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Synthesis, Characterization and Evaluation of Novel substituted benzo-indolylidene acetohydrazide Derivatives as Anti-Microbial Agents

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Abstract

A series of benzo-indolylidene acetohydrazides were synthesized by condensation of 2-chloro-N-[2-oxo-1,2-dihydro-3H-benzo[g]indol-3-ylidene]-acetohydrazide with various amines and tested for their antibacterial activities against various Gram-positive and Gram-negative bacteria and antifungal activity against the fungus Candida albicans and yeast. The synthesized compounds 6a-6f was characterized by spectral data (IR, NMR and mass) and elemental analysis. All compounds were screened for their antibacterial and antifungal activity by Cup-plate method (disc method). The Compounds 6e and 6f, showed considerable enhanced antibacterial activity against all microorganisms. Microbiological results indicated that the synthesized compounds possessed a broad spectrum of activity against the tested microorganisms.

Keywords: Isatin, benzo-indolylidene acetohydrazide, Antibacterial activity, Antifungal activity.

INTRODUCTION

Drug resistance is a steadily increasing process that is reaching alarming level in the treatment of infectious diseases caused by pathogenic bacteria, fungi, parasites and viruses. Over the past few decades, steadily increasing drug resistance in the treatment of infectious disease pose a serious problem in antimicrobial therapy and necessitates continuing research into novel classes of antimicrobials [1]. Isatin (1H-indole-2,3-dione) is an endogamous compound identified in humans and its effect has been studied in variety of systems and its analogues have proved to be versatile starting materials for the synthesis of heterocyclic, and non-cyclic, natural products, and

analogues, as well as for the synthesis of potentially important compounds with biological activities [2]. Due to the importance of indole back bone have shown a variety of biological activities such as antibacterial [3, 4], antifungal [4, 5], anti-HIV [6, 7] and anticonvulsant [8]. N-aryl and heteroary hydrazone derivatives play a vital role in biological fields. Hydrazide-hydrazones compounds are not only intermediate but they are also very effective organic compounds in their own right. Hydrazones have been demonstrated to possess, among other, antimicrobial, antimycobacterial, antidepressant, anticonvulsant, analgesic-anti-inflammatory, anticancer, antiviral , antimalarial, leishmanicidal and vasodilator activities[9-19]. In recent years, schiffs and Mannich bases of isatins are reported to exhibit broad spectrum chemotherapeutic properties such as antiviral [20-22], anti TB [23,24], antifungal and antibacterial [25,26]. Keeping the above facts in view, we considered it of interest to synthesize some novel benzo-indolylidene acetohydrazide derivatives for their antimicrobial properties.

MATERIALS AND METHODS

Melting points were determined using Thermonik Melting Point Apparatus (Campbell electronics, India) by open capillary method and are uncorrected. Infrared (IR) spectra were taken on a Fourier Transform Infrared Spectrophotometer IR-Prestige 21 (Shimatzu Corporation, Japan) from 4000-400 cm-1 using KBr discs. ^{1}H-NMR spectra were recorded at 400 MHz in DMSO-d_6 using a Bruker Avance 400 instrument (Bruker Instruments Inc., USA). Chemical shifts were measured at δ units (ppm) relative to tetramethylsilane (TMS). Fast-atom bombardment (FAB) mass spectra were recorded on a Jeol SX 102/DA-6000 mass spectrometer (Jeol Ltd. Akishima, Tokyo, Japan) using argon/xenon (6 kV, 10 mA) as FAB gas, m-nitrobenzyl alcohol as matrix, and 10 kV as accelerating voltage at room temperature. Elemental analysis was performed on a Vario EL III Elemental Analyser (Elementar, Germany). All chemicals were purchased from Merck, Spectrochem or CDH, India. Solvents were of reagent grade and were purified and dried by standard procedure. Reactions were monitored by thin-layer chromatography on silica gel plates in either iodine or UV chambers. Intermediates were characterized by IR spectroscopic analysis and Elemental Analysis for CHN. In the elemental analysis, the observed values were within ±0.3% of the calculated values. Final compounds were characterized by ^{1}H NMR and FAB mass spectrometry (MS). The final yields and the physicochemical data of the compounds 6a to 6h are presented in Table 1.

Experimental Part*:*

Synthesis of 2-(hydroxyimino)-N-(naphthalene-1- yl) ethanamide (2):

In a 5 L round-bottomed flask are placed chloral hydrate (0.54 mole) and 1200 mL of water. To this solution are then added, in order: 1300 g crystallized sodium sulphate, a solution of a-naphtyl amine (0.5 mole) in 300 mL of water to which concentrated hydrochloric acid (0.52 mole, sp.gr.1.19) is added to dissolve the amine and finally, a solution of hydroxylamine hydrochloride (1.58 moles) in 500 mL water. The contents of the flask are heated over wire gauze so that vigorous boiling begins and the heating is continued till the reaction is completed. The product solidifies, filtered under suction and air dried and this intermediate was used in step-2.

Synthesis of 1H-benzo[g]indole-2, 3-dione (3):
Concentrated Sulfuric acid (600 g, 326mL sp.gr.1.84) is warmed to 50°C in a 1 L, round-bottomed flask fitted with a mechanical stirrer and to this, finely powdered **2** (0.46 mol) was added at such a rate so as to maintain the temperature between 60 and 70 ^{0}C, but not higher to complete the reaction. External cooling was applied at this stage so that the reaction could be carried but more rapidly. After the addition of isonitroso compound was completed the temperature of the solution was raised to 80 ^{0}C 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 (2.5 kg). 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 recrystallisation from methanol and yield was 68%.

Synthesis of 3-hydrazinylidene-1, 3-dihydro-2H-benzo[g]indol-2-one (4) :
To a mixture of 1H-benzo[g]indole-2,3-dione (3, 0.01 mole) and hydrazine hydrate (0.02 mole), methanol (30 ml) was added and refluxed for 30 minutes. The excess of solvent was removed by evaporation under vacuum and the obtained solid was filtered then recrystallised from methanol and the yield of the product was 70%.

Synthesis of 2-chloro-N-[2-oxo-1, 2-dihydro-3H-benzo[g]indol-3-ylidene]aceto-hydrazide :(5)
To a mixture of (3)-3-hydrazinylidene-1, 3-dihydro-2H-benzo[g]indol-2-one ((**4,** 0.01 mol) and chloro acetyl chloride (0.01 mol) were dissolved in 10ml of acetone and refluxed for three hours. After standing for approximately 24 hr at room temperature the product was separated by filtration. The excess of solvent was removed by evaporation under vacuum and recrystallized from alcohol and the yield 67%, M.P 260 – 262 ^{0}C. IR (KBr, cm^{-1}): 3182 (N-H), 1692 (C=O, lactam), 1518 (C=N); MS: m/z [M$^+$]$^{+1}$ 288.

General method for the synthesis of Synthesis of 2-substituted 4-yl-N-[2-oxo-1, 2-dihydro-3H-benzo[g]indol-3-ylidene] acetohydrazides (6a – 6f):
A mixture of 2-chloro-N-[2-oxo-1, 2-dihydro-3H-benzo[g]indol-3-ylidene] aceto-hydrazide (5, 0.01mol), the appropriate secondary amine (0.01mol) in dry benzene (10ml) was heated and refluxed for 3-4 hrs. Progress of reaction was monitored by TLC. At the end of reaction, the solvent was evaporated to one quarter of its volume under vacuum; then the obtained solid products were filtered, dried, purified via column chromatography (hexane:ethyl acetate; 1:0.5) to give compounds 6a–6f.

2-(morpholin-4-yl)-*N*'-[2-oxo-1,2-dihydro-3*H*-benzo[*g*]indol-3-ylidene]aceto-hydrazide (6a):
Elemental analysis (Found): C, 63.09; H, 5.42; N, 16.42; (Calculated): C, 63.89; H, 5.36; N, 16.56; Mol. Formula: $C_{18}H_{18}N_4O_3$; IR (KBr, cm^{-1}): 3364(N-H, lactam), 2974(NH-CO-), 1629(C=O, lactam), 1652 (C=O), 1514 (C=N), 1368(C-N), 1103(C-O-C); ^{1}H NMR (δ ppm): 12.15(s, 1H, CONH lactam), 10.02(s, 1H, NH-N=), 6.82-7.38 (m, 6H, Ar-H), 3.24(t,4H, CH_2-O-CH_2), 2.93 (t,4H, CH_2-N-CH_2), 2.78 (S, 2H, CH_2CO); MS: m/z [M$^+$]$^{+1}$ 339.

2-[diphenylamino]-*N*'-[2-oxo-1,2-dihydro-3*H*-benzo[*g*]indol-3-ylidene]aceto-hydrazide (6b):
Elemental analysis (Found): C, 74.36; H, 4.56; N, 13.12; (Calculated): C, 74.27; H, 4.79; N, 13.33; Mol. Formula: $C_{26}H_{20}N_4O_2$; IR (KBr, cm^{-1}): 3282(N-H, lactam), 3018(NH-CO-),

1692(C=O, lactam), 1642 (C=O), 1518 (C=N), 1398(C-N); ^{1}H NMR (δ ppm): 12.26(s, 1H, CONH lactam), 10.16(s, 1H, NH-N=), 6.62-8.28 (m, 16H, Ar-H), 2.74 (S, 2H, CH_2CO); MS: m/z $[M^+]^{+1}$ 421.

2-(4-methylpiperazin-1-yl)-*N*'-[2-oxo-1,2-dihydro-3*H*-benzo[*g*]indol-3-ylidene]aceto-hydrazide(6c): Elemental analysis (Found): C, 65.2; H, 6.12; N, 19.82; (Calculated): C, 64.94; H, 6.02; N, 19.93; Mol. Formula: $C_{19}H_{21}N_5O_2$; IR (KBr, cm^{-1}): 3374(N-H, lactam), 2967(NH-CO-), 1652(C=O, lactam), 1682 (C=O), 1524 (C=N), 1386 (C-N); ^{1}H NMR (δ ppm): 12.10(s, 1H, CONH lactam), 10.08(s, 1H, NH-N=), 6.88-7.48 (m, 6H, Ar-H), 2.94(t,4H, CH_2-N-CH_2), 2.88 (t,4H, CH_2-N-CH_2), 2.78 (S, 2H, CH_2CO), 1.23 (s, 3H, N-CH_3); MS: m/z $[M^+]^{+1}$ 352.

***N*'-[2-oxo-1,2-dihydro-3*H*-benzo[*g*]indol-3-ylidene]-2-(piperazin-1-yl)aceto-hydrazide (6d):** Elemental analysis (Found): C, 64.18; H, 5.42; N, 20.54; (Calculated): C, 64.08; H, 5.68; N, 20.76; Mol. Formula: $C_{18}H_{19}N_5O_2$; IR (KBr, cm^{-1}): 3367(N-H, lactam), 2972(NH-CO-), 1660(C=O, lactam), 1688 (C=O), 1534 (C=N), 1381 (C-N), 1295 (C-N); ^{1}H NMR (δ ppm): 12.17(s, 1H, CONH lactam), 10.12(s, 1H, NH-N=), 6.84-7.38 (m, 6H, Ar-H), 2.96(t,4H, CH_2-N-CH_2), 2.85 (t,4H, CH_2-N-CH_2), 2.78 (S, 2H, CH_2CO), 1.86(t, 1H, NH, piperazine); MS: m/z $[M^+]^{+1}$ 338.

2-(dicycohexylamino)-*N*'-[-2-oxo-1,2-dihydro-3*H*-benzo[*g*]indol-3-ylidene]aceto-hydrazide (6e): Elemental analysis (Found): C, 72.44; H, 7.36; N, 12.78; (Calculated): C, 72.19; H, 7.46; N, 12.95; Mol. Formula: $C_{26}H_{32}N_4O_2$; IR (KBr, cm^{-1}): 3288(N-H, lactam), 2998(NH-CO-), 1622(C=O, lactam), 1656 (C=O), 1528 (C=N), 1362(C-N); ^{1}H NMR (δ ppm): 12.14(s, 1H, CONH lactam), 10.12(s, 1H, NH-N=), 6.78-7.58 (m, 6H, Ar-H), 2.69 (S, 2H, CH_2CO), 1.54-2.59 (m, 22H, N-cyclohexyl); MS: m/z $[M^+]^{+1}$ 433.

***N*'-[2-oxo-1,2-dihydro-3*H*-benzo[*g*]indol-3-ylidene]-2-(piperidin-1-yl)acetohydrazide (6f):** Elemental analysis (Found): C, 67.14; H, 5.80; N, 16.22; (Calculated): C, 67.84; H, 5.99; N, 16.66; Mol. Formula: $C_{19}H_{20}N_4O_2$; IR (KBr, cm^{-1}): 3364(N-H, lactam), 2964(NH-CO-), 1634(C=O, lactam), 1662 (C=O), 1514 (C=N), 1368(C-N); ^{1}H NMR (δ ppm): 12.20(s, 1H, CONH lactam), 10.22(s, 1H, NH-N=), 6.56-7.40 (m, 6H, Ar-H), 2.84 (t,4H, CH_2-N-CH_2), 2.78 (S, 2H, CH_2CO), 1.76 (q, 4H, CH_2-C-CH_2), 1.54 (t, 2H, C-CH_2-C); MS: m/z $[M^+]^{+1}$ 337.

Anti-microbial activity[27-29]

The antimicrobial properties of the compounds were investigated against bacterial strains i.e., *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 722), *and Proteus vulgaris* (MTCC 109) were selected and obtained from the Institute of Microbial Technology, Chandigarh, India and fungal strains i.e., *yeast and Candida albicans* using disk diffusion method. (Table II). Amipicillin and flucanzole were used as standard drug for antibacterial and antifungal studies respectively. Nutrient Agar [9] (beef extract 1g, Yeast extract 2g, peptone 5g, sodium chloride 5g, Agar 15g and distilled water q. s. to 1,000 ml) was employed as culture media for antibacterial studies. For antifungal evaluation against yeast and Candida albicans, Sabourad dextrose agar medium (SDA) (Mycological peptone 10 g, Dextrose 14 g, Agar 17 g and distilled water q. s. to 1,000 ml) was employed. The sterilization of the culture medias, petridishes and other glasswares was done by autoclaving at 15 1b/sq inch pressure for 30 min. For antibacterial studies, incubation was carried out at 37 ± 1ºC for 24 h and

antifungal at 30 ± 1ºC for 72hr. During antimicrobial evaluation the medium after sterilization was poured into sterile petridishes under aseptic conditions in a laminar flow chamber. When the medium in the plate solidified, 0.5 ml of culture of test organism was inoculated and uniformly spread over the agar surface using a sterile L-shaped glass rod. Solutions of the test compound were prepared by dissolving the test compound in DMSO. In each plate, three discs of 6 mm diameter were made with a sterile borer and test solutions at concentrations (300, 150 and 100 µg/ml) was added to respective disc aseptically and labeled accordingly. The plates were kept undisturbed for 1 hour at room temperature to allow the diffusion of the solution properly in the nutrient agar medium. After incubation of the plates, the diameter of zone of inhibition was measured with the help of an antibiotic zone reader and the diameter was calculated in millimeters. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1 ml of DMSO to observe the solvent effects and the results were represented in Table-II

Table I. Physico-chemical data of compounds 6a-6f

Compd	R	MF	MW	MP (°C)	%Yield	R*$_f$
6a	morpholin-4-yl	$C_{18}H_{18}N_4O_3$	338	184-186	70	0.47
6b	Diphenyl amino	$C_{26}H_{20}N_4O_2$	420	188-190	80	0.41
6c	4-methylpiperazin-1-yl	$C_{19}H_{21}N_5O_2$	351	218-220	68	0.47
6d	piperazin-1-yl	$C_{18}H_{19}N_5O_2$	337	214-216	67	0.42
6e	Dicyclohexyl amino	$C_{26}H_{32}N_4O_2$	432	202-204	92	0.46
6f	piperidin-1-yl	$C_{19}H_{20}N_4O_2$	336	170-172	84	0.51

* Hexane:Ethyl acetate (2:1)

Scheme-I: Synthetic scheme for the title compounds

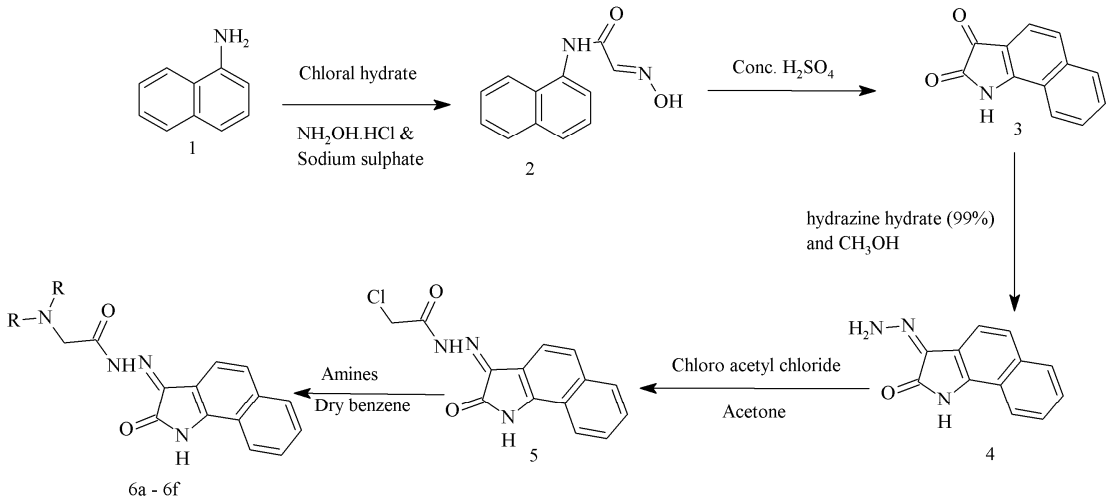
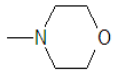
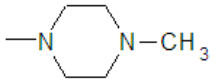
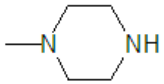
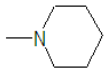


Table II. Diameter of zone of inhibition by individual compounds against gram +ve, gram -ve bacteria and fungus

Compd	R	Conc in µg/ml	Antibacterial Activity				Antifungal Activity	
			B. subtilis	***S. arurus***	***E.coli***	***P. vulgaris***	**C. albicans**	**Yeast**
Contol	--	--	--	--	--	--	--	--
STD*	--	10	22	20	18	20	22	22
6a		100	8	7	8	9	9	9
		150	10	9	10	11	12	10
		300	12	11	11	13	14	12
6b	$-N(Ph)_2$	100	13	10	9	12	10	11
		150	15	12	12	14	12	13
		300	17	14	15	16	15	16
6c		100	11	15	13	9	12	13
		150	12	16	15	11	13	15
		300	13	18	17	13	16	17
6d		100	12	11	14	16	14	13
		150	14	13	16	18	16	15
		300	16	15	18	20	18	17
6e	$-N(C_6H_{11})_2$	100	14	15	16	12	15	14
		150	16	17	18	15	17	16
		300	18	19	20	13	19	18
6f		100	16	17	18	15	18	20
		150	18	19	20	17	20	22
		300	22	23	22	19	22	24

*

Antibacterial – Amipicillin, Antifungal – Flucanzole

RESULTS AND DISCUSSION

The target compounds were synthesized according to the representative scheme 1. The required starting material, 1H-benzo[g]indole-2, 3-dione 3 was prepared in good yield from α-naphthyl amine chloral hydrate and hydroxyl amine, which on hydrazinolysis afforded the corresponding of 3-hydrazinylidene-1, 3-dihydro-2H-benzo[g]indol-2-one (4). The subsequent N-carbamate formation by the reaction of 4 with chloro acetyl chloride in acetone gave the 2-chloro-N-[2-oxo-1, 2-dihydro-3H-benzo[g]indol-3-ylidene]aceto-hydrazide(5) The title compounds 6a-6f was finally synthesized by compound 5 treated with appropriate amine, resulted in the formation of title compounds 6a-6f. The yields of all the synthesized compounds were found to be in the range of 67-92%.

Assignments of selected characteristic IR band positions provide significant indication for the formation of 2-substituted-4-yl-N-[2-oxo-1, 2-dihydro-3H-benzo[g]-indol-3-ylidene] acetohydrazide derivatives. All products were confirmed by IR (KBr, cm^{-1}) spectral data showing characteristic peak in the range 3282-3374 indicated the N-H, lactam, the peak appeared at 3018-2964 shows the presence of NH-CO-, the two peaks appeared in the range 1629-1692 and 1642 1688 indicated that the presence of C=O, lactam and C=O group respectively, peaks in the range 1362-13983202-3393 cm^{-1} indicating the presence of two –NH groups and sharp peak at 1514-1534 indicated the presence of C=N group in the products. The ^{1}HNMR spectra showed singlet in the range δ 12.10-12.20 correspond to one proton of CONH lactam of isatin and at δ 10.02-10.22 assigned to one proton of NH-N= appeared as singlet. The two protons of CH_2CO appeared in the range of δ 2.69- 2.78 as singlet. The protons of CH_2-O-CH_2 and CH_2-N-CH_2 of morpholin ring appeared as triplets at the region of δ 3.24 and 2.93 respectively and the piperazine ring protons of CH_2-N-CH_2 proton appeared in the range of δ 2.94-2.96 as triplets, CH_2-N-CH_2 proton appeared as triplets at δ 2.84-2.88, the methyl proton of N-CH_3 in piperazine appeared at δ 1.23 as singlet and NH proton of piperazine appeared at δ 1.86 as triplets. The aryl protons are appeared as multiplate in the range of δ 6.56-7.58, cyclohexnyl proton are appeared at δ 1.54-2.59 as multiplets and the piperidinyl proton of CH_2-N-CH_2, CH_2-C-CH_2 and C-CH_2-C appeared at δ 2.84 as triplet, 1.76 as quarterate and 1.54 as tripet respectively. Mass spectrum exhibited a molecular ion $[M^+]^{+1}$ peak corresponding to its molecular weight. The mass spectral data of all the titled compounds were found to be in correlation with the expected structure.

All the title compounds were screened for their antibacterial and antifungal activity by Cup-plate method (disc method). The Compounds 6e and 6f, showed considerable enhanced antibacterial activity against all microorganisms. Microbiological results indicated that the synthesized compounds possessed a broad spectrum of activity against the tested microorganisms (Table II).

CONCLUSION

In conclusion, a series of novel benzo-indolylidene acetohydrazides derivatives were synthesized, characterized and evaluated for their antibacterial activities against various Gram-positive and Gram-negative bacteria and antifungal activity against the fungus *Candida albicans* and yeast. The results of antimicrobial data revealed that the compounds possess significant activity which is on a par with the standard. It is convincing that this class of compounds certainly holds great promise towards the pursuit to discover novel classes of antimicrobial agents. Further studies to acquire more information concerning structural activity relationships are in progress.

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