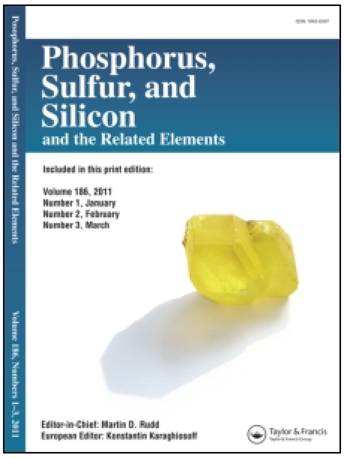

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Synthesis and Reactions of New Fused Heterocycles Derived from 5-Substituted-4-Amino-3-Mercapto-(4H)-1,2,4-Triazole with Biological Interest

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Synthesis and Reactions of New Fused Heterocycles Derived from 5-Substituted-4-Amino-3-Mercapto-(4H)-1,2,4-Triazole with Biological Interest

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The reaction of thiocarbohydrazide with carboxylic acids at the melting temperature allows an improved preparation of 5-substituted-4-amino-3-mercapto 1,2,4-triazoles $\mathbf{1}_{a-g}$. *Compound* $\mathbf{1}_a$ *reacted with 2-bromopropionic acid to give acid derivative* **2**. *The latter was reacted with a mixture of acetic anhydride and triethylamine to afford the mesoionic compound* **3**. *Heating of compound* **3** *in ethanol gave the ester derivative* **4**, *which on alkaline hydrolysis in methanol gave ketone derivative* **5**. *Substituted 1,2,4-triazolo [3,4-b]-6H-1,3,4-thiadiazine* $\mathbf{6}_{h,i}$ *and* **7** *were synthesized by reaction of* $\mathbf{1}_a$ *with acetylacetone, ethyl acetoacetate and chloroacetamide. Heterocyclic systems* **8** *and* **9** *were prepared through the reaction of* $\mathbf{1}_a$ *with 2,3-dichloro-1,4-naphthoquinone and 2,3-dichloroquinoxaline. In addition, thenoyl isothiocyanate, thenoyl chloride, 2-thiophenecarbaldehyde, and p-chlorophenyl isocyanate reacted with compound* $\mathbf{1}_a$ *to afford 1,2,4-triazolo[3,4-b]-1,3,4-thiadiazole ring system* **10**, **11**, *and urea derivative* **12**. *1,2,4-Triazolo[3,4-b]-5H-pyrazole derivatives* $\mathbf{14}_{j,k}$ *were prepared through the reaction of compound* $\mathbf{1}_a$ *with 3-chloro-2,4-pentandione and ethyl-2-chloroacetoacetate. Compound* $\mathbf{14}_j$ *was treated with hydrazine to afford products* **15**, **16**, *and* **17** *depending on the type of hydrazine derivative and reaction conditions. Compound* **19** *was synthesized by refluxing of compound* $\mathbf{14}_j$ *with hydroxylamine hydrochloride to afford the corresponding oxime derivative* **18** *followed by treatment with thenoyl chloride.*

Keywords Pyrazoles; 5-substituted-4-amino-3-mercapto-(4H)-1,2,4-triazole; 1,3,4-thiadiazines; 1,3,4-thiadiazoles

INTRODUCTION

Heterocyclic condensed derivatives posses pharmacological properties such as analgesic, antibacterial,[1] antitumor,[2] antineoplastic agent,[3] and radioprotective activities.[4] 1,2,4-Triazolo[3,4-b]-1,3,4-thiadiazole, 1,2,4-triazolo [3,4-b]-6H-1,3,4-thiadiazine, and 1,2,4-triazolo [3,4-b]

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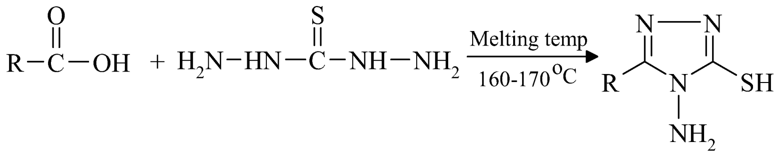
pyrazole derivatives represent a class of compounds hitherto scarcely studied. From a biological point of view, it has been reported that 1,2,4-triazolo [3,4-b]-1,3,4-thiadiazole derivatives possess antibacterial[5,7] and antiinflammatory activities[8] and interesting CNS-depressant actions.[9] Previously, the preparation and pharmacological evaluation of some 6-substituted-3-(pyridine-4-yl)-1,2,4-triazolo [3,4-b]-1,3,4-thiadiaz-oles and 1,2,4-triazolo [3,4-b]-1,3,4-thiadiazine have been reported, with some of them exhibiting antitumor activities.[10–12]

RESULTS AND DISCUSSION

The reaction of thiocarbohydrazide with carboxylic acids at melting temperature resulted in the formation of 5-substituted-4-amino-3-mercapto-1,2,4-triazole heterocycles.[13] Thus, when equimolar amounts of carboxylic acids and thiocarbohydrazide were mixed together and warmed at the melting temperature for 15–60 min, the corresponding 5-substituted-4-amino-3-mercapto-1,2,4-triazoles **1**$_{\mathbf{a-g}}$ were obtained in good to excellent yield (71–96%; Scheme 1, Table I). The Heindel and Reid synthesis[14] was used as a second method for preparation of **1**$_{\mathbf{a}}$. This methodology involved the condensation of a carboxylic acid hydrazide with carbon disulfide and potassium hydroxide to yield potassium dithiocarbazates salt, which was converted to compound **1**$_{\mathbf{a}}$ by reaction with an excess of hydrazine. Melting point depression was not observed in a mixed melting point experimental with an authentic sample from the first method.

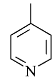
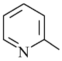
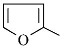
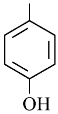
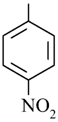
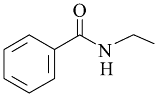
4-Amino-3-mercapto-(4H)-1,2,4-triazole derivatives have been widely utilized as starting materials for the preparation of heterocyclic compounds[15] due to the presence of two adjacent reactive functional groups as reported for similar compounds,[16–20] and this aminothiol derivative proved to be a versatile intermediate for the preparation of bridgehead nitrogen heterocycles. The aminothiol derivative **1**$_{\mathbf{a}}$ reacted in benzene at room temperature in the presence of triethylamine with 2-bromopropionic acid to give the acid derivative **2**. This derivative



1$_{\mathbf{a\text{-}g}}$

SCHEME 1 **1a** = C_5H_4N, **1b** = C_5H_4N, **1c** = C_4H_3O, **1d** = C_6H_4-OH-P, **1e** = C_6H_4-NO_2-P, **1f** = C_6H_5CO NH CH_2 CH_3; **1g** = CH_2BrCH_2.

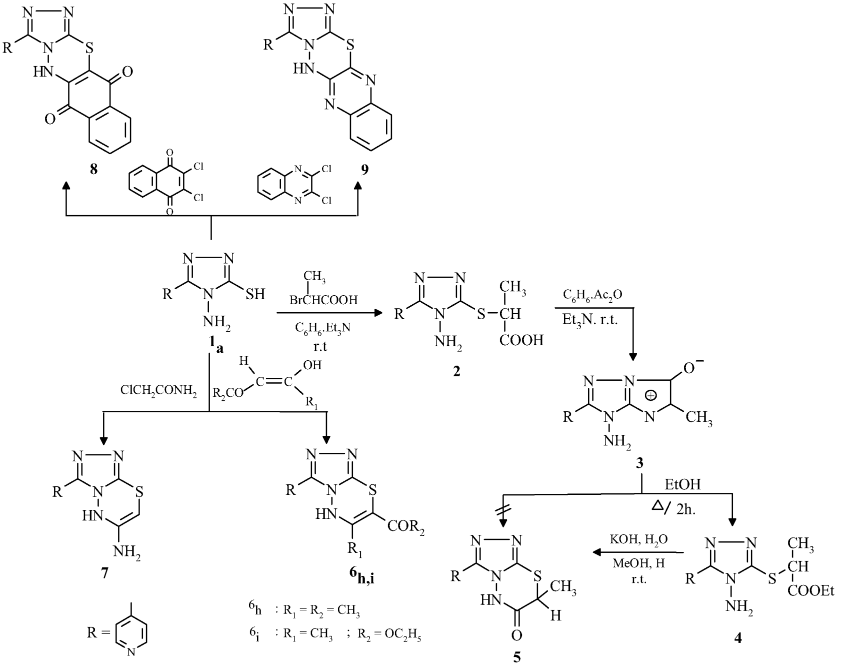
TABLE I Characterization of the Newly Synthesized Compounds

Comp. no.	R	Mp (°C)	Yield (%)	Molecular formula	Analysis % Calcd./Found C	H	N	S
1$_a$		280	96	$C_7H_7N_5S$	43.52	3.62	36.26	16.58
				193	43.10	3.70	36.40	16.30
1$_b$		220	72	$C_7H_7N_5S$	43.52	3.62	36.26	16.58
				193	43.50	3.13	36.40	16.72
1$_c$		>360	70	$C_6H_6N_4OS$	39.56	3.29	30.76	17.58
				182	39.40	3.11	30.81	17.50
1$_d$		220	80	$C_8H_8N_4OS$	46.15	3.84	26.92	15.38
				208	46.00	4.01	26.90	15.11
1$_e$		210	85	$C_8H_7N_5O_2S$	40.50	2.95	29.53	13.50
				237	40.22	3.01	29.20	13.00
1$_f$		280	75	$C_{10}H_{11}N_5OS$	48.19	4.41	28.11	12.85
				249	48.00	4.40	28.00	12.60
*$\mathbf{1}_g$	CH_2BrCH_2–	>360	75	$C_4H_7BrN_4S$	21.52	3.13	25.11	14.35
				222.98	21.60	3.00	25.00	14.10

* 1_g: Br: Calcd 35.86; Found: 36.01.

2 was stirred at room temperature in benzene with a mixture of acetic anhydride and triethylamine (V/V-1:1)[21] and cyclized to give the mesoionic compound **3**. This mesoionic compound was isolated with a high degree of purity. Prolonged heating in ethanol of the compound **3** gave the ethyl ester **4**. The ketone derivative **5** was only obtained from the methanolic alkaline hydrolysis of **4**. According to their synthetic route, the reaction of the aminothiol derivative **1**$_a$ with the appropriate halo-ester gave a product with a γ -lactam structure.[22,23] The ^{1}H-NMR spectrum of compound **5** showed the presence of an amidic hydrogen as a signal at δ 12.50 ppm; in the IR spectrum, the presence of an amidic hydrogen is confirmed by a broad signal at 3210 cm^{-1}.

Interaction of **1**$_a$ with acetylacetone or ethyl acetoacetate using DMSO (dimethyl sulphoxide) as medium caused cyclization to give substituted 1,2,4-triazole [3,4-b]-6H-1,3-4-thiadiazine **6**$_{h,i}$. The IR spectra of compounds $\mathbf{6_{h,i}}$ showed the absence of an absorption band corresponding to the amino group and appearance of a new band corresponding to NH group and ^{1}H-NMR spectra is in agreement with the proposed structure (Table II). Interaction of $\mathbf{1_a}$ with chloroacetamide either by fusion or using dioxan as solvent and triethylamine as catalyst resulted in the formation of 5-amino-8-(pyridine-4-yl)-1,2,4-triazolo[3,4-b]-4H, 6H-1,3,4-thiadiazine 7 (Scheme 2).



SCHEME 2

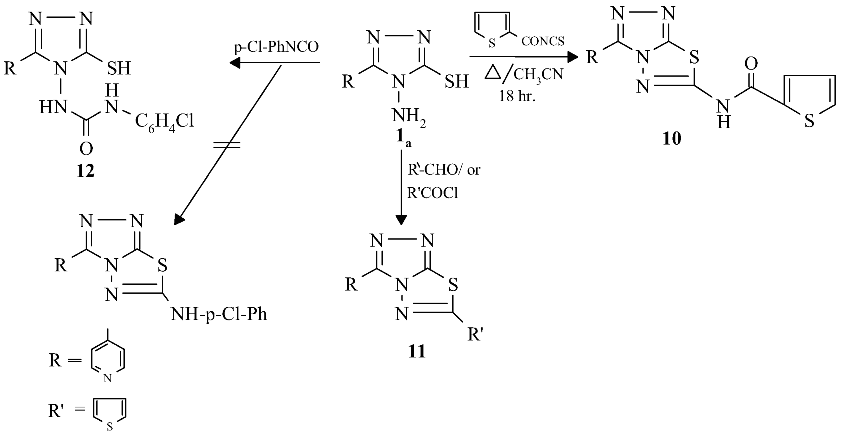
On the other hand, treatment of compound $\mathbf{1_a}$ with 2,3-dichloro-1,4-naphthoquinone and 2,3-dichloroquinoxaline afforded the corresponding naphthoquinon-1,3,4-thiadiazine derivative **8** and quinoxaline-1,3,4-thiadiazine derivative **9** (Scheme 2). The reaction pattern is similar to some known reactions of 2,3-dichloro compounds with binucleophiles such as o-aminothiophenol.[24]

Thenoyl isothiocyanate[25] reacted with 4-amino-5-mercapto-1,2,4-triazole derivative $\mathbf{1_a}$ to give N-[7-(pyridine-4-yl)-1,2,4-triazolo [3,4-b] 1,3,4-thiadiazole-4-yl]-thiopheneamide **10**. The IR spectrum of compound **10** showed the absence of absorption band corresponding to the amino group and appearance of a new band corresponding to NH at 3280 cm^{-1} and ^{1}H-NMR spectrum is in agreement with the proposed

TABLE II Characterization Data of the Newly Synthesized Compounds

Comp. No.	mp (°C)	Yield %	Mol. formula M. W	% Analysis: Calcd/Found C	H	N	S
2	230	82	$C_{10}H_{11}N_5O_2S$	45.28	4.15	26.41	12.07
			265	45.00	4.30	26.60	12.00
3	265	40	$C_{10}H_9N_5OS$	48.58	3.64	28.34	12.95
			247	48.60	3.30	28.11	13.00
4	220	70	$C_{12}H_{15}N_5O_2S$	49.14	5.11	23.89	10.92
			293	49.00	5.15	24.00	11.00
5	>360	50	$C_{10}H_9N_5OS$	48.58	3.64	28.34	12.95
			247	48.60	3.30	28.00	13.01
$\mathbf{6_h}$	200	72	$C_{12}H_{11}N_5OS$	52.74	4.02	25.64	11.72
			273	52.60	4.00	25.50	11.80
$\mathbf{6_i}$	220	65	$C_{13}H_{13}N_5O_2S$	51.48	4.29	23.10	10.56
			303	51.60	4.50	23.00	10.80
7	230	55	$C_9H_8N_6S$ 232	46.55	3.44	36.20	13.79
				46.60	3.33	36.00	14.00
8	210	85	$C_{17}H_9N_5O_2S$	58.78	2.59	20.17	9.22
			347	58.80	2.72	20.00	9.50
9	>360	80	$C_{15}H_9N_7S$ 319	56.42	2.82	30.72	10.03
				56.30	2.70	30.14	10.00
10	260	73	$C_{13}H_8N_6OS_2$	47.56	2.43	25.60	19.51
			328	47.60	2.20	25.61	20.00
11	>360	60	$C_{12}H_7N_5S_2$ 285	50.52	2.45	24.56	22.45
				50.50	2.11	24.60	22.60
12*	240	65	$C_{14}H_{11}ClN_6OS$	48.48	3.17	24.24	9.23
			346.5	48.50	3.20	24.30	9.00
$\mathbf{14_j}$	160	72	$C_{12}H_{11}N_5O$ 241	59.75	4.56	29.04	—
				59.22	4.18	29.18	
$\mathbf{14_k}$	130	40	$C_{13}H_{13}N_5O_2$	57.56	4.79	25.83	—
			271	57.60	4.88	25.55	
15	180	55	$C_{12}H_{15}N_7O$ 273	52.74	5.49	35.89	—
				52.70	5.88	35.60	
16	>360	80	$C_{24}H_{22}N_{12}$ 478	60.25	4.60	35.14	—
				60.00	4.18	35.00	
17*	190	80	$C_{18}H_{16}ClN_7$	59.09	4.37	26.81	—
			365.5	59.00	4.30	26.80	
18	>360	95	$C_{12}H_{12}N_6O$ 256	56.25	4.68	32.81	—
				56.20	4.40	32.60	
19	110	75	$C_{17}H_{14}N_6O_2S$	55.73	3.82	22.95	8.74
			366	55.70	3.65	22.80	8.19

12*: Cl: Calcd. 10.24; Found: 10.11.
17*: Cl: Calcd: 9.71; Found: 9.80.

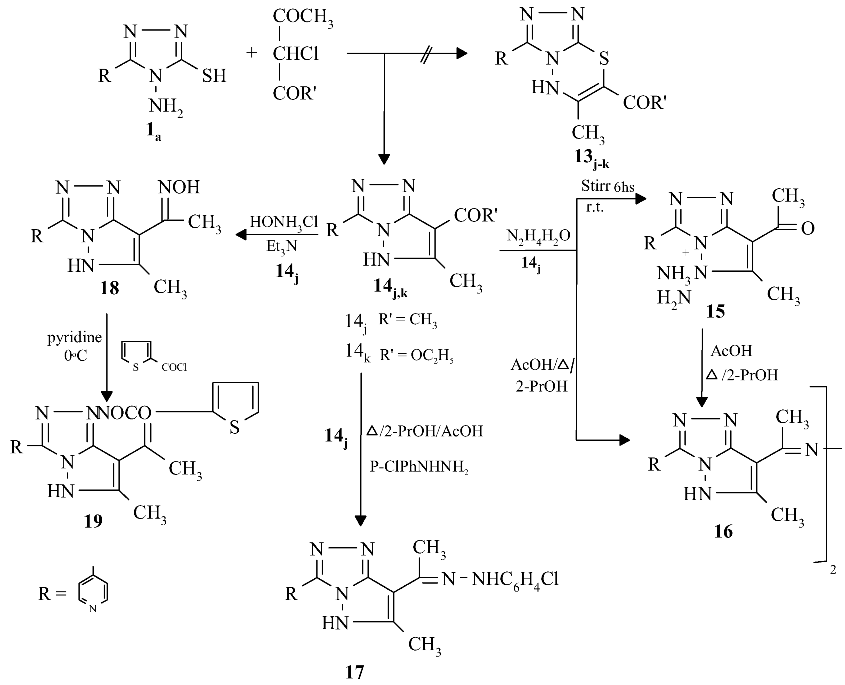


SCHEME 3

structure (Table II, Scheme 3). In contrast, *p*−chlorophenyl isocyanate reacted with 4-amino-5-mercapto-1,2,4-triazole derivative $\mathbf{1}_{\mathbf{a}}^{26}$ to give 5-mercapto 1,2,4-triazole derivative **12**. The IR spectrum of compound **12** revealed the appearance of a CO signal at 1708 cm^{-1}. The behavior of $\mathbf{1_a}$ toward 2-thiophenecarbaldehyde and thenoyl chloride was investigated. Thus, interaction of $\mathbf{1_a}$ with 2-thiophenecarbaldehyde using AcOH as medium[27] caused cyclization to give 7-(pyridine-4-yl)-4-thiophene-1,2,4-triazolo[3,4-b] 1,3,4-thiadiazole **11**. Compound **11** could be also prepared through ring closure reaction between $\mathbf{1_a}$ and thenoyl chloride.[28] The reaction included elimination of one mole of each HCl and H_2O to give **11** (mp, mmp, and TLC; Scheme 3).

In an attempt to synthesize fused 1,3,4-thiadiazine ring with 1,2,4-triazole, I studied the reaction of 5-substituted-4-amino-3-mercapto-(4H)-1,2,4-triazole $\mathbf{1_a}$ with halogenated 1,3-dicarbonyl active methylene compounds. The products from this reaction were shown to be 1,2,4-triazolo[3,4-b] pyrazole derivatives $\mathbf{14_{j-k}}$ rather than the expected 1,2,4-triazolo[3,4-b] 1,3,4-thiadiazine derivative $\mathbf{13_{j,k}}$ (Scheme 4).

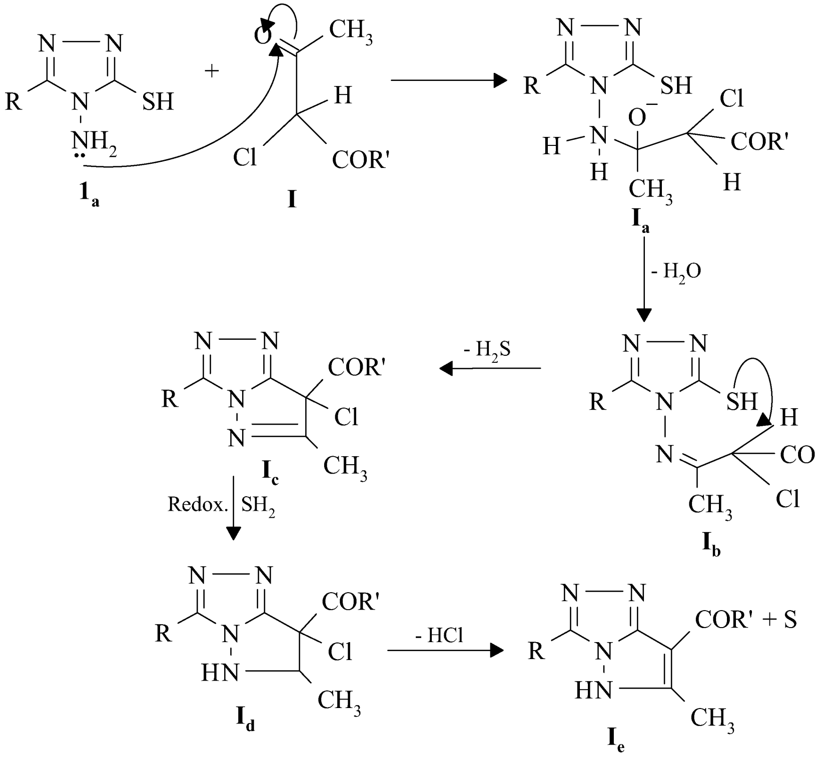
In the IR, ^{1}H-NMR, and ^{13}C-NMR spectra no observable differences are found capable of differentiating between compounds $\mathbf{14_{j,k}}$ and $\mathbf{13_{j,k}}$. The only differences capable of differentiating between them were observed in their mass spectra, which showed the existence of the molecular ion peaks M^+ at m/z 241 and 271 corresponding to $\mathbf{14_j}$ and $\mathbf{14_k}$, respectively, and the elemental microanalysis of these compounds for S gave 0%. The above results confirm that triazolo[3,4-b]-pyrazole structures $\mathbf{14_{j,k}}$ were formed and not the expected 1,2,4-triazolo[3,4-b] 1,3,4-thiadiazine. The mechanism for the formation of compounds $\mathbf{14_{j,k}}$ is



SCHEME 4

postulated as shown in Scheme 5. It proceeds by nucleophilic attack of the amino group of compounds $\mathbf{1_a}$ on the carbonyl group of active methylene compound **I** to give $\mathbf{I_a}$, followed by elimination of water from $\mathbf{I_a}$ to give $\mathbf{I_b}$, which subsequently undergoes internal nucleophilic attack accompanied by the elimination of hydrogen sulfide to give $\mathbf{I_c}$. Compound $\mathbf{I_c}$ undergoes a redox reaction with eliminated hydrogen sulfide to afford $\mathbf{I_d}$ and elemental sulfur. Elimination of hydrogen chloride from I_d afforded the 1,2,4-triazolo [3,4-b] pyrazole derivative $\mathbf{I_e}$. Compounds $\mathbf{14_{j,k}}$ were prepared directly in good yield by the reaction of $\mathbf{1_a}$ with 3-chloro-2,4-pentandione and/or ethyl-2-chloroaceto-acetate in refluxing ethanol (Scheme 4).

Treatment of $\mathbf{14_j}$ with hydrazine hydrate in 2-propanol at room temperature afforded the hydrazinium salt **15**, which upon refluxing in 2-propanol in the presence of a few drops of acetic acid furnished N,N′ -bis[4-methyl-7-pyridine-4-yl] 1,2,4-triazolo[3,2-b]-3-yl-ethylidene) hydrazine-5H-pyrazole **16**. The mass spectrum of compound **16** showed a molecular ion peak M^+ at 478 m/z and the base peak (1/2 M^+) at m/z 239, which is strong evidence for the structure



SCHEME 5

of **16**. Compound **16** was prepared directly by the reaction of $\mathbf{14_j}$ with hydrazine hydrate in refluxing 2-propanol in the presence of a few drops of acetic acid. The ^{1}H-NMR spectrum of compound **15** showed the presence of a broad singlet signal (five protons) at δ 6.90 ppm corresponding to the hydrazinium ion ($N_2H_5^+$). The reaction of $\mathbf{14_j}$ with *p*-chlorophenyl hydrazine in the presence of a few drops of acetic acid in refluxing 2-propanol yielded the corresponding hydrazono derivative **17**. The ^{1}H-NMR spectrum of compound **17** showed the presence of a singlet corresponding to the NH-hydrazono at δ 9.17 ppm and singlet corresponding to pyrazolo NH at δ 13.00 ppm (Table III). The mass spectrum of compound **17** showed a molecular ion peak M^+ at 364 m/z.

Condensation of $\mathbf{14_j}$ with hydroxylamine hydrochloride in refluxing 2-propanol in the presence of triethylamine afforded the corresponding oxime derivative **18**, which was reacted with thenoyl chloride in anhydrous pyridine at 0°C to give the corresponding 3-(1-thenoylimino

TABLE III IR, ^{1}H NMR, ^{13}C NMR, and Mass Spectral Data

Compd. no.	IR (cm^{-1})	^{1}H-NMR δ (ppm)/^{13}C-NMR, δ (ppm)/ms
$\mathbf{1_a}$	3320, 3260 (NH_2), 3053 (CHAr), 2562 (SH), 1639 (C=N)	5.50 (s, 2H, NH_2), 7.25–7.69 (m, 4H, pyridine-H), 12.80 (br, 1H, SH); m/z = 193 (10.65%)
$\mathbf{1_b}$	3300, 3280 (NH_2), 3055 (CHAr), 2500 (SH), 1640 (C=N)	5.20 (s, 2H, NH_2), 7.25–7.70 (m, 4H, pyridine-H), 13.01 (br, 1H, SH)
$\mathbf{1_c}$	3310, 3270 (NH_2), 3040 (CHAr), 2530 (SH), 1630 (C=N)	5.00 (s, 2H, NH_2), 7.23, 7.40 (2d, 2H, furan-H, J = 5.80 Hz), 7.66 (s, 1H, furan-H), 13.00 (br, 1H, SH)
$\mathbf{1_d}$	4495 (OH), 3320 (NH_2), 3033 (CHAr), 2500 (SH), 1610 (C=N)	12.10 (s, 1H, OH), 7.22, 7.56 (2d, 4H, phenyl-H) AA′ XX′ system J = 8.44 Hz), 13.04 (br, 1H, SH)
$\mathbf{1_e}$	3340 (NH_2), 3080 (CHAr), 2560 (SH), 1523 (NO_2), 1620 (C=N)	4.80 (s, 2H, NH_2) 7.44, 7.68 (2d, 4H, AA′ XX′ system, J = 10.22 Hz), 13.37 (s, 1H, SH)
$\mathbf{1_f}$	3300, 3240 (NH, NH_2), 3030 (CHAr), 2900 (CH aliph.), 2560 (SH), 1690 (C=O), 1620 (C=N)	4.11 (s, 2H, CH_2), 5.80 (s, 2H, NH_2), 7.22–7.80 (m, 4H-phenyl-H), 10.08 (br, s, 1H, NH), 13.55 (s, 1H, SH)
$\mathbf{1_g}$	3310 (NH_2), 2820–2900 (CH aliph.), 2670 (SH), 1620 (C=N)	2.94 (m, 4H, CH_2–CH_2), 5.57 (s, 2H, NH_2), 13.46 (br.s, 1H, SH)
2	3400 (OH), 3300 (NH_2), 1695 (C=O), 3063 (CH–pyridine–H), 1635 (C=N)	1.30 (d, 3H, CH_3, J = 8.40 Hz), 4.15 (q, 1H, CH, J= 7.00 Hz), 5.70 (s, 2H, NH_2), 12.11 (br.s, 1H, OH)
3	3250, 3180 (NH_2), 3045 (CHAr), 2912 (CH aliph.), 1695 (C–O^-), 1640 (C=N)	1.40 (s, 3H, CH_3), 6.11 (s, 2H, NH_2) 7.20–7.53 (m 4H, pyridine–H); ms: m/z = 247 (50% M^+)
4	3330, 3240 (NH_2), 3055 (CHAr), 2888–2930 (CH aliph.), 1730 (C=O), 1635 (C=N)	1.41 (t, 3H, CH_2CH_3, J = 7.80 Hz), 1.62 (d, 3H, CH_3, J = 8.91 Hz), 4.48 (q, 1H, C–H, J = 7.20 Hz), 4.75 (q, 2H, CH_2CH_3, J = 9.80 Hz), 6.22 (s, 2H, NH_2), 7.22–7.56 (m, 4H, pyridine–H)
5	3210 (NH), 3030 (CHAr), 2888 (CH aliph), 1700 (C=O), 1630 (C=N)	1.40 (d, 3H, CH_3, J = 7.50 Hz), 4.30 (q, 1H, CH, J = 9.00 Hz), 7.30–7.52 (m, 4H, pyridine–H), 12.50 (br s, 1H, NH); ms: m/z 247 (11.13% M^+)
$\mathbf{6_h}$	3230 (NH), 3060 (CHAr) 2940 (CH aliph), 1710 (C=O), 1640 (C=N), 1590 (C=C)	2.30 (s, 3H, CH_3), 2.60 (s, 3H, $COCH_3$) 7.22–7.36 (m, 4H, pyridine–H), 12.50 (s, 1H, NH); ms: m/z = 273 (22.40% M^+)
$\mathbf{6_i}$	3280 (NH), 3080 (CHAr), 2777–2910 (CH-aliph.), 1725 (C=O), 1635 (C=N), 1590 (C=C)	1.33 (t, 3H, CH_2CH_3, J = 8.11 Hz), 1.82 (s, 3H, CH_3), 4.80 (q, 2H, CH_2CH_3, J = 10.01 Hz), 7.30–7.52 (m, 4H, pyridine–H), 13.11 (s, 1H, NH); ms: m/z = 303 (18.07)

(Continued on next page)

TABLE III **(***Continued)*

Compd. no.	IR (cm^{-1})	^{1}H-NMR δ (ppm)/^{13}C-NMR, δ (ppm)/ms
7	3380, 3260, 3210 (NH, NH$_2$), 3062 (CHAr), 1630 (C=N), 1580 (C=C)	5.44 (s, 1H, NH$_2$), 7.22–7.58 (m, 4H, pyridine–H), 8.24 (s, 1H, CH), 12.08 (s, 1H, NH); ms: m/z = 232 (36.18% M$^+$)
8	3275 (NH), 3055 (CHAr), 1680 (2C=O), 1640 (C=N), 1570 (C=C)	7.22–7.86 (m, 8H, Ar–H), 10.01 (s, 1H, NH); ^{13}C:179.9 (C=O),178.6 (C=O), 153.50, 153.90, 154.20, 155.00, 158.20, 159.00, 160.70
9	3210 (NH), 3056(CHAr), 1630 (C=N), 1600 (C=C)	7.28–7.88 (m, 8H, Ar–H), 12.05 (s, 1H, NH); ms: m/z 305 (15.11M$^+$)
10	3280 (NH), 3055 (CHAr), 1710 (C=O), 1640 (C=N)	7.22–7.52 (m, 5H, Ar–H), 7.63 (d, 1H, Ar–H, J = 5.15 Hz), 8.10 (d, 1H, Ar–H, J = 6.02 Hz), 13.11 (br, s, 1H, NH); ms: m/z = 328 (28.17% M$^+$)
11	3088 (CHAr), 1640 (C=N)	7.24–7.43 (m, 5H. Ar–H), 7.60 (d, 1H, J= 8.00Hz), 7.98 (d, 1H, J = 7.99 Hz); ms: m/z=285 (32.00% M$^+$).
12	3281(NH), 3045 (CHAr), 1708 (C=O), 1635 (C=N)	7.63, 8.60 (2d, 4H, p-Cl phenyl, AA′ XX′ , J = 8.22 Hz), 7.22–7.51 (m, 4H, pyridine–H), 9.93 (br, s, 1H, NH), 10.04 (br, s, 1H, NH); ms: m/z = 346 (25.00% M$^+$)
14$_j$	3389 (NH), 3050 (CHAr), 2920 (CH aliph), 1710 (COCH$_3$), 1644 (C=N), 1560 (C=C)	2.30 (s, 3H, CH$_3$), 2.60 (s, 3H, COCH$_3$), 7.22–7.54 (m, 4H, pyridine–H), 13.80 (s, 1H, NH); ^{13}C: 15.20 (CH$_3$), 29.50 (COCH$_3$), 139.90, 140.22, 141.00, 146.08, and 168 (C=O); ms: m/z = 241 (11.80 M$^+$)
14$_k$	3400 (NH), 3055 (CHAr), 2887–2900 (CH-aliph.), 1730 (C=O) ester, 1640 (C=N), 1590 (C=C)	2.33 (s, 3H, CH$_3$), 1.30 (t, 3H, CH$_3$CH$_2$, J = 11.01 Hz), 4.30 (q, 2H, CH$_2$CH$_3$, J = 9.88 Hz) 12.80 (s, 1H, NH); ^{13}C: 14.0 (CH$_3$CH$_2$), 16.30 (CH$_3$), 60.60 (OCH$_2$), 162.50 (C=O), 139.90, 140.22, 141.00, 146.08. ms: m/z = 271 (30.08% M$^+$)
15	3425, 3247 (NH$_2$), 1711 (C=O), 1631 (C=N), 1541 (C=C)	2.50 (s, 3H, CH$_3$), 2.6 (s, 3H, COCH$_3$), 6.90 (br S, 5H, hydrazinium–Hs), 7.20–7.36 (m, 4H, pyridine–H);^{13}C: 16.40 (CH$_3$), 29.50 (COCH$_3$), 139.50, 149.81, 153.11, 155.70, 191.80 (C=O)
16	3278 (NH), 3055 (CHAr), 2995 (CH aliph), 1570 (C=C), 1640 (C=N)	2.1 (s, 3H, CH$_3$), 2.50 (s, 3H, hydrazono-CH$_3$), 7.22–7.77 (m, 8H, pyridine–H); ms: m/z = 478 (8.12% M$^+$) and base peak at m/z = 239 (50%)

(Continued on next page)

TABLE III (*Continued*)

Compd. no.	IR (cm^{-1})	^{1}H-NMR δ (ppm)/^{13}C-NMR, δ (ppm)/ms
17	3437, 3318 (2NH), 2900(CH aliph.) 1635 (C=N), 1599 (C=C)	2.30 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 7.22–7.50 (m, 8H, Ar-H) 7.82, 8.00 (2d, 4H, AA′ XX′ - system J = 8.10 Hz), 9.17 (s, 1H, hydrazono-NH), 13.0 (s, 1H, pyrazoloe NH), ^{13}C: 14.70 (CH_3), 16.10 (hydrazono–CH_3), 113.90, 119.90, 130.10, 137.44, 138.80, 139.18, 140.00, 141.33, 141.90 (C=N–NH); ms: m/z = 364 (17.02% M^+)
18	3227 (NH), 3042 (CHAr), 2940 (CH aliph.), 1636 (C=N), 1558 (C=C)	2.00 (s, 3H, CH_3), 2.20 (s, 3H, CH_3–C=NOH), 10.90 (s, 1H, OH); ^{13}C: 14.62 (CH_3), 28.11(CH3) 127.70, 129.44, 129.99, 136.50, 142.60
19	3461 (NH), 2990 (CH aliph.), 1713 (C=O), 1609 (C=N), 1543 (C=C)	2.3 (s, 3H, CH_3), 2.6(s, 3H, CH_3–C=N–$OCOC_4H_3$), 7.60–8.13 (m, 7H, Ar–H), 13.00 (s, 1H, NH-pyrazole). ms: m/z = 366 (18.02 M^+)

ethyl)-4-methyl-7-(pyridine-4-yl)-1,2,4-triazolo [3,2-b]-5H-pyrazole **19** through protection of the OH group. A singlet corresponding to the OH proton at δ 10.90 ppm was observed in the ^{1}H-NMR spectrum of compound **18**. The ^{1}H-NMR spectrum of compound **19** shows that the OH group is no longer present, and the singlet at δ 13.00 ppm shows that the NH group is present, which confirmed that protection occurred on the OH group, not the NH group.

Experimental

Melting points were recorded on a Gallen Kamp Melting apparatus and are uncorrected. Infrared spectra were obtained on a Nexus 470-670, and ^{1}H-NMR spectra and ^{13}C-NMR were run on JEOL-400 MHz spectrometer using DMSO-d_6 as a solvent and TMS as an internal standard. The mass spectra were recorded on Ms-S988 operating at 70eV. Microanalysis was performed using a Perkin-Elmer 2400 CHN analyzer. The newly synthesized compounds were screened for cytotoxic activity (*in vitro* study) and antitumor activity (*in vitro* study) at Cairo University, National Cancer Institute, Cancer Biology Department, Pharmacology Unit.

General Procedure for the Preparation of 5-Substituted-4-Amino-3-Mercapto-(4H)-1,2,4-Triazoles 1_{a-h}

A mixture of thiocarbohdyrazide (0.1 mmol) and the appropriate carboxylic acid (0.1 mmol) was warmed carefully at 160–170°C until melting occurred,[29] and then it was warmed again after melting occurred for 15 min. The reaction mixture was cooled, mixed with water (80 mL), and acidified with concentrated hydrochloric acid (2 mL). The precipitate was filtered, washed with water, oven-dried, and recrystallized from ethanol.

2-[(1-Amino-5-(Pyridine-4-yl)-1,2,4-Triazole-2-yl) Thio]-Propionic Acid 2

A mixture of aminothiole derivative $\mathbf{1_a}$ (1.8 mmol), 2-bromopropionic acid (1.8 mmol) and triethylamine (0.25 mL) in benzene (30 mL) was stirred at room temperature for 30 h. After filtration, the resulting solution was extracted with a sodium bicarbonate 5% solution (30 mL). The aqueous solution was acidified with drop-wise addition of hydrochloric acid to pH 5-6; the solid was collected, washed with water, dried, and recrystallized from ethanol to give **2**.

1-Amino-5-Hydroxy-6-Methyl-2-(Pyridine-4-yl)-Thiazolo [3,2-b]-1,2,4-Triazolium Inner Salt 3

A mixture of acetic anhydride (0.5 mL) and triethylamine (0.5 mL) was added to a stirred suspension of the acid derivative **2** (0.43 mmol) in benzene (2 mL); the mixture was stirred at room temperature for 2 h. The yellow resulting mixture was diluted with diethyl ether (10 mL) and the solid collected, washed with diethyl ether, and dried to give **3** and recrystallized from dioxan/benzene.

Ethyl Ester of 2-[(1-Amino-5-(Pyridine-4-yl) 1,2,4-Triazole-2-yl) Thio-Propionic Acid 4

The mesoionic compound **3** (35 mg) in ethanol (25 mL) was heated at reflux for 30 min. The solution was concentrated under vacuum and the residue was collected, washed with methanol, dried, and recrystallized from ethanol to give **4**.

4-Methyl-4H,6H-8-(Pyridine-4-yl)-1,2,4-Triazolo [3,4-b]-1,3,4-Thiadiazine-5-one 5

A suspension of ethyl ester **4** (1.0 mmol) in a solution of sodium hydroxide (1.0 mmol, 10 mL) in methanol (10 mL) and water (2 mL) was stirred at room temperature for 24 h; the mixture was poured into water (100 mL); by acidification of the resulting solution with hydrochloric acid until pH 4–5 a white solid separated. This solid was collected, washed with water, dried, and recrystallized from ethanol to give **5**.

4-Acetyl (or Ethoxycarbonyl)-5-Methyl-6H-8(Pyridine-4-yl) -1,2,4-Triazolo[3,4-b]-1,3,4-Thiadiazine $6_{h,i}$

To a stirred suspension of active methylene compounds (0.01 mol, acetylacetone or ethyl acetoacetate) in DMSO (5 mL) was added of aminothiol derivative $\mathbf{1_a}$ and the solution refluxed for 2 h, concentrated, and cooled to room temperature. The solid separated out was washed with a small amount of methanol and recrystallized from methanol to give pure compounds $\mathbf{6_{h,i}}$.

5-Amino-4H,6H-8(Pyridine-4-yl)-1,2,4-Triazolo [3,4-b]-1,3,4-Thiadiazine 7

- **Method A:** A mixture of aminothiol derivative $\mathbf{1_a}$ (0.01 mmol), chloro-acetamide (0.01 mol), and triethylamine (0.25 mL) in dioxan (30 mL) was refluxed for 6 h. After filtration the resulting solid was collected, dried, and recrystallized from ethanol to give **7**.
- **Method B:** The same reactants were fused together at 170°C for 1 h and then triturated with ethanol and recrystallized to give 7 (mp, mmp, and TLC).

12-(Pyridine-4-yl)-10-(H)-1,2,4-Triazolo[3,4-b]-1,3,4-Thiadiazino [2,3-d]-4,9-(Naphthoquinone or Quinoxaline) 8 and 9

Aminothiol derivative $\mathbf{1_a}$ (10 mmol) and 2,3-dichloro-1,4-naphthoquinone or 2,3-dichloroquinoxaline (10 mmol) was refluxed in ethanol for 6 h. After cooling, the yellow solid was collected and washed with water and then with ethanol to give **8** and **9**.

N-[7-(Pyridine-4-yl)-1,2,4-Triazolo[3,4-b] 1,3,4-Thiadiazol-4-yl]-Thiopheneamide 10

Thenoyl isothiocyanate (0.005 mol) was added to a solution of aminothiol derivative $\mathbf{1_a}$ (0.005 mol) in 20 mL of acetonitrile. The resulting solution was stirred and heated under reflux for 18 h (a solid formed during the course of the reaction). The reaction mixture was cooled and the solid was removed by filtration. The crude product was crystallized from tetrahydrofuran containing a few drops of dimethyl formamide.

7-(Pyridine-4-yl)-4-Thiophene-1,2,4-Triazolo [3,4-b]-1,3-4-Thiadiazole 11

- **Method A:** Aminothiol derivative $\mathbf{1_a}$ (0.01 mol) was refluxed with 2-thiophene carbaldehyde (0.01 mol), followed by the addition of glacial acetic acid (5 mL). The mixture was heated for 3 h and then diluted with ethanol and cooled. The precipitate was collected and recrystallized from ethanol.

- **Method B:** A solution of $\mathbf{1_a}$ (0.01 mol) in thenoyl chloride (10 mL excess) was refluxed for 1 h. Decomposition over ice-cold water gave a solid that recrystallized from ethanol to give **11**. Melting point depression was not observed in a mixed melting point experiment with an authentic sample prepare by method A.

N-p-Chlorophenyl-N′ -[3-Mercapto-5-(Pyridine-4-yl) -1,2,4-Triazole-4-yl] Urea 12

A mixture of aminothiol derivative $\mathbf{1_a}$ (0.01 mol) and p-chlorophenyl isocyanate (0.01 mol) was fused at 140°C for 2 h. The reaction mixture was cooled, triturated with light pet. (40–60), washed with ether, and then recrystallized with ethanol to give **12**.

3-Acetyl-4-Methyl-7-(Pyridine-4-yl)-1,2,4-Triazolo-[3,4-b]-5H-Pyrazole 14$_j$. Ethyl-(4-Methyl-5H-7-(Pyridine-4yl)-1,2,4-Teriazolo [3,4-b] Pyrazole-3-Carboxylate 14k

To a solution of $\mathbf{1_a}$ (0.02 mol) in ethanol (80 mL), 3-chloro-2,4-pentandione (0.02 mol) was added in one portion and refluxed for 2 h, at which time the starting material was consumed (TLC). On cooling, the solid that separated out was isolated by filtration, dried, and recrystallized from ethanol.

Hydrazinium-3-Acetyl-4-Methyl-7-(Pyridine-4-yl) -1,2,4-Teriazolo [3,2-b] Pyrazole-on-5-ide 15

Compound $\mathbf{14_j}$ (0.01 mol) and hydrazine hydrate 80% (0.015 mol) in 2-propanol (50 mL) were stirred at room temperature for 6 h, after which time the starting material had been consumed (TLC). The solid product that formed was separated and recrystallized from ethanol.

N,N-bis-(4-Methyl-7-(Pyridine-4-yl)-5H-1,2,4-Triazolo [3,2-b]-3-yl) Alkylidene-Hydrazine-5H-Pyrazole 16

- **Method A:** Compound **15** (0.005 mol) and glacial acetic acid (0.5 mol) were refluxed in 2-propanol (50 mL) for 2 h until the starting material was consumed (TLC). The solid product that formed was separated and recrystallized from dimethyl formamide.
- **Method B:** Compound $\mathbf{14_j}$ (0.01 mol) and hydrazine hydrate (0.0052 mol) in 2-propanol (50 mL) in the presence of acetic acid (1 mL) was refluxed for 3 h until the starting material was consumed (TLC). The solid product that formed was separated out and recrystallized from

dimethyl formamide. Melting point depression was not observed in a mixed melting point experimental with authentic sample from method A.

4-Methyl-3[1-p-Chlorophenylhydrazono]-Ethyl-7-(Pyridine-4-yl)-1,2,4-Triazolo [2,3-b]-5H-Pyrazole 17

Compound $\mathbf{14_j}$ (0.01 mol) and p-chlorophenyl hydrazine (0.01 mol) in 2-propanol (50 mL) in the presence of acetic acid (1 mL) was refluxed for 2 h, until the starting material was consumed (TLC). The solid product that formed was separated out and recrystallized from ethanol.

3-(1-Hydroxy Iminoethyl)-4-Methyl-7-(Pyridine-4-yl)-1,2,4-Triazolo [3,2-b]-5H-Pyrazole 18

Compound $\mathbf{14_j}$ (0.01 mol) and hydroxylamine hydrochloride (0.01 mol) in 2-propanol (60 mL) in the presence of triethyl amine (0.2 mL) was heated gradually to 90°C followed by refluxing for 3 h until the starting material was consumed (TLC). The solid product that formed was separated and recrystallized from methanol.

3(1-Thenoyloxyiminoethyl)-4-Methyl-7-(Pyridine-4-yl)-1,2,4-Triazolo [3,2-b]-5H-Pyrazole 19

A mixture of **18** (0.005 mol) and thenoyl chloride (0.007 mol) was stirred in anhydrous pyridine (25 mL) at 0°C for 6 h until the starting material was consumed (TLC). The reaction mixture was poured on ice-cold water. The solid product was isolated by filtration and recrystallized from methanol.

Biological Results

Antitumor Activity (In Vitro Study)

EAC were obtained by needle aspiration of ascetic fluid from pre-inoculated mice under aseptic conditions. The cells were tested for viability and contamination by staining a certain cell volume of this fluid by an equal volume of the working solution of Trypan blue dye and examined under a microscope.[30] In a set of sterile test tubes, 0.1 mL of tumor cell suspension, 0.8 mL RPMI 1640 media, and 0.1 mL of each test compound (corresponding to 100, 50, and 25 μg/mL). The later test tubes were carried out to calculate the percentage of nonviable cells. Compounds producing more than 70% nonviable cell are considered active[31] (Table IV).

$$\%\text{ of nonviable cells} = \frac{\text{no of nonviable}}{\text{Total No. of cell}} \times 100$$

TABLE IV ***In Vitro* Cytotoxic Activity of Synthesized Compounds**

	Nonviable cells (%) concentration (μg/mL)		
Compound no.	100	50	25
$\mathbf{1_a}$	90%	85%	80%
2	70%	65%	55%
3	85%	80%	70%
4	60%	50%	35%
5	40%	30%	20%
7	−ve	−ve	−ve
8	−ve	−ve	−ve
9	−ve	−ve	−ve
10	80%	75%	60%
11	50%	40%	20%
12	80%	70%	50%
$\mathbf{14_j}$	60%	55%	45%
15	60%	50%	35%
16	70%	60%	45%
17	90%	85%	80%
18	50%	35%	10%
19	−ve	−ve	−ve
Doxorubicin[32] as reference	100%	55%	20%

Biological Results and Structure Activity Correlation

Antitumor Activity (In Vitro Study)

Potential cytotoxicity of the compounds was tested using the method of Skehan et al.[32] Cells were plated in a 96-well plate (10 cells/well) for 24 h before treatment with the compound to allow attachment of the cells to the wall of the plate. Different concentrations of the compound under test (0, 10, 25, 50, and 100 μg/mL) were added to the cells. Monolayer cells were incubated with the compounds for 48 h at 37°C and in an atmosphere of 5% CO_2. After 48 h, the cells were fixed, washed, and stained with sulfo-rhodamine-B stain. Excess stain was washed with acetic acid and the attached stain was recovered with Tris EDTA buffer. The color intensity was measured in an ELISA reader.

The relation between surviving fraction and drug concentration was plotted to obtain this survival curves of tested cell, the response parameter calculated was IC_{50} value. The data for the tested compounds are summarized in Table V.

Compound $\mathbf{1_a}$, which has a thiol group, was more effective than the positive control (doxorubicin)[33] and was able to reduce the magnitude of activity to 90%, whereas alkylation of compound $\mathbf{1_a}$ lowered this

TABLE V *In Vitro* Anti-HEPG2[a] Testing Results

Comp.	$IC_{50}\mu g/mL$[b]
1_a	14.07
2	50.48
3	18.55
10	14.00
12	25.81
16	60.27
17	8.12
Doxorubicin[c]	43.6

[a] Liver carcinoma cell line.
[b] Concentration of compound that causes 50% inhibition of cell growth.
[c] Positive control.

activity to 70%. Compound **3** showed the highest activity among the tested compounds and it was able to reduce the activity of EAC to 85%. Compounds **10** and **12** were hypothesized to be active through intramolecular hydrogen bonding[34] and were able to reduce the activity of cells to 80%. Condensation of compound **14_j** with hydrazine or *p*-chlorophenyl hydrazine afforded compounds **16** and **17**, which proved to be active toward the use tumor cell lines. Compounds **7**, **8**, **9**, and **19** did not show any activity at different concentrations, probably because of a solubility problem in the culture media used. Compounds **4**, **5**, **11**, **14_j**, **15**, and **1** possess moderate activity against use tumor cell lines (Table III).

The selectivity of compounds **1_a**, **2**, **3**, **10**, **12**, **16**, and **17** were examined against liver carcinoma cell line (HEPG2) (Table V). On the basis of the structure of tested compounds, it can be concluded that structure-activity relationships provided evidence that geometry, size, and shape of the compound are as important as their substituents. These heterocycles could be considered as useful templates for future development and further derivatization or modification to obtain more potent and selective antitumor agents.

REFERENCES

[1] M. M. Ghorab and A. Y. Hassan, *Phosphorus, Sulfur, and Silicon*, **141,** 251–260 (1998).
[2] M. M. Ghorab, A. Y. Hassan, and O. M. Nassar, *Phosphorus, Sulfur, and Silicon*, **134/135,** 447–462 (1998).
[3] M. M. Ghorab, O. M. Nassar, and A. Y. Hassan, *Phosphorus, Sulfur, and Silicon*, **134/135,** 57–76 (1998).

[4] A. Y. Hassan, M. M. Ghorab, and O. M. Nassar, *Phosphorus, Sulfur, and Silicon*, **134/135,** 77–86 (1998).

[5] J. Mohan and G. S. R. Anjaneyulug, *Curr. Sci.*, **58,** 1028 (1989).

[6] A. M. M. E. Omar and O. M. Aboulwafa, *J. Heterocycl. Chem.*, **23,** 1339 (1986).

[7] M. A. Ghannoum, N. F. Eweiss, A. A. Bahajaj, and M. A. Quereshi, *Microbios*, **37,** 151 (1983).

[8] A. R. Rasad, T. Ramalingamat, A. B. Rao, P. W. Diwas, and P. B. Sattur, *Indian J. Chem.*, **25B,** 556 (1986).

[9] A. A. Deshmukh, M. K. Mody, T. Ramalingamt, and P. H. Sattur, *Indian. J. Chem.*, **25B,** 793 (1985).

[10] F. P. Invidiata, S. Grimaudo, P. Giammanco, and L. Giammanco, *Farmaco*, **46,** 1489 (1991).

[11] A B. Herrero, M. Castellanous, E. Marco, F. Gago, and S. Moreno, *Cancer Res.*, **66,** 8155–8162 (2006).

[12] E. Catarzi, *J. Med. Chem.*, **47,** 262–272 (2004).

[13] F. P. Invidiata, G. Furno, I. Lampronti, and D. Simon, *J. Heterocycl. Chem.*, **34,** 1255 (1997).

[14] J. R. Reid and N. D. Heindel, *J. Heterocycl. Chem.*, **13,** 925 (1976).

[15] E. Hoggarth, *J. Chem. Soc., Perkin Trans. 1*, 4811 (1952).

[16] U. Urleb, B. Stanovnik, and M. Tisler. *J. Heterocycl. Chem.*, **27,** 407 (1990).

[17] A. Santagati, M. Santagati, and M. Modica, *Heterocycles*, **36,** 1315 (1993).

[18] A. Santagati, M. Modica, M. Santagati, A. Caruse, and V. Cutuli, *Pharmazie*, **49,** 64 (1994).

[19] A. Santagati, M. Modica, and M. Santagati, *J. Heterocycl. Chem.*, **31,** 1141 (1994).

[20] A. Santagati, M. Modica, L. Monsu, and M. Santagati, *J. Chem. Res. (S)*, 86 (1999).

[21] A. Santagati, M. Modica, and M. Santagati, *J. Heterocycl. Chem.*, **37,** 1161 (2000).

[22] P. B. Talukdar, S. K. Sengupta, and A. K. Datta, *Indian Chem.*, **20B,** 538 (1981).

[23] P. B. Talukudar, S. K. Sengupta, and A. K. Datta, *Indian Chem.*, **18B,** 39 (1979).

[24] W. B. Kang, S. Nanya, Y. Yamaguchi, E. Maekawa, and Y. Ueno, *J. Heterocycl. Chem.*, **24,** 93 (1987).

[25] F. T. Coppo and M. M. Fawzi, *J. Heterocycl. Chem.*, **34,** 1351 (1997).

[26] E. Besk, L. Kuruc, V. Konecny, and Varkonda, *Chem. Abstr.*, 106 (1987).

[27] A. R. Katritzk and W. O. Fan, *J. Heterocycl. Chem.*, **25,** 901 (1988).

[28] A. M. SH. El-Sharief, Y. A. Ammar, Y. A. Mohamed, and M. S. A. El-Gaby, *Phosphorus, Sulfur, and Silicon*, **148,** 117–130 (1999).

[29] H. Beyer and C. F. Kroger, *Liebigs Ann. Chem.*, **637,** 144 (1960).

[30] D. Raffa, G. Daidone, B. Maggio, S. Cascioferro, F. Plescig, and D. Schillaci, *Il Farmaco*, **59,** 515–521 (2004).

[31] M. M. El-Merzabani, A. A. El-Aaser, A. K. El-Dueini, and A. M. Ghazal, *Planta Med.*, **36,** 150–155 (1979).

[32] P. Skehan and R. Storeng, *J. Natl. Cancer Inst.*, **82,** 1107–1112 (1999).

[33] F. A. Fornair, J. K. Randolph, J. C. Yalowich, M. K. Ritke, M. Gewirtz, and D. A. *J. Mol. Pharm.*, **456,** 649 (1999).

[34] R. W. Carling, K. W. Moore, C. R. Moyes, E. A. Jones, K. B. F. Emms, R. Marwood, S. Patel, A. E. Fletcher, M. Beer, B. Andrew, and P. D. Lesson, *J. Med. Chem.*, **42,** 2706–2715 (1999).