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Synthesis, Characterization and Anti-microbial Evaluation of Some 2-Iodo-*N*'-[(1*E*)-Substituted Phenylmethylidene] Benzohydrazide Analogues

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ABSTRACT
Reaction of 2-iodo benzohydrazide (1) with appropriately substituted aromatic aldehyde in glacial acetic acid yielded corresponding 2-Iodo-*N*'-[(1*E*)-Substituted Phenylmethylidene] Benzohydrazides (2). Structures of all the compounds (2) were established on the basis of elemental analysis and spectral data. These compounds were screened for anti-bacterial and anti-fungal activities at 200µg/0.1ml (T_1) and 400µg/0.1ml (T_2). Results obtained illustrates that the compounds F, C, D and G showed highly significant response while compound H showed less significant response for anti-microbial activity at both concentrations 200µg/0.1ml (T_1) and 400µg/0.1ml (T_2).

Keywords: Phenylmethylidene benzohydrazides, glacial acetic acid, anti-bacterial, anti-microbial.

INTRODUCTION

Resistance of pathogenic bacteria to available antibiotics is quickly becoming a major problem in the community and hospital based healthcare settings. The search for novel agents to combat resistant bacteria has become one of the most important areas of antibacterial research today. [1] So it is quite clear from the spectrum of use that these categories of drugs are very important from medical point of view. But microbial resistance towards the drug creates a very serious problem; because of development of resistance, many drugs are now useless which were very effective before. Moreover, the toxic effects produced by these antibiotics are also reducing their significance. [2] So there is need for new antimicrobial agents for resistant microbial infections. Molecules with Azomethine group (–CH=N–), typically known as Schiff bases, have been synthesized by the condensation of primary amines with active carbonyls. Compounds with the azomethine linkage were shown to possess biological effects such as anti-fungal, anti-bacterial and anti-inflammatory activities. [3-4]

In view of this all observations, here an attempt is made to synthesize a new series of hydrazide Schiff's base derivatives of *o*-iodo benzoic acid as potent anti-microbial agents by reaction of 2-iodo benzohydrazide (1) with appropriately substituted aromatic aldehyde in glacial acetic acid. Thus the present investigation describes the synthesis of 2-Iodo-*N*'-[(1*E*)-Substituted Phenylmethylidene] Benzohydrazides and their subsequent anti-microbial evaluation showed better results in comparison with standard drugs.

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MATERIALS AND METHODS

Unless otherwise noted, starting materials were procured from commercial suppliers and were used without further purification. All the melting points of newly synthesized compounds were determined on 'Veego' VMP-D apparatus and were found uncorrected. Silica gel G plates of 3x8 cm (Sigma-Aldrich) were used for TLC and spots were located by iodine chamber. The structures of the synthesized compounds were confirmed by spectral data. [5-6] The IR spectra were recorded on FTIR 8400 F- Shimadzu spectrometer using KBr disc pellet method. ^{1}H NMR spectra were recorded on Varian Mercury (300MHz) using DMSO as solvent and TMS as internal standard, values are expressed in δ ppm. GC-MS spectra were recorded on GCMS-QP-5050 Schimadzu.

Synthesis of o-iodo benzoic acid

Anthranillic acid was dissolved in water and concentrated sulphuric acid. Diazotization was carried by gradual addition of cold solution of sodium nitrite checked with starch iodide paper at end point. Into the clear solution added the solution of potassium iodide in sulphuric acid. Reaction mixture was heated, filtered and recrystallized from hot water. [7-8] Yield 85 % w/w, M. P 161°C.

Synthesis of 2-iodo benzohydrazide (1)

In methanol *o*-iodo benzoic acid was dissolved & added 2-3 drops of concentrated sulphuric acid, refluxed for 4-5 hours to form ester. Added hydrazine hydrate and refluxed for 4 hours. Removed methanol to obtain residue and recrystallized by mixture of cold water and alcohol. [7-9] Yield 75 % w/w, M. P. 260°C.

Formation of 2-iodo-N'-[(1E)-substituted phenylmethylidene] benzohydrazides (2)

General procedure

2-iodo benzohydrazide (1) (0.0025 mole) was dissolved in methanol, added (0.0025 mole) aromatic aldehyde and 3 drops of glacial acetic acid, mixture was refluxed for 5 h. Recrystallized by mixture of cold water and alcohol. [8-9]

Scheme 1

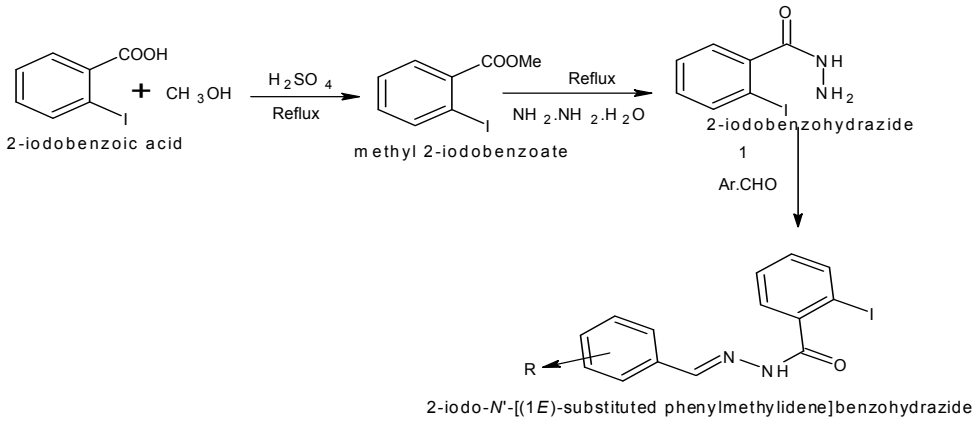

Fig. 1: Method of Synthesis for 2-iodo-*N*'-[(1*E*)-substituted phenylmethylidene] benzohydrazide analogues.

Table 1: Physical data for 2-iodo-*N*'-[(1*E*)-substituted phenylmethylidene] benzohydrazides

Compound	Ar	Mol. formula	Mol. weight	M.P	Yield (%)
A	*p*-methoxy phenyl	$C_{15}H_{13}N_2O_2I_1$	279.9	240°C	36 %
B	*m*-nitro phenyl	$C_{14}H_{10}N_2O_1I_1$	380.9	245°C	41 %
C	Furan	$C_{12}H_9N_2O_2I_1$	339.9	95°C	55 %
D	*o*-hydroxy phenyl	$C_{14}H_{11}N_2O_2I_1$	365.9	220°C	32 %
E	Phenyl	$C_{14}N_2H_{11}I_1$	249.9	290°C	94 %
F	Cinnamaldehyde	$C_{16}N_2H_{13}I_1O_1$	375.9	140°C	68 %
G	*p*-hydroxy phenyl	$C_{14}N_2H_{11}O_2I_1$	365.9	240°C	66 %
H	*p*-dimethyl amino phenyl	$C_{16}H_{16}N_3O_1I_1$	378.9	255°C	55 %

Table 2: Sophisticated analytical data for 2-iodo-*N*'-[(1*E*)-substituted Phenylmethylidene benzohydrazides

Compound	Ar	IR, ^{1}H NMR and GC-MS spectra
A	*p*-OCH_3 phenyl	**IR:** 2677(w, SH, Str),2358 (s,CH_2, Str), 1596(w, C=N, Str) **^{1}H NMR:** 7.6-7.9(s, 2H each for Ph) 8.3(s, 1H, Imino), 8.0(s, 1H hydrazide) 7.8(for Ph), 3.8(s, 3H methoxy gr.) **GC-MS:** M^{+1} 279.9, 261.96, 230.93,202.94,177.07,149.93,120.06
B	*m*-nitro phenyl	**IR:** 2677 (w, SH, Str), 2358 (s, CH_2, Str), 1596 (w, C=N, Str) **^{1}H NMR:** 7.6-7.9(s, 2H each for Ph) 8.3(s, 1H, Imino), 8.0(s, 1H hydrazide) 7.8(for Ph), 8.2(s, for nitro gr.)
C	Furan	**IR:** 2677(w, SH, Str),2358(s, CH_2,Str),1596 (w, C=N, Str) **^{1}H NMR:** 6.5-7.8 (s, Ph), 8.4(s,1H,imino), 7.0(s,Hydrazide),6.5-7.7(furan)
D	*o*-OH phenyl	**IR:** 2677 (w, SH, Str), 2358 (s, CH_2, Str), 1596 (w, C=N, Str) **^{1}H NMR:** 7.6-7.9(s, 2H each for Ph) 8.3(s,1H, Imino), 8.0(s,1H hydrazide) 6.8-7.7(for Ph) 5.3(s, 1H hydroxy gr.)
E	phenyl	**IR:** 2677 (w, SH, Str), 2358 (s, CH_2, Str), 1596 (w, C=N, Str) **^{1}H NMR:** 7.6-7.9(s, 2H each for ph) 8.3(s, 1H, Imino), 8.0(s, 1H hydrazide) 7.8(Ph).
F	Cinnama-ldehyde	**IR:** 2677(w, SH,Str), 2358(s, CH_2,Str),1596 (w, C=N, Str) **^{1}H NMR:** 6.5-7.8(s,Ph),7.0 (s, hydrazide) 7.5(s,1H imino gr.)7.2(s, 2H ethylene gr.), 6.8-7.7(for Ph)
G	*p*-OH phenyl	**IR:** 2677 (w, SH, Str), 2358 (s,CH_2, Str), 1596 (w, C=N, Str) **^{1}H NMR:** 7.6-7.9(s, 2H each for Ph) 8.3(s,1H, Imino),8.0(s,1H hydrazide), 6.8-7.7(for Ph), 5.3(s,1H,hydroxy)
H	*p*-dimethyl amino phenyl	**IR:** 2677 (w, SH, Str), 2358 (s, CH_2, Str), 1596 (w, C=N, Str) **^{1}H NMR:** 7.6-7.9(s, 2H each for Ph) 8.3(s,1H, Imino),8.0(s,1H hydrazide) 7.8(for Ph), 3.0(s, 6H dimethyl)

Anti-bacterial activity

Different strains of bacteria were used as *Bacillus subtilis* (NCIM2711), *Staphylococcus aureus* (NCIM2079), *Kleibsella pneumoniae* (ATCC 4352), and *Pseudomonas aeruginosa* (ATCC27853). Cup-plate Agar method was used for evaluation of anti-bacterial activity. The nutrient agar medium is used. The medium with bacteria was poured into sterilized Petri dishes under aseptic condition. Standard drug used was Norfloxacin (50µg/0.1ml) and test compounds at concentration of 200ug/0.1ml (T_1) & 400µg/0.1ml (T_2). Solvent used was Dimethyl formamide (DMF). Plates were incubated at 37°C for 24 hours. After incubation the average zone of inhibition was recorded in mm. [10-23]

Anti-fungal activity

The antifungal activity was carried out by using Cup-plate method using Sabraud's agar as medium. Fungal strains used were *Aspergillus niger* (NCIM515), *Candida albicans (*ATCC60193*)* with incubation period of 48 hours at temperature 28°C. The standard drug used was Griseofulvin (50µg/0.1ml) and the test compounds at concentration of

200µg/0.1ml (T_1) & 400µg/0.1ml (T_2) by using Dimethyl formamide (DMF) as solvent. [10-23]

RESULT AND DISCUSSION

We had planned for synthesis of 2-iodo-*N*'-[(1*E*)-substituted phenylmethylidene] benzohydrazides and were confirmed by spectral data. Compounds synthesized in this series were tested for anti-microbial activity using Norfloxacin and Griseofulvin as standards. Compound F was highly significant at both concentration T_1 (200µg/0.1ml) and T_2 (400µg/0.1ml). Compound C, D and G were highly significant at T_2 (400µg/0.1ml) and less significant at T_1 (200µg/0.1ml) against tested bacteria as well as fungi. Compound H was less significant at both concentration T_1 (200µg/0.1ml) and T_2 (400µg/0.1ml). Compounds A and B were found less significant at T_2 (400µg/0.1ml) and were poor active at T_1 (200µg/0.1ml) against tested pathogenic micro-organism.

Table 3: Anti-microbial activity of 2-iodo-*N*'-[(1*E*)-substituted Phenylmethylidene benzohydrazides

Compound	Zone of inhibition (mm)											
	B. S		S. A		P. A		K. P		C. A		A. N	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
A	04	13	07	13	08	16	09	15	05	10	08	12
	06	11	10	14	10	16	10	12	06	13	09	14
B	07	13	04	15	07	09	06	14	12	14	13	17
	10	14	05	14	09	15	11	15	08	14	13	16
C	09	18	15	18	12	18	15	18	11	17	15	18
	10	18	12	19	12	17	16	17	15	18	13	16
D	10	16	15	18	16	18	18	19	10	16	15	17
	11	18	14	18	16	19	15	17	10	14	12	16
E	12	14	16	19	13	18	15	10	12	18	13	14
	13	15	12	18	16	17	16	12	13	16	14	15
F	16	19	11	15	12	17	16	19	12	15	14	16
	13	18	11	16	11	16	16	18	14	16	13	17
G	09	14	09	15	11	13	14	17	15	18	16	16
	12	17	11	18	10	15	16	18	16	19	15	17
H	08	15	12	15	08	10	13	16	13	15	14	16
	11	16	16	19	12	13	12	14	09	16	11	16
Norfloxacin 50µg/0.1ml							20 mm					
Griseofulvin 50µg/0.1ml							19 mm					

B.S=*Bacillus subtilis* (NCIM 2711), S.A=*Staphylococcus aureus* (NCIM 2079), P.A= *Pseudomonas aeruginosa* (ATCC 27853), K.P=*Kleibsella pneumonia* (ATCC 4352), C. A=*Candida albicans* (ATCC 60193), A. N= *Aspergillus niger* (NCIM 515). T_1= 200µg/0.1ml, T_2=400µg/0.1ml. All Tests were performed in triplicate. Zone of Inhibition 15 to 20mm= highly significant, between 7 to 14mm=less significant, below 7mm=poor active.

All the 2-iodo-*N*'-[(1*E*)-substituted phenylmethylidene] benzohydrazides compounds tested showed good antimicrobial activity. So, we can conclude from observed result that compounds that we synthesized and evaluated are potent anti-microbial agents against pathogenic bacteria as well as fungi. So, we can use these agents as anti-biotic for resistant microbial infections.

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