Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 6, 2014

Original Article

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SOME NOVEL SCHIFF AND MANNICH BASES OF ISATIN

K. MEENAKSHI, N. GOPAL, M. SARANGAPANI

Department of Pharmaceutical Chemistry, Balaji Institute of Pharmaceutical Sciences, Laknepally (V), Narsampet (M), Warangal (Dt), Andhra Pradesh. Email: k.meenakshi13@gmail.com

Received: 23 Apr 2014 Revised and Accepted: 22 May 2014

ABSTRACT

Objective: Synthesis and antimicrobial evaluation of some novel Schiff and Mannich bases of Isatin.

Methods: A series of novel Schiff bases of Isatin (V) and (X) were synthesized by refluxing Isatin with p-amino ethyl benzoate (IV) and 4-(4[l]-amino phenyl)-6-substituted phenyl pyrimidine-2-thiol (IX). Mannich bases of ethyl-4-(2-oxindolin-3-ylidene amino) benzoate (VI) were synthesized by using various aromatic secondary amines. The chemical structures of synthesized compounds were confirmed by IR, ^{1}HNMR, Mass and elemental analysis. These compounds were also screened for their *in vitro* antibacterial and antifungal activities.

Results: The results of antibacterial and antifungal activities showed that some of the synthesized compounds were exhibited promising antimicrobial activities.

Conclusion: All the newly synthesized compounds were screened for antimicrobial activities by turbidity method using Ampicillin and Clotrimazole as standard against gram positive, gram negative bacteria and fungi.

Keywords: Schiff bases, Mannich Bases, Isatin, Antibacterial Activity, Antifungal Activity.

INTRODUCTION

The indole nucleus is found to be very active nucleus in pharmacy field, as several natural alkaloids having indole as their basic ring are found to be therapeutically active agents. Isatin (indole-2, 3-dione) is an indole derivative, an endogenous compound, widely distributed in mammalian tissues and body fluids [1]. Isatins are synthetically versatile substances that are employed for the synthesis of a very large variety of heterocyclic compounds and possess broad spectrum of biological activities like antibacterial [2], antiviral [2[l], antifungal [2], anti-inflammatory [3], analgesic [4], anti-tubercular [5], antidepressant [6]. In view of these facts, we contemplated to synthesize some new Schiff and Mannich bases of Isatin and planned to screen for their *in vitro* antibacterial and antifungal activities.

MATERIALS AND METHODS

All the chemicals used for this study were in analytical grade only. The melting points were determined by open capillary using Toshniwal melting point apparatus and are uncorrected. Purity of compounds was checked by TLC on Silica Gel precoated plates. IR spectra were recorded in KBr on FTIR Brucker spectrophotometer and frequencies are expressed in cm^{-1}. The ^{1}HNMR spectra were recorded on 400MHz Brucker DPX using $CDCl_3$ and DMSO as solvent. Chemical shift values are reported as values in ppm relative to TMS as internal standard. Mass spectra were recorded on VG AUTOSPEC using EI-Ms mode. Elemental analysis was performed on Perkin-Elmer series-2400.

Isatin (III) and ethyl p-amino benzoate (IV) were synthesized by the methods available in the literature [6, 7]. The synthetic strategies adopted to obtain target compounds are depicted in Figure 1 and 2.

Synthesis of ethyl-4-(2-oxoindolin-3-ylidene amino) benzoate (V)

An appropriate quantity of indole-2, 3-dione (III) (0.01mol) was dissolved in alcohol (20ml) and added ethyl p-amino benzoate (IV) (0.01mol) and few drops of glacial acetic acid. The reaction mixture was stirred well and refluxed for 3 hrs. The resultant yellow crystalline solid was filtered and washed repeatedly with small quantity of methanol. The product was dried and purified by recrystalization from chloroform. The yield was found to be 78%. The melting point of the product was found to be 180-182ºC, M.W:294.

Synthesis of 1-(substituted amino methyl)-4-ethyl-4-(2-oxoindolin-3-ylideneamino) benzoate (VI)

Compound (V) ethyl-4-(2-oxoindolin-3-ylidene amino) benzoate (0.001mol) suspended in minimum quantity of DMF. To that, formaldehyde (1ml, 37%) and various secondary amines (0.001mol) were added with vigorous stirring. Warm the solution on a water bath for 2min.and stirred for an hour. Then left at room temperature over night. By the addition of water, the compound was separated, filtered, washed thoroughly with water, dried and purified by recrystallization from ethanol. The yield was found to be 70%.

Spectral data of compound (V)

IR (KBr in cm^{-1}): 3187(NH stretch), 2984(CH aliphatic stretching), 1654 (C=N stretching), 1751 (C=O ester stretching), 1272 (C-O ester stretching). ^{1}H NMR ($CDCl_3$) (δppm): 1.4(t, 3H, CH_3), 4.4 (q, 2H, CH_2), 6.5-8.1 (m, H, Aromatic), 9.2 (s, 1H, NH). MS: (m/z) 294.2 (M^+). Analysis ($C_{17}H_{14}N_2O_3$) Cal. (Found)%: C 69.38 (69.35), H 4.79 (4.22), N 9.52 (9.05), O 16.31 (16.28).

Spectral data of compound (VIa)

IR (KBr in cm^{-1}): 3356 (NH stretching), 2992 (aliphatic CH stretching), 1728 (C=O ester stretching), 1274 (C-O ester stretching), 1598 (C=N stretching), 1469 (C=C aromatic stretching). ^{1}HNMR ($CDCl_3$) (δppm): 1.4 (t, 3H, CH_3), 2 (s, 1H, NH- piperazino), 2.4 (t, 4H, CH_2 piperazino), 2.7 (t, 4H, CH_2 piperazino), 3.9 (s, 2H, -N-CH_2-N-), 4.4 (q, 2H, CH_2 of ester), 6.5-8.1 (m, 8H, Ar). MS: (m/z) 392 (M^+). Analysis ($C_{22}H_{24}N_4O_3$) Cal. (Found)%: C 67.33 (68.05), H 6.16 (6.15), N 14.28 (13.05), O 12.23 (12.68).

Spectral data of compound (VI b)

IR (KBr in cm^{-1}): 2980(aliphatic CH stretching), 1729 (C=O ester stretching), 1296 (C-O ester stretching), 1608 (C=N stretching), 1470 (C=C aromatic stretching).

^{1}HNMR ($CDCl_3$) (δppm): 1.2 (t, 3H, CH_3), 1.4 (t, 6H, CH_3 of diethyl amino group), 3.5 (s, 2H, N-CH_2-N), 4.1 (q, 2H, CH_2 of ester), 4.3 (q, 4H, CH_2 of diethyl amino group), 6.5-7.4 (m, 8H, Ar). MS: (m/z) 379

(M^+). Analysis ($C_{22}H_{25}N_3O_3$) Cal. (Found)%: C 69.64 (68.55), H 6.64 (6.22), N 11.07 (11.19), O 11.31 (11.88).

Spectral data of compound (VI c)

IR (KBr in cm^{-1}): 2936 (aliphatic CH stretching), 1733 (C=O ester stretching), 1271 (C-O ester stretching), 1605 (C=N stretching), 1469 (C=C aromatic stretching)

^{1}HNMR ($CDCl_3$) (δppm): 1.3 (t, 3H, CH_3), 2.6 (s, 6H, CH_3 of dimethyl amino group), 4.5 (s, 2H, N-CH_2-N), 4.3 (q, 2H, CH_2 of ester), 6.5-8 (m, 8H, Ar). MS: (m/z) 351 (M^+). Analysis ($C_{20}H_{21}N_3O_3$) Cal. (Found)%: C 68.36 (68.25), H 6.02 (6.26), N 11.96 (12.07), O 13.66 (13.16).

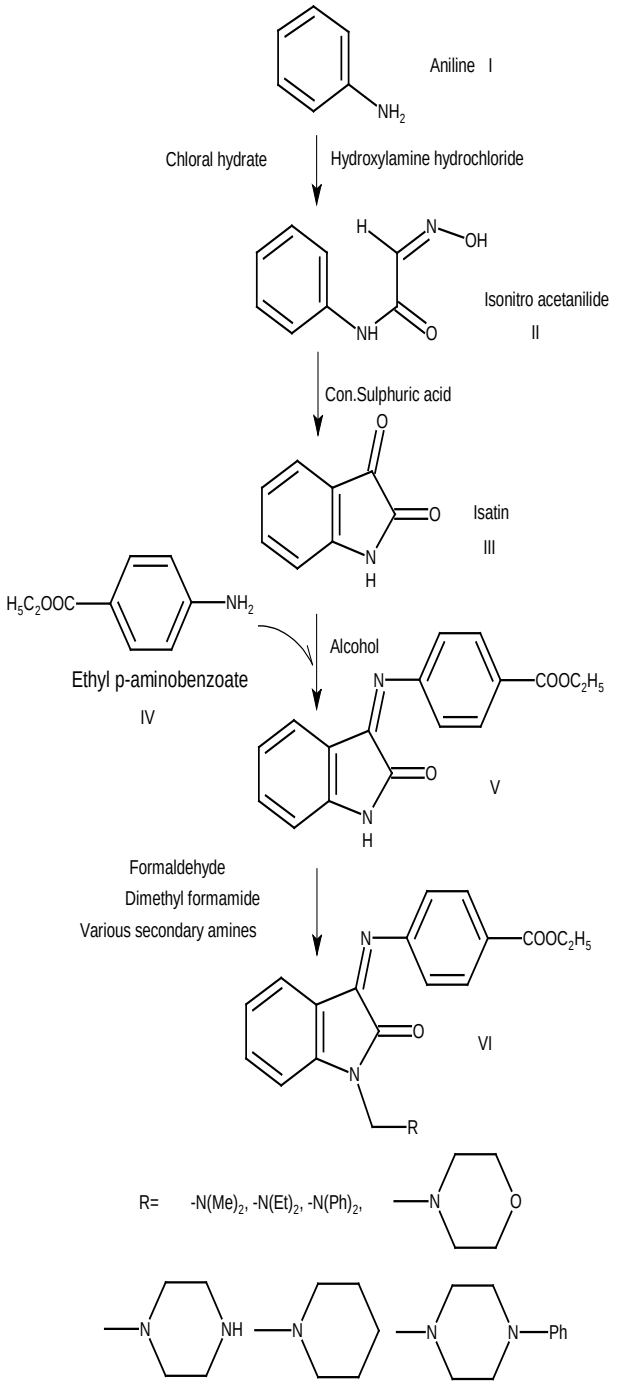


Fig. 1: Experimental scheme (I) for the synthesis of 1-(substituted amino methyl)-4-ethyl-4-(2-oxoindolin-3-ylidene amino) benzoate (VI a-g)

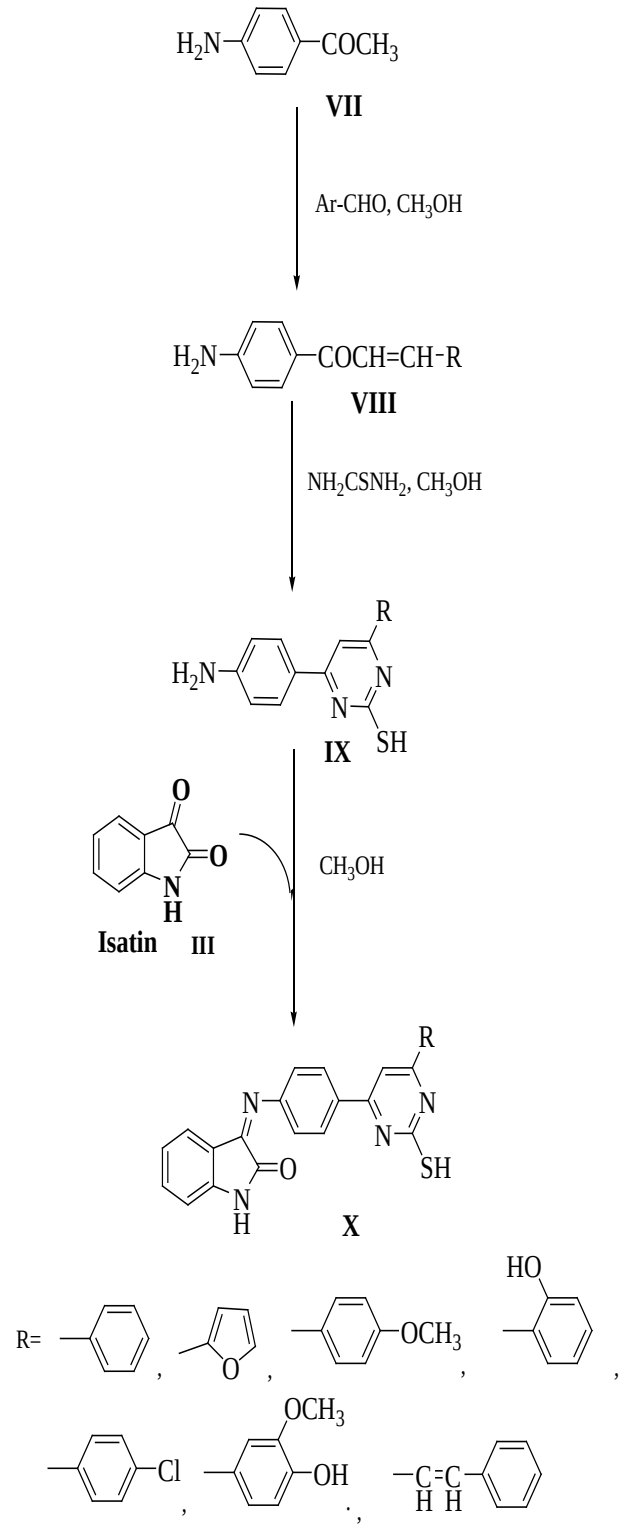


Fig. 2: Experimental scheme (II) for the synthesis of Isatin-3-(4'-(6''-substituted phenyl-2-thiopyrimidin-4-yl) phenyl) imine (Xa-g).

Spectral data of compound (VI e)

IR (KBr in cm^{-1}): 2906 (aliphatic CH stretching), 1728 (C=O ester stretching), 1274 (C-O ester stretching), 1598 (C=N stretching), 1469 (C=C aromatic stretching).

^{1}HNMR ($CDCl_3$) (δppm): 1.3 (t, 3H, CH_3), 2.1 (t, 4H, CH_2 of morpholino), 3.6 (t, 4H, CH_2 of morpholino), 4 (s, 2H, N-CH_2-N), 4.3

(q, 2H, CH_2 of ester), 6.5-8.1 (m, 8H, Ar). MS: (m/z) 393 (M^+). Analysis ($C_{22}H_{23}N_3O_4$) Cal. (Found)%: C 67.16 (67.35), H 5.89 (5.18), N 10.68 (10.95), O 16.27 (16.88).

Synthesis of 1-(4-amino phenyl)-3- substituted phenyl prop-2-en-1-one (VIII)

An appropriate quantity (0.01 moles) of p-amino acetophenone (VII) was refluxed with equimolar quantity (0.01 moles) of aromatic substituted aldehydes in methanol for 24 hrs. The completion of the reaction was checked by TLC. The solvent has been evaporated; the product was washed with ice cold water, dried and purified by recrystallization from ethanol.

Synthesis of 4-(4'-amino phenyl)-6- substituted phenyl pyrimidin-2-thiol (IX)

Compound 1-(4-amino phenyl)-3- substituted phenyl prop-2-en-1-one (VIII) (0.01 mole) and thiourea (0.01 mole) were dissolved in methanol and few drops of conc. HCl was added. The mixture was refluxed for 5 hrs. The crystalline solid separated out on cooling, which was collected by filtration and washed with ice cold water, dried. The product was recrystallised from ethanol.

Synthesis of Isatin-3-(4'-(6''-substituted phenyl-2-thiopyrimidin-4-yl) phenyl) imine (X)

Compound 4-(4'-amono phenyl)-6- substituted phenyl pyrimin-2-thiol (IX) and Isatin (III) were dissolved in methanol in equimolar quantities and refluxed for 5 hrs. The crystalline solid separated out on cooling was collected by filtration and purified by recrystallization from ethanol.

Spectral data of compound (IX)

IR (KBr in cm^{-1}): 3442 (NH stretching), 2916 (aliphatic CH stretching), 2548 (SH stretching), 1460 (C=C aromatic stretching) ^{1}HNMR ($CDCl_3$) (δppm): 3 (s, 1H, SH), 3.7 (s, 3H, OCH_3), 4.0 (s, 2H, NH_2), 6-8 (m, 9H, Ar). MS: (m/z) 309 (M^+). Analysis ($C_{17}H_{15}N_3OS$) Cal. (Found)%: C 66.16 (66.55), H 4.89 (4.43), N 13.58 (13.62), O 5.17. (5.18), S 10.36 (10.66).

Spectral data of compound (Xa)

IR (KBr in cm^{-1}): 3442 (NH stretching), 3010 (CH aromatic stretching), 2542 (SH stretching), 1730 (C=O stretching), 1617 (C=C aromatic stretching), 1331 (C-N stretching)

^{1}HNMR ($CDCl_3$) (δppm): 3.9 (s, 1H, SH), 6-9 (m, 14H, Ar), 11 (s, 1H, NH). MS: (m/z) 408 (M^+). Analysis ($C_{24}H_{16}N_4OS$) Cal. (Found) %: C 70.57 (70.52), H 3.95 (4.02), N 13.72 (13.97), O 3.92. (3.85), S 7.85(7.77)

Spectral data of compound (Xc)

IR (KBr in cm^{-1}): 3450 (NH stretching), 2929 (CH aliphatic stretching), 2540 (SH stretching), 1725 (C=O stretching), 1591 (C=C aromatic stretching), 1353 (C-N stretching), 1097 (C-O stretching). ^{1}HNMR ($CDCl_3$) (δppm): 3.1 (s, 1H, SH), 3.7 (s, 3H, OCH_3), 6-9 (m, 13H, Ar), 11 (s, 1H, NH). MS: (m/z) 438 (M^+). Analysis ($C_{25}H_{18}N_4O_2S$) Cal. (Found)%: C 68.48 (68.52), H 4.14 (4.23), N 12.78 (12.92), O 7.30. (7.08), S 7.31(7.44).

***In Vitro* Antimicrobial Activity**

The newly synthesised compounds ethyl-4-(2-oxoindolin-3-ylideneamino) benzoate (V), 1-(substituted amino methyl)-4-ethyl-4-(2-oxoindolin-3-ylideneamino) benzoate (VI), Isatin-3-(4'-(6''-substituted phenyl-2-thio pyrimidin-4-yl) phenyl) imine (X) were screened for antimicrobial activity studies[8-15] as primary screening in six sets at different concentrations against gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative (*Escherichia coli* and *Proteus vulgaris*) bacteria and fungi (*Candida albicans* and *Aspergillus niger*) by turbidity method in Muller Hinton broth medium and compared with that of standard drugs Ampicillin and Clotrimazole respectively.

Table : Physical properties of 1-(substituted amino methyl)-4-ethyl-4-(2-oxoindolin-3-ylideneamino) benzoate (V&VIa-g))

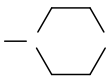
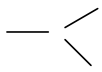
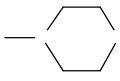
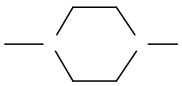
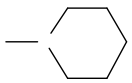
Compoud No	R Group	Molecular Formula	Molecular Weight	Percentage % Yield	Melting Point ºC	R_f Value
V	—	$C_{17}H_{14}N_2O_3$	294	80%	180-182°C	0.56
VI a		$C_{22}H_{24}N_4O_3$	392	70%	300-301ºC	0.62
VI b		$C_{22}H_{25}N_3O_3$	379	68%	240-242ºC	0.61
VI c		$C_{20}H_{21}N_3O_3$	351	60%	295-296ºC	0.51
VI d		$C_{30}H_{25}N_3O_3$	475	65%	160-161ºC	0.51
VI e		$C_{22}H_{23}N_3O_4$	393	72%	220-2220ºC	0.58
VI f		$C_{28}H_{28}N_4O_3$	468	70%	145-146ºC	0.70
VI g		$C_{23}H_{25}N_3O_3$	391	67%	98-100 ºC	0.80

Table 2: Physical properties of Isatin-3-(4'-(6''-substituted phenyl-2-thiopyrimidin-4-yl) phenyl) imine (Xa-g)

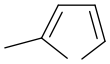
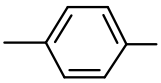
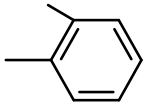
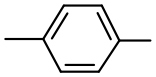
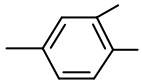
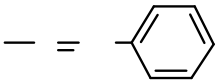
Compoud No	R Group	Molecular Formula	Molecular Weight	Percentage % Yield	Melting Point ºC	R_f Value
Xa		$C_{24}H_{16}N_4OS$	408	70%	200-201°C	0.56
Xb		$C_{22}H_{14}N_4O_2S$	398	65%	215-217ºC	0.68
Xc		$C_{25}H_{18}N_4O_2S$	438	65%	210-212ºC	0.69
Xd		$C_{24}H_{16}N_4O_2S$	424	69%	222ºC	0.44
Xe		$C_{24}H_{16}N_4OSCl$	442	71%	198-200ºC	0.73
Xf		$C_{25}H_{18}N_4O_3S$	458	68%	218-220ºC	0.72
Xg		$C_{26}H_{18}N_4OS$	434	70%	215-216ºC	0.68

Table 3: Antimicrobial activity of 1-(substituted amino methyl)-4-ethyl-4-(2-oxoindolin-3-ylideneamino) benzoate (V &VIa-g) by turbidity method

COMPOUNDS	ANTIBACTERIAL ACTIVITY (Minimum Inhibitory Concentration) (µg/ml)				ANTIFUNGAL ACTIVITY (Minimum Inhibitory Concentration) (µg/ml)	
	B.subtilis	*S.aureus*	*E.coli*	*P.vulgaris*	*C.albicans*	*A.niger*
V	25±1.45**	12.5±2.12***	12.5±1.11***	25±2.28**	25±1.15**	12.5±2.54***
VI-a	12.5±2.60***	12.5±2.21***	12.5±1.80***	25±3.12**	25±1.18**	25±2.65**
VI-b	12.5±1.10***	50±1.28*	25±3.12**	100±2.12*	12.5±1.54***	25±1.95**
VI-c	25±1.25**	25±1.68**	12.5±1.96***	100±1.35*	12.5±1.76***	25±2.24**
VI-d	12.5±1.92***	12.5±1.36***	12.5±2.66***	100±1.45*	25±1.86**	12.5±1.89***
VI-e	12.5±2.12***	12.5±1.44***	12.5±1.28***	50±2.63*	12.5±2.21***	50±2.22*
VI-f	12.5±2.29***	50±2.56*	25±1.94**	50±1.95*	12.5±2.28***	12.5±3.14***
VI-g	50±1.96*	12.51.96***	25±2.02**	12.5±1.28***	12.5±3.12***	25±1.88**
AMPICILLIN	12.5±2.53***	12.5±2.22***	12.5±1.70***	12.5±2.94***	-	-
CLOTRIMAZOLE	-	-	-	-	12.5±1.78***	12.5±1.64***

Values are expressed as Mean ± SD (n=6). ***P <0.001, **P <0.01, *P <0.05. All significant differences are considered from control value 0.00

Table 4: Antimicrobial activity of Isatin-3-(4'-(6''-substituted phenyl-2-thiopyrimidin-4-yl) phenyl) imine (X a-g) by turbidity method

COMPOUNDS	ANTIBACTERIAL ACTIVITY (Minimum Inhibitory Concentration) (µg/ml)				ANTIFUNGAL ACTIVITY (Minimum Inhibitory Concentration) (µg/ml)	
	B.subtilis	*S.aureus*	*E.coli*	*P.vulgaris*	*C.albicans*	*A.niger*
X-a	50 ±1.02*	50±2.02*	50±0.88*	100±1.12*	50±2.12*	25±0.98**
X-b	12.5 ±0.98***	12.5±1.98***	12.5±1.96***	100±0.89*	50±0.91*	25±1.22**
X-c	12.5 ±0.45***	25±1.13**	25±0.75**	25±0.458**	12.5±0.8***	25±1.33**
X-d	50 ±1.24*	50±1.96*	50±1.58*	100±0.58*	50±1.66*	50±1.62*
X-e	12.5 ±0.96***	12.5±1.46***	12.5±1.40***	50±0.88*	12.5±1.46***	12.5±2.09***
X-f	50 ±0.88*	50±1.45*	50±2.02*	100±1.11*	50±1.22*	50±2.12*
X-g	25 ±1.34**	25±2.24**	50±2.12*	50±1.37*	25±0.98**	25±1.67**
AMPICILLIN	12.5 ±1.88***	12.5±2.12***	12.5±1.34***	12.5±1.87***	-	-
CLOTRIMAZOLE	-	-	-	-	12.5±1.99***	12.5±1.41***

Values are expressed as Mean ± SD (n=6). ***P <0.001, **P <0.01, *P <0.05. All significant differences are considered from control value 0.00

Two fold serial dilutions of the test compounds and reference drugs were prepared in broth. Test compounds and standard drugs Ampicillin and Clotrimazole (10 mg) were dissolved in dimethyl formamide (10ml). Further progressive dilutions with Muller Hinton broth were performed to obtain the required concentrations of 200,100,50,25 and 12.5 µg/ml. The test tubes were inoculated with bacteria and fungi and incubated at 37°C for 24 h. The minimum inhibitory concentration (MIC) was the lowest concentration of the tested compound that yields no visible growth in the tube. To ensure that the solvent had no effect on the bacterial growth, a control was performed.

Statistical Analysis

Values are expressed as mean ± standard deviation and statistical analysis was carried out by one way ANOVA. $P < 0.05$ is considered as significant.

RESULTS AND DISCUSSION

A novel series of VIa-g and Xa-g derivatives have been synthesized and screened for their *in vitro* antibacterial and antifungal activities. The results of the physical data of the final synthesized compounds are presented in table 1 and 2.

The antibacterial activity results revealed that all compounds showed significant activity against Bacillus subtilis, staphylococcus aureus and Escherichia coli. Compounds VIa, VIe, VIf, Xb, Xc showed excellent activity against all gram positive organisms and gram negative organism E.coli. In the antifungal activity, compounds VIf and Xe showed highly significant activity which was comparable with the standard drug clotrimazole. The results are presented in table 3 and 4.

The structures of the newly synthesized compounds were established on the basis of spectral data and elemental analysis. The compounds were purified by recrystallization from appropriate solvents. The completion of the reactions were monitored by TLC. The antibacterial activity of the compounds showed excellent activity against gram positive organisms and *E.coli*. The compounds also displayed significant activity against fungal organisms.

CONCLUSION

From the above results it can be concluded that the Mannich bases of isatin with heterocyclic secondary amines (VIa, VIe, VIf) exhibited more activity. In the Schiff bases of isatin, furfuryl derivative (Xb) and Chlorophenyl derivative (Xe) showed more antimicrobial activity.

ACKNOWLEDGEMENTS

The author is thankful to Dr. A. Rajendraprasad Reddy, Chairman, Balaji group of institutions, Narsampet, Warangal for providing all the facilities to carryout the study.

REFERENCES

1. Pandeya SN. Sriavastava, Anupam, Indole" a versatile nucleus in pharmaceutical field. International journal of current pharmaceutical review and research Nov Jan 2011;1:31-17.
2. Pandeya SN, Sriram D, Nath G, DeClercq E. Synthesis, antibacterial, antifungal and anti-HIV activities of Schiff and Mannich bases derived from isatin derivatives and N-[4-(4'-chlorophenyl)thiazol-2-yl] thiosemicarbazide. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 1999;9(1):25-31.
3. Swathi M. K.Sarangapani. Synthesis and anti-inflammatory activity of a novel series of Isatin hydrazone and Isatin thio semicarbazone derivatives. World Journal of Pharmacy and Pharmaceutical sciences 2014;3(2):2070-8.
4. Khan SA, Siddiqui AA, Bhatt S. Analgesic activity of Isatin derivatives. Asian Journal of Chemistry 2002;14:417-18.
5. Tran VH, Nguyen QD, Le NV. Study on the antituberculosis effect of some thiosemicarbazones and isonicotinyl hydrazone derivatives of Isatin and 5-haloisatin. TapChiDou Hoc 2000;8:15-7.
6. Popp FD, Parson R, Donigan BE. Synthesis of potential anticonvulsants: condensation of isatins with acetone and related ketones. Journal of pharmaceutical sciences 1980;69(10):1235-7.
7. S. B, J. A, G. PW, R. A. V. Rogers, and Vogel's Textbook of Practical Organic Chemistry.
8. S. P, V. R. Shradha.S.Binani, Synthesis, Characterization and invitro antimicrobial evaluation of novel 2-Mercapto-4,6-disubstituted phenyl pyrimidine derivatives. International Journal of Pharmacy and Pharmaceutical sciences 2014;6(1):461-3.
9. Harpreet singh, Arvind kumar, Ankita Verma. Evaluation of antimicrobial activity of ethanolic extract of Aesculus indicus seeds. World Journal of Pharmacy and Pharmaceutical Sciences. 2013;2(5):3045-57.
10. Pandeya SN, Sriram D, Nath G, de Clercq E. Synthesis, antibacterial, antifungal and anti-HIV evaluation of Schiff and Mannich bases of isatin and its derivatives with triazole. Arzneimittel-Forschung 2000;50(1):55-9.
11. Sampath Ayyappa G, Raja. S, Jayaveera K.N, Yogananda Reddy.K. Design and synthesis of 1,3,4-oxadiazole Derivatives as Antimicrobial agents. Journal of Pharmacy and Chemistry. 2012;6(4);19-23.
12. P., M., S., A., Mannich S. Synthesis and Evaluation of 1, 3 Di-substituted and SpiroIsatin derivatives. Journal of Young Pharmacist 2010;2(2):169-72.
13. Panneerselvam P, Ravisankar R, Kumarasamy M, Kumar N. Ramesh Synthesis, analgesic, anti-inflammatory and antimicrobial activities of some novel Schiff's bases of 5-substituted Isatin. Der Pharma Chemica 2010;2(1):28-37.
14. Blessi M, Maharaj J, Krishnaveni G, Brahmeshwari M, Sarangapani G. Sammaiah. Synthesis and Antimicrobial activity of some new Isatin derivatives. Journal of Advanced Pharmaceutical Sciences 2011;1(1):20-33.
15. B. P, V. P. Synthesis of substituted Schiff's bases and their antimicrobial activity. Der Pharm Chemica 2014;6(1):262-6.