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

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Abstract □ Preliminary antiviral, antibacterial, and antifungal screening results of a series of isatin *N*-Mannich bases are provided.

Keyphrases □ Isatin *N*-Mannich bases—synthesis, screened for antiviral, antibacterial, and antifungal activities □ Antiviral activity—synthesis and screening of isatin *N*-Mannich bases □ Antibacterial and antifungal activities—synthesis and screening of isatin *N*-Mannich bases

Isatin *N*-Mannich bases (1–3) were synthesized by the condensation of isatin(s), formaldehyde, and a suitable amine in equimolar proportions. Preliminary screening results for antiviral, antibacterial, and antifungal activities are described in this report. Antiviral activity in isatin *N*-Mannich bases was observed previously (2).

EXPERIMENTAL

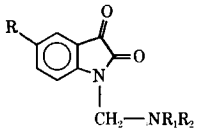
Antiviral screening was done using a modified[1] plaque suppression method (4, 5). Viruses used were poliomyelitis type II (RNA type), herpes simplex (DNA type), measles (RNA type), and parainfluenza-3 (RNA type). The viruses stock cultures were maintained in cultures of HeLa cells grown in Eagle's minimal essential medium. Confluent sheets of HeLa cells (a stable line of human adenocarcinoma cells) in petri dishes were heavily infected with the test virus and then overlaid with agar according to the Siminoff (5) procedure.

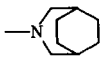
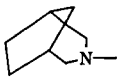
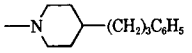
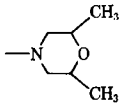
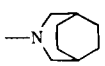
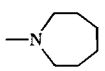
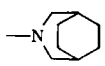
Filter paper disks (6.35 mm) saturated with 2 drops of a suspension of the test compound (20 mg/ml in ethanol) were then placed on the agar. After incubation for 2–4 days, the cells were stained with either neutral red or 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride, which stained living cells only. Killing of the carcinoma cells was indicated by an unstained zone around the paper disk. Inhibition of the virus was indicated by a suppression of viral plaques in the vicinity of the chemical-saturated disk. Positive antiviral tests are shown in Table I. All other tests with these compounds showed no antiviral activity.

Antibacterial and antifungal screening was done with similar paper disks containing the test compounds. These disks were placed on plates of agar that had been freshly smeared with the test organism. Nutrient agar for bacteria and Sabouraud dextrose agar for yeasts and other fungi were used. Twelve[2] representative microbial types were chosen to observe possible drug activity against a wide spectrum of different organisms. The test organisms included Gram-positive human pathogens, Gram-negative human pathogens, acid-fast bacteria (representing the leprosy/tuberculosis group), common yeasts, and pathogenic fungi.

A clear zone around any test disk in a petri plate in which growth of the test microbe was confluent was interpreted to mean that the chemical on that disk had antimicrobial activity. The width of the zone of inhibition was not considered to be significant, since diffusion in agar varies with the solubility and other characteristics of the test compound. Results of the antibacterial and antifungal tests are given in Table II.

Table I—Antiviral Activity[a] of Isatin *N*-Mannich Bases



Compound	R	—NR_1R_2	Toxicity to Cancer Cells	Polio II
1	H		+	+
2	H		−	+
3	H		+	+
4	H		+	+
5	H		+	+
6	CH_3			−
7	CH_3		+	+
8	CH_3		+	−
9	Br			−
10	Br		+	+
11	Br		+	+
12	Br		+	+

[a] + = antiviral activity (or toxicity to cancer cells); − = no activity. Compound 1 was also active against parainfluenza-3, herpes simplex, and measles viruses. A blank indicates that this particular assay was not performed. All other antiviral tests gave negative results.

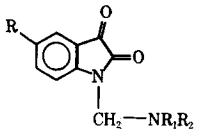
DISCUSSION

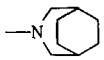
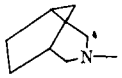
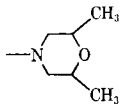
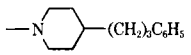
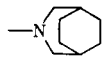
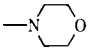
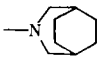
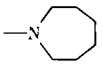
Twelve isatin *N*-Mannich bases incorporating dimethylamino, morpholino, piperidino, 3-azabicyclo[3.2.2]nonano, and 3-azabicyclo[3.2.1]octano moieties were subjected to antiviral screening procedures (Table I). Nine compounds showed activity against polio II virus. Compound 1 displayed activity against four different viruses; its effect was much more pronounced than that produced by

[1] Modifications suggested in a personal communication from Dr. Richard Stewart, Virology Laboratory, Smith Kline and French Co., Philadelphia, Pa.

[2] The microbial spectrum consisted of *Staphylococcus aureus* (K257)(+), *Escherichia coli* (ATCC 4157)(−), *Pseudomonas aeruginosa* (LBa 160)(−), *Klebsiella pneumoniae* (ATCC 8052)(−), *Proteus vulgaris* (LBa 155)(−), *Mycobacterium smegmatis* (Carolina Biological Supply Co.)(+), *Neisseria catarrhalis* (LB130)(−), *Saccharomyces* sp., *Candida albicans* (ATCC 10231), *Trichophyton mentagrophytes* (ATCC 9129), *Staphylococcus epidermidis*, and *Aspergillus niger* organisms. All cultures without identification numbers or source are from the University of Mississippi Department of Biology.

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



Compound	R	—NR_1R_2	Acid-Fast Bacteria	Gram-Positive Bacteria	Gram-Negative Bacteria	Fungi and Yeasts
1	H		−	+	+	+
2	H		−	−	−	−
3	H		−	+	+	+
4	H		−	+	+	+
5	H		−	−	−	+
6	H		−	+	+	−
7	H		−	+	+	+
8	H		+	+	+	+
9	CH_3		−	+	+	+
10	CH_3		−	+	+	+
11	CH_3		−	+	+	−
12	Br		−	+	+	+
13	Br		+	+	+	+
14	Br	—$N(CH_3)_2$	−	+	+	+
15	Br		+	+	+	+
16	Br		−	+	+	+

[a] + = inhibition; − = no inhibition.

any other compound studied. Compounds showing toxicity to cancer cells may have anticancer significance.

Antibacterial and antifungal activities of 16 isatin *N*-Mannich bases are described in Table II. Three compounds (Compounds 8, 13, and 15) showed activity against all four types of organisms. Compound 2 did not show any activity.

REFERENCES

(1) R. S. Varma and W. L. Nobles, *J. Heterocycl. Chem.*, **3**, 462(1966).

(2) R. S. Varma and W. L. Nobles, *J. Med. Chem.*, **10**, 510(1967).

(3) *Ibid.*, **10**, 972(1967).

(4) E. C. Herrmann, Jr., J. Gabliks, C. Engle, and P. L. Perlman, *Proc. Soc. Exp. Biol. Med.*, **103**, 625(1960).

(5) P. Siminoff, *J. Appl. Microbiol.*, **9**, 66(1961).

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