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Synthesis of some Mannich bases of 1-cyclohexylidene-*N*(1,2-dihydro-2-oxo-3H-indol-3-ylidene) thiosemicarbazones and their antibacterial activity

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Abstract

Some Mannich bases of 1-cyclohexylidene-*N*(1,2-dihydro-2-oxo-3H-indol-3-ylidene) thiosemicarbazones have been prepared by employing formaldehyde and morpholine and piperidine as secondary amines. These Mannich bases have been characterised on the basis of different physico chemical evidences. Like some alkaloids, they also form reineckate complexes which serve for their estimation. Antibacterial activity of the synthesised Mannich bases has been studied by employing nine bacterial strains. Chloro group at position 5 broadened the spectrum of activity. Compounds with piperidine showed better activity than the compounds with morpholine, against almost all the organisms used (except 1 or 2 occasions).

Keywords: Mannich bases; Antibacterial activity; Thiosemicarbazones; Reineckate complex; Isatin

1. Introduction

The *N*(1,2-dihydro-2-oxo-3H-indol-3-ylidene) thiosemicarbazone was found to be active against DNA viruses and RNA viruses principally the ortho pox viruses, both in vitro and in vivo (Bauer et al., 1970; Gupta and Srivastava, 1985; Rajendra and Lewis, 1967). The *N*[1-methyl 1,2-dihydro-2-oxo-3H-indol-3-ylidene] thiosemicarbazone (Methisazone) is being used successfully as prophylactic against small pox (Bauer, 1960). Several Mannich bases of *N*(1,2-dihydro-2-oxo-3H-indol-3-ylidene) thiosemicarbazone have been prepared and were found to be more active against small pox, cow pox, alastrim and variola viruses and bacteriae (Gupta et al., 1985). Anticipating a pronounced activity against bacteriae, we have recently synthesised and investigated the antibacterial activity of *N*-Mannich bases of 1-cyclohexylidene-*N*(1,2-dihydro-2-oxo-3H-indol-3-ylidene) thiosemecarbazones.

2. Experimental

2.1. Preparation of 1-cyclohexylidene thiosemicarbazone

An alcoholic solution (25 ml) of thiosemicarbazide (0.01 M) was treated with cyclohexanone (0.01 M) in the

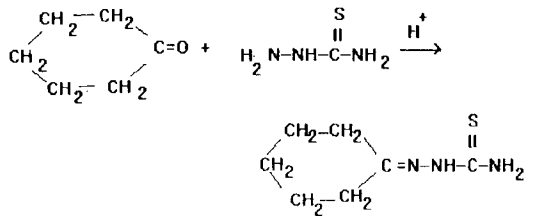

Fig. 1.

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presence of a few drops of concentrated HCl and refluxed for 2–3 min. The resulting product was filtered and dried in a desiccator (yield 57.71%, m.p. 165°C, mol. wt. 171) (Fig. 1).

2.2. Preparation of 1-cyclohexylidene-N(1,2-dihydro-2-oxo-3H-indol-3-ylidene) thiosemecarbazones

1-cyclohexylidene thiosemicarbazone (0.01 M) dissolved in alcohol (100 ml) was mixed with the solution of indole 2,3-dione (0.01 M) in water (150 ml). The mixture was refluxed on a water bath for 10 min. The product obtained after cooling was filtered (m.p. 27°C, yield 88%).

2.3. Preparation of N-Mannich base

To a slurry of 1-cyclohexylidene-*N*(1,2-dihydro-2-oxo-3H-indol-3-ylidene) thiosemicarbazone (0.01 M) in alcohol (15 ml) was added formaldehyde (0.01 M) and while cooling the secondary amine (0.01 M) was added to it and stirred vigorously for half an hour and allowed to stand for 24 h. The Mannich base obtained was recrystallised from chloroform:petroleum ether (2:1) mixture. Molecular formulae of the Mannich bases were checked by the standard reineckate complex method (Beckett and Stenlake, 1962). Melting points were determined on a Toshniwal melting point apparatus. Elemental analyses were done on a Coleman analyser. IR spectra were recorded on JASCO Infrared spectrophotometer IR report 100 as nujol mulls. H^1 NMR spectra were recorded on a VARIAN A-60 D NMR spectrometer in $CDCl_3$ with TMS as internal standard.

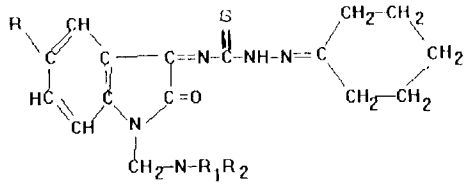

Fig. 2. NR_1R_2 = morpholine for cases I, II, III, IV and NR_1R_2 = piperidine for cases V, VI, VII (see Table 1).

2.4. Antibacterial activity testing

The prepared compounds were tested for their antibacterial activity by diffusion technique (Gupta et al.,

Table 1
Structural and physico chemical details of 1-cyclohexylidene-*N*-[1-(*N*,*N*-dialkylamino methyl)-1,2-dihydro-2-oxo-3H-indol-3-ylidene] thiosemicarbozones (Fig. 2)

No.	R	Yield (%)	M.p. (°C)	Elements (%)		Infrared (Cm^{-1})			TLC (R_f)
				Calculated	Found	NH	C=O	C=S	
I	Cl	72.65	219	C 57.46 H 5.74 N 16.76	57.26 5.52 16.54	3410	1690	1465	0.85
II	Br	73.2	233	51.94 5.19 15.19	51.79 5.07 15.03	3394	1696	1463	0.87
III	NO_2	72.89	244	56.07 5.6 16.355	55.99 5.5 16.13	3394	1695	1465	0.82
IV	OCH_3	72.89	221	61.16 6.5 13.59	60.89 6.3 13.37	3390	1696	1465	0.80
V	Cl	72.7	215	58.4 6.02 16.21	58.1 5.88 6.10	3400	1696	1465	0.83
VI	Br	72.85	235	52.94 5.46 14.7	52.73 5.13 14.48	3406	1690	1465	0.84
VII	NO_2	72.89	227	57.01 5.88 19.0	56.89 5.66 18.88	3400	1690	1465	0.87

TLC was performed with silica gel in acetone:water (70:30) system.

Table 2
Antibacterial activity of 1-cyclohexylidene-*N*-[1-(*N*,*N*-dialkylamino methyl)-1,2-dihydro-2-oxo-3H-indol-3-ylidene] thiosemicarbozones

No.	Diameter of zone of inhibition (mm)								
	E. coli	*S. typhimurium*	*V. cholerae*	*Enterobacter*	*Pr. mirabilis*	*Ps. aeruginosa*	*S. typhi*	*S. para typhi*	*Klebsiella*
I	6	8	16	6	5	6	9	8	5
II	—	—	5	—	—	—	—	—	9
III	9	8	10	8	8	9	10	—	—
IV	—	—	16	5	6	5	—	—	—
V	7	17	9	8	8	8	17	8	10
VI	—	—	18	9	9	—	6	—	—
VII	10	10	5	6	6	10	11	—	—

1985), with filter paper discs dipped in 2.5 mg/ml solutions of the compounds in dimethyl formamide.

3. Results and discussion

The structures of the prepared compounds (see Fig. 2) were confirmed on the basis of H^1 NMR data and the data in Table 1.

3.1. H^1 NMR data

3.1.1. For compounds with morpholine as secondary amine

1. 1.58 δ-broad singlet C–CH_2 (10 H) of cyclohexane ring.
2. 2.6 δ-broad N–CH_2 (4 H) of morpholine ring.
3. 3.7 δ-broad O–CH_2 (4 H) of morpholine ring.
4. 4.66 δ-singlet N–CH_2–N (2 H).
5. 6.88-7.5 δ-multiplet-aromatic protons (3 H).
6. 9.88 δ-NH proton (disappeared in D_2O exchange).

3.1.2. For compounds with piperidine as secondary amine

1. 1.6 δ-broad C–CH_2 (16 H) 10 H of cyclohexane and 6 H of piperidine ring.
2. 2.65 δ-broad N–CH_2 (4 H) of piperidine ring.
3. 4.65 δ-singlet N–CH_2–N (2 H).
4. 6.8 δ-multiplet-aromatic protons (3 H).
5. 9.85 δ-NH proton (disappeared in D_2O exchange).

3.1.3. Compound IV showed a sharp singlet at 3.8δ-OCH_3 at position 5 (3H) in addition to the peaks in Section 3.1.1

It is seen from Table 2 that all the compounds synthesised possess antibacterial activity. All compounds are active against *V. cholerae*, but compounds I, IV and VI are more potent than others.

Against *Enterobacter* and *Pr. mirabilis*, all are active except II. Compounds I and V are active against all nine bacterial strains. So it is worth noting that chloro group might enhance the range of antibacterial activity. Compounds II and IV do not have any activity against *Salmonella* species.

Compounds with piperidine showed better activity than those with morpholine, against almost all the organisms used (except 1 or 2 occasions). This may be explained on the basis of their superior ability to cross the lipoid membrane and binding with the receptor sites, because of the lipophilic nature of the Mannich bases as is evident from TLC R_f values.

Thus from the above discussion it may be conjectured that Mannich bases are playing their due role in enhancing the antibacterial activity of 1-cyclohexylidene-*N*(1,2-dihydro-2-oxo-3H-indol-3-ylidene) thiosemecarbazone group.

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