Synthesis and Antibacterial Activity of Some 5-Nitro-3-phenyliminoindol-2(3*H*)-ones and Their *N*-Mannich Bases

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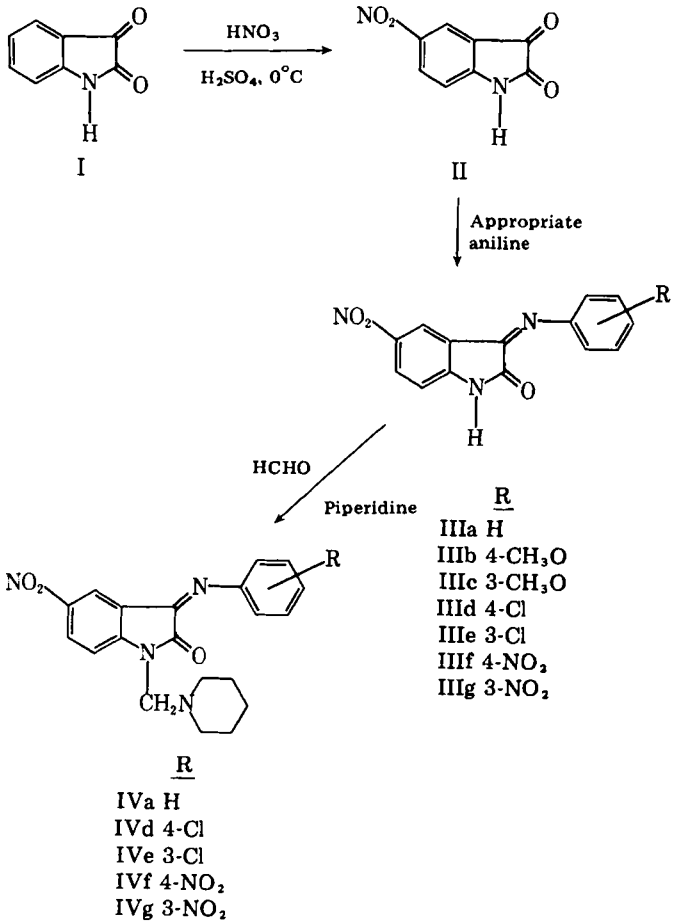
Abstract □ The antimicrobial and antifungal activities of a series of 5-nitro-3-phenyliminoindol-2(3*H*)-ones and their 1-piperidinomethyl analogues (*N*-Mannich bases) were investigated. Growth inhibition of Gram-positive bacteria was observed with little or no activity against Gram-negative bacteria. Antifungal activity was absent. The syntheses were accomplished from 5-nitroindol-2,3-dione by condensation with the appropriate aniline followed by formation of the *N*-Mannich base.

Keyphrases □ 5-Nitro-3-phenyliminoindol-2(3*H*)-ones—synthesis, antimicrobial and antifungal activity □ *N*-Mannich bases—5-nitro-3-phenyliminoindol-2(3*H*)-ones, synthesis, antimicrobial and antifungal activity □ Antimicrobial agents—potential, 5-nitro-3-phenyliminoindol-2(3*H*)-ones and their *N*-Mannich bases, synthesis

Interest in biologically active indol-2,3-dione (isatin) derivatives has increased rapidly over the past few years. Mannich bases derived from indol-2,3-diones have shown antimicrobial (1, 2), antiviral (3), cytostatic, and antimitotic (4, 5) activities. The corresponding substituted 3-thiosemicarbazones or 3-hydrazones have similarly shown antimicrobial (2), antiviral (6), cytostatic, and antimitotic (4, 5) activities. Recently a series of 3-aryliminoindol-2(3*H*)-ones and their *N*-Mannich bases have been shown to possess antibacterial and antifungal (7) activity and have shown promise as excystment and cysticidal agents against *Schizopyrenus russelli* (8, 9). CNS activity has recently been demonstrated for some *N*,*N*-disubstituted 1-(aminomethyl)-5-alkyl-3-(aryloxyacetylhydrazono)indol-2(3*H*)-ones (10).

The 3-*o*-nitrophenylhydrazone of indol-2,3-dione has shown activity against Walker carcinosarcoma 256 (11). Because of the well-known antibacterial activity of other nitro heterocycles (12) and our interest in this area (13), we decided to prepare and test a series of 5-nitro substituted 3-phenyliminoindol-2(3*H*)-ones and their *N*-Mannich bases for antimicrobial activity.



Scheme I—Synthesis of 5-nitro-3-phenylimino-1-piperidinomethylindol-2(3H)-ones.

RESULTS AND DISCUSSION

The synthetic procedure followed is outlined in Scheme I. The starting material was 5-nitroindol-2,3-dione (II) prepared by the nitration of indol-2,3-dione (I) using a modification of the method reported by Calvery (14) which prevented the formation of resinous side products. Direct condensation of the appropriate aniline with 5-nitroindol-2,3-dione (II) in a boiling mixture of ethanol and dimethyl sulfoxide furnished the corresponding 3-phenylimino derivatives (IIIb–d). The remaining compounds (IIIa, e–f) were prepared similarly, but required the addition of a few drops of glacial acetic acid to the reaction medium. Using dimethyl sulfoxide containing a few drops of glacial acetic acid as solvent allowed the reaction to proceed to completion by standing at room temperature overnight.

The 1-piperidinomethyl derivatives (*N*-Mannich bases) (IVa, d–g) were prepared from the 3-phenylimino derivatives using standard methods (7). The reaction failed for IVb and IVc.

All compounds gave satisfactory elemental analysis for C, H, and N (within ±0.4%), the UV, IR, ^{1}H-NMR, and mass spectra were consistent with the assigned structures. Physical constants of the 3-phenylimino

Table I—Physical Properties of 5-Nitro-3-phenyliminoindol-2(3*H*)-ones (III) and 5-Nitro-3-phenylimino-1-piperidinomethylindol-2(3*H*)-ones (IV)

Compound	R	Method	Yield, %	mp, °C	Formula
IIIa	H	C	90	242–243[a]	$C_{14}H_9N_3O_3$
IIIb	4-CH_3O	A	91	222–224[a]	$C_{15}H_{11}N_3O_4$
IIIc	3-CH_3O	A	62	168–170	$C_{15}H_{11}N_3O_4 \cdot H_2O$
IIId	4-Cl	A	77	220–221[a]	$C_{14}H_8ClN_3O_3 \cdot H_2O$
IIIe	3-Cl	B	89	130–131	$C_{14}H_8ClN_3O_3$
IIIe	3-Cl	C	83	129–131	—
IIIf	4-NO_2	B	77	151–152	$C_{14}H_8N_4O_5 \cdot H_2O$
IIIf	4-NO_2	C	80	151–153	—
IIIg	3-NO_2	B	51	235–237	$C_{14}H_8N_4O_5$
IIIg	3-NO_2	C	64	237–238	—
IVa	H	–	66	145–147	$C_{20}H_{20}N_4O_3$
IVd	4-Cl	–	68	165–167	$C_{20}H_{19}ClN_4O_3$
IVe	3-Cl	–	66	174–176	$C_{20}H_{19}ClN_4O_3$
IVf	4-NO_2	–	24	185–187	$C_{20}H_{19}N_5O_5$
IVg	3-NO_2	–	31	188–189	$C_{20}H_{19}N_5O_5$

[a] Kallmayer (15) reports mp 245–247°C for IIa, and 266–270°C and 238–240°C, respectively, for the anhydrous forms of IIIb and IIId.

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Table II—Antibacterial and Antifungal Activities of 5-Nitro-3-phenyliminoindol-2(3*H*)-ones (III) and 5-Nitro-3-phenylimino-1-piperidinomethylindol-2(3*H*)-ones (IV)

Compound	Diameter of Inhibition Zones[a], mm × 10					
	E. Coli (NCTC 5933)		*S. aureus* (NCTC 8532)		*C. albicans* (NCTC 45348)	
	Carpet	Pour	Carpet	Pour	Carpet	Pour
II	–	–	–	–	–	–
IIIa	–	–	–	–	–	–
IIIb	–	–	±	±	–	–
IIIc	–	–	130	120	–	–
IIId	–	–	170	142	–	–
IIIe	–	–	186	162	–	–
IIIf	–	–	–	–	–	–
IIIg	–	–	170	150	–	–
IVa	–	–	–	–	–	–
IVd	±	±	250	240	–	–
IVe	–	–	170	142	–	–
IVf	–	–	–	–	–	–
IVg	–	–	202	176	–	–
Dimethyl sulfoxide	–	–	–	–	–	–

[a] Average of six determinations. Key: (–) no inhibition; (±) minimally observable inhibition.

and corresponding 1-piperidinomethyl derivatives are listed in Table I.

All the compounds synthesized were evaluated for potential antimicrobial activity against Gram-negative *Escherichia coli*, Gram-positive *Staphylococcus aureus*, and *Candida albicans* using the cup–plate technique with either a pour plate or surface-innoculated (carpet) plate. None of the compounds tested showed any activity against *C. albicans*, and only IVd exhibited slight activity against *E. coli* (Table II). Compounds II, IIIa, IIIf, IVa, and IVf were inactive and IIIb showed only slight inhibitory activity against *S. aureus*. However, IIIc–e, IIIg, IVd–e, and IVg were active, with IVd the most active, followed by IVg.

The Mannich bases were usually more active than the 3-phenylimino compounds, with the exception that IIIe was more active than IVe. This is consistent with the observation of Varma (7) and demonstrates the necessity of the 3-phenylimino group, as 5-nitro-3-piperidinomethylindol-2,3-dione has been shown to be devoid of antimicrobial activity (2).

EXPERIMENTAL

Antimicrobial Evaluation—The organisms used were *E. coli* (NCTC 5933), *S. aureus* (NCTC 8532), and *C. albicans* (NCTC 45348). The cup–plate technique was used with either a pour plate or a surface-innoculated (carpet) plate using nutrient agar for the bacteria and malt extract for *C. albicans*.

The compounds were tested at 1 mg/mL in dimethyl sulfoxide, and 100-μl portions were transferred to 9-mm diameter holes cut into the plates. The agar plates were then incubated at 37°C for 24 h and the malt extract plates at 25°C for 72 h, after which the zones of inhibition around each hole were measured. Table II shows the average of six determinations for each zone of inhibition. Dimethyl sulfoxide showed no inhibitory effects on the test organisms.

Chemistry—Melting point values were determined[1] and are corrected. The ^{1}H-NMR[2] spectra were obtained in DMSO-d_6 using 1% tetramethylsilane as internal standard. IR[3] and UV[4] spectra were recorded on double-beam spectrophotometers. Mass spectra[5] were also recorded. TLC was carried out using plates coated with silica gel, and products were visualized with UV light at 254 nm. All compounds submitted for elemental analysis[6] gave spectral data consistent with the proposed structure and were pure by TLC.

5-Nitroindol-2,3-dione (II)—Indol-2,3-dione (44.2 g, 0.33 mol) was dissolved in concentrated sulfuric acid (208 mL), and the solution was kept at 0°C while nitric acid (d = 1.50, 15.5 mL, 0.34 mol) was added dropwise over a period of 3 h. After the addition was complete, the solution was allowed to stand for 1 h and then poured into ice (1.5 kg). The yellow precipitate was collected and recrystallized from aqueous methanol to yield 5-nitroindol-2,3-dione (II), 48.3 g (85%), mp 254–255°C [lit. (14) mp 254–255°C].

5-Nitro-3-phenyliminoindol-2(3H)-ones (III)—Compounds IIIa–g were prepared by one of the following methods:

1. 5-Nitroindol-2,3-dione (1.92 g, 0.01 mol) and the appropriate aniline (0.01 mol) were suspended in a mixture of ethanol (15 mL) and dimethyl sulfoxide (15 mL). The mixture was heated at reflux for 1 h, an equal volume of warm water was added, and the mixture was allowed to stand at room temperature overnight. The material was removed by filtration, washed with aqueous ethanol (50% v/v), and dried. Analytical and microbiological samples were recrystallized from aqueous methanol (50% v/v) (method A).

2. Identical to method A except that a few drops of glacial acetic acid were added to the reaction mixture (method B).

3. 5-Nitroindol-2,3-dione (1.92 g, 0.01 mol) and the appropriate aniline (0.01 mol) were stirred overnight in dimethyl sulfoxide (25 mL) containing a few drops of glacial acetic acid. The products were isolated by dilution with an equal quantity of warm water as described for method A (method C).

5-Nitro-3-phenylimino-1-piperidinomethylindol-2(3H)-ones (IV)—The appropriate 5-nitro-3-phenyliminoindol-2(3*H*)-one (0.01 mol) was treated with piperidine (0.01 mol), formaldehyde solution (2 mL, 37% w/v), ethanol (10 mL), and dimethyl sulfoxide (10 mL). The mixture was then heated on a water bath until a clear solution was obtained. An equal volume of warm water was then added and the resulting mixture was allowed to stand at room temperature overnight; the product was removed by filtration and then recrystallized from aqueous methanol (50% v/v).

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[1] Mettler model FP61.
[2] Perkin-Elmer model R32.
[3] Perkin-Elmer model 157G in KBr disks.
[4] Perkin-Elmer model 554.
[5] VG MM 16F spectrometer with DS 2135 data system.
[6] Carried out by Dr. J. Baker, Department of Pharmacy, Brighton Polytechnic.