WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 5.210
Volume 4, Issue 09, 905-915 Research Article ISSN 2278 – 4357

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL ISATIN DERIVATIVES

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Article Received on
28 June 2015,

Revised on 19 July 2015,
Accepted on 10 Aug 2015

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ABSTRACT

Isatins and its derivatives are useful for the synthesis of several chemical and pharmacological classes of therapeutic agents. Therefore, in the present investigation it has been considered worthwhile to synthesize some new Isatins derivatives by conventional methods. The chemical structures of the synthesized compounds were confirmed by using spectral and elemental analysis methods. Screening for their Antimicrobial, *In-vitro* anti-helmenthic and *In-vitro* anti-inflammatory activities. All the novel Isatin derivatives exhibited mild to moderate activity. Compounds 3, 4, 9 & 12 exhibited better activity than the other test compounds.

KEYWORDS: Isatin anti-helmenthic, anti-inflammatory, *In-vitro*.

INTRODUCTION

Isatin and several of their derivatives have been generally associated with various biological and pharmacological properties such as antibacterial[1-3], antifungal[4-6], antiprotozoal[7-8], antiviral[9-11], anthelmintic[12-13] and CNS activities.[14-15] The synthesis of a large number of Isatin derivatives have been described to obtain biologically potent compounds. In the present investigation, involving reactions of Isatin with a view to synthesize some biologically active compounds, it has been felt worthwhile to study the condensation reaction of 3-hydrazone-1H-benzo[g]indol-2(3H)-one with different carbonyl compounds and also to evaluate the products, biologically. The chemical structures of synthesized compounds were confirmed by means of ^{1}HNMR, IR, mass spectral data and elemental analysis. All the synthesized

compounds were screened for their Antimicrobial, *In-vitro* anti-helmenthic and *In-vitro* anti-inflammatory activity.

EXPERIMENTAL METHODS

All the chemicals used in the synthesis were obtained from standard commercial sources. Reactions were monitored by TLC using silica gel-G (Merck grade) as the adsorbent and the solvent systems are indicated at appropriate places. Silica gel (100-200 mesh, Merck grade) has been used for column chromatography. All the melting points were determined in open capillaries using Boitus melting point apparatus, expressed in ^{o}C and are uncorrected. The ^{1}H NMR spectra of the compounds were recorded on Bruker spect, 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm.

1. Synthesis of 3-(substuited hydrazono)-1H-benzo indol-2(3H)-one derivatives

The synthesis of title compounds could be achieved by the condensation of 3-hydrazone-1H-benzo[g]indol-2(3H)-one with different carbonyl compounds (Scheme).

1.1 Synthesis of 2-(hydroxyimino)-N-(naphthalen-4-yl) acetamide (II) – General Procedure

In a 5 lit. R.B. flask were placed chloralhydrate (0.54 mol) and 1200 ml of water. To this solution, were then added crystallized sodium sulphate (1300 g) followed by a solution of an alpha-Naphthylamine (I) (0.5 mol) in 300 ml of water and concentrated hydrochloric acid (0.52 mol). Finally a solution of hydroxylamine HCl (1.58 mol) in 500 ml of water was added. The contents of flask were heated over a wire-guage by a mecker burner. So that vigorous boiling begins in about 45 minutes. After 1-2 minutes of vigorous boiling the reaction was completed. On cooling under the current of water the entire product was solidified. It was filtered under suction, air dried and purified by recrystallization from suitable solvent.

1.2 Synthesis of 1H-benzo[g]indole-2,3-dione (III) – General Procedure

Sulphuric acid (600 g, d. 1.84, 326 ml) was warmed to 50^{0} C in a one-litre R.B. flask fitted with an efficient mechanical stirrer and to this, finely powdered and (2)-2-(hydroxyimino)-N-(naphthalen-1-yl) ethanamide (0.46 mole). After the addition of isonitroso compound was completed the temperature maintained for 10 mins at 80^{0}c 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 recrystallization from methanol.

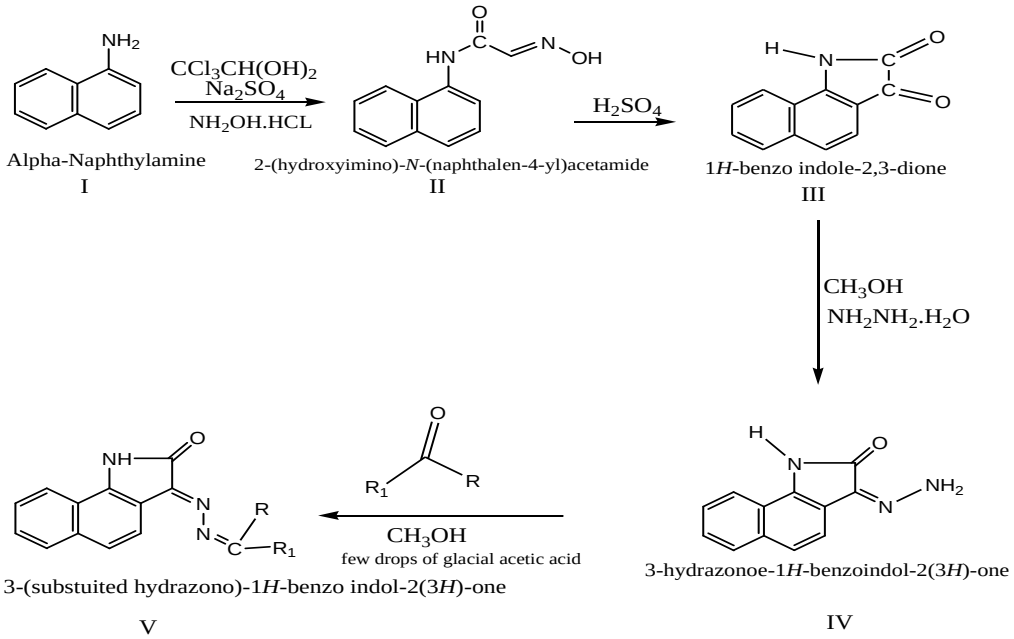
1.3 Synthesis of 3-hydrazonoe-1H-benzoindol-2(3H)-one[16]

Equimolar quantity (0.004mol) of 1H-benzo[g]indole-2,3-dione and hydrazine were dissolved in 10ml of warm methanol and refluxed for 30mins, after standing for approximately 24hours at room temperature the products were separated by filtration, vacuum dried and recrystallized from warm ethanol.

1.4 Synthesis of 3-(substuited hydrazono)-1H-benzo indol-2(3H)-one derivatives

Equimolar quantity of 3-hydrazonoe-1H-benzo indol-2(3H)-one, an appropriate aromatic carbonyl compounds in 10 ml of warm methanol and refluxed for 4hrs. After standing for approximately 24hrs at room temperature. The products were separated by filtration vacuum dried and recrystallized from warm methanol. The synthesized compounds have been characterized by the physical and spectral data.

Scheme



Alpha-Naphthylamine
I

2-(hydroxyimino)-*N*-(naphthalen-4-yl)acetamide
II

1*H*-benzo indole-2,3-dione
III

3-hydrazonoe-1*H*-benzoindol-2(3*H*)-one
IV

3-(substuited hydrazono)-1*H*-benzo indol-2(3*H*)-one
V

Table 1: Physical characterization data of the compounds (PI1-PI13)

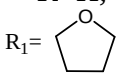
S.no	compound	Substuient	Molecular Formula	Molecular Weight	Melting Point (^{0} C)	Percentage Yield
1	PI-1	R=H, 	$C_{17}H_{11}N_3O_2$	289.29	128-131	61
2	PI-2	R=H, $R_1=C_6H_5$	$C_{19}H_{13}N_3O$	299.33	140-144	67
3	PI-3	R=H, $R_1=C_6H_4$-CH_3	$C_{20}H_{15}N_3O$	313.35	148-152	63
4	PI-4	R=H, $R_1= C_6H_4$-Br	$C_{19}H_{12}BrN_3O$	378.22	122-125	55
5	PI-5	R=H,$R_1=C_6H_3N(CH_3)_2$	$C_{21}H_{18}N_4O$	342.39	158-160	84
6	PI-6	R=H,$R_1=C_6H_3$-$(OCH)_3$	$C_{21}H_{17}N_3O_3$	359.38	124-126	46
7	PI-7	R=H, $R_1=C_6H_3(OH)_3$	$C_{19}H_{13}N_3O_4$	347.32	149-153	53
8	PI-8	R=H, $R_1=C_6H_3(OH)_3$	$C_{19}H_{13}N_3O_4$	347.32	152-156	61
9	PI-9	R=H, $R_1=C_6H_4$-OH	$C_{19}H_{13}N_3O_2$	315.33	142-146	72
10	PI-10	R=CH_3, $R_1=CH_3$	$C_{15}H_{13}N_3O$	251.28	122-125	46
11	PI-11	R=CH_3, $R_1=C_6H_5$	$C_{20}H_{15}N_3O$	313.35	148-151	65
12	PI-12	R=C_6H_5, $R_1=C_6H_5$	$C_{25}H_{17}N_3O$	375.42	159-161	57
13	PI-13		$C_{18}H_{17}N_3O$	291.35	147-150	65

Table II: IR Spectral data of the compounds (PI1 – PI13)

Compound	IR (KBr, cm-1)
PI-1	3398.26(N-H),158.30(C=0),,1630.37(C=N),1018.28(C-O), 1463.30(C=C)
PI-2	3441.59(N-H),1707.57(C=O),1623.75(C=N), 1558.56(C=C)
PI-3	3396.13(N-H), 1710.08(C=O), 1641.43(C=N), 1574.14(C=C),2971.55(C-H)
PI-4	3394.22(NH),1677.54(C=O),1573.36(C=N),1550.20(C=C), 560.22(C-Br)
PI-5	3366.01(N-H), 1678.74(C=O), 1626.17(C=N), 1573.10(C=C), 1348.41(C-N), 2919.46(N-H)
PI-6	3398.60(N-H), 1711.17(C=O), 1649.64(C=N), 1598.61(C=C), 3186.12, 3003.64, 2930.32(C-H)
PI-7	3418.81(NH),1711.47(C=O),1629.54(C=N),3843.69,3829.54, 3768.96(O-H),2921.06(C-H)
PI-8	3420.09(N-H),1732.13(C=O),1630.93(C=N),3743.35,3732.13,3645.95(O-H),2921.02(C-H)
PI-9	3442.03(N-H),1710.21(C=O),1622.92(C=N), 2922.55(C-H),3626.51(O-H)
PI-10	3398.43(N-H),1708.81(C=O),1649.91(C=N), 1580.40(C=C),3177.87, 3069.9,2921.09(C-H)
PI-11	3398.11(N-H),1678.15(C=O),1649.96(C=N),1572.71(C=C),1090.26(C-C),3064.08,3167.14,2921.54(C-H)
PI-12	3149.57(N-H),1682.18(C=O),1652(C=N),1556.32(C=C), 3067.35,3585.73(C-H)
PI-13	3449.56(N-H),1657.29(C=O),1630.76(C=N), 1528.38(C=C),1000.88(C-C)

BIOLOGICAL ACTIVITIES

In view of varied biological and pharmacological importance of different Isatins, it has been prompted us to evaluate for their antimicrobial, antifungal activity, antihelmintic activity and *In-vitro* anti-inflammatory activity for the compounds and shown in Table-1.

Antibacterial Activity

The 3-(substuited hydrazeno)-1H-benzo indol-2(3H)-one derivatives were studied for antibacterial activity[17,18] on microorganisms by using cup plate method.

The test organisms were subculture using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37 °C± 1°C for 18 hrs, they were stored in the refrigerator. Into each sterilized petriplate (20 cm diameter), 125 ml of molten nutrient agar medium was poured which was already inoculated with the respective strain bacteria (5ml of inoculum to 250 ml of nutrient agar medium) aseptically. The plates were left at room temperature aseptically to allow the solidification.
Each test compound (100 & 150 mg) was dissolved in dimethyl sulfoxide (5 ml, AnalR grade) at a concentration of 1000 µg/ml. Ciprofloxacin solution was also prepared at a concentration of 1000 µg/ml in dimethyl sulfoxide. The solutions of each test compound, control and reference standard (0.1ml and 0.15 ml) was added separately in the cups and the plates were kept undisturbed for at least 2 hours in the refrigerator to allow the diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37 ± 1°C for 24 hrs. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicate. The results are presented in Table-III.

Table III: Antibacterial activity of 3-(substuited hydrazono)-1H-benzo indol-2(3H)-one derivatives

S. No	Compound No	Concentration (µg/ml)	*Zone of inhibition (mm)*					
			B. subtilis	***K. pneumonia***	***P. vulgaris***	***S. aureus***	***S. lutues***	***E. coli***
1	PI-1	100	12	16	15	12	13	13
		150	18	19	16	14	14	14
2	PI-2	100	11	10	10	10	13	13
		150	10	10	11	10	14	14

3	PI-3	100	14	11	13	10	11	14
		150	18	12	15	10	12	15
4	PI-4	100	10	10	11	16	13	10
		150	11	10	11	18	13	10
5	PI-5	100	12	13	12	12	15	10
		150	13	15	12	13	16	10
6	PI-6	100	10	10	10	13	12	12
		150	10	10	10	14	12	12
7	PI-7	100	13	18	16	10	14	13
		150	22	20	21	10	16	15
8	PI-8	100	10	10	10	10	10	10
		150	10	10	10	11	10	10
9	PI-9	100	13	12	10	11	11	11
		150	14	14	14	10	10	11
10	PI-10	100	14	12	11	13	12	13
		150	13	12	11	11	11	12
11	PI-11	100	11	14	13	11	11	12
		150	12	14	13	12	10	11
12	PI-12	100	13	11	12	12	13	14
		150	15	11	12	10	13	13
13	PI-13	100	12	13	12	14	12	11
		150	11	13	11	13	12	14
14	STD	10	25	24	23	26	21	22

NOTE: - Average zone diameter of triplicates in mm.

Antifungal Activity

The antifungal activity was tested by cup-plate method and compared with the standard Flucanazole (10 µg/ml). Dimethylsulfoxide (DMSO) was used as a solvent and control.

The test organisms were subcultured using Sabourad dextrose agar medium (SDA) medium. The tubes containing sterilized medium were inoculated with test fungi and kept at 24^0c temperature for obtaining growth. After that they were stored at 4 °C in a refrigerator. The inoculum was prepared by taking a loopful of stock culture to about 5 ml of sabourad dextrose broth in a test tube. The tubes were incubated at 25º C for 48 hrs before use.

The solution of test compound was prepared by a similar procedure described under the antibacterial activity. A reference standard solution of Flucanazole (10µg/ml) was prepared by dissolving 10 mg of Flucanazole in 10 ml of dimethylsulfoxide (DMSO).

Accurately 50 µl of 200 µg, 150 µg, 100 µg concentrations of test solution was transferred to the respective Petri-plates aseptically and labeled accordingly. The reference standard 50µl was also added to the discs in each plate. The plates were kept in refrigerator for one hour to allow the solution to diffuse properly into the SDA medium. Then the plates were incubated at 25º C for 48 hours at inverted position. The diameter of zone of inhibition was read with help of an antibiotic zone reader. The experiment was performed in triplicate and the results were represented in Table-IV.

Table IV: Antifungal activity of *3-(substuited hydrazono)-1H-benzo indol-2(3H)-one derivatives*

S. No	Compd No	Concentration (µg/ml)	*Zone of Inhibition (mm)*	
			Candida albicans	*Aspergillus niger*
01	PI-1	100	11	12
		150	13	13
02	PI-2	100	12	11
		150	14	13
03	PI-3	100	11	13
		150	12	14
04	PI-4	100	11	11
		150	14	13
05	PI-5	100	12	12
		150	10	13
06	PI-6	100	13	12
		150	14	14
07	PI-7	100	11	13
		150	13	14
8	PI-8	100	11	12
		150	13	13
9	PI-9	100	12	11
		150	13	11
10	PI-10	100	13	12
		150	14	10
11	PI-11	100	11	12
		150	13	13
12	PI-12	100	13	13
		150	15	13
13	PI-13	100	12	12
		150	14	12
14	STD	10	16	15

Anti-Helmenthic Activity

The antihelmintic activity was performed according to the method of Ghosh *et al.*,[19] On adult Indian earthworm *Pheritima posthuma* as it has anatomical and physiological

resemblance with the intestinal roundworm parasites of human beings. The earthworms in each group were released into desired formulation. Observations were made for the time taken to paralyze and death of individual worms. Paralysis was said to occur when the worms do not receive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color. The results are presented in Table-V

Table V: Anti-helmenthic activity of 3-(substuited hydrazono)-1h-benzo indol-2(3h)-one derivatives

S. No	Compound	Time taken for paralysis (min)				Time taken for death (min)			
		Dose (µg/ml)				Dose (µg/ml)			
		50	75	125	250	50	75	125	250
1	PI-1	4.66± 0.81	3.3±1.03	2.5± 0.54	0.52± 0.34	65±0.89	57.5±1.04	42.5±1.64	14±0.89
2	PI-2	3.5 ±1.04	3.0±0.63	2.33±.516	1.02±1.75	60.83±0.75	52.33±0.816	37±1.41	9.5±0.83
3	PI-3	4.88±0.75	2.5±0.54	2.6±1.03	0.49±0.27	58.33±0.81	50.05±1.08	28.66±1.03	7.33±1.03
4	PI-4	5.8 ± 0.75	2.66±0.516	2.33±0.516	0.82±0.816	62.5±1.04	59.6±1.21	50.66±0.86	47±0.89
5	PI-5	7.83±0.75	3.5±1.04	2.58±0.37	0.85±1.63	68.8±0.75	61.3±1.03	51.66±1.63	49±1.41
6	PI-6	6±0.894	4.66±0.81	4.16±0.752	1.75±0.52	58.53±0.75	52.83±0.75	27.66±0.51	15.16±1.21
7	PI-7	7.33±0.516	5±0.89	4.83±0.75	2.7±0.63	59.83±1.16	52.83±0.75	30.5±0.54	12.83±0.98
8	PI-8	6.16±0.75	4.5±0.83	4.83±0.75	2.5±0.44	67.66±1.21	51.83±0.63	30.66±2.1	17.83±0.98
9	PI-9	9.16±0.75	5.16±0.75	4.66±0.816	3.58±0.58	66.5±1.04	51.5±1.04	27.83±0.75	15.66±0.81
10	PI-10	9.5±0.55	5.16±0.75	3.5±0.54	4.5±0.55	65.37± 0.81	53.0±0.63	27.88±0.75	12.83±0.75
11	PI-11	7.33± 0.51	5.83±0.75	4.5±0.547	3.66±0.81	65.33±0.81	57.33±1.36	49.33±1.03	10.5±1.51
12	PI-12	8.5±0.54	4.5±0.54	4.66±0.516	3.58±0.58	64.33±0.51	59.16±0.75	41±0.89	12.83±1.6
13	PI-13	9±0.89	4.66±0.816	3.16±1.16	2.7±0.63	67.13±0.75	58±0.89	41.16±0.75	15.66±1.16
14	Flubendazole	3.55±0.56	2.1±0.59	1.9±0.48	0.32±1.03	48.2±0.59	53.55±0.75	24.55±.52	5.22±1.1

Results expressed as Mean + SEM from 6 observations

***In-Vitro* Anti-Inflammatory Activity**

The human red blood cell (HRBC) membrane stabilization method

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution(2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (200 and 400 µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added.

It was incubated at 37^0C for 30 min and centrifuged at 3,000 rpm for 20 min. and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 and 200 µg/ml) was used as reference standard and a control was prepared by omitting the extracts. The percentage of HRBC membrane stabilization or protection was calculated by using the following Formula. The results are presented in Table-VI

Formula = 100 - (Abs of test solution - Abs of product control / Abs of test control) x100

Table VI : In-Vitro anti-inflammatory activity of 3-(substuited hydrazono)-1h-benzo indol-2(3h)-one derivatives

S.No	Compound	% of Inhibition			
		Doses (µg/ml)			
		50	***75***	***125***	***250***
1	PI-1	17.42	33.71	49.41	63.00
2	PI-2	23.26	40.43	52.24	63.36
3	PI-3	33.71	40.31	50.24	62.85
4	PI-4	51.42	33.14	38.85	64.12
5	PI-5	28.57	40.00	49.14	73.71
6	PI-6	30.85	38.85	45.71	62.28
7	PI-7	33.71	41.14	51.42	62.28
8	PI-8	22.28	33.71	51.42	62.85
9	PI-9	41.14	60.57	71.42	80.00
10	PI-10	22.28	30.28	38.85	50.28
11	PI-11	17.14	34.28	51.42	57.14
12	PI-12	33.71	38.85	50	74.28
13	PI-13	40	46.28	62.85	69.71
14	Diclofenac	62.85	74.28	80	91.42

RESULTS AND DISCUSSION

All synthesized compounds were tested for their *Invitro* antibacterial activity by using the agar diffusion method. It has been observed that all the tested compounds showed mild to moderate activity against the bacteria. Whereas compound PI -1, PI-3, PI-4 and PI-7 were found to be most promising antibacterial activity among the series of compounds and compound PI-4 shows highest activity at 150µg/ml.

All synthesized compounds were tested for *Invitro* antifungal activity by the agar diffusion method. It has been observed that all the tested compounds showed mild to moderate activity against the fungi, among them, Compound PI-12 (150µg/ml) were found to be most active against the both *C. albicans* and *A. niger*.

The compounds were tested for anti-helmenthic activity. It has been observed that all the tested compounds showed mild to moderate anti-helmenthic activity. Compound PI-3 and PI-1 was found to be most active agents among the series of compounds.

All synthesized compounds were tested for *In-vitro* anti-inflammatory activity, the tested compounds showed mild to moderate *In-vitro* anti-inflammatory activity, compound PI-9 was found to be most active agents among the series of all compounds.

ACKNOWLEDGEMENT

The authors thank the Gitam Institute of Pharmacy, Gitam University, Visakhapatnam, A.P for providing the necessary facilities to carry out this research work.

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