replacement reactions[18]. Moreover, Schiff bases derived from various heterocycles have been reported to possess cytotoxic[19], anticonvulsant[20], antiproliferative[21], antimicrobial[22], anticancer[23], and antifungal activities[24]. In generally Schiff bases are reported to possess antimicrobial activities. The literature survey revealed that introduction of electron- withdrawing groups at positions 5, 6, and 7 greatly increased activity from that of isatin, with substitution at the 5th position being most favorable. This is not surprising, as C-5 substitution has previously been associated with increased biological activity for a

range of indole-based compounds[25,26] and the presence of substituted aromatic ring at 3rd position has been reported to be associated with antimicrobial properties[27,28]. The various substituent at 3rd position of the isatin which were reported, are various substituted phenyl ring moieties[29,30], heterocyclic rings[31-33] and aliphatic system[34].These observations led to the conception that a series of some different novel Schiff bases of substituted isatin were synthesized using different aromatic aldehydes by condensation with imesatin and their chemical structure were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectroscopy and Elemental analysis. These compounds were screened for their antibacterial activity against four Gram-positive bacteria (*Staphylococcus aureus* ATCC 9144*, Staphylococcus epidermidis* ATCC 155*, Micrococcus luteus* ATCC 4698 and *Bacillus cereus* ATCC 11778), three Gram-negative bacteria (*Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 2853, *and Klebsiella pneumoniae* ATCC 11298*)* and antifungal (*Aspergillus niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645) activities by paper disc diffusion technique. The minimum inhibitory concentrations (MICs) of the compounds were also determined by agar streak dilution method.

EXPERIMENTAL

Chemistry

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on ABB Bomem FT-IR spectrometer MB 104 with KBr pellets. The ^{1}H (400 MHz) and ^{13}C-NMR (400 MHz) spectra were recorded on a Bruker 400 NMR spectrometer (with TMS for ^{1}H and DMSO-d6 for ^{13}C as internal references). Mass spectroscopy was recorded on Shimadzu GC MS QP 5000. Microanalyses were obtained with an elemental analyses system GmbH VarioEL V300 element analyzer. The purity of the compounds was checked by TLC on pre-coated SiO_2 gel (HF_{254}, 200 mesh) aluminium plates (E Merk) using ethyl acetate: *n*-hexane (2:3) and visualized in UV chamber. IR, ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy and elemental analysis were consistent with the assigned structures.

General method of synthesis of Substituted Isatin (3):

Substituted isatin was prepared according to reported literature [35]. In the present study 4-Nitro aniline **1** was treated with chloral hydrate and anhydrous sodium sulphate to form Substituted isonitrosoacetanilide **2.** Then this intermediate undergoes to cyclization with sulphuric acid to form 5-Nitro isatin (**3).**

General method of synthesis of Substituted imesatin (4):

Equimolar quantities of 5-Nitro isatin (**3)** reacted with *p* – Phenylenediamine, resulting in the formation of substituted imesatin **4**[9]**.**

General method of synthesis of Schiff bases (5a-5l)

Equimolar quantities (0.01 mol) of substituted imesatin (**4**) and various aromatic aldehydes were dissolved in ethanol and refluxed for 8 h. After standing for approximately 24 – 48 h at room temperature the product of different substituted derivatives of isatin (**5a-5l**) which separated out as a mixture of isomers was filtered, dried and recrystallised from absolute ethanol. All the synthesized compounds were soluble in dimethylformamide (DMF).

Antimicrobial activity

Bacterial resistance to the antibiotic is a big blow to humanity and continual search for newer chemotherapeutic agent is the only way to fortify against this awful throat. All the synthesized compounds were screened for *in vitro* antibacterial and antifungal activities by sabouraud dextrose agar medium. The antibacterial activity of the compounds were evaluated against four Gram-positive bacteria (*Staphylococcus aureus* ATCC 9144, *Staphylococcus epidermidis* ATCC 155*, Micrococcus luteus* ATCC 4698 and *Bacillus cereus* ATCC 11778) and three Gram-negative bacteria (*Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 2853, *and Klebsiella pneumoniae* ATCC 11298*)*. The antifungal activities of the synthesized compounds were evaluated against two fungi (*Aspergillus niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645). The observed data on the antimicrobial activity of the synthesized compounds, control and standard drugs are given in Table **1**.

Paper disc diffusion technique

Preliminary antimicrobial activities of **5a-5l** compounds were tested by paper disc diffusion method. The sterilized [36] (autoclaved at 120 °C for 30 min) medium (40-50 °C) was inoculated (1 mL/100 mL of medium) with the suspension (10^5 cfu.mL^{-1}) of the microorganism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3-4 mm. The paper impregnated with all the synthesized compounds as its isomeric mixture forms **5a-5l** (100 µg mL^{-1} in dimethylformamide) was placed on the solidified medium. The plates were incubated at 37°C and the diameter of the growth inhibition zones were measured after 24 h in case of bacteria and after 48 h in case of fungi. Ciprofloxacin (100 µg/disc) and Ketoconazole (100 µg/disc) were used as standard for antibacterial and antifungal activities, respectively. The observed zone of inhibition is presented in Table-**1**.

Minimum inhibitory concentration (MIC)

MIC [37] of the compounds was determined by agar streak dilution method. A stock solution of different substituted isatin compound (100 µg.mL^{-1}) in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for antibacterial activity and sabouraud dextrose agar medium for antifungal activity). A specified quantity of the medium (40-50 °C) containing the synthesized compound was individually poured into a petridish to give a depth of 3-4 mm and allowed to solidify. Suspension of the microorganism were prepared to contain approximately 10^5 cfu.mL^{-1} and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37°C for 24 and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate.

RESULTS AND DISCUSSION

Chemistry:

3-(4-(benzylideneamino) phenylimino)-5-nitro indoline-2-one (5a):

Bright yellow crystals; Yield: 85%; mp. 322-324 °C; IR: 3177 (N-H), 3050 (Ar-CH), 1690 (C=O), 1597 (C=N), 1580 (C=C) cm^{-1}; ^{1}H-NMR (DMSO): δ 8.29 (s, 1H, -N=CH-), 8.02 (s, 1H, -NH-), 7.01-7.68 (m, 12H, H-4, H-6, H-7, H-2', H-3', H-5', H-6', H-2", H-3", H-4", H-5", H-6", Ar-H); ^{13}C-NMR (DMSO): δ 167.2 (C-2), 163.5 (C-3), 160.3 (-N=CH-), 151.6 (C-1' and C-4'), 133.5 (C-9'), 132.5 (C-6), 130.4 (C-8), 131.2 (C-4"), 129.5 (C-4), 129.2 (C-2" and C-6"), 129.2 (C-5), 128.7 (C-3" and C-5"), 126.4(C-1"), 123.4 (C-2', C-3', C-5' and C-6'), 121.9 (C-7); EI-MS (m/z, %): 370(M$^+$, 21), 235(14), 120(100), 105(24), 69(44); (Calcd. for $C_{21}H_{14}N_4O_3$: 370.36); Anal. Calcd. for $C_{21}H_{14}N_4O_3$: C, 68.10; H, 3.81; N, 15.13; Found: C, 68.15; H, 3.88; N, 15.19.

3-(4-(4-chlorobenzylideneamino) phenylimino) -5-nitro indoline-2-one (5b):

Pale yellow crystals; Yield: 78%; mp. 356-358 °C; IR : 3130 (N-H), 2988 (Ar-CH), 1613 (C=N), 1700 (C=O), 1599 (C=C), 744 (C-Cl) cm^{-1}; ^{1}H-NMR (DMSO): δ 8.25 (s, 1H, -N=CH-), 7.92 (s, 1H, -NH-), 7.03-7.60 (m, 11H, H-4, H-6, H-7, H-2', H-3', H-5', H-6', H-2", H-3", H-5", H-6", Ar-H); ^{13}C-NMR (DMSO): δ 166.9 (C-2), 164.2 (C-3), 160.1 (-N=CH-), 151.2 (C-1' and C-4'), 136.2 (C-4"), 133.5 (C-9), 131.2 (C-6), 130.6 (C-8), 130.5 (C-2" and C-6"),129.8 (C-4), 129.1 (C-3" and C-5"), 126.6 (C-1"), 124.4 (C-5), 123.1 (C-2',C-3',C-5' and C-6'), 121.6 (C-7); EI-MS (m/z, %): 406(M+2), 404(M$^+$**,** 20), 264(22), 91(100), 77(22), 69(44); (Calcd. for $C_{21}H_{13}ClN_4O_3$: 404.80); Anal. Calcd. for $C_{21}H_{13}ClN_4O_3$: C, 62.31; H, 3.24; N, 13.84; Found: C, 62.36; H, 3.30; N, 13.81.

3-(4-(4-hydroxybenzylideneamino) phenylimino) -5-nitro indoline-2-one (5c):

Pale yellow solid; Yield: 88%; mp. 342-344 °C; IR : 3529 (Ar-OH), 3130 (N-H), 3011 (Ar-CH), 1680 (C=O), 1615 (C=C), 1591 (C=N) cm^{-1}; ^{1}H-NMR (DMSO): δ 8.28 (s, 1H, -N=CH-), 8.01 (s, 1H, -NH-), 7.01-7.48 (m, 7H, H-4, H-6, H-7, H-2', H-3', H-5', H-6', Ar-H), 7.42 (d, J=7.2 Hz, 1H, C-2" Ar-H), 7.47 (d, J=6.5 Hz,1H, C-6" Ar-H), 6.62 (d, J=5.9 Hz,1H, C-3" Ar-H), 6.67 (d, J=7.8 Hz, 1H, H-5" Ar-H), 5.14 (s, 1H, Ar -OH); ^{13}C-NMR (DMSO): δ 166.9 (C-2), 163.2 (C-3), 160.8 (C-4"), 160.1(-N=CH-), 151.4 (C-1' and C-4'), 133.4 (C-9), 132.0 (C-6), 130.5 (C-2" and C-6"), 130.3 (C-8), 129.4 (C-4), 126.4 (C-1"), 124.5 (C-5), 123.5 (C-2',C-3',C-5' and C-6'), 121.7 (C-7), 116 (C-3" and C-5"); EI-MS (m/z, %): 386(M$^+$,

26), 222(66), 149(74), 121(100), 57(74), 69(44); (Calcd. for $C_{21}H_{14}N_4O_4$: 386.36); Anal. Calcd. for $C_{21}H_{14}N_4O_4$: C, 65.28; H, 3.65; N, 14.50; Found: C, 65.33; H, 3.70; N, 14.52.

3-(4-(4-methoxybenzylideneamino) phenylimino) -5-nitro indoline-2-one (5d):

Lemon yellow crystals; Yield: 65%; mp. 332-334 °C; IR: 3146 (N-H), 3079 (Ar-CH), 1688 (C=O), 1647 (C=C), 1567 (C=N), 1270 (C-O-C) cm^{-1}; ^{1}H-NMR (DMSO): δ 8.39 (s, 1H, -N=C<u>H</u>-), 8.01(s, 1H, -N<u>H</u>-), 7.51(d, J=6.3 H_Z, 1H, C-6" Ar-H), 7.47 (d, J=5.9 Hz, 1H, C-2" Ar-H), 6.99-7.31 (m, 7H, H-4, H-6, H-7, H-2', H-3', H-5', H-6' Ar-H), 6.81 (d, J=7.2 Hz, 1H, H-5" Ar-H), 6.77(d, J=6.5 Hz, 1H, H-3" Ar-H), 3.70 (s, 3H, -OC<u>H</u>$_3$); ^{13}C-NMR (DMSO): δ 167.6 (C-2), 163.5 (C-3), 163.1 (C-4"), 160.5(-N=<u>C</u>H-), 151.64 (C-1' and C-4'), 133.6 (C-9), 132.0 (C-6), 130.6 (C-8), 130.2 (C-2" and C-6"), 129.4 (C-4), 126.1 (C-1"), 124.5 (C-5), 123.6 (C-2',C-3',C-5' and C-6'), 121.5 (C-7), 114.3 (C-3" and C-5"), 55.8 (-O<u>C</u>H$_3$); EI-MS (m/z, %): 400(M^+, 18), 282(20), 121(100), 91(42), 55(94); (Calcd. for $C_{22}H_{16}N_4O_4$: 400.39); Anal. Calcd. for $C_{22}H_{16}N_4O_4$: C, 66.00; H, 4.03; N, 13.99; Found: C, 66.02; H, 4.07; N, 13.89.

3-(4-(4-nitrobenzylideneamino) phenylimino) -5-nitro indoline-2-one (5e):

Creamy crystals; Yield: 74%; mp. 352-354 °C; IR : 3132 (N-H), 3012 (Ar-CH), 1690 (C=O), 1603 (C=C), 1590 (C=N), 1515 & 1310 (N=O) cm^{-1}; ^{1}H-NMR (DMSO): δ 8.29 (s, 1H, -N=C<u>H</u>-), 8.21 (d, J=7.1 Hz, 1H, H-5" Ar-H), 8.17 (d, J=6.8 Hz, 1H, H-3" Ar-H), 8.10 (s, 1H, -N<u>H</u>-), 7.77(d, J=7.5 Hz, 1H, H-2' Ar-H), 7.69 (d, J=6.2 Hz, 1H, H-6" Ar-H), 6.99-7.70 (m, 7H, H-4, H-6, H-7, H-2', H-3', H-5', H-6' Ar-H); ^{13}C-NMR (DMSO): δ 166.7 (C-2), 162.9 (C-3), 159.6 (-N=<u>C</u>H-), 151.6 (C-1' and C-4'), 150.2 (C-4"), 139.6 (C-1"), 133.1 (C-9), 131.2 (C-6), 130.2 (C-8), 130.0 (C-2" and C-6"), 128.3 (C-4), 124.6 (C-5), 123.4 (C-2', C-3', C-5' and C-6'), 121.7 (C-7), 121.23 (C-3" and C-5"); EI-MS (m/z, %): 415(M^+, 58), 324(18), 235(100), 120(18), 77(42). (Calcd. for $C_{21}H_{13}N_5O_5$: 415.36); Anal. Calcd. for $C_{21}H_{13}N_5O_5$: C, 60.72; H, 3.15; N, 16.86; Found: C, 60.75; H, 3.16; N, 16.89.

3-(4-(2-hydroxybenzylideneamino) phenylimino) -5-nitro indoline-2-one (5f):

Creamy crystals; Yield: 83%; mp. 328-330 °C; IR : 3467(Ar-OH), 3210 (N-H), 3065 (Ar-CH), 1678 (C=O), 1649 (C=C), 1575 (C=N) cm^{-1}; ^{1}H-NMR (DMSO): δ 8.22 (s, 1H, -N=C<u>H</u>-), 7.06-7.67 (m, 7H, H-4, H-6, H-7, H-2', H-3', H-5', H-6' Ar-H), 6.75-7.40 (m, 4H, H-3", H-4", H-5" and H-6" Ar-H), 6.01 (s, 1H, -N<u>H</u>-), 5.20 (s,1H, Ar-OH); ^{13}C-NMR (DMSO): δ 167.2 (C-2), 163.2 (C-3), 161.2 (C-2"), 160.2 (-N=<u>C</u>H-), 151.7 (C-1' and C-4'), 133.8 (C-9), 132.3 (C-4"), 131.4 (C-6), 130.1 (C-8), 130.5 (C-6"), 129.3 (C-4), 126.2(C-1"), 124.5 (C-5), 123.5 (C-2', C-3', C-5' and C-6'), 121.5 (C-7), 121.3 (C-5"), 116.0 (C-3"). EI-MS (m/z, %): 386(M^+, 36), 282(6), 242(34), 131(100), 89(26), 77(30). (Calcd. for $C_{21}H_{14}N_4O_4$: 386.36); Anal. Calcd. for $C_{21}H_{14}N_4O_4$: C, 65.28; H, 3.65; N, 14.50; Found: C, 65.25; H, 3.68; N, 14.54.

3-(4-(4-methylbenzylideneamino) phenylimino) -5-nitro indoline-2-one (5g):

Pale yellow crystals; Yield: 71%; mp. 322-326 °C; IR: 3198 (N-H), 3144 (Ar-CH), 1696 (C=O), 1618 (C=C), 1518 (C=N) cm^{-1}; ^{1}H-NMR(DMSO): δ 8.21 (s, 1H, -N=C<u>H</u>-), 8.01 (s, 1H, -N<u>H</u>-), 7.01-7.50 (m, 11H, H-4, H-6, H-7, H-2', H-3', H-5', H-6', H-2",H-3", H-5", H-6" Ar-H), 2.30 (s, 3H, -C<u>H</u>$_3$); ^{13}C-NMR (DMSO): δ 166.2 (C-2), 163.2 (C-3), 159.6 (-N=<u>C</u>H-), 151.2 (C-1' and C-4'), 140.6 (C-4''), 133.2 (C-9), 131.2 (C-6), 130.9 (C-8), 130.8 (C-1''), 129.5 (C-4), 129.3 (C-3" and C-5"), 129.1 (C-2" and C-6"), 124.6 (C-5), 123.5 (C-2', C-3', C-5' and C-6'), 121.6 (C-7), 24.1 (-CH_3); EI-MS (m/z, %): 384(M^+, 28), 235(40), 222(80), 104(92), 55(100). (Calcd. for $C_{22}H_{16}N_4O_3$: 384.39); Anal. Calcd. for $C_{22}H_{16}N_4O_3$: C, 68.74; H, 4.20; N, 14.58; Found: C, 68.78; H, 4.22; N, 14.59.

3-(4-(3, 4, 5,-trimethoxy benzylideneamino) phenylimino) -5-nitro indoline-2-one (5h):

Pale yellow powders; Yield: 81%; mp. 318-322 °C; IR: 3186 (N-H), 3061 (Ar-CH), 1682 (C=O), 1672 (C=C), 1574 (C=N), 1283 (C-O-C) cm^{-1}; ^{1}H-NMR(DMSO): δ 8.35 (s, 1H,-N=C<u>H</u>-), 7.99 (s, 1H, -N<u>H</u>-), 6.99-7.29 (m, 7H, H-4, H-6, H-7, H-2', H-3', H-5', H-6' Ar-H), 6.51 (s, 1H, H-2" Ar-H), 6.58 (s, 1H, H-6'' Ar-H), 3.70 (s, 9H, [OC<u>H</u>$_3$]$_3$); ^{13}C-NMR (DMSO): δ 167.2 (C-2), 163.2 (C-3), 160.2 (-N=<u>C</u>H-), 151.6 (C-1' and C-4'), 150.5 (C-3'' and C-5''), 141.3 (C-4"), 132.9 (C-9), 131.2 (C-6), 131.0 (C-8), 129.4 (C-4), 128.1 (C-1"), 124.5 (C-5), 123.5 (C-2', C-3', C-5' and C-6'), 121.9(C-7), 106.6 (C-2" and C-6"), 56.3 ([O<u>C</u>H$_3$]$_3$); EI-MS (m/z, %): 460(M^+, 28), 324(18), 263(8), 167(100), 125(58), 69(30); (Calcd. for $C_{24}H_{20}N_4O_6$: 460.14); Anal. Calcd. for $C_{24}H_{20}N_4O_6$: C, 62.60; H, 4.38; N, 12.17; Found: C, 62.58; H, 4.40; N, 12.19.

3-(4-(4-hydroxy-3-methoxylbenzylideneamino) phenylimino) -5-nitro indoline-2-one (5i):
Yellow crystals; Yield: 77%; mp. 310-314 °C; IR: 3523 (Ar-OH), 3210 (N-H), 3023 (Ar-CH), 1698 (C=O), 1631 (C=C), 1595 (C=N), 1127 (C-O-C) cm^{-1}; ^{1}H-NMR(DMSO): δ 8.29 (s, 1H, -N=CH-), 8.02 (s, 1H, -NH-), 7.03-7.68 (m, 7H, H-4, H-6, H-7, H-2', H-3', H-5' , H-6' Ar-H), 7.00 (d, J=7.8 Hz 1H, H-6" Ar-H), 6.95-6.97 (s, 1H, H-2" Ar-H), 6.64 (d, J=6.6 Hz 1H, H-5" Ar-H), 5.06 (s, 1H, Ar-OH), 3.73 (s, 3H, OCH_3); ^{13}C-NMR (DMSO): δ 167.2 (C-2), 162.3 (C-3), 160.6 (-N=CH-), 151.6 (C-1' and C-4'), 151.6 (C-3"), 148.5 (C-4"), 133.3 (C-9), 132.3 (C-8), 132.2 (C-6), 129.4 (C-4), 126.8 (C-1"), 124.6 (C-5), 123.6 (C-2', C-3', C-5' and C-6'), 122.9 (C-6"), 121.6 (C-7), 117.0 (C-5"), 114.6 (C-2"), 56.8 (-OCH_3); EI-MS (m/z, %): 416(M^+, 72), 324(8), 242(28), 235(100), 177(28), 95(12); (Calcd. for $C_{22}H_{16}N_4O_5$: 416.39); Anal. Calcd. for $C_{22}H_{16}N_4O_5$: C, 63.46; H, 3.87; N, 13.46; Found: C, 63.45; H, 3.89; N, 13.49.

3-(4-(3-nitrobenzylideneamino) phenylimino) -5-nitro indoline-2-one (5j):
Creamy solid; Yield: 82%; mp. 318-322 °C; IR: 3175 (N-H), 3055 (Ar-CH), 1686 (C=O), 1650 (C=N), 1652 (C=C), 1491 & 1373 (C-NO_2) cm^{-1}; ^{1}HNMR(DMSO): δ 8.55 (s,1H, H-2" Ar-H), 8.23(d, J=8.1 Hz, 1H, H-4" Ar-H), 8.19(s,1H, -N=CH-), 8.10 (s, 1H, -NH-), 8.03 (d, J=6.5 Hz, 1H, H-6" Ar-H), 7.54 (dd, J=7.3, Hz, 1H, H-5" Ar-H), 7.01-7.30(m, 7H, H-4, H-6, H-7, H-2', H-3', H-5', H-6' Ar-H); ^{13}C-NMR (DMSO): δ 167.2 (C-2), 164.1 (C-3), 160.4 (-N=CH-), 151.5 (C-1'and C-4'), 148.2 (C-3"), 135.3 (C-6"), 134.6 (C-1"), 133.8 (C-9), 131.3 (C-6), 130.9 (C-8), 129.7 (C-5"), 129.4 (C-4), 124.6 (C-5), 124.1(C-2"), 123.7 (C-2', C-3', C-5' and C-6'), 123.4 (C-4"), 121.6 (C-7); EI-MS (m/z, %): 415(M^+ , 40), 324(16), 242(38), 173(72), 122(100), 77(22); (Calcd. for $C_{21}H_{13}N_5O_5$: 415.36); Anal. Calcd. $C_{21}H_{13}N_5O_5$: C, 60.72; H, 3.15; N, 16.86; Found: C, 60.68; H, 3.19; N, 16.87.

3-(4-(4-dimethylaminobenzylideneamino) phenylimino) -5-nitro indoline-2-one (5k):
Yellow crystals; Yield: 83%; mp. 336-338 °C; IR : 3150 (N-H), 3055 (Ar-CH), 3019 (C-H), 1698 (C=O), 1613 (C=C), 1568 (C=N) cm^{-1}; ^{1}H-NMR(DMSO): δ 8.21 (s, 1H, -N=CH-), 8.02 (s, 1H, -NH-), 7.42 (dd, J=5.9 Hz, 2H, H-2" and H-6" Ar-H), 7.03-7.68 (m, 7H, H-4, H-6, H-7, H-2', H-3', H-5', H-6' Ar-H), 6.61 (dd, J=7.2 Hz, 2H, H-3" , H-5" Ar-H), 2.85 (s, 6H, -N[CH_3]$_2$); ^{13}C-NMR (DMSO): δ 168.1 (C-2), 162.9 (C-3), 160.2 (-N=CH-), 151.9 (C-4"), 151.7 (C-1' and C-4'), 133.5 (C-9), 131.3 (C-6), 131.0 (C-8), 130.2 (C-2" and C-6"), 129.3 (C-4), 124.6 (C-5), 123.6 (C-2', C-3', C-5' and C-6'), 123.3 (C-1"), 121.7 (C-7), 114.3 (C-3" and C-5"), 40.2 (-N[CH_3]$_2$); EI-MS (m/z, %): 413(M^+, 6), 324(14), 242(38), 133(100), 91(20). (Calcd. for $C_{23}H_{19}N_5O_3$: 413.43); Anal. Calcd. $C_{23}H_{19}N_5O_3$: C, 66.82: H, 4.63; N, 16.94; Found: C, 66.77: H, 4.68; N, 16.96.

3-(4-(3-phenylallylideneamino) phenylimino) -5-nitro indoline-2-one (5l):
Creamy crystals; Yield: 64%; mp. 316-320 °C; IR : 3168 (N-H), 3090 (Ar-CH), 1700 (C=O), 1591 (C=N), 1498 (C=C) cm^{-1}; ^{1}H-NMR (DMSO): δ 8.01 (s, 1H, -NH-), 7.51 (s, 1H, -N=CH-), 6.99-7.32 (m, 12H, H-4, H-6, H-7, H-2', H-3', H-5', H-6', H-2", H-3", H-4", H-5", H-6" Ar-H), 6.62 (d, 1H, J=7.1 Hz; C_6H_5-CH=CH-), 5.63 (d, 1H, J=8.2 Hz, C_6H_5-CH=CH-); ^{13}C-NMR (DMSO): δ 167.2 (C-2), 163.7(-N=CH-), 163.3 (C-3), 151.6 (C-1'), 147.6 (C-4'), 138.4 (C_6H_5-CH=CH-), 135.2 (C-1"), 133.3(C-9), 131.3(C-6), 131.2(C-8), 129.3(C-4), 128.7 (C-5" and C-6"), 128.0 (C-4"), 126.4 (C-2" and C-6"), 124.6 (C-5), 123.4 (C-2', C-3', C-5' and C-6'), 119.8 (C_6H_5-CH=CH-), 121.7 (C-7); EI-MS (m/z, %): 396(M^+,26), 300(24), 243(10), 221(8), 179(18), 109(100), 60(32); (Calcd. for $C_{23}H_{16}N_4O_3$: 396.4); Anal. Calcd. for $C_{23}H_{16}N_4O_3$: C, 69.69; H, 4.07; N, 14.13; Found: C, 69.60; H, 4.77; N, 14.17.

Antimicrobial activity:
The preliminary antimicrobial results of the final compounds are shown in Table-**1**. The results revealed that majority of the synthesized compounds showed varying degrees of inhibition against the tested microorganisms with the range of MIC values for *S.aureus* (9.4-25.4 μg.mL^{-1}), *S.epidermidis* (10.0-24.2 μg.mL^{-1}), *M.luteus* (11.4-28.0 μg.mL^{-1}), *B.cereus* (9.8-21.4 μg.mL^{-1}), *E.coli* (12.3-22.1 μg.mL^{-1}), *P.aeruginosa* (11.0-21.8 μg.mL^{-1}), *K.pneumoniae* (10.8-23.8 μg.mL^{-1}), *A.niger* (12.3-29.3 μg.mL^{-1}) and *A.fumigatus* (13.1-30.8 μg.mL^{-1}). The compound 3-(4-(4-nitrobenzylidene amino)-phenylimino) -5-nitro indoline-2-one **5e** was found to exhibit the most potent *in vitro* antimicrobial activity with the MIC range of 9.4, 10.4, 11.8, 9.8, 12.3, 12.0, 10.8, 12.3 and 14.7 μg.mL^{-1} against *S.aureus, S.epidermidis, M.luteus,*

B.cereus, E.coli, P.aeruginosa, K.pneumoniae, A.niger and *A.fumigatus* respectively. Compound **5b** and **5j** exhibited significant antimicrobial activity when compared to standard drugs Ciprofloxacin and Ketoconazole. Other compounds **5a, 5c, 5d, 5f, 5g, 5h, 5i, 5k** and **5l** showed mild to moderate antibacterial and antifungal activity. The results revealed that most of the synthesized compounds exhibited significant antibacterial activity. The most potent antibacterial and antifungal activity exhibited by compound **5e** might be due to the presence of electron withdrawing substituent -NO_2 group at 4'' position of the phenyl ring and nitro group at 5th position in the isatin. Similarly compound **5b** and **5j** also exhibited significant antimicrobial activity due to the presence of electron withdrawing substituent -Cl and -NO_2 groups at either 4'' and 3'' position of the phenyl ring in the 5-nitro isatin. While other compounds, though they contain electron donating substituents like methoxy, hydroxyl, methyl and dimethylamino groups do not exhibit significant *in vitro* antimicrobial activity (Scheme **1**).

The MIC values were determines as the lowest concentration that completely inhibited visible growth of the microorganisms (Table-**1**). The structure antimicrobial activity relationship of the synthesized compounds revealed that the compounds having electron releasing moiety exhibited least activity when compared with compounds having electron withdrawing moieties.

In conclusion, a new class of Schiff bases combination with substituted isatin, *p*-Phenylenediamine and different substituted aromatic aldehydes are developed by adopting simple, elegant and well-versed methodologies. We have also evaluated *in vitro* antimicrobial activity for all the synthesized compounds. The results obtained from antifungal and antibacterial tests together showed that all compounds tested are more active towards bacteria than fungi. The antimicrobial activity of the synthesized compounds may be due to the presence of electron withdrawing substituents in the phenyl ring in 3rd position and also presence of nitro group at 5th position, which might increase the lipophilic character of the molecule, which facilitate the crossing through the biological membrane of the micro-organism and thereby inhibit their growth.

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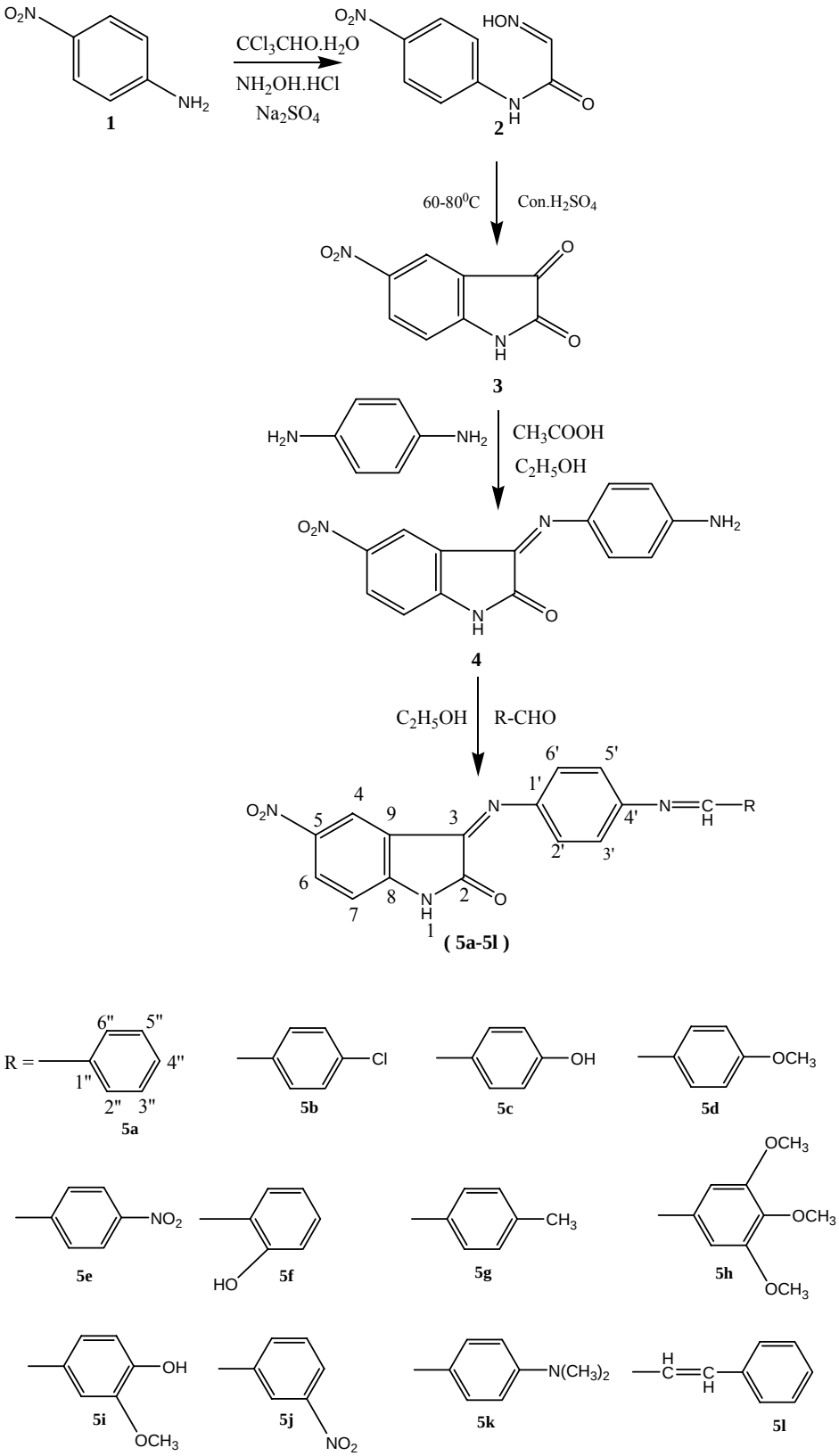

Scheme-1: Synthesis of Schiff bases (5a–5l)

Table-1: Antimicrobial activity of the synthesized compounds (100 μg.mL^{-1})

ɔunds	*In vitro* activity - zone of inhibition in mm (MIC in μg.mL^{-1})								
	S.aureus	*S.epidermidis*	*M.luteus*	*B.cereus*	*E.coli*	*P.aeuriginosa*	*K.pneumoniae*	*A.niger*	*A.fumigatus*
5a	22(13.0)	20(18.8)	22(17.8)	18(20.4)	24(14.3)	18(21.6)	24(15.9)	26(13.0)	19(18.8)
5b	23(12.7)	27(10.5)	24(14.3)	20(10.8)	23(13.6)	23(12.0)	25(13.0)	24(12.4)	22(13.1)
5c	24(10.8)	26(10.6)	23(12.3)	20(13.9)	24(13.4)	21(13.6)	24(14.5)	24(14.3)	17(21.0)
5d	19(23.0)	16(24.2)	20(19.9)	17(20.9)	21(20.4)	16(21.8)	18(19.1)	17(22.0)	15(25.0)
5e	24(9.40)	29(10.4)	26(11.8)	23(09.8)	25(12.3)	23(12.0)	27(10.8)	26(12.3)	23(14.7)
5f	17(25.4)	21(20.6)	15(28.0)	18(19.4)	20(22.1)	19(20.0)	16(23.8)	15(29.3)	13(30.8)
5g	21(19.5)	20(21.4)	22.(16.5)	19(15.5)	20(19.4)	20(18.8)	17(22.0)	21(18.1)	18(20.1)
5h	22(13.4)	22(17.8)	19(20.8)	20(16.9)	19(19.2)	19(19.9)	19(19.8)	20(21.5)	19(19.0)
5i	21(13.8)	25(15.0)	22(12.6)	19(17.3)	22(14.6)	22(12.8)	26(12.0)	25(14.0)	20(14.4)
5j	23(10.6)	27(10.0)	25(11.4)	21(12.3)	23(15.0)	22(11.0)	24(14.1)	22(13.5)	21(13.8)
5k	21(18.4)	22(14.0)	21(18.9)	19(18.8)	22(19..3)	18(21.0)	19(18.8)	20(17.9)	20(16.3)
5l	24(12.0)	20(20.4)	19(19.8)	18(21.4)	21(18.8)	18(18.8)	22(17.8)	21(20.4)	20(18.1)
Ciprofloxacin	28(0.2)	31(0.4)	29(0.1)	25(0.3)	31(0.2)	27(0.3)	29(0.1)	-	-
Ketoconazole	-	-	-	-	-	-	-	31(6.1)	28(0.2)
DMF	-	-	-	-	-	-	-	-	-