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SYNTHESIS AND SCREENING FOR ANTIBACTERIAL ACTIVITY OF SCHIFF'S AND MANNICH BASES OF ISATIN AND ITS DERIVATIVES

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Schiff's bases of Isatin and its derivatives were synthesized by reacting isatin and its derivatives with trimethoprim. The N-Mannich bases of the above isatin Schiff's bases were synthesized by condensing acidic 'NH' group of isatin with formaldehyde and secondary amines and screened for their antibacterial activity.

All the synthesized compounds showed good activity against Vibrio cholerae non-O_1, Shigella boydii, Enterococcus faecalis and Edwardsiella tarda with MIC in the range of 10-25 μg/ml. Some compounds were found to be active against Salmonella typhi [MIC 130-160 μg/ml] and against Vibrio cholerae-O_1 [MIC: 75–150 μg/ml].

Keywords : *Isatin, Schiff's base, Mannich base, Trimethoprim, Antibacterial activity*

Introduction

Isatin is an endogeneous compound identified in humans and rat tissues for the first time in 1988[1]. Isatin has a range of actions in the CNS-MAO inhibition, anticonvulsant[2-6], anxiogenic[7] and antimicrobial activity[8]. Some Schiff's and Mannich bases of isatin appear to act as antibacterial agents[9-12]. Trimethoprim and sulphamethoxazole are used in combination as antimicrobial agents. In the present study these two drugs were incorporated into the single molecule with isatin using Schiff's and Mannich reactions. This report deals with the synthesis of Schiff's bases by reacting Isatin and its derivatives with Trimethoprim. The N-Mannich bases of the above Schiff's bases were synthesized by condensing acidic imino group of isatin with formaldehyde and secondary or primary amines [Dimethylamine, diethylamine, piperidine, pyrrolidine, morpholine and sulphamethoxazole] (Scheme 1). All compounds (Table 1) gave satisfactory elemental analysis. IR and NMR spectra constant with the assigned structure. All the synthesized compounds were screened for their antibacterial activity by agar dilution method.

The Selection of the bacterial strains was made because the Sulfonamides possessed anti-microbial spectrum which included all Gram positive bacilli. Therefore, with altered structure, it was envisaged to see whether this modified structure could as well inhibit Gram (-) bacteria.

Materials and Methods

Melting points were taken in open capillary tubes on a Thomas Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on Jasco infrared spectrometer in KBr NMR spectra were recorded at 90 MHz on a Jeol PX 900 FT-NMR spectromess using tetramethyl silane as the internal reference. A synthesis of 3 [4'amino, 5'-(3",4",5"-trimethoxybenzyl) pyrimidinyl] imino isatin.

Equimolar quantities (0.06 moles) of isatin (8.82 gm) and trimethoprim (17.4 gm) were dissolved in 75 ml of warm alcohol containing 1 ml of glacial acetic acid. The reaction mixture was refluxed for 4 hours and set aside. The resulting solid was washed with dilute alcohol, dried and recrystallized from ethanol: chloroform mixture. Yield-24.5 gm (96%); m.p.-180-185°C; IR (KBr)-1660 (C=N), 3050 (C-H), 1580 (C=C), 1620 (C=O), 3300 (NH); NMR ($CDCl_3$) δ ppm -(4,5,6,7 H) 7.0 (3H) 6.8, (6'H) 6.2, ($4NH_2$) 5.8, 5(CH_2) 4.2, (3",5" OCH_3) 3.4, (4"OCH3) 3.3 Anal ($C_{22}H_{21}N_5O_4$) C,H,N.

I. Synthesis of 1(N,N-dimethylamino)methyl 5 methyl 3-[4'amino (3",4",5"-trimethoxybenzyl) pyrimidinyl] imino isatin.

To a slurry consisting of 5-methyl 3-[4'amino 5'-(3",4",5"-trimethoy benzyl) pyrimidinyl] imino isatin (0.04 moles), 50% ethanol and 37% formalin 1 ml was added to the dimethyl amine (0.04 moles) dropwise with cooling and shaking. The reaction mixture was allowed to stand at room temperature for one hour with occasional shaking. The solid which separated out was filtered and recrystallised from ether.

Yield (92%); m.p.-230-232°C; IR (KBr)-2850 (C-H str of CH_2) 1650 (C=N), 1120 (C-O-C); NMR ($CdCl_3$) δ ppm -(1-$2CH_3$) 2.1, (1 CH_2) 1.8, ($5CH_3$) 1.1, (4'NH_2) 5.8, (2",6"H) 6.42 Anal ($C_{26}H_{30}N_6O_4$) C,H,N.

Microbiological materials and methods

The compounds were evaluated for antibacterial activity against Vibrio cholerae non-0_1, Shigella boydii, Enterococcus faecalis, Edwardsiella tarda, Salmonella typhi, Vibrio cholerae-0_1, Klebsiella pneumoniae, Vibrio

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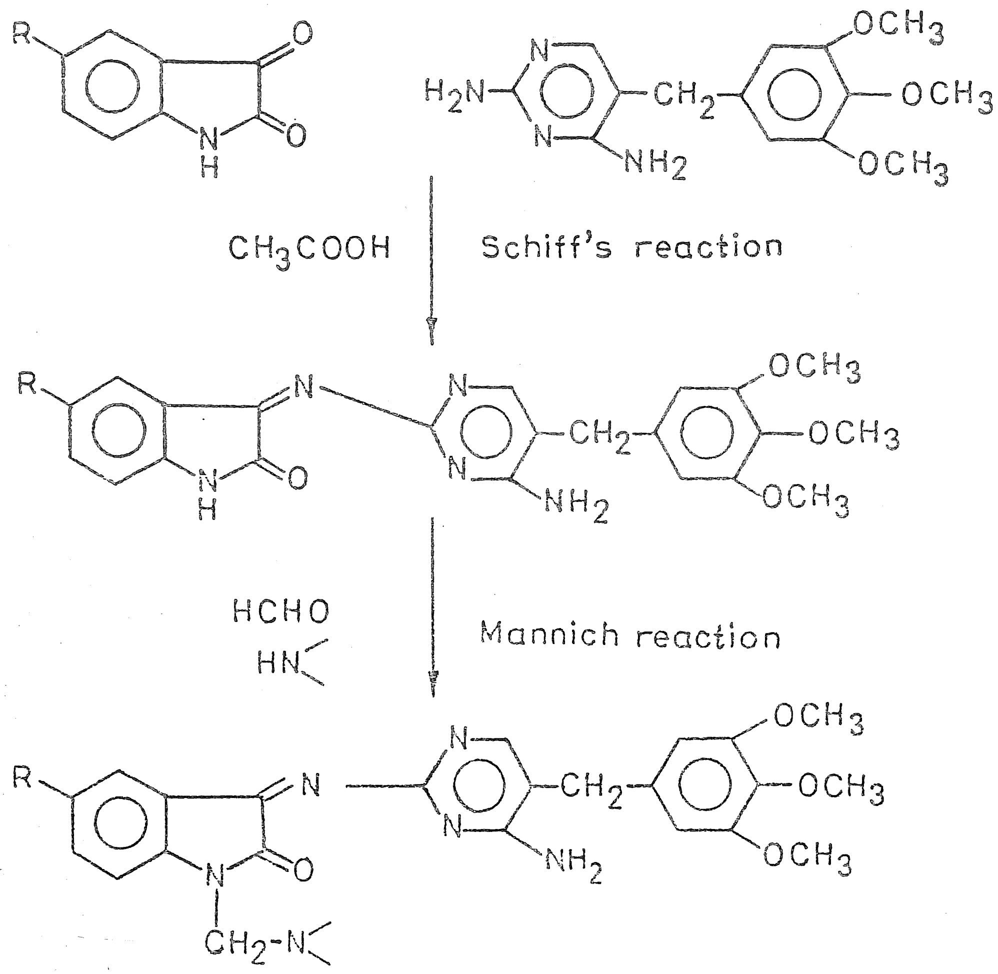

R = H , CH_3 , Br

Scheme-1

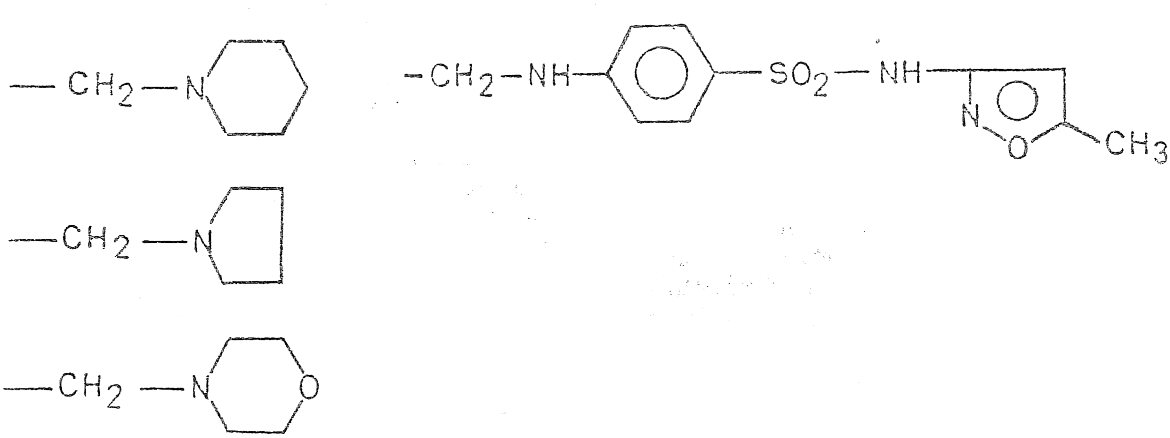

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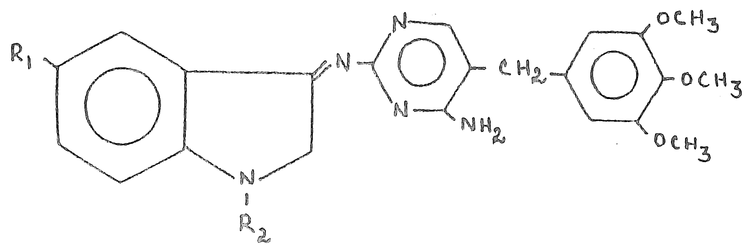
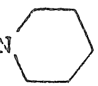
Table 1



Code (1)	R_1 (2)	R_2 (3)	M.P. °C (4)	Yield % (5)	Molecular formula (6)	Molecular weight (7)	R_f (8)
A	H	H	180–185	96.0	$C_{22}H_{21}N_5O_4$	419	0.7786
B	CH_3	H	230–232	94.1	$C_{23}H_{23}N_5O_4$	433	0.8091
C	Br	H	138–143	68.2	$C_{22}H_{20}N_5O_4Br$	498	0.7938
D	H	$-CH_2N(CH_3)(CH_3)$	205	88.6	$C_{25}H_{28}N_6O_4$	476	0.702
E	H	$-CH_2-N(C_2H_5)(C_2H_5)$	140	86.2	$C_{27}H_{32}N_6O_4$	504	0.6274
F	H	$-CH_2-N$ 	142–145	88.0	$C_{28}H_{32}N_6O_4$	516	0.5185
G	H	$-CH_2-N$ 	120–125	79.7	$C_{27}H_{30}N_6O_4$	502	0.7580

(Contd....

Table 1 Contd.

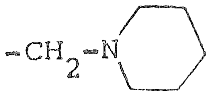
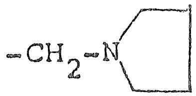
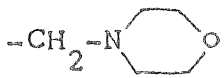
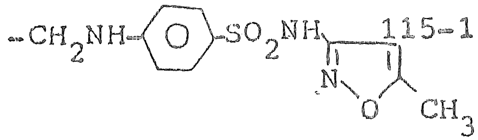
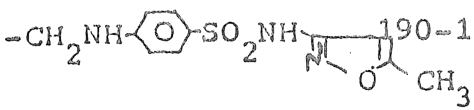
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
H	H		120–125	84.0	$C_{27}H_{30}N_6O_5$	518	0.7500
I	CH_3	$-CH_2-N(CH_3)_2$	95–102	92.0	$C_{26}H_{30}N_6O_4$	490	0.8085
J	CH_3	$-CH_2-N(C_2H_5)_2$	103–105	95.57	$C_{28}H_{34}N_6O_4$	518	0.6603
K	CH_3		107–109	91.0	$C_{29}H_{34}N_6O_4$	530	0.4285
L	CH_3		92–96	74.7	$C_{28}H_{32}N_6O_4$	516	0.5370
M	CH_3		108–109	82.1	$C_{28}H_{32}N_6O_5$	532	0.6363
N	H		115–121	88.4	$C_{33}H_{32}N_8O_7$	652	0.5400
O	CH_3		190–195	92.0	$C_{34}H_{34}N_8O_7$	666	0.7368

Table 2. Antibacterial activity MIC's IN μg/ml

Compound	Vibrio Cholerae non-O_1	Shiegella boydii	Enterococcus faecalis	Edwardsiella torda	Salmonella typhi	Vibrio Cholerae -O_1	Klebsiella Pneumoniae	Vibrio parahaemolyticus
A	9.96	9.96	9.96	14.94	138.98	138.98	29.88	138.98
B	10.06	10.06	10.06	20.12	142.24	-	30.18	-
C	15.18	10.12	10.12	15.18	75.90	75.90	112.31	40.48
D	14.56	9.71	14.56	19.42	153.90	72.82	-	-
E	10.31	10.31	15.46	20.62	154.65	77.32	-	123.72
F	10.21	10.21	15.31	15.31	150.39	76.57	-	120.31
G	9.86	14.79	9.86	14.79	119.52	73.95	119.52	119.52
H	10.60	10.60	10.60	15.90	120.36	79.50	120.36	120.36
I	16.44	10.96	10.96	16.44	153.90	-	-	-
J	11.02	11.02	16.53	16.53	-	-	-	-
K	9.95	9.95	19.90	19.90	-	-	-	-
L	10.36	10.36	10.36	15.54	-	114.51	-	-
M	20.30	10.15	20.30	15.22	151.80	-	15.22	-
N	9.98	9.98	9.98	14.97	149.58	139.60	109.69	-
O	10.12	10.12	10.12	15.18	153.15	-	112.31	-
Trimethoprim	10.62	10.62	10.62	15.93	-	-	121.10	151.38
Sulpha methoxazole	10.36	10.36	10.36	15.54	-	-	15.54	-

parahaemolyticus, Staphylococcus aureus, Escherchia coli, Aeromonas hydrophile and Pseudomonas aeruginosa by agar dilution method.

Agar Dilution Method

Several dilutions in DMSO (10-160 μg/ml approximately) of the synthesised compounds were made in nutrient agar medium. They were innoculated with the test organisms and incubated. The lowest concentration of the substance which causes apparently complete inhibition of growth of the organisms was taken to be the minimum inhibitory concentration. The activity of the compound was computed from the MIC and was reported as μg/ml of substance per ml (Table II). The organisms were procured from Institute of Medical Sciences, B.H.U..

Discussion

All the compounds showed good activity against Vibrio cholerae non-O_1 and Shigella boydii with MIC in the range of 10-15 μg/ml.

The compounds A, B, C, G, H, I, L, O, P showed activity against Enterococcus faecalis with MIC in the range of 10-15 μg/ml. While the MIC of the compounds D, E, F was in the range 15-20 mg/ml and the compounds, J, K, M with 20-25 μg/ml.

All the compounds showed activity against Edwardsiella trada with MIC in the range of 15-25 μg/ml.

Trimethoprim and Sulphamethoxazole were inactive against Salmonella typhi in the concentration upto 10-160 μg/ml whereas among the synthesized compounds, compound C showed activity with MIC in the range of 75-85 μg/ml and compounds A,B,D,E,F,G,H,I with 130-160 μg/ml.

Trimethoprim and sulphamethoxazole were inactive against vibrio cholerae-0_1 in the concentration upto 10-160 μg/ml. Whereas compounds, C, D, E, F, G, H showed activity with MIC in the range of 75-85 μg/ml and compounds A, L, N with 125-150 μg/ml.

The compounds A, B, M showed good activity against Klebsiella pneumoniae with MIC in the range of 20-40 μg/ml while the MIC of the compounds C, G, H, N, O with 100-130 μg/ml.

Compounds C showed activity against Vibrio paraheemolyticus with MIC in the range of 40-50 μg/ml while MIC of the A, E, F, G, H with 120-140 μg/ml.

All the synthesized compounds screened in the concentration of 10-160 μg/ml showed no activity against Staphylococcus aureus, Escherchia coli, Aeromonas hydrophile and Pseudomonas aeruginosa.

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