New Isatin Derivatives and their Antimicrobial Activity

[1]ATTA-UR-REHMAN, [1]ABDUL SUBHAN IJAZ, [2]M.IQBAL CHOUDHARY, [2]ZAREEN AMTUL AND [2]ATTA-UR-RAHMAN

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan*

[2]*H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan*

(Received 30th April, 1997, revised 26th July, 1997)

Summary: Various derivatives of isatin (**1**) such as *N-p*-anisoylisatin (**2**), *N-p*-anisoylisatin-3-semicarbazone (**3**), *N-p*-anisoylisatin-3-hydrazone (**4**), *N-p*-anisoylisatin-3-thiosemicarbazone (**5**), *N-p*-anisoylisatin-3-(2,4-dinitro)phenylhydrazone (**6**), *N-p*-anisoylisatin-3-phenylhydrazone (**7**) and *N-p*-anisoylisatin-3-isonicotinohydrazone (**8**) were prepared. Spectroscopic methods were used for the characterization of these derivatives. These compounds also showed antibacterial and antifungal activities against a number of human pathogenic bacteria and fungi.

Introduction

Isatin (**1**) and its derivatives are important from the pharmacological and biological point of view, since they have been reported to exhibit antiviral, antimicrobial, cytotoxic, antiinflammatory and anticonvulsant activities [1-6]. 1-Methyl-3-thiosemicarbazone appeared to be an exteremely valuable prophylactic agent against small pox, and provides the only example of prophylaxis, a viral disease by a synthetic compound [7]. Isatin has recently been identified in rat tissues and normal urine, where it constitutes the major part of the endogenous monoamine oxidase inhibitor (MOI), tribulin [8]. The present paper describes the preparation of seven new derivatives, *N-p*-anisoylisatin (**2**), *N-p*-anisoylisatin-3-semicarbazone (**3**), *N-p*-anisoylisatin-3-hydrazone (**4**), *N-p*-anisoylisatin-3-thiosemi-carbazone (**5**), *N-p*-anisoylisatin-3-(2',4'-dinitro)-phenylhydrazone (**6**), *N-p*-anisoyli- satin-3-phenylhydrazone (**7**) and *N-p*-anisoyli- satin-3-isonicotinohydrazone (**8**) by substitution of anisoyl group at *N-1* and then condensation of hydrazine and related derivatives at C-3 of isatin. All these derivatives were identified by spectros-copic methods. They exhibited antimicrobial activity against many human pathogenic bacteria and fungi.

Results and Discussion

N-p-Anisoylisatin (**2**) was prepared by treatment of isatin (**1**) with *p*-anisoyl chloride in the presence of pyridine which acts as a solvent as well as a catalyst because of its basic nature.

The IR spectrum of **2** displayed intense absorptions at 1755, 1712 and 1672 cm^{-1} due to the carbonyl functionalities at C-3, C-2 and N-C=O positions. The absence of N-H stretching band in the IR spectrum indicated that the N-H had been replaced by an anisoyl group. Absorption bands at 1161 and 1590 cm^{-1} in the IR spectrum were due to C-O-C (methoxy group) and C=C moieties, respectively.

The mass spectrum of compound **2** showed the molecular ion at *m/z* 281 which was in agreement with the molecular formula $C_{16}H_{11}NO_4$. The base peak at *m/z* 135 was due to the loss of an anisoyl group. Other ions at *m/z* 254, 226, 146, 107, 77 and 64 were also observed in the mass spectrum of **2**.

The ^{1}H-NMR spectrum of **2** showed a three-proton singlet at δ 3.87 due to the methoxy protons. Two doublets at δ 7.08 and 7.93 (J = 8.8 Hz) integrating for two protons each were due to the aromatic protons of the anisoyl moiety, while two multiplets centered at δ 7.36 (1H) and 7.75 (3H) were assigned to the aromatic protons of the isatin moiety. These spectroscopic data helped to characterize compound **2** as *N-p*-anisoylisatin.

N-p-Anisoylisatin-3-semicarbazone (**3**) was synthesized by condensation of semicarbazide HCl with *N-p*-anisoylisatin (**2**). Its IR spectrum showed strong absorptions at 3330-3188 (NH and free NH_2), 1705-1685 (C=O), 1610 (C=N) and 1140 (C-O-C) cm^{-1}.

The mass spectrum of compound **3** showed the molecular ion at *m/z* 338 corresponding to the molecular formula $C_{17}H_{14}N_4O_4$. The base peak at *m/z* 135 was due to the anisoyl group. The ion at *m/z* 279 arose by the loss of $NHCONH_2$ moiety from the M^+ ion. Other ions characteristic of *N-p*-anisoylisatin (**2**) were also observed. The ^{1}H-NMR spectrum of **3** was distinctly similar to that of compound **2** except that it featured a two-proton singlet at δ 11.61 assigned to the NH_2 protons of the semicarbazone group. These spectral data served to identify compound **3** as *N-p*-anisoylisatin-3-semicarbazone, a new derivative of isatin.

N-p-Anisoylisatin-3-hydrazone (**4**) was also synthesized by the reaction of *N-p*-anisoylisatin (**2**) with hydrazine hydrate in the presence of glacial acetic acid. The IR spectrum of **4** was distinctly similar to that of **3** except that only one absorption band at 3280 cm^{-1} was observed due to the NH_2 group.

The EI mass spectrum of compound **4** showed the M^+ at *m/z* 294 which is in accord with the molecular formula $C_{16}H_{13}N_3O_3$. The mass fragmentation pattern was similar to that of compound **3**.

The ^{1}H-NMR spectrum of **4** was also similar to those of **2** and **3** except that it showed the resonance of the NH_2 protons as a sharp singlet at δ 11.61. These spectral observations led to the structure *N-p*-anisoyl-isatin-3-hydrazone (**4**) for the new isatin derivative.

N-p-Anisoylisatin-3-thiosemicarbazone (**5**) was prepared by the reaction of thiosemicarbazide with *N-p*-anisoylisatin. The IR spectrum showed strong absorptions at 3400 (free NH_2), 3154 (NH), 1700, 1680 (C=O), 1698 (C=N), 1225 (C=S) and 1176 (C-O-C) cm^{-1}.

The mass spectrum of compound **5** showed the molecular ion at *m/z* 354 which is in agreement with the molecular formula $C_{17}H_{14}N_4O_3S$. The mass fragmentation pattern was similar to those of the previously discussed compounds. The ^{1}H-NMR spectrum of this derivative was also similar to those of compounds **3** and **4**. Based on these spectroscopic studies compound **5** was characterized as *N-p*-anisoylisatin-3-thiosemicarbazone.

N-p-Anisoylisatin-3-(2,4-dinitro)-phenylhydrazone (**6**) was prepared by the reaction of 2,4-dinitrophenyl hydrazine with *N-p*-anisoylisatin (**2**) in the presence of conc. H_2SO_4 as a catalyst. The IR spectrum displayed strong absorptions at 3100 (NH), 1735, 1675 (C=O), 1144 (C-O-C) and 1530 and 1335 (NO_2) cm^{-1}.

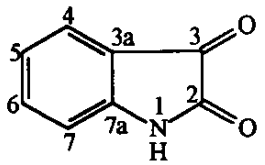
The mass spectrum of compound **6** showed the M^+ at *m/z* 461 corresponding to the molecular formula $C_{22}H_{15}N_5O_7$. The mass fragmentation of this compound was also similar to those of the previously discussed compounds. *N-p*-Anisoylisatin-3-(2',4'-dinito)-phenylhydrazone (**6**) was identified as the new derivative of *N-p*-anisoylisatin based on spectroscopic studies.

Our seventh compound, *N-p*-anisoylisatin-3-phenylhydrazone (**7**) was synthesized by the condensation of *N-p*-anisoylisatin (**2**) with phenylhydrazine in the presence of a few drops of conc. H_2SO_4. The IR spectrum of this derivative displayed strong absorptions at 3300 (NH), 1708, 1675 (C=O), 1603 (C=N) and 1158 (C-O-C) cm^{-1}.

The EIMS showed the molecular ion at *m/z* 371 ($C_{22}H_{17}N_3O_3$). The ion at *m/z* 280 was due to the loss of C_6H_5NH from the M^+. Other fragments in the mass spectrum were similar to those of compound discussed previously.

The ^{1}H-NMR spectrum of compound **7** was similar to those of the previously described compounds except that two multiplets resonated at δ 7.36 and 7.72 integrating for three- and two-proton respectively due to the aromatic protons of the phenylhydrazone moiety. These spectroscopic studies led to the identification of this new isatin derivative as *N-p*-anisoylisatin-3-phenylhydrazone (**7**).

N-p-Anisoylisatin-3-isonicotinohydrazone **8** was obtained by reaction of *N-p*-anisoylisatin (**2**)



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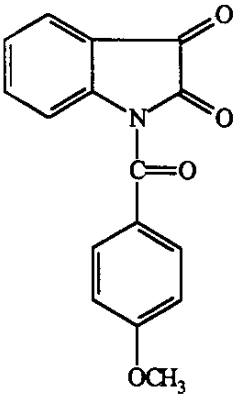

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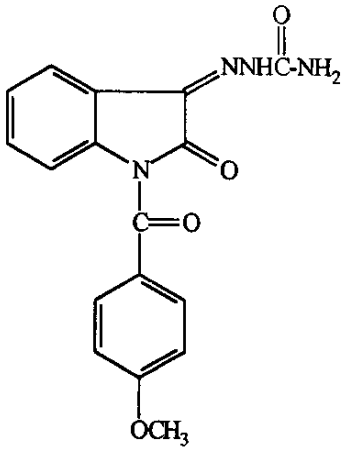

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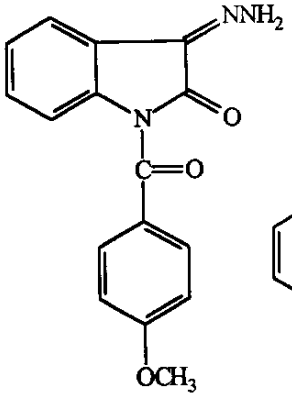

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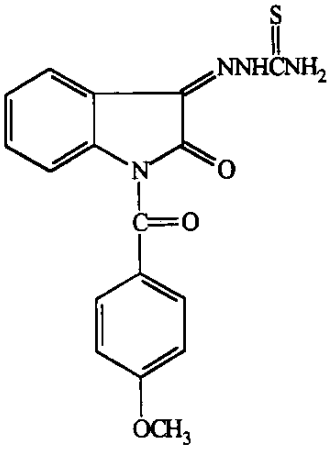

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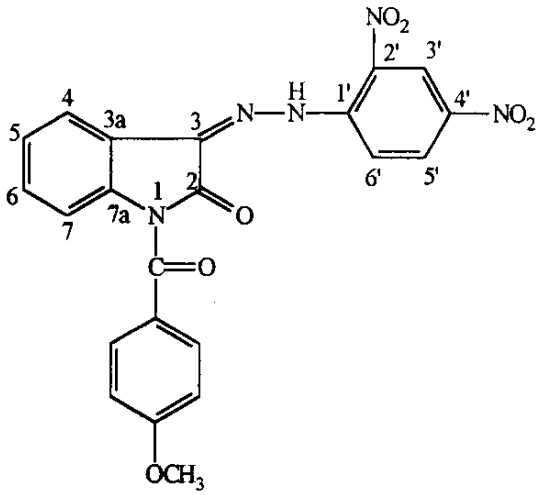

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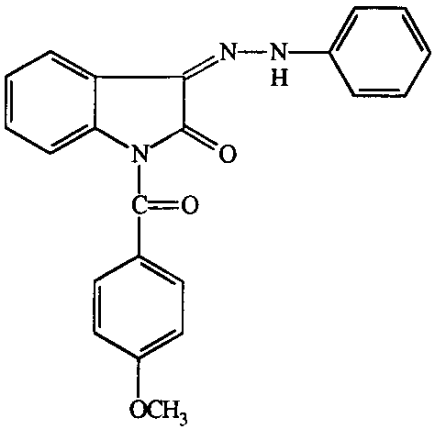

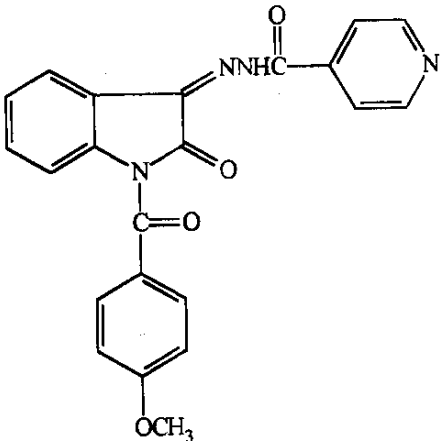

with isonicotinic acid hydrazide. The IR spectrum afforded strong absorptions at 3200 (NH), 1698, 1665, 1640 (C=O), 1600 (C=C), 1580 (C=N) and 1165 (C-O-C) cm^{-1}. The mass spectrum of compound **8** showed the molecular ion at *m/z* 400 ($C_{22}H_{16}N_4O_4$). The ion at *m/z* 281 was due to the loss of a $NHCOC_6H_5$ group from the M^+. Other ions found in the mass spectrum were similar to earlier described compounds **1-7.** Peak at *m/z* 266 was due to loss of a anisoyl group from the M^+.

The ^{1}H-NMR spectrum of derivative **8** was also similar to that of compound 7 except that ^{1}H-NMR spectrum of this compound featured two additional doublets at δ 7.96 and 8.62 and each integrating for two protons, which were assigned to the aromatic protons of the isonicotinohydrazone moiety. These spectroscopic studies led to structure **8** (*N-p*-anisoylisatin-3-isonicotinohydrazone) for the substance.

Compound **2** was found to be strongly active against *Aspergillus niger, Microsporum canis, Trichophyton simii* and *Trichophyton mentagrophytes* whereas weakly active against *Pseudallescheria boydii.*

Compound **3** showed somewhat good activity against *Aspergillus niger, Trichophyton mentagrophytes* and *Microsporum canis* at 150 μg/ml but weak activity below 150 μg/ml.

Compound **4** similarly compound 4 was found moderately active against *Aspergillus niger, Curvularia lunata* and *Trichophyton simii.*

Compound **5** is strongly active against *Aspergillus niger, Trichophyton mentagrophytes* and *Trichophyton schoenleinii.*

Compound **6** is moderately active against *Pseudallescheria boydii* and *Trichophyton mentagrophytes.*

Compound **7** is strongly active against *Aspergillus niger, Microsporum canis* and *Trichophyton schoenleinii* at 150 μg/ml concentration only.

Compound **8** significantly fungicidal against *Microsporum canis* and *Trichophyton mentagrophytes* but weakly active against *Pseudallescheria boydii* and *Nigrospora oryzae.*

All compounds **2-8** exhibited weak bactericidal activity against all bacteria tested.

Experimental

Melting points were determined in open capillaries and uncorrected. The IR spectra were recorded in KBr disc on Shimadzu IR 460 spectrophotometer. Mass spectra were recorded on Varian MAT 311-A mass spectrophotometer at 70 ev. ^{1}H-NMR were recorded on Bruker AM-300 NMR spectrophotometer in DMSO-d_6 at 300 MHz. Purity was checked by T.L.C. using pet. ether (40-60): acetone: methanol (6:3.5:0.5) mixture as solvent with ammonia vapors.

N-p-Anisoylisatin (2)

A mixture of isatin (**1**) (1.47 g; 0.01) and *p*-anisoyl chloride (1.71 g, 0.01 mol) in pyridine (10 ml) was heated under reflux conditions for 30 minutes, cooled and poured into cold water. Yellow precipitate appeared which were filtered and recrystallized with methanol to yield 2.5 g (88%), m.p. 165°.

N-p-Anisoylisatin-3-semicarbazone (3)

Semicarbazide-HCl (1.12 g, 0.01 mol) and 1.5 g of crystalline sodium acetate were dissolved in 1 ml of methanol, shaken and then added to *N-p*-anisonylisatin (**2**) (2.81 g, 0.01 mol) in methanol (50 ml) with a few drops of glacial acetic acid. The mixture was refluxed for a few minutes and then allowed to stand for a few hours until white precipitates appeared. These crystals were filtered and washed with a little cold water and recrystallized with absolute ethanol-water mixture (50:50), yield 1.25 g (37%), m.p. 122°.

N-p-Anisoylisatin-3-hydrazone (4)

A mixture of *N-p*-anisoylisatin (**2**) (2.81 g, 0.01 mol) and hydrazine hydrate (0.01 mol) was refluxed in methanol (50 ml) for 20 minutes in the presence of few drops of glacial acetic acid, cooled and kept for overnight. A white slurry of crystals was collected washed with water and recrystallized with absolute ethanol, yield 2.00 g (67%), m.p. 158°.

Table-1: *In vitro* fungicidal activity of compounds **2-8** (Growth inhibition in percentage at three different concentrations:

Compounds	Concentration (μg/ml)	A.n	C.l	M.c	T.s	P.b	N.o	T.sh	T.m
2	150	81	-	85	86	72	-	84	-
	100	79	-	82.5	82.5	68	-	82.5	-
	50	76	-	80	82.5	55	-	76	-
3	150	85	-	78	-	66	-	70.6	78
	100	84	-	48	-	23	-	62	78
	50	65	-	-	-	-	-	52	40
4	150	87	70.9	-	82	-	44	-	-
	100	69.8	36	-	80	-	26	-	-
	50	38	-	-	11	-	20	-	-
5	150	80	-	-	-	-	-	88	86
	100	78	-	-	-	-	-	87	86
	50	63	-	-	-	-	-	76	73
6	150	-	-	-	-	70	-	70	-
	100	-	-	-	-	80	-	51	-
	50	-	-	-	-	-	-		-
7	150	81	-	88	-	70	-	-	88
	100	48	-	82	-	68	-	-	81.5
	50	34	-	11	-	-	-	-	78
8	150	-	-	82.4	-	66	72	86	-
	100	-	-	82.4	-	66	69	84	-
	50	-	-	-	-	50.7	25	82	-
Miconazole and Ketocanazole (standard)	150	100	100	100	100	100	100	100	100
	100	100	100	100	100	100	100	100	100
	50	100	100	100	100	100	100	100	100

Key:

A.n. = *Aspergillus niger*
C.l. = *Curvularia lunata*
M.c. = *Microsporum canis*
T.s. = *Trichophyton simii*
P.b. = *Pseudallescheria boydii*
N.o. = *Nigrospora oryzae*
T.m. = *Trichophyton mentagrophytes*
T.sh. = *Trichophyton schoenleinii*

Table-2: *In vitro* bactericidal activity of compounds **2-8** (Zones of inhibition in mm at 200 μg/100 μl of DMSO).

Compounds	S.a	St.p	C.d	B.s	E.c	S.b	S.t	P.m	K.p
2	7	8	7	9	8	8	-	-	-
3	-	7	7	9	8	-	-	-	-
4	-	7	7	-	8	7	6	-	6
5	6	6	7	-	8	7	-	7	-
6	6	6	-	6	8	6	6	7	-
7	-	7	6	6	7	6	6	-	-
8	-	6	-	-	8	8	6	-	-
Ampicillin$(H_2O)_3$ (Standard)	20	21	18	21	22	18	17	19	19

Key:

S.a. = *Staphylococcus aureus*
St.p = *Streptococcus pyogenes*
B.s = *Bacillus subtilus*
C.d = *Corynebacterium diphtheriae*
S.b. = *Shigella boydii*
E.c = *Escherichia coli*
P.m = *Proteus mirabilis*
S.t = *Salmonella typhi*
K.p. = *Klebsiella pneumoniae*

N-p-Anisoylisatin-3-thiosemicarbazone (5)

A mixture of *N-p*-anisoylisatin (**2**) (2.81 g, 0.01 mol) and thiosemicarbazide (0.91 g, 0.01 mol) in methanol was refluxed for 30 minutes in the presence of a few drops of glacial acetic acid. The pale yellow colored precipitates appeared which were filtered and recrystallized with absolute ethanol, yield 3.00 g (84%), m.p. 220°.

N-p-Anisoylisatin-3-(2,4-dinitro) phenylhydrazone (6)

A solution of *N-p*-anisoylisatin (**2**) (2.81 g, 0.01 mol) in methanol was heated for a few minutes under reflux conditions and then a hot solution of 2,4-dinitrophenylhydrazine (1.98 g, 0.01 mol) in methanol containing 1 ml of conc. H_2SO_4 was added. This mixture was refluxd for 15 minutes. The precipitates which appeared were cooled and filtered. Recrystallization from absolute ethanol yield an orange-red compound, yield 3.2 g (69%), m.p 310°.

N-p-Anisoylisatin-3-phenylhydrazone (7)

A mixture of *N-p*-anisoylisatin (**2**) (2.81 g, 0.01 mol) and phenyl hydrazine (1.08 g, 0.01 mol) in methanol with a few drops of conc. H_2SO_4 was refluxed for one hour. The reaction mixture containing a yellow solid was then cooled to room temperature filtered and recrystallized with absolute ethanol, yield 1.75 g (47%), m.p. 195°.

N-p-Anisoylisatin-3-isonicotinohydrazone (8)

To *N-p*-anisoylisatin (**2**) (2.81 g, 0.01 mol) and isoniazied (1.37 g, 0-001 mol) in methanol were added few drops of glacial acetic acid and the solution refluxed for 2 hrs. The yellow precipitates which appeared were then cooled at room temperature filtered and recrystallized with absolute ethanol, yield 1.55 g (38%), m.p. 220°.

Antimicrobial testing

Fungicidal bioassay (in vitro)

This test was performed by incorporating the test compounds with Sabouraud Dextrose Agar at different concentrations (50 μg/ml upto 200 μg/ml) in DMSO and diluted with distilled water. Non-solidified Sabouraud Dextrose Agar media were poisoned with compounds of desired concentrations prepared from the stock solutions and allowed to solidify. Each tube was inoculated with a 5 mm diameter disk of inoculum removed from 7 day old cultures of different fungi (Table-1). Other media were supplemented with DMSO serving as control. Treatments were replicated 3 times and the tubes incubated at 25°-29°C for seven days. Growth on the compound containing media was determined by measuring linear growth (mm) and growth inhibition calculated with reference to the control [9] and compared with reference drug i.e. miconazole and ketoconazole.

Bectericidal bioassay (in vitro)

Antibacterial activity was determined by agar well diffusion method [10]. In this method 24 hour-old cultures containing approximately 10^4-10^6 CFU was spread on the surface of Mueller Hinton Agar plates. Wells were dug in the media with the help of sterile metallic borer. Test samples of different concentrations were added in their respective wells. Experimental plates were incubated at 37°C for 24 hours and zones of inhibition were measured and compared with standard antibiotic [ampicillin$(H_2O)_3$].

The sensitivity of the bacteria was found to differ from compound to compound. The zones of inhibition are given in Table-**2**.

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