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Research Article

SYNTHESIS AND EVALUATION OF NOVEL ISATIN DERIVATIVES FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Final compound 3-({substituted phenyl [phenyldiazenyl] methylidene} hydrazinylidene)-1,3-dihydro-2*H*-indol-2-ones (VIa – VIe) which were prepaped by treatment of 3-(benzylidenehydrazinylidene)-1,3-dihydro-2*H*-indol-2-one(V) with hydrochloric acid, sodium nitrite and aryl amines (like anilines) in good yields. The structures of all compounds were confirmed by IR, ^{1}HNMR and mass spectral analysis. All the compounds were screened for their antibacterial and antifungal activities by using cup – plate method. Out of these compounds VIa and VId exhibited maximum activity. All the text compounds showed mild to moderate antibacterial and antifungal activities.

Keywords: Isatin Derivatives, ^{1}HNMR, IR, Antibacterial Activity and Antifungal Activity.

INTRODUCTION

Isatin was chemically 1H- indole – 2,3 – dione[1] and was a versatile lead molecule for potential bioactive agents and its derivatives were reported to possess wide variety of important biological activities like antibacterial[2,3], antifungal[2,3], anticonvulsant[4], anti HIV[2], antiturberculosis[5], antiviral[7], anti-inflammatory[6] and antidepressant[7] activity etc, keeping in view an array of applications. In view of their facts we hereby report the synthesis of some isatin derivatives using Schiff's and diazotization reaction (VI).

The present study deals with the synthesis of isatin schiff's bases (V) by reacting isatin hydrazide with substituted aromatic aldehydes and diazotization reaction of these Schiff's bases treated with aryl amine, hydrochloric acid and sodium nitrite. The chemical structures of the synthesized compounds were confirmed by means of their IR, ^{1}HNMR and mass spectral data. The synthesized compounds were tested for their antimicrobial activity by cup plate method.

MATERIALS AND METHODS

Melting points of all the synthesized compounds were determined in open capillary tubes (table1). The purity of the compounds was checked by TLC using silica gel G as stationary phase. The structure of the synthesized compounds was elucidated using FTIR spectrophotometer in KBR disc and ^{1}HNMR spectra was taken on bruker.

Synthesis of 1*H*-Indole-2,3-dione[3]

Synthesis of 2-(hydroxyimino)-*N*-phenylacetamide[2] – General Procedure

In a 5-litre R.B. flask were placed chloral hydrate (0.54 mole) and 1200ml of water. To this solution, were then added crystallized sodium sulphate (1300gm) followed by a solution of aniline (0.5mole) (I) in 300ml of water and concentrated hydrochloric acid (0.52mole). Finally a solution of hydroxylamine HCl (1.58mole) in 500ml of water was added. The contents of the flask were heated over a heating mantle so that vigorous boiling begins in about 45 minutes. After 1-2 minutes of vigorous boiling the reaction was complete. During the heating period itself the crystals of isonitrosoacetanilide started separating out. On cooling undercurrent of water the entire product was solidified. It was filtered under suction, air dried and purified by recrystallization from suitable solvent(s).

Synthesis of 1*H*-Indole-2,3-dione (III) – General Procedure:

Sulphuric acid (600gm, d.1.84, 326ml) was warmed to 50ºC in a one-litre R.B. flask fitted with an efficient mechanical stirrer and to this, finely powdered 2-(hydroxyimino)-*N*-phenylacetamide(II)(0.46mole) was added at such a rate so as to maintain the temperature between 60 and 70ºC, but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly. After the addition of isonitrosoacetanilide was completed, the temperature was raised to 80ºC and maintained at that temperature for 10 minutes, to complete the reaction. Then, the reaction mixture was cooled to room temperature and poured on crushed ice (2.5kg) after standing for about half-an-hour the product separated was filtered, washed several times with small portions of cold water and dried. Purification of the compound was effected by recrystallization from suitable solvent(s).

Synthesis of 3-hydrazinylidene-1,3-dihydro-2*H*-indol-2-one[4]

A mixture of 1*H*-indole-2,3-dione(III, 0.006mole) and excess amount of hydrazine hydrate in 15ml of methanol was refluxed for 30 minutes. The separated crystals were filtered, washed with a little amount of methanol, dried and recrystallized with chloroform solvent(s), m.p. 2040^{0}C, yield 82%.

Mobile phase –Chloroform:Ethylacetate - 1.5:0.5ml

Synthesis of 3-(benzylidenehydrazinylidene)-1,3-dihydro-2*H*-indol-2-one[5]

Equimolar quantity (0.05mole) of 3-hydrazinylidene-1,3-dihydro-2*H*-indol-2-one(IV), an appropriate aromatic aldehyde (0.05mole) and few drops of glacial acetic acid (0.05mole) were dissolved in 10ml of warm methanol and refluxed for four hours. After standing for approximately 24 hours at room temperature, the product was separated by filtration. The product obtained was vacuum dried and recrystallized with chloroform solvent(s). yield 75%

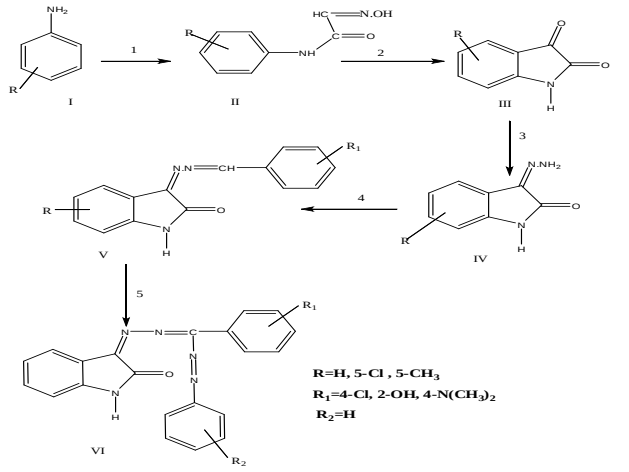
Mobile phase –Chloroform:Ethylacetate - 1.5:0.5ml

Synthesis of 3-({phenyl [phenyldiazenyl] methylidene} hydrazinylidene)-1,3- dihydro-2*H*-indol-2-ones [6]

1.5ml of arylamine was dissolved in 10ml of 2N hydrochloric acid in beaker (1) and cooled to 0ºC by keeping in an ice bath. 10ml of 10% sodium nitrite solution was cooled in beaker (2). 1gm of 3-

(benzylidenehydrazinylidene)-1,3-dihydro-2*H*-indol-2-one(V) was dissolved in 10ml methanol and 10% sodium hydroxide solution was added to this and cooled in beaker (3). After all the solutions were cooled to 0ºC, the contents of beaker (2) were added gradually to beaker (1) with vigorous shaking while maintaining extreme cold conditions and kept aside. After 10 minutes, the contents in beaker (1) were added to beaker (3) with continuous stirring while maintaining extreme cold conditions. During this addition, the compound was started separating out. After complete addition, the beaker was kept in ice bath for about one hour. The compound was filtered and washed with water and alcohol. The product was purified by recrystallization from suitable solvent(s). As many different compounds were prepared by above mentioned procedure and their physical data is presented in Table-1.

Scheme



1. Chloralhydrate,Hydroxylamine hydrochloride and Sodiumsulphate

2. Conc.H2SO43. Hydrazine hydrate (99%) and Methanol

4. Various aromatic aldehydes,Methanol, and few drops of glacial acetic acid.

5.Aniline,10% Sodium nitrite,2N HCl, and 10% Sodium hydroxide solution.

RESULTS AND DISCUSSION

Compound VI a

{(4-chlorophenyl)[(*E*)-phenyldiazenyl] methylidene} hydrazinylidene] -1,3-dihydro-2*H*-indol-2-one

IR (KBr, cm-1): 3060(C-H aromatic), 2932 (C-H str in CH_2), 1592 (C=N), 1059 (N-N);

1432 (N=N): MS (EI). m/z 388 (M+1)

^{1}H-NMR ($CDCl_3$) δ: 7.0 – 7.9 (triplet and doublet, 13H, aromatic-H); 8.1(s,1H,indole-H);

Compound VI b

{(2-hydroxyphenyl)[(*E*)- phenyldiazenyl] methylidene} hydrazinylidene]-1,3-dihydro-2*H*-indol-2-one

IR (KBr, cm-1): 3250 (OH), 3100(C-H aromatic), 2982 (C-H str in CH_2), 1582 (C=N), 1029 (N-N), 1452 (N=N).

^{1}H-NMR ($CDCl_3$) δ: 7.0 – 7.8 (triplet and doublet, 13H, aromatic-H); 8.0(s,1H,indole-H), 5.35 (s, H, OH).

MS (EI). m/z 369 (M+1)

Compound VI c

{(4- dimethyl aminophenyl) [(*E*)-phenyldiazenyl] methylidene} hydrazinylidene]-1,3-dihydro-2*H*-indol-2-one

IR (KBr, cm-1): 3080(C-H aromatic), 2952 (C-H str in CH_2), 1572 (C=N), 1079 (N-N), 1425 (N=N).

^{1}H-NMR ($CDCl_3$) δ: 7.0 – 7.8 (triplet and doublet, 13H, aromatic-H); 8.1(s,1H,indole-H); 2.3 (s, 6H, N $(CH_3)_2$)

MS (EI). m/z 396 (M+1)

Compound VI d

5-chloro-3-[2-{(4-chlorophenyl)[phenyldiazenyl] methylidene} hydrazinylidene] -1,3-dihydro-2*H*-indol-2-one

IR (KBr, cm-1): 3060(C-H aromatic), 2962 (C-H str in CH_2), 1592 (C=N), 1059 (N-N), 1500 (N=N)

MS (EI). m/z 421 (M+1)

Compound VI e

5-chloro-3-[(2-{(2-hydroxyphenyl)[phenyldiazenyl] methylidene} hydrazinylidene]-1,3-dihydro-2*H*-indol-2-one

IR (KBr, cm-1): 3300 (OH), 3100(C-H aromatic), 2992 (C-H str in CH_2), 1582 (C=N), 1029 (N-N), 1472 (N=N).

MS (EI). m/z 403 (M+1)

ANTIMICROBIAL ACTIVITY

All the newly synthesized compounds were evaluated for their antimicrobial activities against various microorganisms representing Gram – Positive bacteria (S. aureus), Gram – negative bacteria (E. coli) and fungus (C. arbicans) were carried out by cup – plate agar diffusion method using nutrient agar. The compounds were tested in – vitro for their antibacterial activity against two microorganisms viz. Escherichia coli and Staphylococcus aureus which are pathogenic to human beings. The anti fungal screening was carried out by cup – plate agar diffusion method using nutrient agar. Inoculation of the test organism Candida albicans fungal cultures were made in the Sabouraud – Dextrose agar and then incubated at 37 ^{o}C for 18 – 24 hrs standard drugs Norfloxacin and griseofalvin were used. The concentration was 200 µg/ml.

RESULTS AND DISCUSSION

Formation of 3-({substituted phenyl [phenyldiazenyl] methylidene} hydrazinylidene)-1,3-dihydro-2*H*-indol-2-ones (VI a-e) was confirmed by recording their IR, NMR and Mass spectral analysis. IR spectrum of compound VIa showed absorption bands at 3060, 2932, 1592, 1059 and 1432 cm-1 which is due to the C-H aromatic, C-H str in CH_2, C=N, N-N and N=N groups, respectively. The 1HNMR spectrum of VI a showed a triplet and doublelet at δ: 7.0 – 7.8 corresponds to aromatic CH(13H) and A singlet observed at δ 8.1 due to indole N-H. Similarly the mass spectrum was recorded and reported as (M + 1) values. For the compound IV a molecular weight 387 is consistent with the molecular formula $C_{22}H_{14}ClN_4O$. The values for the remaining compounds have been presented under the experimental part.

All the newly synthesized compounds were screened for their antibacterial and antifungal activities. For antibacterial studies, microorganisms employed were S. aureus and E. coli. For antifungal studies, microorganism employed was C.albicans. Both antimicrobial studies were assessed by cup – plate method.

From the antimicrobial activity study, it was found that compounds VIa and VId exhibited the promising activity as that of the standard drug norfloxacin against S.aureus and E.coli and VIb, VIc and VIe exhibited the moderate activity as that of standard drugs. The antifungal activity of compounds VIa and VId against C.albicans was found to be higher than that of the VIb, VIc and VIe. The observed activity in VIa and VId was may be due to the presence of chloro group.

Table 1: Physical data of 3-({phenyl[phenyldiazenyl]methylidene}hydrzinylidene)-1,3-dihydro-2H-indol-2-one

S.NO	COMPOUND	SUBSTITUENTS R	R_1	R_2	MOLECULAR FORMULA	MOL. WEIGHT (G/MOL)	MELTING POINT (0^0C)	PERCENTAGE YIELD (%)
1	VIa	H	4-Cl	H	$C_{22}H_{14}ClN_4O$	385.83	78-80	75
2	VIb	H	2-OH	H	$C_{22}H_{15}N_4O_2$	367.38	79-82	70
3	VIc	H	$4N(CH_3)_2$	H	$C_{24}H_{20}N_5O$	394.45	80-82	68
4	VId	5-Cl	4-Cl	H	$C_{22}H_{13}Cl_2N_4O$	420.27	79-83	70
5	VIe	5-Cl	2-OH	H	$C_{22}H_{14}ClN_4O_2$	401.83	79-81	72

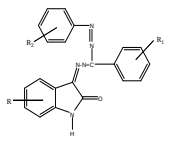


Table 2: Antibacterial activities of the synthesized compounds.

S.NO	COMPOUNDS	ZONE OF INHIBITION AT 200 Mg/ml		
		E.Coli	S. aureus	C.albicans
1	VIa	24	23	23
2	VIb	20	20	19
3	VIc	19	19	21
4	VId	23	22	23
5	VIe	18	20	20
6	Norflaxacin	26	25	----
7	griseofulvin	-----	-----	24

Compounds VIa and IVd have shown promising antibacterial and antifungal activity. Norfloxacin and griseofulvin were used as standard drugs.

CONCLUSION

A novel series of indole derivatives ring systems were synthesized. These were characterized by IR, NMR, TLC and Mass spectrometry. All the compounds were screened for their antibacterial and antifungal activity by cup – plate method. Compounds VIa and IVd exhibited promising activity as that of the standard drug norfloxacin and griseofulvin.

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