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

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Abstract

Different novel Schiff bases derivatives have been synthesized by a series of reactions. 5-nitroisatin (1) has been condensed with thiosemicarbazide (2) to yield different thiosemicarbazone (3) which were further cyclised to form corresponding Thia-3, 4, 9-triaza-fluoren-2-ylamines (4). These were subjected to react with substituted aldehydes to give corresponding Schiff bases (A1-A6). All the synthesized compounds were characterized by spectral analysis (IR, MS and NMR). These compounds were screened for their antibacterial activity against Gram-positive bacteria and Gram negative bacteria. Antifungal activity was also performed using agar cup plate method. The minimum inhibitory concentrations (MICs) of the compounds were also determined by agar streak dilution method. Among the synthesized compounds; (6-nitro-1-thia-3, 4, 9-triaza-fluoren-2-yl)-naphthalen-1-ylmethylene-amine (A1) was found to exhibits the most potent in-vitro antimicrobial activity with the MICs of 3.131, 1.6, 22 µg/ml against E. coli, P. aeruginosa, B. pumilus respectively. Compound (6-nitro-1-thia-3, 4, 9-triaza-fluoren-2-yl)- pyridine-2-ylmethylene-amine (A6) was found to exhibit the most potent in-vitro anti-fungal activity with MICs 0.81 and 0.095 µg/ml against A. niger and P. chrysogenum.

Keywords: - Antibacterial, MICs, Isatin, Schiff bases.

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Introduction

Isatin (1H-indoline-2, 3-dione, 13) is an endogenous indole found in the mammalian brain, peripheral tissues, and body fluids. It exhibits many neurophysiological and neuropharmacological effects (Fedchenko, 2008), It is a versatile compound with diversity of effects including antibacterial (Pandeya & Sriram, 1998; Sarangapani & Reddy, 1994; Varma & Nobles, 1975), anticonvulsant (Küçükgüzel, 2003), antifungal (Lon-cle, 2004; Vicini, 2002), antiviral (Varma & Nobles, 1967; Singh, 1983), anticancer (Holla, 2000), antimycobacterial (Pandeya, 2005), antimalarial (Pal, 1991) and anti-inflammatory activities (Gaston, 1996). Isatin was first obtained by Erdman and Laurent in 1841 as a product from the oxidation of indigo by nitric and chromic acids. In nature, isatin is found in plants of the genus *Isatis* in *Calanthe discolor* and in *Couroupi-ta guianensis*. It has also been found as a component of the secretion from the parotid gland of *Bufo frogs*, and in humans as it is a metabolic derivative of adrenaline. Substituted isatins are also found in plants, for example the melosatin alkaloids.

In recent years, Schiff and Mannich bases of isatin are reported to exhibit broad-spectrum chemotherapeutic properties such as antiviral (Sriram & Yogeeswari, 2003), anti-TB (Karah, 1998), antifungal and antibacterial activities

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(Pandeya, 1999). Recently it has been reported that a bis-imine of isatin has antimicrobial properties [Bacchi, 2005] and affects cell viability [Cerchiaro, 2005]. Present study aimed synthesis of the compact structure of Schiff bases and investigation of their possible antimicrobial activities.

Experimental

Materials and Method

All the chemicals and solvents used in the synthesis of Schiff bases were purchased as LR grade from S. D. Fine Chem. Ltd., Mumbai and Sigma-Aldrich Chemical Co., Lancaster and were used directly without any further purification. Melting points were determined by open capillary method and were uncorrected. Infrared spectra of synthesized compounds were recorded on Shimadzu FTIR-8400s in the range of 400-4000 cm^{-1}. ^{1}HNMR spectra (ppm, δ) were recorded on Brucker spectrometer with TMS as the internal standard.

1. General Procedure of thiosemicarbazone derivative (3)

Equimolar quantities (0.004 mol) of isatin and substituted isatin (5-nitro) were dissolved in 90% ethanol with thiosemicarbazide separately and refluxed for 1 hr in the presence of few drops of glacial acetic acid. The completion of reaction was checked by TLC using solvent system chloroform: methanol (95:5). Excess ethanol was distilled off and residue was poured into ice water. Solid product was filtered washed with water, dried and recrystallized using ethanol.

1.1 5- nitro-3-thiosemicarbazido-indole-2, 3,-dione (3)

IR (KBr) ν_{max} in cm^{-1}:1215 (C=S), 1620 (C=C of aromatic ring), 1675 (C=N), 1745 (C=O), 3130 (C-H aromatic), 3430 (NH_2).

2. General Procedure of Thia-3, 4, 9-triaza-fluoren-2-ylamine derivative (4)

Equimolar quantities of 5-chloroisatin-3-thiosemicarbazone (3) and 4-5 drops of cold con. H_2SO_4 were dissolved in ethanol and refluxed for about 8 hrs. The completion of reaction was checked by TLC. The reaction mixture was cooled and neutralized with ammonia. The neutralized mixture was then poured into ice-water, filtered, dried and recrystallized using rectified spirit.

2. 6 -nitro-1-thia-3, 4, 9-triaza-fluoren-2-ylamine (4)

IR (KBr) ν_{max} in cm^{-1}: 1665 (C-S-C), 1625 (C=C of aromatic ring), 1535 (C=N), 3130 (C-H aromatic), 3425 (NH_2).

3. General Procedure of Schiff base derivatives (A1-A6)

Equimolar quantities of thia-3, 4, 9-triaza-fluoren-2-ylamine derivative (4) and appropriate aldehyde were dissolved in 20 ml of absolute ethanol in the presence of 5-6 drops of glacial acetic acid and reaction mixture was refluxed till the completion. The completion of reaction was checked by TLC using different solvent systems. After completion of reaction, the hot mixture was poured onto crushed ice. Then the crude product was purified by recrystallization using ethanol.

3.1 (6-nitro-1-thia-3, 4, 9-triaza-fluoren-2-yl)-napthayl-1-ylmethylene-amine (A1) IR (KBr) ν_{max} in cm^{-1}: 1440 (C=C), 1570 (C=N), 1110 (C S), 750, 730 (Ar-H) 1555–1485/1355–1320 (Aromatic nitro compounds) 2000–1660 (Aromatic combination). **^{1}H-NMR ($CDCl_3$, δ, ppm)**: 1.34-1.68 (s, 3H); 6.14-7.19 (Ar-H) 8.18-8.75 (m, 7H, Naphthalene).

3.2 (6-nitro-1-thia-3, 4, 9-triaza-fluoren-2-yl)-2-methoxyphenol-1-ylmethylene-amine (A2)

IR (KBr) ν_{max} in cm^{-1}: 3270 (N-H), 1435 (C=C), 1540 (C=N), 1170 (N-H), 1120 (C=S), 740, 735 (Ar-H) 1555–1485/1355–1320 (Aromatic nitro compounds) 2850–2815 (Methoxy). **^{1}H-NMR**

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($CDCl_3$, δ, ppm): 5.76-5.98 (s, 1H, CH); 7.3-7.8 (m, 4H, Ar- H)

3.3 (6-nitro-1-thia-3, 4, 9-triaza-fluoren-2-yl)-benzo-1-ylmethylene-amine (A3)

IR (KBr) ν_{max} in cm^{-1}: 3260 (N-H), 1430 (C=C), 1545 (C=N), 1190 (N-H), 1140 (C=S), 745, 730 (Ar-H), 1555–1485/1355–1320 (Aromatic nitro compounds) 1510–1450 (Aromatic ring stretch). **^{1}H-NMR ($CDCl_3$, δ, ppm)**: 5.70-5.88 (s, 1H, CH); 7.3-7.7 (m, 4H, Ar-H).

3.4 (6-nitro-1-thia-3, 4, 9-triaza-fluoren-2-yl)-toluidineidene-amine (A4)

IR (KBr) ν_{max} in cm^{-1}: 3240 (N-H), 1445 (C=C), 1555 (C=N), 1130 (N-H), 1100 (C=S), 755, 725 (Ar-H) 1555–1485/1355–1320 (Aromatic nitro compounds) 900–670 (Aromatic C-H). **^{1}H-NMR ($CDCl_3$, δ, ppm)**: 1.29-1.59 (s, 3H, CH_3); 6.74-7.19 (m, 4H, Ar-H).

3.5 (6-nitro-1-thia-3, 4, 9-triaza-fluoren-2-yl)-nitrobenzo-1-ylmethylene-amine (A5)

IR (KBr) ν_{max} in cm^{-1}: 3280 (N-H), 1450 (C=C), 1530 (C=N), 1140 (N-H), 1160 (C=S), 745, 740 (Ar-H), 1555–1485/1355–1320 (Aromatic nitro compounds). **^{1}H-NMR ($CDCl_3$, δ, ppm)**: 5.54-5.68 (s, 1H, CH); 7.54-7.87 (m, 4H, Ar- H).

3.6 (6-nitro-1-thia-3, 4, 9-triaza-fluoren-2-yl)-pyridine-2-ylmethylene-amine (A6)

IR (KBr) ν_{max} in cm^{-1}: 3240 (N-H), 1425 (C=C), 1525 (C=N), 1155 (N-H), 1170 (C=S), 755, 765 (Ar-H), N-H 3490–3430 (Heterocyclic amine). **^{1}H-NMR ($CDCl_3$, δ, ppm)**: 5.65-5.78 (s, 1H, CH); 7.56-7.77 (m, 4H, Ar-H); 8.42-8.64 (m, 4H, Pyridine).

***In-vitro* Antimicrobial Screening:**

The antibacterial activities of the synthesized compounds were screened against the following standard bacterial strains: *Bacillus pumillus* (MTCC 1456), *Pseudomonas fluorescens* (MTCC 2421), *Micrococus luteus* (MTCC 1538), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 1573), *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 1430). For antifungal screening *Penicillium chrysogenum* (MTCC 161), *Aspergillus niger* (MTCC 2546) were used.

Cylinder plate method:

A definite volume of the microbial suspension (inoculums) was poured into the sterilized nutrient agar media (cooled at 40ºC) and mixed thoroughly. About 20 ml of this suspension was poured aseptically in the petri plates and kept till the solidification. The surface of agar plates was pierced using a sterile cork borer. The prepared wells were filled with equal volume of a solution of synthesized compounds and standard drugs; separately. After a period of pre-incubation diffusion, the plates were incubated face up for a definite time under specified conditions. The zones of inhibition were measured as a parameter of antimicrobial properties of synthesized derivatives.

Minimum inhibitory concentration (MIC)

A series of glass tubes containing different concentrations of the synthesized compounds (In Dimethyl Sulphoxide) with Mueller Hinton broth was inoculated with the required amount of the inoculum to obtain a suspension of microorganism which contains 10^5 colony forming units per milliliter. Growth control tube was prepared with the addition of the compound and blank was prepared without the addition of microorganism. The tubes were incubated at 37 °C for 24 h. The turbidity produced in each tube was recorded by using a UV–visible spectrometer.

Result and Discussion

Novel Schiff bases derivative of Isatin were synthesized by fusion of two heterocyclic moieties **(Figure 1.).** Antifungal & antibacterial activities were also performed as *in-vitro* antimicrobial screening against fungal strains &

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bacterial strain respectively. The minimum inhibitory concentrations (MICs) values for all active compounds were determined by agar streak dilution method **(Table 2, Table 3)** Synthesized compounds characterized using FT-IR and ^{1}H-NMR. The IR spectrum of the synthesized compounds revealed the presence of C =O stretching at 1615-1655 cm^{-1} and C=N stretching at 1515-1655 cm^{-1}. In ^{1}H-NMR spectra δ value of various synthesized compounds was found in the range of. 129-1.68 for methyl proton and 6.14-7.87 for benzyl proton. Physiochemical properties of synthesized compounds were determined in terms of melting point & % yield **(Table 1)**. According to preliminary antibacterial screening by paper disc method all compounds were found to have comparable antibacterial activity against *S. aureus*, *B. subtilis B. pumillus*, *E. coli* compared to Norfloxacin as a standard drug, and for antifugal screening all compounds were found to active against *A. niger* and *P. chrysogenum* using Fluconazole as a standard drug.

The antimicrobial screening revealed that the compound **A1** & compound **A6** exhibited potent antibacterial & antifungal activity respectively as compared to other derivatives **(Table 2, Table 3).**

Conclusion

Present research work involves combination of Indole with Schiff bases moiety together as both known to have antimicrobial properties in order to explore their antimicrobial activity. Compound **A1** exhibited highest antibacterial activity against *E. coli* MTCC 1573 (MIC: 3.125 μg mL^{-1}) *P. aeruginosa* MTCC 424 (MIC: 3.125 μg mL^{-1}) *B. Pumillus* MTCC 1456 (MIC: 25 μg mL^{-1}). Compound **A6** exhibited highest antifungal activity against *A. niger* MTCC 2546 (MIC: 0.78 μg mL^{-1}) and *P. Chrysogenum* MTCC 161 (MIC: 0.097 μg mL^{-1}). Present work may prove to be a lead for the development of new agents against resistant strain for the treatment of bacterial strain as well as fungal strain.

Table 1. Physico-chemical properties of the compounds

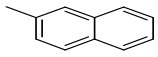
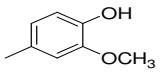
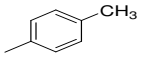
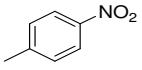
Products ↓	R	M. P (^{o}C)	Yield (%)	R_f Value
A1		231	87	0.539
A2		339	65	0.877
A3		295	91	0.994
A4		261	69	0.620
A5		378	62	0.612
A6		329	79	0.827

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Table 2. Antibacterial Activity (Minimum Inhibitory Concentration)

Compounds	Zone of Inhibition (Mm)						
	Gram negative bacteria			Gram positive bacteria			
	Escherichia coli (MTCC 1573)	*Pseudomonas aeruginosa* (MTCC 424)	*Pseudomonas fluorescens* (MTCC 2421)	*Staplococc aureus* (MTCC 1430)	*Bacill subtilis* (MTCC 441)	*Bacillus pumillus* (MTCC 1456)	*Micrococcus luteus* (MTCC 1538)
A1	3.131	1. 6	145	91	194	22	61
A2	62	51	76	136	195	42	147
A3	56	41	109	202	161	49	-
A4	37	-	41	165	106	84	23
A5	119	144	94	111	84	31	57
A6	81	66	-	194	29	158	66
Norfloxacin	2.91	1.19	3.6	13	13	11	3.2

Table 3. Antifungal Activity (Paper Disc Diffusion Method)

Compounds	Antifungal Activity (Paper Disc Diffusion Method)			
	Zone of Inhibition (mm)		Minimum Inhibitory Concentration ($\mu g\ mL^{-1}$)	
	Fungal Strain			
	Penicillium chrysogenum (MTCC 161)	*Aspergillus niger* (MTCC2546)	*Penicillium chrysogenum* (MTCC 161)	*Aspergillus niger* (MTCC 2546)
A1	24	29	37	84
A2	24	31	51	61
A3	21	19	62	108
A4	27	18	21	92
A5	21	22	61	91
A6	32	31	0.81	0.095
Fluconazole	-	-	0.8	0.07

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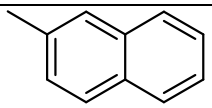
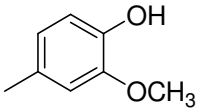
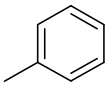
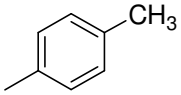
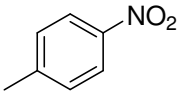
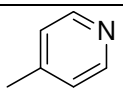
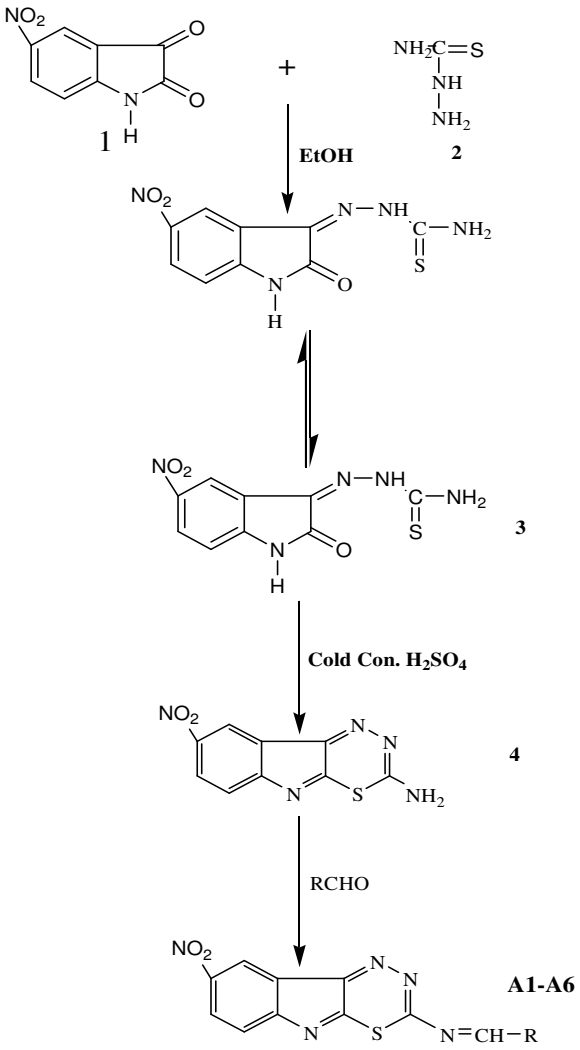
Products ↓	R_1
A1	
A2	
A3	
A4	
A5	
A6	



Scheme 1 Synthesis of various Schiff Base derivatives

Fig. 1. Scheme for the synthesis of various Schiff Base derivatives

Reference:

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