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SYNTHESIS AND ANTIMICROBIAL SCREENING OF SOME MANNICH BASES OF ISATIN

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ABSTRACT
A series of Mannich bases have been synthesized by treating thiourea, isatin with formaldehyde and different aromatic primary and secondary amines. The structures of compounds were established based on IR, ^{1}H NMR and Mass spectral data. The title compounds were screened for antibacterial and antifungal activities. The compounds S_4, S_6 and S_{10} with 1-benztriazolyl, 1-diethylamino and 1-dimethylamino substituted derivatives exhibited significant antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebesiella pneumonia*, whereas the compounds S_4, S_9 and S_{10} with 1-benztriazolyl, 1-piperazinyl and 1-dimethylamino substituted derivatives exhibited better antifungal activity against *Aspergillus niger.*

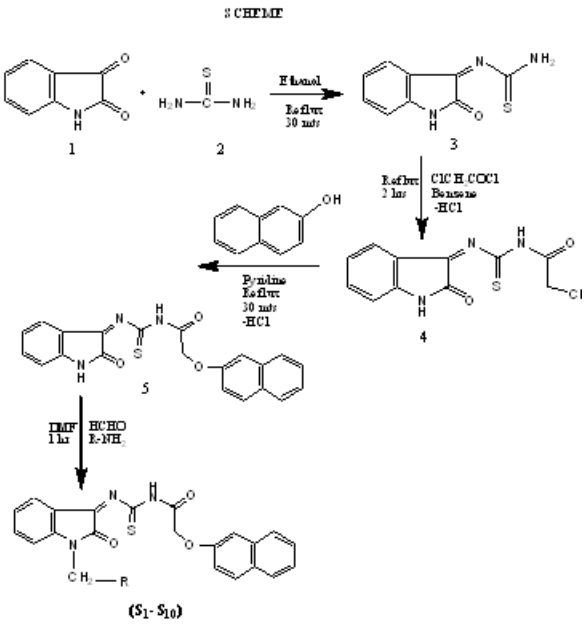
Keywords: *Mannich base; Isatin; Antimicrobial.*

INTRODUCTION

Many natural and synthetic compounds containing heterocyclic rings such as Isatin (Indoline-2, 3-dione) like oxindole and endogenous polyfunctional heterocyclic compounds, to exhibit biological activity in mammals. Schiff bases and Mannich bases of isatin are known to possess a wide range of pharmacological activities like antibacterial[1], antifungal[2], anti-HIV[3], anticonvulsant[4], antiviral[5], anticancer[6] and antimalarial activities[7]. Bis-schiff bases can act as inhibitors of human á-thrombin. Some of the new bis-schiff bases of isatin, benzylisatin and 5-fluroisatin, reported as potential biologically active compounds[8]. It was identified in animals as a major component of the endogenous mono amino oxidase tribulin[9]. Isatin is a versatile lead molecule for potential bioactive compounds and its derivatives were reported to possess anticancer activity[10]. Indole derivatives are an important class of organic heterocycles because of their potential bioactivity[11]. The pharmacological activity of isatin varies from a range of actions in the brain and to be protective against certain types of infections[12].

EXPERIMENTAL

Melting points were determined in open capillary tubes using Veego digital melting point apparatus VMP-D and are uncorrected. The compounds purity was checked using TLC precoated plates. IR spectra were recorded in KBr on a Perkin Elmer-RX I grating spectrophotometer (cm^{-1}). ^{1}H NMR spectra were recorded on Bruker FT NMR (300MHz) spectrometer with TMS as internal standard. Mass spectra were recorded on a LC-MS D-TRAP-SL 2010A Shimadzu mass spectrophotometer



METHODS

General method of synthesis of 1-(2-Oxoindolin-3-ylidene)thiourea.

Isatin **1** (1.47gm, 0.01mol) thiourea **2** (0.76gm, 0.01mol) and 25ml of ethanol were refluxed for 30 minutes at 90-95°c. The mixture was cooled and kept aside for 24 hours at room temperature. The crystalline product obtained was separated and dried, recrystallized from methanol. Yield (85%), m.p: 175-177°c.

General method of synthesis of 1-(2-Chloroacetyl)-3-(2-oxoindolin-3-ylidene)thiourea

To 1-(2-oxoindolin-3-ylidene) thiourea **3** (2.06gm, 0.01mol) in benzene was added with

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chloroacetylchloride (1.12ml, 0.01mol) refluxed for 2 hours at 40-50°c. The product obtained was filtered and washed with benzene and recrystallized from acetone. Yield (80%), m.p: 155-157°c.

General method of synthesis of 1-(2-(Napthalen-2-yloxy)acetyl)-3-(2-oxoindolin-3-ylidene)thiourea

To 1-(2-Chloroacetyl)-3-(2-oxoindolin-3-ylidene) thiourea **4** (2.81gm, 0.01mol) in pyridine, â-naphthal (1.44gm, 0.01mol) was added and refluxed for 30 minutes. Then filtered and collect the residue, recrystallized from methanol. Yield (65%), m.p: 110-113°c.

Synthesis of Mannich base of Isatin derivatives (S_1-S_{10})

To 1-(2-(Napthalen-2-yloxy) acetyl)-3-(2-oxoindolin-3-ylidene) thiourea **5** (3.53gm, 0.001mol) was suspended in minimum quantity of dimethyl formamide various primary or secondary amines (0.001mol) were added and 1ml of 37% formaldehyde was added with vigorous stirring. Then the solution was warmed on a water bath for 2 minutes and stirred for 1 hour. Then kept over night at room temperature. The content was then poured into ice cold water, the product was obtained filtered and collected, recrystallized from methanol and dried. The physical data of the synthesized compounds (S_1-S_{10}) were summarized in Table 1.

Table 1. *Physical data of Mannich bases of Isatin (S_1-S_{10})*

Compounds	R	Molecular Formula	R_f value	Melting range (°c)	Percentage Yield (%)
S_1	-C_8H_7N (Indolyl)	$C_{33}H_{28}N_4O_3S$	0.92	125-127	80
S_2	4-NO_2-C_6H_4N (4-Nitroaminophenyl)	$C_{33}H_{27}N_5O_5S$	0.87	127-129	83
S_3	-$C_7H_5N_2$ (Benzimidazolyl)	$C_{33}H_{27}N_5O_3S$	0.75	215-217	82
S_4	-$C_7H_4N_3$ (Benztriazolyl)	$C_{32}H_{26}N_6O_3S$	0.76	205-207	69
S_5	3-NO_2-C_6H_4N (3-Nitroaminophenyl)	$C_{33}H_{27}N_5O_5S$	0.85	100-102	82
S_6	-$N(C_2H_5)_2$ (Diethylamino)	$C_{31}H_{33}N_5O_3S$	0.84	170-172	80
S_7	4-Br-C_6H_4N (4-Bromoaminophenyl)	$C_{33}H_{27}N_4O_3SBr$	0.66	105-107	73
S_8	-C_4H_8NO (Morpholyl)	$C_{31}H_{31}N_4O_4S$	0.84	136-138	83
S_9	-$C_5H_{10}N$ (Piperadinyl)	$C_{32}H_{33}N_4O_3S$	0.80	165-167	79
S_{10}	-$N(C_2H_5)_2$ (Dimethylamino)	$C_{29}H_{29}N_4O_3S$	0.81	170-172	70

Antimicrobial activity

The *in vitro* antibacterial and antifungal activity was carried out against 24 hours old culture of four bacteria and one fungus. The bacteria used were *E. coli, S. aureus, P. aeuroginosa* and *K. pneumonia* and fungus used was *A.niger.* Serial dilution method was used for determining minimum inhibitory concentration (MIC) [13]. Nutrient broth was used as growth medium for bacteria and Sabouraund's medium was used for growth of fungus. Dimethylformamide (DMF) was used as solvent. The result obtained was compared with standard drug Ciprofloxacin for antibacterial activity and Ketoconazole for antifungal activity. The result of MIC value was summarized in Table 2.

Table 2. *In vitro antimicrobial activity of the compounds S_1-S_{10}*

Compounds	MIC values (µg/ml)				
	E. coli	*K. pneumonia*	*S. aureus*	*P. aeruginosa*	*A niger*
S_1	50	50	12.5	50	50
S_2	200	200	50	200	100
S_3	100	50	100	50	50
S_4	25	12.5	50	25	12.5
S_5	50	25	25	50	25
S_6	25	50	25	12.5	50
S_7	100	100	200	100	100
S_8	100	100	100	100	200
S_9	50	12.5	50	12.5	12.5
S_{10}	25	50	12.5	12.5	12.5
Ciprofloxacin	6.25	12.5	12.5	25	-
Ketoconazole	-	-	-	-	12.5

RESULTS

1-((H-Indol-1-yl)methyl)-2-oxoindolin-3-ylidene)-3-(2-(napthalen-2-yloxy)acetyl)thiourea(S_1): IR (KBr) cm^{-1}: 3445.47 (N-H), 1640.31 (CONH), 1610.89 (C=N), 1352.72 (C-N), 1210.33 (C-O-C), 1049.34 (C=S); ^1H NMR ($CDCl_3$, DMSO-d_6) äppm: 7-7.7 (m, 17H, Ar-H), 4.3 (s, 2H, N-CH_2), 4.1(s, 2H, OCH_2), 6.1 (s, 1H, NH); EI-MS (m/z): 519 (M^+, 34.8%).

1-(2(Napthalen-2-yloxy)acetyl)-3-(1-((4-nitrophenylamino)methyl-2-oxoindolin-3-ylidene)thiourea (S_2): IR (KBr) cm^{-1}: 3446.37 (N-H), 1638.42 (CONH), 1610.14 (C=N), 1352.39 (C-N), 1202.04 (C-O-C), 1049.93 (C=S); ^1H NMR ($CDCl_3$, DMSO-d_6) äppm: 7-7.8 (m, 15H, Ar-H), 4.3 (s, 2H,OCH_2), 5.1(s, 2H, N-CH_2), 5.4 (s, 1H, NH), 4.0 (S, 1H, Ar-NH); EI-MS (m/z): 526 (M^+, 33.3%).

1-(1-((1H-Benzo[d]imidazol-1-yl)methyl)-2-oxoindloin-3-ylidene)-3-(2-napthalen-2-yloxy) acetyl)thiourea (S_3): IR (KBr) cm^{-1}: 3238.23 (N-H), 1639.61 (CONH), 1613.40 (C=N), 1324.80 (C-N), 1210.35 (C-O-C), 1030.59 (C=S); ^1H NMR ($CDCl_3$, DMSO-d_6) äppm: 7-7.9 (m, 16H, Ar-H), 4.9 (s, 2H,OCH_2), 5.1(s, 2H, N-CH_2), 5.4 (s, 1H, NH); EI-MS (m/z): 520 (M^+, 33.8%)

1-(1-((1H-Benzo[d][1,2,3]triazol-1-yl)methyl)-2-oxoindolin-3-ylidene)-3-(2-(napthalen-2-yloxy)acetyl)thiourea (S_4): IR (KBr) cm^{-1}: 3228.06 (N-H), 1641.71 (CONH), 1611.19 (C=N), 1353.77 (C-N), 1227.96 (C-O-C), 1030.69 (C=S); ^1H NMR ($CDCl_3$, DMSO-d_6) äppm: 7-7.7 (m, 15H, Ar-H), 4.2 (s, 2H, OCH_2), 4.8 (s, 2H, N-CH_2), 5.3 (s, 1H, NH); EI-MS (m/z): 521 (M^+, 32.7%).

1-(2-(Napthalen-2-yloxy)acetyl)-3-(1-((3-nitrophenylamino)methyl)-2-oxoindolin-3-ylidene)thiourea (S_5): IR (KBr) cm^{-1}: 3448.84 (N-H), 1639.41 (CONH), 1613.96 (C=N), 1350.25 (C-N), 1254.22 (C-O-C), 1046.84 (C=S); ^1H NMR ($CDCl_3$, DMSO-d_6) äppm: 7-7.7 (m, 16H, Ar-H), 4.9 (s, 2H, OCH_2), 5.2 (s, 2H, N-CH_2), 5.5 (s, 1H, NH); EI-MS (m/z): 523 (M^+, 33.3%).

1-(1-((Diethylamino)methyl)-2-oxoindolin-3-ylidene)-3-(2-(napthalen-2-yloxy)acetyl)thiourea (S_6): IR (KBr) cm^{-1}: 3256.05 (N-H), 1641.31 (CONH),

1613.81 (C=N), 1354.15 (C-N), 1254.15 (C-O-C), 1053.02 (C=S); ^{1}H NMR ($CDCl_3$, DMSO-d_6) äppm: 7-7.9 (m, 11H, Ar-H), 4.7 (s, 2H, OCH_2), 5.3 (s, 2H, N-CH_2), 5.43 (s, 1H, NH), 1.37 (s, 4H, $N(CH_2)_2$), 0.86 (s, 6H, $N(CH_3)_2$); EI-MS (m/z): 475 (M^+, 30.5%).

1-(1-((4-Bromophenylamino)methyl)-2-oxoindolin-3-ylidene)-3-(2-(napthalen-2-yloxy)acetyl) thiourea (S_7): IR (KBr) cm^{-1}: 3424.76 (N-H), 1639.32 (CONH), 1610.05 (C=N), 1356.21 (C-N), 1253.52 (C-O-C), 1058.85 (C=S); ^{1}H NMR ($CDCl_3$, DMSO-d_6) äppm: 7-7.8 (m, 16H, Ar-H), 4.9 (s, 2H, OCH_2), 5.2 (s, 2H, N-CH_2), 5.4 (s, 1H, NH); EI-MS (m/z): 653 (M^+, 34.2%).

1-(1-(Morpholinomethyl)-2-oxoindolin-3-ylidene)-3-(2-(napthalen-2-yloxy)acetyl)thiourea (S_8): IR (KBr) cm^{-1}: 3448.13 (N-H), 1637.45 (CONH), 1617.02 (C=N), 1315.64 (C-N), 1267.30 (C-O-C), 1115.90 (C=S); ^{1}H NMR ($CDCl_3$, DMSO-d_6) äppm: 7-7.8 (m, 15H, Ar-H), 4.23 (s, 2H, OCH_2), 4.84 (s, 2H, N-CH_2), 5.3 (s, 1H, NH), 3.67 (s, 4H, $(CH_2)_2$); EI-MS (m/z): 489 (M^+, 30.8%).

1-(2-(Napthalen-2-yloxy)acetyl)-3-(2-oxo-1-(piperazin-1-ylmethyl)indolin-3-ylidene)thiourea (S_9): IR (KBr) cm^{-1}: 3463.23 (N-H), 1640.22 (CONH), 1613.69 (C=N), 1354.14 (C-N), 1254.17 (C-O-C), 1161.10 (C=S); ^{1}H NMR ($CDCl_3$, DMSO-d_6) äppm: 7-7.9 (m, 15H, Ar-H), 4.91 (s, 2H, OCH_2), 5.2 (s, 2H, N-CH_2), 5.5 (s, 1H, NH), 2.65 (s, 4H, $(CH_2)_2$), 2.0 (S, 1H, NH); EI-MS (m/z): 488 (M^+, 30.5%).

1-(1-((Dimethylamino)methyl)-2-oxoindolin-3-ylidene)-3-(2-(napthalen-2-yloxy)acetyl)thiourea (S_{10}): IR (KBr) cm^{-1}: 3247.25 (N-H), 1642.21 (CONH), 1610.70 (C=N), 1355.75 (C-N), 1192.30 (C-O-C), 1053.37 (C=S); ^{1}H NMR ($CDCl_3$, DMSO-d_6) äppm: 7-7.8 (m, 11H, Ar-H), 4.9 (s, 2H, OCH_2), 5.1 (s, 2H, N-CH_2), 5.23 (s, 1H, NH), 1.3 (s, 6H, N-$(CH_3)_2$); EI-MS (m/z): 447 (M^+, 28.2%).

DISCUSSION

The titled compounds Mannich bases of Isatin derivatives were characterized using IR and ^{1}H NMR spectral data. Compounds S_1-S_{10} exhibited characterized infrared absorption in the ranges 3448-3228 cm^{-1} (N-H), 1642-1637 cm^{-1}(CONH), 1350, 1210, 1053 cm^{-1} for C-N, C-O-C and C=S groups respectively. The ^{1}H NMR exhibited chemical shift at 4.3 äppm (s, 2H, N-CH_2), 6.1 (s, 1H, NH), 4.9 (s, 2H, OCH_2) and multiplets at 7.0-7.9 for aromatic protons confirms the presence of functional group present in the synthesized compounds. Further the titled compounds were confirmed by mass spectrum.

The antibacterial screening put in evidence that all the synthesized compounds (S_1-S_{10}) exhibited a wide spectrum of antibacterial profile *in vitro* against the tested organism. The MIC value of the target compounds indicates that the compounds S_4, S_6 and S_{10} are showing promising activity against *E. coli*. The compounds S_4 and S_9 are highly active against the organism *K. pneumonia* and S_{10} is active against *S. aureus,* where as S_6, S_9 and S_{10} exhibited better antibacterial activity against *P. aeruginosa* and the results of the antibacterial activity was compared with the standard drug Ciprofloxacin. The compounds S_4, S_9 and S_{10} show high efficacy against the fungal organism *A. niger* and the results are compared with the standard with Ketoconazole.

CONCLUSION

It can be concluded from the above study that among all the compounds synthesized the compounds S_4, S_9 and S_{10} with 1-benztriazolyl, 1-piperazinyl and 1-dimethylamino substituted derivatives of Mannich bases of Isatin exhibited better antibacterial and antifungal activities against the bacteria and fungus screened.

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