Potential Antimicrobial Agents-I: Structural Modifications and Antimicrobial Activity of some Isatin Derivatives

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New isatin-3-isonicotinylhydrazones, isatinazine and its Mannich bases and spiro (indoline-3, 2'-thiadiazoline)-2-one have been synthesized. These compounds have been screenced for their antibacterial activity against the Gram-positive and Gram-negative bacteria.

Key words: Synthesis, Isatin-derivatives, Antimicrobial activity

INTRODUCTION

In our previous communication, we have reported the biopharmaceutical activity of a number of 2-oxo-3-indolyl derivatives (Khan *et al.*, 1986; Alam *et al.*, 1990, 1991, 1992). The importance of indole nucleus is well established in the field of pharamceutical chemistry (Barbara *et al.*, 1974, Popp, 1980; Singh, 1988; Pisocopo *et al.*, 1987). In this paper, the synthesis of some isatin derivatives as potent antibacterial agents has been described.

MATERIALS AND METHODS

Organisms

Seven bacteria: *Bacillus pumilus, Bacillus subtilis, Bordetella bronchoseptica, Escherichia coli, Micrococcus flavis, Proteus vulgaris, Staphylococcus aureus* were obtained from Drug Testing Laboratory of Health Department. All the bacterial strains were maintained on nutrient agar medium (N.A. medium) at 37℃.

Test Compounds

The antibacterial activity of 2-oxo-3-indolyl derivatives was evaluated in this study. Erythromycin and Polymyxin were used for comparisons.

Testing Procedure

Antibacterial activity of the synthetic compounds along with erythromycin and polymyxin (control) were determined against bacteria mentioned above. Either drug was insoluble in water; therefore, dimethyl sulfoxide (DMSO) was empolyed to dissolve the compounds.

The bacterial suspensions were prepared by suspending a loopful of pure culture in 10 ml of sterile distilled water. One ml of bacterial suspensions were separately mixed with 15 ml of sterile molten N.A. medium in different sterile petri dishes (already labelled with bacterial name/compound under study). The media of the petri dishes were divided into four equal parts after solidification, and uniform holes of 8 mm in diameter, were made with a cork borer. Each hole was filled with 0.08 ml solution of the test compound and the fourth part was kept as control. These dishes were incubated at 37℃ for 24 hours. The zones of inhibition were measured by vernier calipers.

Synthesis of 2-Oxo-3-Indolyl Derivatives

Melting points were uncorrected. Infra-red spectra were run as KBr disces on a Perkin-Elmer Model-283 spectropholtometer. NMR spectra were recorded on Bruker AM-300 instrument using tetramethylsilane as internal standard. Mass spectra were measured on MAT-1125 spectrometer at H.E.J. Research Institute of Chemistry, Karachi (Pakistan).

N-Chloroacetyl isatin (1): Monochloroacetyl chloride (24 g) was added slowly with stirring to a solution of isatin (14.7 g) in toluene (100 ml) and heated under reflux for 4 hrs. On cooling to room temperature solid product which separated out was collected and recrystallised from toluene to furnish 18.5 g (82%) of **1**, m.p. 205-206℃ [Found M (Mass spectroscopy) 223 requires for $C_{10}H_6NO_3Cl$, M, 223]. IR absorption at 1780, 1710

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cm^{-1}. NMR ($CDCl_3$): δ 4.2 (2H, s, $COCH_2$), 7.0-8.0 (4H, m, aromatic).

Isatin-3-isonicotinylhydrazone (2): Isatin (14.7 g) and isoniazide (15.0 g) in dimethylformamide (100 ml) were refluxed for 4 hrs. On cooling at room temperature orange crystals separated out, which were collected and recrystallized from dimethylformamide to furnish 20.0 g (89%) of **2**, m.p. 289-291°C [Found M (mass spectroscopy) 266 requires for $C_{14}H_{10}N_4O_2$, M, 266]. IR absorption at 3220 (NH), 1760 and 1670 (amide C=O) cm^{-1}. NMR, δ (DMSO), 8.85 (2H, d, 2-H), 7.77 (2H, d, H-4, H-7), 7.6 (1H, s, NH-1), 7.5 (1H, s, H-2), 7.4 (1H, m, H-6), 7.1 (1H, m, H-5) and 9.95 (2H, d, 1H).

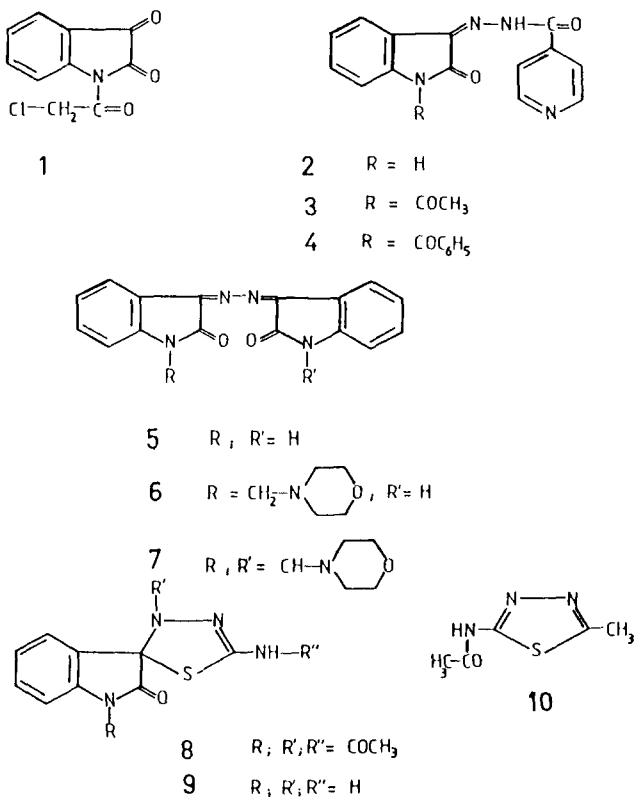
1-Acetyl isatin-3-isonicotinylhydrazone (3): Isoniazide (13.7 g) and 1-acetyl isatin (19.0 g) in ethanol (50 ml) were refluxed for 4 hrs. On cooling orange crystalline product separated out, was collected and recrystallized from dimethylformamide to give 30 g (82 %) of **3**, m.p. 285°C [Found M (mass spectroscopy) 308 requires for $C_{16}H_{12}N_4O_3$, M, 308]. IR absorption in the carbonyl region at 1780, 1745 and 1710 cm^{-1}. The NMR spectrum of **3** showed disappearance of resonance signal due to >NH proton in spectrum of **2**. Besides, additional $-CH_3$ resonance signal of $-CO-CH_3$ group appears as singlet at δ 2.5 ppm.

1-Benzoyl isatin-3-isonicotinylhydrazone (4): The analog **4** was prepared under similar conditions as described above for compound **3**. The product was obtained in 87% yield recrystallized from ethanol to furnish **4**, m.p. 195-195°C.

Isatinazine (5): Isatin (11.2 g), isatin-3-hydrazone (12.8 g) and dimethylformamide (100 ml) were heated under reflux for 2 hrs. On cooling, the red crystalline product was collected and recrystallized from dimethylformamide to give 20.0 g (90%) of **5**, m.p. 221-222 °C [Found M, 290 (mass spectroscopy) requires for $C_{16}H_{10}N_4O_2$, M, 290]. IR, 3400 (NH) and 1720 (amide C=O). The product was identical with a synthetic sample of isatinazine **5** (spectra and mixed m.p.) (Rahman *et al.*, 1987).

1-Morpholinomethyl isatinazine (6): Isatin-3-hydrazone (14.7 g), 1-morpholinomethyl isatin (24.6 g) and absolute ethanol (125 ml) were refluxed for 3 hrs. On cooling the resulting red crystalline product was collected and recrystallized from ethanol to give 38.7 g (91%) of **6**, m.p. 18-151°C [Found: C, 64.5; H, 5.3; N, 17.4% requires for $C_{21}H_{19}N_5O_3$: C, 64.8: H, 4.9; N, 17.9%].

1,1'-Morpholinomethyl isatinazine (7): A mixture of isatinazine (18.0 g), formaldehyde (30 g) and dimethylformamide (150 ml) was placed in two neck flate bottom flask and morpholine (25 ml) was added dropwise at 20-25°C with constant stirring. The mixture was stirred for two hours. The product separated out was filtered and washed with dimethylformamide (3×3 ml) and recrystallized from ethanol to furnish 27.7 g (90%) of Mannich base 7, m.p. 170°C [Found: C, 64.4; H, 5.6; N, 16.9%; $C_{26}H_{28}N_6O_4$ requires C, 63.9; H, 5.8; N, 17.2%]. IR absorption in the carbonyl region at 1730 and 1700 cm^{-1}.



3'-Acyl-5'-(acetylamino)-1-acetyl-spiro(indoline-3,2'-thiadiazoline)-2-one (8): Isatin-3-thiosemicarbaznone (11.0 g) and acetic anhydride (65 ml) were heated to reflux for two hours. On cooling, the resulting crystalline product was collected by filteration and recrystallized from ethanol to furnish 25.0 g (80%) of **8**, m.p. 243°C [Found: C, 51.9; H, 4.5; N, 16.1%; requires for $C_{15}H_{14}N_4O_4S$: C, 52.0; H, 4.0; N, 16.2%]. IR in the carbonyl region at 1800, 1720 and 1650 cm^{-1}.

5-Amino-spiro(indoline-3,2'-thiadiazoline)-2-one (9): Compound **3** (16.5 g) and ammonium hydroxide (100 ml) were heated to reflux for 1.5 hours and cooled to room temperature, the resulting crystalline product was collected and recrystallized from ethanol to give 10.2 g (90%) of **9**, m.p. 206°C [Found C, 48.6; H, 4.3; N, 24.3%; requires for $C_9H_8N_4OS$; C, 49.1; H, 3.9; N, 24.6%]. IR shows absorption due to -NH group at 3300-3060 and in the carbonyl region at 1720 cm^{-1}.

Table I. Zone of inhibition (mm) obtained by various derivatives of isatin against different bacteria

Culture	1	2	3[a]	4[a]	5	6	7	8	9	10
Bacillus pumilus	14	17	26	23	14	17	14	30	16	19
Bacillus subtilis	–	12	11	14	16	20	–	–	–	–
Bordetella bronchoseptica	–	14	16	19	–	19	24	27	–	–
Escherichia coli	–	12	13	17	16	–	–	21	–	–
Micrococcus flavis	15	30	27	20	–	–	16	23	14	–
Proteus vulgaris	15	12	15	18	12	15	18	16	19	13
Staphylococcus aureus	–	17	12	15	13	16	20	24	11	12

[a]In concentration of 400 μg/ml only as compared to rest of compounds which were taken as 5 mg/ml.

2-Acylamino-5-methyl-1,3,4-thiadiazoline (10): Thiosemicarbazide (15 g) and acetic anhydride (30 ml) were heated to reflux for 2 hrs. On cooling the resulting crystalline product was collected and recrystallized from ethanol to give 15.6 g of **10**, m.p. 290°C [Found: M (mass spectroscopy) 157 requires for $C_5H_7N_3OS$; M, 157]. IR absorption at 3230, 1700 and 610 cm $^{-1}$. NMR ($CDCl_3$) δ, 7.3 (1H, s, NH), 2.7 (3H, s, $COCH_3$) and 2. 4 (3H, s, CH_3).

RESULTS AND DISCUSSION

Synthesis of Compounds

Isatin-3-isonicotinylhydrazones **2**, **3** and **4** were prepared by the condensation of isoniazide hydrochloride with appropriate N-substituted isatin. The compound isatinazine **5** and its Mannich bases **6** and **7** were also prepared. Isatin-3-thiosemicarbazone was treated with acetic anhydride to furnish 3'-acyl-5'(acetylamino)-1-acetyl-spiro(indoline-3,2'-thiadiazoline)-2-one **8**.

Antibacterial Activity

All the synthesized compounds described above were screened for antibacterial activity against Gram-positive and Gram-negative bacteria by the agar-diffusion method.

All the compounds showed antibacterial activity (Table I). Isatin-3-isonicotinylhydrazone **2** and its 1-acetyl and 1-benzoyl derivatives **3** and **4** were found to be the most effective. The zones of inhibition obtained by compounds **2**, **3**, and **4** against the different bacteria were maximum and could not be measured in petri dish (100 mm) as it was used for other compounds. Thus the concentration of these compounds was gradually reduced upto 400 μg/ml to obtain the results. The control group of Erythromycin (A) and Polymyxin (B) also indicated zones of inhibition ranging from 22 mm to 30 mm and 13 mm to 18 mm respectively.

The compound isatinazine **5** and its Mannich bases **6** and **7** exhibited broad antibacterial spectrum. In these compounds the presence of two equivalent 2-oxo-3-indolinylidene systems are probably responsible to enhance the activity. It is of interest that 1-acetyl spiro (indoline-3,2'-thiadiazoline)-2-one **8** exhibited higher activity, when compared with its deacylated analog **9**; whereas the compound **1** and **10** were less active.

The results obtained in this investigation indicated a structure-activity relationships of the test compounds.

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