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Original Research Article

SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF MANNICH BASES OF ISATIN DERIVATIVES

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ABSTRACT

Some Mannich bases of isatin derivatives were synthesized and evaluated for antimicrobial activity. The target compounds (**4a-4f**) were obtained by the reaction of isatin with various substituted anilines in the presence of glacial acetic acid in methanol afforded the Schiff bases (3a-3d), which further reacts with phenylpiperazines in the presence of formaldehyde in ethanol at room temperature followed by refrigeration for 48 hours afforded the target compounds (**4a-4f**). The structures of target compounds (**4a-4f**) were established on the basis of IR and ^{1}H-NMR spectral analysis. Target compounds (**4a-4f**) were screened for their antimicrobial activity against *Escherichia coli, Bacillus subtilis and Candida albicans*, respectively. The compound **4c** showed most promising effect whereas compound **4d & 4f** were found to be least effective against *E.coli*. The compounds **4c & 4f** were found to have maximum zone of inhibition and compounds **4a** was found to have minimum zone of inhibition against *B. subtilis*. The compound **4d** showed most promising effect whereas compound **4b** were found to be least effective against *C. albicans*.

Key words: Isatin, Schiff bases, Mannich bases, Phenylpiperazines, Antimicrobial activity.

INTRODUCTION

The chemistry of isatin and its derivatives is particularly interesting because of their potential applications in medicinal chemistry. Literature survey revealed that Schiff bases and Mannich bases of isatin derivatives possess a broad spectrum of biological activities like, antibacterial [1-2], anti-convulsant[3], anti-HIV[4], analgesic[5] antitubercular[6] and anti-viral activities[7]. Literature survey informs that piperazines and substituted piperazines are important family of heterocyclic compounds as they have attracted significant interest in medicinal chemistry due to their antimicrobial activities[8-10]. The present paper deals with synthesis and antimicrobial activity of some new Mannich bases of isatin derivatives bearing piperazines motif in their structure.

EXPERIMENTAL: MATERIALS AND METHODS

Melting points of the synthesized compounds were determined by open capillary method and are uncorrected. The IR spectra of synthesized compounds were recorded in potassium bromide discs on FT-IR spectrophotometer MODEL-8300 of SHIMADZU. ^{1}H-NMR spectra were recorded using a Bruker AV 400MHz spectrometer using DMSO-d6 as solvent and TMS as an internal standard. The reactions progress was monitored by thin-layer chromatography (TLC) using silica gel G and spots were visualized in an iodine chamber.

General Procedure for the synthesis of Schiff bases (3a-3d)

0.01 moles of isatin and substituted anilines were dissolved in 50 ml methanol in presence of glacial acetic acid (2-3 drops) and refluxed for 1 hour. Then kept aside for 2 hours, the product was separated out, filtered, dried and recrystallized from chloroform to afford the Schiff bases (**3a-3d**). The reactions are outlined in Scheme 1.

3-(Phenyl imino) indolin-2-one (3a)

Yield: 75%. Mp. 200-205 ^{0}C. IR (KBr, cm-1): 3164, 3081, 1716, 1610, 1286. ^{1}H NMR (400 MHz; DMSO-d6, δ ppm): 11.52 (br s, NH), 8.06-6.31(9H, Ar-H), R_f 0.80 (Hexane: Ethylacetate 1:2).

3-((4-chlorophenyl) imino) indolin-2-one (3b)

Yield: 78%. Mp. 275-280 ^{0}C. IR (KBr, cm-1): 3174, 3081, 1717, 1609, 1285, 750. ^{1}H NMR (400

MHz; DMSO-d6, δ ppm): 11.53 (br s, NH), 8.11-6.42 (8H, Ar-H), R_f 0.72 (Hexane: Ethylacetate 1:2).

3-((4-bromophenyl)imino) indolin-2-one (3c)

Yield: 70%. Mp. 288-292 ^{0}C. IR (KBr, cm-1): 3168, 3077, 1717, 1608, 1285, 770. ^{1}H NMR (400 MHz; DMSO-d6, δ ppm): 11.55 (br s, NH), 8.12-6.42(8H, Ar-H), R_f 0.83 (Hexane: Ethylacetate 1:2).

3-((2-chlorophenyl)imino) indolin-2-one (3d)

Yield: 74%. Mp. 198-200 ^{0}C. IR (KBr, cm-1): 3189, 3080, 1720, 1611, 1286, 740. ^{1}H NMR (400 MHz; DMSO-d6 , δ ppm): 11.59 (br s, NH), 8.08-6.18 (8H, Ar-H), R_f 0.92 (Hexane: Ethylacetate 1:2).

General Procedure for the synthesis of Mannich bases (4a-4f)

Mannich bases were synthesized by stirring equimolar proportions of Schiff's bases (0.01mol) and phenylpiperazine (0.01mol) in formaldehyde in 50 ml of ethanol at room temperature followed by refrigeration for 48 hours. The product thus obtained were separated by suction filtration and recrystallized from ethanol to afford the Mannich bases **(4a-4f).** The reactions are outlined in Scheme1.

3-(Phenylimino)-1-((4-phenylpiperazine-1-yl) methyl) indolin-2-one (4a)

Yield: 45%. Mp. 94-98 ^{0}C. IR (KBr, cm^{-1}): 3036, 2820, 1669, 1599,1238. ^{1}H NMR (400 MHz; DMSO-d6, δ ppm): 7.28-6.83 (14H, Ar-H), 3.21-2.67 (8H, pipring), 3.03(s, 2H, CH_2). R_f 0.14 (Hexane: Ethylacetate 1:2).

3-((4-Chlorophenyl) imino)-1-((4-phenylpiperazine-1-yl) methyl) indolin-2-one (4b)

Yield: 50%. Mp. 100-112^{0}C. IR (KBr, cm^{-1}): 3036, 2820, 1669, 1599,1238 ,767. ^{1}H NMR (400 MHz; DMSO-d6, δ ppm): 7.27-6.82 (13H, Ar-H), 3.20-2.67 (8H, pipring), 3.02(s, 2H, CH_2). R_f 0.22 (Hexane: Ethylacetate 1:2).

3-((4-Bromophenyl) imino)-1-((4-phenyl piperazine-1-yl) methyl) indolin-2-one (4c)

Yield: 35%. Mp. 102-105 ^{0}C. IR (KBr, cm^{-1}): 3056, 2819, 1672, 1599, 1237,790. ^{1}H NMR (400 MHz; DMSO-d6, δ ppm): 7.33-6.88 (13H, Ar-H), 3.26-2.72 (8H, pipring), 3.08(s, 2H, CH_2). R_f 0.46 (Hexane: Ethylacetate 1:2).

3-((2-Chlorophenyl) imino)-1-((4-phenyl piperazine-1-yl) methyl) indolin-2-one (4d)

Yield: 40%. Mp. 75-80 ^{0}C. IR (KBr, cm^{-1}): 3036, 2819, 1668, 1599,

1237,740. ^{1}H NMR (400 MHz; DMSO-d6, δ ppm): 7.80-6.49 (13H, Ar-H), 3.26-2.73 (8H, pipring), 3.09(s, 2H, CH_2). R_f 0.23 (Hexane: Ethylacetate 1:2).

3-((4-Chlorophenyl) imino)-1-((4-p-toly) piperazine-1-yl) methyl) indolin-2-one (4e)

Yield: 42%. Mp. 121-123 ^{0}C. IR (KBr, cm^{-1}): 3029, 2813, 1673, 1599, 1239,756. ^{1}H NMR (400 MHz; DMSO-d6, δ ppm): 7.04-6.74 (12H, Ar-H), 3.36-2.58 (8H, pipring), 3.04 (s, 2H, CH_2). 2.18 (s, 3H, CH_3). R_f 0.20 (Hexane: Ethylacetate 1:2).

3-((4-bromophenyl) imino)-1-((4-p-toly) piperazine-1-yl) methyl) indolin-2-one (4f)

Yield: 46%. Mp.129-133 ^{0}C. IR (KBr, cm^{-1}): 3029, 2813, 1672, 1598, 1239, 780. ^{1}H NMR (400 MHz; DMSO-d6, δ ppm): 7.22-6.83 (12H, Ar-H), 3.24-2.66 (8H, pipring), 3.02(s, 2H, CH_2). 2.27 (s, 3H, CH_3). R_f 0.30 (Hexane: Ethylacetate 1:2).

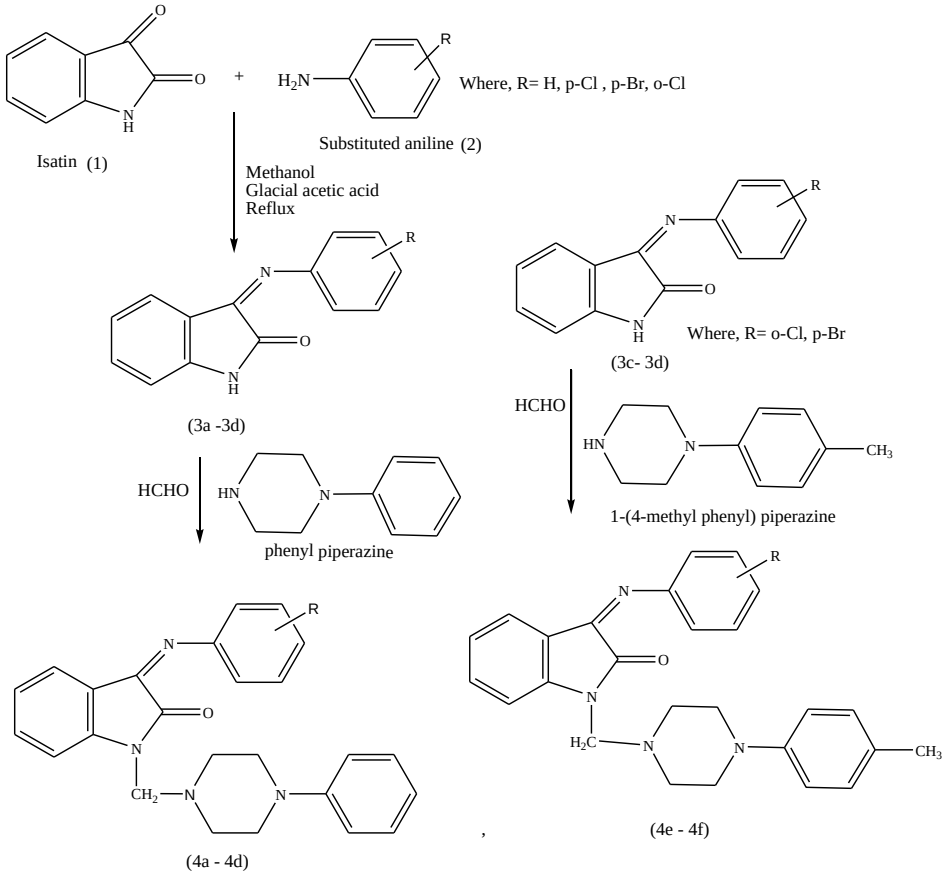

Fig.1.Scheme: Synthesis of Mannich bases (4a-4f)

Antimicrobial activity

The disc diffusion method[11] was employed to study the antibacterial and antifungal activity of synthesized compounds (**4a-4f**) against *B. subtilis*, *E. coli* and *C. albicans*. The synthesized compounds, as 1 mg/ml solutions in dimethylformamide (DMF), were prepared. Ampicillin was used as a standard antibacterial agent and ketoconazole was used as a standard antifungal agent. Dimethylformamide was used as a control. Sterile nutrient agar was inoculated with the test organisms (each 100 mL of the medium received 1 mL of 24 h broth culture), and then seeded agar was poured into sterile petri dishes. Cups (8 mm in diameter) were cut in the agar, and each cup received 0.1 mL of the test compound solution. The plates were then incubated at 37 °C for 24 hr. The activities were estimated as zones of inhibition in mm diameter (Table 1).

Antimicrobial activity of Target compounds (4a-4f)

Table.1.Antibacterial activity of synthesized compounds against *E.Coli*

Compounds	Zone of inhibition (mm) *E. coli* (ESS 2231) 100µg/ml
Control	-
Ampicillin	20
4a	14
4b	10
4c	15
4d	11
4e	13
4f	11

Table.2.Antibacterial activity of synthesized compounds against *B. subtilis*

Compounds	Zone of inhibition (mm) *B. Subtilis* (ACC-132) 100µg/ml
Control	-
Ampicillin	20
4a	8
4b	13
4c	14
4d	11
4e	10
4f	14

Table.3.Antifungal activity of synthesized compound against *C. albicans*

Compounds	Zone of inhibition (mm) *C.albicans* 100µg/ml
Control	-
Ketokonazole	20
4a	11
4b	8
4c	13
4d	14
4e	10
4f	10

RESULT AND DISCUSSION

We have synthesized some Mannich bases of isatin derivatives (Scheme 1) and evaluated for their antimicrobial activity. The synthesized compounds were characterized by their physical and spectral studies. IR spectra of target compounds (**4a-4f**) showed the presence of characteristic absorption peaks around 3029–3056 cm^{-1} (C–H, Ar Str.), 2813–2820 cm^{-1} (C–H, Alp. Str.), 1668-1673 cm^{-1} (C=O Str.), 1516–1599 cm^{-1} (C=N Str.), 1237–1239 cm^{-1} (C-N Str.), and 740–767cm^{-1} which correspond to (C–Cl Str.), 780-790 (C–Br Str.), respectively. The ^{1}HNMR spectra of target compounds(**4a-4f**) have shown absence of singlet peak in the region of 11.52 to 11.59 corresponding to secondary amino group (–NH). The methylene protons (CH_2) showed the singlet peak in the region of 3.02 to 3.09. The aromatic protons resonate in the region of 6.49 to 7.80 contributing the confirmation of compounds. The synthesized compounds were evaluated for their in-vitro antimicrobial activity by disk fusion method. The results of the antibacterial study were summarized in Table. From the results of antibacterial studies it was concluded that the tested compounds exhibited significant antibacterial activities against both gram positive and gram negative organisms. The compound **4c** showed most promising effect whereas compound **4d** & **4f** were found to be least effective against *E.coli*. Similarly the compounds **4c** & **4f** were found to have maximum zone of inhibition and compounds **4a** was found to have minimum zone of inhibition

against *B. subtilis*. Among the tested compounds, compound substituted with electron withdrawing group in isatin residue preferably at para position showed promising antibacterial activities; this may be attributed to their enhanced electronic character which favors greater penetration through microbial membrane. The results of the antifungal study were summarized in Table 1. The compound **4d** showed most promising effect whereas compound **4b** were found to be least effective against *C. albicans*.

CONCLUSION

This paper describes the synthesis,spectral characterization and screening of antimicrobial activity of Mannich bases of isatin derivatives bearing piperazines motif in their structure. The synthesized compounds showed a wide range of potentially promising antimicrobial activities.

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