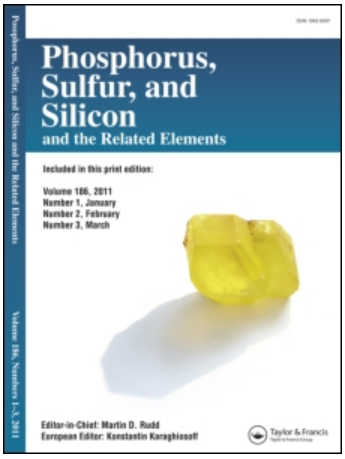

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Synthesis of Novel Heterocyclic Compounds for Antitumor and Radioprotective Activities

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SYNTHESIS OF NOVEL HETEROCYCLIC COMPOUNDS FOR ANTITUMOR AND RADIOPROTECTIVE ACTIVITIES

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Some novel pyridothienoxazine (5); pyridothienopyrimidines (6), (8), (9), (14); pyridothienoimidazole (16); isothiazolopyridine (17), and pyridoisothiazolopyrimidines (18–20) were synthesized. The structural assignment of the prepared compounds were based on microanalytical and spectroscopic evidences. Some prepared compounds were tested in vitro for their antitumor and radioprotective activities. Compounds (7), (12), (17) and (19) showed significant activities against EAC cells, at a concentration of 250μg/ml; while the isothiazolopyridine (17) exhibited radioprotective activity.

Keywords: Pyridothienopyrimidines; isothiazolopyridine; pyridoisothiazolopyrimidines; antitumor and radioprotection activities

INTRODUCTION

Pyridothienopyrimidines, an example of triheterocyclic system, have attracted much attention because of their promising biological activities[1]. The various derivatives of 3-cyano-2(1*H*)pyridinethione are of some interest as strategic intermediates for the production of physiologically active substances, for the protection of plants,[2–6]. On the other hand many pyridine, pyrimidine, imidazole, isothiazole derivatives incorporating a sulfur are widely used as antitumor and radioprotective drugs[7–13]. Therefore, it seemed of interest to synthesize a series of fused ring pyridothieno-hetero-

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cyclic, an isothiazolopyridine and pyridoisothiazolopyrimidines to evaluate their antitumor and radioprotection activities.

RESULTS AND DISCUSSION

Interaction of (1)[14] with some active methylene chloro compounds, namely chloroacetone and ethyl chloroacetate, furnished 2-acetyl-methylthio-6-phenyl-4-methyl-3-pyridinecarbonitrile (2a) and/or ethyl (3-cyano-6-phenyl-4-methylpyridinylthio)acetate (2b), respectively. IR spectrum of (2a) showed bands at 2920 Cm^{-1} (CH aliphatic), 2210 Cm^{-1} (C≡N), 1690 cm^{-1} (C=O). IR spectrum of (2b) exhibited bands at 2950 Cm^{-1} (CH aliphatic), 2225 Cm^{-1} (C≡N), 1750 Cm^{-1} (C=O). ^{1}H-NMR spectrum of (2a in DMSO-d_6) showed signals at 2.4 [s, 3H, CH_3], 3.4 [s, 3H, $COCH_3$], 4.1 [s, 2H, SCH_2], 7.1–7.8 [m, 6H, Ar-H]. ^{1}H-NMR spectrum of (2b in DMSO-d_6) exhibited signals at 1.1 [t, 3H, CH_3, ethyl], 2.8 [s, 3H, CH_3], 4.0 [s, 2H, SCH_2], 4.2 [q, 2H, CH_2, ethyl], 7.3–8.1 [m, 6H, Ar-H].

Cyclocondensation of (2a,b) in boiling ethanol in the presence of sodium ethoxide gave 2-acetyl-3-amino-6-phenyl-4-methylthieno[2,3-b]-pyridine (3a) and/or ethyl-3-amino-6-phenyl-4-methylthieno[2,3-b]-pyridine-2-carboxylate (3b). IR spectrum of (3a) showed disappearance of (C≡N) band and presence of bands at 3450, 3330 Cm^{-1} (NH_2), 2900 Cm^{-1} (CH aliphatic), 1640 Cm^{-1} (C=O). IR spectrum of (3b) showed absence of (C≡N) band and presence of bands at 3400, 3320 Cm^{-1} (NH_2), 2910 Cm^{-1} (CH aliphatic), 1700 cm^{-1} (C=O). ^{1}H-NMR spectrum of (3b in DMSO-d_6) exhibited signals at 1.8 [t, 3H, CH_3, ethyl], 2.8 [s, 3H, CH_3], 4.1 [q, 2H, CH_2, ethyl], 6.3 [s, 2H, NH_2]; 7.4–8.1 [m, 6H, Ar-H].

Saponification of the amino ester (3b) using alcoholic sodium hydroxide furnished sodium-3-amino-6-phenyl-4-methylthieno[2,3-b]-pyridine-2-carboxylate (4), which afforded the 7-phenyl-2,9-dimethyl-pyrido [3`,2`:4,5]thieno[3,2-d] –3,1-oxazine-4(3H)-one (5), upon heating under reflux in acetic anhydride. IR spectrum of (5) showed the absence of (NH_2) bands and presence of (C=O) at 1690 Cm^{-1}.^{1}H-NMR spectrum of (5 in DMSO-d_6) exhibited signals at 2.2 [s, 3H, CH_3], 2.9 [s, 3H, CH_3, pyridine], 7.2–8.1 [m, 6H, Ar-H].

Treatment of (5) with ammonium acetate in boiling acetic acid led to formation of 7-phenyl-2,9-dimethylpyrido[3',2':4,5]thieno[3,2-d]-pyrimidine-4(3H)-one (6). The same product (6) was obtained also when (7) was heated under reflux in acetic anhydride. IR spectrum of (6) gave bands at 3365 Cm^{-1} (NH), 1675 Cm^{-1} (C=O). ^{1}H-NMR spectrum of (6 in DMSO-d_6) exhibited signals at 2.4 [s, 3H, CH_3, pyrimidine], 2.9 [s, 3H, CH_3, pyridine], 7.2–7.8 [m, 6H, Ar-H], 10.5 [s, 1H, NH].

6-Phenyl-3-amino-4-methylthieno[2,3-b]pyridine-2-carboxamide (7) was obtained via reaction of (1) with chloroacetamide in presence of sodium ethoxide. IR spectrum of (7) showed the absence of (C≡N) band and presence of bands at 3400, 3340 Cm^{-1} (NH_2), 1680 Cm^{-1} (C=O). ^{1}H-NMR spectrum of (7 in DMSO-d_6) showed signals at 2.8 [s, 3H, CH_3], 3.5 [s, 2H, $CONH_2$], 7.2 [s, 2H, NH_2], 7.5–8.1 [m, 6H, Ar-H].

Interaction of (3b) with formamide gave 7-phenyl-9-methylpyrido-[3`,2`:4,5]thieno[3,2-d]pyrimidine-4(3H)-one (8). IR spectrum of (8) exhibited bands at 3380 Cm^{-1} (NH), 1700 Cm^{-1}(C=O). ^{1}H-NMR spectrum of (8 in DMSO-d_6) showed signals at 2.8 [s, 3H, CH_3], 6.6 [s, 1H, CH, pyrimidine], 7.4–8.1 [m, 6H, Ar-H], 10.1 [s, 1H, NH].

Interaction of (3b) with phenyl isothiocyanate in dry pyridine for 48hr gave 2-thioxo-3,7-diphenyl-9-methylpyrido[3`,2`:4,5]thieno-[3,2-d]pyrimidine-4(3H)-one (9). IR spectrum of (9) showed bands at 3300 Cm^{-1} (NH), 1700 Cm^{-1} (C=O), 1600 Cm^{-1} (C=N), 1280 Cm^{-1} (C=S). ^{1}H-NMR spectrum of (9 in DMSO-d_6) exhibited signals at 2.7 [s, 3H, CH_3], 7.0–7.9 [m, 11H, Ar-H], 10.7 [s, 1H, NH]. Mass spectrum of (9) showed a molecular ion peak m/z 401 (0.1%) with a base peak at 194 (100%) and other significant peaks appeared at 373 (0.5%), 301 (0.26%), 289 (1.85%), 288 (16.62%), 286 (27.75%), 228 (6.87%), 210 (10.15%), 167 (8.34%), 105 (0.79%), 93 (74.71%), 77 (45.11%), 65 (24.64%).

Although a number of papers have been published concerning the synthesis of 2(1*H*)-pyridinethione derivatives, those containing a styryl group have not yet been reported. Now we report the synthesis of some new 3-cyano-2(1*H*)-pyridinethiones containing styryl group and the corresponding polyfunctionally substituted-3-aminothieno-[2,3-b]pyridine derivatives. Thus, interaction of (1) with aromatic aldehydes afforded 3-cyano-6-phenyl-4-styryl-2-(1*H*) -pyridinethione (10), which was converted into ethyl-3-amino-6-phenyl-4-styrylthieno [2,3-b]pyridine-2-carboxylate (11) via reaction of (10) with ethyl chloroacetate in dimethylformamide in presence of anhydrous potassium carbonate. IR

spectrum of (10a) showed bands at 3400 Cm^{-1} (NH), 2220 Cm^{-1} (C≡N), 1290 Cm^{-1}(C=S). IR spectrum of (11a) showed the absence of (C≡N) band and presence of bands at 3320, 3280 Cm^{-1} (NH_2), 2950 Cm^{-1}(CH aliphatic), 1680 Cm^{-1} (C=O).^{1}H-NMR spectrum of (10a in DMSO-d_6) showed signals at 7.3 [d, 1H, CH (b)], 7.9 [d, 1H, CH (a)], 7.4–7.8 [m, 11H, Ar-H], 8.4 [s, 1H, NH]. ^{1}H-NMR spectrum of (11a in DMSO-d_6) showed signals at 1.3 [t, 3H, CH_3, ethyl], 4.4 [q, 2H, CH_2, ethyl], 6.7 [br, 2H, NH_2], 7. 1 [d, 1H, CH(b)], 7.3–7.8 [m, 11H, Ar-H], 7.9 [d, 1H, CH (a)].

3-Amino–4-methylthieno[2,3-b]pyridine-2-carboxhydrazide (12) was obtained upon treatment of the amino ester (3b) or its precursor (2b) with hydrazine hydrate. IR spectrum of (12) exhibited bands at 3400, 3360, 3280 Cm^{-1} (NH, NH_2), 1640 Cm^{-1} (C=O).

The interaction of (12) with acetylacetone furnished 3-amino-4-methyl-2-(3,5-dimethylpyrazol-1-yl)carbonylthieno-[2,3-b]pyridine (13). IR spectrum of (13) showed bands at 3200, 3150 Cm^{-1} (NH_2), 1700 Cm^{-1}(C=O). Mass spectrum of (13) showed a molecular ion peak m/z 362 (0.50%), with a base peak at 317 (100%) and other significant peaks appeared at 334 (50.71%), 332 (97.44%), 289 (85.16%), 274 (31.84%), 189 (12.52%), 158 (71.45%), 144 (93.25%), 94 (10.30%), 75 (2.61%).

3-Formylamino-7-phenyl-9-methylpyrido[3`,2` : 4,5]thieno[2,3-d]-pyrimidine-4(3H)-one (14) was obtained upon treating the amino hydrazide (12) with formic acid. The interaction of (12) with nitrous acid gave 3-amino-6-phenyl-4-methylthieno[2,3-b]pyridine-2-carboxyazide (15), which was subjected to Curtius rearrangement in refluxing xylene[15] to give 2-oxo-6-phenyl-8-methylpyrido[3`,2`:4,5]thieno[3,2-d]imidazole (16). IR spectrum of (14) showed bands at 3380 Cm^{-1} (NH), 2930 Cm^{-1} (CH aliphatic), 1700, 1650 Cm^{-1} (2C=O). IR spectrum of (15) showed bands at 3230, 3190 Cm^{-1} (NH_2), 2180 Cm^{-1} (CON_3), 1690 Cm^{-1} (C=O). IR spectrum of(16) showed the absence of (CON_3) band and presence of bands at 3420, 3360 Cm^{-1} (2NH), 1650 Cm^{-1} (C=O). ^{1}H-NMR spectrum of (14 in DMSO-d_6) showed signals at 2.7 [s, 3H, CH_3],7.4–8.2 [m, 6H,Ar-H], 8.5 [s, 1H, CH, pyrimidine], 8.6 [s, 1H, CHO], 11.6 [s, 1H, NH].

Treatment of (1) with chloramine furnished 3-amino-4-methyl-6-phenyl-isothiazolo[5,4-b]pyridine (17), which was reacted with, methyl acrylate, diethylmalonate and/or ethyl acetoacetate to give (18), (19) and (20), respectively. IR spectrum of (17) showed the absence of (C≡N) band and

presence of bands at 3420, 3300 Cm^{-1} (NH_2), 3100 Cm^{-1} (CH aromatic), 1630 cm^{-1} (C=N). IR spectra of (18, 19, 20) showed the absence of (NH_2) bands. Mass spectrum of (17) showed a molecular ion peak m/z 241 (100%, base peak), and other significant peaks appeared at 243 (29.10%), 212 (21.28%), 192 (26.20%), 166 (27.86%), 127 (17.07%), 102 (15.08%), 77 (21.13%), 63 (10.52%). ^{1}H-NMR spectrum of (18 in DMSO-d_6) exhibited signals at 2.7 [s, 3H, CH_3], 3.9 [t, 2H, CH_2CO], 4.3 [t, 2H, N-CH_2], 7.2–7.8 [m, 6H, Ar-H]. Mass spectrum of (19) showed a molecular ion peak m/z 309 (100%, base peak) and other significant peaks appeared at 310 (18.36%), 281 (27.59%), 267 (54.78%), 241 (50.83%), 226 (17.31%), 193 (21.65%), 140 (28.07%), 77 (18.35%), 69 (94.94%), 63 (7.39%). Mass spectrum of (20) showed a molecular ion peak m/z 307 (1.12%), with a base peak at 241 (100%) and other significant peaks appeared at 308 (2.37%), 309 (2.44%), 268 (35.66%), 192 (31.70%), 139 (24.51%), 85 (21.34%), 69 (15.06%).

EXPERIMENTAL

All melting points are uncorrected. The IR spectra were recorded on potassium bromide disks on a JASCO FT IR spectrometer. ^{1}H-NMR spectra were measured on a Varian GEMINI 200 instrument (200 MHz, ^{1}H-NMR), using DMSO-d_6 as a solvent and TMS as internal standard. Chemical shifts are expressed as δ ppm units. Mass spectra were obtained using GCMS qp 1000 ex Scheimadzu instrument (70eV). Elemental analyses were checked on a Perkin-Elmer CHN 240 A analyzer in Cairo University (ARE). The irradiation tool was a Canadian Cesium 137 irradiation unit installed at National Centre for Radiation Research and Technology.

Alkylation of 3-cyano –6-phenyl –4-methyl-2(1H)-pyridinethione:

General Procedure

Compound (1, 0.005 mol) was dissolved in ethanolic solution of sodium hydroxide (20 ml, 10%), then chloroacetone or ethyl chloroacetate (0.05 mol) was added and the mixture was heated under reflux for 15 minutes. After cooling, the reaction mixture was poured into cold water, and the

solid product was collected and recrystallized from ethanol to give 2a or 2b (Table I).

Cyclization of 2-alkylthio-6-phenyl-4-methylpyridine-3-carbonitrile

The mercapto derivatives (2a or 2b; 0.005 mol) was heated under reflux in ethanolic sodium ethoxide solution (0.5 g, 0.02 g-atom of sodium in 25 ml of absolute ethanol) for 30 minutes. After cooling, the solid product was filtered and recrystallized from ethanol to give 3a or 3b (Table I).

Formation of 4

The amino ester (3b, 0.01 mol) was heated under reflux in methanolic sodium hydroxide (30 ml, 10%) for 3hr. The solid product obtained after cooling was filtered, washed with ethanol and dried. This compound was used as such in the next procedure.

Formation of oxazine derivative 5

The sodium salt (4, 0.01 mol) was heated under reflux in acetic anhydride (20 ml) for 3hr. The solid precipitate obtained on cooling was filtered and recrystallized from dioxane to give 5 (Table I).

Formation of 6

Method A

A mixture of (5, 0.01 mol), ammonium acetate (0.02 mol) was heated under reflux in acetic acid (20 ml) for 3hr. The solid product obtained after cooling was filtered and recrystallized from acetic acid to give 6 (Table I).

Method B

A solution of (7, 0.01 mol) in acetic anhydride (5 ml) was heated under reflux for 12hr. After cooling the solid formed was collected and recrystallized from acetic acid to give 6.

Formation of 7

To a solution of (1; 0.005 mol) in ethanolic sodium ethoxide solution (0.5 g, 0.02 g-atom of sodium in 25 ml of absolute ethanol), chloroaceta-

mide (0.005 mol) was added and the mixture was heated under reflux for 1hr. After cooling, the solid product was collected and recrystallized from ethanol to give 7 (Table I).

Formation of 8

A mixture of (3b, 0.01 mol) and formic acid (20 ml) was heated under reflux for 8 hr. The solid product formed on cooling was collected and recrystallized from ethanol to give 8 (Table I).

Formation of 9

A mixture of (3b, 0.01 mol) and phenyl isothiocyanate (0.01 mol) in pyridine (20 ml) was refluxed in an oil-bath for 48 hr (tlc). The reaction mixture was cooled and triturated with aqueous ethanol. The resulting solid was filtered, dried and recrystallized from dimethylformamide to give 9 (Table I).

Formation of 10

To a solution of (1, 0.01 mol) in dioxane (20 ml), benzaldehyde or 2-thienaldehyde (0.011 mol) and a catalytic amount of piperidine were added. The reaction mixture was refluxed for 45hr. After cooling, the precipitate was filtered and recrystallized from acetic acid to give 10a or 10b (Table I).

Formation of 11

To a solution of (10a,b; 0.01 mol) in dimethylformamide (50 ml), potassium carbonate anhydrous (2.76 g, 0.02 mol) and ethyl chloroacetate (0.01 mol) were added. The reaction mixture was stirred at room temperature for 7hr and then diluted with cold water (50 ml). The resulting solid product was collected by filtration, washed with water, dried and recrystalized from ethanol to give 11a or 11b (Table I).

Formation of 12

A mixture of (2b; 0.01 mol) and hydrazine hydrate (0.1 mol) in ethanol were heated under reflux for 2 hr and was then allowed to cool. The solid

precipitate was filtered, washed with water and recrystallized from dioxane to give 12 (Table I). Alternatively, it was obtained in 95% yield using compound 3b instead of 2b.

Formation of 13

A mixture of (12; 0.01 mol) and acetylacetone (0.01 mol) was heated under reflux in ethanol (20 ml) for 6 hr and was then allowed to cool. The solid precipitate was filtered and recrystallized from ethanol to give 13 (Table I).

Formation of 14

A mixture of (12, 0.01 mol) and formic acid (10 ml) was heated at reflux for 6 hr., and allowed to cool. The solid precipitate was collected and recrystallized from ethanol-dimethylformamide to give 14 (Table I).

Formation of 15

A solution of sodium nitrite (0.01 mol) in water (10 ml) was added with stirring to a solution of compound 12 (0.01 mol) in acetic acid (15 ml) during 5 minutes at room temperature. The solid product was filtered, washed with cold water and air dried to give 15 (Table I).

Formation of 16

The carboxyazide (15, 0.01 mol) was heated at reflux in xylene (30 ml) for 30 minutes and then was allowed to cool. The solid product was filtered, washed with petroleum ether, dried and recrystallized from dioxane to give 16 (Table I).

Formation of 17

Aqueous chloramine [made by mixing 14% w/w sodium hypochlorite (150 g) and aqueous ammonia (d 0.88; 12g) at 0°C was added in one portion to a stirred solution of (1, 0.044 mol) in M sodium hydroxide (100 ml) at 70°C. The mixture was allowed to cool, the colourless precipitate was collected, washed well with water, and recrystallized from ethanol to give 17 (Table I).

Formation of 18

A suspension of 17 (0.01 mol) and (0.01 mol) of methyl acrylate in benzene (30ml) was heated under reflux. In 5 minutes the solid disappeared and instantaneously a new white solid precipitated, which was filtered and recrystallized from dioxane to give 18 (Table I).

Formation of 19

A suspension of 17 (0.01 mol) and (0.01 mol) of diethyl malonate in o-dichlorobenzene (20ml) was heated under reflux for 3 hr. When the solution was cooled a white solid crystallized which was purified from o-dichlorobenzene to give 19 (Table I).

Formation of 20

A mixture of 17 (0.01 mol), ethyl acetoacetate (0.01 mol) and 20 ml of ethanol was refluxed for 3 hr. Once the solution cooled, a white solid crystallized which was recrystallized from methanol to give 20 (Table I).

TABLE I Characterization data of newly synthesized compounds (2-20)

Compd. No.	*M. P °C*	*Yield %*	*Mol.-formula*	*Analysis % Required / (Found)*		
				C	*H*	*N*
2a	124–26	64	$C_{16}H_{14}N_2OS$	68.08	4.96	9.92
				(68.20)	(4.60)	(10.10)
2b	135–37	58	$C_{17}H_{16}N_2O_2S$	65.38	5.12	8.97
				(65.60)	(5.30)	(8.60)
3a	> 300	82	$C_{16}H_{14}N_2OS$	68.08	4.96	9.92
				(68.30)	(4.70)	(9.70)
3b	276–78	86	$C_{17}H_{16}N_2O_2S$	65.38	5.12	8.97
				(65.80)	(5.40)	(8.70)
4	> 300	79	$C_{15}H_{11}N_2O_2SNa$	58.82	3.59	9.15
				(59.10)	(3.20)	(9.40)
5	280–82	65	$C_{17}H_{12}N_2O_2S$	66.23	3.89	9.09
				(66.50)	(3.60)	(8.80)
6	> 300	61	$C_{17}H_{13}N_3OS$	66.44	4.23	13.68
				(66.80)	(4.10)	(13.30)
7	> 300	85	$C_{15}H_{13}N_3OS$	63.60	4.59	14.84
				(63.20)	(4.20)	(14.40)

Compd. No.	*M. P °C*	*Yield %*	*Mol.-formula*	*Analysis % Required / (Found)*		
				C	*H*	*N*
8	> 300	63	$C_{16}H_{11}N_3OS$	65.52	3.75	14.33
				(65.80)	(3.50)	(14.10)
9	220–22	58	$C_{22}H_{15}N_3OS_2$	65.83	3.74	10.47
				(65.50)	(3.90)	(10.80)
10a	> 300	61	$C_{20}H_{14}N_2S$	76.43	4.45	8.91
				(76.10)	(4.10)	(8.60)
10b	160–62	65	$C_{18}H_{12}N_2S_2$	67.50	3.75	8.75
				(67.90)	(3.50)	(8.50)
11a	> 300	51	$C_{24}H_{20}N_2O_2S$	72.00	5.00	7.00
				(72.30)	(4.80)	(7.20)
11b	> 300	56	$C_{22}H_{18}N_2O_2S_2$	65.02	4.43	6.89
				(64.80)	(4.10)	(6.60)
12	287–89	81	$C_{15}H_{14}N_4OS$	60.40	4.69	18.79
				(60.60)	(4.40)	(18.40)
13	218–20	67	$C_{20}H_{18}N_4OS$	66.29	4.97	15.46
				(66.10)	(4.60)	(15.10)
14	248–50	71	$C_{17}H_{12}N_4O_2S$	60.71	3.57	16.66
				(60.40)	(3.20)	(16.30)
15	158–60	84	$C_{15}H_{11}N_5OS$	58.25	3.55	22.65
				(58.60)	(3.70)	(22.40)
16	> 300	78	$C_{15}H_{11}N_3OS$	64.05	3.91	14.94
				(63.80)	(3.50)	(14.70)
17	133–35	90	$C_{13}H_{11}N_3S$	64.73	4.56	17.42
				(64.40)	(4.20)	(17.20)
18	165–67	82	$C_{16}H_{13}N_3OS$	65.08	4.40	14.23
				(65.30)	(4.10)	(14.50)
19	240–42	74	$C_{16}H_{11}N_3O_2S$	62.13	3.55	13.59
				(62.40)	(3.80)	(13.20)
20	202–204	86	$C_{17}H_{13}N_3OS$	66.44	4.23	13.68
				(66.10)	(4.40)	(13.90)

BIOCHEMICAL STUDIES

In-vitro studies

- *Determination of LD_{50} of the synthesized compounds*

Various concentrations (25, 50, 100, 250 and 500 µg/ml)of the selected compounds (1, 2b, 7, 12, 17 and 19) were incubated with Ehrlich Ascites

Carcinoma cells (2.5×10^6 cells/ml) for 2 hr. The cytotoxic effect of the tested compounds were determined according to the method of Lin. et al (1978)[16], (Table II).

TABLE II In-vitro cytotoxic activity of the synthesized compounds against Ehrlich Ascites Carcinoma cells

Compd. No.	*% viability**					
	conc μg/ml					
	0	*25*	*50*	*100*	*250*	*500*
1	100	91	84	70	76	71
2b	100	96	82	83	71	63
7	100	71	78	55	7	0
12	100	93	92	41	23	0
17	100	100	100	59	23	0
19	100	74	94	66	41	23

* % of non viable cells = $\frac{\text{No. of non viable}}{\text{Total No. of cells}} \times 100$

Effect of the synthesized compounds on growth rate of EAC cells

Compounds (1, 2b, 7, 12, 17 and 19) were incubated for 2 hr with EAC cells (2.5×10^6 cells/ml) in a concentration of 100 μg/ml. At the end of incubation the viable cells were counted in aheomocytometer using trypan blue dye according to the method of Takemoto, et al (1982)[17]. These results were compared with those obtained by irradiation with a dose of 5 Gy after incubation.

TABLE III Effect of the synthesized compounds[a] on viability of EAC cells with or without radiation exposure

Compd. No.	*Non-Irradiated Group*		*Irradiated Group*[b]	
	No. of viable cells mean[c] ± *SE*	*% Mortality*	*No. of viable cells Mean* ± *SE*	*% Mortality*
Control	$2.6 \times 10^6 \pm 0.06 \times 10^6$	-	$2.26 \times 10^6 \pm 0.01 \times 10^6$*	13
1	$1.75 \times 10^6 \pm 0.01 \times 10^6$*	30	$0.416 \times 10^6 \pm 0.02 \times 10^6$*	84
2b	$2.087 \times 10^6 \pm 0.03 \times 10^6$*	16.5	$0.37 \times 10^6 \pm 0.01 \times 10^6$*	85.7

Compd. No.	*Non-Irradiated Group*		*Irradiated Group*[b]	
	No. of viable cells mean[c] ± *SE*	*% Mortality*	*No. of viable cells Mean ± SE*	*% Mortality*
7	$1.375 \times 10^6 \pm 0.01 \times 10^6$*	45	$2.593 \times 10^6 \pm 0.01 \times 10^6$	100
12	$1.06 \times 10^6 \pm 0.02 \times 10^6$*	58.5	$0.150 \times 10^6 \pm 0.01 \times 10^6$*	94.2
17	$1.487 \times 10^6 \pm 0.03 \times 10^6$	40.5	$2.37 \times 10^6 \pm 0.09 \times 10^6$*	8
19	$1.73 \times 10^6 \pm 0.06 \times 10^6$*	33.5	$2.61 \times 10^6 \pm 0.08 \times 10^6$	100

a- Compound concentration = 100 µg/ml
b- Radiation dose = 5 Gy as single dose
c- Mean of count for at least four sterile test tubes containing tumor cells suspension per each group.
* significant $P<0.05$.

- Effect of the synthesized compounds on nucleic acids of EAC cells.

A- Quantitative determination of DNA

DNA content in Ehrlich Ascites carcinoma cells after the incubation period was evaluated by the method of Schnieder (1957)[18].

B- Quantitative determination of RNA

RNA was estimated according to the method of Bobin (1953)[19], (Table IV).

TABLE IV Effect of the synthesized compounds[a] on DNA and RNA of EAC cells with or without radiation exposure[b]

Compd. No.	*DNA content (µg/ml ascites)*				*RNA content (µg/ml ascites)*			
	Non-Irradiated Group		*Irradiated Group*		*Non-Irradiated Group*		*Irradiated Group*	
	Mean ± SE	*% of change*	*Mean ± SE*	*% of change*	*Mean ± SE*	*% of change*	*Mean ± SE*	*% of change*
Control	57.331 ± 1.59	100	57.116 ± 1.59	100	21.403 ± 23.2	100	22.56 ± 1.53	100
1	57.54 ± 1.37	100.5	65.915 ± 2.16	98	19.206* ± 1.16	89.7	17.99* ± 1.35	79.7
2b	52.95 ± 1.19	88.26	57.90* ± 0.791	86.3	12.89*	60.20	16.045* ± 1.09	71.1
7	51.998 ± 2.15	85.71	58.00* ± 0.87	86.4	15.48*	72.3	15.94* ± 1.73	70.5

Compd. No.	DNA content (µg/ml ascites)				RNA content (µg/ml ascites)			
	Non-Irradiated Group		Irradiated Group		Non-Irradiated Group		Irradiated Group	
	Mean ± SE	% of change	Mean ± SE	% of change	Mean ± SE	% of change	Mean ± SE	% of change
12	60.77 ± 0.9	106.5	61.00* ± 1.679	90.8	21.20 ± 0.739	99	14.789* ± 3.03	65.5
17	55.944 ± 2.15	96.28	31.25* ± 1.325	47.9	22.55 ± 1.019	106.8	14.019*	62.9
19	60.55 ± 0.99	108.6	40.40* ± 1.491	60.2	21.76 ± 0.799	101.6	13.236* ± 2.21	58.5

a- Compound concentration = 100 µg/ml
b- Radiation exposure at dose level 5 Gy
* Significant P<0.05

- Effect of the synthesized compounds on protein content of EAC cells

The protein content of the incubation mixture of tumor cells and the tested compounds (1, 2b, 7, 12, 17 and 19) was determined according to Lowry (1951)[20] using Folin-Ciocalteu reagent, (Table V).

TABLE V Protein content of control EAC cells and cells treated with the synthesized compounds[a] with or without radiation exposure[b]

Compd. No.	Protein content (mg/ml ascites)			
	Non-Irradiated Group		Irradiated Group	
	Mean ± SE	% of change	Mean ± SE	% of change
Control	21.35 ± 0.20	100	22.35 ± 2.03	100
1	23.42 ± 1.3	109.7	19.5 ± 0.15	87.6
2b	15.33 ± 0.4*	71.80	15.33 ± 1.06*	68.5
7	12.48 ± 0.7*	58.45	12.48 ± 0.4*	55.8
12	22.32 ± 0.31*	104.74	10.68 ± 0.03*	47.7
17	19.5 ± 0.09	91	23.26 ± 0.09	100
19	16.2 ± 0.12*	75.8	16.26 ± 0.05*	72.2

a- Compound concentration = 100 µg/ml
b- Radiation exposure dose = 5 Gy
* Significant P<0.05

Biochemical studies of tested compounds were concerned with their effect, with or without radiation, on the growth rate, DNA, RNA and protein contents of EAC cells.

The results (Table II) showed that the percentage of viability cells reached about 50% in compounds (7, 12, 17 and 19) at a dose level of 100 μg/ml of EAC cells, thus the LD_{50} for these compounds was considered 100 μg/ml.

The thienopyridines, with a carboxamido group (7) and carbohydrazide group (12), in addition to the isothiazolopyridine (17) and the pyridoisothiazolopyrimidine (19) showed a certain antitumor activity at a concentration of 250 μg/ml, (Table II).

Compounds (7, 12 and 17) showed the highest percentage of mortality in non-irradiated group of cells at a dose of (100 μg/ml). Compounds (7) and (19) increase the mortality percentage to 100% in the group of cells exposed to 5 Gy at a dose of (100 μg/ml), which reflect the sensitization effects of compounds (7) and (19), (Table III). On the other hand the isothiazolopyridine (17) decreases the mortality percentage of cells to 8% in irradiated group of cells, which indicates that this compound has a protection properties against radiation damage, (Table III).

(Tables IV) and (Table V) showed that, in non-irradiated group of cells compounds (2b) and (7) exhibited pronounced depression in RNA and protein contents. In non-irradiated group of cells all compounds caused a significant decrease in RNA, and protein content, except for compounds (1) and (17).

Compounds (17) and (19) revealed a significant reduction in DNA content.

From these results we can conclude that compounds (17) and (19) revealed a potential activity against Erlich Ascites Carcinoma cells.

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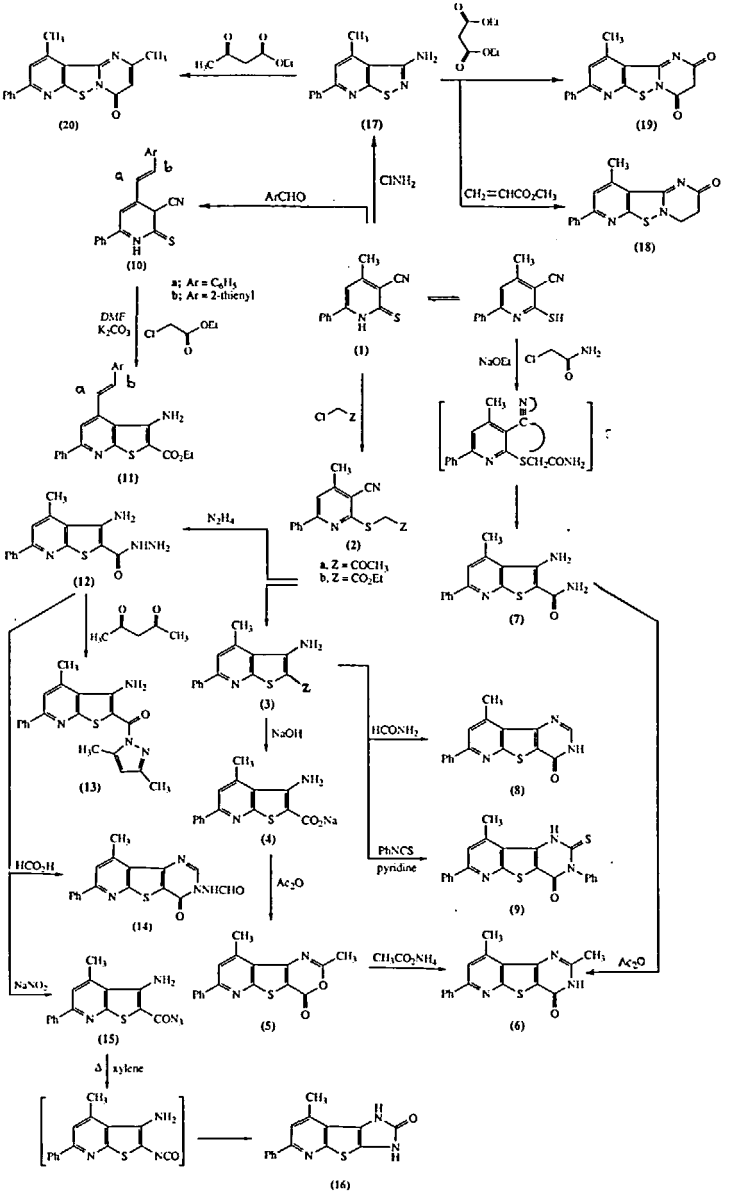

SCHEME 1