and that the introduction of the electron-attracting chloro atom, at variance of the electron-releasing methyl group, at C-2 position of the indole ring decreases the activity. The reduced in vitro activity of the investigated compounds, compared with that of miconazole used as drug standard, has not encouraged further *in vivo* experiments, although *in vitro* and *in vivo* antifungal activities are sometimes poorly correlated[4,5].

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# Novel Phenanthridine Thiosemicarbazone and Thiazoline Derivatives: Syntheses and Evaluation for Anticancer and Antimicrobial Activities

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Two novel series of thio compounds utilizing 6-formylphenanthridine as a carrier for various thiosemicarbazone moieties, which were partly combined with variously substituted thiazoline functions, were synthesized as potential anticancer and antimicrobial agents. The thiosemicarbazones were found to be inactive against P 388 lymphocytic leukemia and the thiazolines were ineffective in the suppression of various micro-organisms.

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**Neue Phenanthridin-Thiosemicarbazon- und Thiazolin-Derivate: Synthese und Prüfung auf cytostatische and antimikrobielle Wirkung**

Es wurden zwei neue Serien von Thioverbindungen synthetisiert, wobei 6-Formylphenanthridin als Carrier für verschiedene Thiosemicarbazon-Reste verwendet wurde und zwar allein oder kombiniert mit unterschiedlich substituierten Thiazolin-Derivaten. Die neuen Verbindungen wurden auf ihre cytostatische und antimikrobielle Wirksamkeit geprüft: Die Thiosemicarbazone haben sich als unwirksam gegen P388 lymphozytische Leukämie erwiesen. Die Thiazoline zeigten gegen Mikroorganismen keine antimikrobielle Wirkung.

The phenanthridine nucleus is among a number of heterocyclic nuclei which proved to be efficient in inducing anticancer[1-10] and antimicrobial[11-17] properties when suitably substituted by proper functional groups. Concerned with these properties, the phenanthridine-6-formylthiosemicarbazones **3–8** and the corresponding thiazoline derivatives **9–22** were synthesized with the objective of checking compounds **3–8** for anticancer properties and compounds **9–22** for bacteriostatic activity.

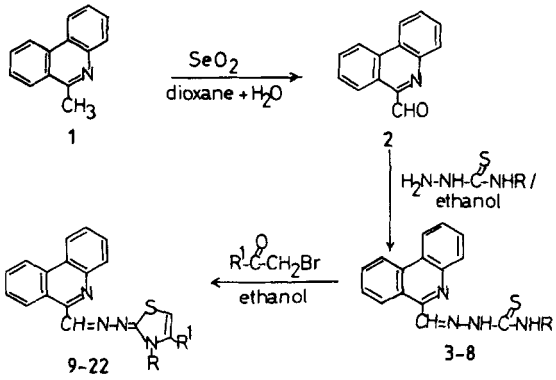
The 6-methylphenanthridine **(1)**, obtained by cyclization of 2-acetyl-aminobiphenyl with phosphorus oxychloride[18], was oxidized with selenium dioxide in aqueous dioxane to give the 6-formylphenanthridine **(2)** in a high yield as reported[14]. The treatment of compound **2** with the equivalent amount of thiosemicarbazide or the appropriately 4-substituted 3-thiosemicarbazides in boiling ethanol produced the required phenanthridine-6-formylthiosemicarbazone derivatives **3–8** in variable yields as shown in table 1. These on reaction with the selected phenacyl bromides under reflux in ethanol underwent cyclization into the corresponding phenanthridine-6-(3′,4′-disubstituted)(2′,3′-dihydrothiazol-3′-ylidene)formylhydrazones **9–22**), (scheme 1, table 2).

The products were identified by elemental analysis, infrared and $^1$HNMR spectra. In the $^1$HNMR spectra of compounds **3–8**, the phenanthridine protons were identified as a multiplet at 7.56–8.16 ppm for the C-2, C-3, C-8 and C-9-protons, a singlet at $\delta$ = 8,05–8.48 ppm for the C-7-proton, a multiplet at $\delta$ = 8.41–9.96 ppm for the C-1 and C-10-protons and another multiplet at $\delta$ = 9.10–9.76 ppm for the C-4-protons[19-21]. The other protons resonated at various chemical shifts depending on their shielded and deshielded nature (table 1). The $^1$HNMR of the thiazolines lacked the signals for the NH protons but showed the singlet of the thiazoline proton at $\delta$ = 6.2–6.31 ppm (Exp. Part). For more concerted evidence, the mass spectra of 1-(phenanthridine-6′-formyl)-4-phenyl-3-thiosemicarbazone **(4)** and the corresponding 4-bromophenyl derivative **(8)** were measured and the structure of the prominent ions formed, under electron impact, was assigned. The molecular ion peaks were shown at m/e 357 and 434 (436, for M + 2) for compound **4** and **8**, resp. Their fragmentation (chart 1) was found to be following a common pathway involving the elimination of the N-4-aryl substituent giving the unsubstituted phenanthridine-6-formylthiosemicarbazone ion **A**, at m/e 280, which cleaved an amino group, carbon monosulfide, an NH function and hydrogen to give 6-cyanophenanthridine ion **B** at m/e 204. The complete elimination of the thiosemicarbazone function from the products gave the dibenzoazatropylium[6] ion **C**, at m/e 192, which on successive loss of acetylene produced the azatropylium ions **D** and **E** at m/e 166 and 92, while on losing a cyano and a methylene function gave the biphenylene ion **F** at m/e 152. The base peak at m/e 93 in compound **4** and at 171 (173) for compound **8** corresponded to the anilino and p-bromoanilino ions. The spectra of the thiazolines **12** and **17** showed the molecular ion peaks at 395 and 490 (492). They indicated that the compounds have eliminated the aryl substituents from the thiazoline ring giving the phenanthridine-6-(2′,3′-dihydro-thiazol-2′-ylidene)formylhydrazone, as a common ion, at m/e 303, which cleaved an acetylene and accepted hydrogen to produce the phenanthridine-6-methylthiosemicarbazide ion at m/e 282. The

**Table 1:** *1-(Phenanthridine-6'-formyl)-4-substituted 3-thiosemicarbazones* **3–8**

| Compd. | R | Yield % | M.P.°C Cryst. solvent | Molecular formula | Calcd. | Found | $^1$H-NMR (δ) ppm in DMSO-$d_6$ |
|---|---|---|---|---|---|---|---|
| 3 | H | 77 | 258–260 A | $C_{15}H_{12}N_4S$ | C: 64.3<br>H: 4.32<br>N: 20.0 | 63.9<br>3.95<br>19.6 | 7.64–8.12(m, 2H, $CSNH_2$, mixed with phenanthridine protons), 8.60(s, 1H, CH=N), 10.0 (s, 1H, NH–CS). |
| 4 | $C_6H_5$ | 71 | 199–200 B | $C_{21}H_{16}N_4S$ | C: 70.8<br>H: 4.53<br>N: 15.7<br>S: 9.0 | 70.8<br>4.50<br>15.3<br>8.9 | 7.08–7.44(m, 5H, Ar-H), 8.72(s, 1H, CH=N), 9.90 (s, 1H, N–NH–CS), 11.95 (s, 1H, Ar-NH). |
| 5 | $C_6H_4CH_3$(p) | 83 | 188–190 | $C_{22}H_{18}N_4S$ | N: 15.1 | 15.4 | |
| 6 | $C_6H_4OCH_3$(o) | 88 | 236–237 C | $C_{22}H_{18}N_4OS$ | N: 14.5<br>S: 8.3 | 14.4<br>8.0 | 3.86(s, 3H, $OCH_3$), 6.82–7.16 (m, 4H, Ar-H), 8.70(s, 1H, CH=N), 9.89 (s, 1H, N–NH–CS), 12.10 (s, 1H, Ar–NH). |
| 7 | $C_6H_4Cl$(p) | 64 | 215–217 A | $C_{21}H_{15}ClN_4S$ | C: 64.5<br>H: 3.84<br>N:14.3 | 64.5<br>3.80<br>14.1 | 7.28–7.34(m, 4H, Ar–H), 8.72 (s, 1H, CH=N), 10.58 (s, 1H, N–NH–CS), 12.04 (s, 1H, Ar–NH). |
| 8 | $C_6H_4Br$(p) | 83 | 218–220 A | $C_{21}H_{15}BrN_4S$ | C: 57.9<br>H: 3.45<br>N:12.9 | 57.8<br>3.30<br>12.8 | |

Crystallization solvents: A = washing with hot ethanol, B = from ethanol/benzene mixture, C = from benzene.

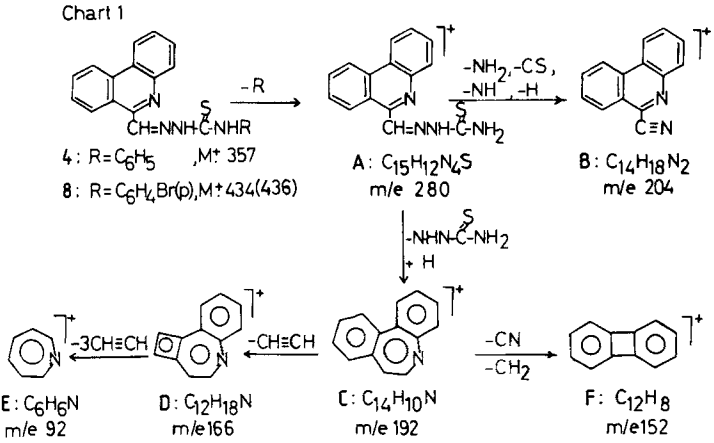



Scheme 1

**Table 2:** *Phenanthridine-6-(3',4'-disubstituted)(2',3'-dihydrothiazol-2'-ylideno)formylhydrazone Derivatives* **9–22**

| Compd. | R | $R^1$ | Yield % | M.P.°C | Molecular formula | Calcd. | Found |
|---|---|---|---|---|---|---|---|
| **9** | $C_6H_5$ | $C_6H_5$ | 82 | 202–203 | $C_{29}H_{20}N_4S$ | C: 76.3<br>H: 4.42<br>N: 12.3 | 75.9<br>4.60<br>12.6 |
| **10** | $C_6H_5$ | $C_6H_4Cl$(p) | 87 | 240–242 | $C_{29}H_{19}ClN_4S$ | C: 70.9<br>H: 3.87<br>N: 11.4 | 71.0<br>4.20<br>11.0 |
| **11** | $C_6H_5$ | $C_6H_5C_6H_4$(p) | 77 | 249–250 | $C_{35}H_{24}N_4S$ | N: 10.5<br>S: 6.0 | 10.4<br>6.3 |
| **12** | $C_6H_4CH_3$(p) | $C_6H_5$ | 91 | 202–203 | $C_{30}H_{22}N_4S$ | C: 76.6<br>H: 4.71<br>N: 11.9 | 76.6<br>4.60<br>11.5 |
| **13** | $C_6H_4CH_3$(p) | $C_6H_4Cl$(p) | 81 | 275–276 | $C_{30}H_{21}ClN_4S$ | C: 71.4<br>H: 4.16<br>N: 11.1<br>S: 6.3 | 71.3<br>4.20<br>11.1<br>6.5 |
| **14** | $C_6H_4CH_3$(p) | $C_6H_5C_6H_4$(p) | 81 | 231–233 | $C_{36}H_{26}N_4S$ | N: 10.3<br>S: 5.9 | 10.0<br>6.2 |
| **15** | $C_6H_4OCH_3$(o) | $C_6H_5$ | 80 | 238–239 | $C_{30}H_{22}N_4OS$ | C: 74.1<br>H: 4.56 | 74.5<br>4.70 |
| **16** | $C_6H_4OCH_3$(o) | $C_6H_4Cl$(p) | 74 | 232–233 | $C_{30}H_{21}ClN_4OS$ | C: 69.2<br>H: 4.03<br>N: 10.8 | 69.2<br>3.90<br>10.8 |
| **17** | $C_6H_4Cl$(p) | $C_6H_5$ | 92 | 195–196 | $C_{29}H_{19}ClN_4S$ | N: 11.4<br>S: 6.5 | 11.3<br>6.0 |
| **18** | $C_6H_4Cl$(p) | $C_6H_4Cl$(p) | 86 | 219–221 | $C_{29}H_{18}Cl_2N_4S$ | N: 10.7<br>S: 6.1 | 11.2<br>6.4 |
| **19** | $C_6H_4Cl$(p) | $C_6H_5C_6H_4$(p) | 97 | 276–277 | $C_{35}H_{23}ClN_4S$ | N: 9.9<br>S: 5.7 | 10.1<br>6.0 |
| **20** | $C_6H_4Br$(p) | $C_6H_5$ | 94 | 200–202 | $C_{29}H_{19}BrN_4S$ | N: 10.5 | 10.1 |
| **21** | $C_6H_4Br$(p) | $C_6H_4Cl$(p) | 84 | 252–253 | $C_{29}H_{18}BrClN_4S$ | C: 61.1<br>H: 3.16<br>N: 9.8 | 61.1<br>3.20<br>9.4 |
| **22** | $C_6H_4Br$(p) | $C_6H_5C_6H_4$(p) | 96 | 279–281 | $C_{35}H_{23}BrN_4S$ | N: 9.2 | 9.3 |

Chart 1




spectra also showed the ions at m/e 204, 192, 166, 152 and 92, thus confirming that the fragmentation of the phenanthridine part of the products proceeded in the same pattern as proposed above for the thiosemicarbazone derivatives. A similar pattern was reported for the fragmentation of quinoline and isoquinoline derivatives[22,23]. The structures due to the additional ions in the spectra of all examined products were found to agree with the structures proposed for the fragmentation of such functional groups previously reported from this laboratory[24].

## Biological Screening, Results and Discussion

*Anticancer properties*: The thiosemicarbazones **3–8** in doses of 400, 200 and 100 mg/kg, suspended in hydroxypropylcellulose (HPC, Klucel), were given as intraperitoneal injections in female C57BL/6 mice inoculated with P 388 lymphocytic leukemia. The assays were performed in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda, MD 20014, U.S.A. and the activities were measured as the ratio of the mean survival time of the test animals to that of the control animals and expressed as (% T/C). The activity of the products **3–8** ranged between 90 and 99 with is far below the minimum ratio-requirement of 125.

*Antimicrobial properties*: The products **9–22** in concentration of 100, 10 and 1 μg/ml were evaluated against Escherichia coli (NCTC L0418), Klebsiella aerogenes A, Pseudomonas aeruginosa (NCTC 10662), Serratia marcescens (US 32), Staphylococcus aureus Oxford, Candida albicans (W 97), Bacteroides fragilis (BC 4), Bacteroides fragilis (NCTC 8560) and Bacteroides fragilis (B 3), using the serial dilution method in *Meller Hinton* agar. The tests were performed in accordance with the protocol of antimicrobial acreening of the Chemotherapeutic Research Centre, Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey, BH3 7AJ, United Kingdom. All compounds were inactive in the tests performed except for compound **20** which showed activity against Bacteroides fragilis (B 3) at the highest concentration of 100 μg/ml.

The lack of antileukemic activity of the thiosemicarbazones **3–8**, although unexpected when considering the potent activity exhibited by various 6-formylthiosemicarbazones derived from pyridine[25], quinoline[26] and isoquinoline[27] and the ability of the products to form the tridentate ligands which blocks the DNA synthesis[28], reflects the effect of the bulk of the phenanthridine nucleus in preventing the penetration of the compounds to reach the target tissues. Similarly, the failure of any compound to show antimicrobial

activity against various types of bacteria, although the compounds contain the bacteriostatic phenanthridine[11–17] and thiazoline[29] nuclei as structural elements, is suggested to be due to the bulk induced by the substituted thiazoline ring on the whole molecules.

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## Experimental Part

*MP*: not corr. *Elemental analyses*: Laboratory for Microanalysis of the Faculty of Sciences, Cairo University, Egypt. *IR spectra*: Beckman 4210 IR (Nujol mull technique). *$^1$HNMR spectra*: Varian EM 360L. *Mass spectra*: Finnigan 3200.

*1-(Phenanthridine-6'-formyl)-4-substituted-3-thiosemicarbazones* **3–8**

A solution of 1 g (4.8 mmole) phenanthridine-6-carboxaldehyde **(2)**[14] in 25 ml hot ethanol was treated with the equivalent amount of thiosemicarbazide or the properly 4-substituted 3-thiosemicarbazide and the mixture was heated under reflux for 1 h. The products, separated during the reflux time or after cooling the reaction mixture to room temp., were crystallized from the proper solvents giving the required products (table 1). IR (Nujol): 3390–3310 and 3260–3060 (NH), 1605–1590 (C=N and C=C aromatic), 1515–1490 (C=C aromatic), 1540–1515, 1350–1340, 1185–1155 and at 915–910 $cm^{-1}$. Mass spectrum of compound **4**: m/e (relative abundance %): 356 (17), 296 (8), 284 (10), 283 (17), 282 (24), 280 (2), 264 (9), 263 (16), 254 (26), 237 (11), 236 (28), 235 (31), 225 (17), 224 (24), 221 (16), 220 (45), 206 (20), 205 (27), 204 (65), 193 (24), 192 (33), 191 (26), 190 (27), 180 (15), 179 (36), 178 (39), 177 (15), 166 (13), 165 (42), 163 (18), 153 611), 152 (22), 151 (34), 150 (36), 141 (15), 139 (11), 137 (14), 136 (20), 135 (66), 126 (15), 125 (13), 123 (15), 118 (27), 108 (15), 105 (10), 103 (14), 102 (21), 100 (10), 99 (12), 94 (22), 93 (100), 92 (32).

*Phenanthridine-6-(3',4'-disubstituted)(2',3'-dihydrothiazol-2'-ylidino)formylhydrazones* **9–22**

A mixture of the thiosemicarbazone derivatives **3–8** (250 mg) and the equivalent amount of the selected phenacyl bromide in 25 ml absol. ethanol was heated under reflux while stirring for 1 h. The reddish brown solutions so produced were left to cool to room temp., diluted with enough water, to produce a permanent turbidity, and set aside for complete separation of the products which were crystallized from ethanol-benzene mixture (table 2). IR (Nujol): 1600–1585 (C=N and C=C aromatic), 1510–1490 (C=C aromatic) and at 695–690 $cm^{-1}$ (C-S). $^1$HNMR ($CDCl_3$): δ (ppm). For compound **11**: 6.31 (s, 1H, thiazoline-H), 7.1–7.52 (m, 14H, Ar-H), 8.81 (s, 1H, CH=N). For compound **13**: 2.36 (s, 3H, tolyl-$CH_3$), 6.23 (s, 1H, thiazoline-H), 6.98–7.26 (m, 8H, Ar-H), 8.78 (s, 1H, CH=N). For compound **16**: 3.64 (s, 3H, $OCH_3$), 6.2 (s, 1H, thiazoline-H), 6.8–7.42 (m, 8H, Ar-H), 8.74 (s, 1H, CH=N). For compound **19**: 6.30 (s, 1H, thiazoline-H), 7.08–7.58 (m, 13H, Ar-H), 8.80 (s, 1H, CH=N). For compound 21: 6.29 (s, 1H, thiazoline-H), 6.96–7.58 (m, 8H, Ar-H), 8.79 (s, 1H, CH=N).

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