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SYNTHESIS AND EVALUATION OF NEW ISATIN DERIVATIVES FOR POSSIBLE BIOLOGICAL ACTIVITIES

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ABSTRACT

Isatin Derivatives have been reacted with 2-(phenylamino)acetohydrazide to form Mannich bases of these compounds were synthesized by reacting them with formaldehyde and secondary amines. Their chemical structures have been confirmed by IR, 1H-NMR data and by elemental analysis Investigation of Antibacterial Activity of compounds done by cup plate method. Among the synthesized compounds Ie (R=F) showed the most favourable activity.

KEY WORDS

Isatin; Mannich bases; Antibacterial.

1. INTRODUCTION

Isatin (indole-2,3-dione), its Mannich bases are reported to show a variety of biological activities, such as Antibacterial [1], antifungal [2], antiviral [3], anti-inflammatory [4], analgesic [5], anticonvulsant [6], anticancer [7], anti-tubercular [8], anti-depressant [6], anti-HIV [9]. In view of these facts and as a continuation of our work in the laboratory, prompted us to synthesize new isatin derivatives of (1-((bis (2 - chloroethyl) methyl) - 2 - oxoindolin - 3- ylidene)- 2 -(phenylamino) acetohydrazide. All the synthesized compounds were screened for their Antibacterial Activity.

2. Experimental

Melting points were determined on a Thomas-Hoover Melting point apparatus and are uncorrected. IR spectra were recorded in KBr on FTIR 8400S Shimadzu IR Spectrophotometer. ^{1}H NMR Spectra were recorded on 300MHz Bruker DRX-300 Using DMSO With TMS as internal standard.

2.1 Synthesis of 1-{[bis(2-chloroethyl) amino] methyl}-1H-indoline-2,3-dione(III):

The mannich condensation was done by the following procedure. A mixture of equimolar concentration of Isatin (0.01mol), Formaldehyde (0.01 mol), Bis(2-chloroethyl) amine hydrochloride (0.010 moles; 1.42g) was refluxed in ethanol with stirring for 12hrs at 70-80 °C. After completion of reflux the compound was filtered and evaporated. The residue was recrystallized from petroleum ether gave pure material.

2.2 Synthesis of ethyl 2-(phenylamino)acetate (VI):

Ethyl chloroacetate (0.012 mol) was refluxed with aniline (0.01 mol) on water bath in dry acetone (25 mL) and anhydrous potassium carbonate (0.01 mol) for 6 h. The solvent was evaporated, and the reaction mixture was poured into crushed ice to get the respective ethyl phenyl amino acetate. The solid thus separated was filtered, dried and recrystallized from petroleum ether. The compound was characterized by the physical constant available in literature. m.p. 55 °C.

2.3 Synthesis of 2-(phenylamino) acetohydrazide (VIII):

Ethyl 2-(phenylamino) acetate (**VI**) (0.01 mol) was refluxed on water bath with excess of hydrazine hydrate (0.02 mol) in alcohol (25 mL) for 4 h. The solvent was evaporated, the product thus obtained was washed

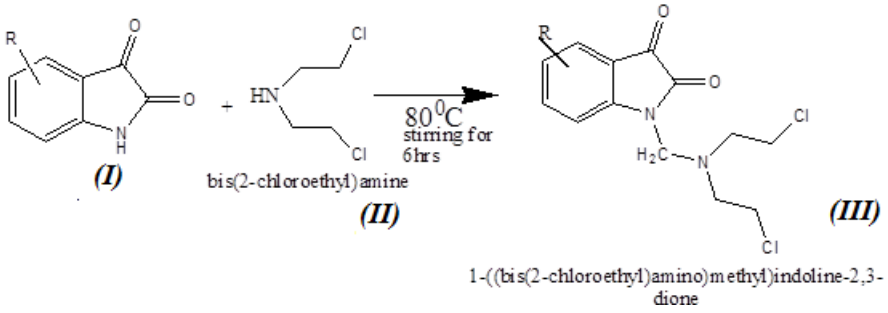
with cold water, dried and purified by recrystallization with suitable solvent(s). The compound was characterized as a 2-(phenylamino) acetohydrazide (**VIII**) by physical data and m.p. 125-127 ^{0}C.

2.4 Synthesis of (1-((Bis (2-chloroethyl) amino) methyl)-2-oxoindolin- 3 - ylidene) -2- (phenylamino) acanthodrilids:

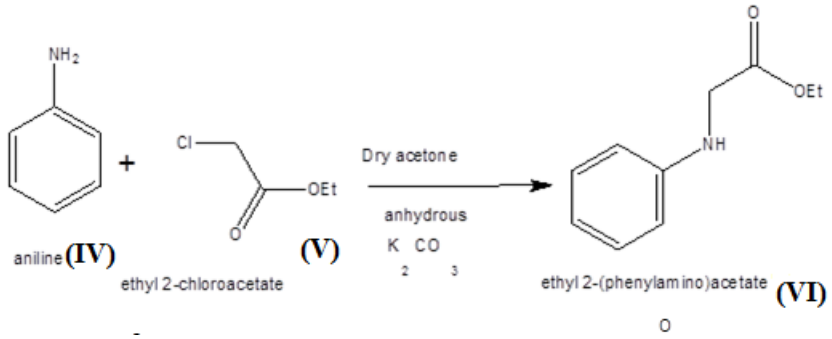
Compound(III) (0.001mol) was condensed with 2-(phenylamino) acetohydrazide (VIII) (0.001 mol)) in alcohol (25 mL) containing traces of acetic acid on heating with stirring for 24hrs to get the respective (1-((Bis(2-chloroethyl) amino) methyl)-2-oxoindolin-3-ylidene)-2-(phenylamino)acetohydrazide. The solvent was evaporated, and the reaction mixture was poured into crushed ice. The product thus separated was filtered, dried and recrystallized from alcohol. Similarly, all the compounds were prepared, and the purity was checked by TLC. The compounds were characterized by physical data and m.p.167^{0}C.

Scheme

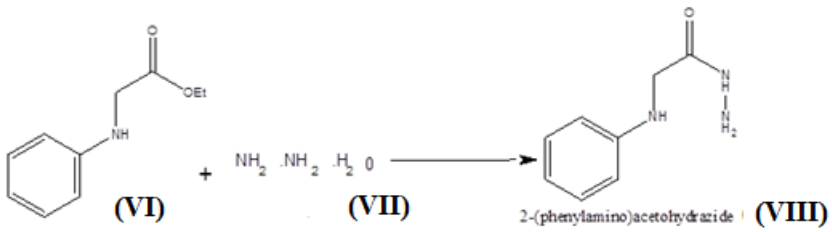
2.1 Synthesis Of 1-((Bis(2-chloroethyl) amino) methyl) indoline-2,3-dione(III) :



2.2 Synthesis of ethyl 2-(phenylamino)acetate (VI):



2. 3 Synthesis of 2-(phenylamino)acetohydrazide (VIII):



2.4 (1-((Bis(2-chloroethyl) amino) methyl)-2-oxoindolin-3-ylidene)-2-(phenylamino)acetohydrazide(IX)

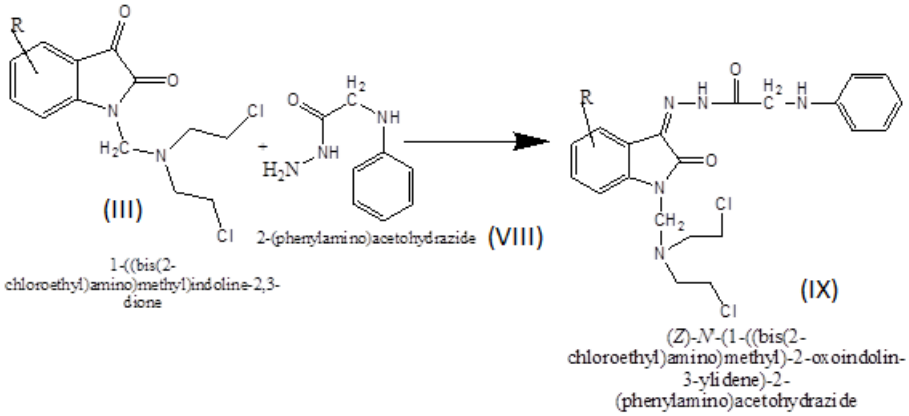
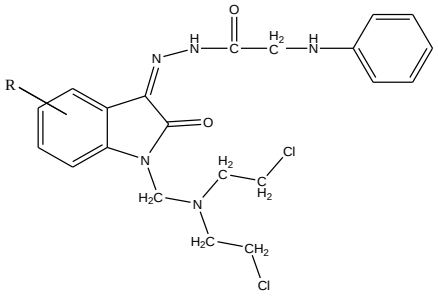


Table 1

Physical Data



S.No	Compound	R	Molecular formula	molecular weight	M.P	% yield
1	IX a	H	$C_{21}H_{23}Cl_2N_5O_2$	448	167	45
2	IXb	NO_2	$C_{21}H_{22}Cl_2N_6O_4$	493	172	56
3	IXc	Br	$C_{21}H_{22}BrCl_2N_5O_2$	527	159	51
4	IXd	Cl	$C_{21}H_{22}Cl_3N_5O_2$	482	181	49
5	IXe	F	$C_{21}H_{22}FCl_2N_5O_2$	466	176	41
6	IXf	CH_3	$C_{22}H_{25}Cl_2N_5O_2$	462	184	52

Spectral Data:

1. 1-((bis(2-chloroethyl) amino) methyl)-2-oxoindolin-3-ylidene)-2(phenylamino)acetohydrazide (IX-a):

IR Spectrum: (KBr, cm^{-1}) : 3178(NH), 1686 (NH-C=O), 1604 (C=N),1340(C-N), 1508(Ar C=C), 794 (Ar C-H), 748 (C-Cl) Mass spectrum: M+2 peak was observed at 450, M+4 peak was observed at 452 ; ^{1}H NMR 2.84(t, 4H), 3.5(d, 2H, CH_2-NH), 3.94(t, 4H, C-Cl),4.4(s, 2H), 6.87(t, 1H, Ar-H), 7.30(t, 2H, Ar-H), 7.35(d, 1H, Ar-H), 7.40(d, 2H, Ar-H),7.41-7.42(m, 2H, Ar-H), 7.96(t,1H, Ar-H), 8.67(t, NH), 8.98(s, NH-CO)

2.1-((bis(2-chloroethyl) amino) methyl)-5-methyl-2-oxoindolin-3-ylidene)-2-(phenylamino)acetohydrazide(IXf)

IR Spectrum: (KBr, cm^{-1}) : 3340(NH), 1672 (CO), 1602(C=N),1321(C-N), 1486(Ar C=C), 769 (Ar C-H), 769.4(C-Cl) ;) Mass spectrum: M+2 peak was observed at 464 and m+4 peak was observed at 466 ; ^{1}H NMR 2.3(s, 3H, CH_3), 2.94(t, 4H), 2.99(s, 2H, CH_2) 3.3(d, 2H, CH_2-NH), 3.9(t, 4H, C-Cl), 6.78(t, 1H, Ar-H), 7.05(t, 2H, Ar-H), 7.19(d, 1H, Ar-H), 7.44(t, 2H, Ar-H),7.50(s, 1H, Ar-H), 7.68(s,1H, Ar-H), 8.55(t, NH), 8.76(s, NH-CO)

Evaluation of Synthesized Compounds:

Cup-Plate Agar Diffusion Method:

Materials:

Nutrient Broth medium, Nutrient Agar medium, Dimethyl Sulfoxide (DMSO), Ciprofloxacin, Distilled Water

Evaluation for Antibacterial Activity:

The antibacterial activity of the substituted synthesized 1-((bis(2-chloroethyl) amino) methyl)-2-oxoindolin-3-ylidene)-2(phenylamino)acetohydrazidederivatives (IXa – IXf) had been assayed against four different strains of bacteria by cup-plate agar diffusion method by measuring the zone of inhibition in mm.

Organisms selected: -	
Gram-positive bacteria,	**Gram-negative bacteria**
Staphylococcus aureus	*Escherichia coli*
Bacillus subtilis	*Pseudomonas aurgunousa*

Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar media. The bacterial growth inhibition can be measured by two methods.

a) Cup plate method

b) Serial dilution method

- **Bacterial Culture medium**:

Nutrient broth has been used for the preparation of inoculums of the bacteria and nutrient agar is used for the evaluation of antibacterial activity.

Composition of the nutrient agar medium:

Peptone	5gm
Sodium chloride	5gm
Beef extract	1.5gm
Yeast extract	1.5gm
Agar	1.5gm
Distilled water up to	1000mL
pH	7.4±0.2

Procedure:

The test organisms were sub cultured using nutrient broth medium. The tubes containing sterilized medium were inoculated with respective bacterial strains. After incubation at 37±1^{0}c for 24 hours, they were stored in refrigerator. The stock cultures were maintained. Bacterial inoculum was prepared by transferring a loopful of culture to nutrient broth in conical flasks. The flasks were incubated at 37±1^{0}c for 48 hours before the experimentation.

Solution of the test compound was prepared by dissolving the sample in DMSO.A reference standard for both Gram-positive and Gram-negative bacteria was made by dissolving accurately weighed quantity of Ciprofloxacin in sterile distilled water.

The nutrient agar medium was sterilized by autoclaving at 121^{0}c for 15 min. The petri plates, tubes and flasks plugged with cotton were sterilized in hot air oven at 160^{0}c for an hour. Into each sterilized petri plate, about 25 ml of molten nutrient agar medium inoculated with the respective strains of bacteria was transferred, aseptically. The plates were left at room temperature to allow solidification. In each plate, cups of 10mm diameter were made with sterile borer. Then 100µl of the test solution was added to the respective cups aseptically and labelled accordingly. The plates were kept undisturbed for at least 2 hours in refrigerator to allow diffusion of the solution properly into the nutrient agar medium. After the incubation of the plates at 37±1^{0}c for 24 hours, the diameter of the zone of inhibition surrounding each of the cups was measured with the help of the scale and tabulated. All the experiments were carried out in triplicate. Simultaneously controls were maintained employing 0.1ml of DMSO to observe the solvent effects. Results of anti-bacterial activity of compounds are presented in Table 3.03 (Gram-negative bacteria), Table No.3.04 (Gram-positive bacteria).

Concentration of test sample solutions: 50 µg/µL, 100 µg/µL, 1000µg/µL

Antibacterial activity:

Antibacterial activity of substituted' - (1-((bis(2-chloroethyl) amino) methyl)-2-oxoindolin-3-ylidene)-2-(phenylamino) acetohydrazide (1_a-1_f) against Gram-negative and Gram-positive bacteria at various concentrations on different strains of bacteria.

Compounds	R	E.Coli			Pseudomonas aeruginosa			Streptococcus aureus			Bacillus subtilis		
		50µg/ml	100µg/ml	1000µg/ml	50µg/ml	100µg/ml	1000µg/ml	50µg/ml	100µg/ml	1000µg/ml	50µg/ml	100µg/ml	1000µg/ml
1a	H	6mm	10mm	12mm	5 mm	9 mm	14 mm	6mm	10mm	14mm	8 mm	10 mm	15 mm
1b	NO_2	7mm	10mm	14mm	10 mm	12 mm	18 mm	**12mm**	**14mm**	**18mm**	12 mm	16 mm	20 mm
1c	Br	8mm	11mm	12mm	6 mm	10 mm	15 mm	6mm	8mm	15mm	6 mm	10 mm	15 mm
1d	Cl	10mm	12mm	16mm	**10 mm**	**12 mm**	**14 mm**	8mm	10mm	14mm	10 mm	12 mm	16 mm
1e	F	12mm	14mm	18mm	6 mm	10 mm	16 mm	**10mm**	**12mm**	**16mm**	12 mm	14 mm	16 mm
1f	CH_3	6mm	8mm	10mm	10 mm	12 mm	15 mm	8mm	10mm	15mm	10 mm	12 mm	16 mm
Ciprofloxacin		14mm	18mm	24mm	14 mm	18 mm	22 mm	**14mm**	**18mm**	**22mm**	14 mm	18 mm	20 mm

RESULTS AND DISCUSSION

All the synthesized compounds showed good activity at 100µg/ml concentration. The compound 1e, (R=F), showed maximum activity against *bacillus subtilis* at 100 µg/ml with zone of inhibition 14mm, when compare with standard Ciprofloxacin. The compound (IXe), (R=F), showed maximum activity against *Staphylococcus aureus* at 100µg/ml with zone of inhibition 14mm when compare with standard Ciprofloxacin. The compounds (IXe), (R=F), showed maximum activity against *E. coli*, *at* 100µg/ml with zone of inhibition 14mm, when compare with standard Ciprofloxacin. The compound (IXe), (R=F), showed maximum activity against *P.aurgunosa* at 100µg/ml with zone of inhibition 14mm, when compare with standard Ciprofloxacin. The compounds (IXb), (R=-NO_2) showed maximum activity against Gram positive bacteria *Staphylococcus aureus, Bacillus subtilis* at 100µg/ml with zone of inhibition 16mm, 14mm.but are least active against gram negative (*E. coli and pseudomonas aureus*) at at 100µg/ml with zone of inhibition 10mm. The compound (IXa), (R=H), showed moderate activity against *S.aureus* and *B.subtilis* at 100µg/ml with zone of inhibition 10mm. all those compounds, substituted with fluoro ((IXe),), chloro(IXd), and bromo (IXc), and –NO_2 (IXb), showed potent activity against Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria compared to standard drug ciprofloxacin.

CONCLUSION

The synthesized derivatives (IXe)-(IXe) has been evaluated for their antimicrobial activity against Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria by measuring the zone of inhibition. The results have been compared with a broad spectrum antibacterial agent ciprofloxacin as standard drug.

REFERENCES

1. Alagarsamy V, Meena S and Revathi I*ndian J.pharma.sci.,*2004,4,459-62
2. Verma R S and Nobles W.L *J. pharma.sci.,*1975, 69, 881-882
3. PandeyaSN, Sriram D, Nath G and DeClercq E.*Eur. J.pharma.sci.,*1999, 9, 25-31.
4. Alagarsamy V and Ramseshu KV. *Pharmazie,* 2003,58, 233-36
5. Sridhar SK and Sreenivasulu M. *Indian drugs*, 2001, 38, 531-34
6. Popp FD, Parson R and Donigan BE. *J.pharma.sci.1980, 69, 1235-1237.*
7. Yogeeswari P, Sriram D, Kavya R,Tiwari S. Synthesis and in vitro cytotoxicity evaluation of Gatifloxacin Mannich bases. Biomed and pharmacother. 2005; 59; 501-510
8. Tran VH, Nguyen QD and Le NV. *Tap. Chi. Dou Hoc.* 2002, 8, 15-17.
9. PandeyaSN, Sriram D, Nath G and De Clercq Synthesis Antibacterial, antifungal and anti-HIV activity of schiff's and Mannich bases of Isatin with N- [6-Chlorobenzen thiazole-2-yl] thiosemicarbazide Indian J.pharma.sci.,1999; 61: 358-361.

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