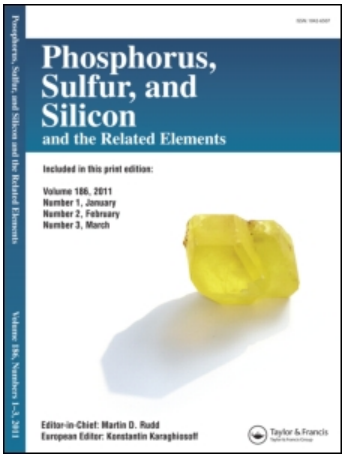

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The Utility of Isothiocyanato Thiophenes in the Synthesis of Thieno[2,3-*d*]pyrimidine Derivatives as Possible Radioprotective and Anticancer Agents

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The Utility of Isothiocyanato Thiophenes in the Synthesis of Thieno[2,3-*d*]pyrimidine Derivatives as Possible Radioprotective and Anticancer Agents

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*The synthesis of novel thioureido derivatives **3**, **8**, and **10**; biscompounds **7**, **9**, and **11**; and tetracyclic compounds **5**, **6**, and **16** utilizing 5-isothiocyanato-3-methyl-thiophene-2,4-dicarboxylic acid diethyl ester **2** are reported. The structures of these compounds were confirmed by microanalyses and IR, ^{1}H NMR, and mass spectroscopy. Preliminary biological studies of some of the synthesized compounds showed promising radioprotective and anticancer activities.*

Keywords Anticancer agents; new thiophene and thieno[2,3-d]pyrimidine derivatives; radioprotective

INTRODUCTION

Thieno[2,3-d]pyrimidines have been a subject of chemical and biological studies due to their pharmacological activities, such as antitumor,[1–6]

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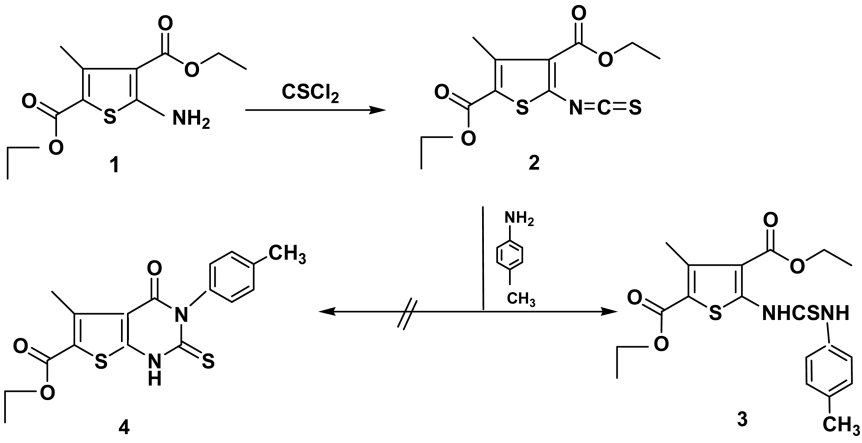
radioprotective activities,[7–10] hypnotic,[11] and analgesic and antiinflammatory agents.[12–14] Thienopyrimidine as a purine analog will be expected to exhibit similar activity as an antitumor and antileukemic agent.[15,16] In addition, sulfur-containing compounds are widely used as radioprotective drugs,[17–19] In light of these facts and as a continuation of our investigations on sulfur-containing bis compounds and tetracyclic systems, we synthesized some new thiophene and thienopyrimidine derivatives to evaluate their radioprotective and anticancer activities.

INVESTIGATION, RESULTS, AND DISCUSSION

Chemistry

The starting material **2** was synthesized through the reaction of the oami-noester **1**[20] with thiophosgene according to the reported method.[21]

The reactivity of isothiocyanate **2** toward some nitrogen nucleophiles was investigated; thus, reaction of isothiocyanate **2** with p-toluedine afforded a 5-[3-(4-methyl-phenyl thioureido)]-3-methyl-thiophene-2, 4-dicarboxylic acid diethyl ester **3** instead of the cyclized expected compound **4**. This was proved by analytical and spectral data; the ^{1}H NMR spectrum of **3** showed the presence of a multiplet peak due to the two ester groups (Scheme 1).

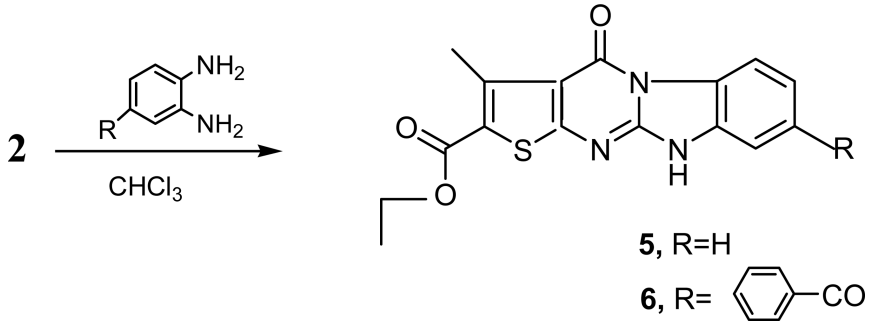


SCHEME 1

The IR spectrum of compound **3** exhibited the absence of a (NCS) band and the presence of bands at 3390 cm^{-1} (br, 2 NH), 2960 cm^{-1} (CH aliph.) 1700, and 1680 cm^{-1} (2C=O). The ^{1}H NMR spectrum of (**3** in DMSO-d_6) revealed signals at 1.2–1.28 [m, 6H, 2CH_3 ester], 1.93 [s,

3H, CH_3 thiophene], 2.48 [s, 3H, CH_3 tolyl], 3.56 [s, 2H, 2NH], 4.15–4.19 [m, 4H, $2CH_2$ ester], and 7.6–8.0 [m, 4H, Ar-H].

Our investigation was extended to include the reactivity of isothiocyanate **2** toward binucleophiles, such as 1,2-phenylenediamine derivatives, in chloroform to yield the corresponding benzimidazothienopyrimidine derivatives **5** and **6**, respectively. The reaction progress was tested by the evolution of hydrogen sulfide gas using lead acetate paper. Analytical and spectral data are compatible with the structure of benzimidazothienopyrimidine derivatives **5** and **6** (Scheme 2).

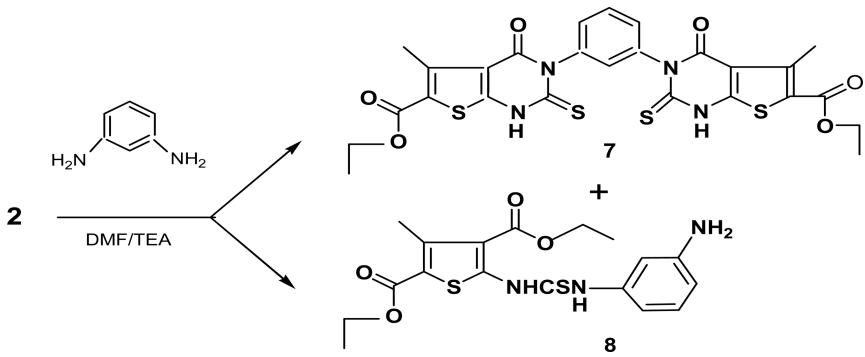


SCHEME 2

The IR spectrum of compound **5** showed bands at 3320 cm^{-1} (NH), 2960 cm^{-1} (CH aliph.), 1740, 1690 cm^{-1} (2C=O), and 1600 cm^{-1} (C=N). The mass spectrum of compound **5** exhibited a molecular ion peak m/z 327 (M^+, 5.75%) with a base peak at 108 (100%), and other significant peaks appeared at 299 (39.22%), 255 (5.90%), 197 (7.38%), 182 (7.68%), 76 (6.56%), and 56 (2.44%). The IR spectrum of compound **6** revealed bands at 3360 cm^{-1} (NH), 2950 cm^{-1} (CH aliph.), 1710, 1670, 1660 cm^{-1} (3 C=O), and 1610 cm^{-1} (C=N).

It is observed from the literature that bisheterocyclic compounds displayed a remarkable biological activity.[22,23] Thus, two molecules of isothiocyanate **2** reacted with one molecule of 1,3 phenylenediamine to yield both compounds **7** and **8**. Compound **7** was precipitated while hot, and obtained upon filtration of the reaction mixture, while compound **8** was isolated from the filtrate of the reaction mixture (Scheme 3).

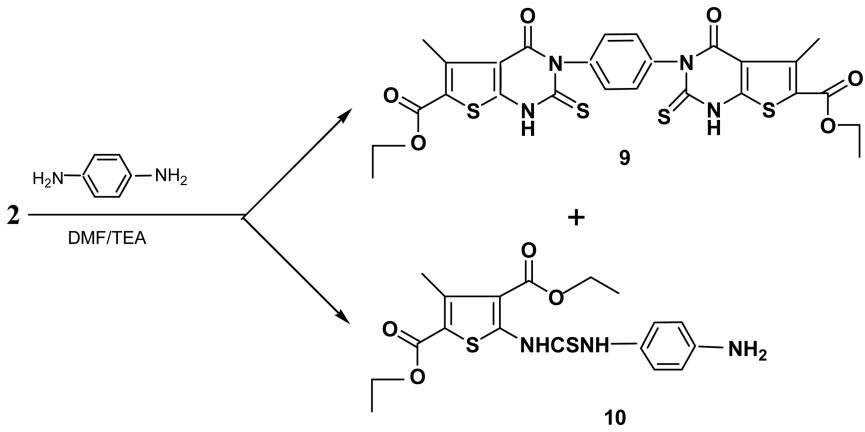
The assignment of their structures was confirmed by elemental analysis, and IR, ^1H NMR, and mass spectral data. The IR spectrum of compound **7** showed bands at 3370 cm^{-1} (br, 2NH), 2930 cm^{-1} (CH aliph.), 1730, 1710, 1690, and 1680 cm^{-1} (4C=O). The mass spectrum of compound **7** revealed a molecular ion peak m/z at 614 (M^+, 0.8%), with a base peak at 52 (100%), and other significant peaks appeared at 607 (60.0%), 347 (80.0%), and 57 (60.0%). The IR spectrum of compound **8**



SCHEME 3

exhibited bands at 3300, 3320, 3280 cm^{-1} (NH, NH_2), 2960 cm^{-1} (CH aliph.), 1720, and 1700 cm^{-1} (2C=O).

Also, the interaction of the isothiocyanate **2** with 1,4-phenylenediamine (2:1 mole) furnished a very pure biscompound **9** while hot while compound **10** was obtained from the filtrate of the reaction mixture (Scheme 4).

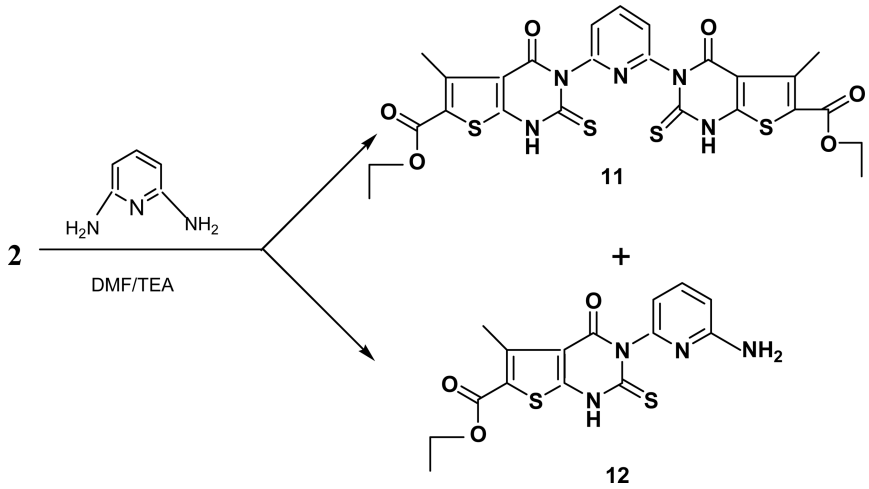


SCHEME 4

The IR spectrum of compound **9** revealed bands at 3400 cm^{-1} (NH), 2920 cm^{-1} (CH aliph.) 1730, 1710, 1680, and 1670 cm^{-1} (4C=O). The mass spectrum of compound **9** showed a molecular ion peak m/z 614 (M^+, 47.83%), and other significant peaks appeared at 445 (47.83%), 227 (56.52%), 218 (52.17%), 176 (52.17%), and 77 (56.52%), with a base peak at 57 (100%). The IR spectrum of compound **10** revealed bands

at 3400, 3300, 3250 cm^{-1} (NH, NH_2) 2940 cm^{-1} (CH aliph.), 1710, and 1690 cm^{-1} (2C=O). The ^1H NMR spectrum of (**10** in DMSO-d_6) exhibited signals at 1.1–1.5 [m, 6H, $2CH_3$ ester], 2.55 [s, 3H, CH_3], 2.6 [s, 2H, NH_2], 4.1–4.3 [m, 4H, $2CH_2$ ester], 7.0–8.0 [m, 4H, Ar-H], and 8.55 [s, 2H, 2NH]. The mass spectrum of compound **10** showed a molecular ion peak m/z 408 (M^+ 1, 18.22 %) with a base peak at 210 (100%), and other significant peaks appeared at 280 (18.13%), 257 (82.80%), 166 (55.81%), and 80 (25.81%).

Repeating the same reaction with 2,6 diaminopyridine in dimethylformamide in the presence of a catalytic amount of triethylamine afforded compounds **11** and **12**. The pure bis compound **11** was isolated by filtration of the reaction mixture on hot while compound **12** was obtained from the mother liquor of the reaction mixture (Scheme 5).

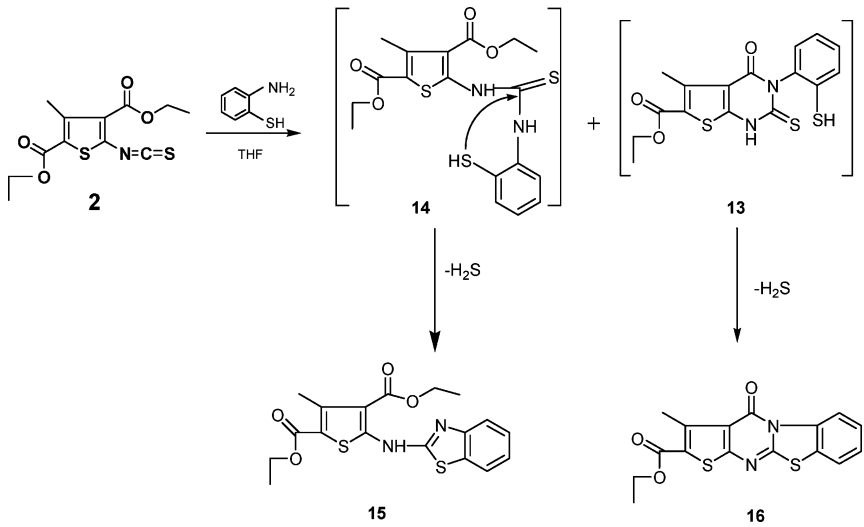


SCHEME 5

The IR spectrum of compound **11** showed bands at 3470 cm^{-1} (br, 2NH), 2950 cm^{-1} (CH aliph.), 1700, 1680, 1670, 1650 cm^{-1} (4C=O), and 1580 cm^{-1} (C=N). The mass spectrum of compound **11** revealed a molecular ion peak m/z at 615 (M^+, 52.38%) with a base peak at 104 (100%), and other significant peaks appeared at 609 (52.38%), 322 (57.14%), 256 (80.95%), 211 (85.71%), 127 (76.19%), 93 (76.19%), and 57 (76.19%). The IR spectrum of compound **12** exhibited bands at 3400, 3290, 3230 cm^{-1} (NH, NH_2), 2980, 2860 cm^{-1} (CH aliph.), 1700, 1680 cm^{-1} (2C=O), and 1600 cm^{-1} (C=N). The mass spectrum of compound **12** revealed a molecular ion peak at m/z 362 (M^+,

0.14%), and other significant peaks appeared at 283 (0.27%), and 257 (80.34%). A base peak appeared at 211 (100%), 95 (5.95%), and 77 (2.25%).

In the same manner, the reaction of 2-aminothiophenol with compound **2** in tetrahydrofuran gave compounds **15** and **16**. The formation of **15** on hot was probably proceeded via an initial formation of **14** followed by the intramolecular cyclization through a loss of hydrogen sulfide (lead acetate paper). Compound **16** was obtained from the filtrate of the reaction mixture. The formation of benzothiazolothienopyrimidine **16** was assumed to proceed via the formation of **13** followed by an intramolecular cyclization by the elimination of H_2S (lead acetate paper) to give **16**.



SCHEME 6

The IR spectrum of compound **15** showed bands at 2970 cm^{-1} (CH aliph.), 1720, and 1690 cm^{-1} (2C=O). The mass spectrum of compound **15** revealed a molecular ion peak m/z 390 (M^+, base peak, 100%), and other significant peaks appeared at 391 (M^+1, 20.73%), 345 (31.71%), 315 (93.90%), 299 (56.10%), 272 (39.02%), 243 (23.17%), 160 (63.41%), 134 (31.71%), 82 (36.59%), and 65 (29.27%). The ^1H NMR spectrum of (**15** in DMSO -d_6) exhibited signals at 1.2–1.4 [m, 6H, $2CH_3$ ester], 2.80 [s, 3H, CH_3], 4.2–4.4 [m, 4H, $2CH_2$ ester], 7.5–8.6 [m, 4H, Ar-H], and 10.5 [s, 1H, NH]. The IR spectrum of compound **16** showed bands at 2980 cm^{-1} (CH aliph.), 1720, 1685 cm^{-1} (2C=O), and 1580 cm^{-1}(C=N).

The mass spectrum of compound **16** revealed a molecular ion peak m/z 344 (M^+, base peak 100%), and other significant peaks appeared at 345 ($M^{-1}1$, 77.27%), 211 (77.27%), 116 (59.09%), 84 (77.27%), 78 (68.18%), and 52 (72.73%).

EXPERIMENTAL

Melting points were uncorrected and were determined on a Stuart melting point apparatus. Elemental analyses were carried out at the Micro-Analytical Laboratories, at Cairo University (National Center for Radiation and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt). IR spectra (KBr) were measured on Shimadzu IR 110 spectrophotometer; ^{1}H NMR spectra were obtained on a BRUKER proton NMR-Avance 300 (300, MHZ) spectrometer in DMSO-d_6 as a solvent, using tetramethylsilane (TMS) as an internal standard. Mass spectra were run on an HP Model MS-5988 spectrometer.

The Synthesis of 5-[3-(4-Methylphenyl)-thioureido] -3-methyl-thiophene-2,4-dicarboxylic Acid Diethyl Ester (3)

A mixture of **2** (0.01 mol) and p-toludine (0.01 mol) in dioxane (20 mL) in the presence of catalytic amounts of triethylamine was refluxed for 3 h. The obtained solid was recrystallized from dioxane to give (**3**, Table I).

The Formation of Benzimidazolothienopyrimidine Derivatives (5 and 6)

A mixture of **2** (0.01 mol) and 1,2 phenylenediamine or 5-benzoyl-1,2-phenylenediamine (0.012 mol) in chloroform was refluxed for 8 h. The solid obtained while hot was recrystallized from ethanol to give **5** and **6**, respectively (Table I).

The Preparation of 1,3-Bis[5-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydro-thieno[2,3-d] primidine-6-carboxylic Acid Ethyl Ester-3,5-Di-Yl]-Benzene (7) and 5-[3-(3-Amino-phenyl-thioureido)]-3-methyl-thio-phene-2,4-di-caroxylic Acid Diethyl Ester (8)

A mixture of **2** (0.02 mol) and 1,3-phenylenediamine (0.01 mol) in dimethylformamide containing 3 drops of triethylamine was refluxed for 10 h. The reaction mixture was filtered while hot to give the

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

Compound no.	M.P. C°	Yield %	Mol. formula (mol. wt.)	Analysis rquired/(found)% C	H	N
3	238–240	68	$C_{19}H_{22}N_2O_4S_2$ (406)	56.16 (56.40)	5.42 (5.60)	6.90 (7.20)
5	186–188	73	$C_{16}H_{13}N_3O_3S$ (327)	58.72 (58.50)	3.98 (3.70)	12.84 (13.10)
6	178–180	59	$C_{23}H_{17}N_3O_4S$ (431)	64.04 (64.20)	3.94 (3.60)	9.74 (10.00)
7	>300	43	$C_{26}H_{22}N_4O_6S_4$ (614)	50.81 (50.60)	3.58 (3.80)	9.12 (8.90)
8	110–112	47	$C_{18}H_{21}N_3O_4S_2$ (407)	53.07 (52.80)	5.16 (5.40)	10.32 (10.10)
9	>300	39	$C_{26}H_{22}N_4O_6S_4$ (614)	50.81 (50.50)	3.58 (3.40)	9.12 (8.80)
10	95–97	40	$C_{18}H_{21}N_3O_4S_2$ (407)	53.07 (53.30)	5.16 (5.30)	10.32 (10.60)
11	>300	34	$C_{25}H_{21}N_5O_6S_4$ (615)	48.78 (48.40)	3.41 (3.20)	11.38 (10.70)
12	88–90	39	$C_{15}H_{14}N_4O_3S_2$ (362)	49.72 (49.90)	3.87 (4.10)	15.47 (15.60)
15	237–239	56	$C_{18}H_{18}N_2O_4S_2$ (390)	55.38 (55.70)	4.62 (4.30)	7.18 (6.80)
16	156–158	41	$C_{16}H_{12}N_2O_3S_2$ (344)	55.81 (55.50)	3.49 (3.70)	8.14 (7.80)

biscompound **7** while compound **8** was obtained from the filtrate by evaporation and treated with ice/water and recrystallized from ethanol (Table I).

1,4-Bis[5-methyl-4-oxo-2-thioxo-1,2,3,4-tetrahydro-thieno[2,3-d]pyrimidine-6-carboxylic Acid Ethyl Ester-3,5-di-yl]-benzene (9) and 5-[3-(4-Amino-phenyl Thioureido)]-3-methyl-thiophene-2,4-dicarboxylic Acid Diethyl Ester(10)

A mixture of **2** (0.02 mol) and 1,4phenylenediamine (0.01 mol) was refluxed in dimethylformamide containing 3 drops of triethylamine for 10 h. The reaction mixture was filtered while hot to separate pure biscompound **9,** while compound **10** was obtained when the filtrate was poured onto ice/water, and the obtained solid was recrystallized from ethanol (Table I).

The Synthesis of 2,6-Bis-5-methyl-4-oxo-3-pyridin-2-yl-2-thioxo-1,2,3,4-tetrahydro-thioxo[2,3-d]-pyrimidine-6-carboxylic Acid Ethyl Ester (11) and 3-(6-Amino-2-pyridyl)-5-methyl-2-thioxo-4-oxo-1,2,3,4-tetrahy-dro-thieno[2,3-d]pyrimidin-6-carboxylic Acid Ethyl Ester (12)

A mixture of **2** (0.02 mol) and 2,6-diaminopyridine (0.01 mol) was refluxed in dimethylformamide containing 3 drops of triethylamine for 10 hr. The reaction mixture was filtered while hot to separate pure bis compound **11**, while compound **12** was obtained when the reaction mixture was poured onto ice/water and recrystallized from ethanol (Table I).

The Formation of 5-(Benzothiazole-2-yl-amino)-3-methyl-thiophene-2,4-dicarboxylic acid diethyl ester (15) and Benzothiazolothienopyrimidine (16)

A mixture of **2** (0.01 mol) and o-aminothiophenol (0.01 mol) in tetrahydrofuran (20 mL) was refluxed for 6 hr. The reaction mixture was filtered while hot to give benzothiazole derivative **15**, while compound **16** was obtained by evaporating the solvent under reduced pressure and recrystallized from dioxane (Table I).

BIOCHEMICAL ANALYSIS

A Radioprotection Activity "In Vivo Study"

Material and Methods

Experimental Animals. Thirty-six female albino rats (100–120 g), were used throughout the present experiment.

Treatment

The tested compounds were suspended in carboxymethyl cellulose and administered i.p. to rats in a concentration of 300 mg/kg body weight/dose for twenty minutes preradiation 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. Compounds **5** and **15** were selected to be evaluated for their radioprotection activity.

Experimental Design

Animals were randomly divided into six groups each of six rats. Group (1): control group Group (2): animals administered with compound **5**. Group (3): animals administered with compound **15**. Group (4): Animals were exposed to whole body γ-irradiation. Group (5): Animals administered with compound **5** then subjected to whole-body γ-irradiation. Group (6): Animals administered with compound **15** then subjected to whole body γ-rays.

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). The lipid peroxide content was indicated in plasma and liver homogenates,[24] reduced Glutathione (GSH) content was estimated in whole blood and liver homogenate,[25] and superoxide dismutase activity was estimated in whole blood and liver homogenate.[26] The results were presented as mean values ± standard error, and groups were compared using a two ways ANOVA method (F-test).[27]

Results and Discussion

Several studies postulated that Reactive Oxygen Species (ROS) participated in the etiology of many chronic health problems. Free radicals can cause tissue damage by reacting with polyunsaturated fatty acids in cellular membrane and nucleotides in DNA and critical sulfhydryl bonds in.[28] 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.[29,30] In health, under normal conditions, a delicate balance exists between the generation of ROS and the cellular antioxidant defense systems.[31]

The present study was designed to evaluate the radioprotective role of new synthesized compounds (**5** and **15**); they were selected because they contain benzimidazole and benzothiazole moieties respectively known to have radioprotective activity.[32] Compounds **5** and **15** were administrated 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, Thiobarbituric Acid Reactive Substances (TBARS) content in plasma and liver tissues was estimated. The concentration of reduced glutathione (GSH) and the activities 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. The administration of compounds **5** and **15** resulted in insignificant 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** or compound **15** prerradiation resulted in significant improvement in the levels of lipid peroxidation when compared to irradiated groups.

Levels of the reduced form GSH in both blood and hepatic tissue, which is represented in Table III, showed that the administration of either compound **5** or **15** revealed insignificant elevations in GSH in both blood and liver tissue when compared with the control level. Animals exposed to γ-irradiation revealed significant decrease in GSH content detected in blood and liver tissue. The treatment of animals with compound **5** preradiation exposure showed an improved glutathione profile as compared with the 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 that occurred in the SOD activity levels in blood and liver tissue.

From these results, it is observed that the radiation exposure revealed a high level in lipid peroxide content accompanied with a depression in the levels of GSH concentration and SOD activity levels.

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

	Blood (mg/dL packed cells)		Liver (mg/g protein)	
Groups	Mean ± SE	Percentage of change	Mean ± SE	Percentage of change
Control	28.50 ± 0.80	100	157.27 ± 3.3	100
Compound **5**	25.65 ± 0.90*	90.0	152.25 ± 3.5	96.8
Compound **15**	28.70 ± 0.90	100.7	156.15 ± 2.9	99.2
Radiation (Rad.)	47.52 ± 1.16*	160.7	213.57 ± 3.9*	135.8
Compound **5** + Rad.	32.50 ± 0.90*	114.0	183.89 ± 3.80*	116.9
Compound **15** + Rad.	33.37 ± 1.10*	117.0	180.90 ± 3.40	115.0

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

*Significance 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 Glutathione Contents in Different Groups of Animals[†]

Groups	Blood (mg/dL packed cells)		Liver (mg/g protein)	
	Mean ± SE	Percentage of change	Mean ± SE	Percentage of change
Control	47.50 ± 1.0	100	22.7 ± 0.45	100
Compound 5	48.40 ± 1.12	102	22.65 ± 0.60	99.5
Compound 15	47.46 ± 1.11	99.9	20.50 ± 0.55	90.3
Radiation (Rad.)	39.36 ± 0.95*	82.8	17.40 ± 0.55*	76.6
Compound 5 + Rad.	45.18 ± 1.11	95.1	18.70 ± 0.45*	82.4
Compound 15 + Rad.	40.40 ± 1.00*	85.4	18.80 ± 0.48*	82.8

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

*Significance of change between groups and control.

LSD of blood groups at 0.05 = 4.662.

LSD of liver tissue groups at 0.05 = 3.81.

The administration of compound **5** or **15** preradiation exposure showed some improvement in the damage induced by radiation exposure. From the present data, we found that compound **5** is more effective than compound **15**.

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

Groups	Blood (U/g Hb)		Liver (U/mg Protein)	
	Mean ± SE	Percentage of change	Mean ± SE	Percentage of change
Control	48.26 ± 4.78	100	277.12 ± 22.96	100
Compound 5	49.90 ± 4.12	103.4	273.91 ± 21.02	98.8
Compound 15	49.37 ± 4.56	102.3	278.02 ± 22.85	100.3
Radiation (Rad.)	42.71 ± 3.33*	88.5	263.55 ± 21.90*	95.1
Compound 5 + Rad.	48.54 ± 3.90	100.5	280.45 ± 25.85	101.2
Compound 15 + Rad.	42.83 ± 2.16*	88.7	270.88 ± 23.45*	97.7

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

*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 Carcnoma cells

Compound no.	Nonviable cells (%) Concentration (μg/mL)							
	0	25	50	75	100	125	250	300
5	0	0	3	10	35	40	45	55
6	0	0	10	20	50	50	50	50
10	0	5	10	40	100	100	100	100
15	0	0	0	0	0	0	10	50
16	0	5	10	15	20	30	60	80
Doxorubicin	0	20	55	75	100	100	100	100

Anticancer Study "In Vitro Studies"

Experimental

Various concentrations (300, 250, 125, 100, 75, 50 and 25) μg/mL of the selected compounds (**5**, **6**, **10**, **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.[33]

Results and Discussion

Invitro studies for the synthesized compounds revealed that compound **10** induced the greatest effect on Ehrlich Ascites Carcinoma (EAC) cells, which recorded 100% nonviable cells at a concentration of 100 μg/mL, which is nearly as active as doxorubicin as a reference drug.

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