Scientific paper

New Approaches for the Synthesis and Ctytotoxicity of Thiazoles Derived from Cyclohexanone

Nermeen S. Abbas[1],*and Ebtsam A. Ahmed[1]

[1] *Department of Chemistry, Faculty of Science, Helwan University, Cairo, A. R. Egypt*

** Corresponding author: E-mail: nermeen_gomaa@yahoo.com*
Tel: 01017942034

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Abstract

A new class of substituted thiazole derivatives **6a–c** which showed promising anticancer activity was synthesized via the reaction of cyclohexanone with phenylisothiocyanate with a good yield. The latter compounds reacted with benzendiazonium chloride to form corresponding 5-phenylazothiazole derivatives **8a** and **8b**, respectively. Moreover, the reaction of thiazole derivatives **6a–c** with each of elemental sulfur and either of malononitrile or ethyl cyanoacetate gave the thiophene derivatives **10a–e**, respectively. Compounds **10a–e** were subjected to a series of heterocylclization reactions to give heterocyclic derivatives. Their cytotoxicity against four human tumor cells lines was measured and showed promising anticancer activity.

Keywords: Cyclohexanone; thiophene; thiazole; phenylazo

1. Introduction

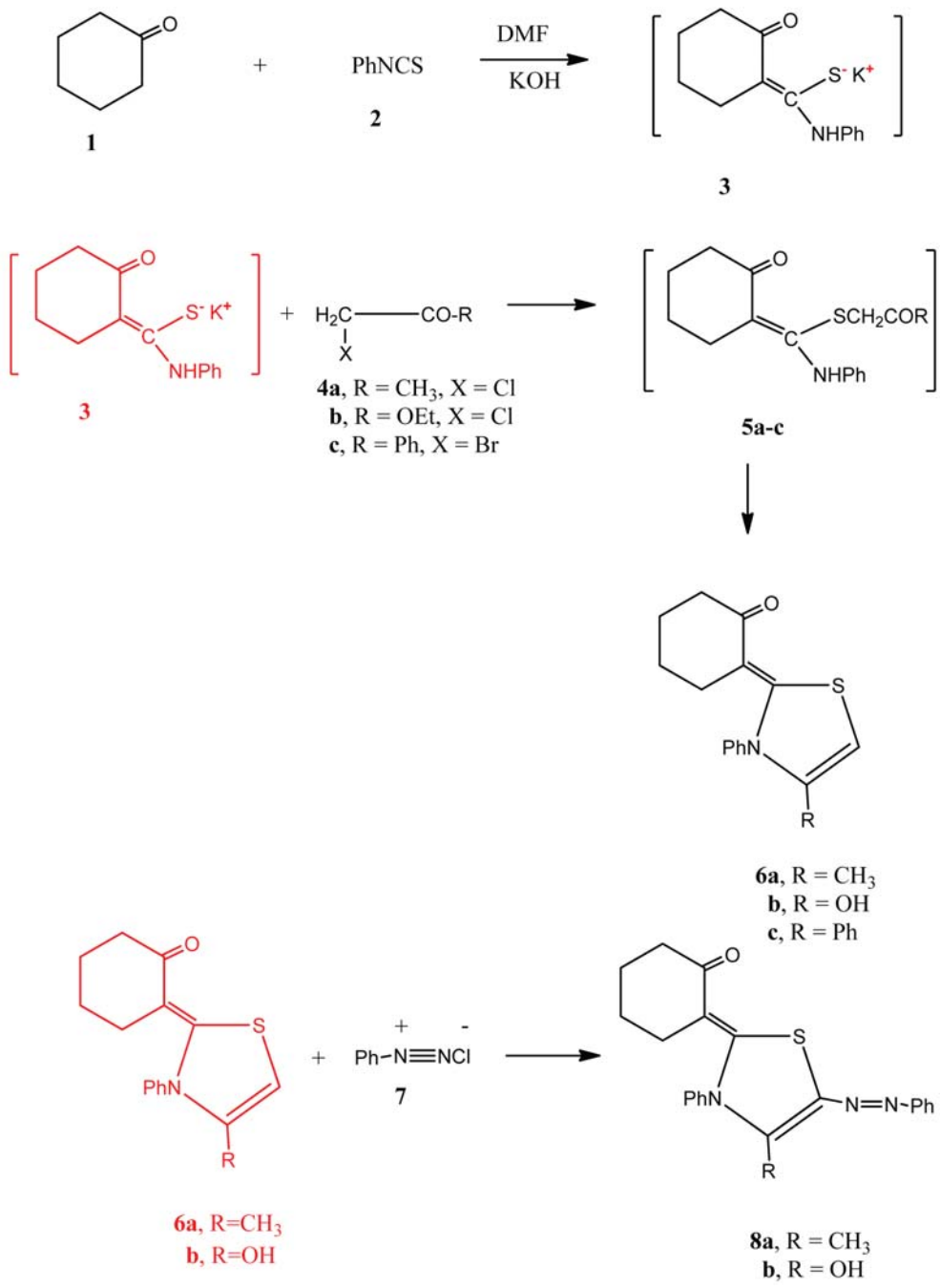
Thiazole is a core structural motif present in a variety of natural products, such as vitamin B1 (thiamine) and penicillin. Thiazole derivatives also exhibit a broad spectrum of medicinal and biological properties, such as antibacterial, antifungal,[1] anti-inflammatory,[2] antiviral,[3] antimalarial[4] and anti-HIV activities.[5] Thiazole analogs have also been reported as ligands at estrogen receptors,[6] neuropeptide Y5,[7] adenosine receptors,[8] and act as inhibitors of human platelet aggregation factor,[9] urokinase[10] and poly (ADP-Ribose) polymerase-1.[11] Selena-zoles have been reported to possess antibacterial,[12] and superoxide anion scavenging activity,[13] and exhibit cytotoxicity and DNA fragmentation effects in human HT-1080 fibrosarcoma cells.[14] In this work, we present the Hantsch reaction of cyclohexanone that involves its reaction with phenyl isothiocyanate in basic solution followed by heterocyclization reactions.

2. Results and Discussion

2. 1. Chemistry

The reaction of cyclohexanone **1** with phenyl isothiocyanate in dimethylformamide solution containing potassium hydroxide gave the intermediate potassium sulphide salt **3**. The latter intermediate interact with either α-chloroacetone (**4a**), ethyl 2-chloro acetate (**4b**) or 2-bromo-1-phenylethanone (**4c**) to give the intermediate thioether derivatives **5a–c**, respectively which was cyclized spontaneously to the thiazole derivatives **6a–c**. The structures of the latter products were assigned on the basis of analytical and spectral data. Thus, the ^{1}H NMR of **6a** showed the presence of a multiplet at δ 1.81–1.89 ppm corresponding to the presence of three CH_2 groups, a triplet at δ 2.45–2.49 indicating the presence of CH_2 group, a doublet at δ 2.51 ppm corresponding to the presence of methyl group, a singlet at δ 6.96 ppm indicating the presence of thiazole H-3 and a multiplet at δ 7.09–7.51 ppm corresponding to the presence of the of benzene ring's protons. The high yields of compounds **6a,b** encouraged us to make further work . Thus, either of compound **6a** or **6b** reacted with benzenediazonium chloride to form the corresponding 5-phenylazothiazole derivatives **8a** and **8b**, respectively (Scheme 1).

Compounds **6a–c** showed interesting activity towards Gewald's thiophene reaction. Thus, the reaction of either **6a**, **6b** or **6c** with each of elemental sulfur and either of malononitrile (**9a**) or ethyl cyanoacetate (**9b**) gave the thiophene derivatives **10a–e**, respectively. The

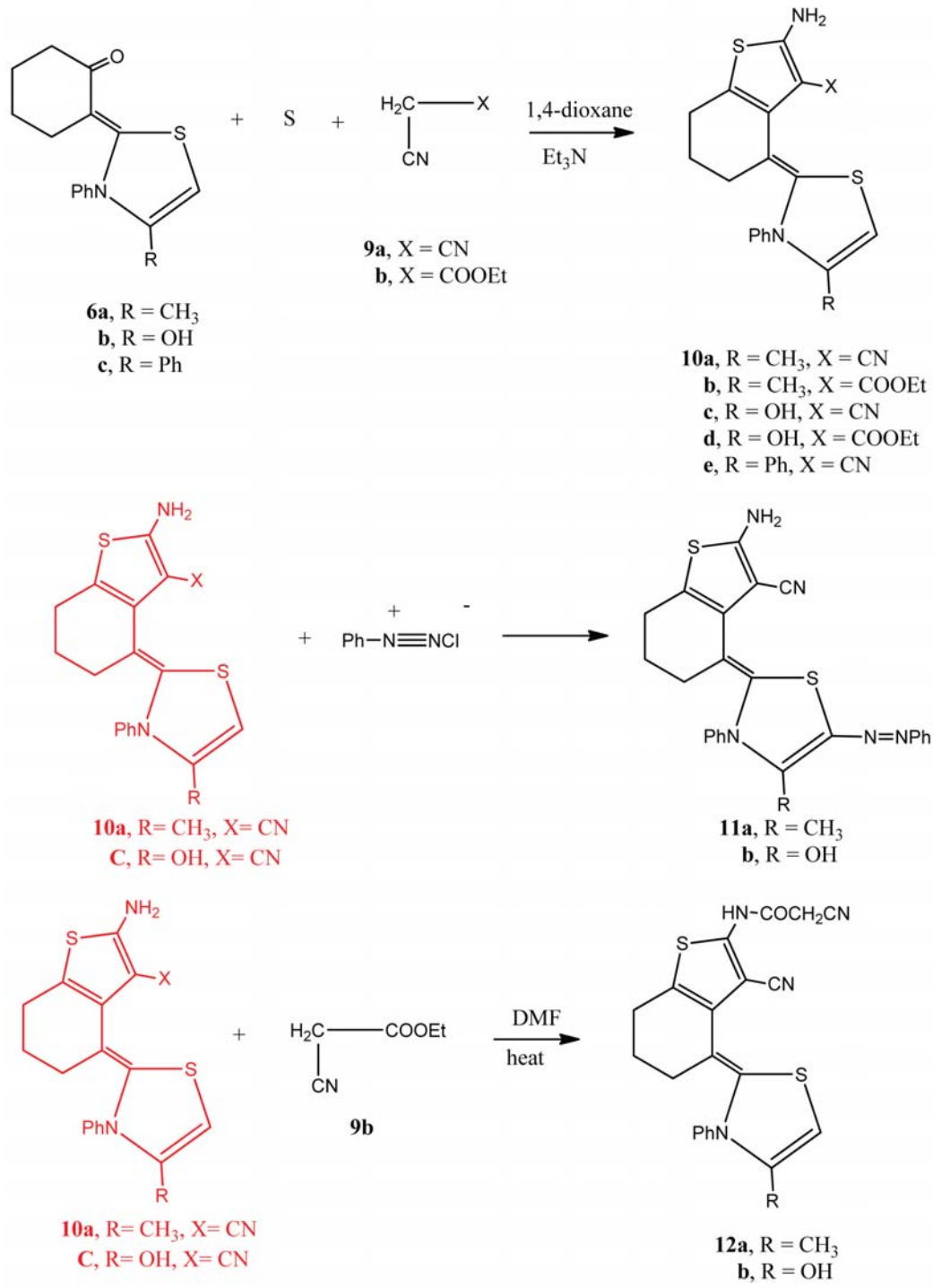


Scheme 1. Synthesis of the thiazole derivatives **6a–c** and 5-phenylazothiazole derivatives **8a,b**.

structures of the latter products were established on the basis of their respective analytical and spectral data. Thus, the ^{1}H NMR spectrum of **10a**, as an example, showed beside the expected signals, singlet at δ 4.16 ppm (D_2O exchangeable) corresponding to the presence of the NH_2 and a singlet at δ 6.87 ppm for the presence of of the thiazole H-3. Compounds **10a,c** reacted with benzenediazonium chloride to give the phenylazo derivatives **11a** and **11b**, respectively. On the other hand, the 2-aminothieno group present in **10a** and **10c** reacted with ethyl cyanoacetate to give the amido derivatives **12a** and **12b**, respectively. The analytical and spectral data of the latter products were the tools of their structural elucidation (Scheme 2).

Moreover, the reaction of compounds **10a,c** with benzaldehyde gave the Schiff's bases **14a,b**, respectively.

Finally we moved towards the uses of the cyclohexanone (**1**) to form tetrahydrobenzothiazole derivatives. Thus, the reaction of **1** with each of elemental sulfur and



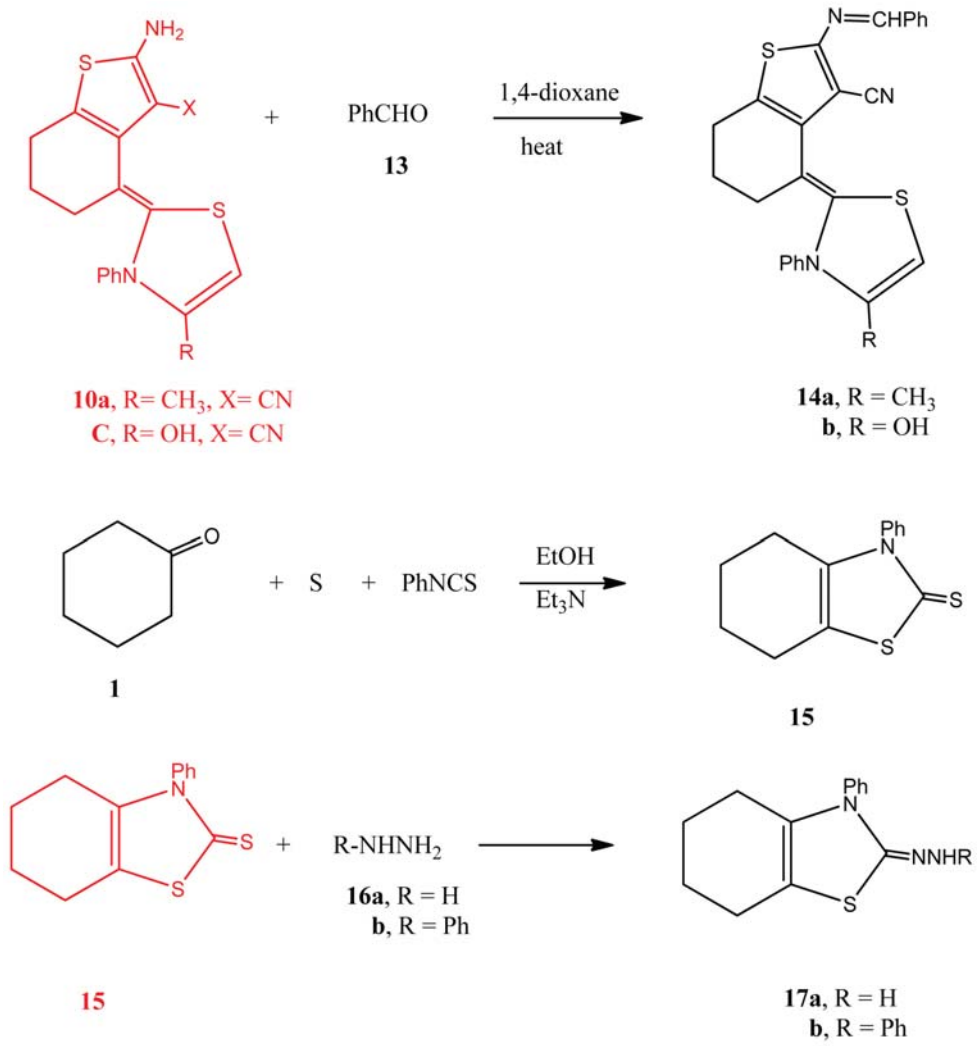
Scheme 2. Synthesis of the thiazole derivatives **10a–e** and the reaction of **10a,c** with benzendiazonium chloride and ethyl cyanoacetate to give phenylazo derivatives **11a,b** and amido derivatives **12a,b**.

phenylisothiocyanate gave the 4,5,6,7-tetrahydro-3-phenylbenzo[*d*]thiazole-2(3H)-thione (**15**). The latter reacted with either hydrazine hydrate (**16a**) or phenylhydrazine (**16b**) to give the hydrazone derivatives **17a** and **17b**, respectively (Scheme 3).

2. 2. Antitumor and Normal Cell Line Activity Tests

2. 2. 1. Chemicals

Fetal bovine serum (FBS) and L-glutamine, were purchased from Gibco Invitrogen Co. (Scotland, UK).



Scheme 3. Synthesis of the Schiff's bases **14a,b**, the 4,5,6,7-tetrahydro-3-phenylbenzo[*d*]thiazole-2(3H)-thione **15** and the hydrazone derivatives **17a,b**.

RPMI-1640 medium was purchased from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (Saint Louis, USA).

2. 2. 2. 1. Cell cultures

Was obtained from the European Collection of cell Cultures (ECACC, Salisbury, UK) and human gastric cancer (NUGC), human liver cancer (HA22T and HEPG2), human breast cancer (MCF) and normal fibroblast cells (WI38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 lg/mL), at 37 °C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for the seven human cancer cell lines including cells derived from 0.75×10^4 cells/mL followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

The heterocyclic compounds, prepared in this study, were evaluated according to standard protocols for their in vitro cytotoxicity against four human cancer cell lines including cells derived from human gastric cancer (NUGC), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), and a normal fibroblast cells (WI38). All of IC_{50} values were listed in Table 1. Some heterocyclic compounds was observed with significant cytotoxicity against most of the cancer cell lines tested (IC_{50} = 10–1000 nM). Normal fibroblasts cells (WI38) were affected to a much lesser extent (IC50>10,000 nM). The reference compound used is the CHS-828 which is a pyridyl cyanoguanidine anti-tumor agent.

Table 1. Cytotoxicity of novel pregnenlone derivatives against a variety of cancer cell lines [IC_{50}[b] (nM)]

Compd	Cytotoxocity (IC_{50} in nM)				
	NUGC	HA22T	HEPG2	MCF	WI38
6a	2276	3276	2711	1790	Na
6b	512	1120	420	338	Na
6c	3342	3310	1328	3243	Na
8a	2228	2100	1140	3860	Na
8b	2120	1140	2250	325	Na
10a	1655	2167	2799	3270	Na
10b	2101	2258	2166	2330	Na
10c	38	35	2577	255	Na
10d	1255	230	2177	288	Na
10e	2544	2176	2179	380	Na
11a	1222	2063	2528	1140	Na
11b	38	120	337	3148	Na
12a	122	59	2326	1264	Na
12b	84	233	250	338	Na
14a	2165	3266	3330	2281	Na
14b	3270	760	1285	180	Na
15	1355	262	1186	3230	Na
17a	1135	1278	2359	2555	Na
17b	22	3063	84	2711	Na
CHS 828	25	2067	1245	18	Na

[a]NUGC, gastric cancer, HA22T, liver cancer, HEPG2, liver cancer; MCF, breast cancer; WI38, normal fibroblast cells.

2. 2. 2. Structure Activity Relationship

From table 1 it is clear that the thiazoles moiety was found to be crucial for the cytotoxic effect of cyclic compounds **6–17**. Compounds **6b,10c,11b,12a,12b** and**17b** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range. Comparing the cytotoxicity of compounds **6a** and **6b**, it is obvious that the cytotoxicity of **6b** is higher than that of **6a**. Thus, substituting the methyl group by the hydroxyl group increase the cytotoxicity. Morever, 5-phenyl azothiazole derivatives **8a** and **8b** remarkable that compound **8b** showed high cytotoxicity against MCF cell line with IC_{50} value 325 nM. Similarly, Comparing the cytotoxicity of thiophene compounds **10a–e**, one can say that compound **10c** with R=OH and X=CN showed the highest cytotoxicity among the five thiophene compounds against NUGC, HA22T, MCF cell lines with IC50 values 38, 35 and 255 nM respectively. This is attributed to the presence of the hydroxyl group and the introduction of the cyano group. Similarly comparing of **11a** and **11b**, it is obvious that the presence of OH group in **11b** is responsible for its reactivity against NUGC, HA22T and HEPG2 over **11a**.

Considering the amido derivatives **12a** and **12b**, it is clear that compound **12b** bearing the OH group show much cytotoxicity over **12a**. Considering the 4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile **14a,b** against MCF cell line is higher than that of **14a**. Such high cytotoxicity of **14b** is attributed to the presence of OH group in the thiazole moiety together with 3-cyano group of the thiophene moiety.

It is obvious that the presence of phenyl group associated with nitrogen atom in tetrahydrobenzo[*d*]thiazol-2(3*H*)-thione moiety **15** is responsible for the high cytotoxicity against HA22T with IC_{50} value 262 nM.

Similarly, considering the 2,3,4,5,6,7-hexahydrobenzo[d]thiazole derivatives **17a–b** where compound **17b** show high cytotoxicity against NUGC and HEPG2 cell lines with IC_{50} values 22 and 84 nM, respectively.

3. Experimental

3. 1. General

All melting points are uncorrected. IR spectra were recorded as KBr discs on a Pye Unicam SP-1000 spectrophotometer. ^1H and ^{13}C NMR spectra were measured on a Varian EM-390-200 MHZ in CD_3SOCD_3 as the solvent using TMS as the internal standard and chemical shifts are expressed as δ. Analytical data were obtained from the micro analytical Data Unit at Cairo University, Giza, Egypt. Physical and spectral data of compound **15** was reported in the litrature.[15–21]

3. 1. 1. General Procedure for the Synthesis of Thiazole Derivatives 6a–c

To a solution of cyclohexanone (9.8 g, 0.1 mol) in DMF (40 ml) containing potassium hydroxide (5.6 g, 0.1 mol), phenylisothiocyanate (13 mL, 0.1 mol) was added. The reaction mixture was stirring overnight then either chloroacetone (9.2 mL, 0.1 mol), ethyl chloroacetate (12.3 mL, 0.1 mol) or phenacyl bromide (19.9 g, 0.1 mol) was added. The reaction mixture was stirring overnight then poured onto ice/water containing a few drops of HCl. The formed solid product was collected by filtration and crystallized from the proper solvent.

6-(4-Methyl-3-phenylthiazol-2(3*H*)-ylidene)cyclohex-1-enol (6a)

Reddish brown crystals from ethanol, yield 85% (23 g), m.p. 137–140 °C. *Anal*. Calculated for $C_{16}H_{17}NOS$ (271.38): C, 70.81; H, 6.31; N, 5.16; S, 11.82. Found: C, 71.02; H, 6.52; N, 5.32; S, 11.90. MS: *m/e* 271 (M^+, 11%), IR, ν: 3131 (CH, aromatic), 2960, 2855 (CH_3, CH_2), 1720 (C=O), 1656 (C=C), ^1H-NMR, δ: 1.81–1.89 (m, 6H, $3CH_2$), 2.45–2.49 (t, 2H, CH_2), 2.51 (d, 3H, CH_3), 6.96 (s, 1H, , thiazole H-3), 7.09–7.51(m, 5H, C_6H_5), ^{13}C-NMR, δ: 19.1 (CH_3), 21.3, 23.4, 26.4, 38.9 ($4CH_2$), 105.9 (thiazole C-3), 115.7 (C=C), 120.5, 121.0, 129.4, 137.9 (Ar), 139.8 (C-CH_3), 145.2 (thiazole C-5), 183.5 (C=O).

2-(2-Hydroxycyclohex-2-en-1-ylidene)-3-phenylthiazolidin-4-one (6b)

Yellow crystals from ethanol, yield 83% (22.6 g), m.p.

164–167 °C. *Anal*. Calculated for $C_{15}H_{15}NO_2S$ (273.35): C, 65.91; H, 5.53; N, 5.12; S, 11.73. Found: C, 65.88; H, 5.56; N, 5.18; S, 11.78. MS: *m/e* 273 (M^+, 66.6%), IR, ν: 3452-3328 (OH), 3044 (CH, aromatic), 2954 (CH_2), 1635 (C=C), ^{1}H-NMR, δ: 1.83–1.89 (m, 6H, $3CH_2$), 2.45–2.49 (t, 2H, CH_2), 6.96 (t, 1H, thiazole H-3), 7.12–7.50 (m, 5H, C_6H_5), 9.76 (s, 1H, OH), ^{13}C-NMR, δ: 22.7, 23.8, 24.9, 39.7 ($4CH_2$), 71.2 (thiazole C-3), 116.1 (C=C), 120.5, 123.1, 129.5, 140.9(Ar), 149.9 (thiazole C-5), 165.2 (C-OH), 183.7 (C=O).

6-(3,4-Diphenylthiazol-2(3*H*)-ylidene)cyclohex-1-enol (6c)

Yellow crystals from ethanol, yield 70% (23.3 g), m.p. 174–176 °C. *Anal*. Calculated for $C_{21}H_{19}NOS$ (333.45): C, 75.64; H, 5.74; N, 4.20; S, 9.62. Found: C, 75.70; H, 5.71; N, 4.22; S, 9.65. MS: *m/e* 333 (M^+, 24%), IR, ν: 3056 (CH, aromatic), 2856 (CH_2), 1725 (C=O), 1648 (C=C), ^{1}H-NMR, δ: 1.81–1.90 (m, 6H, $3CH_2$), 2.45-2.48 (t, 2H, CH_2), 6.89 (s, 1H, thiazole H-3), 7.00–7.29 (m, 10H, $2C_6H_5$), ^{13}C-NMR, δ: 21.8, 22.5, 25.4, 40.1 ($4CH_2$), 108.9 (thiazole C-3), 115.9 (C=C) , 121.9, 123.2, 127.8, 129.4, 129.6, 130.8, 133.0, 138.5 (Ar), 141.2 (thiazole C-2), 148.3 (thiazole C-5), 184.5 (C=O).

3. 1. 2. General Procedure for the Synthesis of 5-phenylazothiazole Derivatives 8a,b

To a cold solution of either **6a** (2.71 g, 0.01 mol) or **6b** (2.73 g, 0.01 mol) in ethanol (40 mL) containing sodium acetate (2.5 g) a cold solution of the diazonium salt [prepared by the addition of sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution of aniline (0.94 g, 0.01 mol) in concentrated hydrochloric acid (12 mL) with continuous stirring] was added while stirring. The formed solid product, upon stirring at room temperature was collected by filtration and crystallized using a suitable solvent.

6-(4-Methyl-3-phenyl-5-(phenyldiazenyl)thiazol-2(3*H*) -ylidene)cyclohex-1-enol (8a)

Yellow crystals from ethanol, yield 80% (30 g), m.p. 123–125 °C. *Anal*. Calculated for $C_{22}H_{21}N_3OS$ (375.49): C, 70.37; H, 5.64; N, 11.19; S, 8.54. Found: C, 70.35; H, 5.67; N, 11.16; S, 8.55. MS: *m/e* 375 (M^+, 61.9%), IR, ν: 3032 (CH, aromatic), 2930, 2815 (CH_3, CH_2), 1712 (C=O), 1636 (C=C), 1497 (N=N), ^{1}H-NMR, δ: 1.80–1.87 (m, 6H, $3CH_2$), 2.45–2.48 (t, 2H, CH_2), 2.51 (s, 3H, CH_3), 6.99–7.88 (m, 10H, $2C_6H_5$), ^{13}C-NMR, δ: 14.3 (CH_3), 23.2, 25.9, 26.3, 38.6 ($4CH_2$), 105.1 (thiazole C-3), 118.0 (C=C), 120.5, 121.0, 123.1, 126.8, 128.2, 129.4, 139.8, 140.1 (Ar), 142.1 (C-CH_3), 148.2 (thiazole C-5), 183.5 (C=O).

2-(2-Hydroxycyclohex-2-en-1-ylidene)-3-phenyl-5-(2-phenylhydrazono)-thiazolidin-4-one (8b)

Brown crystals from ethanol, yield 80% (32.7 g), m.p. 123–125 °C. *Anal*. Calculated for $C_{21}H_{19}N_3O_2S$ (377.46): C, 66.82; H, 5.07; N, 11.13; S, 8.49. Found: C, 66.80; H, 5.10; N, 11.16; S, 8.52. MS: *m/e* 377 (M^+, 24%), IR, ν: 3431–3295 (OH), 3046 (CH, aromatic), 2861 (CH_2), 1724 (C=O), 1636 (C=C), 1490 (N=N), ^{1}H-NMR, δ: 1.81–1.89 (m, 6H, $3CH_2$), 2.44–2.48 (t, 2H, CH_2), 7.09–7.58 (m, 10H, $2C_6H_5$), 9.98 (s, 1H, OH), ^{13}C-NMR, δ: 21.5, 23.9, 28.1, 38.6 ($4CH_2$), 86.0 (thiazole C-3), 115.1 (C=C), 120.7, 122.4, 124.6, 129.2, 129.4, 133.1, 140.3, 145.8 (Ar), 146.9 (thiazole C-5), 166.3 (C-OH), 185.9(C=O).

3. 1. 3. General Procedure for the Synthesis of Tetrahydrobenzo[*b*] Thiophene Derivatives 10a–e

To a solution of either **6a** (2.7g, 0.01 mol), **6b** (2.8 g, 0.01 mol) or **6c** (3.3 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine(1 mL) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added followed by elemental sulfur (0.32 g, 0.01 mol). The reaction mixture was heated under reflux for 3 h then poured onto an acidified crushed ice and filtrated off. The solid product, formed in each case was crystallized from suitable solvent.

2-Amino-4-(4-methyl-3-phenylthiazol-2(3*H*)-ylidene)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carbonitrile (10a)

Reddish brown crystals from ethanol, yield 71% (25 g), m.p. 113–115 °C. *Anal*. Calculated for $C_{19}H_{17}N_3S_2$ (351.49): C, 64.91; H, 4.87; N, 11.95; S, 18.25. Found: C, 64.90; H, 4.84; N, 11.98; S, 18.27. MS: *m/e* 351(M^+-1, 20%), IR, ν: 3436–3315 (NH_2), 3046 (CH, aromatic), 2935, 2810 (CH_3, CH_2), 2210 (CN), 1636 (C=C), ^{1}H-NMR, δ: 2.45–2.48 (m, 6H, $3CH_2$), 2.52 (d, 3H, CH_3), 4.16 (s, 2H, NH_2), 6.87 (s, 1H, thiazole H-3)), 7.11–7.23 (m, 5H, C_6H_5), ^{13}C-NMR, δ: 18.9 (CH_3), 26.9, 28.4, 30.2 ($3CH_2$), 96.0 (C-CN), 111.9 (thiazole C-3), 114.5 (C=C), 116.0 (CN), 120.5, 122.3 (Ar), 124.6 (thiophene C-4), 129.2 (Ar), 133.1 (thiophene C-5), 139.4 (Ar), 140.3 (thiazole C-5)) 145.9 (C-CH_3), 149.0 (C-NH_2).

Ethyl-2-amino-4-(4-methyl-3-phenylthiazol-2(3*H*)-ylidene)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylate (10b)

Brown crystals from ethanol, yield 60% (23.8 g), m.p. 195–197 °C. *Anal*. Calculated for $C_{21}H_{22}N_2O_2S_2$ (398.54): C, 63.29; H, 5.56; N, 7.03; S, 16.09. Found: C, 63.30; H, 5.58; N, 7.00; S, 16.07. MS: *m/e* 398 (M^+, 12%), IR, ν: 3426–3320 (NH_2), 3058 (CH, aromatic), 2930, 2813 (CH_3, CH_2), 1758 (C=O), 1639 (C=C), ^{1}H-NMR, δ: 1.15 (t, 3H, CH_3 ester), 2.44–2.49 (m, 6H, $3CH_2$), 2.51 (d, 3H, CH_3), 4.03 (q, 2H, CH_2 ester), 4.16 (s, 2H, NH_2), 6.88 (s, 1H, thiazol H-3), 7.25–7.52 (m, 5H, C_6H_5), ^{13}C-NMR, δ:

14.2, 17.2 ($2CH_3$), 23.3, 27.2, 31.3 ($3CH_2$), 61.9 (O-CH_2), 103.9 (thiazole C-3), 113.2 (C=C), 120.5, 122.6 (Ar), 124.6 (thiophene C-4), 125.3 (Ar), 128.9 (thiophene C-5), 133.1 (thiophene C-3), 138.4 (Ar), 139.4 (thiazole C-5), 141.0 (C-CH_3), 147.2 (C-NH_2), 165.6 (C=O).

2-Amino-4-(4-oxo-3-phenylthiazolidin-2-ylidene)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carbonitrile (10c)

Reddish brown crystals from ethanol, yield 74% (26 g), m.p. 143–145 °C. *Anal*. Calculated for $C_{18}H_{15}N_3OS_2$ (353.46): C, 61.16; H, 4.28; N, 11.89; S, 18.14. Found: C, 61.13; H, 4.30; N, 11.85; S, 18.19. MS: *m/e* 353 (M^+-1, 41%), IR, ν: 3435–3323 (NH_2), 3046 (CH, aromatic), 2838 (CH_2), 2208 (CN), 1643 (C=C), ^{1}H-NMR, δ: 2.45-2.49 (m, 6H, $3CH_2$), 4.16 (s, 2H, NH_2), 6.81 (s, 1H, thiazole H-3), 7.12–7.50 (m, 5H, C_6H_5), 9.76 (s, 1H, OH), ^{13}C-NMR, δ: 22.0, 27.3, 34.5 ($3CH_2$), 72.2 (C-CN), 108.2 (thiazole C-3), 113.7 (C=C), 116.9 (CN), 121.0, 123.1(Ar), 125.6 (thiophene C-4), 129.4 (Ar), , 133.0 (thiophene C-5), 137.9 (Ar), 142.9 (thiazole C-5), 149.9 (C-NH_2), 163.9 (C-OH).

Ethyl 2-amino-4-(4-oxo-3-phenylthiazolidin-2-ylidene)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylate (10d)

Brown crystals from ethanol, yield 60% (24 g), m.p. 133–135 °C. *Anal*. Calculated for $C_{20}H_{20}N_2O_3S_2$ (400.51): C, 59.98; H, 5.03; N, 6.99; S, 16.01. Found: C, 60.01; H, 5.07; N, 7.03; S, 15.99. MS: *m/e* 400 (M^+, 10.7%), IR, ν: 3455–3329 (OH, NH_2), 3061 (CH, aromatic), 2970, 2815 (CH_3, CH_2), 1757 (C=O), 1644 (C=C), ^{1}H-NMR, δ: 1.16 (t, 3H, CH_3 ester), 2.45-2.48 (m, 6H, $3CH_2$), 4.06 (q, 2H, CH_2), 4.16 (s, 2H, NH_2), 6.79 (s, 1H, thiazole H-3), 7.25–7.52 (m, 5H, C_6H_5), 9.80 (s, 1H, OH), ^{13}C-NMR, δ: 15.2 (CH_3), 21.9, 23.4, 29.8 ($3CH_2$), 61.2 (OCH_2), 110.9 (thiazole C-3), 113.1 (C=C), 122.1, 124.6 (Ar), 125.9 (thiophene C-4), 128.6 (Ar), 129.5 (thiophene C-5), 134.6 (thiophene C-3), 137.5 (Ar), 141.5 (thiazole C-5), 149.5 (C-NH_2),159.9 (C-OH), 166.5 (C=O).

2-Amino-4-(3,4-diphenylthiazol-2(3*H*)-ylidene)4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carbonitrile (10e)

Brown crystals from ethanol, yield 60% (24.7 g), m.p. 193–195 °C. *Anal*. Calculated for $C_{24}H_{19}N_3S_2$ (413.56): C, 69.70; H, 4.63; N, 10.16; S, 15.51. Found: C, 69.68; H, 4.69; N, 10.19; S, 15.55. MS: *m/e* 413 (M^+, 100%), IR, ν: 3433–3358 (NH_2), 3052 (CH, aromatic), 2852 (CH_2), 2367 (CN), 1616 (C=C), ^{1}H-NMR, δ: 2.44–2.47 (m, 6H, $3CH_2$), 4.16 (m, 2H, NH_2), 6.88 (s, 1H, thiazole-H-3), 7.03–7.35 (m, 10H, $2C_6H_5$), ^{13}C-NMR, δ: 23.3, 28.7, 32.4 ($3CH_2$), 80.1 (C-CN), 108.7 (thiazole C-3), 112.3 (C=C), 115.7 (CN), 120.9, 122.8 (Ar), 125.6 (thiophene C-4), 128.5 (Ar), 128.9 (thiophene C-5), 129.2, 129.4, 130.5, 132.7, 134.2 (Ar), 136.9 (thiazole C-5), 137.8 (thiazole C-2), 141.2 (C-NH_2).

3. 1. 4 General Procedure for the Synthesis of Phenylazo Derivatives 11a,b

To a cold solution of either **10a** (3.5 g, 0.01 mol) or **10c** (3.5 g, 0.01 mol) in ethanol (40 mL) containing sodium acetate (2.5 g) a cold solution of the diazonium salt [prepared by the addition of sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution of aniline (0.94 g, 0.01 mol) in concentrated hydrochloric acid (12 mL) with continuous stirring] was added while stirring. The formed solid product, upon stirring at room temperature was collected by filtration and crystallized using a suitable solvent.

2-Amino-4-(4-methyl-3-phenyl-5-(phenyldiazenyl) thiazol-2(3*H*)-ylidene)-4,5,6,7-tetrahydrobenzo[*b*] thiophene-3-carbonitrile (11a)

Reddish brown crystals from ethanol, yield 85% (38.6 g), m.p. 85–87 °C. *Anal*. Calculated for $C_{25}H_{21}N_5S_2$ (455.60): C, 65.91; H, 4.65; N, 15.37; S, 14.08. Found: C, 69.94; H, 4.69; N, 15.33; S, 14.04. MS: *m/e* 455 (M^+, 11%), IR, ν: 3457–3325 (NH_2), 3059 (CH, aromatic), 2990, 2813 (CH_3, CH_2), 2204 (CN), 1644 (C=C), 1492 (N=N), ^{1}H-NMR, δ: 2.43-2.46 (m, 6H, $3CH_2$), 2.55 (s, 3H, CH_3), 4.16 (s, 2H, NH_2), 7.38-7.64 (m, 10H, $2C_6H_5$), ^{13}C-NMR, δ: 14.0 (CH_3), 23.3, 26.7, 31.3 ($3CH_2$), 72.9 (C-CN), 106.9 (C-N=N), 112.9 (C=C), 116.0 (CN), 120.5, 121.3, 123.1 (Ar), 124.5 (thiophene C-4), 128.7, 129.2, 129.7 (Ar), 133.1 (thiophene C-5), 137.8 (thiazole C-5), 140.8 (Ar), 141.3 (C-CH_3), 147.5 (Ar), 149.9 (C-NH_2).

2-Amino-4-(4-oxo-3-phenyl-5-(phenyldiazenyl)thiazolidin-2-ylidene)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (11b)

Reddish brown crystals from ethanol, yield 85% (38.8 g), m.p. 109–111 °C. *Anal*. Calculated for $C_{24}H_{19}N_5OS_2$ (457.57): C, 63.00; H, 4.19; N, 15.31; S, 14.02. Found: C, 62.97; H, 4.21; N, 15.33; S, 14.06. MS: *m/e* 457 (M^+, 25.7%), IR, ν: 3480–3327 (OH, NH_2), 3047 (CH, aromatic), 2833 (CH_2), 2210 (CN), 1639 (C=C), 1496 (N=N), ^{1}H-NMR, δ: 2.43–2.48 (m, 6H, $3CH_2$), 4.16 (s, 2H, NH_2), 7.09–7.56 (m, 10H, $2C_6H_5$), 9.98 (s, 1H, OH), ^{13}C-NMR, δ: 22.4, 27.1, 30.9 ($3CH_2$), 73.9 (C-N=N), 82.4 (C-CN), 113.5 (C=C), 116.0 (CN), 120.3, 121.9, 122.4 (Ar), 123.9 (thiophene C-4), 124.6, 128.7, 129.2 (Ar), 131.5 (thiophene C-5), 137.4 (thiazole C-5), 140.7, 144.5 (Ar), 146.9 (C-NH_2), 168.7 (C-OH).

3. 1. 5. General Procedure for the Synthesis of Amido Derivatives 12a,b

To a solution of either **10a** (3.5 g, 0.01 mol) or **10c** (3.5 g, 0.01 mol) in DMF (40 mL) ethyl cyanoacetate (1.13 mL, 0.01 mol) was added. The reaction mixture in each case was heated under reflux for 3 h then pour into an acidified crushed ice and the precipitated solid product was filtrated and crystallized from the suitable solvent.

2-Cyano-*N*-(3-cyano-4-(4-methyl-3-phenylthiazol-2(3*H*)-ylidene)-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)acetamide (12a)

Reddish brown crystals from ethanol, yield 65% (27.2 g), m.p. 188–190 °C. *Anal*. Calculated for $C_{22}H_{18}N_4OS_2$ (418.53): C, 63.13; H, 4.33; N, 13.39; S, 15.32. Found: C, 63.16; H, 4.29; N, 13.35; S, 15.29. MS: *m/e* 418 (M^+, 60%), IR, ν: 3465-3324 (NH), 3049 (CH, aromatic), 2992, 2810 (CH_3, CH_2), 2345-2206 (2CN), 1723 (C=O),1643 (C=C), ^{1}H-NMR, δ: 2.43–2.46 (s, 6H, $3CH_2$), 2.73 (s, 3H, CH_3), 2.88 (s, 2H, CH_2), 6.88 (s, 1H, thiazole H-3), 7.24–7.57 (m, 5H, C_6H_5), 10.23 (s, 1H, NH). ^{13}C-NMR, δ: 16.9 (CH_3), 23.1, 24.9, 26.2, 31.1 ($4CH_2$), 86.9 (C-CN), 112.4 (thiazole C-3), 115.9 (C=C), 119.1, 121.4 (2CN), 124.7, 125.9, 129.8 (Ar), 132.9 (thiophene C-5), 133.3 (thiophene C-4), 137.3 (thiazole C-5), 140.4 (Ar), 141.5 (C-CH_3), 146.8 (C-NH), 166.2 (C=O).

2-Cyano-*N*-(3-cyano-4-(4-oxo-3-phenylthiazolidin-2-ylidene)-4,5,6,7-tetrahydro-benzo[*b*]thiophen-2-yl)acetamide (12b)

Brown crystals from ethanol, yield 75% (27.2 g), m.p. 188–190 °C. *Anal*. Calculated for $C_{21}H_{16}N_4O_2S_2$ (420.51): C, 59.98; H, 3.84; N, 13.32; S, 15.25. Found: C, 60.01; H, 3.80; N, 13.35; S, 15.29. MS: *m/e* 420 (M^+, 45.30%), IR, ν: 3475–3323 (OH, NH), 3047 (CH, aromatic), 2854 (CH_2), 2342–2205 (2CN), 1724 (C=O), 1642 (C=C), ^{1}H-NMR, δ 2.43–2.47 (m, 6H, $3CH_2$), 2.73 (s, 2H, CH_2), 6.86 (s, 1H, thiazole H-3), 7.07–7.54 (m, 5H, C_6H_5), 9.93 (s, 1H, OH), 10.17 (s, 1H, NH), ^{13}C-NMR, δ: 23.2, 24.8, 26.8, 30.9 ($4CH_2$), 72.3 (thiazole C-3), 90.4 (C-CN), 113.9 (C=C), 115.2, 119.6 (2CN), 122.2, 122.9, 124.6 (Ar), 129.5 (thiophene C-5), 132.8 (thiophene C-4), 137.9 (thiazole C-5), 141.2 (Ar), 148.6 (C-NH), 162.5 (C=O), 168.2 (C-OH).

3. 1. 6. General Procedure for the Synthesis of Shiff's Bases 14a,b

To a solution of either **10a** (3.5 g, 0.01 mol) or **10c** (3.5 g, 0.01 mol) in 1,4-dioxane (40 mL) containing 3 drops of piperidine, benzaldehyde (1.06 mL, 0.01 mol) was added. The reaction mixture in each case was heated under reflux for 2 h then poured onto ice/water containing a few drops of HCl. The formed solid product was collected by filtration and crystallized from the proper solvent.

2-(Benzylideneamino)-4-(4-methyl-3-phenylthiazol-2(3*H*)-ylidene)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (14a)

Brown crystals from ethanol, yield 60% (26.3 g), m.p. 100–103 °C. *Anal*. Calculated for $C_{26}H_{21}N_3S_2$ (439.60): C, 71.04; H, 4.82; N, 9.56; S, 14.59. Found: C, 70.99; H, 4.80; N, 9.55; S, 14.60. MS*: m/e* 441 (M^+-2, 2%), IR, ν: 3046 (CH, aromatic), 2978, 2811 (CH_3, CH_2), 2274 (CN), 1636 (C=C), 1587 (C=N), ^{1}H-NMR, δ: 2.44–2.47 (m, 6H, $3CH_2$), 2.54 (d, 3H, CH_3), 6.88 (s, 1H, thiazole-H-3), 7.07 (s, 1H, N=CH), 7.10–7.52 (m, 10H, $2C_6H_5$), ^{13}C-NMR, δ: 17.8 (CH_3), 21.3, 25.6, 30.9 ($3CH_2$), 79.2 (C-CN), 108.4 (thiazole C-3), 112.9 (C=C), 115.9 (CN), 121.3, 122.4 (Ar), 124.5 (thiophene C-4), 125.0, 128.7, 129.2, 131.9, 134.2 (Ar), 133.4 (thiophene C-5), 137.6 (Thiazole C-5), 140.2 (Ar), 141.9 (C-CH_3), 150.9 (thiophene C-2), 158.9 (N=C).

2-(Benzylideneamino)-4-(4-oxo-3-phenylthiazolidin-2-ylidene)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (14b)

Yellow crystals from ethanol, yield 75% (33 g), m.p. 120–123 °C. *Anal*. Calculated for $C_{25}H_{19}N_3OS_2$ (441.57): C, 68.00; H, 4.34; N, 9.52; S, 14.52. Found: C, 68.03; H, 4.36; N, 9.55; S, 14.55. MS: *m/e* 441 (M^+, 21%), IR, ν: 3425-3343 (OH), 3051 (CH, aromatic), 2810 (CH_2), 2208 (CN), 1646 (C=C), 1553 (C=N), ^{1}H-NMR, δ: 2.45-2.48 (m, 6H, $3CH_2$), 6.85 (s, 1H, thiazole-H-3), 6.98 (s, 1H, N=CH), 7.13–7.53 (m, 10H, $2C_6H_5$), 9.82 (s, 1H, OH), ^{13}C-NMR, δ: 23.2, 26.3, 31.1 ($3CH_2$), 80.1 (C-CN), 107.9 (thiazole C-3), 114.1 (C=C), 116.1 (CN), 122.2, 123.1 (Ar), 124.9 (thiophene C-4), 128.6, 129.1, 129.9, 131.1, 136.7 (Ar), 137.8 (thiophene C-5), 139.9 (thiazole C-5), 141.1 (Ar), 153.2 (C-OH), 155.3 (thiophene C-2), 159.9 (N=C).

3. 1. 8. General Procedure for the Synthesis of Hydrazone Derivatives 17a,b

To a solution of **15** (2.5 g, 0.01 mol) in ethanol (20 mL) either of hydrazine hydrate (0.5 mL, 0.01 mol) or phenyl hydrazine (1.08 mL, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h. The reaction mixture was left a side at room temperature to cool, The solid product was collected by filtration and crystallized from a proper solvent.

2-Hydrazono-3-phenyl-2,3,4,5,5,6,7-hexahydrobenzo[*b*]thiazole (17a)

Yellow crystals from ethanol, yield 60% (14.7 g), m.p. 153–155 °C. *Anal*. Calculated for $C_{13}H_{15}N_3S$ (245.34): C, 63.64; H, 6.16; N, 17.13; S, 13.07. Found: C, 63.61; H, 6.12; N, 17.10; S, 13.10. MS: *m/e* 245 (M^+, 17%), IR, ν: 3340–3315 (NH_2), 3021 (CH, aromatic), 2815 (CH_2), 1630 (C=C), 1545 (C=N), ^{1}H-NMR, δ: 1.81-1.93 (m, 4H, $2CH_2$), 2.44–2.49 (m, 4H, $2CH_2$), 5.02 (s, 2H, NH_2), 7.13–7.45 (m, 5H, C_6H_5), ^{13}C-NMR, δ: 20.1, 21.3, 23.7, 25.9 ($4CH_2$), 93.3 (S-C=C), 122.4, 125.9, 129.5, 140.9 (Ar), 147.8 (N-C=C), 150.0 (C=N).

3-Phenyl-2-(2-phenylhydrazono) -2,3,4,5,5,6,7-hexahydrobenzo[*b*]thiazole (17b)

Yellow crystals from ethanol, yield 65% (23.4 g), m.p. 150–152 °C. *Anal*. Calculated for $C_{19}H_{19}N_3S$ (321.44): C, 70.99 ; H, 5.96; N, 13.07; S, 9.98. Found: C, 71.00; H,

5.99; N, 13.10; S, 10.00. MS: m/e 321 (M^+, 83%), IR, ν: 3432–3310 (NH), 3032 (CH, aromatic), 2820 (CH_2), 1632 (C=C), 1556 (C=N), ^{1}H-NMR δ: 1.84–1.93 (m, 4H, $2CH_2$), 2.44–2.49 (m, 4H, $2CH_2$), 7.13–7.45 (m, 10H, $2C_6H_5$), 8.70 (s, 1H, NH), ^{13}C-NMR, δ: 20.3, 21.1, 22.3, 25.6 ($4CH_2$), 95.6 (S-C=C), 118.6, 122.4, 122.9, 123.6, 125.7, 128.9, 139.9, 142.0 (Ar), 144.5 (N-C=C), 148.2 (C=N).

4. Conclusion

In summary, we have shown here in that our strategy is compatible with the synthesis of new class of substituted thiazole derivatives. Cytotoxicity of newly synthesized products was evaluated against the cancer cell lines. The results showed that compounds **6b**, **10c**, **11b**, **12a**, **12b** and **17b** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM rang.

5. References

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Povzetek

Z reakcijo med cikloheksanonom in fenilizotiocianati smo z dobrimi izkoristki pripravili nov razred substituiranih tiazolnih derivatov **6a–c**, ki kažejo obetavno aktivnost proti raku. Spojine **6** smo reagirali z benzendiazonijevim kloridom in pripravili 5-fenilazotiazolna derivata **8a** in **8b**. Tiazolne produkte **6a–c** smo ob prisotnosti elementarnega žvepla in malononitrila oz. etil cianoacetata pretvorili v tiofenske derivate **10a–e**. Spojine **10a–e** smo z vrsto heterociklizacijskih reakcij pretvorili v različne heterociklične derivate. Ugotovili smo, da mnoge izmed teh spojin kažejo obetavno aktivnost proti človeškim tumorskim celičnim linijam.