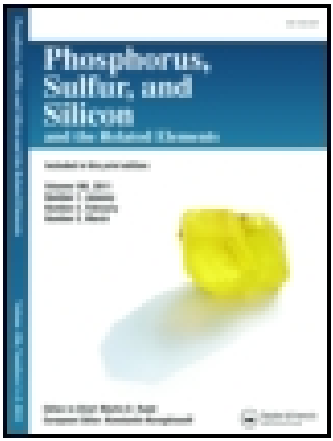

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Radioprotective and Antitumor Activity of Some Novel Amino Acids and Imidazoles Containing Thieno[2,3-*d*]pyrimidine Moiety

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*A variety of novel thieno[2,3-*d*]pyrimidine derivatives, comprising amino acids **3a–l**, imidazothieno-pyrimidines **4A**, **4b–h**, and **7**, were obtained via the reaction of 4-chloro-5,6-dimethylthieno[2,3-*d*]pyrimidine **1** with a variety of reagents. The structures of these compounds were confirmed by microanalysis, IR, ^{1}H NMR, and mass spectrometry. Some of the obtained compounds showed promising radioprotective and antitumor activities.*

Keywords Amino acids; antitumor; radioprotective; thieno[2,3-*d*]pyrimidines

INTRODUCTION

The pharmacological activities of thieno[2,3-*d*]pyrimidine derivatives are of great interest in the field of medicinal chemistry.[1] They display antibacterial,[2] antifungal,[3] antitumor,[4–6] and radioprotective characteristics.[7–9] In addition, compounds having amino acid moieties are also known to possess a wide range of biological and pharmacological activities, such as antitumor and radioprotective capacity.[10] This perception

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has inspired us to find an efficient way to synthesize a new class of heterocyclic ring systems containing a thienopyrimidine nucleus pendentto an amino acid and imidazole moiety for biological screening. We performed a series of reactions using 4-chlorothieno[2,3-*d*]pyrimidine **1** as a precursor for the versatile functionalized amino acids and imidazoles bearing thienopyrimidines as possible new antitumor and radioprotective agents.

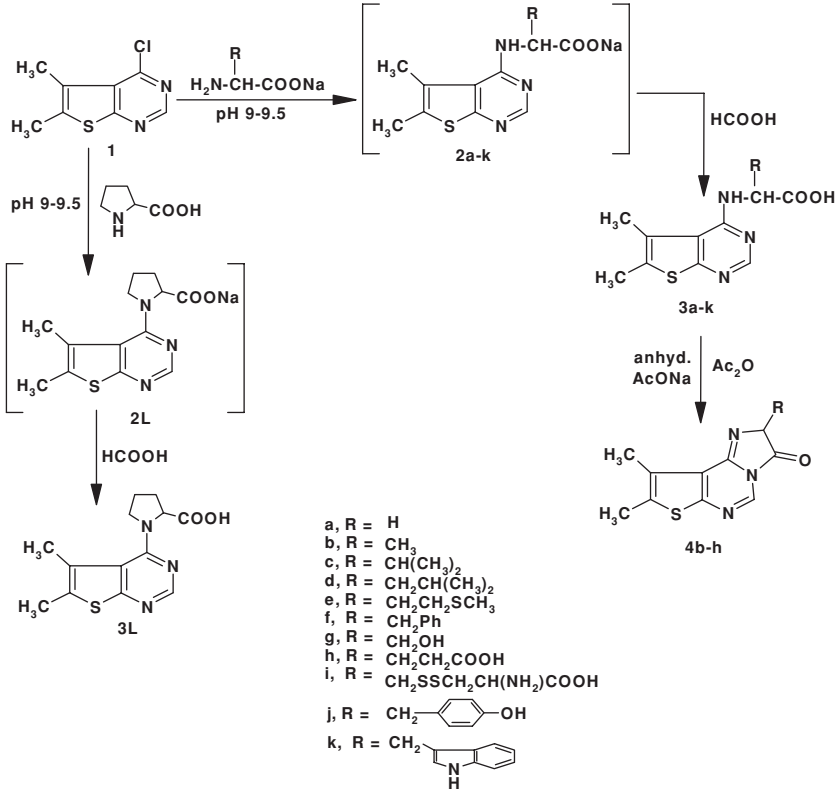
RESULTS AND DISCUSSION

Chemistry

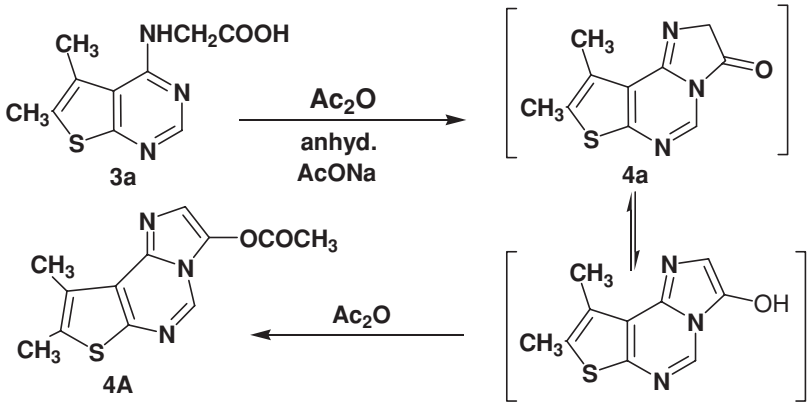
The present work was to design and synthesize some new amino acids containing biologically active thieno[2,3-*d*]pyrimidine derivatives expected to have radioprotective and antitumor activities. When chloro derivative **1**[11] was treated with the sodium salt of various amino acids under reflux at pH 9.0–9.5, each reaction afforded a single product identified as *N*-(5,6-dimethylthieno[2,3-*d*]pyrimidin-4-yl)amino acids **3a–l**. (Scheme 1). The imidazopyrimidine derivatives having the thiophene nucleus (purine analogues) were obtained in good yields via reaction of the amino acid derivatives **3b–h** with acetic anhydride in the presence of anhydrous sodium acetate[12] (Scheme 1). In the case of **3a**, when reacted with acetic anhydride in presence of anhydrous sodium acetate, the acetyl imidazopyrimidine **4A** was obtained instead of the expected imidazopyrimidine **4a** (Scheme 2). Also, the interaction of chloro compound **1** with ethanolamine or 3-aminopropanol in pyridine yielded the hydroxy compounds **5** and **6,** respectively (Scheme 3). Cyclization of compound **5** with thionyl chloride afforded **7** (Scheme 3). 5,6-Dimethylthieno[2,3-*d*]pyrimidine-4-yl-isothiocyanate **8** was obtained via the reaction of compound **1** with ammonium thiocyanate in dry acetone (Scheme 3). It was reported that the condensation of isothiocyanate with active methylene compounds such as malononitrile was exploited in the synthesis of heterocyclic derivatives.[2] Thus, interaction of the isothiocyanate **8** with malononitrile in presence of sodium ethoxide gave the pyrimidinethione derivative **10**. This reaction proceeded via the initial formation of intermediate **9** followed by intramolecular cyclization to give pyrimidinethione derivative **10** (Scheme 3). Physical data for **3a–10** are shown in Table I.

Antitumor Activity

The results of antitumor activity for the synthesized compounds (Table II) indicated that compounds **3c**, **3d**, **3e**, **3j**, **3k**, and **8** showed a significant in vitro activity toward Ehrlich Ascites Carcinoma



SCHEME 1 Synthesis of compounds **3a–k**, **3L**, and **4b–h**.

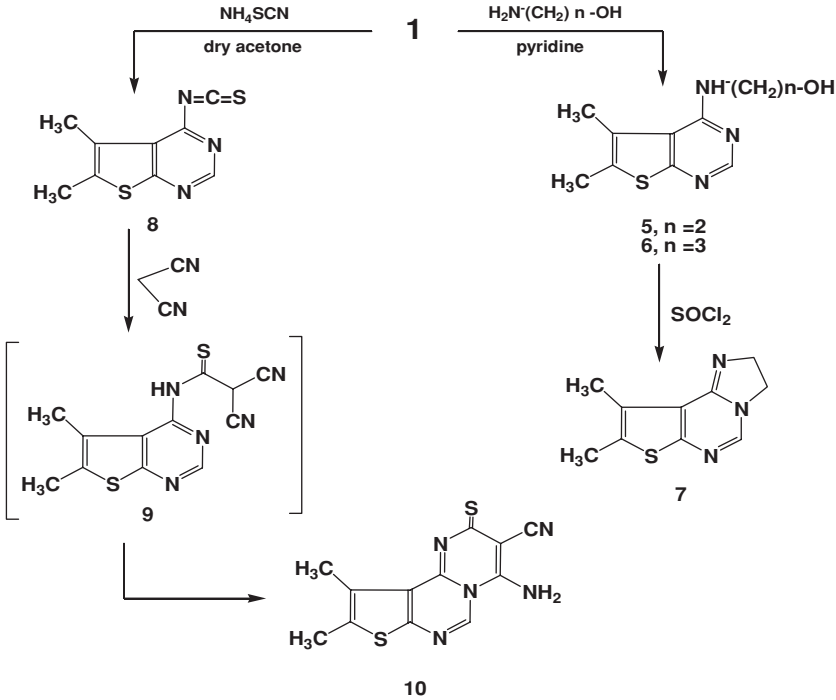


SCHEME 2 The postulated mechanism for the formation of compound **4A**.

TABLE I Physical Data of the Newly Synthesized Compounds (3a–10)

Cpd. No.	Solvent	Mp (°C)	Yield%	Mol. Formula	% Analyses: Calcd. (found). C	H	N
3a	D	228–230	81	$C_{10}H_{11}N_3O_2S$	50.62(50.31)	4.67(4.35)	17.71(17.58)
3b	D	184–186	74	$C_{11}H_{13}N_3O_2S$	52.57(52.33)	5.21(4.98)	16.72(16.50)
3c	D	209–211	78	$C_{13}H_{17}N_3O_2S$	55.89(55.68)	6.13(5.89)	15.04(15.32)
3d	D	190–191	86	$C_{14}H_{19}N_3O_2S$	57.31(57.15)	6.53(6.70)	14.32(14.29)
3e	D	200–202	77	$C_{13}H_{17}N_3O_2S_2$	50.14(50.36)	5.50(5.14)	13.49(13.65)
3f	D	162–163	79	$C_{17}H_{17}N_3O_2S$	62.36(62.17)	5.23(5.57)	12.83(12.66)
3g	D	177–178	85	$C_{11}H_{13}N_3O_3S$	49.43(49.70)	4.90(4.66)	15.72(15.54)
3h	D	185–187	87	$C_{13}H_{15}N_3O_2S$	56.30(56.59)	5.45(5.18)	15.15(14.85)
3i	D	170–171	85	$C_{14}H_{16}N_4O_4S_3$	41.99(41.65)	4.03(4.35)	13.99(14.18)
3j	D	160–162	88	$C_{17}H_{16}N_3O_3S$	59.63(59.50)	4.71(4.42)	12.27(12.30)
3k	D	280–282	79	$C_{19}H_{18}N_4O_2S$	62.28(62.47)	4.95(4.71)	15.29(15.46)
3l	D	160–162	86	$C_{13}H_{12}N_3O_2S$	56.92(56.73)	4.41(4.19)	15.32(15.65)
4A	A	> 300	85	$C_{12}H_{11}N_3O_2S$	55.16(55.46)	4.24(4.17)	16.08(16.29)
4b	A	110–111	87	$C_{11}H_{10}N_3OS$	56.88(56.59)	4.34(4.67)	18.09(17.75)
4c	A	135–136	86	$C_{13}H_{14}N_3OS$	59.98(59.65)	5.42(5.13)	16.14(16.37)
4d	A	178–180	85	$C_{14}H_{17}N_3OS$	61.06(61.40)	6.22(5.95)	15.26(15.56)
4e	A	> 300	87	$C_{13}H_{14}N_3OS_2$	53.40(53.57)	4.83(4.50)	14.37(14.51)
4f	A	224–226	88	$C_{17}H_{15}N_3OS$	66.00(65.82)	4.89(5.02)	13.58(13.70)
4g	A	> 300	86	$C_{11}H_{10}N_3O_2S$	53.21(53.60)	4.06(4.35)	16.92(17.08)
4h	A	75–77	89	$C_{13}H_{11}N_3O_3S$	53.97(54.12)	3.83(3.65)	14.52(14.40)
5	D	163–165	76	$C_{10}H_{13}N_3OS$	53.79(53.52)	5.87(5.53)	18.82(18.73)
6	D	217–218	81	$C_{11}H_{15}N_3OS$	55.67(55.81)	6.37(6.67)	17.71(17.58)
7	E	> 300	83	$C_{10}H_{11}N_3S$	58.51(58.81)	5.40(5.17)	20.47(20.25)
8	E	134–136	78	$C_9H_7N_3S_2$	48.85(48.60)	3.19(3.25)	18.98(19.23)
10	D	215–217	68	$C_{12}H_9N_5S_2$	44.53(44.35)	14.01(13.8)	21.46(21.36)

Solvent: A = acetic acid; D = dioxane; E = ethanol.



SCHEME 3 Synthesis of compounds **5–8** and **10**.

cells (EAC). The active compounds having amino acids L-valine-**3c**, L-leucine-**3d**, DL-methionine-**3e**, tyrosine-**3j**, DL-tryptophan-**3k**, and isothiocyanate group **8** were all found to have more powerful activity than the other compounds and displayed a significant percentage of nonviable tumor cells to about 100%, 80%, 80%, 95%, 90%, and 95%, respectively, at a concentration of 50 μg/mL. A common factor in these compounds is the presence of thienopyrimidine moieties. Compounds **3c**, **3d**, **3e**, **3k**, and **8** are more potent than doxorubicin. Considering the effect of the tested compounds on tumor volume (TV), treatment of the animals with compound **8** caused a marked suppression of the tumor growth where the TV was significantly decreased compared to corresponding EAC group. γ-Irradiation to mice bearing tumors and treated with the same compound showed marked synergistic inhibition in the TV. Generally, the combined therapy showed better suppression in TV as compared with single treatment either by compound **8** or irradiation alone. Compound **3k** did not show significant reduction in TV compared to corresponding EAC group.

TABLE II In Vitro Antitumor Activity of Some Newly Synthesized Compounds

	% of Nonviable cells Conc. (μg / ml)									
Compd. No.	400	200	150	100	90	80	75	50	25	10
Doxorubicin	100	100	100	100	90	85	75	55	20	10
3b	100	100	100	100	10	10	0	0	0	0
3c	100	100	100	100	100	100	100	100	80	0
3d	100	100	100	100	100	100	95	80	30	20
3e	100	100	100	100	100	100	100	80	40	20
3f	100	100	100	70	70	60	60	50	30	30
3g	100	100	100	100	50	40	20	5	0	0
3h	100	100	100	100	50	40	30	30	30	0
3i	100	90	80	55	50	50	50	30	5	0
3j	100	100	100	100	100	100	100	95	90	20
3k	100	100	100	100	100	100	100	90	10	0
3l	100	100	100	100	80	50	30	20	0	0
4A	100	100	100	100	90	90	85	0	0	0
4d	100	80	0	0	0	0	0	0	0	0
4f	100	100	100	90	90	90	85	75	60	50
4g	100	100	80	50	40	40	10	5	0	0
5	100	100	100	65	0	0	0	0	0	0
6	100	100	100	70	10	0	0	0	0	0
7	100	100	100	100	100	100	80	30	0	0
8	100	100	100	100	100	100	100	95	90	70

Radioprotective Activity

EAC and radiation exposure significantly increase lipid peroxide content (LPx). Such an increase seemed to be due to the result of inactivation of scavenger enzyme activities induced by reactive oxygen species (ROS). Oxidative stress occurs in living organisms when the production of ROS exceeds their ability to prevent their accumulation.[13,14] Such elevation of LPx is accompanied by a decline in glutathione level (GSH) content and in the activity of related antioxidant enzyme super oxide dismutase (SOD). Additionally, LPx can be initiated by hydrogen abstraction from lipid molecules by lipid radiolytic products. This leads to permeability changes, secondary alteration in membrane proteins, and other sequences.[15,16] Also, it was postulated that the decline in GSH level accompanied by cancer growth is due to reduction in the glutathione redox status (GSH/GSSG) in the blood of tumor-bearing mice, which is mainly due to an increase in blood GSSG levels as a result of oxidative stress.[17] On the other hand, the decline in SOD activity

in tumor-bearing mice is worthy of mentioning. SOD activity plays an important role in the antitumor effects of radiation therapy and active oxygen-forming anticancer agents. However, when the oxidative damage is extreme as a result of tumor growth and/or irradiation, ROS scavenging enzymes such as SOD are degraded.[18]

In view of minimizing the toxicity of ionizing radiation on normal organs of Ehrlich carcinoma-bearing mice, the present study was applied. The evaluation of agents regarding antitumor effect alone or in combination with irradiation was also considered. The current investigation demonstrated that administration of the tested compounds in tumor-bearing mice almost prevented LPx in plasma and significantly normalized GSH content and SOD efficiently up to normal values. In addition, compounds administrated to irradiated mice groups exhibited general amelioration to a great extent the levels of LPx, GSH, and related antioxidant scavenger enzymes activity in blood. On the other hand, our reference drug doxorubicin revealed unexpected deterioration of GSH and LPx levels. It also showed disruption of the antioxidant enzyme system SOD to a lesser extent. Mice injected with doxorubicin and subjected to irradiation showed slight improvement in the enzymes' levels, yet still less than our tested compounds **3k** and **8**.

Effect of Tested Compounds and/or γ-Irradiation on Lipid Peroxidation and Antioxidant Status

Glutathione level in blood (GSH). As summarized in Table III, a very high, significant depletion in GSH level was observed in the blood of tumor-bearing mice (ER group) as compared to the corresponding controls. The same pattern of GSH depletion was recorded in irradiated group and in the ER+Rad group as well. Treatment with compound **3k** 10 days after tumor inoculation (ER+compd **3k**) showed a very high, significant depletion of GSH content in blood. Similar trends were seen in animals pretreated with compound **3k** prior irradiation (ER + Compd **3k** + Rad). Treatment with compound **8** 10 days after tumor inoculation ER+compd **8** showed a very high significant elevation of GSH content in blood. Compound **8** treatment prior irradiation (ER + compd **8** + Rad) for a total of 3 treatments every other day showed higher elevation, yet still very highly significant in GSH content in blood.

Lipid peroxidation content (MDA) in plasma. The effects of compounds **3k** and **8** treatment and/or irradiation on the level of lipid peroxidation measured in terms of malondialdehyde (MDA) in plasma of tumor-free or tumor-bearing mice are shown in Table III.

TABLE III Effect of Compounds 3k and 8 Administration on Blood Glutathione (GSH) Content, Activity Levels of Superoxide Dismutase (SOD), and Plasma Lipid Peroxide (LP) Concentrations of Normal, Tumor-Bearing, and Irradiated Mice

Groups Means ± SE % of change[#] SE	GSH mg/mL	SOD mg/mL	LP μmol/mL
Control	78.47 ± 0.60(100%)	7.79 ± 0.1(100%)	77 ± 0.53(100%)
CMC	75.45 ± 0.28**(96.15%)	7.65 ± 0.9(98.20%)	80 ± 1.18*(103.9%)
Irradiated	57.56 ± 0.93***(73.33%)	5.71 ± 0.2***(73.29%)	80 ± 1.44(103.9%)
EAC	53.22 ± 0.18***(67.8%)	5.65 ± 0.9*(72.53%)	113 ± 2.18***(146.8%)
EAC+Rad	68.5 ± 0.20***(87.3%)	6.77 ± 0.34*(87%)	95.5 ± 4.7**(124%)
EAC+CMC +Rad	69.2 ± 0.14***(88.19%)	8.57 ± 0.45(110%)	97 ± 4.44**(126%)
ER+Doxo	59.31 ± 0.3***(75.58%)	6.95 ± 0.1***(89.35%)	112 ± 2.13***(145.45%)
EAC+D 3k	72.1 ± 0.19***(92%)	7.01 ± 0.1***(90%)	85 ± 1.41***(110.4%)
EAC+ D 3k+ Rad	72.1 ± 0.19***(92%)	7.01 ± 0.1***(90%)	85 ± 1.41***(110.4%)
EAC+D 8	82.2 ± 0.4***(104.7%)	8.4 ± 0.6(107.8%)	80 ± 1.26(103%)
EAC + D8+ Rad	86.2 ± 0.4***(109.8%)	8.53 ± 0.6(109.5%)	80 ± 1.44(103%)

Each value is the mean of six mice ± SE (standard error).
[#]Percentage of change from control group.
*Significant difference from control at $P < 0.05$.
**Highly significant at $P < 0.01$.
***Very highly significant at $P < 0.001$.
CMC: carboxy methyl cellulose; ER: Ehrlich carcinoma. Doxo: doxorubicin.

Tumor-bearing mice (ER group) showed a very high significant elevation in MDA level in plasma compared with the control values. MDA levels in plasma of tumor-bearing mice and exposed to irradiation (ER + Rad group) showed high significant increase from the control group. Treatment with compound **3k** 10 days after tumor inoculation (ER + Compd **3k**) lowered MDA levels, which was still a very high, significant elevation with respect to the control group. Similar trends were seen in animals pretreated with compound **3k** and prior irradiation (ER + Compd **3k** + Rad). Treatment with compound **8** 10 days after tumor inoculation (ER + compd **8**) lowered MDA levels and showed insignificant differences from control group. Similar trends were seen in animals pretreated with compound **8** and prior irradiation (ER + compd **8** + Rad) when compared with the control group.

Superoxide ismutase (SOD) activity in blood. As shown in Table III, SOD activity in the ER group showed a significant decline in blood. Very high significant inhibition of SOD activity was observed in the blood of animals exposed to γ-irradiation. Treatment with compound **3k** 10 days after tumor inoculation (ER + Compd **3k**) revealed very high and significant reduction in SOD activity in blood. Similar trends were seen in animals pretreated with compound **3k** prior to irradiation (ER + Compd **3k** + Rad) when compared with control group. Treatment with compound **8** 10 days after tumor inoculation (ER + Compd **8**) revealed insignificant difference from the control group. Similar trends were seen in animals pretreated with compound **8** prior to irradiation (ER + compd **8** + Rad) when compared with the control group.

CONCLUSIONS

It is clear from these results that compounds **3c, 3d, 3e, 3k,** and **8** are more potent than doxorubicin as a reference drug. In addition, the administration of compounds **3k** and **8** to solid tumor-bearing mice not only protected the animals against γ-irradiation induced toxicity to normal organs, but also exhibited oncolytic activity and acted in synergy with γ-irradiation to suppress Ehrlich carcinoma tumor.

MATERIALS AND METHODS

General Considerations

Melting points were uncorrected and were determined on a Stuart melting point apparatus (Stuart Scientific, Redhill, UK). Elemental

analyses (C, H, N) were performed on Perkin-Elmer 2400 analyser (Perkin-Elmer, Norwalk, CT, USA) at the Microanalytical Laboratories of the Faculty of Science, Cairo University. The 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), in DMSO-d_6 as a solvent, using tetramethylsilane (TMS) as internal standard. Mass spectra were run on an HP model MS-5988.

Synthesis

2-(5,6-Dimethylthieno[2,3-d]pyrimidin-4-ylamino)acetic Acid (3a), Propionic Acid (3b), 3-Methylbutyric acid (3c), 4-Methylpentanoic Acid (3d), 4-Methylsulfanylbutyric Acid (3e), 3-Phenylpropionic Acid (3f), 3-Hydroxy-propionic Acid (3g), Pentanedioic Acid (3h), 2-Amino-3-disulfide-3,4-dicarboxylic Acid (3i), 2-(5,6-Dimethyl-thieno[2,3-d]pyrimidin-4-ylamino)-3-(4-hydroxyphenyl)propionic Acid (3j), 3-(2,3-Dihydro-1H-indol-2-yl)-2-(5,6-dimethylthieno[2,3-d] pyrimidin-4-ylamino)propionic Acid (3k), and 1-(5,6-Dimethyl-thieno[2,3-d]pyrimidin-4-yl)-pyrrolidine-2-carboxylic Acid (3l)

An amino acid (0.0096 mol) and sodium carbonate (0.57 g, 0.0054 mol) were dissolved in water (10 mL), and the solution was adjusted to pH 9.0–9.5. The chloro derivative **1** (0.95 g, 0.0048 mol) was then added, and the mixture was stirred at 100°C for 6 h. The reaction mixture was left overnight at room temperature and then treated with formic acid (88%). The solid product obtained was filtered off, washed with water, and crystallized from the proper solvent (Table I).

IR (KBr, cm^{-1}) 3a: 3420 (NH, OH), 2860 (CH aliph.), 1710 (C = O), 1550 (C=N), 1244 (COOH); δ **3a:** 2.4, 2.5 [2s, 6H, 2CH_3], 3.4[s, 1H, OH], 4.2 [d, 2H, CH_2, J = 7.3 Hz], 7.1 [s, 1H, NH], 8.3 [s, 1H, CH pyrimidine].

IR (KBr, cm^{-1}) 3b: 3423 (NH, OH), 2925 (CH aliph.), 1661 (C = O), 1600 (C=N), 1248 (COOH); δ **3b:** 1.5[d, 3H, CH_3, J = 7.3 Hz], 2.3, 2.4[2s, 6H, 2 CH_3], 6.6[q, 1H, CH], 8.3[s, 1H, CH pyrimidine].

IR (KBr, cm^{-1}) 3c: 3410 (br, NH, OH), 2965 (CH aliph.), 1711 (C = O), 1600 (C=N), 1272 (COOH); δ **3c:** 1.1[t, 6H, γ-CH_3], 2.3[m, 1H, β-CH], 2.4[s, 6H, 2CH_3], 4.3[m, 1H, α-CH], 6.3[s, 1H, NH], 6.4[s, 1H, OH], 8.3[s, 1H, CH pyrimidine]; **MS (*m/z*) 3c:** 279 (M^+,15.07%), 179 (100%), 280 (3.38%), 281 (1.08%), 234 (32.52%), 220 (44.20%), 163 (59.22%), 136 (8.39%), 92 (10.40%), 55 (18.41%).

IR (KBr, cm^{-1}) 3d: 3400 (NH, OH), 2940, 2840 (CH aliph.), 1700 (C = O), 1241 (COOH); δ **3d:** 0.9 [d, 6H, 2CH_3, J = 7.4 Hz], 1.8 [t, 2H, β-CH_2], 2.1, 2.4 [2s, 6H, 2CH_3],3.3[s, 1H, OH], 4.6 [m, 1H, β-CH], 6.4

[m, 1H, α-CH], 8.3 [s, 2H, CH pyrimidine + NH]; **MS (*m/z*) 3d:** 293 (M^+, 12.57%), 206 (100%).

IR (KBr, cm^{-1}) 3e: 3413 (NH, OH), 2916, 2857 (CH aliph.), 1659 (C = O), 1600 (C=N), 1263 (COOH); δ **3e:** 2.2 [m, 2H, β-CH_2], 2.35, 2.38 [2s, 6H, $2CH_3$], 2.4 [s, 3H, SCH_3], 4.7[m, 2H, γ-CH_2], 6.7 [m, 1H, α-CH], 8.0 [s, 1H, NH], 8.3 [s, 1H, CH pyrimidine], 12.2 [s, 1H, OH]; **MS (*m/z*) 3e:** 311 (M^+, 10.31%), 237 (100%), 312 (3.26%), 313 (1.86%), 250 (38.75%), 191 (52.59%), 163 (91.35%), 136 (12.74%), 92 (16.07%), 61 (61.30%).

IR (KBr, cm^{-1}) 3f: 3418 cm^{-1} (NH, OH), 2926, 2859 (CH aliph.), 1718 (C = O), 1573 (C=N), 1245 (COOH); δ **3f:** 2.3, 2.4 [2s, 6H, $2CH_3$], 4.7 [m, 1H, α-CH], 6.45 [d, 2H, β-CH_2, J = 7.2 Hz], 7.2–7.4 [m, 5H, Ar-H], 8.5 [s, 2H, CH pyrimidine + NH], 8.6 [s, 1H, OH].

IR (KBr, cm^{-1}) 3g: 3430 (OH), 3186 (NH), 2922, 2865 (CH aliph.), 1662 (C = O), 1580 (C=N), 1230 (COOH); δ **3g:** 2.3 [s, 6H, $2CH_3$], 3.9 [s, 2H, 2OH], 4.7 [m, 1H, α-CH], 6.6 [d, 2H, β-CH_2, J = 7.3 Hz], 8.3 [s, 2H, NH+CH pyrimidine].

IR (KBr, cm^{-1}) 3h: 3563 (OH), 3412 (NH), 2934 (CH aliph.), 1714 (C = O), 1581 (C=N), 1247 (COOH). δ **3h:** 2.2 [m, 2H, β-CH_2], 2.3, 2.4 [2s, 6H, $2CH_3$], 4.8 [t, 2H, γ-CH_2], 6.6 [m, 1H, α-CH], 8.2 [s, 2H, NH+CH pyrimidine], 8.3 [s, 2H, 2OH].

IR (KBr, cm^{-1}) 3i: 3416 (br, NH, NH_2, OH), 2980, 2922 (CH aliph.), 1666 (C = O), 1576 (C=N), 1298 (COOH); δ **3i:** 1.4 [m, 1H, β-CH], 2.4 [s, 6H, $2CH_3$], 2.7 [d, 4H, 2 SCH_2, J = 7.4 Hz], 4.9 [m, 1H, α-CH], 6.8 [d, 2H, NH_2, J = 7.4 Hz], 8.1 [s, 1H, NH], 8.15 [s, 1H, pyrimidine-CH], 8.2, 8.3 [2s, 2H, 2OH].

IR (KBr, cm^{-1}) 3j: 3429 (OH), 3208 (NH), 2930 (CH aliph.), 1722 (C = O), 1570 (C=N), 1240 (COOH); δ **3j:** 2.25, 2.37 [2s, 6H, $2CH_3$], 4.7 [d, 2H, ß-CH_2, J = 7.5 Hz], 6.5 [m, 1H, α-CH], 6.6–7.0[m, 4H, Ar-H], 8.2[s, 1H, NH], 8.3[s, 1H, pyrimidine-CH], 8.45, 9.3[2s, 2H, 2OH]; **MS (*m/z*) 3j:** 342 (M-1, 0.33%), 107 (100%), 323 (2.15%), 295 (1.38%), 236 (0.51%), 219 (19.56%), 163 (48.27%), 132 (13.20%), 77 (37.34%).

IR (KBr, cm^{-1}) 3k: 3444 (OH), 3350 (NH), 3073 (CH arom.), 2922 (CH aliph.), 1725 (C = O), 1556 (C=N), 1269 (COOH); δ **3k:** 2.2, 2.3 [2s, 6H, $2CH_3$], 4.9 [d, 2H, ß-CH_2, J = 7.3 Hz], 6.35[m, 1H, α-CH], 6.9–7.3 [m, 4H, Ar-H], 7.47, 7.50 [2s, 2H, 2NH], 8.26 [s, 1H, pyrimidine-CH], 10.9 [s, 1H, OH].

IR (KBr, cm^{-1}) 3l: 3449 (OH), 2966, 2922 (CH aliph.), 1721 (C = O), 1550 (C=N), 1212 (COOH); **MS (*m/z*) 3l:** 277 (M^+, 3.44%), 70 (100%), 232 (58.74%), 204 (20.72%), 190 (67.42%), 163 (51.76%), 109 (9.94%), 92 (14.58%).

*7,8-Dimethyl-4-oxoacetylimidazo[1,5:4,5]thieno[2,3-*d*]pyrimidine (4A), 5,7,8-Trimethyl-4-oxo (4b), 5-Isopropyl (4c), 5-Isobutyl (4d), 5-(2-Methylsulfanyl-ethyl) (4e), 5-Benzyl (4f), 5-Hydroxymethyl (4g), 5-Propionic Acid (4h), Imidazo[1,5:4,5] thieno[2,3-*d*]pyrimidine*

A mixture of **3a–h** (0.01 mol) and anhydrous sodium acetate (2 g) in acetic anhydride (30 mL) was refluxed for 3 h. The reaction mixture was filtered while hot, the solvent was concentrated, and the solid obtained was crystallized from the proper solvent (Table I).

IR (KBr, cm^{-1}) 4A: 2930 (CH aliph.), 1710 (C = O), 1640 (C=N); δ **4A:** 2.2 [s, 6H, 2 CH_3], 2.5 [s, 3H, $COCH_3$], 8.1, 8.4 [2s, 2H, CH imidazole + CH pyrimidine]; **MS (*m/z*) 4A:** 261 (M^+, 92.92%), 179 (100%).

IR (KBr, cm^{-1}) 4b: 2900 (CH aliph.), 1740 (C = O), 1600 (C=N). δ **4b:** 2.08 [d, 3H, CH_3, J = 7.3 Hz], 2.5 [s, 6H, $2CH_3$], 3.2 [m, 1H, CH imidazole], 8.1 [s, 1H, CH pyrimidine].

IR (KBr, cm^{-1}) 4c: 2900 (CH aliph.), 1700 (C = O), 1610 (C=N). δ **4c:** 1.612, 1.621 [2s, 6H, $2CH_3$], 2.07 [d, 1H, CH imidazole, J = 7.2 Hz], 2.5 [2s, 6H, $2CH_3$ thiophene], 3.3 [m, 1H, CH], 8.1 [hump, 1H, CH pyrimidine].

IR (KBr, cm^{-1}) 4d: 2952, 2868 (CH aliph.), 1714 (C = O), 1576 (C=N); **MS (*m/z*) 4d:** 275 (M^+, 56.33%), 69 (100%).

IR (KBr, cm^{-1}) 4e: 2940 (CH aliph.), 1700 (C = O), 1630 (C=N); **MS (*m/z*) 4e:** 293 (M^+, 12.28%), 55 (100%), 287 (10.53%), 278 (31.58%), 240 (28.07%), 213 (42.11%), 180 (26.32%), 161 (38.60%), 127 (28.07%), 102 (38.60%).

IR (KBr, cm^{-1}) 4f: 2920 (CH aliph,), 1720 (C = O), 1640 (C=N). **MS (*m/z*) 4f:** 309 (M^+, 12.73%), 163 (100%), 279 (4.97%), 218 (59.47%), 190 (16.14%), 136 (5.19%), 91 (52.29%), 65 (23.69%).

IR (KBr, cm^{-1}) 4g: 3500–3300 (br, OH), 2930 (CH aliph.), 1670 (C = O), 1620 (C=N).

IR (KBr, cm^{-1}) 4h: 3500–3000 (br, OH), 2900 (CH aliph,), 1720, 1700 (2C = O), 1590 (C=N). δ **4h:** 1.9, 2.1 [2s, 6H, $2CH_3$], 3.4 [t, 2H, CH_2], 4.0 [t, 2H, CH_2CO], 7.8 [s, 1H, CH pyrimidine], 8.2 [s, 1H, OH].

*2-(5,6-Dimethylthieno[2,3-*d*]pyrimidin-4-ylamino)ethanol (5) and 3-(5,6-Dimethylthieno[2,3-*d*]pyrimidin-4-ylamino)propan-1-ol (6)*

A mixture of **1** (1.99 g, 0.01 mol) and ethanolamine or propanolamine (0.01 mol) in pyridine (20 mL) was refluxed for 12 h. The reaction mixture was cooled and acidified with dil. HCl and the solid obtained was crystallized from proper solvent (Table I).

IR (KBr, cm^{-1}) 5: 3390 (OH), 3150 (NH), 2940 (CH aliph.), 1595 (C=N). δ **5:** 0.9 [t, 2H, CH_2OH], 1.2 [t, 2H, NCH_2], 2.3, 2.4 [2s, 6H, $2CH_3$], 6.7 [s, 1H, NH], 8.3 [s, 1H, CH pyrimidine].

IR (KBr, cm^{-1}) 6: 3500–3000 (br, OH+NH), 2920 (CH aliph.), 1600 (C=N). δ **6:** 1.8 [t, 2H, NCH_2], 2.3, 2.4 [2s, 6H, $2CH_3$], 3.6–3.8 [m, 4H, OCH_2CH_2], 5.7 [hump, 1H, OH], 8.1 [t,1H, NH], 8.7 [s, 1H, CH pyrimidine].

*7,8-Dimethyl-4,5-dihydroimidazo[1,5:4,5]thieno[2,3-*d*] pyrimidine (7)*

A solution of **5** (2.23 g, 0.01 mol) and thionyl chloride (20 mL) was refluxed for 5 h. The solvent was evaporated under vacuum, and the solid obtained was crystallized from the proper solvent (Table I).

IR (KBr, cm^{-1}): 2970 (CH alpih.), 1630 (C=N). **MS (*m/z*):** 205 (M^+, 77.89%), 57 (100%), 204 (93.54%), 98 (54.42%) 83 (81.97%).

*4-Isothiocyanato-5,6-dimethylthieno[2,3-*d*]pyrimidine (8)*

A mixture of **1** (1.99 g, 0.01 mol) and ammonium thiocyanate (0.76 g, 0.01 mol) in dry acetone (20 mL) was refluxed for 1 h. The reaction mixture was filtered while hot then poured into ice water, and the solid obtained was crystallized from the proper solvent (Table I). **IR (KBr, cm^{-1}):** 2940 (CH aliph.), 2150 (N=C=S), 1620 (C=N). **MS (*m/z*):** 221 [M^+, 100%, base peak].

*4-Amino-5-cyano-8,9-dimethyl-6-thioxopyrimido[1,6:4,5] thieno[2,3-*d*]pyrimidine (10)*

A mixture of **8** (2.21 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol), and sodium ethoxide (0.68 g, 0.01 mol) in ethanol (30 mL) was refluxed for 6 h. The reaction mixture was poured into ice water, and the solid product was crystallized from the proper solvent (Table I).

IR (KBr, cm^{-1}): 3320, 3210 (NH_2), 2940 (CH aliph.), 2210 (C≡N), 1600 (C=N).

Biological Testing

Animals, Chemicals, and Facilities

Female Swiss albino mice weighing 25–30 g (the holding company for biological products and vaccines, VACSERA, Cairo, Egypt) were housed at a constant temperature (24 ± 2°C) with alternating 12 h light and dark cycles and were fed standard laboratory food and water. All chemicals and reagents were of the highest commercially available grade. Facilities including animal house, biochemical equipment,

and γ-irradiation were made available by the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. Whole body irradiation was performed using Gamma cell-40 (Caesium-137 source). All animal experiments were conducted in and approved by NCRRT.

Antitumor Activity

Ehrlich Ascites Carcinoma (EAC) cells were obtained by needle aspiration of ascitic fluid from the preinoculated mice under aseptic conditions.[19] A suspension of tumor cells (2.5×10^6 per mL) was prepared in saline. Tested compounds were prepared with various dilutions by dissolving 1.0, 0.75, 0.5, and 0.25 mg of the test compounds in DMF (1 mL).

In a set of sterile test tubes, 0.1 mL of tumor cells suspension, 0.8 mL saline, and 0.1 mL of each tested compound (corresponding to 400, 200, 150, 100, 90, 80, 75, 50, 25, and 10 μg) were mixed. The test tubes were incubated at 37°C for 2 h. The Trypan blue exclusion test was carried out to calculate the percentage of nonviable cells after 2 h of incubation.[20] Compounds producing more than 70% nonviable cells are considered active.[21] The results of in vitro cytotoxic activity experiments are presented in Table II.

Radioprotective Activity

This study was conducted to evaluate the potency of some of the synthesized compounds **3k** and **8** as protective agents against γ-irradiation–induced toxicity, which may extend to affect normal organs in mice bearing solid Ehrlich tumor. Also, to evaluate their antitumor effect, alone or in combination with irradiation, was accomplished by measuring the change of tumor volume (TV). Female Swiss albino mice were injected intraperitoneally with a suspension of the tested compounds in carboxy methylcellulose at the maximum tolerated dose of 150 mg/kg body weight 10 days after tumor inoculation, then once every other day for a total of three injections during 7 days. Each injection was given 30 min prior to exposure to a single dose of whole body γ-irradiation at a dose level of 2 Gy delivered at a dose rate of 0.86 Gy/min. Lipid peroxide content (LPx), glutathione level (GSH), and the activity of the antioxidant scavenger enzyme system super oxide dismutase (SOD) were estimated in blood of animals after the end of the experiment.

Experimental Tumor Cells and Tumor Transplantation

A line of EAC was used in this study. The parent line was kindly supplied by the National Cancer Institute, Cairo University, Egypt. The tumor cells were maintained by weekly intraperitoneal transplantation of 2.5×10^6 cells. Solid tumors were produced by intramuscular inoculation with 0.2 mL of EAC in the right thigh of the lower limb of each mouse. Mice with a palpable solid tumor mass (100 mm^3) that developed within 10 days after inoculation were used in the study. The change in tumor volume (TV) was measured at the end of the experiment using a Vernier caliper (Hangzhou Jinnan Tools & Measures, Hangzhou, China) and calculated by the following formula according to Osman et al.[22]: TV (mm^3) = $0.52\,\boldsymbol{AB^2}$, where $\boldsymbol{A}$ is the minor axis and $\boldsymbol{B}$ is the major axis.

Experimental Design

From the beginning of the experiment, mice were divided into 10 groups. All experimental animals were categorized as follows:

1. **Control**: Animals served as untreated control group.
2. **CMC**: Animals were treated by i.p. injection of carboxy methylcellulose.
3. **Rad:** Animals were subjected to 3 doses; every other day of whole body γ-irradiation at a dose level of 2 Gy starting from day 10.
4. **ER**: Mice bearing solid Ehrlich tumors without any treatment.
5. **ER** + **Rad**: Mice bearing solid Ehrlich tumors and subjected to whole body γ-irradiation starting from day 10.
6. **ER** + **CMC** + **Rad**: Mice bearing solid Ehrlich tumor were injected intraperitoneally with carboxy methylcellulose and subjected to whole body γ-irradiation starting from day 10.
7. **ER** + **Compound 3k**[3-(2,3-Dihydro-1*H*-indol-2-yl)-2-(5,6-dimethyl-thieno[2,3-*d*]pyrimidin-4-ylamino)-propionic acid]**:** Mice bearing solid Ehrlich tumor wer injected i.p. with compound **3k**.
8. **ER** + **Compound 8** (4-Isothiocyanato-5,6-dimethyl-thieno[2,3-*d*]pyrimidine): Mice bearing solid Ehrlich tumor were injected i.p. with compound **8.**
9. **ER** + **Compound 3k** + **R:** Mice bearing solid Ehrlich tumor and injected i.p. with compound **3k** were subjected to whole body γ-irradiation.
10. **ER** + **Compound 8** + **R:** Mice bearing solid Ehrlich tumor and injected i.p. with compound **8** were subjected to whole body γ-irradiation.

Samples Collection

Animals were fasted for 16 h prior to each sampling. Samples were collected after 1 day post last irradiation dose. Whole blood was collected by heart puncture after light anesthesia using heparinized syringes. One part was used for glutathione (GSH) and superoxide dismutase (SOD) estimations. The separated plasma from heparinized blood was used for the determination of lipid peroxide as malondialdehyde (MDA).

Analytical Procedures

Lipid peroxide (Lpx) content in plasma was ascertained by the formation of MDA and measured as described by Yoshioka et al.[23] GSH content was determined according to Beutler et al.,[24] and SOD was quantized according to Minami and Yoshikawa.[25]

Statistical Analysis

ANOVA test[26] was used for the evaluation of tumor volume (TV) and other biochemical parameters.

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