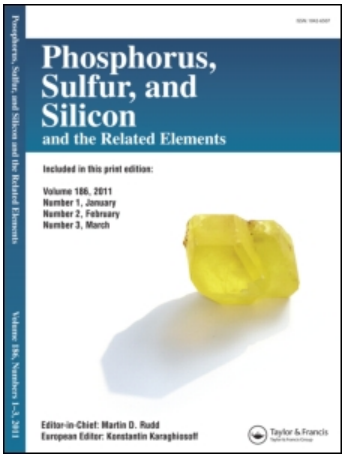

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BIOLOGICAL AND BIOCHEMICAL SCREENING OF SOME NEW AMINO ACID THIENOPYRIMIDINONE DERIVATIVES FOR POTENTIAL RADIOPROTECTIVE CHARACTER

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BIOLOGICAL AND BIOCHEMICAL SCREENING OF SOME NEW AMINO ACID THIENOPYRIMIDINONE DERIVATIVES FOR POTENTIAL RADIOPROTECTIVE CHARACTER

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Ethyl-4,5,6,7-tetrahydro-2-isothiocyanato-1-benzothiophene-3-carboxylate **1** reacts with α-amino acids to give thienopyrimidinone derivatives **12–21** with the nitrogen of the amino acid component being incorporated into the fused pyrimidine ring at position three. The synthesized compounds were characterised by elemental analysis, IR, and in typical instances also by ^{1}H-NMR and mass spectra. The compounds were screened for their antimicrobial activity and most of them showed a remarkably good activity against the tested microorganisms. The preliminary biochemical screening of the prepared compounds **12–21** revealed no appreciable changes of serum triglycerides and phospholipids at doses (0.004, 0.04, 0.4 mg/100 g body weight). Only the glycine **12**, serine **18**, methionine **19** and glutamic **20** derivatives showed significant changes in serum triglycerides, while at doses 1.0 and 2.0 mg/100 g body weight most of the compounds caused variable and significant changes in both triglycerides and phospholipids. The methionine **19** and glutamic **20** derivatives showed the most radioprotective effect on serum albumin.

Keywords: Thienopyrimidinone; amino acids; radioprotective agents

INTRODUCTION

Some thienopyrimidine-4-ones of general formula **I** and **II** have been found to exhibit analgesic, antiinflammatory, diuretic and CNS depressant activities,[1,2] while 2-mercapto-3,4-dihydrothienopyrimidine-4-one **III** has been found to be a potent hypocholesterolemic.[3] On the other hand, compounds having amino acid moieties are known to possess a wide range of biological and pharmaco-

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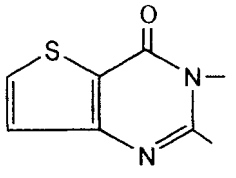
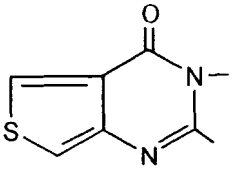


I



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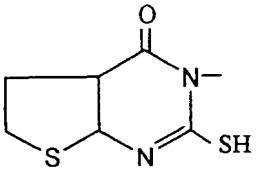


III

logical activity.[4] The amino acids in addition to their role as protein monomeric units, are energy metabolites, important intermediates in various metabolic processes and precursors of many biologically important nitrogen containing compounds notably heme, physiologically active amines, glutathione and nucleotide coenzymes.[5] They also act as chemical messengers in the communications between cells, and some of them function as detoxicating agents.[6] Many investigators reported that sulfur containing compounds have a radioprotective capacity, since a free SH group which acts as -OH scavenger or H-donating agent, reacts with primary lesions thus leading to a chemical restitution of the damaged site.[7]

Based on the foregoing, a symbiotic approach was utilized via the combination of the thienopyrimidinone, amino acid and mercapto moieties in one structural formula aiming to produce new compounds of possible biological and radioprotector activities.

MATERIAL AND METHODS

Melting points were taken on electrothermal melting point apparatus and are uncorrected. Microanalysis were performed by the Microanalytical Center, University of Cairo, Egypt. IR spectra were determined on a Pye Unicam SP 1000 spectrophotometer in KBr. ^{1}H-NMR was carried out using a JEOL FXQ 90 MHz NMR spectrometer. Mass spectra were run using HP MODEL: MS 5988.

Constants of the prepared compounds are given in Table I. The following illustrates the general preparation procedure.

A General Preparation Procedure

To a solution of the required amino acid (0.0025 mol) in a mixture of water (5 ml), dioxan (5 ml) and sodium hydroxide (1 M, 3 ml), ethyl-4,5,6,7-tetrahydro- 2 -isothiocyanato-1-benzothiophene-3-carboxylate **1**[8] (0.66 g; 0.0025 mol) was added and the mixture was stirred at 50°C. The volatile components

TABLE I Physicochemical data of the synthesized compounds 12–21[a].

Amino acid	*0.0025 mol*	*Time of reaction*[b]	*Compd. No.*	*m.p. °C*	*Cryst. Solvent*[c]	*Yield %*	*Mol. formula*	*Analysis % Required/(Found)*		
								C	*H*	*N*
Glycine	190 mg	5	12	>280	E-DMF	75	$C_{12}H_{12}N_2S_2O_3$	48.61 (48.70)	4.08 (4.00)	9.45 (9.60)
L-Alanine	220 mg	20	13	174	E	39	$C_{13}H_{14}N_2S_2O_3$	50.28 (50.20)	4.54 (4.60)	9.00 (9.10)
L-Phenylalanine	410 mg	20	14	142	H	78	$C_{19}H_{18}N_2S_2O_3$	59.03 (58.90)	4.69 (4.60)	7.25 (7.30)
L-Valine	290 mg	20	15	158	H	87	$C_{15}H_{18}N_2S_2O_3$	53.21 (53.10)	5.36 (5.20)	8.28 (8.20)
L-Leucine	320 mg	5	16	160	H	91	$C_{16}H_{20}N_2S_2O_3$	54.50 (54.40)	5.72 (5.60)	7.95 (7.90)
L-Isoleucine	320 mg	5	17	122	H	94	$C_{16}H_{20}N_2S_2O_3$	54.50 (54.60)	5.72 (5.60)	7.95 (8.10)
L-Serine	260 mg	20	18	152	E	31	$C_{13}H_{14}N_2S_2O_4$	47.82 (47.70)	4.32 (4.40)	8.58 (8.50)
L-Methionine	370 mg	20	19	162	H	77	$C_{15}H_{18}N_2S_3O_3$	48.60 (48.70)	4.90 (5.00)	7.56 (7.60)
L-Glutamic	370 mg	20	20	166	E	65	$C_{15}H_{16}N_2S_2O_5$	48.88 (48.80)	4.38 (4.30)	7.60 (7.70)
B-Alanine	220 mg	20	21	228	DMF	74	$C_{13}H_{14}N_2S_2O_3$	50.28 (50.20)	4.54 (4.60)	9.00 (9.10)

a) Prepared by the reaction of the amino acid with **1**.
b) Hour at 50°C.
c) E = ethanol; DMF = dimethylformamide; H = n-hexane.

were evaporated in vacuo, water was added to the residue and acidified with hydrochloric acid (10%) to pH **3**. The precipitate was collected by filtration and recrystallized from suitable solvent Table I.

B Biochemical Analysis

1 Materials

Male albino rats weighing 100–120 gm were divided into groups of four. Each of the tested compounds **12–21** was injected at different doses, (0.004, 0.04, 0.4, 1,2 mg/100 g body weight), dissolved in dimethylsulfoxide (DMSO) which was chosen as a solvent for its higher solubility and permeability effect, with the addition of its lower toxicity action at lower concentrations. All compounds showed an LD_{50} of more than 1000 mg/kg.[9] Three of the tested compounds **12**, **19** and **20** were selected to be tested as radioprotectors and injected, 30 minutes, before exposure to gamma irradiation in a dose of 1 mg/100 g body weight dissolved in 0.2 ml of DMSO.

2 Irradiation

The animals injected with three selected compounds were exposed to 4 Gy of gamma irradiation at dose rate 0.82 Gy/min. Irradiation process was performed by Gamma Cell-40 (^{137}Cs) achieved by Egypt's National Center for Radiation Research and Technology, NCRRT, Cairo.

3 Methods

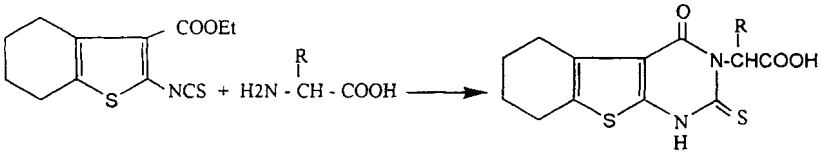
Triglycerides and phospholipids levels were determined in the serum of rats one day after injection intraperitoneally with the different doses of the tested compounds by enzymatic methods.[10,11]

Total protein and albumin were assayed colorimetrically, after one day of irradiation, using the method reported.[12,13] The data were statistically compared with corresponding values of control rats injected with 0.2 ml of DMSO.[14] Globulins are determined by substracting albumin from total protein.

RESULTS AND DISCUSSION

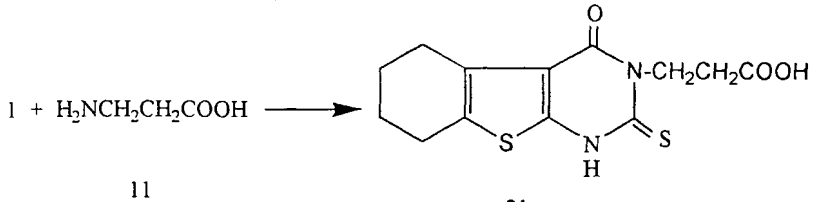
The compounds were designed in the aim of exploring their biological, biochemical and radioprotection efficiency (Scheme 1).

Hence, condensation of ethyl-4,5,6,7-tetrahydro-2-isothiocyanato-1-benzothiophene-3-carboxylate, **1**[8] with glycine **2** gave 2-thiooxo-3-carboxymethyl-



1 **2 - 10** **12 - 20**

	R
2, 12	H
3, 13	CH_3
4, 14	CH_2PH
5, 15	$CHMe_2$
6, 16	CH_2CHMe_2
7, 17	$CH(CH_3)\,CH_2CH_3$
8, 18	CH_2OH
9, 19	$(CH_2)_2SCH_3$
10, 20	$(CH_2)_2COOH$



11 21

Scheme 1

tetrahydrobenzeno[b]thieno [2,3-d] pyrimidine-4(3H) one **12**. The IR spectrum of **12** revealed the disappearance of the (-NCS) band and the presence of the (OH) band at 3500 cm^{-1}, (NH) at 3250 cm^{-1}, and two overlapping bands (2CO) at 1730–1700 cm^{-1}. In a similar way **1** reacted with L-alanine **3** to obtain the corresponding **13**; the IR spectrum showed the absence of the (-NCS) band. The mass spectrum of **13** showed the molecular ion peak at m/z 310 (11.14%), together with a base peak at 64 (100%), other significant peaks appeared at 225 (9.84%); 193 (16.84%), 179 (64.51%) and 91 (34.46%).

The reaction of **1** with L-phenylalanine **4**, L-valine **5**, L-leucine **6**, L-isoleucine **7** and L-serine **8** resulted in the formation of compounds **14**, **15**, **16**, **17** and **18** respectively. IR spectra of these compounds revealed the disappearance of the (-NCS) band characteristic for the parent compound **1**. Their structures were confirmed by elemental analysis and mass spectra which showed a molecular ion peaks m/z 386 and 352 for compounds **14** and **16**, respectively.

Upon reacting **1** with L-methionine **9** compound **19** was obtained. The IR spectrum revealed the presence of (OH) band at 3500 cm^{-1}, (NH) band at 3325 cm^{-1}, (CH) aliphatic at 2900 cm^{-1} and (2CO) at 1730, 1660 cm^{-1}. Mass spectrum of **19** showed a molecular ion peak m/z 369 (1.29%) together with a base peak at 58 (100%); other significant peaks appeared at 358 (2.27%), 322 (1.94%), 258 (4.53%), 242 (4.21%), 183 (2.59%), 128 (8.74%) and 88 (95.15%).

The reaction of **1** with glutamic acid **10** resulted in the formation of **20**. Structure elucidation of **20** was based on elemental analysis, ^{1}H-NMR spectrum in ($CDCl_3$) exhibited signals at 1.9, 2.4 [2s, 8H, $4CH_2$ cyclo], 3.9 [t, 2H, CH_2CO], 4.2 [t, 2H, CH_2-CH], 6.4 [s, 1H, NH, exchangeable D_2O], 7.7 [s, 1H, CH], 11.2 [s, 1H, C-COOH] and 12.2 ppm [s, 1H, CH_2-COOH].

Reaction of **1** with B-alanine **11**, resulted in the formation of the product **21**. IR revealed the absence of (-NCS) band and presence of (OH) at 3500 cm^{-1}, (NH) at 3280 cm^{-1}, (CH) aliphatic at 2920 cm^{-1} and (2CO) at 1710, 1690 cm^{-1}. ^{1}H-NMR spectrum of **21** in ($CDCl_3$) showed signals at 1.9, 2.4 [2s, 8H, $4CH_2$ cyclo], 3.9 [t, 2H, CH_2-CO], 4.2 [t, 2H, CH_2-N], 7.5 [s, 1H, NH-exchangeable-D_2O] and 11.2 ppm [s, 1H, OH].

Biological Screening

1 Antimicrobial activity

All the synthesized compounds were tested for their antimicrobial activity by the cup-diffusion technique.[15] Ampicillin and mycostatine were used as the reference compounds. Several microorganisms from stock cultures were used. *Bacillus cereus; Bacillus megaterium; Escherichia coli; Pseudomonas putida; Aspergillus niger* and *Penicillium janthinellum*. The compounds were dissolved in DMF, and the concentration of each one was 1 mg/1 ml.

The resulting inhibition zones were measured for six replicates and their means were determined. No inhibition zones were observed with DMF. Most of the tested compounds showed higher activity against all the tested organisms; this indicates that incorporation of the amino acid moiety and mercapto group with the thienopyrimidinone nucleus introduces a remarkable activity compared to ampicillin and mycostatine against the tested organisms. The results are given in Table II.

II Biochemical analysis

Since the present work has been designed to synthesize new compounds having some biological activity, it was necessary to undergo some of the preliminary biochemical analysis at different doses on serum lipids.

TABLE II Diameter of inhibition zones [mm] as a criterion of antimicrobial activity of the newly synthesized compounds at a concentration level of mg/ml.

Compd. No.	*B. cereus (MIC)*	*B. megaterium (MIC)*	*E. coli (MIC)*	*Ps. putida (MIC)*	*Asp. niger (MIC)*	*Pen. janth (MIC)*
12	–	–	8	8	10	8
13	–	–	–	10	8	8
14	8	12	12	15	10	8
15	10	8	10	8	10	10
16	10	12	12	15	8	10
17	12	12	12	–	8	10
18	–	–	–	8	8	8
19	–	10	12	15	12	8
20	–	8	–	8	8	8
21	–	–	8	8	10	10
DMF	–	–	–	–	–	–
Ampicillin	–	–	8	18	–	–
Mycostatine	–	–	–	–	8	8

Figure 1 revealed that all the tested compounds at the lower doses (0.004, 0.04, 0.4 mg/100 g body weight) did not show any significant changes in serum triglycerides, except for the compounds **12**, **18**, **19** and **20** which showed significant changes; while at dose of 1 mg, the compounds caused variable changes, only four compounds **13**, **15**, **19** and **20** showed significant decreases in serum

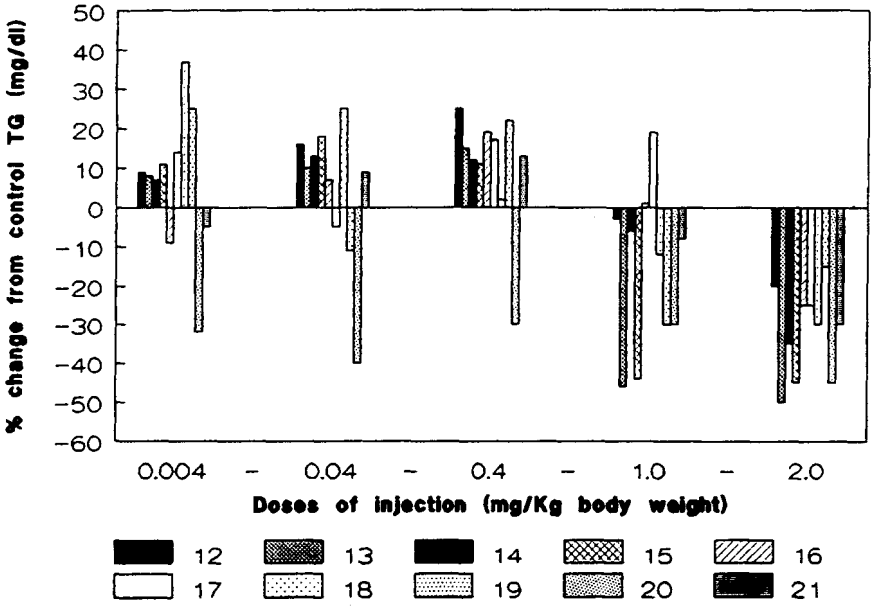


FIGURE 1 Effect of different doses of the synthesized compounds (12–21) on serum triglyceride.

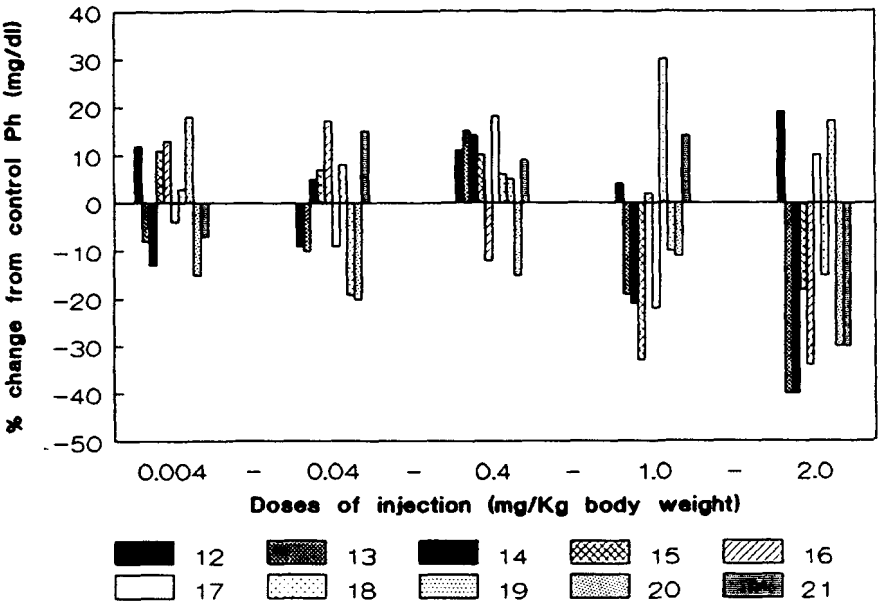


FIGURE 2 Effect of different doses of the synthesized compounds 12–21 on serum phospholipids.

triglycerides. It was also observed that the injection of 2 mg/100 g body weight of the tested compounds induced significant decreases.

Figure 2 revealed non-appreciable changes in serum phospholipids at lower concentration of the tested compounds. Only compound **18** caused a significant increase while compounds **14**, **15** and **17** showed a significant decreases at dose 1 mg/100 g body weight.

Significant decreases were observed after injection of 2 mg/100 g body weight of compounds **13**, **14**, **16**, **20** and **21**.

The data presented in Table III showed nonappreciable changes in serum total proteins, albumin and globulins when the tested compounds were injected alone.

Administration of the methionine **19** and glutamic **20** derivatives prior to gamma-irradiation showed values of serum albumin close to control values indicating partial radioprotection, while the glycine derivative **12** showed no protection.

The data peresented in Figure 1 and 2 revealed the ability of the synthesized compounds at large doses to reduce serum triglycerides and phospholipids. Furthermore the data presented in Table III indicated that irradiation induced a decrease in serum albumin and the administration of compound **12** before irradiation had no effect, while compounds **19** and **20** were the most effective compounds as radioprotectors on serum albumin.

TABLE III Effect of compounds 12, 19 and 20 on serum total protein (g/dL) of irradiated adult rats.

Compound No.	*Total protein*	*Albumin*	*Globulins*
		X ± S.D.	
Injected "DMSO"	7.51 ± 0.25	2.99 ± 0.26	4.52 ± 0.13
Radiat. rats	6.93 ± 0.43	2.33** ± 0.18	4.60 ± 0.53
DMSO + Radiat.	7.69 ± 0.25	2.53 ± 0.35	5.16 ± 0.50
Compd. **12** alone	7.54 ± 0.26	2.81 ± 0.33	4.72 ± 0.59
Compd. **12** + Radiat.	7.33 ± 0.26	2.00** ± 0.22	5.30* ± 0.48
Compd. **19** alone	7.60 ± 0.22	3.05 ± 0.21	4.55 ± 0.40
Compd. **19** + Radiat.	7.71 ± 0.23	2.70 ± 0.26	5.00 ± 0.49
Compd. **20** alone	7.71 ± 0.23	3.14 ± 0.32	4.57 ± 0.39
Compd. **20** + Radiat.	7.32 ± 0.25	3.10 ± 0.29	4.42 ± 0.54

Each value represents mean (g/dL) ± S.D. of 4 rats.
*Significantly different from control (injected DMSO) at $p < 0.05$
**Significantly different from control (injected DMSO) at $p < 0.01$

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