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Synthesis, Characterization and Antimicrobial Activity of Some Novel Mannich Bases of Indole-2,3-dione

SHOBHIT SHRIVASTAVA* and DHARMENDRA AHUJA

Department of Pharmaceutical Science, Jayoti Vidyapeeth Women's University, Jaipur-303122, India

*Corresponding author: E-mail: shrivastava.shobhit@gmail.com

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Indole-2,3-dione (isatin) and its 5-substituted derivatives have been reacted with N^2-benzylidenepyridine-2,6-diamine to form Schiff bases and the Mannich bases of these compounds were synthesized by reacting them with some secondary amines in presence of formaldehyde. Their chemical structures have been confirmed by mean of their IR and ^{1}H NMR. Antimicrobial screening of synthesized compounds was done by well diffusion method against 4 pathogenic bacteria and 2 pathogenic fungi. Amongst the tested compounds 3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-(piperazin-1-ylmethyl)-5-fluoro-indolin-2-one and 3-{[6-(benzylideneamino)pyridin-2-yl]-imino}-1-(morpholin-4-ylmethyl)-5-fluoro-indolin-2-one showed significant antibacterial activity and 3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-(piperazin-1-ylmethyl)-5-methyl-indolin-2-one and 3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-(morpholin-4-ylmethyl)-5-methyl-indolin-2-one showed the favourable antifungal activity.

Keywords: Isatin, Mannich base, Antimicrobial activity, 2,6-Diaminopyridine.

INTRODUCTION

Indole-2,3-dione (isatin), an endogenous heterocyclic moiety widely present in human tissues and body fluids [1]. Indole-2,3-dione versatility allows the synthesis of many Schiff and Mannich bases with large variety of heterocyclic compounds. Many of its derivatives were outlined for broad spectrum pharmacodynamic activities like antimicrobial [2-11], antiviral [12,13], anti-inflammatory and analgesic [14-18], anticonvulsant [19,20], antituberculosis [21-24], anti-helminthic [25], anticancer [26-28] and anti-HIV [29-32], *etc.* In present work, we have target to achieve a better antimicrobial profile by synthesizing Mannich bases with indole-2-3-dione and its 5-substituted derivatives.

EXPERIMENTAL

All chemicals were of AR grade and used without further purification. Completion of reaction was confirmed by thin layer chromatography plates using chloroform:methanol (9:1) and spots were visualized in iodine chamber. Open capillary tube apparatus was used for melting points determination and are uncorrected. IR spectra were recorded on JASCO FTIR 460+ using KBr dispersion diffuse reflectance technique. ^{1}H NMR Spectra were recorded in DMSO-d_6 solvent on 400 MHz JEOL JNM ECS 400 using TMS (Me_4Si) as internal standard. All spectra were consistent with the assigned structure.

Synthesis of isatin and its derivative (S1-5): Aniline/substituted aniline (0.05 mol) was treated with chloral hydrate (0.05 mol) and sodium sulphate (0.05 mol) in presence of hydroxylamine hydrochloride to get intermediate isonitrosoacetanilide (**1**). Finally isatin and substituted isatin was obtained by cyclization of intermediate (**1**) using H_2SO_4 and recrystallized them with suitable solvent.

Synthesis of N^2-benzylidenepyridine-2,6-diamine (M): Dissolved the equimolar (2 mmol) quantity of 2,6-diaminopyridine and benzaldehyde in ethanol and refluxed the mixture for 2 h using few mL of glacial acetic acid. Cooled it and filtered to get solid, finally re-crystallized it with methanol.

Synthesis of Schiff base (MS1-5): Dissolved the equimolar quantity of N^2-benzylidenepyridine-2,6-diamine and isatin/5-substituted isatin (2 mmol) in 25 mL ethanol containing 1 mL of glacial acetic acid. Refluxed it on steam bath for 4-5 h. Cooled the resulting mixture at room temperature and filtered, then washed with cold ethanol. Dried it in air and then re-crystallized from appropriate solvent.

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Synthesis of Mannich base (MM1-20): Mannich bases were synthesized [3,8] using slurry consisting of 0.002 mol of Schiff's base (**MS1-5**), 5 mL of tetrahydrofuran and 2 mL of 37 % formalin. To this, secondary amines (0.002 mol) was added dropwise with cooling and shaking. The reaction mixture was allowed to stand at room temperature for 1 h with occasional shaking then it was warmed on a steam bath for 15 min. The mixture was then cooled and the product was recrystallized from suitable solvent.

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-(piperazin-1-ylmethyl)-indolin-2-one (MM01): Yield: 79%, m.p.: 262-264 ºC; IR (KBr, ν_{max}, cm^{-1}): 3387 (N-H), 3054 (Ar-H), 2933 (N-CH_2-N), 1709 (C=O), 1592 (CH=N), 1491 (C=C Ar), 1348 (C-N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.86-3.75 (8H, m, piperazine), 4.61 (2H, s, N-CH_2-N), 6.70-7.80 (8H, m, Ar), 8.27 (1H, s, CH=N), 9.54 (1H, s, H-piperazine).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-(morpholin-4-ylmethyl)-indolin-2-one (MM02): Yield: 80%, m.p.: 269-271 ºC; IR (KBr, ν_{max}, cm^{-1}): 3058 (Ar-H), 2937 (N-CH_2-N), 1720 (C=O), 1595 (CH=N), 1490 (C=C Ar), 1348 (C-N), 1112 (C-O-C); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.09-2.11 (4H, d, CH_2-N-CH_2), 3.46-3.67 (4H, d, CH_2-O-CH_2), 4.61 (2H, s, N-CH_2-N), 6.68-8.10 (8H, m, Ar), 8.26 (1H, s, CH=N).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-[(diethylamino)methyl]-indolin-2-one (MM03): Yield: 59%, m.p.: 258-260 ºC; IR (KBr, ν_{max}, cm^{-1}): 3061 (Ar-H), 2984 (N-CH_2-N),1718 (C=O), 1596 (CH=N), 1490 (C=C Ar), 1348 (C-N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.89-0.99(6H, t, CH_3), 2.26-2.68 (4H, q, CH_2), 4.61 (2H, s, N-CH_2-N), 7.17-8.16(8H, m, Ar), 8.26 (1H, s, CH=N).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-[(dimethylamino)methyl]-indolin-2-one (MM04): Yield: 73%, m.p.: 248-251 ºC; IR (KBr, ν_{max}, cm^{-1}): 3058 (Ar-H), 2892 (N-CH_2-N), 1721 (C=O), 1595 (CH=N), 1490 (C=C Ar), 1345 (C-N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.86 (6H, s, CH_3), 4.61 (2H, s, N-CH_2-N), 6.70-7.80 (8H, m, Ar), 8.27 (1H, s, CH=N).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-(piperazin-1-ylmethyl)-5-fluoro-indolin-2-one (MM05): Yield: 74%, m.p.: 268-270 ºC; IR (KBr, ν_{max}, cm^{-1}): 3384 (N-H), 3061 (Ar-H), 2946 (N-CH_2-N), 1718 (C=O), 1588 (CH=N), 1485 (C=C Ar), 1334 (C-N), 1185 (C-F); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.86-3.75 (8H, m, piperazine), 4.61 (2H, s, N-CH_2-N), 6.60-7.80 (8H, m, Ar), 8.27 (1H, s, CH=N), 9.47 (1H, s, H-piperazine).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-(morpholin-4-ylmethyl)-5-fluoro-indolin-2-one (MM06): Yield: 62%, m.p.: 276-278 ºC; IR (KBr, ν_{max}, cm^{-1}): 3061 (Ar-H), 2964 (N-CH_2-N), 1725 (C=O), 1587 (CH=N), 1491 (C=C Ar), 1331 (C-N), 1114 (C-O-C), 1186 (C-F); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.12-2.28 (4H, d, CH_2-N-CH_2), 3.52-3.96 (4H, d, CH_2-O-CH_2), 4.61 (2H, s, N-CH_2-N), 6.89-7.88 (8H, m, Ar), 8.27 (1H, s, CH=N).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-[(diethylamino)methyl]-5-fluoro-indolin-2-one (MM07): Yield: 66%, m.p.: 270-273 ºC; IR (KBr, ν_{max}, cm^{-1}): 3061 (Ar-H), 2980 (N-CH_2-N),1721 (C=O), 1586 (CH=N), 1488 (C=C Ar), 1331 (C-N), 1186 (C-F); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.99-1.19 (6H, t, CH_3), 2.28-2.65 (4H, q, CH_2), 4.61 (2H, s, N-CH_2-N), 7.00-8.15(8H, m, Ar), 8.27 (1H, s, CH=N).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-[(dimethylamino)methyl]-5-fluoro- indolin-2-one (MM08): Yield: 76%, m.p.: 278-280 ºC; IR (KBr, ν_{max}, cm^{-1}): 3061 (Ar-H), 2953 (N-CH_2-N), 1718 (C=O), 1594 (CH=N), 1487 (C=C Ar), 1327 (C-N), 1185 (C-F); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.86 (6H, s, CH_3), 4.61 (2H, s, N-CH_2-N), 7.20-8.10 (8H, m, Ar), 8.27 (1H, s, CH=N).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-(piperazin-1-ylmethyl)-5-chloro-indolin-2-one (MM09): Yield: 77%, m.p.: 275-278 ºC; IR (KBr, ν_{max}, cm^{-1}): 3388 (N-H), 3061 (Ar-H), 2937 (N-CH_2-N), 1725 (C=O), 1586 (CH=N), 1490 (C=C Ar), 1327 (C-N), 815(C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.09-2.49 (8H, m, piperazine), 4.61 (2H, s, N-CH_2-N), 7.23-7.88 (8H, m, Ar), 8.27 (1H, s, CH=N), 9.52 (1H,7 s H-piperazine).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-(morpholin-4-ylmethyl)-5-chloro-indolin-2-one (MM10): Yield: 79%, m.p.: 284-286 ºC; IR (KBr, ν_{max}, cm^{-1}): 3060 (Ar-H), 2957 (N-CH_2-N), 1726 (C=O), 1587 (CH=N), 1490 (C=C Ar), 1338 (C-N), 1113 (C-O-C), 749 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.21-2.25 (4H, d, CH_2-N-CH_2), 3.94-3.98 (4H, d, CH_2-O-CH_2), 4.61 (2H, s, N-CH_2-N), 7.12-7.88 (8H, m Ar), 8.27 (1H, s CH=N).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-[(diethylamino)methyl]-5-chloro-indolin-2-one (MM11): Yield: 58%, m.p.: 270-272 ºC; IR (KBr, ν_{max}, cm^{-1}): 3058 (Ar-H), 2971 (N-CH_2-N), 1732 (C=O), 1590 (CH=N), 1491 (7C=C Ar), 1327 (C-N), 816 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.96-0.99 (6H, t, CH_3), 2.20-2.40 (4H, q, CH_2), 4.61 (2H, s, N-CH_2-N), 6.89-8.15(8H, m, Ar), 8.27 (1H, s, CH=N).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-[(dimethylamino)methyl]-5-chloro-indolin-2-one (MM12): Yield: 63%, m.p.: 279-281 ºC; IR (KBr, ν_{max}, cm^{-1}): 3061 (Ar-H), 2939 (N-CH_2-N), 1731 (C=O), 1578 (CH=N), 1491 (C=C Ar), 1331 (C-N), 815 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.86 (6H, s, CH_3), 4.61 (2H, s, N-CH_2-N), 6.60-7.88 (8H, m, Ar), 8.27 (1H, s, CH=N).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-(piperazin-1-ylmethyl)-5-nitro-indolin-2-one (MM13): Yield: 82%, m.p.: 274-276 ºC; IR (KBr, ν_{max}, cm^{-1}): 3376 (N-H), 3061 (Ar-H), 3027 (N-CH_2-N), 1734 (C=O), 1606 (CH=N), 1486 (C=C Ar), 1338 (C-N), 1450 and 1229 (C-NO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.28-2.95 (8H, m, piperazine), 4.61 (2H, s, N-CH_2-N), 6.90-8.40 (8H, m, Ar), 8.23 (1H, s, CH=N), 9.54 (1H, s, H-piperazine).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-(morpholin-4-ylmethyl)-5-nitro-indolin-2-one (MM14): Yield: 80%, m.p.: 271-273 ºC; IR (KBr, ν_{max}, cm^{-1}): 3065 (Ar-H), 2957 (N-CH_2-N), 1735 (C=O), 1585 (CH=N), 1497 (C=C Ar), 1332 (C-N), 1114 (C-O-C), 1530 and 1358 (C-NO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.09-2.40 (4H, d, CH_2-N-CH_2), 3.47-3.51 (4H, d, CH_2-O-CH_2), 4.61 (2H, s, N-CH_2-N), 6.78-8.42 (8H, m, Ar), 8.27 (1H, s, CH=N).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-[(diethylamino)methyl]-5-nitro-indolin-2-one (MM15): Yield: 68%,

m.p.: 268-270 ºC; IR (KBr, ν_{max}, cm^{-1}): 3061 (Ar-H), 2973 (N-CH_2-N), 1736 (C=O), 1589 (CH=N), 1489 (C=C Ar), 1335 (C-N), 1443 and 1298 (C-NO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.05-1.14 (6H, t, CH_3), 2.07-2.11(4H, q, CH_2), 4.61 (2H, s, N-CH_2-N), 7.10-7.98 (8H, m, Ar), 8.27 (1H, s, CH=N).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-[(dimethylamino)methyl]-5-nitro-indolin-2-one (MM16): Yield: 67%, m.p.: 266-268 ºC; IR (KBr, ν_{max}, cm^{-1}): 3058 (Ar-H), 2950 (N-CH_2-N), 1742 (C=O), 1589 (CH=N), 1490 (C=C Ar), 1332 (C-N), 1520 and 1335 (C-NO_2); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.86 (6H, s, CH_3), 4.62 (2H, s, N-CH_2-N), 7.20-8.10 (8H, m, Ar), 8.27 (1H, s, CH=N).

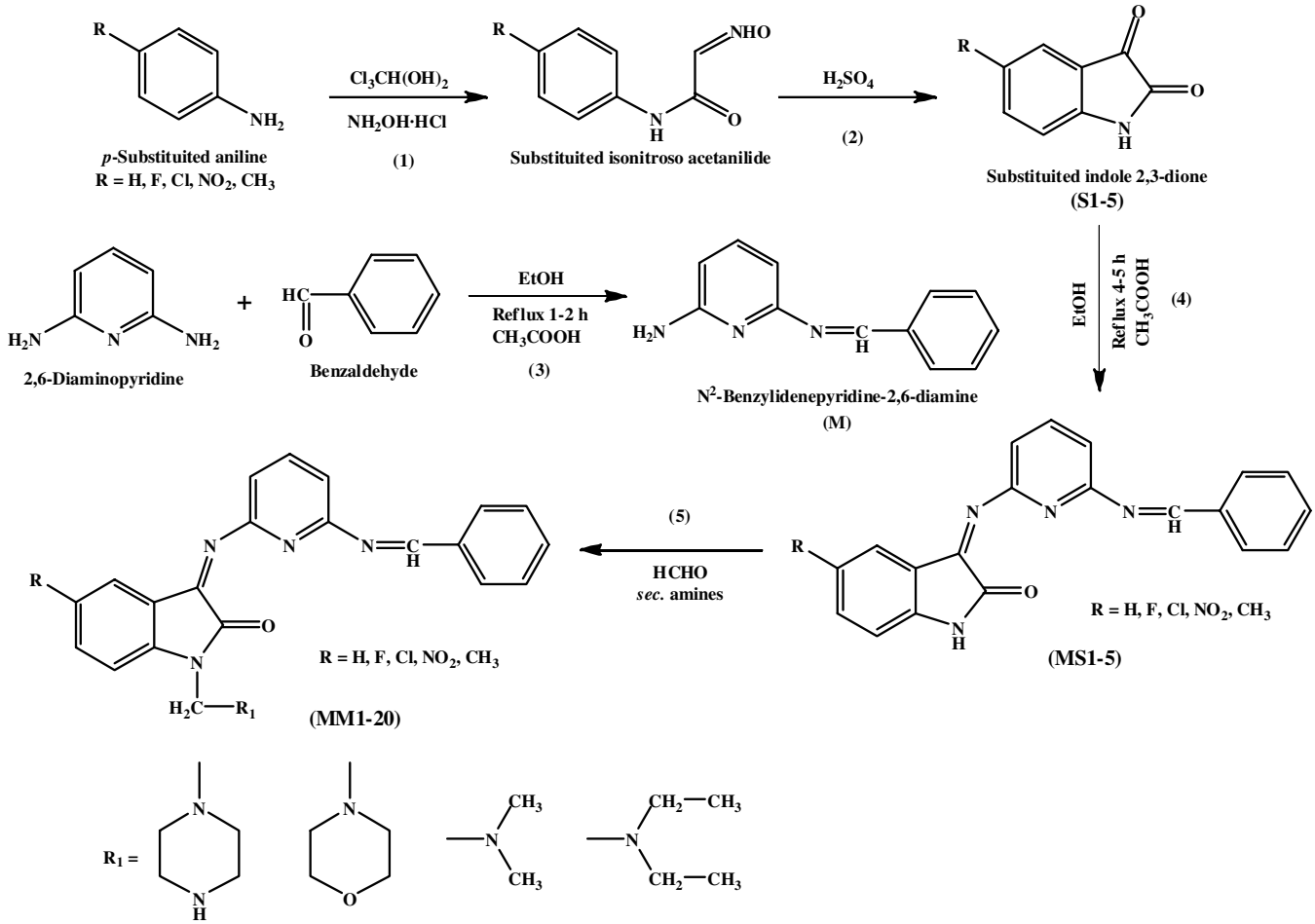
3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-(piperazin-1-ylmethyl)-5-methyl-indolin-2-one (MM17): Yield: 82%, m.p.: 269-271 ºC; IR (KBr, ν_{max}, cm^{-1}): 3396 (N-H), 3061 (Ar-H), 2946 (N-CH_2-N), 2885 (C-H), 1720 (C=O), 1590 (CH=N), 1490 (C=C Ar), 1334 (C-N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.19(3H, s, CH_3), 2.08-2.66 (8H, m, piperazine), 4.61 (2H, s, N-CH_2-N), 7.00-8.15 (8H, m, Ar), 8.27 (1H, s, CH=N), 9.52 (1H, s, H-piperazine).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-(morpholin-4-ylmethyl)-5-methyl-indolin-2-one (MM18): Yield: 75%, m.p.: 261-263 ºC; IR (KBr, ν_{max}, cm^{-1}): 3058 (Ar-H), 2956 (N-CH_2-N), 2861 (C-H), 1723 (C=O), 1589 (CH=N), 1492 (C=C Ar), 1331(C-N), 1110 (C-O-C); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.51(3H, s, CH_3), 2.29-2.47 (4H, d, CH_2-N-CH_2), 3.49-3.53 (4H, d, CH_2-O-CH_2), 4.61 (2H, s, N-CH_2-N), 7.10-7.98 (8H, m, Ar), 8.27 (1H, s, CH=N).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-[(diethylamino)methyl]-5-methyl-indolin-2-one (MM19): Yield: 66%, m.p.: 270-273 ºC; IR (KBr, ν_{max}, cm^{-1}): 3061 (Ar-H), 2973 (N-CH_2-N), 2909 (C-H), 1718 (C=O), 1589 (CH=N), 1494 (C=C Ar), 1327 (C-N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ δ 0.94-0.99 (6H, t, CH_3), 1.19 (3H, s, CH_3), 2.40-2.60 (4H, q, CH_2), 4.61 (2H, s, N-CH_2-N), 6.89-7.88 (8H, m, Ar), 8.27 (1H, s, CH=N).

3-{[6-(Benzylideneamino)pyridin-2-yl]imino}-1-[(dimethylamino)methyl]-5-methyl-indolin-2-one (MM20): Yield: 61%, m.p.: 274-276 ºC; IR (KBr, ν_{max}, cm^{-1}): 3061 (Ar-H), 2946 (N-CH_2-N), 2879 (C-H), 1720 (C=O), 1588 (CH=N), 1492 (C=C Ar), 1338 (C-N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.51 (3H, s, CH_3), 1.86 (6H, s, CH_3), 4.61 (2H, s, N-CH_2-N), 7.25-8.02 (8H, m, Ar), 8.27 (1H, s, CH=N).

Antimicrobial activity: The *in vitro* antimicrobial activity of newly synthesized Mannich bases of isatin were carried out as per reported well diffusion method [33]. The synthesized Mannich bases were screened for antibacterial activity against *Staphylococcus aureus* (MTCC-3160), *Bacillus subtilus* (MTCC-441), *Escherichia coli* (MTCC-452), *Klebsiella pneumonia* (MTCC-432) and antifungal activity against *Candida albicans*



Scheme-I: Synthesis of Mannich bases of substituted isatin [(1) and (2): synthesis of substituted isatin; (3) synthesis of N^2-benzylidenepyridine-2,6-diamine; (4) synthesis of Schiff bases; (5) synthesis of Mannich bases]

(MTCC-183), *Aspergillus niger* (MTCC-282). The 10, 20 and 30 µg/mL concentration of tested sample were used against both the bacterial and fungal strains. Ciprofloxacin and fluconazole were used as standard drugs and zone of inhibition was examined after 24 h of incubation at 37 ºC.

RESULTS AND DISCUSSION

Newly synthesized Mannich bases of isatin, 3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1-(substituted-4-ylmethyl)-5-substituted-indolin-2-one **(MM01-20)**, were obtained according to the reaction sequences depicted in **Scheme-I**. Firstly, substituted isatins were synthesized by reacting substituted anilines with chloral hydrated, sodium sulphate and hydroxylamine hydrochloride. Resultant intermediate reacted with sulphuric acid to get desired compounds (**S1-5**). Acid catalyzed condensation of 2,6-diaminopyridine with benzaldehyde resulted in the formation of N^2-benzylidenepyridine-2,6-diamine (**M**). Later on Schiff bases of isatin were obtained by condensing the N^2-benzylidenepyridine-2,6-diamine with substituted isatins in presence of acid (**MS1-5**). It was then converted into the targeted Mannich bases of isatin (**MM1-20**) using formaldehyde with various secondary amines. The synthesized compounds were confirmed by IR and ^{1}H NMR Spectral analyses.

In IR spectrum, a sharp and strong band at 1730-1720 cm^{-1} was observed, which confirmed the presence of carbonyl C=O group. An absorption band at 1590-1570 cm^{-1} was observed which confirmed the CH=N (imine) group of Schiff base. Methylene bridge (N-CH_2-N) of Mannich bases was confirmed by the absorption band at 2970-2950 cm^{-1}. In ^{1}H NMR spectrum, characteristic singlet at δ 8.26-8.27 ppm and δ 4.60-4.62 ppm could be attributed for the proton of imine group of Schiff base and –CH_2- group of Mannich bases, respectively.

Antibacterial activity: All newly synthesized compounds showed antibacterial activity, however some did not show similar bioactivity against all tested bacterial strains (Table-1). Among the tested compounds, **MM05** and **MM06** exhibited the inhibition against *B. subtilus* as that of standard ciprofloxacin. Whereas, compounds **MM07**, **MM08**, **MM13**, **MM14** and **MM15** showed mild to moderate inhibition as compared to standard drug against *B. subtilus*. Compounds **MM05**, **MM06** and **MM07** exhibited the good antibacterial activity against both *S. aureus* and *K. pneumonia* whereas **MM13** against *S. aureus* and **MM14** against *K. pneumonia* showed moderate activity. Compounds **MM06** and **MM09** at 10 and 20 µg/mL while **MM05** and **MM07** at 10 and 30 µg/mL showed the similar antibacterial activity against *E. coli*. Compounds **MM03** and **MM04** showed inhibition against all bacterial strains at 20 µg/mL MIC value.

Compound **MM07**, **MM08**, **MM13**, **MM14** and **MM15** showed mild to moderate inhibition against all bacterial strains with 10 µg/mL MIC value. Whereas on similar MIC value **MM05** and **MM06** showed better activity, which may be due to strong electron withdrawing group present on Mannich bases, which enhance the antibacterial activity.

TABLE-1
ANTIMICROBIAL ACTIVITY OF SOME MANNICH BASES OF INDOLE-2-3-DIONE (**MM01-20**)

Sample code	Conc. (µg/mL)	Zone of inhibition (mm)					
		Gram-positive bacteria		Gram-negative bacteria		Fungi	
		S. aureus	*B. subtilus*	*E. coli*	*K. pneumoniae*	*C. albicans*	*A. niger*
MM-01	10	09 ± 0.47	07 ± 0.86	06 ± 0.0	08 ± 0.47	06 ± 0.00	06 ± 0.00
	20	12 ± 0.70	09 ± 0.47	07 ± 0.47	11 ± 0.47	06 ± 0.00	06 ± 0.00
	30	14 ± 0.47	11 ± 0.47	09 ± 0.47	13 ± 0.47	08 ± 0.47	06 ± 0.00
MM-02	10	09 ± 0.0	07 ± 0.81	07 ± 0.81	07 ± 0.00	06 ± 0.00	06 ± 0.00
	20	11 ± 0.81	10 ± 0.47	10 ± 0.47	10 ± 0.81	07 ± 0.00	06 ± 0.00
	30	15 ± 0.47	12 ± 0.47	12 ± 0.47	12 ± 0.47	09 ± 0.47	07 ± 0.00
MM-03	10	06 ± 0.0	06 ± 0.00	06 ± 0.0	06 ± 0.0	06 ± 0.00	06 ± 0.00
	20	08 ± 0.47	07 ± 0.70	08 ± 0.47	09 ± 0.47	06 ± 0.00	07 ± 0.00
	30	10 ± 0.47	09 ± 0.47	10 ± 0.47	11 ± 0.47	08 ± 0.47	08 ± 0.00
MM-04	10	06 ± 0.0	06 ± 0.00	06 ± 0.0	06 ± 0.0	06 ± 0.00	06 ± 0.00
	20	07 ± 0.0	08 ± 0.00	07 ± 0.0	08 ± 0.0	08 ± 0.00	06 ± 0.00
	30	09 ± 0.47	10 ± 0.47	09 ± 0.47	10 ± 0.47	08 ± 0.47	06 ± 0.00
MM-05	10	10 ± 0.47	12 ± 0.45	10 ± 0.5	10 ± 0.47	06 ± 0.00	07 ± 0.47
	20	13 ± 0.74	14 ± 0.25	13 ± 0.47	13 ± 0.47	07 ± 0.47	08 ± 0.74
	30	17 ± 0.47	20 ± 0.10	16 ± 0.47	16 ± 0.47	09 ± 0.47	10 ± 0.47
MM-06	10	11 ± 0.47	11 ± 0.25	11 ± 0.5	12 ± 0.47	06 ± 0.00	07 ± 0.47
	20	15 ± 0.70	13 ± 0.47	14 ± 0.47	14 ± 0.47	08 ± 0.47	09 ± 0.47
	30	18 ± 0.47	19 ± 0.47	19 ± 0.47	18 ± 0.47	09 ± 0.47	11 ± 0.47
MM-07	10	10 ± 0.47	09 ± 0.86	10 ± 0.5	11 ± 0.00	06 ± 0.00	06 ± 0.47
	20	12 ± 0.74	11 ± 0.47	13 ± 0.51	13 ± 0.81	08 ± 0.47	09 ± 0.00
	30	15 ± 0.47	17 ± 0.47	15 ± 0.47	15 ± 0.74	10 ± 0.47	10 ± 0.47
MM-08	10	09 ± 0.47	09 ± 0.00	09 ± 0.5	08 ± 0.00	07 ± 0.00	07 ± 0.47
	20	10 ± 0.70	13 ± 0.47	13 ± 0.47	10 ± 0.47	08 ± 0.47	09 ± 0.47
	30	13 ± 0.47	18 ± 0.47	15 ± 0.81	12 ± 0.47	09 ± 0.47	10 ± 0.47
MM-09	10	08 ± 0.47	08 ± 0.86	11 ± 0.5	07 ± 0.47	09 ± 0.47	09 ± 0.00
	20	11 ± 0.74	10 ± 0.47	15 ± 0.47	09 ± 0.47	11 ± 0.47	10 ± 0.00
	30	15 ± 0.47	11 ± 0.47	19 ± 0.47	11 ± 0.47	14 ± 0.47	11 ± 0.47

	10	09 ± 0.47	08 ± 0.00	09 ± 0.5	09 ± 0.5	09 ± 0.00	09 ± 0.00
MM-10	20	12 ± 0.74	10 ± 0.47	12 ± 0.47	11 ± 0.47	12 ± 0.47	11 ± 0.00
	30	16 ± 0.47	12 ± 0.47	14 ± 0.47	13 ± 0.47	14 ± 0.47	13 ± 0.47
	10	08 ± 0.47	07 ± 0.00	08 ± 0.5	07 ± 0.5	08 ± 0.47	08 ± 0.00
MM-11	20	10 ± 0.25	09 ± 0.47	10 ± 0.47	10 ± 0.47	11 ± 0.00	10 ± 0.47
	30	12 ± 0.25	11 ± 0.47	12 ± 0.81	12 ± 0.81	14 ± 0.47	12 ± 0.00
	10	06 ± 0.0	06 ± 0.00	07 ± 0.0	07 ± 0.0	09 ± 0.00	09 ± 0.47
MM-12	20	08 ± 0.81	08 ± 0.47	09 ± 0.47	09 ± 0.81	11 ± 0.00	10 ± 0.00
	30	10 ± 0.47	11 ± 0.47	10 ± 0.47	11 ± 0.47	13 ± 0.47	11 ± 0.47
	10	11 ± 0.47	09 ± 0.81	09 ± 0.47	07 ± 0.00	06 ± 0.0	06 ± 0.00
MM-13	20	13 ± 0.74	12 ± 0.47	13 ± 0.47	09 ± 0.47	08 ± 0.47	08 ± 0.74
	30	15 ± 0.47	17 ± 0.47	16 ± 0.47	13 ± 0.47	11 ± 0.47	09 ± 0.47
	10	09 ± 0.47	09 ± 0.00	07 ± 0.15	11 ± 0.47	08 ± 0.0	06 ± 0.00
MM-14	20	11 ± 0.47	13 ± 0.47	10 ± 0.25	14 ± 0.47	10 ± 0.47	09 ± 0.47
	30	14 ± 0.47	18 ± 0.47	12 ± 0.15	17 ± 0.47	12 ± 0.47	11 ± 0.74
	10	08 ± 0.47	08 ± 0.00	06 ± 0.0	08 ± 0.0	07 ± 0.0	06 ± 0.00
MM-15	20	11 ± 0.81	12 ± 0.47	09 ± 0.47	10 ± 0.00	09 ± 0.47	08 ± 0.81
	30	13 ± 0.47	15 ± 0.47	11 ± 0.47	13 ± 0.47	11 ± 0.47	09 ± 0.47
	10	08 ± 0.00	08 ± 0.47	06 ± 0.0	07 ± 0.47	08 ± 0.0	06 ± 0.00
MM-16	20	10 ± 0.81	11 ± 0.70	09 ± 0.47	09 ± 0.00	09 ± 0.47	08 ± 0.47
	30	12 ± 0.47	13 ± 0.81	12 ± 0.47	12 ± 0.47	11 ± 0.47	10 ± 0.74
	10	09 ± 0.47	07 ± 0.00	07 ± 0.5	06 ± 0.00	10 ± 0.47	08 ± 0.47
MM-17	20	11 ± 0.74	09 ± 0.47	09 ± 0.47	06 ± 0.00	12 ± 0.47	11 ± 0.50
	30	15 ± 0.47	12 ± 0.47	11 ± 0.47	07 ± 0.47	15 ± 0.47	15 ± 0.47
	10	07 ± 0.47	08 ± 0.00	09 ± 0.47	09 ± 0.47	11 ± 0.47	07 ± 0.00
MM-18	20	09 ± 0.47	10 ± 0.47	11 ± 0.47	10 ± 0.47	13 ± 0.74	10 ± 0.47
	30	12 ± 0.47	12 ± 0.47	13 ± 0.47	12 ± 0.47	16 ± 0.47	14 ± 0.47
	10	07 ± 0.50	07 ± 0.00	06 ± 0.0	07 ± 0.50	09 ± 0.00	07 ± 0.00
MM-19	20	10 ± 0.47	09 ± 0.47	08 ± 0.47	09 ± 0.47	11 ± 0.0	09 ± 0.50
	30	13 ± 0.81	11 ± 0.47	11 ± 0.47	11 ± 0.81	14 ± 0.47	12 ± 0.47
	10	07 ± 0.50	06 ± 0.00	06 ± 0.0	07 ± 0.00	08 ± 0.47	08 ± 0.00
MM-20	20	09 ± 0.81	08 ± 0.00	07 ± 0.47	09 ± 0.00	11 ± 0.81	08 ± 0.47
	30	12 ± 1.5	08 ± 0.47	10 ± 0.47	11 ± .0.74	13 ± 0.47	11 ± 0.47
	10	25 ± 0.47	12 ± 0.5	22 ± 0.47	23 ± 0.47	–	–
Ciprofloxacin	20	29 ± 0.47	17 ± 0.74	26 ± 0.47	26 ± 0.47	–	–
	30	34 ± 0.47	20 ± 0.15	30 ± 0.47	28 ± 0.47	–	–
	10	–	–	–	–	26 ± 0.86	17 ± 0.00
Fluconazole	20	–	–	–	–	30 ± 0.74	24 ± 0.5
	30	–	–	–	–	32 ± 0.57	30 ± 0.0

n = 3, Mean ± SD

Amongst the 20 newly synthesized Mannich bases, compounds **MM09**, **MM10**, **MM11**, **MM12**, **MM17**, **MM18**, **MM19** and **MM20** showed better antifungal activity against both strains while other compounds exhibited mild or no activity (Table-1). However, compounds **MM17**, **MM18**, **MM19** and **MM20** expressed better antifungal activity at all concentration level than Cl-substituted Mannich bases of isatin. Compounds **MM17** and **MM18** exhibited almost half antifungal activity as compared to standard drug fluconazole, which may due to the electron releasing group present in the structure, which enhances the antimicrobial activity. Compounds **MM01**, **MM02**, **MM03**, **MM04**, **MM7** and **MM13** did not show any significant antifungal activity against both strains at 10 µg/mL. Rest all other compounds showed greater activity against *Candida albicans* than *Aspergillus niger.*

Conclusion

An effort to new anti-infective agents, few novel Mannich bases of substituted isatin was synthesized as per the prescribed methods using some secondary amines. All compounds were screened for their antibacterial and antifungal activity. Results showed that compound **MM05** and **MM06** showed good antibacterial activity against all tested bacterial strains, whereas **MM07**, **MM08**, **MM13**, **MM14** and **MM15** showed mild to moderate inhibition. Compounds **MM17** and **MM18** with methyl substitution Mannich bases of isatin exhibited the better antifungal activity. Moreover, the results revealed that piperazine and morpholine Mannich bases of isatin showed greater antimicrobial activity than the aliphatic amines.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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