Antibacterial and Antifungal Activities of Isatin *N*-Mannich Bases

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Abstract □ The antimicrobial and antifungal activities of 29 congeneric isatin *N*-Mannich bases were investigated by testing against standard test microorganisms and 21 pathogenic Gram-negative microorganisms. Considerable growth inhibition of Gram-negative bacteria and yeasts and slight inhibition of Gram-positive bacteria resulted when they were treated with the various *N*-Mannich bases of isatin and 5-nitroisatin, respectively.

Keyphrases □ Isatin *N*-Mannich bases—screened for antimicrobial and antifungal activities □ Nitroisatin *N*-Mannich bases—screened for antimicrobial and antifungal activities □ Antimicrobial activity—series of isatin and 5-nitroisatin *N*-Mannich bases screened □ Antifungal activity—series of isatin and 5-nitroisatin *N*-Mannich bases screened

Interest in biologically active isatin (indole-2,3-dione) derivatives, particularly hydrazones, is increasing (1, 2). The biological activities of isatin-3-*o*-nitrophenylhydrazones against the Walker carcinosarcoma 256 were reported (3). Results indicated that all compounds except the parent compound, *i.e.*, isatin-3-*o*-nitrophenylhydrazone, were inactive. Methyl and ethyl derivatives of some isatin-3-thiosemicarbazones, however, were more active biologically than the parent compound (4).

Preliminary screening results for antiviral, antibacterial, and antifungal activities of some isatin *N*-Mannich bases were reported (5). Isatin *N*-Mannich bases with morpholine and piperidine moieties as amino components showed some activity against certain Gram-negative bacteria but were inactive against tested fungi and yeasts.

This study concerns a series of structurally related *N*-Mannich bases of isatin that have not been tested for antimicrobial and antifungal activities. This series includes a group of compounds substituted in position 1 (Mannich bases) and a group substituted in positions 1 and 3 (Mannich base hydrazones)[1] (6). All compounds were tested against various microorganisms.

EXPERIMENTAL

Materials—Isatin *N*-Mannich bases (Table I) were prepared according to the previously described procedure[1] (6).

Test Microorganisms—Compounds I–XXIX were subjected to antibacterial and antifungal screening procedures against the following test microorganisms[2]: *Micrococcus flavus* (ATCC 10240), *Sarcina lutea* (FDA 1001), *Sarcina lutea* (ATCC 9341), *Staphylococcus albus*, *Staphylococcus aureus* (ATCC 209-P), *Staphylococcus aureus* (ATCC 6538-P), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus faecalis* (ATCC 8043), *Brucella bronchiseptica* (ATCC 4617), *Escherichia coli* (99-1), *Escherichia coli* (ATCC 10536), *Klebsiella pneumoniae* (ATCC 10031), *Mycobacterium smegmatis*, *Candida albicans*, and *Candida monosa*.

The following pathogenic microorganisms were used[3]: *Aerobacter aerogenes* (two strains), *Escherichia coli* (13 strains), *Klebsiella* sp. (one strain), *Proteus mirabilis* (two strains), *Proteus morganii* (one strain), and *Pseudomonas aeruginosa* (two strains).

Assay—Preliminary assays were performed with 15 Gram-negative and Gram-positive microorganisms grown on agar[4]. The diffusion technique generally used for antibiotic screening was adopted both for growth inhibition studies and determination of the minimum inhibitory concentration. Stock solutions (1 mg/ml) of the compounds were prepared in 96% ethanol (I–XIII and XVI–XXV) or dimethylformamide (XIV, XV, and XXVI–XXIX). Working solutions of 0.5 and 0.25 mg/ml were prepared by dilution of stock solution with pH 7.4 phosphate buffer. Paper disks were immersed into these solutions, drained, and placed onto the nutrient surface.

The cup–plate technique was used to determine minimum inhibitory concentrations. Several dilutions containing 0.06, 0.125, 0.25, 0.50, 1.0, 2.5, 5.0, 10.0, 20.0, 40.0, 62.5, 125, and 250 μg of compounds/ml were prepared, and 100-μl portions were transferred to 9-mm diameter holes cut into the agar plates with a micropipet. Compounds that had a strong inhibitory effect in preliminary testing were retested against the second group of microorganisms. These tests were made at two concentration levels, 10.0 and 0.125 μg/ml, respectively. In all tests, the cultures were incubated at 37° for 16–18 hr before the diameters of inhibition zones surrounding each disk or hole were measured.

RESULTS AND DISCUSSION

Preliminary values for growth inhibition of different microorganisms treated with isatin and nitroisatin derivatives are given in Tables II and III. Most isatin *N*-Mannich bases strongly inhibited Gram-negative bacteria and fungi but only moderately inhibited the growth of Gram-positive bacteria (Table II). Compounds V, VI, and XIII were more active against *M. flavus*, a Gram-positive microorganism, than against some Gram-negative bacteria, as evidenced by the relative size of the inhibition zones. Compound XIII was particularly active against all test organisms, and VII was active against all test organisms but *M. flavus*.

In several instances, the diameters of the inhibition zones with various concentrations of the test compounds did not differ markedly, *e.g.*, for X against *E. coli* (99-1), *K. pneumoniae*, and *C. monosa*; for XI against *C. monosa*; and for XIII against *M. flavus* and *S. lutea* (ATCC 9341). The inhibition zones increased with a decrease in concentration for some compounds, *e.g.*, V against *E. coli* (ATCC 10536 and 99-1), *B. bronchiseptica*, and *C. albicans*; VII against *S. lutea* (ATCC 9341), *E. coli* (ATCC 10536), *C. albicans*, and *C. monosa*; X against *M. smegmatis* and *C. albicans*; XI against *M. flavus* and *K. pneumoniae*; XIII against *S. lutea* (ATCC 9341), *E. coli* (99-1); and XV against *E. coli* (ATCC 10536 and 99-1).

Table II also shows that most isatin nitrogen mustards (IV, VIII, XII, and XVII) were least active against all tested microorganisms. Compound IV showed slight inhibition against *K. pneumoniae* except at the highest concentration (1 mg/ml). Compounds VIII, XII, and XVII were active at the highest concentrations against the acid-fast *M. smegmatis*. Furthermore, XII showed some activity against *B. bronchiseptica* at both 0.5 and 1 mg/ml. *C. monosa* was inhibited at the lowest concentration by XVII.

Compound III, closely related structurally to nitrogen mustards, showed some activity against *K. pneumoniae* at the maximum concentration, but XVI was inactive. Compound IX was active against Gram-positive *M. flavus* at two concentrations. The observed dependencies of inhibition zones on the concentration of the test compounds could be attributed to effects of solvent interaction with test compounds and/or limiting solubility of the test compounds in agar, as was found with XVI and XVIII, which partially precipitated.

[1] See M. Movrin and M. Medić-Šarić, *Eur. J. Med. Chem.*, **13**, 309 (1978).

[2] Obtained from ATCC, FDA, and the Institute for the Control of Drugs.

[3] Obtained from the Microbiological Laboratory at the hospital "Dr. Ozren Novosel," Zagreb, Yugoslavia.

[4] Difco.

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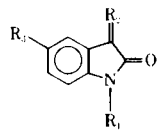

Table I—Synthesized Isatin *N*-Mannich Bases

Compound	R_1	R_2	R_3	Reference
I		O	H	7
II		O	H	7
III	$CH_2N(CH_2CH_2OH)_2$	O	H	—[a]
IV	$CH_2N(CH_2CH_2Cl)_2$	O	H	8
V	H	$NNHCOCH_3$	H	6
VI		$NNHCOCH_3$	H	6
VII		$NNHCOCH_3$	H	6
VIII	$CH_2N(CH_2CH_2Cl)_2$	$NNHCOCH_3$	H	6
IX	H	$NNHCH_3$	H	6
X		$NNHCH_3$	H	6
XI		$NNHCH_3$	H	6
XII	$CH_2N(CH_2CH_2Cl)_2$	$NNHCH_3$	H	6
XIII	H	$NNHCSNH_2$	H	10
XIV		$NNHCSNH_2$	H	5
XV		$NNHCSNH_2$	H	5
XVI	$CH_2N(CH_2CH_2OH)_2$	$NNHCSNH_2$	H	—[a]
XVII	$CH_2N(CH_2CH_2Cl)_2$	$NNHCSNH_2$	H	8
XVIII	H	O	NO_2	9
XIX		O	NO_2	—[a]
XX		O	NO_2	—[a]
XXI	$CH_2N(CH_2CH_2Cl)_2$	O	NO_2	—[a]
XXII	H	$NNHCH_3$	NO_2	—[a]
XXIII		$NNHCH_3$	NO_2	—[a]
XXIV		$NNHCH_3$	NO_2	—[a]
XXV	$CH_2N(CH_2CH_2Cl)_2$	$NNHCH_3$	NO_2	—[a]
XXVI	H	$NNHCSNH_2$	NO_2	—[a]
XXVII		$NNHCSNH_2$	NO_2	—[a]
XXVIII		$NNHCSNH_2$	NO_2	—[a]
XXIX	$CH_2N(CH_2CH_2Cl)_2$	$NNHCSNH_2$	NO_2	—[a]

[a] M. Movrin and M. Medić-Šarić, *Eur. J. Med. Chem.*, 13, 309 (1978).

Table III shows that of 12 5-nitroisatin derivatives, only XXVI–XXIX inhibited considerably the acid-fast bacteria, the Gram-negative bacteria except *B. bronchiseptica*, and the test fungi. The same compounds, except XXVII and XXVIII, were inactive against the Gram-positive bacteria; XXVII and XXVIII showed some activity against *S. aureus* (ATCC 6538-P) and *S. epidermidis*. Compounds XVIII–XXV did not show any inhibition except XVIII, which affected the growth of *K. pneumoniae*. Compounds XX and XXV were completely inactive in all concentrations against any tested microorganism.

The diameter of inhibition zones increased with a decrease in concentration with XXVI against *K. pneumoniae* and *C. monosa*, with XXVII against *E. coli* (ATCC 10536), and with XXVIII against *E. coli* (99-1), *K. pneumoniae*, *C. albicans*, and *C. monosa*. Compounds XXII–XXV did not show any effect against the test microorganisms, except XXII and XXIV very slightly inhibited *B. bronchiseptica* and XXIII inhibited *S. lutea* (ATCC 9341).

Compounds XVIII–XXI did not show any inhibition against *M. smegmatis*, *E. coli*, and fungi. These compounds showed the strongest resistance with *S. lutea* (ATCC 9341), *S. aureus* (ATCC 209-P), *S. albus*, *S. faecalis*, and *M. flavus*.

Based on the results presented in Tables II and III, the 12 most biologically active substances, *i.e.*, V–VII, X, XI, XIII–XVIII, and XXIX, were further tested to determine their minimum inhibitory concentrations. Three Gram-negative bacteria, *i.e.*, *E. coli* (99-1), *E. coli* (ATCC 10536), and *K. pneumoniae*, which were maximally inhibited in the presence of these substances, were used for minimum inhibitory concentration determinations. The results obtained are depicted in Fig. 1.

E. coli (99-1) was inhibited by all compounds at 0.125 μg/ml. The demonstration of strong antibacterial activity by the 12 compounds included in Fig. 1 stimulated interest to test some pathogenic strains of the Gram-negative bacteria. Of the 13 pathogenic *E. coli* strains, seven were strongly inhibited by all compounds (I–XXIX). Five other strains were

Table II—Antibacterial and Antifungal Activities of Isatin *N*-Mannich Bases [a]

Compound	Diameter of Inhibition Zones, mm × 10											
	M. flavus			*S. lutea* (ATCC 9341)			*M. smegmatis*			*E. coli* (ATCC 10536)		
I	–	–	–	–	–	170	–	–	–	–	–	–
II	–	–	–	–	–	–	–	–	–	–	–	–
III	–	–	–	–	–	–	–	–	–	–	–	–
IV	–	–	–	–	–	–	–	–	–	–	–	–
V	190	210	225	–	–	–	240	210	240	155	160	148
VI	223	–	–	–	–	–	170	170	180	–	140	156
VII	–	–	–	148	150	145	210	210	220	158	155	152
VIII	–	–	–	–	–	–	–	–	158	–	–	–
IX	135	142	–	–	–	–	–	–	–	–	–	–
X	179	182	188	–	–	–	220	223	210	170	161	248
XI	272	250	132	–	–	–	200	160	220	±	±	145
XII	–	–	–	–	–	–	–	–	170	–	–	–
XIII	182	180	181	148	145	145	210	220	260	140	147	150
XIV	–	–	–	–	–	–	–	180	201	185	160	180
XV	–	–	–	–	–	–	–	–	302	178	172	155
XVI	–	–	–	–	–	–	–	–	–	–	–	–
XVII	–	–	–	–	–	–	–	–	180	–	–	–
Concentration [b]	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3

Compound	Diameter of Inhibition Zones, mm × 10														
	E. coli (99-1)			*K. pneumoniae*			*B. bronchiseptica*			*C. monosa*			*C. albicans*		
I	–	–	–	–	–	142	–	–	–	–	–	–	–	–	–
II	158	153	159	–	–	149	–	–	162	–	–	–	–	–	–
III	–	–	–	–	–	161	–	–	–	–	–	–	–	–	–
IV	–	–	–	–	–	146	–	–	–	–	–	–	–	–	–
V	300	158	147	181	175	189	180	170	170	195	200	210	180	180	178
VI	208	245	310	177	151	162	–	–	–	177	190	240	148	198	211
VII	>300	300	>300	162	161	182	179	181	190	250	220	212	248	202	160
VIII	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IX	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
X	>300	300	>300	185	182	185	145	160	162	200	201	202	205	202	192
XI	238	208	290	212	192	171	±	162	±	198	190	198	158	160	212
XII	–	–	–	–	–	–	–	170	172	–	–	–	–	–	–
XIII	190	168	148	158	181	175	170	175	180	188	210	210	198	198	180
XIV	280	250	248	201	212	203	–	–	–	200	210	215	170	185	205
XV	255	220	217	201	205	201	–	–	–	–	–	218	–	–	210
XVI	–	–	–	–	–	–	–	–	–	165	170	172	–	–	–
XVII	–	–	–	–	–	–	–	–	–	168	–	–	–	–	–
Concentration [b]	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3

[a] ± = minimally observable inhibition, and – = no inhibition. [b] T_1 = 0.25 mg/ml, T_2 = 0.5 mg/ml, and T_3 = 1 mg/ml.

Table III—Antibacterial and Antifungal Activities of 5-Nitroisatin *N*-Mannich Bases[a]

Compound	Diameter of Inhibition Zones, mm × 10														
	S. lutea (FDA 1001)			*S. aureus* (ATCC 6538-P)			*S. epidermidis*			*M. smegmatis*			*E. coli* (ATCC 10536)		
XVIII	–	–	–	–	165	200	–	–	138	–	–	–	–	–	148
XIX	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
XX	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
XXI	–	–	160	–	–	–	–	–	–	–	–	–	–	–	–
XXII	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
XXIII	–	–	268	–	–	–	–	–	–	–	–	–	–	–	–
XXIV	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
XXV	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
XXVI	–	–	–	–	–	–	–	–	–	160	185	215	168	162	181
XXVII	–	–	–	–	160	180	±	±	148	235	290	310	168	163	151
XXVIII	–	–	–	153	–	–	158	–	–	280	–	–	140	152	171
XXIX	–	–	–	–	–	–	–	–	–	160	192	210	153	160	165
Concentration[b]	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3

Compound	*E. coli* (99-1)			*K. pneumoniae*			*B. bronchiseptica*			*C. monosa*			*C. albicans*		
XVIII	–	–	–	141	162	187	–	–	–	–	–	–	–	–	–
XIX	–	–	–	–	–	150	–	152	158	–	–	–	–	–	–
XX	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
XXI	–	–	–	–	–	151	–	–	159	–	–	–	–	–	–
XXII	–	–	–	–	–	–	170	162	–	–	–	–	–	–	–
XXIII	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
XXIV	–	–	–	–	–	–	–	168	180	–	–	–	–	–	–
XXV	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
XXVI	160	180	185	203	202	196	–	–	–	201	195	188	170	185	202
XXVII	170	190	195	208	212	220	–	–	–	245	260	260	202	220	238
XXVIII	180	175	165	212	191	191	–	–	–	228	168	165	215	160	160
XXIX	148	170	190	206	200	298	–	–	–	188	205	210	170	175	–
Concentration[b]	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3

[a] ± = minimally observable inhibition, and – = no inhibition. [b] T_1 = 0.25 mg/ml, T_2 = 0.5 mg/ml, and T_3 = 1 mg/ml.

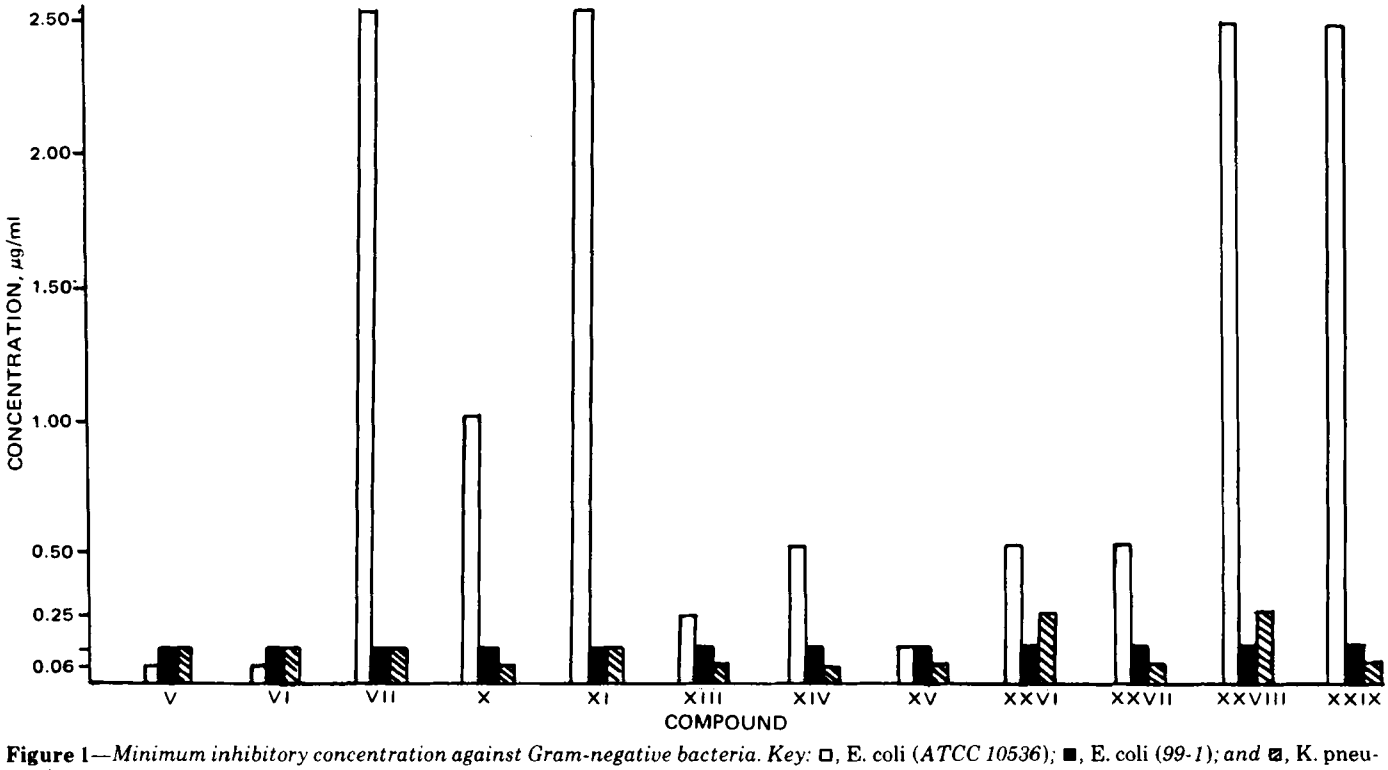

Figure 1—*Minimum inhibitory concentration against Gram-negative bacteria. Key:* □, E. coli (*ATCC 10536*); ■, E. coli (*99-1*); *and* ▨, K. pneumoniae.

moderately resistant and one *E. coli* strain was highly resistant to the action of any isatin derivative at 1 mg/ml.

Compounds I–XXIX also were tested against *P. mirabilis* but antimicrobial activity was not observed at 1 mg/ml or less.

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