Journal of the Pharmaceutical Society of Korea
17, 141—146 (1973)

Syntheses and Antimicrobial and Antitumor Activities of Isatin Derivatives

Yun Sung Chough and Kun Il Kang*

(Received May 5, 1973)

Abstract—Eight isatin N-Mannich bases were synthesized and tested their antimicrobial and antitumor activities. N-Piperidinomethylisatin-(N-cyclohexyl)-thiosemicarbazone is active against *St. aureus* at 10μg/disk and isatin thiosemicarbazone against *P. chrysogenum* at 10μg/disk.

Numerous studies on the antiviral properties of isatin thiosemicarbazones have been carried out.[1-8] Xuong[9] and Koshimura, *et al.*[10] found that isatin hydrazones and isatin thiosemicarbazones demonstrated tuberculostatic activity.

In addition, isatin hydrazone derivatives were shown by Willey [11] and Popp [12,13] to exhibit antitumor activity. In view of the pharmacological properties of N-Mannich bases,[14] Varma[15-17] and Ciba[18] synthesized several N-Mannich base derivatives of isatin and reported that Isatin N-Mannich base thiosemicarbazones demonstrated antitumor, antihistamic, analgesic and antibacterial activitios against *Peseudomonas septica*, *Mycobacterium tuberculosis* and *Staphylococus aureus* and showed also antiviral activity.

In connecting with these interesting facts, eight isatin hydrazone and thiosemicarbazone derivatives were prepared and subjected to pharmacological tests for antimicrobial and antitumor activities.

Interest in the orotic acid hydrazide for the synthesis of isatin hydrazones stemmed from the fact that orotic acid is precursor of nucleic acid and had been tried for the preparation of anticancer agents[19].

Interest, too, was prompted by the fact that orotic acid hyrazide acts as effective antifungal agent[20].

Thiosemicarbazone derivatives are known to have antimicrobial activities[21], and more of

* Department of Pharmacodynamics, College of Pharmacy, Seoul National University, Seoul, Korea.

these compounds have been synthesized in order to find compounds with greater antitumor activities, one of which is 1-formylisoquinoline thiosemicarbazone by Agrawal, *et al.*[22]

Paying attention to Varma's report that among the isatin N-Mannich base derivatives the compounds bearing such amines as piperidine and morpholine are more active, we attempted to synthesize isatin N-Mannich base thiosemicarbazone derivatives furthermore for testing of antimicrobial and antitumor activities.

EXPERIMENTAL

5-Methylisatin—This intermediate was prepared according to the method submitted by Marvel, *et al* [23].

Isatin N-Mannich Bases (general procedure)—According to Varma, these intermediates were prepared [15].

N^4-Substited Thiosemicarbazide (general procedure)—These intermediates were prepared according to Nardi with modifications [24].

N-Piperidinomethyl isatin-(N^4-cyclohexyl) thisemicarbazone (I)—To a solution of 0.01 mole of N-Piperidinomethylisatin in 15ml of absolute ethanol was added, in one portion, 0.01 mle of N-cyclohexylthiosemicarbazide dissolved in 20 ml of ethanol by heating. The reaction mixture was heated to 80°and refluxed at this temperature for an hour. At the end of this time the contents of flask were cooled and the product was collected by filteration. The product was then recrystallized from small amouns of ethanol.

N-Piperidinomethyl-5-methyl isatin-(N^4-cyclohexyl)-thiosemicarbazone (II)— This material was prepared by the same method as for **I.**

N-Morpholinomethyl-5-methyl itatin-(N^4-cyclohexyl)-thiosemicarbazone (III)—To a solution of 0.01 mole of N^4-morpholinomethylisatin in 15 ml ethanol was added, in one portion, 0.01 mole of cyclohexyl thiosemicarbazide. The reaction mixture was stirred overnight at room temperature. The resulting solid was collected by filtration and washed with absolute ethanol.

Orotic Acid Hydrazide—The orotic acid butylester and orotic acid hydrazide were prepared by published method[19].

Isatin-orotic Acid Hydrazide (IV)—Orotic acid hydrazide 0.01mole was dissolved in 100ml of hot ethanol. To this solution, 0.01 mole of isatin in 50 ml ethanol was added. The mixture was refluxed for an hour and allowed to cool to room temperature. The product was removed by filtration and recrystallized from DMF-ethanol. The crystals washed thoroughly with ethanol.

5-Bromoisatin—The procedure of Liebermann [25] was employed to give 5.4g (76%) yield from 4.4 g isatin (0.03 mole).

5-Bromoisation-(N^4-substituted) Thiosemicarbazones (general procedure)—To 0.01 mole of 5-bromoisatin dissolved in 50ml ethanol by heating was added 0.01 mole of N^4-substituted thiosemicarbazide in 50 ml hot ethanol. The mixture was refluxed for an

hour. After cooling to room temperature the crystals which separated were collected on a filter. The crystals were recrystallized from ethanol.

Thiocarbohydrazide—Published method was used[26].

Isatin-thiocarbohydrazone (VIII)—To a solution of thiocarbohydrazide (0.01 mole) in small amount of H_2O was added a solution of isatin (0.01 mole) in ethanol. The mixture was stirred under refluxed for an hour. The precipitation was collected by filtration and recrystallized from ethanol to give yellow crystals.

Antimicrobial Activity—The compounds were tested for the inhibitory effect on the growth of *E. coli*, *St. aureus* and *P. chrysogenum* Q-176, using filter paper disk method. For the culture medium, nutrient agar (pH 7.0) was employed for *E. coli*, peptone-casein agar (pH6.5) for *St. aureus*, Czapeck agar (pH 6.2) for *P. chrysogenum*. All compounds were dissolved in polyethylene glycol 400. Disks (Whatman 1 filter paper) moistend with the samples were placed on the inoculated agar plate. After incubation at regulated conditions (*E. coli* and *St. aureus* at 37° for 16-20 hrs; *P. chrysogenum*, at 25° for 48 hrs), zone of inhibition was measured.

Antitmor Activity—Tissue culture assay method was employed for the testing of cytotoxicity of the compounds. Assay was performed by the monolayer culture method against HeLa-S_3 strain. Stock cultures were cultivated on YLE medium plus 10% calf serum. Cells were removed from the culture bottle with trypsin. The cells were diluted to 40—50 g of cells protein/ml YLE medium plus 10% calf serum. 0.9ml of abovemedium was implanted in a series of replicate 16×122 mm screw cap culture tubes and 0.1ml of the test compounds were added at tenfold dilutions resulting in final concentration at 1 to 100μg/ml of medium. The culture tubes were incubated at 37° at a 10° angle. After incubation for 72 hrs, growth rate of cells was calculated from protein content measured by the method of Oyma and Eagle[27], using bovine serum albumine as a reference. The compounds showing greater than 50% inhibition of cell growth at the concentration of 10 μg/ml of medium were considered active.

RESULTS AND DICSUSSION

The compounds synthesized were shown in Table I.

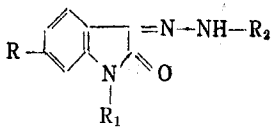
Microanalysis and ir data showed that thiosemicarbazide group of isatin thiosemicarbazone (**VIII**) does not have cyclization.

As shown in Table II, compound **I** is active against *St. aureus*, compound **III** against both *St. aureus* and *P. chrysogenum*, and compound **VIII** against both *E. coli* and *P. chrysogenum*.

Since the compounds to be tested were insoluble in water and the suspensions in other solvents were very turbid, it was impossible to test by tube dilution method and check the growth of origanism by spectrophotometer.

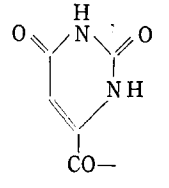
While Worthen[28] used the filter paper disks (12.7mm, diameter) saturated with 0.1ml

Table I—Isatin derivatives.



Comp. No.	R	R_1	R_2	mp	Yield (%)	Recryst. solvent	Formula	Analysis(%)	
								Calcd	Found
I	H	piperidinomethyl	-CS-NH-cyclohexyl	130-132	79	EtOH	$C_{21}H_{29}ON_5S$	C 63.16	C 62.96
								H 7.30	H 7.47
								N 7.54	N 17.43
II	CH_3	piperidinomethyl	-CS-NH-cyclohexyl	180-182	87	EtOH	$C_{22}H_{31}ON_5S$	C 63.90	C 63.42
								H 7.50	H 7.55
III	CH_3	morpholinomethyl	-CS-NH-cyclohexyl	133-134	68	Ethyl-acetate	$C_{21}H_{29}O_2N_5S$	C 60.72	C 59.94
								H 6.98	H 7.14
IV	H	H	A*	300	87	DMF-EtOH	$C_{13}H_9O_4N_5$	C 52.17	C 51.82
								H 3.01	H 3.54
V	CH_3	H	A*	300	93	DMF-EtOH	$C_{14}H_{11}O_4N_5$	C 53.67	C 53.97
								H 3.51	H 3.97
								N 22.36	N 21.95
VI	Br	H	-CS-NH-naphthyl	256-258 (dec.)	92	EtOH	$C_{19}H_{13}ON_4SBr$	C 53.64	C 53.21
								H 3.05	H 3.38
VII	Br	H	-CS-NH-cyclohexyl	258-262 (dec.)	89	EtOH	$C_{15}H_{17}ON_4SBr$	C 47.24	C 47.64
								H 4.46	H 4.99
VIII	H	H	$-CS-NHNH_2$	268 (dec.)	94	EtOH	$C_9H_9ON_5S$	C 45.95	C 46.13
								H 3.82	H 3.71
								N 29.79	N 29.19

A*;



of 1 : 1,000 concentration of the compounds and Benns *et al.*[29], 1cm disks impregnated with 5% solution or suspension, we employed 6.5mm disks saturated with 0.01 ml of a 1 : 10,000 concentration.

N-Piperidinomethyl isatin-(N^4-cyclohexyl) thiosenicarbazone (**I**) showed antibacterial activity against *St. aureus*, while N-piperidinomethylisatinthiosemicarbazone showed antibacterial activity against gram negative organism[17].

It was also interesting that isatin thiocarbohydrazone (**VIII**) had antibacterial activity against *E. coli* and *P. chrysogenum* as had been found out by Lee, *et al.*[30], that some of thiocarbohydrazone Schiff bases showed excellent antimicrobial activities.

The preliminary results obtained from the cytotoxicity testing of compounds were also shown in Table II and the data by microscopic observation were approximately identical with those by protein measurement. 2.5% acacia water and 0.5% PEG 400 did not influence on the cell growth. As shown in Table II, compound **IV**, **VI** and **VII** showed cytotoxicity against HeLa cell at the 10 μg/ml level.

Table II-*In vitro* biological activity.

comp.[a]	Antimicrobial activity[b]			Cytotoxicity
	E. coli	*St. aureus*	*P. chrysogenum*	T/C[c], HeLa cell
I	—	10	—	+
II	—	—	—	17
III	—	50	50	+
IV	—	—	—	60
V	—	—	—	+
VI	—	—	—	70
VII	—	—	—	83
VIII	50	—	10	+

a; Compounds were dissolved in PEG 400 and diluted in 2.5% acacia water.

b; Minimal inhibitory amounts of samples, μg/disk.

c; $\frac{\text{Protein content of the test group}}{\text{Protein content of control group}} \times 100$.

concentration; 10 μg/ml of medium.

—; Inactive up to 100μg/disk of sample.

+; Normal cell growth.

REFERENCES

1. R.L. Thomson, S.A. Minton, J.E. Officer, and GH. Hitchinges, *J. Immunol.*, **70**, 229(1953)
2. D.J. Bauer, *Brit. J. Exptl. Pathol.*, **36**, 105(1955)
3. E. Campaigne, *J. Med. Pharm. Chem.*, **1**, 577(1959)
4. D.J. Bauer, *Brit. J. Pharmcol.*, **15**, 101(1960)
5. P.W. Sadler, *J. Chem. Soc.*, **1961**, p-243

6. D.J. Bauer, *Bull. World Health Organ.*, **26**, 727(1962)
7. G.D. Person and E.F. Zimmerman, *Virology*, **38**, 641(1969)
8. J.H. Hunt, D. Jack, and Z.M.Z. Gladych, *Nature*, **221**, 286(1969)
9. N.D. Xuong, *J.Chem. Soc.*, **1953**, 1358
10. S. Koshimura, *Ann. Rept. Res. Inst. Tuberc. Kanazawa Univ.*, **12**, 5(1954)
11. R.H. Willey, *J. Org. Chem.*, **24**, 1925(1959)
12. F.D. Popp and G. Irick, *J. Med. Chem.*, **10**, 986(1967)
13. F.D. Popp, F.P. Silver, and A.C. Noble, *J.M. Chem.*, **12**, 182(1969)
14. S. Foldeak, *Chem. Abstr.*, **64**, 9670(1966)
15. R.S. Varma, *J. Heterocyclic Chem.*, **3**, 462(1966)
16. R.S. Varma and W.L. Nobles, *J. Med. Chem.*, **10**, 510(1967)
17. *Ibid*, **10**, 972 (1967)
18. Ciba Ltd., Neph. Appl., 5,507,422; *C.A.*, **65**, 2227g(1969)
19. L.O. Ross, L. Goodmanand and B.R. Baker, *J. Org. Chem.*, **25**, 1950(1960)
20. Bristol Lab., U.S. Pat. No.2, 832,782; *C.A.*, **52**, 20863h(1958)
21. D.M. Wiles, *J. Med. Chem.*, **12**, 323(1970)
22. K.C. Agrawal and A.C. Sartorelli, *J. Med. Chem.*, **12**, 771(1969)
23. C.S. Marvel, *Org. Syn.*, Coll. Vol. I, 327(1955)
24. D. Nardi, E. Massarani, A. Tajana, L. Degen, and M.J. Magistretti, *J. Med. Chem.*,**10**, 530(1967)
25. Liebermann, *Ber.*, **40**, 2501(1607)
26. L.H. Audrieth, *J. Org. Chem.*, **19**, 733(1954)
27. V.I. Oyama and H. Eagle, *Proc. Soc. Exptl. Biol. Med.*, **91**, 305(1956)
28. L.R. Worthen and H.W. Bond, *J. Pharm. Sci.*, **59**, 1185(1970)
29. B.G. Benns, *et al.*, *Appl. Microbial.*, **8**, 353(1960)
30. S.H. Lee, *This journal*, **16**, 162(1972)