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Synthesis of some Mannich bases of isatin-3-(4′-phenyl-3′-thiosemicarbazone) and their antibacterial activity

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Abstract. Some Mannich bases of isatin-3-(4′-phenyl-3′-thiosemicarbazone) have been prepared by employing formaldehyde and different secondary amines. These Mannich bases have been characterized on the basis of different physico-chemical evidences. These behave as Lewis-bases and have been estimated accordingly in non-aqueous media. Like some alkaloids they also form the reineckate complexes which serve for their estimation. Antibacterial activity of the synthesized Mannich bases has been studied by employing *Escherichia coli* and *Staphylococcus aureus* as bacterial strains.

Keywords. Mannich bases; reineckate complex; antibacterial activity; *E. coli*; *S. aureus*.

1. Introduction

A large number of N-Mannich bases of isatin and its 3-thiosemicarbazone have been prepared and tested for antiviral activity (Lucka-Sobstel and Zejc 1972, 1973; Lucka-Sobstel *et al* 1974; Porebska *et al* 1973; Borysiewicz *et al* 1973, 1975; Zgorniak-Novosielska *et al* 1973; Varma and Nobles 1967). Antiviral activity was found to depend on the character of the basic moieties. In this work we have prepared a series of Mannich bases of isatin 3-(4′-phenyl-3′-thiosemicarbazone) in the hope of getting compounds of enhanced antimicrobial activity.

The reaction was carried out by employing equimolar quantities of isatin 3-(4′-phenyl-3′-thiosemicarbazone), dissolved in tetrahydrofuran (THF)-formaldehyde ($\approx 38\%$) solution, and an amine. The secondary amines used as the basic nucleophilic components were dimethylamine, diethylamine, dipropylamine, di-isopropylamine, di-iso-butylamine, piperidine, morpholine, pyrrolidine, piperazine, N-phenyl-piperazine, dicyclohexylamine, diphenylamine and dibenzylamine. Isatin forms methylol when its aqueous solution is heated with formaldehyde solution in the presence of potassium carbonate; this suggests that methylol is first formed which is then attacked by amines to give Mannich bases.

Mannich bases are fairly stable in solutions of organic solvents like benzene, alcohol, chloroform and acetone, but get hydrolized when heated with acids and bases. The structure of Mannich bases were confirmed by another route. Isatin was first aminoalkylated followed by condensation with 4-phenyl-3-thiosemicarbazide. The bases thus obtained did not show any depression in melting point upon admixing the bases obtained from direct aminoalkylation of isatin 3-(4′-phenyl-3′-thiosemicarbazone).

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Mannich bases accept a proton on the nitrogen atom of the basic aminoalkyl chain and have been assayed by visual titration in non-aqueous media with acetous perchloric acid using 0·5 % acetous crystal violet as indicator (violet to bluish-green) (Vogel 1957).

Molecular formulae of the Mannich bases were checked by the standard reineckate-complex method (Beckett and Stenlake 1962). This method depends upon the precipitation of a Mannich base-reineckate complex, which is formed on addition of ammonium reineckate to an acidified solution of the sample.

2. Experimental

Melting points reported are uncorrected and were determined on a Toshniwal melting point apparatus. Infrared spectra were determined on a Perkin Elmer 720 infrared spectrometer as nujol mulls. Nuclear magnetic resonance spectra were recorded on a VARIAN A-60D analytical NMR spectrometer with $SiMe_4$ as the internal standard. Microanalyses were performed with a Coleman analyser and molecular weights were determined by non-aqueous titration. The Mannich bases were recrystallized from an acetone-ethanol mixture (3:1).

2.1 *4-Phenyl-3-thiosemicarbazide* (1)

An alcoholic solution of phenyl isothiocyanate (67·6 g, 0·5 M) was treated with a solution of hydrazine hydrate (25·03 g, 0·5 M) to furnish (1) (Pulvermacher *et al* 1893, 1894, from Rodd 1954). The resulting product was filtered and recrystallized from amyl alcohol, m.p. 139° (literature value: 140°) (Tisler *et al* 1956) yield 50·70 g (37·69 %).

2.2 *Isatin* 3-(*4'-phenyl-3'-thiosemicarbazone*) (*IPTS*) (2)

4-Phenyl-3-thiosemicarbazide (16·7 g, 0·1 M) dissolved in ethanol (100 ml) was mixed with the solution of isatin (14·7 g, 0·1 M) in water (150 ml) (Halzbecher 1950). The mixture was refluxed on a waterbath for a few minutes. The resulting product obtained after cooling was filtered—m.p. 240–41°, yield 25·0 g (84·4 %) (found: C-60·81; H-4·06; N-18·86; required for $C_{15}H_{12}N_4OS$: C-60·79; H-4·07; N-18·90 %), ν_{max} (nujol) 3300 (N–H), 1695 (C=O), 1625 (C=N), 1468 cm^{-1} (C=S).

2.3 *N-(dimethylaminomethyl) isatin* 3-(*4'-phenyl-3'-thiosemicarbazone*) *hydrochloride* (3)

To the solution of IPTS (2·96 g, 0·01 M) in THF (35 ml) were added dimethylamine (0·7 ml, ≈ 0·01 M) and 38 % aqueous formaldehyde solution (0·8 ml ≈ 0·01 M). The reaction mixture was refluxed for five hrs. The separated oil was dissolved in acetone and excess of hydrochloric acid added. The crystals obtained on cooling were filtered—m.p. 162–63°, yield 1·8 g (46·27 %) (found: C-55·42; H-5·19; N-17·90; mol. wt., 390·20; required for $C_{18}H_{20}ClN_5OS$: C-55·45; H-5·17; N-17·96 %; mol. wt.-389·89), ν_{max} (nujol) 3310 (N–H), 1685 (C=O), 1615 (C=N), 1465 cm^{-1} (C=S).

2.4 *N-(diethylaminomethyl) isatin* 3-(*4'-phenyl-3'-thiosemicarbazone*) *hydrochloride* (4)

To IPTS (2·96 g, 0·01 M) THF (35 ml) were added diethylamine (1·0 ml, ≈ 0·01 M) and 38 % aqueous formaldehyde (0·8 ml. ≈ 0·01 M). The mixture was refluxed for five hrs.

The oil obtained was dissolved in acetone and excess hydrochloride acid added. The resulting product obtained after cooling was filtered—m.p. 87°, yield 1·5 g (35·9%) (found: C-57·43; H-5·80; N-6·72; mol. wt.-416·32; required for $C_{20}H_{24}ClN_5OS$; C-57·47; H-5·79; N-6·75%; mol. wt.-417·94), ν_{max} (nujol) 3305 (N–H), 1685 (C=O), 1612 (C=N), 1465 cm^{-1} (C=S).

2.5 *N-(dipropylaminoethyl) isatin* 3-(4′-*phenyl*-3′-*thiosemicarbazone hydrochloride* (5)

To a solution of IPTS (2·96 g, 0·01 M) in THF (35 ml) were added dipropylamine (1·4 ml, ≈ 0·01 M) and 38% formaldehyde solution (0·8 ml, ≈ 0.01 M). The reaction mixture was refluxed on a water bath for about five hrs. The oil obtained was dissolved in acetone and excess hydrochloric acid added. After cooling the product obtained was filtered—m.p. 111–13°, yield 1·2 g (26·96%) (found: C-59·31; H-6·35; N-15·76; mol. wt.-446·25; required for $C_{22}H_{28}ClN_5OS$: C-59·24; H-6·33; N-15·70%; mol. wt.-445·99), ν_{max} (nujol) 3300 (N–H), 1685 (C=O), 1610 (C=N), 1643 cm^{-1} (C=S).

2.6 *N-(di-isopropylaminomethyl) isatin* 3-(4′-*phenyl*-3′-*thiosemicarbazone) hydrochloride* (6)

IPTS (2·96 g, 0·01 M) dissolved in THF (35 ml) was treated with a mixture of di-isopropylamine (1·4 ml, ≈ 0·01 M) and 38% formaldehyde solution (0·8 ml, ≈ 0·1 M). The reaction mixture was refluxed for two hrs. on a water bath till half the solvent evaporated. The separated oil was dissolved in acetone and excess hydrochloric acid added. The oil obtained again was crystallized from methanol—m.p. 161° (decomposition-D), yield 1·6 g (35·96%) (found: C-59·31; H-6·37; N-15·53; mol. wt.-443·65; required for $C_{22}H_{28}ClN_5OS$: C-59·24; H-6·33; N-15·70% mol. wt.-445·99), ν_{max} (nujol) 3300 (N–H), 1690 (C=O), 1616 (C=N), 1468 cm^{-1} (C=S).

2.7 *N-(di-isobutylaminomethyl) isatin* 3-(4′-*phenyl*-3′-*thiosemicarbazone) hydrochloride* (7)

To a solution of IPTS (2·96 g, 0·01 M) in THF (35 ml) were added diisobutylamine (1·8 ml, ≈ 0·01 M) and a solution of 38% formaldehyde (0·8 ml, 0·01 M). The resulting reaction mixture was refluxed on a water bath for five hrs. The separated oil was dissolved in acetone and excess hydrochloric acid added. The crystals obtained on cooling were filtered, m.p. 230° (D), yield 0·5 gm (10·54%) (found: C-60·76; H-6·85; N-14·82, mol. wt.-475·26; required for $C_{24}H_{32}ClN_5OS$: C-60·80; H-6·80; N-14·77%, mol. wt.-474·04).

2.8 *N-(Piperidinomethyl) isatin* 3-(4′-*phenyl*-3′-*thiosemicarbazone) hydrochloride* (8)

To a solution of IPTS (2·96 g, 0·01 M) in THF (35 ml) were added piperidine (1·0 ml, ≈ 0·01 M) and 38% formaldehyde (0·8 ml, ≈ 0·01 M). The reaction mixture was refluxed on a water bath for five hrs. The oil obtained was dissolved in acetone and excess hydrochloric acid added. The crystals obtained on cooling were filtered—m.p.-105°, yield-1·0 g (23·26%) (found: C-58·55; H-5·70; N-16·02; mol. wt.-428·25; required for $C_{21}H_{24}ClN_5OS$: C-58·65; H-5·62; N-16·28%, mol. wt.-428·96).

2.9 *N-(Morpholinomethyl) isatin*-3-(4′-*phenyl*-3′-*thiosemicarbazone*) (9)

To a solution of IPTS (2·96 g, 0·01 M) in THF (35 ml) were added morpholine (0·9 ml, ≈ 0·01 M) and formaldehyde (0·8 ml, ≈ 0·01 M). The reaction mixture was refluxed on

a water bath for five hrs., one-third of the solvent was evaporated. It was kept in the refrigerator overnight. The separated product was filtered—m.p.-163°, yield-0·5 g (12·65%) (found: C-60·50; H-5·2; N-17·65; Mol. wt.-393·54; required for $C_{20}H_{21}N_5O_2S$: C-60.47; H-5·31; N-17·71, mol. wt.-395·46).

2.10 *N-(Pyrrolidinomethyl) isatin 3-(4'-phenyl-3'-thiosemicarbazone) hydrochloride* (10)

IPTS (2·96 g, 0·01 M) was dissolved in THF (35 ml) and pyrrolidine (0·9 ml, ≈ 0·01 M) and 38% formaldehyde (0·8 ml, ≈ 0·01 M) were added to it. The resulting mixture was refluxed on a water bath for about five hrs. The oil obtained was dissolved in acetone and excess hydrochloric acid added. The product separated after cooling was filtered, m.p. 81°, yield 0·4 g (9·63%) (found: C-57·63; H-5·23; N-16·45; mol. wt.-416·23; required for $C_{20}H_{22}ClN_5OS$: C-57·75; H-5·33; N-16·83%, mol. wt.-415·93), ν_{max} (nujol) 3300 (N–H), 1685 (C=O), 1615 (C=N), 1465 cm^{-1} (C=S).

2.11 *Bis N-N'-piperazino-methyl-isatin-3-(4'-phenyl-3'-thiosemicarbazone)* (11)

To a solution of IPTS (2·96 g, 0·01 M) in THF (35 ml) were added piperazine (1·0 g, ≈ 0·005 M) and 38% formaldehyde solution (0·8 ml, ≈ 0·01 M). The reaction mixture was refluxed on a water bath for 15 minutes. The product separated during refluxing was filtered—m.p. 209–10°, yield 3·0 g (42·73%) (found: C-61·46; H-4·82; N-19·85; mol. wt.-700·05; required for $C_{36}H_{34}N_{10}O_2S_2$: C-61·52; H-4·88; N-19·93%; mol. wt-702·82), ν_{max} (nujol) 3295 (N–H), 1692 (C=O), 1615 (C=N), 1465 cm^{-1} (C=S).

2.12 *N-(N'-phenyl-piperazinomethyl) isatin 3-(4'-phenyl-3'-thiosemicarbazone)* (12)

To a solution of IPTS (2·96 g, 0·01 M) in THF (35 ml) were added N-phenyl piperazine (1·6 g, ≈ 0·01 M) and 38% formaldehyde solution (0·8 ml, ≈ 0·01 M). The reaction mixture was refluxed for four hrs. on a water bath two-thirds of the solvent was evaporated. It was cooled in the refrigerator overnight and the separated crystals were filtered out—m.p. 154°, yield 1·3 g (27·65%) (found: C-66·16; H-5·47; N-17·58; mol.wt.-468·36; required for $C_{26}H_{26}N_6OS$: C-66·36; H-5·57; N-17·86%, mol. wt.-470·57), ν_{max} (nujol) 3305 (N–H), 1690 (C=O), 1615 (C=N), 1470 cm^{-1} (C=S). NMR $\delta(CDCl_3)$ 2·8–3·4 may be due to the eight methylene protons of the piperazine ring. A sharp singlet at 4·6 may be assigned to the two methylene protons between the two nitrogen atoms and a multiplet at 6·9–7·9 is due to fourteen aromatic protons. A peak at 9·6 δ which disappeared in D_2O exchange may be due to the two N–H protons of the thiosemicarbazide moiety.

2.13 *N-(dicyclohexylaminomethyl) isatin 3-(4'-phenyl-3'-thiosemicarbazone hydrochloride* (13)

IPTS (2·96 g, 0·01 M) was dissolved in THF (35 ml). Dicyclohexylamine (2·0 ml, ≈ 0·01 M) and 38% formaldehyde solution (0·8 ml, ≈ 0·01 M) were added to it. The mixture was refluxed on a water bath for nine hrs., half of the solvent was evaporated. After cooling the oil obtained was dissolved in acetone and hydrochloric acid gas was passed through it. The oil obtained got crystallized when more acetone was added, m.p.-285° (D), yield 1·0 g (19·01%) (found: C-63·20; H-6·84; N-13·41; mol. wt.-525·15 required for $C_{28}H_{36}ClN_5OS$: C-63·92; H-6·90; N-13·31%, mol. wt.-526·12), ν_{max} (nujol) 3300 (N–H), 1690 (C=O), 1615 (C=N), 1465 cm^{-1} (C=S).

2.14 *N-(Diphenylaminomethyl) isatin 3-(4'-phenyl-3'-thiosemicarbazone)* (14)

Diphenylamine (1·5 g, ≈ 0·01 M) and 38 % formaldehyde solution (0·8 ml, ≈ 0·01 M) were mixed to a solution of IPTS (2·96 g, 0·01 M) dissolved in THF (35 ml). The resulting mixture was refluxed on a water bath for an hr, half the solvent evaporated. On cooling the crystals separated and they were filtered—m.p.-237–39 (D), yield 1·7 g (35·60 %) (found: C-70·31; H-4·81; N-14·12; mol. wt.-479·32; required for $C_{28}H_{23}N_5OS$: C-70·42; H-4·85; N-14·66 %; mol. wt.-477·55), ν_{max} (nujol) 3300 (N–H), 1690 (C=O), 1620 (C=N), 1465 cm^{-1} (C=S).

2.15 *N-(Dibenzylaminomethyl) isatin 3-(4'-phenyl-3'-thiosemicarbazone) hydrochloride* (15)

To a solution of IPTS (2·96 g, 0·01 M) in THF (35 ml) were added dibenzylamine (2·0 ml, ≈ 0·01 M) and 38 % formaldehyde solution (0·8 ml, ≈ 0·01 M). The reaction mixture was refluxed on a water bath for about fifteen hrs., half the solvent evaporated. The oil obtained was dissolved in dry ether and hydrogenchloride gas was passed through it. The oil again obtained got crystallized when shaken with acetone—m.p.-208–10°, yield-1·1 g (20·29 %) (found: C-66·51; H-5·22; N-13·01; mol. wt.-541·05; required for $C_{30}H_{28}ClN_5OS$: C-66; H-5·21; N-12·92 % mol. wt.-542·07), ν_{max} (nujol) 3300 (N–H), 1690 (C=O), 1615 (C=N), 1475 cm^{-1} (C=S).

3. Screening for antibacterial activity

Antibacterial activity of the synthesized Mannich bases has been studied by employing *Escherichia coli* and *Staphylococcus aureus* as bacterial strains. The paper disc diffusion method (Cruickshank *et al* 1965) was employed for the evaluation of the antibacterial

Table 1. Antibacterial activity of Mannich bases of isatin 3-(4'-phenyl-3'-thiosemicarbazone)

Compound		Antibacterial activity Diameter of zone of inhibition (mm)	
Name	Number	*E. coli*	*S. aureus*
N-(dimethylaminomethyl) IPTS-HCl	1	15	12
N-(diethylaminomethyl) IPTS-HCL	2	—	9
N-(di-*n*-propylaminomethyl) IPTS-HCl	3	16	14
N-(di-*i*-propylaminomethyl) IPTS-HCl	4	7	8
N-(di-*i*-butylaminomethyl) IPTS-HCl	5	7	—
N-(piperidinomethyl) IPTS-HCl	6	8	7
N-(morpholinomethyl) IPTS	7	9	16
N-(pyrrolidinomethyl) IPTS-HCl	8	8	8
N,N'-piperazino-bis-(methyl IPTS)	9	—	—
N-(N-phenylpiperazinomethyl) IPTS	10	9	7
N-(dicyclohexylaminomethyl) IPTS-HCl	11	14	12
N-(diphenylaminomethyl) IPTS	12	14	9
N-(dibenzylaminomethyl) IPTS-HCl	13	12	11

IPTS denotes isatin 3-(4'-phenyl-3'-thiosemicarbazone).

activity. A heavy suspension of the inoculum was streaked or smeared over the surface of the agar media. Bactosensitivity paper discs of 6·25 mm diameter, containing drugs, (0·25 mg/ml) were placed on the medium suitably spaced apart full aseptic precautions. The plates were incubated at 37°C for 24 hours and the zone of inhibition was measured.

The results of the antibacterial screening of the Mannich bases has been summarised in table 1. It is evident from the results that the majority of Mannich bases showed some measurable zone of bacterial inhibition. The compounds N-(dimethylaminomethyl)-isatin-3-(4′-phenyl-3′-thiosemicarbazone)-hydrochloride; N-(di-*n*-propylamino-methyl)-isatin-3-(4′-phenyl-3′-thiosemicarbazone)-hydrochloride; N-(morpholino-methyl)-isatin-3-(4′-phenyl-3′-thiosemicarbazone); N-(dicyclohexylaminoethyl)-isatin-3-(4′-phenyl-3′-thiosemicarbazone) -hydrochloride; N-(diphenylaminoethyl)-isatin-3-(4′-phenyl-3′-thiosemicarbazone) and N-(dibenzylaminomethyl)-isatin-3-(4′-phenyl-3′-thiosemicarbazone)-hydrochloride have marked activity.

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