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SYNTHESIS AND CHELATING PROPERTIES OF SUBSTITUTED FORMYL PYRIDINE THIOSEMICARBAZONES OF POTENTIAL BIOLOGICAL ACTIVITY

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تم تحضير سلسلة من ٤-محتل ثيوسيمى كاربازونات الـ ٦-ميثيل-٥ -نيتروبيريدين-٢-كربوكسى الدهيد (4a-j) بطريقتين مختلفتين. وقد تم أختبار قابلية هذه المركبات للارتباط مع العناصر الثنائية مثل النحاس والزنبق والزنك وأثبتت جميع المركبات قابلية الارتباط مع أيونات العناصر المذكورة كما نجحت الأربع مركبات المختارة كمضادات للسمية بالنحاس بالمقارنة بالبنسيلامين كترياق لسمية النحاس حيث أثبتت هذه المشتقات فاعلية أكبر من فاعلية البنسيلامين وذلك عند جرعات أصغر. كذلك أظهرت الثيوسيمى كاربازونات فاعلية ضد البكتريا موجبة الجرام بينما أظهر متراكب النحاس رقم ٥ ومتراكب الزنك رقم ٦ فاعلية جيدة ضد البكتريا موجبة وسالبة الجرام.

The synthesis of a series of 4-(un) substituted thiosemicarbazones of 6-methyl-5-nitropyridine-2-carboxaldehyde (4a-j) is reported by two different routes. The prepared compounds were tested for their binding ability against Cu(II), Hg(II) and Zn(II). Four derivatives were tested for their complexing potentials in vivo. These compounds showed promising antidotal activities against Cu(II) relative to D-penicillamine. The compound 4g exhibited marked and significant increase in the mean threshold lethal dose of $CuSO_4$ in dose level (5 mg/kg) equal to the effect of D-penicillamine at dose level (30 mg/kg). The copper chelate 5 and zinc chelate 6 showed good activity against both Gram-positive and Gram-negative bacteria while the ligands showed activity against Gram-positive bacteria only.

INTRODUCTION

Substituted aromatic thiosemicarbazones are known to possess a wide spectrum of numerous pharmacological activities such as, antibacterial,[1-3] antitubercular,[4] antiviral,[5,6] antifungal,[7,8] antimalarial,[9,10] and anticonvulsant.[11] The antineoplastic effect of a variety of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones (HCTs) and their metal chelates have been actively investigated.[12-23] Since the first report that pyridine-2-carboxaldehyde thiosemicarbazone was proved to possess antileukaemic activity in mice,[12] numerous molecular variations were considered. Replacement of the pyridine ring with benzene, furan or thiophene ring systems led to loss of antitumor activity.[13-15]

Furthermore, replacement of the pyridine ring with imidazole, pyrazole, or triazole ring systems also resulted in a decrease or a complete loss of activity.[13,16] On the other hand, α(N)-heterocyclic carboxaldehyde thiosemicarbazone (HCTs) are among the potent known inhibitors of riboneucleoside diphosphate reductase, being 80-5000 times more effective than hydroxyurea; the clinically useful anticancer agent.[24] Moreover, this class of compounds is known to be an excellent co-ordinating agent for a number of transition metals including divalent iron,

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cobalt, nickel, copper, zinc, mercury and manganese.

The present investigation is concerned with the synthesis of 4-(un) substituted thiosemicarbazone of 6-methyl-5-nitropyridine-2-carboxaldehyde in an effort to find out a more efficacious compound as antimicrobial and/or antidote for metal poisoning.

EXPERIMENTAL

All melting points were determined in an open capillary tube apparatus and are uncorrected. Elemental microanalysis was performed by the Microanalysis Unit, Faculty of Science, Assiut University and the Microanalysis Unit, Faculty of Science, Cairo University. IR spectra were recorded on a Shimadzu 740 spectrometer as KBr discs. ^{1}H-NMR spectra were recorded on an EM-360 60 MHz Varian NMR spectrometer and JEOL JNM-EX 270 MHz spectrometer, with tetramethylsilane (TMS) as an internal standard, and the chemical shift values are given in δ ppm. Dimethyl sulfoxide-d_6 (DMSO-d_6) was used as the solvent. Purity of the compounds was checked by TLC silica plates. Mass spectra (MS) were obtained on a JEOL/JMS-HX/HX 110 spectrometer at the Faculty of Pharmaceutical Sciences, Kyoto University, Japan. UV spectral measurements were performed with Unicam SP-1750 spectrophotometer adopted with a Unicam SP-1805 programm controller and AR 55 linear recorder. Metal content of the complex compounds were determined by atomic absorption.

4-Substituted 3-thiosemicarbazides (1)

Were prepared according to the reported method.[25]

6-Methyl-5-nitropyridine-2-carboxaldehyde (2)

Was prepared from 2,6-dimethyl-3-nitropyridine[26] by oxidation with SeO_2 in dioxane.[27,28] m.p. 62-63°C as reported.[27] IR(ν) cm^{-1}: 1364, 1552, 1567, 1716, 3070. ^{1}H-NMR ($CDCl_3$), δ 2.95 (s, 3H, 6-CH_3), 8.01 (d, 1H, J= 8.52 Hz, C-3 H of pyridine), 8.36 (d, 1H, J= 8.52 Hz, C-4 H of pyridine), 10.13 (s, 1H, CHO).

6-Methyl-5-nitropyridine-2-carboxaldehyde hydrazone (3)

To a stirred solution of hydrazine hydrate (80 %) (18.75 ml, 0.3 mole) in ethanol (150 ml), a solution of 2 (16.6 g, 0.1 mole) in ethanol (150 ml) was added dropwise. The mixture was refluxed for 30 minutes and allowed to cool. The separated product was filtered, washed with cold ethanol and dried, m.p. 129-30°C as reported.[29] IR (ν) cm^{-1}: 1358, 1514, 1557. 1592, 3200, 3380.

4-(Un)substituted-6-methyl-5-nitropyridine-2-carboxaldehyde thiosemicarbazones (4a-j)

Method A

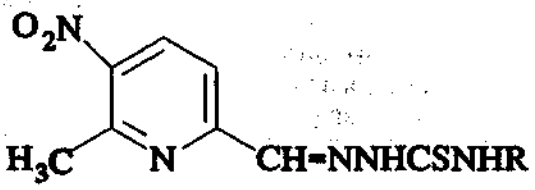
A mixture of 2 (1.66 g, 0.01 mole) and the appropriate thiosemicarbazide 1 (0.01 mole) in absolute ethanol (30 ml) containing 2 drops of conc. HCl was refluxed with stirring for 2 hrs. The reaction mixture was allowed to cool and the precipitated crystals were filtered, washed and re-crystallized from DMF/Ethanol (1:4) (Tables 1 and 2).

Method B

A mixture of 3 (1.80 g, 0.01 mole) and the appropriate isothiocyanate (0.01 mole) in absolute ethanol (50 ml) was refluxed with stirring for 3 hrs. The reaction mixture was allowed to cool. The precipitated crystals were filtered, washed and re-crystallized from DMF/Ethanol (1:4) (Tables 1 and 2).

MS (EI$^+$) data; Compound **4a**, m/z (%): 240 (M^++1, 20.8), 239 (M^+, 100), 179 (54.17), as reported.[30] Compound **4b**, m/z (%): 254 (M+1, 12.28), 253 (M^+, 100), 184 (33.32), 170 (37.77). Compound **4c**, m/z (%): 269 (M^++2, 5.63), 268 (M^++1, 13.45), 267 (M^+, 100), 184 (23.5), 179 (26.26), 166 (72.81). Compound **4d**, m/z (%): 297 (M^++2, 5.27), 296 (M^++1, 13.93), 295 (M^+, 88.75), 184 (26.06), 179 (23.7), 166 (100). Compound **4e**, m/z (%): 322 (M^++1, 10.63), 321 (M^+, 58.95), 184 (6.4), 166 (100). Compound **4f**, m/z (%): 330 (M^++1, 9.37), 329 (M^+, 48.98),

Table 1: Physical constants of 4-(un) substituted-6-methyl-5-nitropyridine-2-carboxaldehyde-thiosemicarbazones (**4a-j**).



Compd. No.	R	Yield% method of prep.	mp. (°C)	Mol. formula (M.W)	Analysis (%), Calc./found		
					C	H	N
4a	H	83 (A)	222-23*a	$C_8H_9N_5O_2S$ (239.25)			
4b	CH_3	79 (B)	237-39	$C_9H_{11}N_5O_2S$ (253.28)	42.68 42.80	4.38 4.16	27.65 27.30
4c	C_2H_5	77 (B)	183-84[a]	$C_{10}H_{13}N_5O_2S$ (267.31)	44.93 45.00	4.90 5.20	26.20 25.78
4d	C_4H_9(n)	80 (A)	215-17	$C_{12}H_{17}N_5O_2S$ (295.36)	48.80 48.80	5.81 5.40	23.73 23.30
4e	C_6H_{11}(c)	86 (A)	225-27	$C_{14}H_{19}N_5O_2S$ (321.40)	52.32 52.70	5.96 6.20	21.79 21.40
4f	$CH_2C_6H_5$	85 (A)	230-31	$C_{15}H_{15}N_5O_2S$ (329.38)	54.70 54.60	4.59 4.40	21.26 21.40
4g	C_6H_5	90 (A)	213-15	$C_{14}H_{13}N_5O_2S$ (315.35)	53.32 53.42	4.16 4.08	22.21 21.80
4h	C_6H_4Br(p)	75 (B)	210-12	$C_{14}H_{12}BrN_5O_2S$ (394.25)	42.65 42.40	3.07 3.40	17.76 17.30
4i	C_6H_4Cl(p)	80 (B)	225-27	$C_{14}H_{12}ClN_5O_2S$ (349.79)	48.07 48.40	3.46 4.00	20.02 19.90
4j	$C_6H_4CH_3$(p)	75 (B)	223-25	$C_{15}H_{15}N_5O_2S$ (329.38)	54.70 54.70	4.59 4.10	21.26 21.30

* as reported[30], [a] Ethanol

Table 2: IR and ^{1}H-NMR spectral data of 4-(un) substituted-6-methyl-5-nitropyridine-2-carboxaldehyde-thiosemicarbazones (**4a-j**).

Compd. No.	IR (KBr, cm^{-1})	^{1}H-NMR (δ ppm, DMSO-d_6)
4a	1338, 1531, 1574, 3150, 3330, 3350	2.75 (s, 3H, 6-CH_3), 8.075 (s, 1H, CH=N), 8.33 (d, 1H, J= 8.43, C_3-H of pyridine), 8.42 (d, 1H, J= 8.43, C_4-H of pyridine), 8.507 (s, broad, 2H, NH_2)*, 11.88 (s, 1H, N^2HCS)*
4b	1321, 1551, 1572, 3184, 3359, 3464	2.79 (s, 3H, 6-CH_3), 3.09 (d, 3H, NH$\underline{CH_3}$), 8.066 (s, 1H, CH=N), 8.27 (d, 1H, C_3-H of pyridine), 8.45 (d, 1H, C_4-H of pyridine), 8.79 (q, 1H, $\underline{N^4H}CH_3$)*, 11.9 (s, 1H, N^2HCS)*
4c	1220, 1329, 1542, 1595, 3135, 3415	1.20 (t, 3H, $CH_2\underline{CH_3}$), 2.707, 2.744 (2s, 3H, 6-CH_3), [27.2%, 72.8%], 3.55 (p, 2H, $\underline{CH_2}CH_3$), 8.06 (2s, 1H, CH=N), 8.28 (d, 1H, C_3-H of pyridine), 8.46, (d, 1H, C_4-H of pyridine), 8.8 (t, 1H, $\underline{N^4H}CH_2$)*, 11.95 (2s, 1H, N^2HCS)*
4d	1249, 1331, 1527, 1592, 3135, 3450	0.99 (t, 3H, CH_3 of n-butyl), 1.3-1.7 (2m, 4H, CH_2CH_2), 2.79 (s, 3H, 6-CH_3), 3.54-3.62 (m, 2H, CH_2 of n-butyl), 8.06 (s, 1H, CH=N), 8.37 (d, 1H, C_3-H of pyridine), 8.43 (d, 1H, C_4-H of pyridine), 8.82 (t, H, $\underline{N^4H}CH_2$)*, 11.9 (s, 1H, N^2HCS).
4e	1262, 1320, 1529, 1590, 3140, 3330	1.18-1.89 (2m, 10H, cyclohexyl), 2.722, 2.772 (2s, 3H, 6-CH_3), [12%, 88%], 4.22 (m, 1H, NH$\underline{CH}$ cyclohexyl), 8.0475, 8.097 (2s, 1H, CH=N) [11%, 89%], 8.2807 (d, 1H $\underline{N^4H}$ cyclohexyl)*, 8.364 (d, 1H, J= 8.5 C_3-H of pyridine), 8.415 (d, 1H, J= 8.57, C_4-H of pyridine), 11.866 (s, 1H, N^2HCS)*
4f	1251, 1344, 1516, 1576, 3120, 3285	2.729, 2.766 (2s, 3H, 6-CH_3), [16.5%, 83.5%], 4.88 (d, 2H, CH_2Ph), 7.24-7.35 (m, 5H, ArH), 8.076, 8.125 (2s, CH=N) [16.5%, 83.5%], 8.369, 8.416 (pd, 2H, C_3-H of pyridine, C_4-H of pyridine), 9.41 (t, 1H, $\underline{N^4H}CH_2$Ph)*, 12.06 (s, 1H, N^2HCS)*
4g	1243, 1331, 1525, 1595, 3115, 3330	2.745, 2.778 (2s, 3H, 6-CH_3), [21%, 79%], 7.22-7.55 (3m, 5H, ArH), 8.186 (2s, 1H, CH=N), 8.44 (d, 1H, J= 8.57, C_3-H of pyridine), 8.58 (d, 1H, J= 8.9, C_4-H of pyridine), 10.423 (2s, 1H, N^4HPh)*, 12.261 (2s, 1H, N^2HCS)*
4h	1249, 1355, 1531, 1584, 3125, 3235	2.8 (s, 3H, 6-CH_3), 7.39-7.73 (s, broad, 4H, ArH), 8.2 (s, 1H, CH=N), 8.3, 8.63 (dd, 2H, C_3-H of pyridine, C_4-H of pyridine), 10.36 (s, 1H, N^4HAr)*, 12.3 (s, 1H, N^2HCS)*
4i	1250, 1324, 1517, 1590, 3157, 3325	2.775 (s, 3H, 6-CH_3), 7.394 (d, 2H, $H_{2'}$, $H_{6'}$, ArH), 7.612 (d, 2H, $H_{3'}$, $H_{5'}$, ArH), 8.198 (s, 1H, CH=N), 8.376 (d, 1H, C_3-H of pyridine), 8.55 (d, 1H, C_4-H of pyridine), 10.33 (s, 1H, N^4HAr)*, 12.264 (s, 1H, N^2HCS)*
4j	1265, 1330, 1532, 1591, 3160, 3320	2.396 (s, 3H, p-CH_3), 2.825 (s, 3H, 6-CH_3), 7.19 (d, 2H, $H_{3'}$, $H_{5'}$, ArH), 7.55 (d, 2H, $H_{2'}$, $H_{6'}$, ArH), 8.17 (s, 1H, CH=N), 8.418 (d, 1H, C_3-H of pyridine), 8.627 (d, 1H, C_4-H of pyridine), 10.313 (s, 1H, N^4HAr)*, 12.18 (s, 1H, N^2HCS)*

* Exchangeable in D_2O.

179 (5.7), 166 (100), 91 (57.26). Compound **4g**, m/z (%): 316 (M^++1, 17.35), 315 (M^+, 100), 254 (15.78), 184 (47.13), 179 (35.16). Compound **4h**, m/z (%): 395 (M^++2, 92.81), 394 (M^++1, 17.91), 393 (M^+, 94.97), 258 (21.65), 184 (100), 179 (63.50). Compound **4i**, m/z (%): 351 (M^++2, 35.81), 350 (M^++1, 17.99), 349 (M^+, 100) 184 (69.03), 179 (50.36). Compound **4j**, m/z (%): 331 (M^++2, 6.32), 330 (M^++1, 18.58), 329 (M^+, 100), 327 (10.24), 184 (43.93), 179 (31.16).

General procedure for the preparation of metal chelates 5 and 6

A hot solution of **4a** (0.239 g, 0.001 mole) in methanol (50 ml) was added to a solution of copper acetate monohydrate (0.199 g,0.001 mole) or zinc acetate (0.182 g, 0.001 mole) in methanol (30 ml) with constant stirring. Stirring was continued for further 1 hr. The reaction mixture was left for an over-night at room temperature. The precipitated product was filtered, wash with methanol several times and dried in vaccum.

Copper chelate compound **5**, m.p. 205°C. IR (KBr, ν cm^{-1}): 1180, 1340, 1436, 1462, 1537, 1557, 2915, 3405. Analysis for $C_8H_8N_5O_2S.Cu(II)$ (301.79), Calcd: C, 31.84; H, 2.67; N; 23.21, Cu(II), 21.05. Found: C, 32.10; H, 3.2; N, 23.8; Cu(II), 20.91.

Zinc chelate compound **6**, m.p. 265-67°C. IR (KBr, ν cm^{-1}), 1321, 1407, 1534, 1557, 3155, 3350. Analysis for $(C_8H_8N_5O_2S)_2.Zn(II).H_2O$ (560.07), Calcd: C, 34.32; H, 3.24; N, 25.01; Zn(II), 11.68. Found: C, 34.6; H, 3.2; N, 24.7; Zn(II), 11.57.

Screening of the metal binding properties of the test compounds 4a-j

a- Solution of the compounds

Accurately weighed amounts of the compounds were dissolved separately in methanol and the volume was adjusted to 100 ml in a volumetric flask to provide a final dilution of $5x10^{-4}$ M. solution.

b- Metal salt solutions

Accurately weighed amounts, equivalent to $5x10^{-4}$ mole of copper acetate monohydrate (0.0995 g), Zinc acetate (0.091 g) and mercuric acetate (0.159 g), were dissolved separately each in 50 ml of methanol by aid of ultrasonic apparatus, then the volume was adjusted to 100 ml. Ten ml were transferred to 100 ml volumetric flask and the volume was adjusted by methanol to give $5x10^{-4}$ M solution.

c- Solutions for spectral measurements

Into volumetric flasks each of 10 ml capacity aliquots of the solution of the compound **4a-j** and the solution of the metal salt were mixed in ratios (1.6:0.4, 1.33:0.67, 1:1, 0.67:1.33 and 0.4:1.6 respectively) and the volume was adjusted by methanol.

d- Spectral scanning

Solutions of the compounds and those for spectral measurement were scanned in the range of 200-800 nm using methanol and ligand solution as a blank respectively. Results are listed in Table (3).

Evaluation of antidotal activity

Compounds **4a, 4d, 4e** and **4g** were ground and sieved, then suspended in (2%) aqueous solution of carboxymethyl cellulose. Adult albino rats (200-250 g) were used to investigate the antidotal action of the tested compounds against toxicity induced by copper sulfate pentahydrate (1% w/v) solution using D-penicillamine as a reference. Rats were divided into groups, each of 6 animals. Rats were anaesthetized with intraperitoneal injection of urethane in a dose of 1.6 g/kg. Then tied on a board and their jugular veins were exposed and cannulated for intravenous infusion. In the control group the threshold lethal dose of $CuSO_4$ was determined half an hour after the intraperitoneal injection of 1 ml of aqueous solution of carboxymethylcellulose (2%). The $CuSO_4.5H_2O$ was dissolved in normal saline to obtain (1%) solution and was infused at a rate of 0.5 ml/min. until the animals developed cardiac standstill as indicated by electrocardiographic monitoring using cardiosuny ECG. The mean threshold lethal dose of $CuSO_4$ was determined and calculated in terms of mg/kg. In the other groups of animals each of tested compounds and D-penicillamine were intraperitoneally injected in different dose level (5, 10, 20 & 30 mg/kg).

Table 3: Ability of Cu(II), Hg(II) and Zn(II) ions to bind the thiosemicarbazone derivatives in methanol.

Compd. No.	λmax [nm]	L:M ratio	Metal ions (λ_{max}) [nm]		
			Cu(II)	Hg(II)	Zn(II)
4a	214, 240, 368	4:1	438	420	428
		2:1	440	420	432
		1:1	440	420	432
		1:2	438	292, 424	430
		1:4	438	290, 438	430
4b	246, 370	4:1	452	420	440
		2:1	452	424	440
		1:1	290, 452	420	440
		1:2	292, 460	278, 420	440
		1:4	290, 460	278, 420	440
4c	216, 244, 370	4:1	440	424	446
		2:1	444	424	446
		1:1	444	424	446
		1:2	446	424	446
		1:4	448	424	446
4d	240, 360	4:1	292, 442	424	444
		2:1	292, 444	424	444
		1:1	292, 444	420	444
		1:2	292, 440	428	444
		1:4	292, 440	428	444
					444
4e	240, 366	4:1	434	430	446
		2:1	w0, 440	428	446
		1:1	290, 448	430	446
		1:2	270, 290, 444	290, 430	446
		1:4	270, 290, 442	290, 430	446
4f	220, 258, 364	4:1	293, 435	420	294, 444
		2:1	293, 440	420	292, 444
		1:1	295, 445	420	292, 444
		1:2	271, 295, 443	290, 434	292, 444
		1:4	262, 291, 442	290, 434	292, 444
4g	220, 254, 371	4:1	286, 442	430	284, 454
		2:1	290, 444	298, 430	284, 454
		1:1	286, 450	298, 430	284, 454
		1:2	292, 448	298, 428	454
		1:4	290, 444	298, 428	454

Table 3: Continued.

Compd. No.	λmax [nm]	L:M ratio	Metal ions (λ_{max}) [nm]		
			Cu(II)	Hg(II)	Zn(II)
4h	220, 234, 370	4:1	290, 446	426	284, 450
		2:1	294, 446	426	284, 450
		1:1	294, 460	300, 420	284, 450
		1:2	292, 452	298, 422	284, 450
		1:4	292, 460	300, 426	450
4i	230, 250, 366	4:1	444	426	448
		2:1	450	424	284, 448
		1:1	458	426	284, 448
		1:2	450	426	284, 448
		1:4	450	426	448
4j	230, 262, 370	4:1	460	430	292, 456
		2:1	460	432	290, 454
		1:1	302, 470	300, 432	290, 452
		1:2	300, 466	296, 426	294, 456
		1:4	300, 470	300, 426	290, 456

Half an hour later, the threshold lethal dose of $CuSO_4$ was determined as previously mentioned in control group. Results are illustrated in Table (4).

Evaluation of antimicrobial activity

The antimicrobial activity of the test compounds was determined by the disc diffusion method[31] against Gram-positive bacteria (*S. aureus*, ATCC 25923, *B. cereus*, DMS 345) and Gram-negative bacteria (*E. coli*, ATCC 25922) and a yeast (*C. albicans*, WT-5). Each test compound was dissolved in dimethyl sulfoxide and added at a concentration of 2.5 mg/disc (Whatman No. 3 filter paper, 0.5 cm diameter). Incubation was carried out at 37±1°C for 24 hrs (for bacteria) or 48 hrs (for yeast), and the diameter of zones of inhibition was measured in mm. Streptomycin sulphate and clotrimazole were used as standards for antibacterial and antifungal activity, respectively. The results are listed in Table (5).

RESULTS AND DISCUSSION

Chemistry

The designed 4-(un) substituted-6-methyl-5-nitropyridine-2-carboxaldehyde thiosemicarbazones (**4a-j**) were synthesized by two different routes as shown in Scheme 1.

6-Methyl-5-nitropyridine-2-carboxaldehyde (**2**)[27,28] was allowed to react with 4-(un) substituted 3-thiosemicarbazide (**1**) in boiling ethanol in presence of HCl drops as a catalyst (method A). Alternatively, the aldehyde **2** was treated with hydrazine hydrate to give the corresponding hydrazone **3**.[29] The latter upon treatment with the appropriate alkyl/aryl isothiocyanate, gave rise to the desired thiosemicarbazones (method B). Both procedures produced high yields of the desired compounds (Table 1). The structures of the final compounds were confirmed by elemental analyses, IR, MS and ^{1}H-NMR spectral data. These data revealed the presence of E/Z geometric isomers. TLC showed that some compounds turned out to be single isomer, while others are not. According to the ^{1}H-NMR spectra, compounds **4c, 4e, 4f, 4g** appeared to be mixtures of unequal proportion of the two isomers as predicted from the measurements of the signals corresponding to C_6-CH_3 and CH=N functions (Table 2).

Chelating properties of the thiosemicarbazones (4a-j)

There is a strong evidence that the inhibition of ribonucleoside diphosphate reductase, the

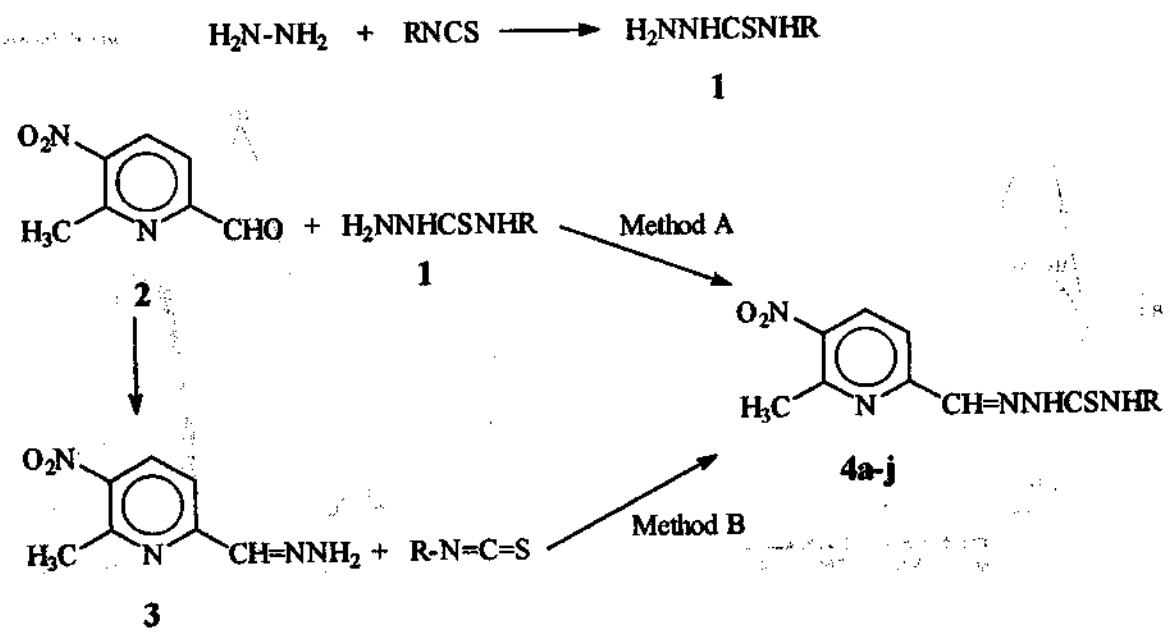
Table 4: Effect of different dose levels of the tested compounds and D-penicillamine on the mean threshold lethal dose[a] of $CuSO_4$ in rats.

Comp. No.	Dose mg/kg	Mean threshold lethal dose (mg/kg)*	% Change
Control		104.00 ± 3.36	0
D-penicillamine	10	177.86 ± 2.14	70.84
	30	207.14 ± 1.84	99.17
4a	5	176.64 ± 2.61	69.84
	10	229.87 ± 2.41	121.03
	20	289.06 ± 3.88	177.94
	30	299.52 ± 3.65	188.00
4d	5	191.36 ± 2.68	84.00
	10	286.72 ± 3.65	175.69
	20	323.84 ± 2.67	211.38
4e	5	191.50 ± 4.71	84.13
	10	266.24 ± 4.17	156
	20	341.12 ± 3.64	228
4g	5	199.47 ± 3.87	91.80
	10	318.40 ± 3.36	206.15
	20	481.60 ± 5.26	363.08

[a] Data represent mean ± S.E of 6 observations.
* Highly significant difference from both control and reference at $p < 0.01$.

Table 5: Antimicrobial activity of the test compounds (Diameter of inhibition zones in mm.).

Compound No.	*S. aureus*	*B. cereus*	*E. coli*	*C. albicans*
4a	15	15	-	-
4b	30	25	-	-
4c	22	16	-	-
4d	18	-	-	-
4e	17	17	-	-
4f	18	-	-	-
4g	27	-	-	-
4h	27	15	-	-
4i	20	18	-	-
4j	12	25	-	-
5	28	25	24	15
6	22	40	28	-
Clotrimazole	-	-	-	25
Streptomycin sulphate	40	45	30	-



R= H, CH_3, C_2H_5, C_4H_9(n), C_6H_{11}(c), $C_6H_5CH_2$, C_6H_5, C_6H_4Br(p), C_6H_4Cl(p) $C_6H_4CH_3$(p)

Scheme 1

obligatory enzyme in the pathway of synthesis of the precursors of DNA by HCTs is taking place either by coordination of iron in the metal-bound enzyme or by a pre-formed iron chelate of these agents.[22,24] Therefore,the heterocyclic carboxaldehyde thiosemicarbazones are considered to be strong metal chelating agents.[13,20]

The co-ordination of the metal by these compounds being through their N^*-N^*-S^* tridentate (i.e., pyridyl nitrogen, azomethine nitrogen and thione sulphur) ligand system.[16,17]

In this work the metal binding ability of the prepared compounds **4a-j** was tested against Cu(II), Hg(II) and Zn(II) at five ligand:metal ratios (4:1, 2:1, 1:1, 1:2, 1:4).[32] The metal binding potential was monitored by spectral scanning of the ligand-metal solution in the range 200-800 nm. The obtained spectrograms displayed the disappearance of the absorption maxima relevant to the ligand with simultaneous emergence of a new maximum at higher wave length. This pattern provides useful criteria to infer complex formation.[33]

In the region of high energy absorption, Cu(II) and Hg(II) ions showed interference with ligand-metal absorption maxima (Fig. 1). Such maxima were neglected on fixing the absorption maxima listed in Table 3. Representative spectrogram of ligand and ligand-metal are illustrated by Figures 2,3. All the tested compounds showed marked chelating properties against the different metals tested (Table 3). Application of Jop's method of continuous variation[34], showed that Cu(II), Hg(II) and Zn(II) reacted with the thiosemicarbazone derivative **4a** in the molar ratios of 1:1, 1:2 and 1:2 respectively.

Two metal chelates of compound **4a** were prepared (**5** and **6**). The structures of the chelates were confirmed by IR and elemental analyses (experimental section).

Biological activity

Four compounds were chosen to be screened for their complexing potentials *in vivo*. Albino rats were used to investigate the antidotal action of the tested compounds and D-penicillamine against toxicity induced by copper sulphate.

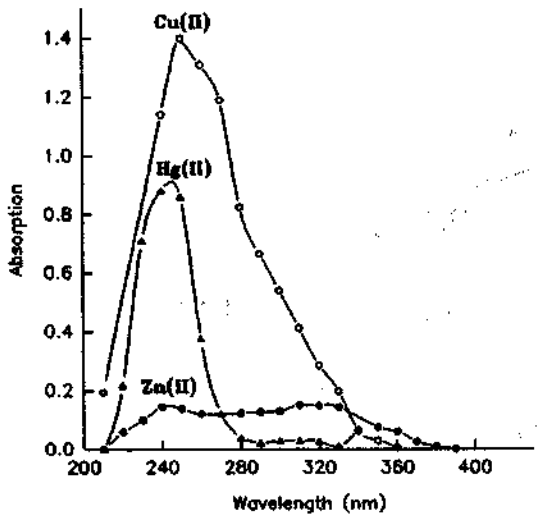


Fig. 1: Absorption spectra of metal ions in methanol at $5x10^{-4}$ M solutions.

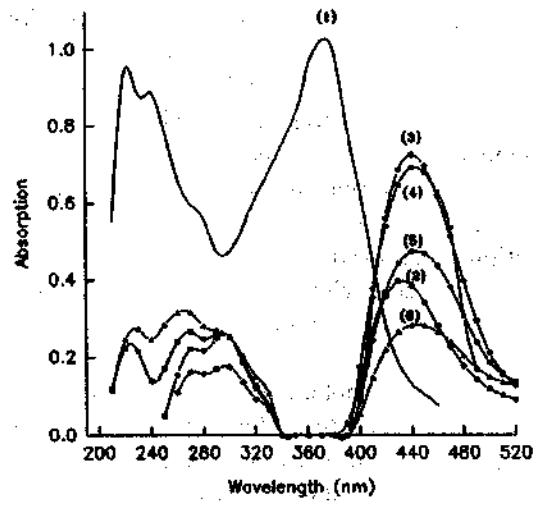


Fig. 2: Absorption spectra of 4f and Cu(II) in methanol 4f : metal ratio 1:0 (1); 4:1 (2); 2:1 (3); 1:1 (4); 1:2 (5); 1:4 (6).

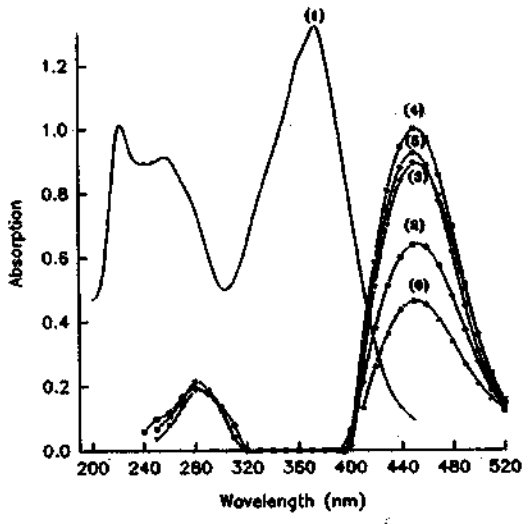


Fig. 3: Absorption spectra of 4g and Zn(II) in methanol 4g : metal ratio 1:0 (1); 4:1 (2); 2:1 (3); 1:1 (4); 1:2 (5); 1:4 (6).

Compound **4a** showed greater activity than that obtained by D-penicillamine when tested at the higher dose level (30 mg/kg) which was chosen parallel to that reported for D-penicillamine.[35] It is clear from Table 4, that copper was more bound by all the test compounds than the reference compound; D-penicillamine even when tested at lower dose levels (5,10 and 20 mg/kg). The most active compound, 4-phenylthiosemicarbazone derivative (**4g**) showed a promising antidotal activity in the dose level (5 mg/kg) comparable with the effect of D-penicillamine in the dose level 30 mg/kg (Table 4).

The antimicrobial activities of the thiosemicarbazones **4a-j** as well as the Cu(II), and Zn(II) complex (**5&6**) were evaluated by means of *in vitro* growth inhibitory activity assay against a variety of Gram-positive and Gram-negative strain bacteria, namely: *S. aureus*, *B. cereus* and *E. coli*, and the yeast *C. albicans*. The disc diffusion method was applied.[31] The zone of inhibition of the test compounds and the reference streptomycin sulfate and clotrimazole were measured. All the tested compounds **4a-j** showed activity against Gram-positive bacteria only. The metal chelates

5 and **6** showed promising antimicrobial activity against Gram-positive and Gram-negative bacteria. Only the copper chelate **5** showed moderate activity against *C. albicans* (Table 5).

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REFERENCES

1- A. S. Dobek, D. L. Klayman, J. P. Scovill and E. T. Jr. Dickson, Chemotherapy, 32, 25 (1986).
2- A. S. Dobek, D. L. Klayman, E. T. Jr. Dickson, J. P. Scovill and C. N. Oster, Arzneim-Forsch, 33, 1583 (1983).
3- R. P. Gupta and N. I. Narayana, Pharm. Acta Helv., 72, 43 (1997).
4- B. Milczarska, H. Foks, Z. Trapkowski, Z. Milzynska-Kolaczek, M. Janowiec, Z. Zevolska and Z. Andrzejczyk, Acta Pol. Pharm., 55, 289 (1998).
5- A. Ferranti, L. Garuti, G. Giovanninetti and E. Katz, Arch. Pharm., 318, 415 (1985).
6- D. Hadjipavlou-Litina, Pharmazie, 51, 468 (1996).
7- O. E. Offiong and S. Martelli, Farmaco, 48, 777 (1993).
8- A. E. Liberta and D. X. West, Biometals, 5, 121 (1992).
9- D. L. Klayman, A. J. Lin, J. M. Hoch, J. P. Scovill, C. Lambross and A. S. Dobek, J. Pharm. Sci., 73, 1763 (1984).
10- D. L. Klayman, C. J. Mason and J. P. Scovill, Arzneim-Forsch, 34, 1701 (1984).
11- S. N. Pandeya and J. R. Dimmock, Pharmazie, 48, 659 (1993).
12- R. W. Brockman, J. R. Thomson, M. J. Bell and H. E. Skipper, Cancer Res, 16, 167 (1956).
13- F. A. French and E. J. Blanz, Jr., J. Med. Chem., 9, 585 (1966).
14- B. A. Booth, E. C. Moore and A. C. Sartorelli, Cancer Res., 31, 228 (1971).
15- M. Mohan, N. S. Gupta, L. Chandra and N. K. Jha, J. Inorg. Biochem., 31, 7 (1987).
16- F. A. French and E. J. Blanz, Jr., J. Med. Chem., 17, 172 (1974).
17- M. C. Liu, T. S. Lin and A. C. Sartorelli, Prog. Med. Chem., 32, 1 (1995).
18- J. Antonini, F. Claudi, P. Frunchetti, M. Grafantini and S. Martelli, J. Med. Chem., 20, 447 (1977).
19- M. C. Miller 3rd, C. N. Stineman, J. R. Vance, D. X. West and I. H. Hall, Anticancer Res., 18, 4131 (1998).
20- W. E. Antholine, J. M. Knight and D. H. Peterring, J. Med. Chem., 19, 339 (1976).
21- A. S. Dimoglo, Y. M. Chumakov, B. N. Dobrova and M. Saracoglu, Arzneim-Forsch, 47, 415 (1997).
22- M. C. Liu, T. S. Lin, J. G. Cory, A. H. Cory and A. C. Sartorelli, J. Med. Chem., 39, 2586 (1996).
23- M. C. Miller 3rd, K. F. Bastow, C. N, Stineman, J. R. Vance, S. C. Song, D. X. West and I. H. Hall, Arch Pharm, 331, 121 (1998).
24- A. C. Sartorelli, K. C. Agrawal, A. S. Tsiftsoglou and E. C. Moore, Adv. Enzyme Regul., 15, 117 (1977).
25- P. L. Klayman, J. F. Bartosevich, T. S. Oriffir, C. J. Mason and J. P. Scovill, J. Med. Chem., 22, 855 (1979).
26- E. Plazek, Ber., 72, 577 (1939).
27- Z. Skrowaczewska and H. Ban, Bull. Acad. Polan, Sa, Ser. Chem., 9, 213 (1961).
28- T. Banas and Z. Shrowaczewska, Rocz. Chem., 43, 739 (1969).
29- H. H. Farag, A. M. Abdel-Aleem and A. F. Abdel Ghaffar, Egypt. J. Pharm. Sci., 20, 393 (1979).
30- H. Y. Hassan, Bull. Pharm. Sci., Assiut Univ., 22, Part 1, 35 (1999).

31- I. P. Garrod and F. O. Grady, "Antibiotics and chemotherapy", Churchill Livingstone, London, 5th, p. 464 (1981).

32- B. S. Lyengar, S. M. Sami, T. Takahashi, E. E. Sikorski, W. A. Rewers and W. T. Bradner, J. Med. Chem., 29, 1760 (1986).

33- Y. Matsushinia, Y. Nagata, Y. Ramano, S. Sugata, T. Fujita, Y. Karubr and A. Kono, Chem. Pharm. Bull, 35, 4695 (1987).

34- P. Jop, Ann. Chim., 11, 97 (1939).

35- Goodman and Gilman's, "The pharmacological basis of therapeutics", MC Graw-Hill Companies, Inc., New York, 9th edn., p. 1667 (1996).