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Original Article

SYNTHESIS AND ANTIMICROBIAL SCREENING OF 2,6-DIAMINOPYRIDINE SCHIFF BASES OF ISATIN DERIVATIVES

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ABSTRACT

Objective: Synthesis and *in vitro* antimicrobial screening of 2,6-diaminopyridine Schiff bases of isatin derivatives.

Methods: Isatin and it's 5-substituted derivatives (S1-5) were prepared by Sandmeyer method and N^2-Benzylidenepyridine-2,6-Diamine (M) was obtained by the reacting 2,6-diaminopyridine with benzaldehyde. Schiff bases (MS1-5) were prepared by reacting isatin derivatives (S1-5) with N^2-Benzylidenepyridine-2,6-Diamine (M). Resultant compound structures were confirmed by some analytical techniques' data. All synthesized compound were screened for *in vitro* antimicrobial activity by broth dilution methods against *Staphylococcus aureus* (MTCC-3160), *Bacillus subtilus* (MTCC-441), *Escherichia coli* (MTCC-452), *Klebsiella pneumoniae* (MTCC-432), *Candida albicans* (MTCC-183), *Aspergillus flavus* (MTCC-277) using ciprofloxacin and fluconazole as standard drugs.

Results: All compounds exhibited better antibacterial activity compared to standard. Among all compounds, MS2 and MS4 were found most effective against all strains of bacteria. Only MS3 and MS5 showed antifungal activity against both fungal strains.

Conclusion: All newly synthesized Schiff bases of isatin showed significant antibacterial activity against the tested strain of bacteria, only a few compounds were found effective as antifungal.

Keywords: Isatin, Schiff base, Antimicrobial activity, 2,6-diaminopyridine

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INTRODUCTION

Microbial resistance against the antimicrobial agents kindles the researcher to arouse the search of new antimicrobial agents. Isatin was reported an endogenous compound [1] and disclosed its role in chemotherapy of infectious microbes [2]. Schiff bases reported enormous promising applications in chemical biology. Schiff bases of isatin were accounted for their broad spectrum activity *viz.* antimicrobial [3-7], anti HIV [3], anti-inflammatory [8], analgesic [8, 9], anticonvulsant [10], anticancer [11, 12] and anti-tuberculosis activity [13, 14]. As per literature in isatin Position C-5 with electron-donating and withdrawing groups were more favaourable for its biological activity. Along with this another substitution at C-3 with aromatic and heterocyclic rings has been reported with antimicrobial profiles. In recent years, derivatives of 2,6-diaminopyridine with aldehydes were reported for their antimicrobial activity [15-20], cytotoxic activity [19] and, anticancer activity [21], DNA binding activity [19]. Prompted by these, in this article a new series of Schiff bases of isatin/substituted isatin with 2,6-diaminopyridine has been reported. All synthesized compounds were characterized and investigated for their antimicrobial activity against some pathogenic gram+ve, gram–ve bacteria and fungus.

MATERIALS AND METHODS

Chemicals were procured from CDH, Chemcho and SRL used without further purification. Melting points were determined by open capillary tube apparatus and are uncorrected. Purity of the compound was checked by TLC on pre-coated SiO_2 gel ($HF_{254,}$ 200 Mesh) using chloroform: methanol (9:1) and spots were visualized by iodine vapour. IR spectra were recorded on JASCO FTIR 460+ using KBr dispersion diffuse reflectance technique. ^1H NMR Spectra were recorded on 400 MHzJEOL JNM ECS 400 using DMSO-d6 solvent and tetramethylsilane (Me_4Si) as internal standard. All spectra were consistent with the assigned structure. The physio-chemical data was presented in table 1.

Method for synthesis

General method of isatin/5-substituted isatin (S 1-5) (Sandmeyer method) [22]

It was prepared by reported method, in which 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 desired isonitrosoacetanilide [1]. Then this intermediate was carried to cyclization to form substituted isatin by the help of sulphuric acid, re-crystallize with suitable solvent [2].

Preparation of N^2-Benzylidenepyridine-2, 6-Diamine (M) [23]

Compound was prepared as per literature by refluxing equimolar (2 mmol) quantity of 2,6-diaminopyridine and benzaldehyde in ethanol using few ml of glacial acetic acid for 2 h. Purity was compound was checked by TLC using chloroform: methanol (9:1). Cool the resulting mixture and filtered the solid obtained, re-crystallized it with methanol to get pure product [3].

General method for synthesis of Schiff base: [24] (MS 1-5)

Equimolar quantity of N^2-benzylidenepyridine-2,6-Diamine and isatin/5-substituted isatin (2 mmol) was taken in 25 ml ethanol containing 1 ml of glacial acetic acid. Resulting mixture was refluxed on steam bath for 4-5 h and then cooled at room temperature. Reaction was monitored by TCL using chloroform: methanol (9:1). Solid was obtained by filtration and washed with cold ethanol, dried in air and then re-crystallized from a suitable solvent [4].

Antimicrobial activity

The antimicrobial screening of all synthesized compounds was done by using the broth dilution technique. All compounds were investigated against two gram-positive, two gram negative bacteria and two pathogenic fungus, *viz. Staphylococcus aureus* (MTCC-3160), *Bacillus subtilus*(MTCC-441), *Escherichia coli* (MTCC-452), *Klebsiella pneumoniae* (MTCC-432), *Candida albicans* (MTCC-183), *Aspergillus flavus* (MTCC-277) using ciprofloxacin and fluconazole as standard drugs. Both standard and tested compounds were dissolved in dimethyl sulphoxide (DMSO) and diluted to get 100, 50, 25, 12.5, 6.25 µg/ml. To each tube i.e. test and standard, 9 ml of sterile broth nutrient after seeding with microbes was added; followed by 1 ml of test and standard solution of different concentrations. Negative and Positive control tubes were also prepared by taking sterile nutrient broth only and nutrient broth with microbes, respectively. All tubes

were incubated at 37 °C for 24 h to observe the antimicrobial activity. After 24 h lowest concentration of the substance was recorded as the MIC value that showed no visible turbidity.

RESULTS AND DISCUSSION

Schiff bases of isatin/substituted isatin with N²-Benzylidenepyridine-2, 6-Diamine

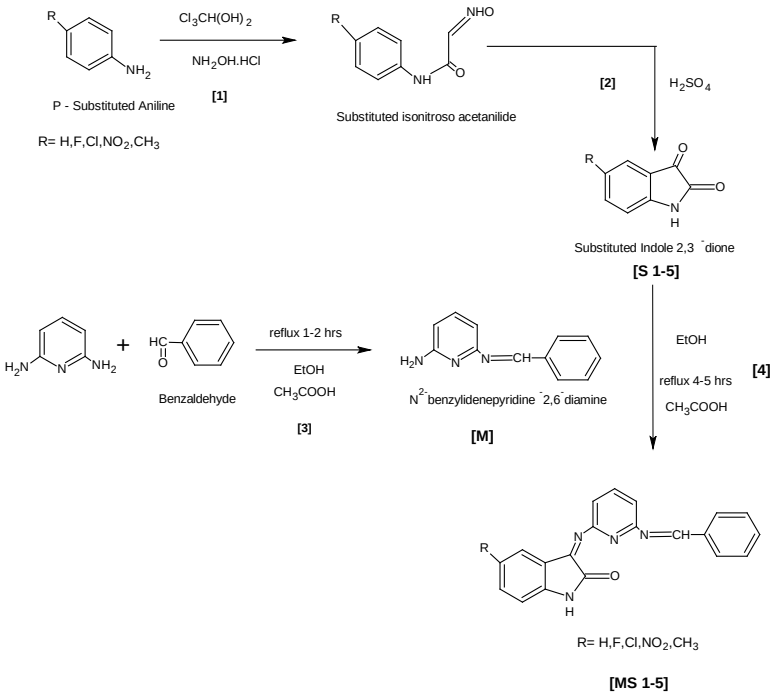
MS1: 3-{[6-(benzylideneamino)pyridin-2-yl]imino}-1,3-dihydro-*2H*-indol-2-one: yield 72%, M. P. 295-298 °C; IR (KBr, cm^{-1}): 3372 (v, N-H), 3024 and 3061 (v, C=CH), 2922 (v, CH Ar), 1723 (v, C=O), 1603 (v, CH=N), 1449 (v, C=C Ar), 1185 (v, C-F); ^{1}H NMR (DMSO-d_6, 400MHz): δ 6.63-7.24 (8H, m Ar), 7.12-7.79 (3H, m, Pyri), 8.38 (1H, s CH=N), 10.89 (1H, s, NH).

MS2: 3-{[6-(benzylideneamino)pyridin-2-yl]imino}-5-fluoro-1,3-dihydro-2*H*-indol-2-one: yield 78%, M. P. 285-288 °C; IR (KBr, cm^{-1}): 3363 (v, N-H), 3200 and 3237 (v, C=CH), 3027 (v, CH Ar), 1718 (v, C=O), 1607 (v, CH=N), 1483 (v, C=C Ar), 1185 (v, C-F); ^{1}H NMR (DMSO-d_6, 400MHz): δ 6.25-7.16 (8H, m, Ar), 7.11-7.26 (3H, m, Pyri), 8.39 (1H, s, CH=N), 10.52 (1H, s, NH).

MS3: 3-{[6-(benzylideneamino)pyridin-2-yl]imino}-5-chloro-1,3-dihydro-2*H*-indol-2-one: yield 66%, M. P. 265-268 °C; IR (KBr, cm^{-1}): 3369 (v, N-H), 3208 (v, C=CH), 3024 (v, CH Ar), 1721 (v, C=O), 1606 (v, CH=N), 1454 (v, C=C Ar), 702 (v, C-Cl); ^{1}H NMR (DMSO-d_6, 400MHz): δ 6.74-7.52 (8H, m, Ar), 7.29-7.59 (3H, m, Pyri), 8.42 (1H, s, CH=N), 11.09 (1H, s, NH).

MS4: 3-{[6-(benzylideneamino)pyridin-2-yl]imino}-5-nitro-1,3-dihydro-2*H*-indol-2-one: yield 73%, M. P. 278-280 °C; IR (KBr, cm^{-1}): 3333 (v, N-H), 3228 and 3101 (v, C=CH), 3024 (v, CH Ar), 1749 (v, C=O), 1605 (v, CH=N), 1456 (v, C=C Ar), 1517 and 1336 (v, C-NO_2); ^{1}H NMR (DMSO-d_6, 400MHz): δ 6.83-7.50 (8H, m, Ar), 8.17-8.40 (3H, m, Pyri), 8.42 (1H, s, CH=N), 11.62 (1H, s, NH).

MS5: 3-{[6-(benzylideneamino)pyridin-2-yl]imino}-5-methyl-1,3-dihydro-2*H*-indol-2-one: yield 69%, M. P. 245-248 °C; IR (KBr, cm^{-1}): 3375 (v, N-H), 3061 and 3024 (v, C=CH), 2912 (v, CH Ar), 1719 (v, C=O), 1616 (v, CH=N), 1454 (v, C=C Ar); ^{1}H NMR (DMSO-d_6, 400MHz): δ 2.21 (3H, s, CH_3) 6.78-7.28 (8H, m, Ar), 7.15-7.88 (3H, m, Pyri), 8.89 (1H, s, CH=N), 10.89 (1H, s, NH).



Scheme 1: Synthesis of schiff bases

Table 1: Physio-chemical properties of the compounds

S. No.	Compound	R	Molecular formula	M. P. (°C)	% Yield	R_f Value
1	MS1	H	$C_{20}H_{14}N_4O$	295-298	72%	0.69
2	MS2	F	$C_{20}H_{13}FN_4O$	285-288	78%	0.79
3	MS3	Cl	$C_{20}H_{13}ClN_4O$	265-268	66%	0.82
4	MS4	NO_2	$C_{20}H_{13}N_5O_3$	278-280	73%	0.75
5	MS5	CH_3	$C_{21}H_{16}N_4O$	245-248	69%	0.66

Table 2: *In vitro* antibacterial activities of synthesized compounds in opposition to selected strains (MICs in µg/ml)

S. No.	Compound	MIC in µg/ml			
		S. aureus	*B. subtilis*	*E. coli*	*K. pneumoniae*
1	MS 1	12.5	25	25	12.5
2	MS 2	12.5	12.5	12.5	6.2
3	MS 3	25	25	12.5	6.2
4	MS 4	12.5	25	12.5	12.5
5	MS 5	12.5	25	25	12.5
6	Ciprofloxacin	6.2	12.5	6.2	6.2

Table 3: *In vitro* antifungal activities of synthesized compounds in opposition to selected strains (MICs in µg/ml)

S. No.	Compound	MIC in µg/ml	
		C. albicans	*A. niger*
1	MS1	12.5	50
2	MS2	12.5	50
3	MS3	25	25
4	MS4	12.5	50
5	MS5	12.5	25
6	Fluconazole	6.2	12.5

All compounds were investigated for their antimicrobial activity and results are shown in table 2 and table 3 for antibacterial and antifungal, respectively. MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganism. All synthesizedcompounds showed varying activity against the tested microorganism. Results revealed that all compounds expressed significant, mild to moderate activity against the tested microorganisms. Compound MS2 (3-{[6-(benzylideneamino) pyridin-2-yl]imino}-5-fluoro-1,3-dihydro-2*H*-indol-2-one) and MS4 3-{[6-(benzylideneamino)pyridin-2-yl]imino}-5-nitro-1,3-dihydro-2*H*-indol-2-one showed significant activity against both gram-positive and negative bacteria. MS4 Compound showed two time more MIC value against most bacteria strain compared to standard. Compound MS2 and MS3 (MIC 6.2 µg/ml) exhibited equipotent antibacterial activity against *K. pneumonia*, compared to standard drug ciprofloxacin (MIC 6.2 µg/ml). Compound MS1, MS4 and MS5 showed equal potency against the *K. pneumonia*. Compounds MS2, MS3 and MS4 exhibited good antimicrobial activity against the *E. coli* while MS1 and MS5 showed mild potency for the same. Compounds MS2 showed equal potency against *B. subtilis* as compared to the reference drug ciprofloxacin. MS1, MS2, MS4 and MS5 exhibited the similar MIC value i.e. 12.5 µg/ml, against the *S. aureus.* Only compound MS3 and MS5 showed just two times more MIC value against *A. niger* as compared to standard. From antifungal activity, it was observed that newly synthesized isatin derivatives are more active in opposition to *C. albicans* as compared to *A. niger.*

CONCLUSION

Present study reported a new Schiff bases of isatin/substituted isatin with N^2-benzylidenepyridine-2, 6-Diamine. New synthesized Schiff bases were screened *in vitro* for their antimicrobial activity against some pathogenic strain of bacteria and fungus. Compound MS2 and MS4 showed significant activity against most gram+ve and gram–ve bacteria. All compound showed activity antifungal activity against *C. albicans***.** Only MS3 and MS5 compound exhibited good antifungal activity against *A. niger*. All newly synthesized Schiff bases were more active against tested bacteria than fungus.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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