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The Synthesis and Cytotoxicity of Novel Thiophene Derivatives Derived from 2-(4-Oxo-4,4-Dihydrothiazol-2-yl) Acetonitrile

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Abstract

The reaction of the 2-(4-oxo-4,4-dihydrothiazol-2-yl)acetonitrile 1 with cyaclopentanone (2) afforded the condensed product 3. The latter underwent a series of heterocyclizations through its reaction with different reagents. Moreover, compound 1 underwent the Gewald's thiophene to afford compounds 15 and 17. The reaction of either hydrazine hydrate or phenylhydrazine with compound 17 gave the hydrazide derivatives 19a and 19b, respectively. The cytotoxicity of the newly synthesized products was measured towards the three cancer cell lines MCF-7, NCI-H460 and SF-268. The study showed that compounds 3, 5, 9c, 11, 13a, 13c, 17 and 19b were the most active compounds towards the three cancer cell lines.

Keywords

Thiazole, Cyclopentanone, Thiophene, Hydrazide, Cytotoxicity

1. Introduction

Although the number of drugs is available in the market, the need of discovering the new anti-tumor drugs with better pharmacokinetic profile and lesser toxicity has become the main objective in the field of medicinal chemistry, and it is also due to the fast microbial resistance to the existing molecules [1]-[3]. A large number of

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compounds containing thiophene system have been investigated because of their broad spectrum of biological activities which include analgesic [4], antibacterial [5], antifungal [6], antiparasitic [7], antiviral [8], anti-inflammatory [9], anticonvulsant [10], anti-nociceptive [11], DNA cleavage [12], herbicidal [13], antitubercular [14], protein kinase inhibition [15], respiratory syndrome protease inactivation [16], an active ester in the peptide synthesis and agonists of peroxisome proliferator activated receptors [17]. In the present work, we study the reactivity of compound **3** resulting from reaction of the 2-(4-oxo-4,4-dihydrothiazol-2-yl)acetonitrile (**1**) with cyclopentanone to produce novel thiophene derivatives together with cytotoxic evaluations of the newly synthesized products towards different cell lines.

2. Chemistry

The reaction of the 2-(4-oxo-4,4-dihydrothiazol-2-yl)acetonitrile (**1**) with cyaclopentanone (**2**) in the presence of ammonium acetate at 120˚C gave the Knoevenagel condensation compound **3**. The structure of the compound **3** was confirmed on the basis of analytical and spectral data. The reaction of compound **3** with elemental sulphur in the presence of ethanol and triethylamine gave the 4,5,6,7-tetrahydrobenzo[*b*]thiophene derivative **4**. The 2-amino group present in compound **4** showed interesting reactivity as primary aromatic amine. Thus, compound **4** reacted with acetic anhydride in presence of acetic acid gave the N-acetyl derivative **5**. On the other hand the reaction of compound **4** with ethyl cyanoacetate **6** gave the N-cyanomethylacetamide derivatives 7. The analytical and spectral data are the tools of the structure elucidation of compound **7**. Thus, the ^{1}H NMR spectrum showed a multiplet at δ1.18 - 1.69 ppm indicating the cyclopentene three CH_2, a singlet at δ2.50 ppm corresponding to the CH_2 group, a singlet at δ4.29 ppm for the thiazol CH_2, and a singlet at δ8.27 ppm for the NH group (**Figure 1**).

The high yield of compound **4** encouraged us to study its further reactivity towards some chemical reagents. Thus, the reaction of **4** with any of benzene diazoniumchloride **8a**, 4-chlorobenzene-diazonium chloride **8b** or 4-methoxybenzene-diazonium chloride **8c** in the presence of ethanol and sodiumhydroxide gave the arylhydrazonederivatives **9a-c**, respectively. The analytical and spectral data of the latter products are consistent with their respective structures. On the other hand, compound **4** is capable for diazotization and coupling. Thus, compound **4** reacted with sodium nitrite in the presence of sodium nitrite and acetic acid at 0˚C - 5˚C gave the non isolable-diazonium salt **10**. The latter coupled with acetylacetone to give the hydrazoderivative **11**.

The reaction of compound **3** with ethyl cyanoacetate **6** in the presence of 1,4-dioxane and triethylamine gave the ethyl 4,6-diamino-7-(4-oxo-4,5-dihydrothiazol-2-yl)-2,3-dihydro-1H-indene-5-carboxylate **12**. The structure of compound **12** was confirmed on the basis of analytical and spectral data. Thus the ^{1}H NMR spectrum showed a triplet at δ1.16 ppm for ester CH_3, a multiplet at δ1.54 - 1.72 ppm indicating the cyclopentene three CH_2, a singlet at δ4.19 ppm corresponding to the NH_2 group, a quartet at δ4.24 ppm for ester CH_2, a singlet at δ4.97 ppm for the NH_2 group, a singlet at δ6.01 ppm for the thiazol CH_2 (**Figure 2**).

Moreover, compound **3** was coupled with any of 4-chlorobenzenediazonium chloride **8b**, 4-methoxybenzene-diazonium chloride **8c** or 4-methyl benzene diazonium chloride **8d** in the presence of ethanol and sodium hydroxide gave the arylhydrazone derivatives **13a-c**, respectively. Compound **1** reacted with elemental sulphur and cyanoacetanilide (**14**) in 1,4-dioxane and the presence of triethylamine to give the ethyl 3,5-diamino-4-(4-oxo-4,5- dihydrothiazol-2-yl)thiophene-2-carboxylate **15**.

Similarly the reaction of compound **1** with elemental sulphur and ethyl acetoacetate **16** gave the ethyl 5-amino-3-methyl-4-(4-oxo-4,5-dihydrothiazol-2-yl)thiophene-2-carboxylate (**17**). Compound **17** reacted with either hydrazinehydrate or phenylhydrazine to give the hydrazide derivatives **19a** and **19b**, respectively (**Figure 3**).

3. Cytotoxicity

3.1. Antitumor and Normal Cell Line Activity Tests

The cytotoxicity of the synthesized compounds was tested for Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) were used. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK), NCI-H460, SF-268 and normal fibroblast cells (WI 38) 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 µg/mL), at 37˚C in a humidified

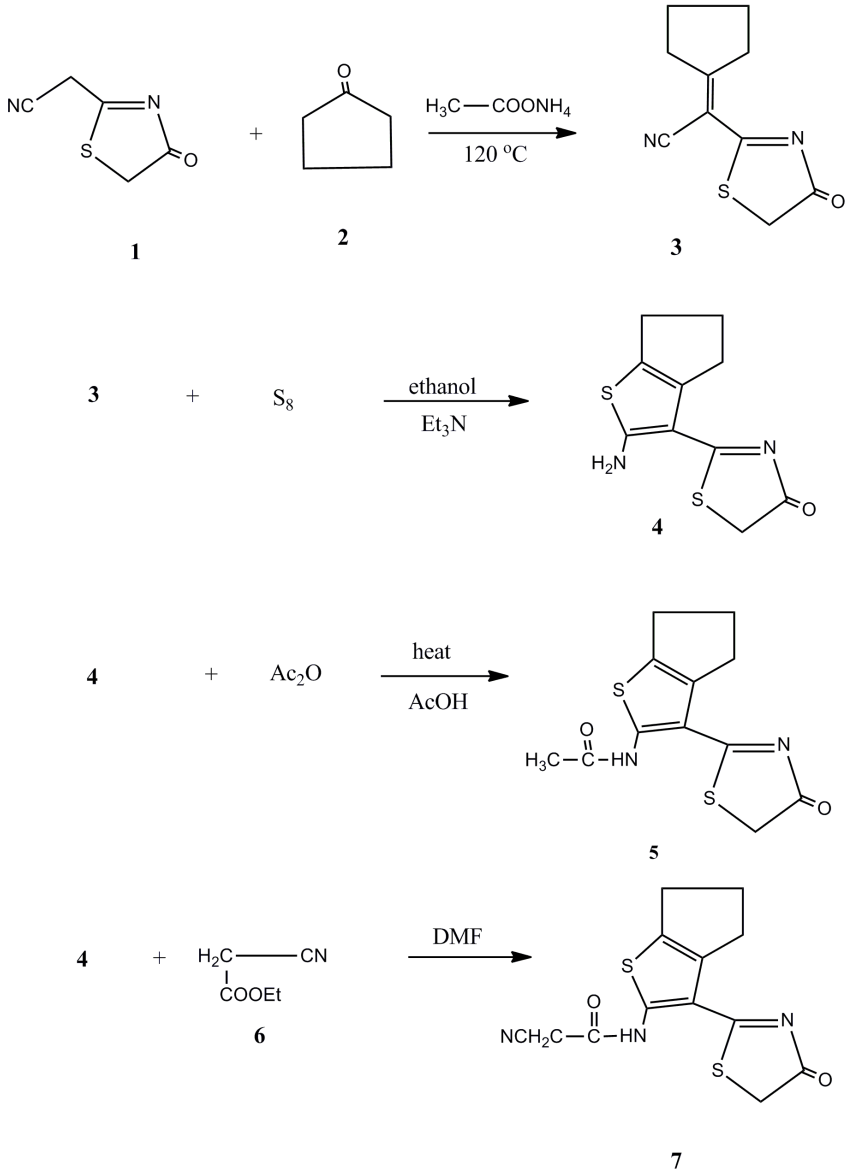

Figure 1. Synthesis of compounds **3**, **4**, **5** and **7**.

atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for MCF-7 and SF-268 and 0.75×10^4 cells/mL for NCI-H460, 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 effects of synthesized compounds on the in vitro growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the "*In Vitro* Anticancer Drug Discovery Screen" that uses the protein-binding dye sulforhodamine B to assess cell growth (12). Briefly, exponentially, cells growing in 96-wellplates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 μM. Following this exposure period adherent cells were fixed, washed, and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Powerwave XS, Wincoski, USA). For each test compound and cell line, a dose-response curve was obtained and the growth inhibition of 50% (IC_{50}), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth was calculated as described elsewhere. Doxorubicin was used as a positive control and tested in the same manner.

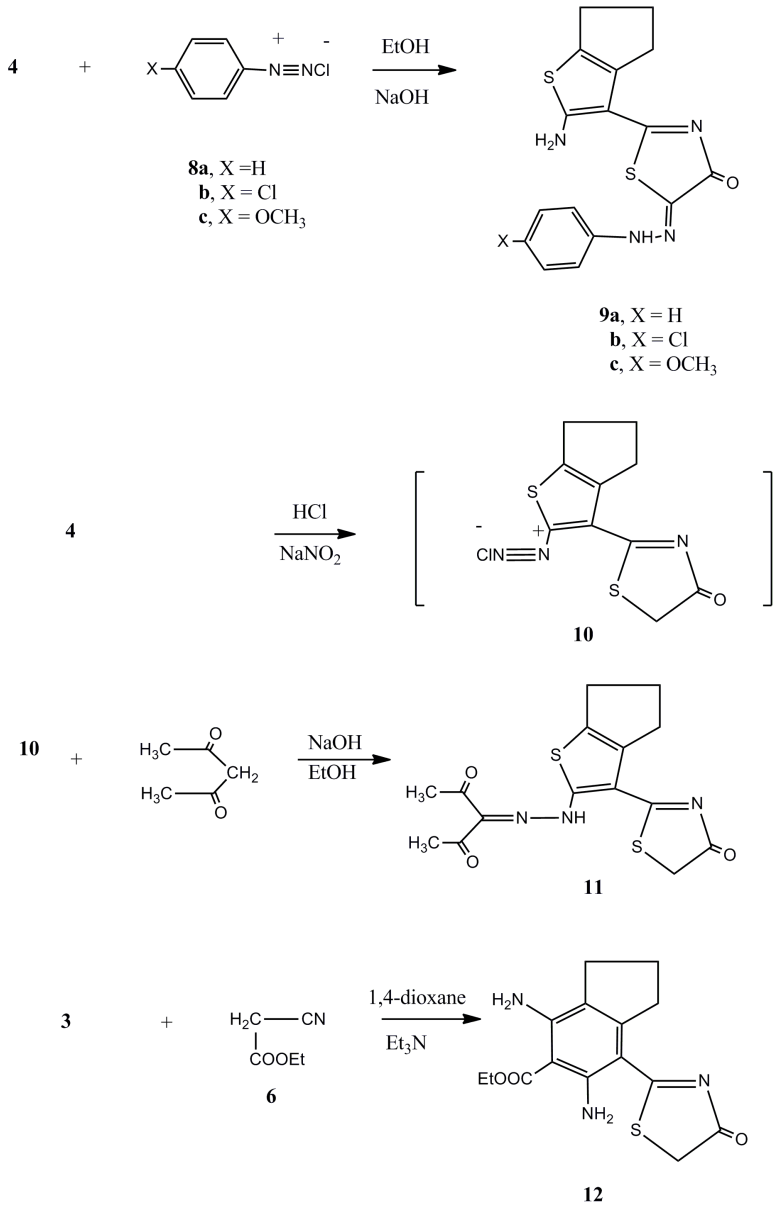

Figure 2. Synthesis of compounds **9a-c**, **11** and **12**.

3.2. Structure Activity Relationship

It is clear from **Table 1** that compounds **3**, **5**, **9c**, **11**, **13a**, **13c**, **17** and **19b** were the most active compounds towards the three cancer cell lines with IC50's against MCF-7 cell line (as an example) 0.02, 0.2, 0.01 1.6, 0.6, 0.4, 0.01 and 0.20, respectively. On the other hand, compounds **12**, **13b** and **19a** were of moderate activities with IC50's against MCF-7 6.1, 11.1 and 10.6, respectively. The rest of compounds showed low activity. Consider compounds **9a-c** it is clear that compound **9c** showed the highest activity among the three compounds which is attributed to the presence of the OCH_3 group. Considering compounds **13a, b** it is clear that compound **13a** with the 4-CH_3 group showed higher activity than **13b** with the 4-Cl group. For the hydrazide derivatives **19a, b** it is obvious that compound **19b** with the phenyl moiety is more potent than compound **19a**.

3.3. Conclusion

We have reported a convenient synthesis of variety compounds from compound **1** to **19b** derivatives. The cyto-

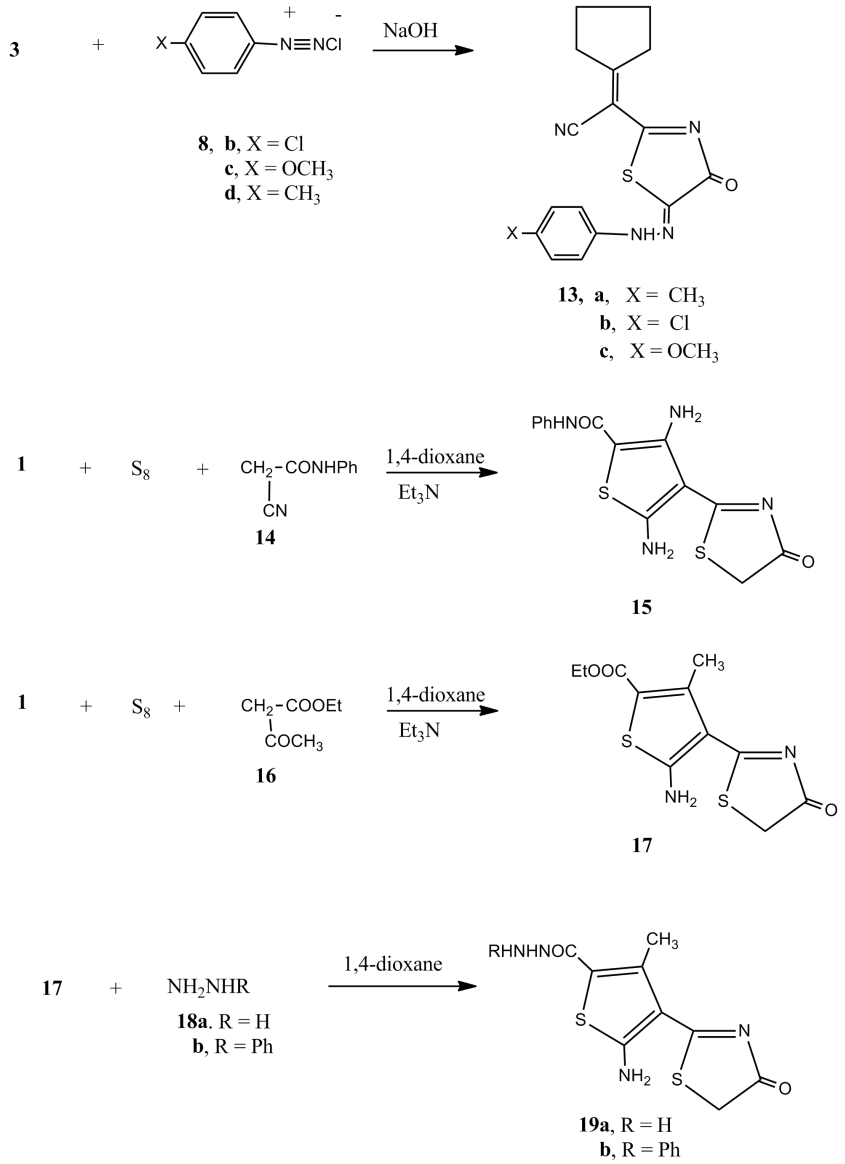

Figure 3. Synthesis of compounds **13a-c**, **15**, **17** and **19a**, **b**.

toxicity of some derivatives towards three types of cancer cell lines were studied most of the synthesized compounds were found to be cytotoxic and hence deserve further pharmacological investigation. The results of these investigation will be published in due time.

4. Experimental

All melting points determined on an electrothermal digital melting point apparatus and are uncorrected. IR spectra (KBr discs) were recorded on a FTIR plus 460 or Pyeunicam SP-1000 spectrophotometer. 1H-NMR spectra were recorded with varian Gemini 200 (200 MHz) (cairo university) instrument in DMSO-d_6 as solvent using TMS as internal standard and chemical shifts are expressed as δ ppm. The mass spectra were recorded with Hewlett Packard 5988 A GC/MS system and GCMS-QP 1000 Ex shimadzu instruments. Analytical data were obtained from the microanalytical data unit at cairo university and were performed on vario El III Elemental CHNS analyzer.

2-*Cyclopentylidene*-2-(4-*oxo*-4,5-*dihydrothiazol*-2-*yl*)*acetonitrile* (3)

To a solution of compound **1** (1.40, 0.01 mol) cyclopentanone **2** (0.84 g, 0.01 mol) was added and the reaction

Table 1. Effect of the synthesized compounds on the growth of three human tumor cell lines.

Compound	IC_{50} (μMmol·L^{-1})		
	MCF-7	NCI-H460	SF-268
1	22.6 ± 2.4	18.9 ± 4.8	16.2 ± 2.6
3	0.02 ± 0.002	0.01 ± 0.002	0.02 ± 0.001
4	18.1 ± 0.8	12.3 ± 2.6	11.3 ± 0.8
5	0.2 ± 0.02	0.3 ± 0.01	0.2 ± 0.08
7	22.2 ± 1.4	16.1 ± 2.4	14.0 ± 1.2
9a	18.6 ± 0.6	14.5 ± 0.8	22.7 ± 8.4
9b	14.4 ± 8.1	20.2 ± 2.8	21.3 ± 4.2
9c	0.01 ± 0.002	0.01 ± 0.004	0.01 ± 0.001
11	1.6 ± 0.4	2.2 ± 0. 8	4.0 ± 0.2
12	6.1 ± 2.4	8.1 ± 2.1	4.2 ± 1.3
13a	0.6 ± 0.2	0.1 ± 0.02	0.5 ± 0.05
13b	11.1 ± 2.2	12.2 ± 1.1	6.20 ± 2.4
13c	0.4 ± 0.2	0.2 ± 0.06	0.5 ± 0.01
15	32.0 ± 1.6	40.0 ± 0.4	10.5 ± 1.2
17	0.01 ± 0.003	0.02 ± 0.001	0.01 ± 0.001
19a	10.6 ± 4.6	8.5 ± 2.8	6.7 ± 1.4
19b	0.2 ± 0.01	0.1 ± 0.02	0.2 ± 0.02
Doxorubicin	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007

Results are given in concentrations that were able to cause 50 % of cell growth inhibition (GI_{50}) after a continuous exposure of 48 h and show means ± SEM of three-independent experiments performed in duplicate.

mixture was heated under fusion with ammonium acetate to 120˚C for 1hr, then cooled and then poured onto ice/water mixture and crystallized from 1,4-dioxane Yellow crystals, yield 1.75 g (85%), m.p. 120˚C - 122˚C; IR (KBr) (υ-cm^{-1}): 2928 - 2385 (CH_2), 2200 - 2195 (2CN), 1604 (C=O), 1580 (C=C). ^{1}H-NMR (DMSO-d_6, δ ppm): 1.55 - 1.75 (m, 8H, 4CH_2), 4.97 (s, 2H, thiazole CH_2). MS m/e = 206 (M^+, 12); Anal. Calcd. for $C_{10}H_{10}N_2OS$: C, 58.23; H, 4.89; N, 13.58; S, 15.55%. Found, C, 58.21; H, 5.01; N, 13.63; S, 15.33%.

2-(2-*Amino*-5,6-*dihydro*-4*H*-*cyclopenta*[*b*]*thiophen*-3-*yl*)*thiazol*-4(5*H*)-*one* (4)

To a solution of **3** (2.06 g, 0.01 mol) in ethanol (35 ml) containing triethylamine (1.00 ml) solid sulfur (0.32 g, 0.01 mol) was added, the reaction mixture was then heated under reflux for 30 min then cooled and neutralized by pouring onto ice/water mixture containing few drops of hydrochloric acid, the solid product formed was collected by filtration and crystallized from 1,4-dioxane Yellow crystals, (1,4-dioxane) yield 1.90 g (80%), m.p. 93˚C - 95˚C; IR (KBr) (υ-cm^{-1}): 3400, 3320 (NH_2), 2930 (CH_2), 2220 (CN), 1686 (C=O), 1620 (C=C). ^{1}H-NMR (DMSO-d_6) δ ppm: 1.62 - 2.55 (m, 6H, 3CH_2), 4.21 (s, 2H, NH_2), 5.73 (s, 2H, thiazole CH_2). MS m/e = 238 (M^+, 18); Anal. Calcd. for $C_{10}H_{10}N_2OS_2$: C, 50.40; H, 4.23; N, 11.75; S, 26.91%. Found, C, 50.22; H, 4.31; N, 11.49; S, 26.94%.

***N*-(3-(4-*Oxo*-4,5-*dihydrothiazol*-2-*yl*)-5,6-*dihydro*-4*H*-*cyclopenta*[*b*]*thiophen*-2-*yl*)*acetamide* (5)**

To a solution of **4** (2.38 g, 0.01 mol), acetic acid/acetic anhydride (10:3 ml) was added, the reaction mixture was heated under reflux 1 hr, the solid product formed upon pouring onto ice/water mixture, collected by filtration then washed with water and crystallized. The solid product formed was collected by filtration and crystallized from 1,4-dioxane. Pale yellow crystals, yield 2.24 g (80%), m.p. 133˚C - 135˚C; IR (KBr) (υ-cm^{-1}): 3853, 3480 (NH), 2858 - 2430 (CH_3, CH_2), 2221 (CN), 1692 (2C=O), 1589 (C=C). ^{1}H-NMR (DMSO-d_6) δ ppm: 1.56 - 1.89 (m, 6H, 3CH_2), 2.66 (s, 3H, CH_3), 4.97 (s, 2H, thiazol CH_2), 12.1 (s, 1H, NH). MS m/e = 280 (M^+, 25); Anal. Calcd. for $C_{12}H_{12}N_2O_2S_2$: C, 51.41; H, 4.31; N, 9.99; S, 22.87%. Found, C, 51.70; H, 4.55; N, 9.72; S, 22.90%.

2-*Cyano-N*-(3-(4-*oxo*-4,5-*dihydrothiazol*-2-*yl*)-5,6-*dihydro*-4*H*-*cyclopenta*-[*b*]*thiohen*-2-*yl*)*acetamide* (7)

To a solution of compound **4** (2.38 g, 0.01 mol) in DMF (20 ml) containing triethylamine (1.00 ml) ethylcyanoacetate (1.13 g, 0.01 mol) was added, the reaction mixture was heated under reflux 30 mins, then cooled and neutralized by pouring onto ice/water mixture containing few drops of hydrochloric acid, the solid product formed in each case was collected by filtration and crystallized from 1,4-dioxane. Brown crystals, (1,4-dioxane) yield 2.44 g (80%), m.p. 164˚C - 166˚C; IR (KBr) (υ-cm^{-1}): 3458 - 3320 (NH), 2884 (CH_2), 2227, 2220 (2CN), 1683, 1679 (2C=O), 1610 (C=C). ^{1}H-NMR (DMSO-d_6) δ ppm: 1.18 - 1.69 (m, 6H, cyclopentene 3CH_2), 2.50 (s, 2H, CH_2), 4.29 (s, 2H, thiazol CH_2), 8.27 (s, 1H, NH). MS m/e =305 (M^+, 84.54); Analy. Calcd. for $C_{13}H_{11}N_3O_2S_2$: C, 51.13; H, 3.63; N, 13.76; S, 21.00%. Found, C, 50.86; H, 3.79; N, 13.83; S, 21.26%.

***Synthesis of diazotized* 2-(2-*amino*-4,5,6,7-*tetrahydrobebzo*[*b*]*thiophen*-3-*yl*)*thiazol*-4 (5*H*)-*one derivatives* (9*a-c*).**

General procedure:

To a cold (0˚C - 5˚C) solution of compound **4** (2.38 g, 0.01 mol) in ethanol (20 mL) containing sodium hydroxide (1.00 g) an equivalent amount of either benzenediazonium chloride, 4-chlorobenzenediazonium chloride, or 4-methoxybenzenediazonium chloride [which was prepared by adding $NaNO_2$ (0.70 g, 0.01 mol) solution to a cold solution of either aniline (1.0 g, 0.01 mol) in HCl (6 mL) or 4-chloroaniline (0.01 mol) or 4-methoxyaniline (0.01 mol)] was gradually added while stirring, the solid product formed upon cooling in an ice bath, collected by filtration and then washed with water.

2-(2-*Amino*-5,6-*dihydro*-4*H*-*cyclopenta*[*b*]*thiophen*-3-*yl*)-5-(2-*phenylhydrazono*)*thiazol*-4(5*H*)-*one* (9*a*)

Orange crystals (1,4-dioxane), yield 2.91 g (85%), m.p. 144˚C - 147˚C; IR (KBr) (υ-cm^{-1}): 3490 - 3320 (NH, NH_2), 3056 (CH aromatic), 2872 (CH_2), 1686 (C=O), 1620 (C=N), 1580 (C=C). ^{1}H-NMR (DMSO-d_6) δ ppm: 1.26 - 1.59 (m, 6H, 3CH_2), 4.8 (s, 2H, NH_2), 6.83 - 7.46 (m, 5H, C_6H_5), 8.12 (s, 1H, NH). MS m/e = 342 (M^+, 18); Analy. Calcd. for: $C_{16}H_{14}N_4OS_2$: C, 56.12; H, 4.12; N, 16.36; S, 18.73%. Found, C, 56.23; H, 4.29; N, 16.47; S, 18.63%.

2-(2-*Amino*-5,6-*dihydro*-4*H*-*cyclopenta*[*b*]*thiophen*-3-*yl*)-5-(2-(4-*chlorophenyl*)*hydrazono*)*thiazol*-4(5*H*)-*one* (9*b*)

Orange crystals (1,4-dioxane), yield 3.20 g (85%), m.p. 188˚C - 190˚C; IR (KBr) (υ-cm^{-1}): 3478 - 3320 (NH, NH_2), 3054 (CH aromatic), 2872 (CH_2), 2200 (CN), 1690 (C=O), 1580 (C=C), 1530 (=N-NH). ^{1}H-NMR (DMSO-d_6) δ ppm: 1.61 - 1.72 (m, 6H, 3CH_2), 2.59 (s, 2H, NH_2), 7.38 - 7.42 (m, 4H, C_6H_4), 8.27 (s, 1H, NH), MS m/e = 376 (M^+, 60); Analy. Calcd. For: $C_{16}H_{13}ClN_4OS_2$: C, 50.99; H, 3.48; N, 14.87; S, 17.02%. Found, C, 50.72; H, 3.62; N, 14.62; S, 17.22%.

2-(2-*Amino*-5,6-*dihydro*-4*H*-*cyclopenta*[*b*`]*thiophen*-3-*yl*)-5-(2-(4-*methoxyphenyl*)*hydrazono*)*thiazol*-4(5*H*)-*one* (9*c*)

Brown crystals (1,4-dioxane) yield: 2.44 g (85%), m.p. 180˚C - 183˚C; IR (KBr) (υ-cm^{-1}): 3478 - 3328 (NH, NH_2), 3053 (CH aromatic), 2873 (CH_2), 2220 (CN), 1690 - 1685 (2C=O), 1603 (C=N), 1588 (C=C). ^{1}H-NMR (DMSO-d_6) δ ppm: 1.56 - 1.69 (m, 6H, cyclopentene, 3CH_2), 2.28 (s, 3H, CH_3), 4.58 (s, 2H, NH_2), 8.29 (s, 1H, NH), 7.28 - 7.43 (m, 4H, C_6H_4) MS m/e = 372 (M^+, 40); Analy. Calcd. for $C_{17}H_{16}N_4O_2S_2$: C, 54.82; H, 4.33; N, 15.04; S, 17.22%. Found, C, 54.66; H, 4.53; N, 15.42; S, 17.42%.

3-(2-(3-(4-*Oxo*-4,5-*dihydrothiazol*-2-*yl*)-5,6-*dihydro*-4*H*-*cyclopenta*[*b*]*thiophen*-2-*yl*)*hydrazono*)*pentane*-2,4-*dione* (11)

To a cold solution (0˚C - 5˚C) of acetyl acetone (1 mL) in ethanol (20 ml) containing sodium hydroxide (1.00 g) the diazotized 2-(2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophen-3-yl)thiazol-4-(5H)-one [which was prepared by adding $NaNO_2$ (0.70 g, 0.01 mol) solution to a cold solution of compound **4** (2.38 g, 0.01 mol) in acetic acid (20 mL), HCl (6 mL)] was gradually added while stirring, the solid product formed upon cooling in an ice bath, collected by filtration then washed with water and crystallized from acetic acid. Red crystals (1,4-dioxane), yield 2.79 g (80%), m.p. 199˚C - 202˚C; IR (KBr) (υ-cm^{-1}): 3498 - 3329 (NH), 2978 - 2850 (CH_3, CH_2), 2221 (CN), 1690 - 1669 (3C=O). ^{1}H-NMR (DMSO-d_6) δ ppm: 1.49 - 1.63 (m, 6H, cyclopentene, 3 CH_2), 2.62, 2.80 (2s, 6H, 2 CH_3), 5.08 (s, 2H, CH_2), 8.25 (s,1H, NH). MS m/e = 349 (M^+, 18); Anal. Calcd. for $C_{15}H_{15}N_3O_3S_2$: C, 51.56; H, 4.33; N, 12.03; S, 18.35%. Found, C, 51.42; H, 5.51; N, 12.27; S, 18.50%.

Ethyl 4,6-diamino-7-(4-oxo-4,5-dihydrothiazol-2-yl)-2,3-dihydro-1H-indene-5-carboxylate (12)

To a solution of compound **3** (2.06 g, 0.01 mol) in 1,4 dioxane (35 ml) containing triethylamine (1.00 ml), ethylcyanoacetate **6** (1.13 g, 0.01 mol) was added, the reaction mixture was heated under reflux for 45 mins, then cooled and neutralized by pouring onto ice/water mixture. Solid product formed was collected by filteration

and crystallized from 1,4-dioxane. Yellow crystals (1,4-dioxane) yield 3.19 g (65%), m.p. 120˚C - 122˚C; IR (KBr) (υ-cm^{-1}): 3477 - 3329 (2NH$_2$), 2969 (CH$_2$), 2220 (CN), 1989-1687 (3C=O), 1610 (C=C). ^{1}H-NMR (DMSO-d$_6$) δ ppm: 1.16 (t, 3H, ester CH$_3$), 1.54 - 1.72 (m, 6H, 3CH$_2$), 4.19 (s, 2H, NH$_2$), 4.24 (q, 2H, ester CH$_2$), 4.97 (s, 2H, NH$_2$), 6.01 (s, 2H, thiazole CH$_2$). MS m/e = 319 (M$^+$, 30); Anal. Calcd. for $C_{15}H_{17}N_3O_3S$: C, 56.41; H, 5.37; N, 13.16; S, 10.04%. Found, C, 56.32; H, 5.42; N, 12.94; S, 9.88%.

***Synthesis of diazotized* 2-*cyclohexylidene*-2-(4-*oxo*-4,5-*dihydrothiazol*-2-*yl*) *acetonitrile derivatives* (13*a-c*)**

General procedure:

To a cold solution of compound **3** (2.06 g, 0.01 mol) in ethanol (20 mL) containing sodium hydroxide (1.00 g), either of the diazo-4-methylaniline, diazo-4-chloroaniline ordiazo-4-methoxyaniline [which was prepared by adding $NaNO_2$ (0.70 g, 0.01 mol) solution to a cold solution of 4-methylaniline (1.07 g, 0.01 mol) 4-chloroaniline (1.27 g, 0.01 mol) or 4-methoxyaniline (1.23 g, 0.01 mol) in concentrated hydrochloric acid (6 ml)] was gradually added while stirring, the solid product formed upon cooling in an ice bath was collected by filtration, washed by water.

2-*Cyclopentylidene*-2-(4-*oxo*-5-(2-*phenylhydrazono*)-4,5-*dihydrothiazol*-2-*yl*)*acetonitrile* (13*a*)

Red crystals (1,4-dioxane), yield 2.05 g (66%), m.p. 177˚C - 179˚C; IR (KBr) (υ-cm^{-1}): 3488 - 3346 (NH), 3054 (CH aromatic) 2979 (CH$_2$), 2227 – 2220 (3CN), 1690 (C=O), 1620 (C=N), 1596 (C=C). ^{1}H- NMR (DMSO-d$_6$) δ ppm: 1.59 - 1.75 (m, 8H, 4 CH$_2$), 3.11 (s, 3H, CH$_3$), 7.29 - 7.38 (m, 4H, C$_6$H$_4$), 8.28 (s, 1H, NH). MS m/e = 324 (M$^+$, 28); Anal. Calcd. for $C_{17}H_{16}N_4OS$: C, 62.94; H, 4.97; N, 12.27; S, 9.88%. Found, C, 62.29; H, 4.86; N, 12.93; S, 10.02%.

2-(5-(2-(4-*Chlorophenyl*)*hydrazono*)-4-*oxo*-4,5-*dihydrothiazol*-2-*yl*)-2-*cyclopentylideneacetonitrile* (13*b*)

Brown crystals (1,4-dioxane), yield 2.24 g (65%), m.p. 210˚C - 212˚C; IR (KBr) (υ-cm^{-1}): 3498 - 3326 (NH), 3056 (CH aromatic), 2920 (CH$_2$), 2228 - 2220 (3CN), 1687 (C=O), 1610 (C=N), 1580 (C=C). ^{1}H-NMR (DMSO-d$_6$) δ ppm: 1.53 - 1.82 (m, 8H, 4CH$_2$), 6.29 - 7.38 (m, 4H, C$_6$H$_4$), 8.29 (s, 1H, NH); MS m/e = 344 (M$^+$, 28); Analy. Calcd. for $C_{16}H_{13}ClN_4OS$: C, 55.73; H, 3.80; N, 16.25; S, 9.30%. Found, C, 55.60; H, 3.72; N, 16.32; S, 9.26%.

2-*Cyclopentylidene*-2-(5-(2-(4-*methoxyphenyl*)*hydrazono*)-4-*oxo*-4,5-*dihydrothiazol*-2-*yl*)*acetonitrile* (13*c*)

Orange crystals (1,4-dioxane), yield 2.62 g (77%), m.p. 167˚C - 170˚C; IR (KBr) (υ-cm^{-1}): 3388 - 3340 (NH), 3050 (CH aromatic), 2850 - 2430 (CH$_3$, CH$_2$), 2200 (3CN), 1690 (C=O), 1580 (C=C), 1530 (C=N). ^{1}H- NMR (DMSO-d$_6$) δ ppm: 1.48 - 1.72 (m, 8H, 4CH$_2$), 3.11 (s, 3H, OCH$_3$), 7.28 - 7.39 (m, 4H, C$_6$H$_4$), 3.45 (s, 1H, NH). MS m/e = 340 (M$^+$, 18); Anal. Calcd. for $C_{17}H_{16}N_4O_2S$: C, 59.98; H, 4.74; N, 16.46; S, 9.42%. Found, C, 60.18; H, 4.62; N, 16.59; S, 9.33%.

General procedure for the synthesis of thiophene derivatives 15 and 17

To solution of compound **1** (1.40 g, 0.01 mol) in 1,4 dioxane (25 mL) containing triethylamine (1.0 mL), either cyanoacetanilide (1.60 g, 0.01 mol) or ethyl acetoacetate (1.30 g, 0.01 mol) was added followed by elemental sulfur (0.32 g, 0.01 mol) and the reaction mixture was heated under reflux for 2 h then poured onto ice/water mixture containing few drops of hydrochloric acid. The formed solid product, in each case, was then collected by filtration and crystallized from 1,4dioxane.

3,5-*Diamino*-4-(4-*oxo*-4,5-*dihydrothiazol*-2-*yl*)-*N*-*phenylthiophene*-2-*carboxamide* (15)

Dark crystals (1,4-dioxane), yield 2.26 g (70%), m.p. 223˚C - 226˚C; IR (KBr) (υ-cm^{-1}): 3428 - 3400 (2NH$_2$), 3056 (CH aromatic), 2978 (CH$_2$), 1723 - 1682 (2 C=O), 1577 (C=C). ^{1}H- NMR (DMSO-d$_6$) δ ppm: 4.35, 5.15 (2s, 4H, 2NH$_2$), 6.06 (s, 2H, thiazole CH$_2$), 7.28 - 7.43 (m, 5H, C$_6$H$_5$), 8.20 (s, 1H, NH). MS m/e = 332 (M$^+$, 20); Anal. Calcd. for $C_{14}H_{12}N_4O_2S_2$: C, 50.59; H, 3.64; N, 16.86; S, 19.29%. Found, C, 50.88; H, 3.83; N, 16.73; S, 19.32%.

***Ethyl* 5-*amino*-3-*methyl*-4-(4-*oxo*-4,5-*dihydrothiazol*-2-*yl*)*thiophene*-2-*carboxylate* (17)**

Yellow crystals (1,4-dioxane), yield 1.99 g (70%), m.p. 166˚C - 168˚C; IR (KBr) (υ-cm^{-1}): 3490, 3341(NH$_2$), 2980, 2880 (CH$_3$, CH$_2$), 1683, 1672 (2C=O). ^{1}H-NMR (DMSO-d$_6$) δ ppm: 1.13 (t, 3H, J = 6.97 Hz, CH$_3$), 2.61 (s, 3H, CH$_3$), 4.21 (q, 2H, J = 6.67 Hz, CH$_2$), 5.15 (s, 2H, NH$_2$), 6.18 (s, 2H, thaizole CH$_2$). MS m/e = 284(M$^+$, 20); Anal. Calcd. for $C_{11}H_{12}N_2O_3S_2$: C, 46.46; H, 4.25; N, 9.86; S, 22.55%. Found, C, 46.70; H, 4.28; N, 9.68; S, 22.71%.

General procedure for the synthesis of the hydrazide derivatives 19a, b

To a solution of compound **17** (2.84 g, 0.01 mol) in 1,4-dioxane (40 mL) either hydrazine hydrate (0.50 g, 0.01 mol) or phenylhydrazine (1.08 g, 0.01 mol) was added. The reaction mixture in each case was heated under reflux for 4 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product

was collected by filtration and crystallized from 1,4-dioxane.

5-Amino-3-methyl-4-(4-oxo-4,5-dihydrothiazol-2-yl)thiophene-2-carbohydrazide (19a)

Yellow crystals (1,4-dioxane), yield 0.43 g (16%), m.p. 122˚C - 125˚C; IR (KBr) (υ-cm^{-1}): 3490 - 3348 ($2NH_2$, NH), 2974, 2883 (CH_3, CH_2), 1689, 1682 (2C=O). ^{1}H-NMR (DMSO-d_6) δ ppm: 2.61 (s, 3H, CH_3), 4.56, 5.11 (2s, 4H, $2NH_2$), 6.18 (s, 2H, thaizole CH_2), 8.15 (s, 1H, NH). MS m/e = 270(M^+, 16); Anal. Calcd. for $C_9H_{10}N_4O_2S_2$: C, 39.99; H, 3.73; N, 20.73; S, 23.72%. Found, C, 40.21; H, 3.82; N, 20.81; S, 24.01%.

5-Amino-3-methyl-4-(4-oxo-4,5-dihydrothiazol-2-yl)-N'-phenylthiophene-2-carbohydrazide (19b)

Yellow crystals (1,4-dioxane), yield 2.28 g (66%), m.p. 142˚C - 145˚C; IR (KBr) (υ-cm^{-1}): 3484 - 3328 (NH_2, 2NH), 2980, 2881 (CH_3, CH_2), 1688, 1684 (2C=O), 1651 (C=N). ^{1}H-NMR (DMSO-d_6) δ ppm: 2.67 (s, 3H, CH_3), 4.58 (s, 2H, NH_2), 6.16 (s, 2H, thaizole CH_2), 7.35 - 7.38 (m, 5H, C_6H_5), 8.20, 8.29 (2s, 2H, 2NH). MS m/e = 346 (M^+, 28); Anal. Calcd. for $C_{15}H_{14}N_4O_2S_2$: C, 52.01; H, 4.07; N, 16.17; S, 18.51%. Found, C, 51.99; H, 3.93; N, 15.86; S, 18.44%.

5. Conclusion

We have reported a convenient synthesis of variety compounds from compound **1** to **19b** derivatives. The cytotoxicity of some derivatives towards three types of cancer cell lines was studied. Most of the synthesized compounds were found to be cytotoxic and hence deserve further pharmacological investigation. Compounds **3**, **5**, **9c**, **11**, **13a**, **13c**, **17** and **19b** were the most active compounds towards the three cancer cell lines. The results of these investigations will be published in due time.

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