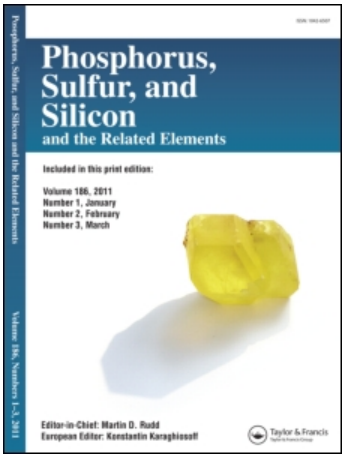

Phosphorus, Sulfur, and Silicon and the Related Elements

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The Synthesis of Some New Sulfur Heterocyclic Compounds as Potential Radioprotective and Anticancer Agents

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The Synthesis of Some New Sulfur Heterocyclic Compounds as Potential Radioprotective and Anticancer Agents

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Some novel 5-subistituted amino-3-methylthiophene-2,4-dicarboxylic acid diethyl ester ***(3–6)****, 3,5-dimethyl-4-oxo-2-thioxo-1,2,3,4-tetra-hydro-thieno[2,3-d]pyrimidine* ***(7)****, imidazothienopyrimidene (****8****), and 1,2,4-triazolo-thienopyrimidine* ***(11)*** *were synthesized via a reaction of the isothiocyanate 2 with different reagents. The identification of the new compounds was established by elemental analysis, and IR,* ^{1}*H NMR, and mass spectral data. Some prepared compounds were tested for their radioprotective and anticancer activities. Compounds* ***7*** *and* ***16*** *showed significant activities against EAC cells, while compound* ***5*** *exhibited radioprotective activity.*

Keywords Radioprotective and anticancer activities; thieno[2,3-d]pyrimidines

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INTRODUCTION

The chemistry of pyrimidine and thieno[2,3-d]pyrimidine derivatives has been of increasing interest since many of these compounds revealed several biological activities and useful applications as antibacterial,[1–6] antifungal,[7–10] anticancer,[11–13] and radioprotective.[14–17] In addition, several nitrogen- and sulfur-containing heterocyclic compounds incorporating sulfhydryl, imidazole, 1,2,4-triazole, and hydrazine moieties were found to posses anticancer activity.[18–21] This prompted us to undertake the synthesis of some new thienopyrimidine derivatives containing the previously mentioned moieties, aiming to produce more potent and less toxic drugs as radioprotective and anticancer agents.

EXPERIMENTAL

Melting points are uncorrected and were determined on a Stuart melting point apparatus. Elemental analyses were carried out at the micro-analytical laboratories, Cairo University, Giza, Egypt. IR spectra (KBr) were measured on a Shimadzu IR 110 spectrophotometer, and ^{1}H NMR spectra were obtained on a BRUKER proton NMR-Avance 300(300,MHZ) spectrometer, in DMSO-d_6 and $CDCl_3$ as a solvent, using tetramethyl-silane (TMS) as an internal standard. Mass spectra were run on an HP Model MS-5988 spectrometer.

The Synthesis of 5-Methoxy and/or 5-Ethoxythiocarbonylamino-3-methyl-thiophene-2,4-dicarboxylic Acid Diethyl Ester (3) and (4)

A solution of **2** (0.01 mol) in anhydrous methanol and/or ethanol (10 mL) was heated under reflux for 24 h. The solvent was evaporated under vacuum, and the solid obtained was recrystallized from ethanol to give **3** and **4**, respectively (Table I).

The Formation of 5-(2-Hydroxy-Ethoxythiocarbonylamino)-3-methyl-thio-phene-2,4-dicarboxylic Acid Diethyl Ester (5)

To a solution of **2** (0.01 mol) in benzene (10 mL), ethylene glycol (0.01 mol) was added, and the mixture was heated under reflux for 10 h. The precipitate formed after cooling was collected by filtration and recrystallized from dioxane to give (**5**, Table I).

TABLE I Physical and Analytical Data of Synthesized Compounds (3–16)

Compound No.	M.P. C°	Yield %	Mol. Formula (mol. wt.)	Analysis Required/(Found)% C	H	N
3	95–97	62	$C_{13}H_{17}NO_5S_2$ (331)	47.13 (46.80)	5.14 (5.40)	4.23 (4.50)
4	110–112	59	$C_{14}H_{19}NO_5S_2$ (345)	48.70 (48.40)	5.51 (5.80)	4.06 (4.20)
5	127–129	56	$C_{14}H_{19}NO_6S_2$ (361)	46.54 (46.80)	5.26 (5.50)	3.88 (3.60)
6	202–204	88	$C_{13}H_{18}N_2O_4S_2$ (330)	47.27 (46.90)	5.45 (5.20)	8.48 (8.30)
7	>300	89	$C_{11}H_{12}N_2O_3S_2$ (284)	46.48 (46.20)	4.23 (4.50)	9.86 (9.60)
8	186–188	48	$C_{12}H_{13}N_3O_3S$ (279)	51.61 (51.30)	4.66 (4.40)	15.05 (15.20)
9	270–272	38	$C_{12}H_{15}N_3O_3S_2$ (313)	46.01 (46.30)	4.79 (4.60)	13.42 (13.70)
11	>300	69	$C_{11}H_{10}N_4O_3S_2$ (310)	42.58 (42.20)	3.23 (3.40)	18.06 (18.30)
12	92–94	71	$C_{18}H_{21}N_3O_4S_2$ (407)	53.07 (53.30)	5.16 (5.40)	10.32 (10.60)
13	208–210	54	$C_{16}H_{15}N_3O_3S_2$ (361)	53.19 (53.40)	4.16 (4.50)	11.63 (11.90)
14	232–234	82	$C_{10}H_{11}N_3O_3S_2$ (285)	35.42 (35.70)	3.32 (3.50)	25.83 (25.60)
15	>300	79	$C_8H_9N_5O_2S_2$ (271)	42.11 (42.40)	3.86 (3.60)	14.74 (14.60)
16	268–270	79	$C_8H_{11}N_7O_2S$ (269)	35.69 (35.50)	4.09 (4.30)	36.43 (36.20)

The Preparation of 3-Methyl-5-(3-methyl-thiourido)-thiophene-2,4-dicarboxylic Acid Diethyl Ester (6)

A mixture of **2** (0.01 mol) and methyl amine (0.01 mol) in dichloromethane (10 mL) was stirred at r.t. for 24 h. The precipitate was filtered, washed with methanol, and recrystallized from ethanol to give **6** (Table I).

The Cyclization of Compound (6): The Formation of 3,5-Dimeth-yl-4-oxo-2-thioxo-1,2,3,4-tetrahydro-thieno[2,3-d]pyrimidine-6-carboxylic Acid Ethyl Ester (7)

A solution of **6** (0.01 mol) in dimethylformamide (10 mL) was heated under reflux for 8 h. The solvent was concentrated, and

the solid obtained was recrystallized from dioxane to give **7** (Table I).

The Synthesis of 6-Methyl-5-oxo-[1H]-2,3-dihydro-imidazo-[3,2-a]thieno-[2,3-d]pyrimidine-7-Carboxylic Acid Ethyl Ester (8) and 3-(2-Aminoethyl)-5-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydro-thieno [2,3-d]pyrimidine-6-yl-ethyl Carboxylic Acid Ester (9)

A mixture of **4** (0.01 mol) and ethylendiamine (0.01 mol) in dry benzene (20 mL) was refluxed for 10 h. The reaction mixture was filtered while hot to give compound **8**, while compound **9** was isolated after cooling the filtrate of the reaction mixture and was recrystallized from ethanol (Table I).

The Formation of 8-Methyl-9-oxo-3-thioxo[1,2,4]triazolo[2,3:5,1]thieno-[2,3-d]pyrimidine-7-carboxylic Acid Ethyl Ester (11)

A mixture of **2** (0.01 mol) and thiosemicarbazide (0.012 mol) in dimethylformamide (10 mL) containing 3 drops of triethylamine was refluxed for 10 h. The reaction mixture was poured on ice water, and the obtained solid was recrystallized from dioxane to give **11** (Table I).

The Synthesis of 3-Methyl-5-(phenylthiosemicarbazide)-thiophene-dicarboxylic Acid Diethyl Ester (12)

A mixture of **2** (0.01 mol) and phenyl hydrazine (0.01 mol) in dichloromethane (10 mL) was stirred at r.t. for 6 h. The solid obtained was recrystallized from methanol to give **12** (Table I).

The Formation of 5-Methyl-4-oxo-3-phenylamino-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrmidine-6 carboxylic acid Ethyl Ester (13)

A solution of **12** (0.01 mol) in dimethylformamide (10 mL) was refluxed for 6 h. The reaction mixture was poured onto ice water, and the solid obtained was recrystallized from ethanol to give **13** (Table I).

The Synthesis of 3-Amino-5-methyl-4-oxo-2-thioxo-1,2,3,4-Tetrahydro-Thieno [2,3-d]pyrimidine-6-carboxylic Acid Ethyl Ester (14)

A mixture of **4** (0.01 mol) and hydrazine hydrate (0.012 mol) in ethanol (20 mL) was stirred at r.t. for 48 h. The obtained solid was recrystallized from ethanol to give **14** (Table I).

The Formation of 3-Amino-5-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydro-thieno[2,3-d]pyrimidin-6-yl-Hydrazide (15)

A mixture of **2** (0.01 mol) and hydrazine hydrate (0.012 mol) in dry benzene (15 mL) was refluxed for 8 h. The solid obtained on heating was recrystallized from benzene to give **15** (Table I).

The Synthesis of 3-Amino-5-methyl-4-oxo-2-hydrazino-1,2,3,4-tetrahydro-thieno[2,3,-d]pyrimidine-6-yl Hydrazide (16)

Method A

A mixture of **14** (0.01 mol) and hydrazine hydrate (0.012 mol) in ethanol (20 mL) was refluxed until an evolution of H_2S ceased (about 8 h). The solid obtained was recrystallized from ethanol to give **16** (Table I).

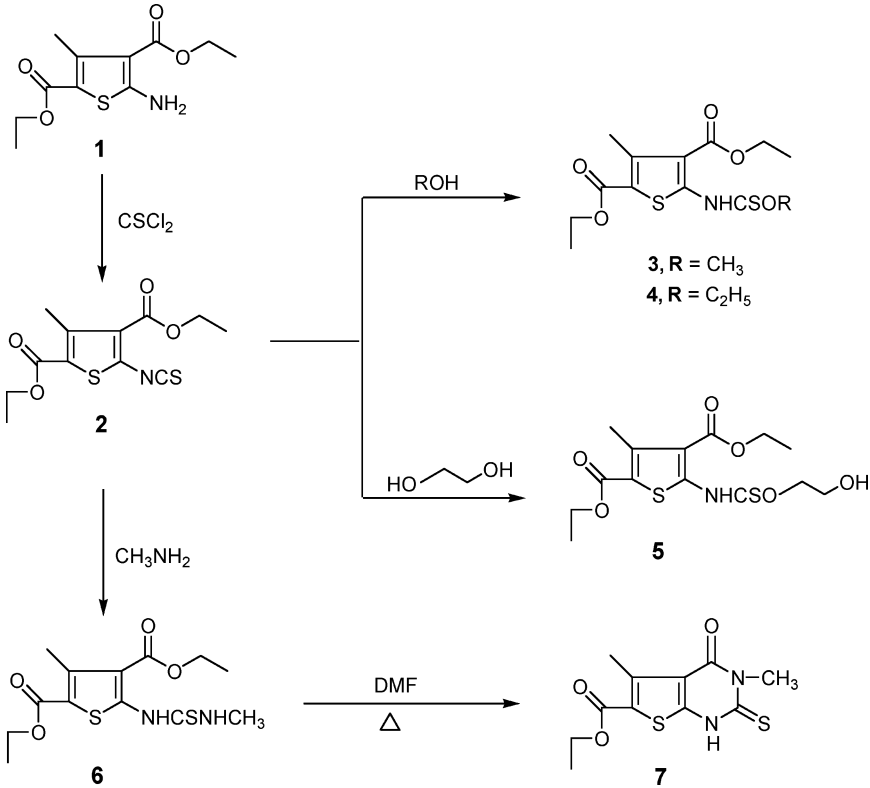
Method B

A mixture of **15** (0.01 mol) and hydrazine hydrate (0.012 mol) in ethanol (20 mL) was refluxed for 10 h, and then filtered while hot. The obtained solid was recrystallized from ethanol to give **16** (m.p. and m.m.p.).

Investigation, Results, and Discussion

Aromatic and heterocyclic o-aminoester derivatives are strategic intermediates for the preparation of various condensed heterocyclic systems. Although the chemistry of methyl-2-isothiocyanatobenzoate has been investigated intensively,[22–25] the number of publications on the synthesis and reactivity of heterocyclic isothiocyanates is very limited.[26] This prompted the authors to undertake the synthesis of **2**[27] from the reaction of **1**[28] with thiophosgene.

Since compound **2** contains two reactive groups at ortho positions, it reacted with some nucleophiles to furnish a series of heterocyclic systems analogous to purine, which is known for its potential cytotoxic activity.[16,29] The sequence of reactions leading to the formation of the



SCHEME 1

target compounds is depicted in Schemes 1–5. Thus, the interaction of compound **2** with anhydrous methanol or ethanol gave the corresponding thiocarbonyl amino derivatives **3,4** (Scheme 1). The IR spectrum of compound **3** revealed the absence of an (NCS) band and the presence of bands at 3120 cm^{-1}(NH), 2960, 2890 cm^{-1} (CH aliph.), 1750, and 1700 cm^{-1} (2C=O). The ^1H NMR spectrum of compound (**3** in DMSO-d_6) exhibited bands at 1.3 [m, 6H, $2CH_3$ ester], 2.2 [s, 3H, CH_3], 2.8 [s, 3H, OCH_3], 4.1 [s,1H,NH], and 4.3 [m, 4H, $2CH_2$ ester]. The IR spectrum of compound **4** showed the absence of an (NCS) band and the presence of an (NH) band at 3120 cm^{-1}, and (CH aliph.) at 2950 cm^{-1}, 1740, and 1700 cm^{-1} (2C=O). The ^1H NMR spectrum of compound (**4** in $CDCl_3$) exhibited signals at 1.1–1.4 [m, 9H, $3CH_3$ ethyl], 2.75 [s, 3H, CH_3], 4.4 [m, 6H, $3CH_2$ ethyl], and 7.3 [s, 1H, NH]. The mass spectrum of compound **4** exhibited a molecular ion peak m/z 345 (M^+, 31.12%) with a base peak at 91(100%), and other significant peaks appeared at

346 (M+1, 19.65%), 347 (M+2, 13.40%), 289 (22.30%), 210 (91.73%), and 110 (63.58%).

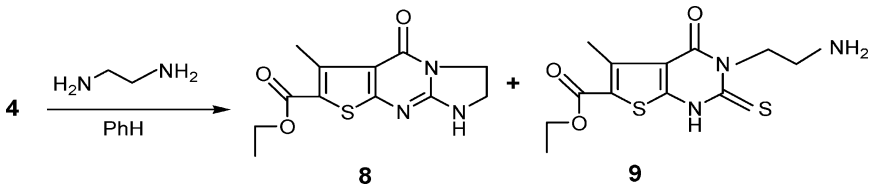
The treatment of compound **2** with ethylene glycol afforded the 5-(2-hydroxy-ethoxythiocarbonylamino)-3-methyl-thiophene-2,4-dicarboxylic acid diethyl ester **5.** The IR spectrum of compound **5** showed the absence of an (NCS) band and the presence of bands at 3500–3000 cm^{-1} (br, OH), 3120 cm^{-1} (NH), 2960, 2850 cm^{-1}(CH aliph.), 1700, and 1660 cm^{-1} (2C=O). The ^1H NMR spectrum of compound (**5** in DMSO-d_6) revealed signals at 1.2–1.4 [m, 6H, $2CH_3$ ester], 2.2 [s, 3H, CH_3], 3.7 [t, 2H, OCH_2], 4.3 [m, 4H, $2CH_2$ ester], 4.2–4.4 [t, 2H, CH_2OH], and 12.3 [s, 2H, NH + OH]. Mass spectrum of compound **5** exhibited a molecular ion peak m/z 361 (M^+, 8.23%) with a base peak at 211 (100%), and other significant peaks appeared at 362 (M+1, 1.48%), 327 (18.83%), 280 (25.34%), 257 (37.34%), 183 (41.09%), 166 (30.58%), 110 (24.70%), and 66 (24.23%).

3-methyl-5-(3-methyl-thiouriedo)-thiophene-2,4-dicarboxylic acid diethyl ester **6** was obtained in a good yield via the reaction of compound **2** with methylamine in dichloromethane. The IR spectrum of compound **6** showed bands at 3340 cm^{-1}(NH), 2940 cm^{-1} (CH aliph.), 1720, 1690 cm^{-1} (2C=O), and 1260 cm^{-1} (C=S). The ^1H NMR spectrum of compound (**6** in DMSO-d_6) revealed signals at 1.1–1.5 [m, 6H, $2CH_3$ ester], 2.3 [s, 3H, CH_3], 2.4 [s, 3H, NCH_3], 4.1–4.5 [m,4H,$2CH_2$ ester], and 7.6 [s, 2H, 2NH]. The mass spectrum of compound **6** exhibited a molecular ion peak m/z 330 (M^+, 1.11%), with a base peak at 257 (100%), and other significant peaks appeared at 301 (3.35%), 241 (1.53%), 226 (31.51%), 153 (5.88%), 80 (1.75%), and 56 (2.45%).

When compound **6** was heated under reflux in dimethylformamide, the corresponding 3,5-dimethyl-4-oxo-2-thioxo-1,2,3,4-tetrahydro-thieno-[2,3-d]pyrimidine-6-carboxylic acid ethyl ester **7** was isolated in a good yield; its IR spectrum showed bands at 3400 cm^{-1} (NH), 1700, 1650 cm^{-1} (2C=O), and 1210 cm^{-1} (C=S). Its mass spectrum exhibited a molecular ion peak m/z 284 (M^+, 12.58%) with a base peak at 129 (100%) and other significant peaks appeared at 285 (M+1, 51.85%), 252 (25.87%), 207 (80.81%), 173 (78.87%), 111 (92.78%), 83 (78.36%), and 55 (98.3%).

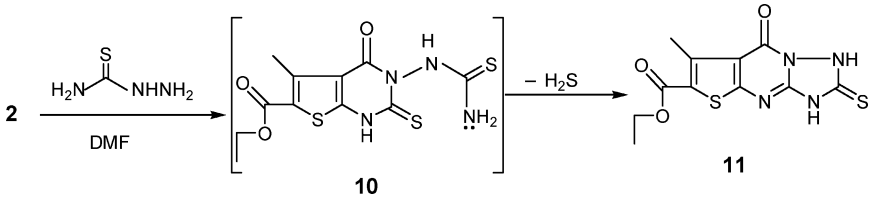
6-methyl-5-oxo[1H]-2,3-dihydro-imidazo[6,5:2′3′]thieno[2,3-d]pyrimidine-7-carboxylic acid ethyl ester **8** was isolated on heating via the reaction of compound **4** with ethylenediamine in refluxing benzene, while 3-(2-aminoethyl)-5-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydro-thieno[2,3-d]pyrimidine-6-yl-ethyl carboxylic acid ester **9** was obtained after cooling the filtrate of the reaction mixture (Scheme 2). This was confirmed on the basis of elemental analysis and spectral data.

The IR spectrum of compound **8** revealed bands at 3390 cm^{-1} (NH), 2940 cm^{-1} (CH aliph.), 1710, 1690 cm^{-1} (2C=O), and 1580 cm^{-1} (C=N). The mass spectrum of **8** exhibited a molecular ion peak m/z at 279 (M^+, 1.77%) with a base peak at 64 (100%), and other significant peaks appeared at 280 (M+1, 0.77%), 182 (0.6%), 137 (1.49%), 123 (0.63%), 95 (14.04%), and 80 (0.66%). The IR spectrum of compound **9** exhibited bands at 3250, 3150, 3125 cm^{-1}(NH, NH_2), 2950 cm^{-1} (CH aliph.), 1700, 1680 cm^{-1} (2C=O), and 1270 cm^{-1}(C=S). The ^1H NMR spectrum of compound (**9** in DMSO-d_6) revealed signals at 1.27 [t, 3H, CH_3 ester], 2.2 [s, 3H, CH_3], 2.7 [s, 2H, NH_2], 3.7 [t, 2H, NCH_2], 4.1 [t, 2H, CH_2NH_2], 4.25 [q, 2H, CH_2 ester], and 7.4 [s, 1H, NH].



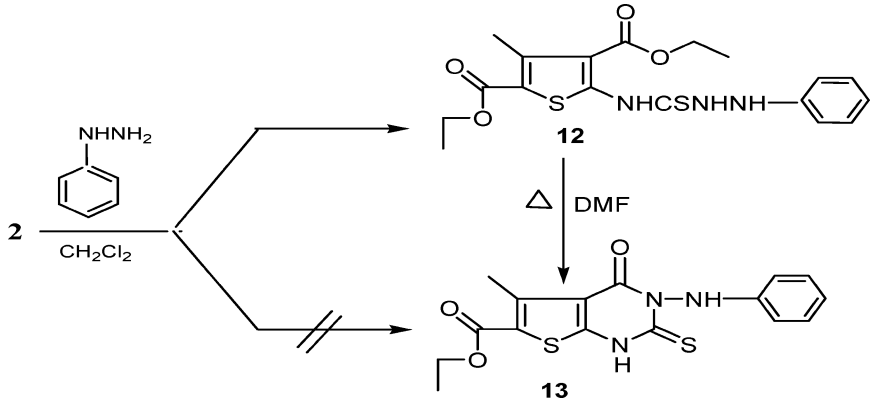
SCHEME 2

This work was extended to cover the reactivity of compound **2** toward thiosemicarbazide. Thus, the condensation of compound **2** with thio-semicarbazide in dimethylformamide in the presence of triethylamine, double cyclization occurred to give the triazolothienopyrimidine derivative **11** probably through the formation of the intermediate **10** (Scheme 3), which lost hydrogen sulfide (lead acetate paper). The IR spectrum of compound **11** revealed bands at 3390, 3250 cm^{-1} (2NH), 2930 cm^{-1} (CH aliph.), 1700, 1650 cm^{-1} (2C=O), 1610 cm^{-1} (C=N), and 1240 cm^{-1} (C=S). Its mass spectrum showed a molecular ion peak m/z 310 (M^+, 19.17%), with a base peak at 60 (100%), and other significant peaks appeared at 311 (M+1, 4.55%), 312 (M+2, 2.87%), 281 (13.64%), 237 (3.26%), 209 (3.16%), 194 (4.94%), 180 (1.68%), 165 (5.24%), 108 (5.73%), 81 (5.14%), and 56 (5.04%).



SCHEME 3

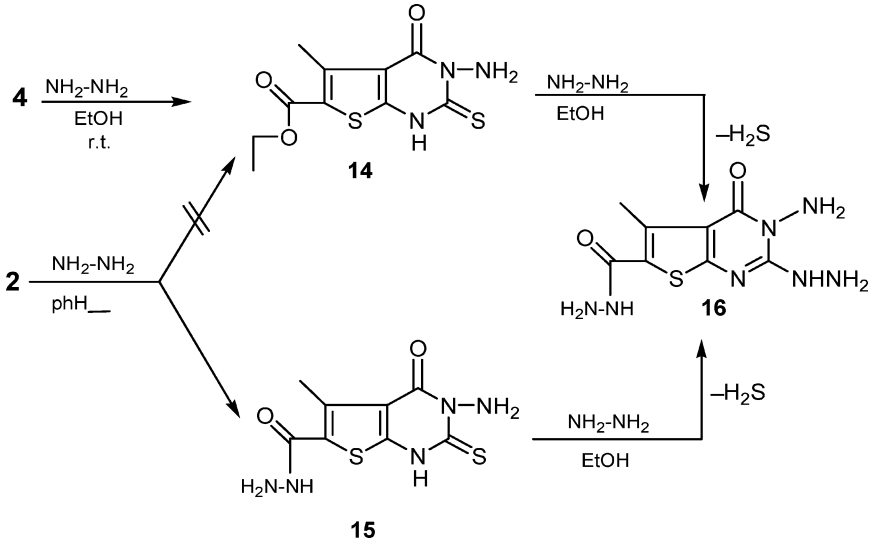
The interaction of compound **2** with phenylhydrazine yielded the corresponding thiosemicarbazide derivative **12**, which, upon heating in dimethylformamide, afforded the expected 3-anilino-thienopyrimidine **13** through intramolecular cyclization via a loss of one mole of ethanol (Scheme 4). The Structure of compounds **12** and **13** was proven on the basis of elemental analysis and spectral data. The IR spectrum of compound **12** revealed bands at 3380 cm^{-1} (NH), 2940 cm^{-1} (CH aliph.), 1730, 1720 cm^{-1} (2C=O), and 1280 cm^{-1} (C=S). The ^1H NMR spectrum of compound (**12** in $CDCl_3$) exhibited signals at 1.3–1.4 [m, 6H, $2CH_3$ ester], 2.2 [s, 3H, CH_3], 2.75, 2.8, 3.45 [3s, 3H, 3NH], 4.2–4.5 [m, 4H, $2CH_2$ester], and 7.2–7.5 [m, 5H, Ar-H]. The mass spectrum of compound **12** revealed a molecular ion peak m/z 407 (M^+, 7.51%), with a base peak 212 (100%), and other significant peaks appeared at 408 (M+1, 2.04%), 362 (1.58%), 317 (3.41%), 302 (2.63%), 273 (1.45%), 213 (9.45%), 122 (3.67%), 91 (13.74%), 80 (6.72%), and 56 (4.56%). The IR spectrum of compound **13** revealed bands at 3400 cm^{-1} (NH), 3000 cm^{-1} (CH arom.), 1780, and 1630 cm^{-1} (2C=O). The Mass spectrum of compound **13** revealed a molecular ion peak m/z 360 (M−1, 0.11%), with a base peak 211 (100%), and other significant peaks appeared at 285 (0.40%), 257 (63.10%), 183 (62.18%), 166 (48.85%), 111 (32.06%), and 66 (89.95%).



SCHEME 4

The behavior of compound **2** toward hydrazine hydrate was also investigated. Thus, when refluxing compound **2** with hydrazine hydrate in dry benzene to obtain the cyclic structure 3-amino-5-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydro-thieno[2,3-d]pyrimidine-6-carboxylic acid ethyl ester **14**, instead 2 moles of hydrazine hydrate were consumed with the elimination of 2 moles of ethanol to give the cyclic structure having the hydrazide moiety **15**. The literature survey revealed that

compound **14** was obtained, in two steps, through the reaction of **2** with hydrazine hydrate.[27] In this article, compound **14** was obtained, in a one step reaction, through the reaction of compound **4** with hydrazine hydrate in ethanol. The structure of compounds **14** and **15** was proven on the basis of elemental analysis and IR, ^{1}H NMR, and mass spectral data. The IR spectrum of compound **14** revealed bands at 3300, 3200 cm^{-1} (NH, NH_2), 1690, and 1670 cm^{-1}(2C=O). The ^{1}H NMR spectrum of compound (**14** in DMSO-d_6) exhibited signals at 1.3 [t, 3H, CH_3 ester], 2.6 [s, 3H, CH_3], 4.3 [q, 2H, CH_2 ester], 5.7 [s, 2H, NH_2], and 8.3 [s, 1H, NH]. The IR spectrum of compound **15** showed bands at 3340, 3220 cm^{-1} (NH, NH_2), 2940 cm^{-1} (CH aliph.), 1710, and 1680 cm^{-1} (2C=O). The ^{1}H NMR spectrum of compound (**15** in DMSO-d_6)) showed the absence of a triplet and quartet due to the ester group in position 6 and the presence of signals at 2.6 [s, 3H, CH_3], and 4.2–5.0 [hump, 6H, $2NH_2 + 2NH$]. The mass spectrum of compound **15** revealed a molecular ion peak m/z 271 (M^+, 22.47%), with a base peak 64 (100%), and other significant peaks appeared at 255 (7.87%), 240 (56.55%), 212 (12.36%), 181 (11.24%), 154 (8.61%), 167 (35.58%), and 139 (17.98%).



SCHEME 5

Finally, compound **16** was isolated while hot in a good yield when compound **14** reacted with hydrazine hydrate in absolute ethanol (Scheme 5). Also, compound **16** was obtained via a reaction of compound **15** with hydrazine hydrate through the elimination of 1 mol of H_2S. The IR spectrum of compound **16** exhibited bands at 3360, 3320, 3200 cm^{-1} (NH, NH_2), 2940 cm^{-1} (CH aliph.), 1700, 1690 cm^{-1} (2C=O), and

1620 cm^{-1} (C=N). The ^1H NMR spectrum of (**16** in DMSO-d_6) showed signals at 2.4 [s, 3H, CH_3], and 4.8 [hump, 8H, 2NH, $3NH_2$].

BIOCHEMICAL ANALYSIS

Radioprotection Activity In-Vivo Study

Material and Methods: Experimental Animals

Twenty four female albino rats (100–120 g) were used throughout the present experiment.

Treatment

The tested compounds were suspended in carboxymethyl cellulose before use and administered i.p. to rats in a concentration of 300 mg/kg body weight/dose 20 min pre-radiation exposure.

Radiation Processing

γ-irradiation was performed by using Cesium 137. The dose rate was 0.86 Gy/min at the time of the experiment. Animals were exposed to whole body γ-rays at a sublethal single dose of 8 Gy.

Experimental Design

Animals were randomly divided into four groups each of six rats. Group (1): control group; Group (2): animals administered with compound **5**; Group (3): animals were exposed to whole body γ-irradiation; Group (4): animals were administered with compound **5** and then subjected to whole body γ-irradiation.

Experimental Parameters

Five animals of each group were sacrificed after seven days from radiation exposure. Blood was collected in a heparinized tube by a heart puncture; the liver was dissected out and homogenized in bidistilled water (10% homogenates). Lipid peroxide content was indicated in plasma and liver homogenates[30]; reduced Glutathione (GSH) content was estimated in whole blood, and liver homogenate[31] and superoxide dismutase activity was estimated in whole blood and liver homogenate.[32] The results were presented as mean values $\pm$ standard error and groups were compared using the two ways ANOVA method (F-test).[33]

Results and Discussion

Several studies postulated that Reactive Oxygen Species (ROS) participate in the etiology of many chronic health problems. Free radicals can cause tissue damage by reacting with polyunsaturated fatty acids

in the cellular membrane, nucleotides in DNA, and critical sulfhydryl bonds in proteins.[34] These highly reactive species can originate endogenously from normal metabolic reactions or exogenously through air pollutants, chemotherapeutics and pesticides as well as through exposure to ionizing radiation.[35,36] In health under normal conditions, a delicate balance exists between the generation of ROS and the cellular antioxidant defense systems.[37] Compound **5** was selected to be evaluated as a radioprotective agent as it contains both a sulfur-containing compound, a thiophene ring, and a hydroxyl group known to enhance radioprotection activity.[38,39] Compound **5** was administered to acute oxidative stress γ-irradiated rats. In order to evaluate the lipid peroxidation that might be induced as a result of exposure to ionizing radiation, and measured as a Thiobarbituric Acid Reactive Substances (TBARS) content in plasma and liver tissues. The concentration of reduced GSH and the activity of Superoxide Dismutase (SOD) were estimated in whole blood and liver tissues.

Lipid Peroxidation

The results for lipoperoxide contents, evaluated as TBARS concentration, are shown in Table II. Compound **5** administration resulted in nonsignificant changes in the TBARS levels. There was a significant increase in lipoperoxidation in the group of animals exposed to γ-radiation. The administration of compound **5** preirradiation resulted in a significant improvement in the levels of lipid peroxidation when compared to irradiated groups.

The levels of the reduced form GSH in both blood and hepatic tissue that is represented in Table III showed that the administration of

TABLE II Lipid Peroxidation (TBARS) Contents in Different Groups of Animals[†]

	Blood (mg/dl Packed Cells)		Liver (mg/g Protein)	
Groups	Mean ± SE	% of Change	Mean ± SE	% of Change
Control	28.50 ± 0.80	100	157.27 ± 3.3	100
Compound **5**	25.56 ± 0.89	89.7	151.15 ± 2.95	96.1
Radiation (Rad.)	47.52 ± 1.16*	160.7	213.57 ± 3.9*	135.8
Compound **5** + Rad.	30.37 ± 0.90	106.6	172.66 ± 3.20	109.8

[†]Each value represents the mean of six-observations ±SE (7 days after radiation exposure).

*A significant of change between groups.

LSD of blood groups at 0.05 = 3.433.

LSD of tissues groups at 0.05 = 4.33.

TABLE III Reduced GSH Contents in Different Groups of Animals[†]

Groups	Blood (mg/dl Packed Cells)		Liver (mg/g Protein)	
	Mean ± SE	% of Change	Mean ± SE	% of Change
Control	47.50 ± 1.0	100	22.7 ± 0.45	100
Compound **5**	51.21 ± 0.98	107.8	21.10 ± 0.50	92.9
Radiation (Rad.)	39.36 ± 0.95*	82.8	17.40 ± 0.55*	76.6
Compound **5** + Rad.	46.88 ± 1.15	98.7	22.00 ± 0.60	96.9

[†]Each value represents the mean of six-observations ±SE (7 days after radiation exposure).

*A significant of change between groups and control.

LSD of blood groups at 0.05 = 7.662.

LSD of tissues groups at 0.05 = 3.81.

compound **5** revealed insignificant elevations in GSH in both blood and liver tissue when compared with the control level. Animals exposed to γ-irradiation revealed a significant decrease in GSH content detected in blood and liver tissue. The treatment of animals with compound **5** preradiation exposure improved their glutathione profile as compared with irradiated group. Table IV shows SOD activity in whole blood and liver for different groups. Irradiation with γ-rays caused some depression in the SOD activity, while the administration of compound **5** before radiation rectified the changes occurred in SOD activity levels in blood and liver tissue.

From these results, it can be concluded that the thiophene derivative **5**, characterized by the presence of a free hydroxyl group, exhibited great amelioration against radiation damage, which is represented in a normalized level of lipid peroxide content and increased levels of GSH

TABLE IV Superoxide Dismutase Activities in Different Groups of Animals[†]

Groups	Blood (U/g Hb)		Liver (U/mg Protein)	
	Mean ± SE	% of Change	Mean ± SE	% of Change
Control	48.26 ± 4.78	100	277.12 ± 22.96	100
Compound **5**	47.51 ± 4.10	98.4	280.51 ± 23.97	101.2
Radiation (Rad.)	42.71 ± 3.33*	88.5	263.55 ± 21.90*	95.1
Compound **5** + Rad.	49.81 ± 4.40	103.2	281.97 ± 24.04	101.7

[†]Each value represents the mean of six-observations ± SE.

*A significant of change between groups and control.

LSD of blood groups at 0.05 = 4.573.

LSD of tissues groups at 0.05 = 5.497.

TABLE V In-Vitro Cytotoxic Activity Against Ehrlich Ascites Carcinoma Cells

	Nonviable cells (%) Concentration (μg/mL)							
Compound No.	0	25	50	75	100	125	250	300
3	0	0	0	0	20	100	100	—
4	0	0	0	0	0	0	0	0
7	0	100	100	100	100	100	—	—
11	0	5	20	20	25	25	100	100
15	0	15	30	50	100	100	100	100
16	0	0	0	0	0	0	0	0
Doxorubicin	0	20	55	75	100	100	100	100

content and SOD activity. These results are in agreement with previous studies,[38] which mentioned that the introduction of hydroxyl groups significantly enhanced the radioprotective properties of nonhydroxylated parent compounds, however, only in the case of intraperitoneally administered.

B-Anticancer Activity In-Vitro Study

Experimental

Various concentrations (300, 250, 125, 100, 75, 50, and 25) μg/mL of the selected compounds (**3**, **4**, **7**, **11**, **15**, and **16**) were incubated with Ehrlich ascites carcinoma cells (2.5×10^6) for 2 h. The cytotoxic effect of the tested compounds were determined[40] (Table V).

Results and Discussion

The results of cytotoxic activity for the synthesized compounds indicated that compounds **(7 and 15)** containing the thienopyrimidine moiety were found to be the most active ones exhibiting a very promising sign as anticancer agents. It was found that compound **7** with 2-thioxo, 3-methyl, 6-ethyl ester, groups directly linked to the thienopyrimidine nucleus induced the greatest effect on EAC cells with a 100% nonviable cell at a concentration of 25 μg/mL, and so it seems to be more potent than doxorubicin as a reference drug. On the other hand the substitution with 3-amino and 6-hydrazido groups, compound **15** showed a 100% nonviable cell at a higher concentration of 100 μg/mL, which is nearly as active as doxorubicin, which displayed a significant percentage of non-viable cells to about 100% at a concentration of 100 μg/mL.

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