Synthesis, ^{13}C-NMR Characterization and Antimicrobial Properties of a Novel Series of 3-(*N*-Substituted thiocarbamoyl)hydrazino-1,2,4-triazino-[5,6-*b*]indole Derivatives

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A series of 3-(*N*-substituted thiocarbamoyl)hydrazino-1,2,4-triazino[5,6-*b*]indole derivatives **3-22** has been synthesized and evaluated for *in vitro* antimicrobial activity. Although some of the products displayed significant activity against *Staphylococcus aureus, Escherichia coli* and *Candida albicans*, their bactericidal and bacterostatic potencies were lower than that of penicillin G. The structure of the products was assigned upon the basis of their infrared, ^{1}H-nmr and ^{13}C-nmr spectra.

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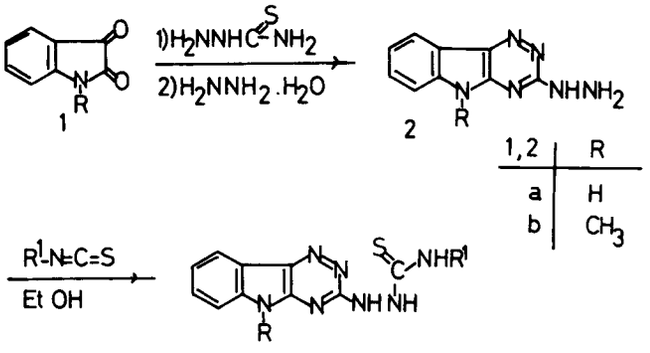
The 1,2,4-triazino[5,6-*b*]indole ring has been successfully used as a carrier for diverse functional groups in the development of several antiviral agents [1-7]. Analogous studies on the efficacy of such ring systems in the production of antibacterial [1,8,9] and antifungal [10] agents have not been well investigated. Recently, we synthesized various 3-arylidene- and heterocyclic-formylidenehydrazino-1,2,4-triazino[5,6-*b*]indole derivatives, and found that the arylidene part displayed a great role in inducing to the products a moderate activity against *Staphylococcus aureus* and *Bacillus cereus* and, in some instances, a significant effect against P388 lymphocytic leukemia [11]. In this paper, we are reporting the synthesis and antimicrobial properties of a new series of 1,2,4-triazino[5,6-*b*]indole derivatives bearing at position 3 a variety of 4-substituted 3-thiosemicarbazide functions. This study constitutes a part of an extensive progarm investigating the pharmacological properties of certain simple [12-21] and condensed [22-24] heterocyclic compounds. It also furnishes the first report on the detailed assignments of the ^{13}C chemical shifts of the various carbons of the 1,2,4-triazino[5,6-*b*]-indole skeleton.

The 3-hydrazino-1,2,4-triazino[5,6-*b*]indoles **2a,2b** were synthesized through the reaction of the indole-2,3-diones **1a,1b** with thiosemicarbazide in an alkaline medium and the hydrazinolysis of the products as previously reported [11] (Scheme I). Treatment of these hydrazino derivatives **2a** and **2b** with the properly substituted alkyl, aryl or aralkylisothiocyanates in refluxing ethanol yielded the desired 3-(*N*-substituted thiocarbamoyl)hydrazino-1,2,4-triazino-[5,6-*b*]indole derivatives **3-22** in high yields (Table I). The infrared spectra of the products showed the bands due to the NH function in addition to the four absorptions characterizing the vibrational coupling of the NCS function of the thiosemicarbazide moiety [25] (experimental). In the ^{1}H-nmr spectra, the C-6, C-7 and C-8 protons of the triazinoindole ring resonated at 6.93-7.89 ppm as a multiplet including the protons of the aryl substituent attached to N-4 of the thiosemicarbazide function. The C-9 proton appeared as a doublet of doublets at 8.17-8.38 ppm [11]. The N-1 and N-2 protons of the thiosemicarbazide moiety were shown as a distorted singlet resonating at 9.39-9.63 ppm. The N-4 proton appeared as a singlet whose chemical shift was dependent on the nature of the substituent in the aryl function. In the phenyl **9** and **10**, *o*-tolyl **12**, *m*-tolyl **13** and **14** and *p*-tolyl **15** derivatives, such a proton resonated at 9.54-9.83 ppm, while in the *p*-chlorophenyl **17** and the

Scheme I



1 2 3-22

1,2	R
a	H
b	CH_3

No.	R	R^1
3:	H	$-(CH_2)_3CH_3$
5:	H	$-CH_2CH=CH_2$
7:	H	$-C_6H_{11}$(cyclo)
9:	H	$-C_6H_5$
11:	H	$-C_6H_4CH_3$(o)
13:	H	$-C_6H_4CH_3$(m)
15:	H	$-C_6H_4CH_3$(p)
17:	H	$-C_6H_4Cl$(p)
19:	H	$-C_6H_4Br$(p)
21:	H	$-CH_2C_6H_5$

No.	R	R^1
4:	CH_3	$-(CH_2)_3CH_3$
6:	CH_3	$-CH_2CH=CH_2$
8:	CH_3	$-C_6H_{11}$(cyclo)
10:	CH_3	$-C_6H_5$
12:	CH_3	$-C_6H_4CH_3$(o)
14:	CH_3	$-C_6H_4CH_3$(m)
16:	CH_3	$-C_6H_4CH_3$(p)
18:	CH_3	$-C_6H_4Cl$(p)
20:	CH_3	$-C_6H_4Br$(p)
22:	CH_3	$-CH_2C_6H_5$

Table I

Synthesized 3-(*N*-Alkyl-, aryl- or aralkylthiocarbamoyl)hydrazino-1,2,4-triazino[5,6-*b*]indole Derivatives **3-22**

Compound No.	Mp (°C)	Yield (%)	Molecular Formula	Analysis, Calcd./Found % C	H	N	S	Inhibition Zone mm [a] S	E	C
3	215-216	89	$C_{14}H_{17}N_7S$	53.31 53.60	5.43 5.10	31.09 31.20	10.17 10.20	22	17	17
4	196-197	89	$C_{15}H_{19}N_7S$	54.69 54.70	5.81 5.50	29.77 29.40	9.73 9.50	27	17	19
5	240 dec	80	$C_{13}H_{13}N_7S$	52.16 52.30	4.38 4.50	32.76 32.50	10.71 10.50	19	16	19
6	212-213	92	$C_{14}H_{15}N_7S$	53.65 53.30	4.83 4.50		10.23 10.00	24	19	17
7	209-210	92	$C_{16}H_{19}N_7S$	56.28 56.20	5.61 5.80	28.72 28.80	9.39 9.70	25	17	17
8	207-208	80	$C_{17}H_{21}N_7S$	57.44 57.40	5.96 6.30	27.59 27.60	9.02 9.10	22	16	21
9	178 dec	96	$C_{16}H_{13}N_7S$	57.30 57.30	3.91 4.10		9.56 9.30	28	18	24
10	199-200	80	$C_{17}H_{15}N_7S$	58.43 58.20	4.33 4.60		9.18 9.00	22	16	21
11	167-168	100	$C_{17}H_{15}N_7S$	58.43 58.50	4.33 4.50		9.18 9.00	25	19	23
12	151-152	90	$C_{18}H_{17}N_7S$	59.48 59.60	4.72 4.50	26.98 27.20	8.82 8.80	21	16	24
13	175-176	97	$C_{17}H_{15}N_7S$	58.43 58.50	4.33 4.20	28.06 27.70	9.18 9.10	25	20	25
14	179-180	92	$C_{18}H_{17}N_7S$	59.48 59.40	4.72 4.70	26.98 26.70	8.82 9.20	16	16	20
15	208 dec	100	$C_{17}H_{15}N_7S$	58.43 58.40	4.33 4.30	28.06 28.40	9.18 8.80	24	19	24
16	218 dec	94	$C_{18}H_{17}N_7S$	59.48 59.30	4.72 4.80		8.82 8.80	—	—	— [b]
17	204 dec	81	$C_{16}H_{12}ClN_7S$	51.96 52.10	3.27 3.20	26.51 26.70	8.67 8.60	29	16	21
18	192-193	84	$C_{17}H_{14}ClN_7S$	53.19 53.30	3.68 3.50	25.54 25.70	8.35 8.50	17	17	17
19	199 dec	94	$C_{16}H_{12}BrN_7S$	46.38 46.40	2.92 3.30	23.67 23.50	7.74 7.60	22	17	22
20	196-197	92	$C_{17}H_{14}BrN_7S$	47.67 47.40	3.30 3.30	22.89 23.00	7.49 7.60	—	—	— [b]
21	217-218	97	$C_{17}H_{15}N_7S$	58.43 58.50	4.33 4.50	28.06 28.20	9.18 8.80	20	16	15
22	209-210	91	$C_{18}H_{17}N_7S$	59.48 59.10	4.72 5.00	26.98 26.60	8.82 8.90	26	19	22

[a] S = *Staphylococcus aureus* (NCTC 4163), E = *Escherichia coli* (NCTC 5933) and C = *Candida albicans* (NCTC 2708). [b] Not tested.

p-bromophenyl **19** and **20** derivatives, it was shown at 9.95-9.98 ppm. In the butyl derivatives **3** and **4**, it was identified as a triplet at 8.13 ppm.

The ^{13}C chemical shift data and assignments of the various carbons of compounds **4**, **10** and **14** are collected in Table II. The assignments were realized by comparison with the data reported for the simpler substituted 1,2,4-triazines [26-28] and the *N*-methylindole ring system [29]. The thiocarbonyl carbon, being the most deshielded, was readily identified downfield as a low intensity signal at 180.9-181.78 ppm. This is in agreement with our previous finding [30] and the calculations reported by Kalinowski and Kessler [31]. By direct comparison of the spectra of the phenyl **10** and *m*-tolyl **14** thiosemicarbazides with that of the butyl derivative **4**, the chemical shifts of the protonated and non-protonated carbons of the triazinoindole skeleton could be distinguished from those of the aryl substituents in the thiosemicarbazide moiety. The quaternary carbons were characterized by line width, signal intensity and off-resonance data. Carbon 3, in view of its similarity to C-3 of 3-amino-5,6-dimethyl-1,2,4-triazine [26] and due to its highly deshielded nature, was assigned to the resonance at 160.36-160.51 ppm. The angular carbons **4a** and 9b, constituting the junction of the pyrrolotriazine system, were identified at different chemical shifts in accordance with their shielded nature. The relatively deshielded C-4a resonated at 147.64-147.66 ppm, while C-9b appeared at 140.89-140.94 ppm. Carbon 5a was shown at 139.18-139.52 ppm, which again reflected its more deshielded nature than the other angular indole carbon 9a which resonated at 117.96-118.03 ppm. The benzenoid tertiary carbons C-8, C-9, C-7 and C-6 were identified at 129.05-129.14, 122.22, 120.08-120.10 and 110.50-110.53 ppm respectively [29,32]. Carbon 1′ of the phenyl **10** and *m*-tolyl **14** moieties of the thiosemicarbazide branch resonated at 138.45 ppm. The other quaternary carbon 3′ was shown at 136.97 ppm while the remainder of the benzenoid carbons as well as the

Chart I

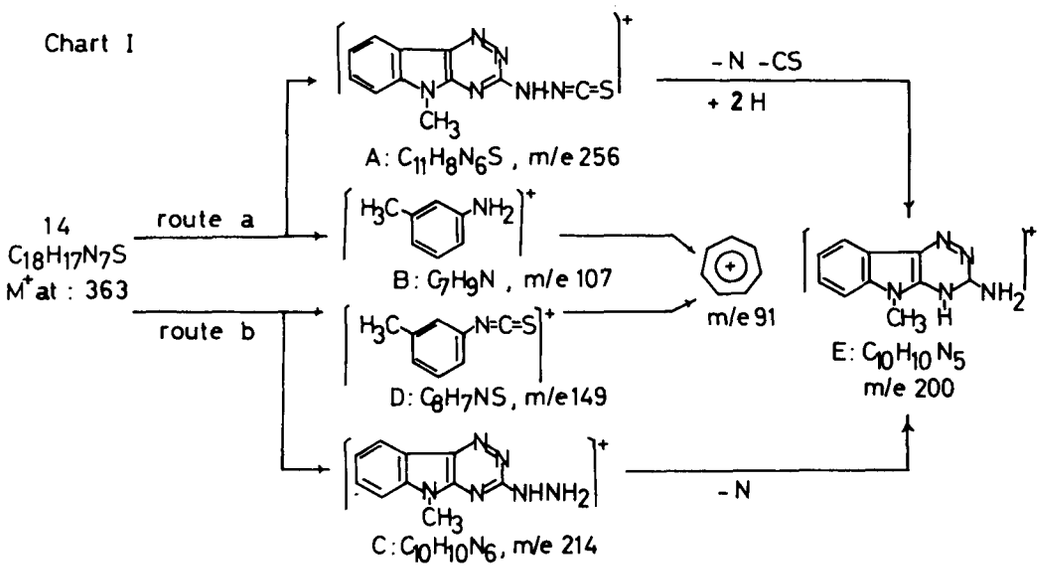
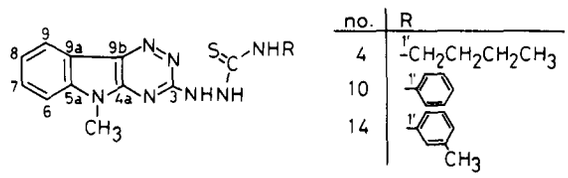


Table II

^{13}C-NMR Chemical Shifts in δ, ppm and Assignments for Some 1,2,4-Triazino[5,6-*b*]indole 3-Thiosemicarbazide Derivatives



Compound No.	Chemical shift (ppm) C-3	C-4a	C-5a	C-6	C-7	C-8	C-9	C-9a	C-9b	C=S	C-1′	N-CH$_3$
4	160.51	147.66	139.52	110.53	120.08	129.05	122.22	118.03	140.89	181.78	43.12	26.89
10	160.36	147.64	139.33	110.50	120.10	129.14	122.22	117.96	140.94	180.90	138.45	26.94
14	160.38	147.66	139.18	110.53	120.10	129.12	122.22	117.98	140.94	181.10	138.45	26.94

methyl and methylene carbons were identified at their expected chemical shifts.

The mass spectrum of the 5-methyl-3-[*N*-(*m*-tolyl)thiocarbamoyl]hydrazino-1,2,4-triazino[5,6-*b*]indole (**14**) showed the molecular ion peak at m/e 363. The ions produced indicated that the molecule underwent two major fragmentation pathways (Chart I). The first (route a) involved the production of the 3-aminoisothiocyanate ion **A**, at m/e 256, and *m*-toluidine ion **B**, as a base peak at m/e 107. The second (route b) yielded 3-hydrazino-5-methyl-1,2,4-triazino[5,6-*b*]indole ion **C**, at m/e 214, and the *m*-tolylisothiocyanate ion **D** at m/e 149. Further fragmentation of ions **A** and **C** led to the 3-amino-5-methyl-3*H*-1,2,4-triazino[5,6-*b*]-indole ion **E**, at m/e 200, whose subsequent fragmentation was found to be consistent with the fragmentation pattern previously reported for such types of compounds [11].

The products **3-22** were *in vitro* evaluated for their antimicrobial properties against *Staphylococcus aureus* (NCTC 4163), *Escherichia coli* (NCTC 5933) and *Candida albicans* (NCTC 2708) using the agar diffusion method. The results as shown in Table I indicated a relatively potent activity of compounds **4, 9, 11, 13, 17** and **22** against *Staphylococcus aureus,* and significant activity of compounds **9, 11, 13** and **22** against *Escherichia coli* and *Candida albicans.* The bacterostatic and bactericidal potencies of the most active compounds **9, 17** and **22**, as determined by the serial dilution method, indicated insignificant activity as compared with Penicillin G, Table III.

Table III

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Compounds **9**, **17** and **22**.

Compound No.	MIC [a] S	E	C [b]	MBC S	E	C
9	31.25	62.5	62.5	62.5	125	125
17	31.25	62.5	125.0	62.5	125	250
22	62.50	62.5	125.0	125.0	125	250

[a] All values are in ug/ml. [b] See footnote [a] in Table I.

EXPERIMENTAL

All melting points are uncorrected. The infrared spectra were measured as Nujol mulls on Beckman 4210 Spectrometer. The ^{1}H-nmr were recorded on a Varian A60 (EM 360L) NMR Spectrometer using TMS as an internal reference. The ^{13}C-nmr spectra were obtained on a JEOL FX-200 spectrometer in the Fourier transform mode with full proton decoupling. All samples were run in DMSO-d_6 solution and chemical shifts are referenced to internal TMS. The mass spectrum was measured on Finnigan 3200 Spectrometer.

3-(*N*-Alkyl, aryl or aralkylthiocarbamoyl)hydrazino-1,2,4-triazino[5,6-*b*]-indole Derivatives **3-22** (General Procedure).

A mixture of the 3-hydrazino-1,2,4-triazino[5,6-*b*]indole (**2a**) or the 5-methyl derivative **2b** [11] (2.5 mmoles) and the appropriate alkyl-, aryl- or aralkylisothiocyanate (2.5 mmoles) in ethanol (15 ml) was heated under reflux, while stirring, for 2 hours. After allowing to cool, the precipitate was filtered, washed with ethanol, dried and crystallized from ethanol to give the required 3-(*N*-substituted thiocarbamoyl)hydrazino-1,2,4-triazino[5,6-*b*]indole derivatives **3-22** as white to pale yellow crystals. Yields, melting points and microanalytical data are recorded in Table I; ir: ν 3370-3320, 3300-3250, 3200-3160 (thiosemicarbazide-NH), 3120-3100, 3090-3040 (indole-NH), 1545-1520, 1345-1305, 1090-1080 and 975-925 cm^{-1} (NCS Amide I, II, III and IV vibrational couplings); ^{1}H-nmr (DMSO-d_6 and deuteriochloroform) for some representative examples: for compound **4**, δ = 0.85 (t, 3, CH_2CH_3), 1.07-1.64 (m, 4, 2 $\times CH_2$), 3.50 (m, 2, N-CH_2), 3.74 (s, 3H, N5-CH_3), 7.40-7.82 (m, 3, H6-H8), 8.22-8.36 (dd, 1, H-9), 8.13 (t, 1, -(C=S)NH-) and 9.40 ppm (s, 2, -(C=S)NHNH-). For compound **9**, δ = 7.17-7.82 (m, 8, H6-H8 and ArH), 8.17-8.32 (dd, 1, H-9), 9.45 (bs, 2, -(C=S)NHNH-), 9.81 (s, 1, -(C=S)NH-) and 12.37 ppm (s, 1, N5-H). For compound **15**, δ = 2.32 (s, 3, CH_3(p)), 7.02-7.66 (m, 7, H6-H8 and ArH), 8.18-8.30 (dd, 1, H-9), 9.58 (bs, 2, -(C=S)NHNH-), 9.74 (s, 1, -(C=S)NH-) and 12.36 ppm (s, 1, N5-H). For compound **20**, δ = 3.78 (s, 3, N5-CH_3), 7.37-7.77 (m, 7, H6-H8 and ArH), 8.20-8.29 (dd, 1, H-9), 9.63 (bs, 2, -(C=S)NHNH-), and 9.95 ppm (s, 1, -(C=S)NH-). Mass spectrum for compound **14**: m/e (relative abundance %) 363 (M^+), 256 (19), 215 (7), 214 (63), 200 (6), 185 (6), 170 (13), 169 (7), 156 (5), 149 (29), 148 (6), 143 (28), 142 (10), 129 (7), 128 (7), 117 (6), 116 (16), 108 (9), 107 (100), 106 (79), 102 (10), 101 (6), 91 (24), 89 (12), 77 (19), 76 (7).

Antimicrobial Screening.

The compounds in a concentration of 0.5 mg/ml in propylene glycol were applied on the nutrient agar plates prepared as reported [21], and the zones produced were measured. In the serial dilution method, for evaluating the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) (Table III), using nutrient broth, each tube was inoculated with 0.1 ml of 1:200 diluted overnight culture of *Staphylococcus aureus, Escherichia coli* or *Candida albicans.* The tubes were incubated and the MIC as well as the MBC values were determined [21.

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