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The Uses of Cyclopentanone for the Synthesis of Biologically Active Pyran, Pyridine and Thiophene Derivatives

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Abstract

In the recent work, a series of novel pyran and pyridine and thiophene derivatives were designed and synthesized starting from 2-benzylidenecyclopentanone. The reactivity of these derivatives towards different chemical reagent was studied. The antitumor evaluations of the newly synthesized products were measured and the results showed that some of the synthesized products showed high cytotoxicity.

Keywords

Cyclopentanone, Pyran, Pyridine, Pyrimidine

Subject Areas: **Medicinal Chemistry**

1. Introduction

Multicomponent reactions (MCRs), an important subclass of tandem reactions, are one-pot processes in which three or four easily approachable components react to form a single product. The methodology has emerged as a powerful synthetic tool for the preparation of biologically active compounds and important drugs [1] [2]. The multi-component reactions have been used frequently inorganic synthesis, and significant attempts have been focused on the design and development of environmentally friendly and less expensive methods for the generation of libraries of heterocyclic compounds [3] [4]. Therefore, academic and industrial research groups have increasingly focused on the development of MCRs that can lead to new, efficient synthetic methodologies to afford several biologically-active compounds. There has been considerable attention in syntheses reactions and biological activities of 4H-pyran-containing molecules. Furthermore, 4H-pyran derivatives also constitute a structural unit of some pharmaceutical agents, and natural products [5] [6]. The 2-amino-3-cyano-4H-pyran derivatives represent a significant class of compounds, viz. used in cosmetics and pigments and utilized as potentially

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biodegradable agrochemicals [7]. Additionally, several poly functionalized 4H-pyran derivatives have been reported to show a variety of biological activities such as antitumor [8] antibacterial [9] and antimicrobial activities [10]. These compounds are structurally similar to the anticancer agent MX58151 and inhibitors of insulin-regulated amino peptidase (IRAP) related to enhancement of memory and learning functions [11] (**Figure 1**). The 4H-pyran derivatives are also used as photoactive materials [12] and as synthetic intermediates for dihydrofurans [13]. In the present work, we are starting with cyclopentanone as the key starting material for the synthesis of pyran, pyridine, thiophene derivatives together with studying their cytotoxicity against six cancer and one human normal cell lines.

Here in, in order to extend our research on anticancer heterocyclic derivatives with high inhibitory effects toward some cancer cell lines, we report the synthesis of new fused pyran, pyridine, Thiophene derivatives derived from cycloprntanone **1**. Moreover, some newly synthesized products were good candidates as anticancer drugs through their screening towards cancer and normal cell lines.

2. Results and Discussion

The reaction of cyclopentanone with benzaldehyde, 4-nitrobenzaldehyde or 4-methylbenzaldehyde in the presence of piperidine in an oil bath at 120˚C gave the 2-arylidenecyclopentanone derivatives **3a-c**, respectively. The structures of the latter products were based on their respective analytical and spectral data. Thus, the ^{1}H NMR spectrum of **3c** showed δ1.58 - 2.78 (m, 6H, $3CH_2$), 3.130 (s, 3H, CH_3), 7.28 - 7.39 (m, 4H, C_6H_4), 7.61 (s, 1H, CH=C). Compounds **3a-c** reacted with malononitrile **4** in absolute ethanol containing a catalytic amount of triethylamine gave the pyran derivatives **5a-c**, respectively. The analytical and spectral data of the latter products were the basis of their structural elucidation. On the other hand, carrying the same reaction but using ammonium acetate instead of triethylamine gave the pyridine derivatives **6a-c**, respectively (**Scheme 1**).

Next, we studied the reactivity of compounds **3a-c** towards thiophene synthesis using the well-known Gewald's thiophene synthesis [14] [15]. Thus, the reaction of either of compounds **3a**, **3b** or **3c** with elemental sulfur and malononitrile**4** gave the thiophene derivatives **7a-c**, respectively. The analytical and spectral data of the latter product are consistent with their respective structures. Thus, the ^{1}HNMR spectrum of compound **7a** showed δ1.52 - 2.83 (m, 4H, $2CH_2$), 4.76 (s, 2H, D_2O exchangeable, NH_2), 7.21 (s, 1H, CH=C), 7.24 - 7.39 (m, 5H, C_6H_5). On the other hand, the reaction of either of compound **3a**, **3b** or **3c** with thiourea in an oil bath at 120˚C gave the 2-(arylidenecyclohexylidene)thiourea derivatives **9a-c**, respectively. Moreover, the multi-component reaction (MCR) of any of compound **3a**, **3b** or **3c** with thiourea and malononitrile in ethanol containing triethylamine gave the pyrimidine derivatives **10a-c**, respectively (**Scheme 2**). The structures of compounds **10a-c** were established on the basis of their analytical and spectral data. Thus, the ^{1}H NMR spectrum of compound **10a** showed δ1.39 - 2.84 (m, 6H, $3CH_2$), 3.84 (s, 2H, CH_2), 5.62 (s, 1H, SH), 6.70 (s, 1H, pyrimidine H-2), 7.28 - 7.40 (m, 5H, C_6H_5).

Next, we studied the reactivity of compounds **3b** and **3c** using ethyl cyanoacetate. Thus, the reaction of either of compound **3b** and **3c** reacted with ethyl cyanoacetate **11** in ethanol containing a catalytic amount of triethylamine gave the pyran derivatives **12a** and **12b**, respectively. On the other hand, the reaction of either **3b** or **3c** with ethyl cyanoacetate using ammonium acetate instead of triethylamine gave the pyridine derivatives **13a** and **13b**, respectively.

The reaction of compound **3c** with elemental sulfur and ethyl cyanoacetate **11** in ethanol containing a catalytic amount of triethylamine gave ethyl4-(methoxybenzylidene)-2-aminoctahydrobenzo[*b*]thiophene-3-carbo-xylate **14** (**Scheme 3**).

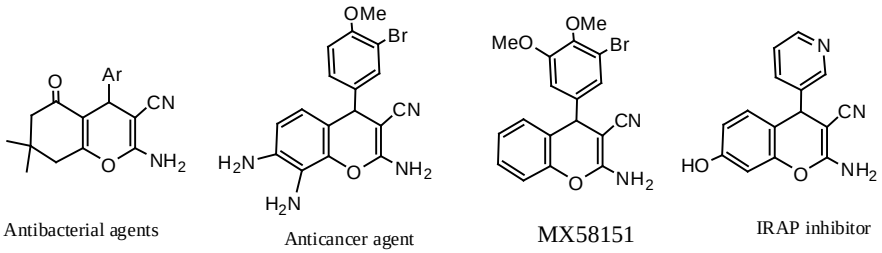
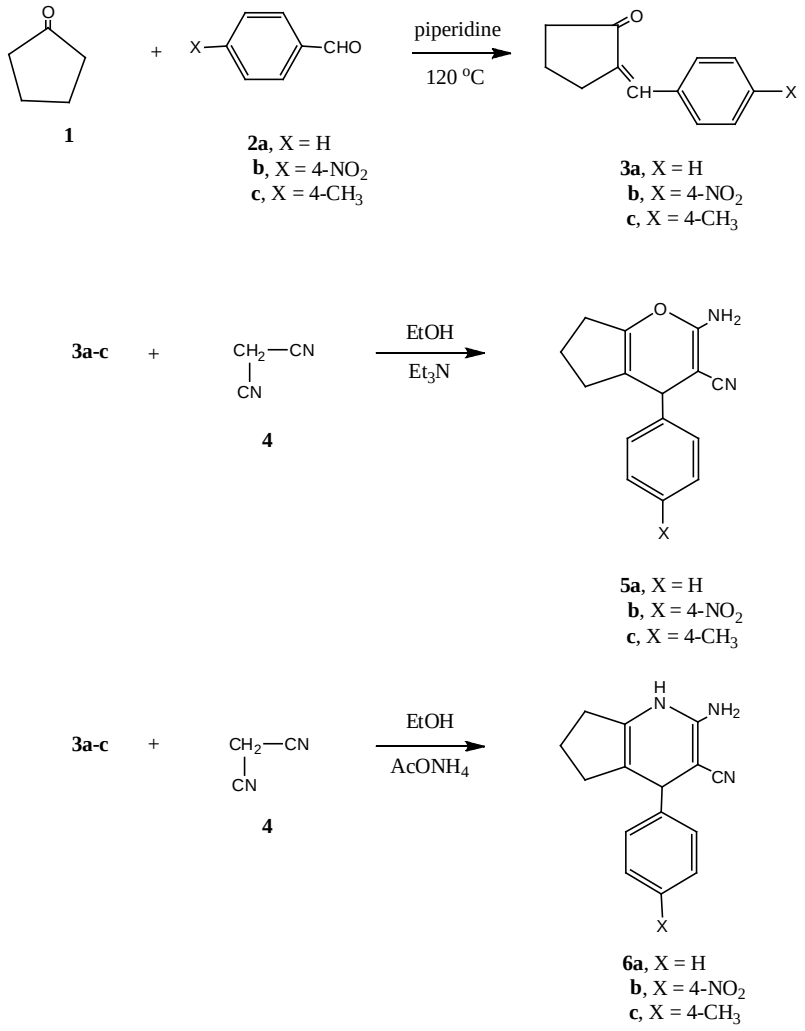


Figure 1. 2-Amino-3-cyano-4H-pyrans containing heterocycles demonstrating pharmacological and biological activity.



Scheme 1. Synthesis of compounds **3a-c**; **5a-c** and **6a-c**.

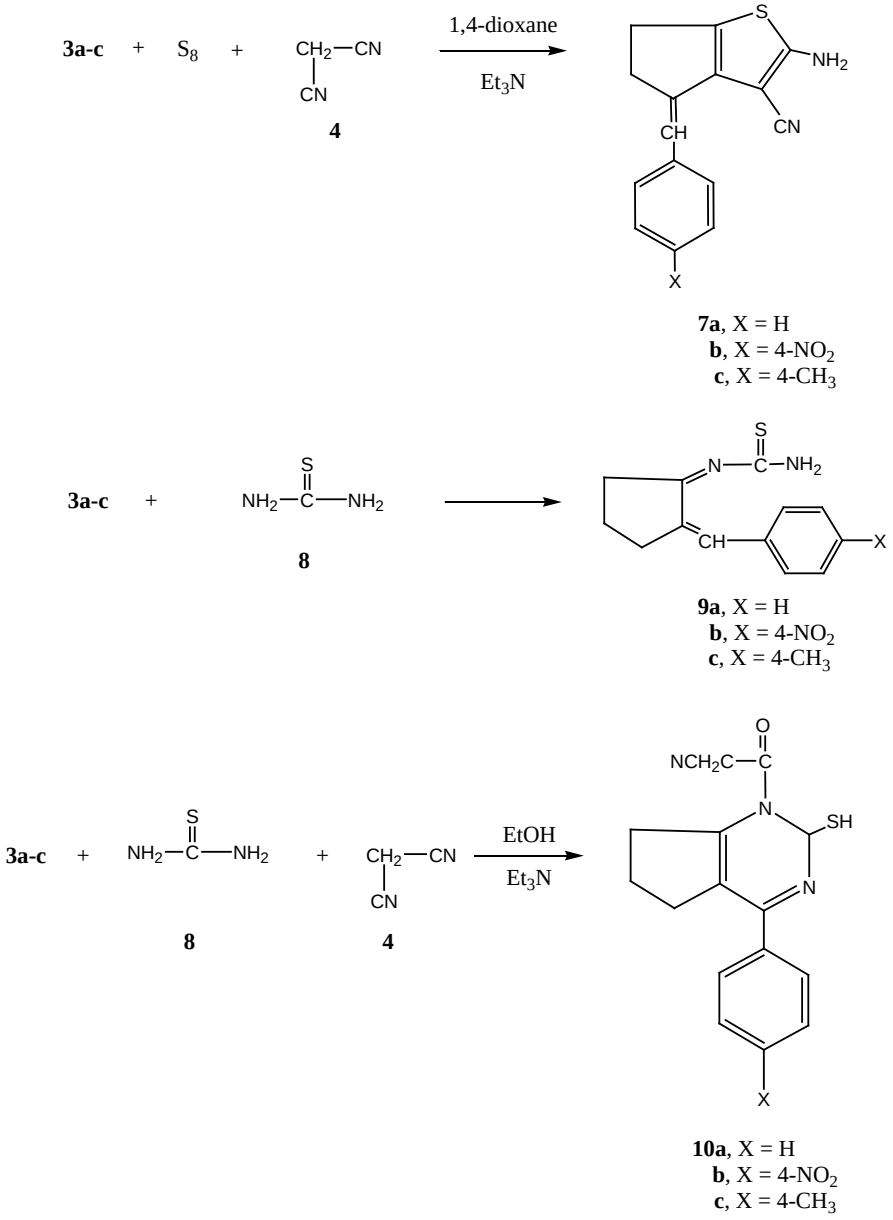
3. Biological Activities

3.1. Chemicals

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

3.2. Cell Cultures

The Cell cultures was obtained from the European Collection of cell Cultures (ECACC, Salisbury, UK) and human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) 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 six 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 maxi-



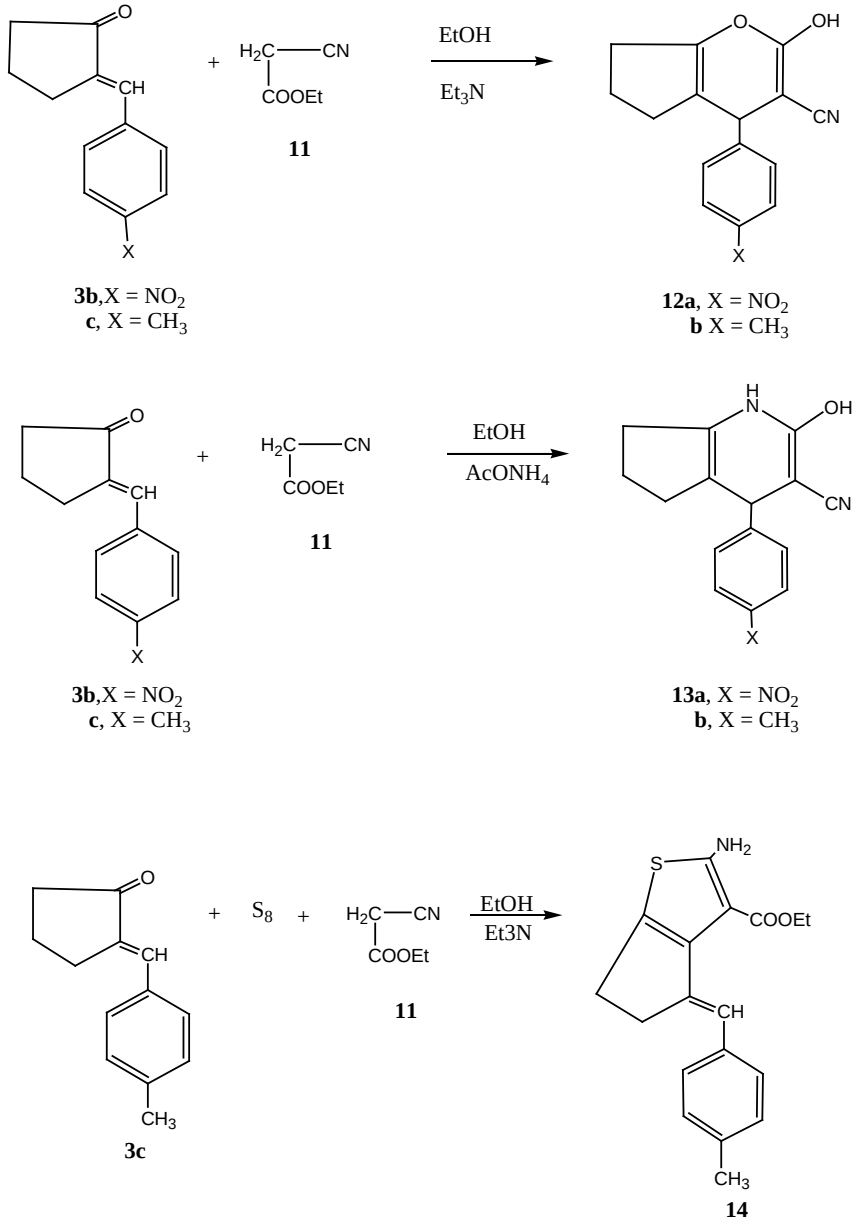
Scheme 2. Synthesis of compounds **7a-c**; **9a-c** and **10a-c**.

mum 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 six human cancer cell lines including cells derived from human gastric cancer (NUGC), human gastric cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and the 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 cytotoxicity against the tumor cell lines were evaluated through the National cancer Institute in Egypt obeying all ethical rules.

3.3. Structure Activity Relationship

From **Table 1**, it is clear that compounds **5a**, **5b**, **6c**, **7b**, **7c**, **9c**, **10c** and **14** are the most potent compounds



Scheme 3. Synthesis of compounds **12a**, **12b**, **13a**, **13b** and **14**.

among the tested compounds. It is clear that compounds **3a-c** showed low potency. Considering the pyran derivatives **5a-c**, it is clear that **5a** with the un-substituted phenyl group and **5b** with the 4-nitrosubstitutent are more potency than compound **5c** with the 4-methyl substituent. On the other hand, for the pyridine derivatives **6a-c** the 4-methyl substituent **6c** showed the higher potency than **6a** and **6b**. For the thiophene derivatives **7a-c**, it is clear that compounds **7b** and **7c** are more potent than **7a**. In addition, for compounds **9a-c**, it is obvious that the 4-methyl substituted compound **9c** is more potent than **9b** and **9c**. The pyrimidine derivatives **10a-c**, compound **10c** with the 4-nitro substituent showed the highest potency among the three compounds. The pyran **12a, b** and pyridines **13a, b** derivatives showed low potency toward the six cancer cell lines. The thiophene derivative **14** showed the maximum potency towards the six cancer cell lines among the tested compounds.

4. Experimental

All melting points determined on an Electrothermal digital meltig point apparatus and are uncorrected. IR spec-

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

Compound	Cytotoxocity (IC_{50} in nM)						
	NUGC	DLDI	HA22T	HEPG2	HONE1	MCF	WI38[c]
3a	1378	2393	2768	3298	2292	2472	Na
3b	3278	2283	2080	2772	2630	2049	Na
3c	1122	1274	2366	1096	1239	2145	Na
5a	190	105	99	2389	1153	2059	Na
5b	44	122	1764	1077	1184	1662	Na
5c	28	1349	1884	1870	1089	887	Na
6a	2376	2370	1259	1163	983	550	Na
6b	2548	2210	2672	1877	1603	1438	Na
6c	77	49	42	59	39	1106	Na
7a	3082	2180	2361	2360	1672	2036	Na
7b	149	113	1277	1398	108	92	Na
7c	48	1274	1449	329	323	120	Na
9a	1321	2318	1163	2318	218	241	Na
9b	2235	2662	2187	2962	1029	1392	Na
9c	893	1280	152	627	831	240	Na
10a	1641	2163	2117	3277	3219	1986	Na
10b	1264	1387	2218	2130	2058	2342	Na
10c	34	120	1276	329	1432	2893	Na
12a	1054	1083	2383	2196	1286	1142	Na
12b	1039	2024	1305	1440	1873	1873	Na
13a	1183	1082	1247	1408	1662	1482	Na
13b	1236	1290	1157	1195	1279	1243	Na
14	29	41	90	44	32	636	Na
CHS 828	25	2315	2067	1245	15	18	378

[a]NUGC, human gastric cancer, DLDI, colon cancer, HA22T, liver cancer, HEPG2, liver cancer; HONEI, nasopharyngeal carcinoma; MCF, breast cancer; WI38, normal fibroblast cells; [b]The sample concentration produces a 50% reduction in cell growth; [c]Na indicating no activity towards the normal cell line.

tra (KBr discs) were recorded on a FTIR plus 460 or Pyeunicam SP-1000 spectrophotometer. ^{1}H NMR spectra were recorded with Mercury-300BB (300 MHz) (Cairo university) instrument in DMSO-d_6 as solvent using TMS as internal standard and chemical shifts are expressed as δ ppm.

General procedure for synthesis of 2*-benzylidenecyclohexanone derivatives* **3*a-c***

Equimolar amounts of **1** (0.84 mL, 0.01 mol) and either benzaldhyde (1.06 g, 0.01 mol), p-nitrobenzaldhyde (1.52 g, 0.01mol) or p-methylbenzaldhyde (1.2 mL, 0.01 mol) containing a catalytic amount of piperidine (0.5 mL) was heated under reflux at 120˚C for 2 hours. The reaction mixture allowed to cool at room temperature and then poured onto ice/water. The mixture was neutralized by adding few drops of concentrated HCl. The solid productformed was collected by filtration and crystallized from ethanol.

2-(*benzylidene*)*cyclopentanone* (**3a**)

Yellow crystals, m.p. 76˚C, yield 64% (1.10 g) IR (KBr) (υ-cm^{-1}): 3056 (CH aromatic), 2876 (CH_2), 1688 (C=O), 1536 (C=C). ^{1}HNMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.49 - 2.46 (m, 6H, $3CH_2$), 7.00 - 7.67 (m, 5H, C_6H_5), 7.56 (s, 1H, CH=C). Analysis Calcd for $C_{12}H_{12}O$ (172.22): C, 83.69; H, 7.02. Found: C, 83.88; H, 7.29.

2-(4-*Nitrobenzylidene*)*cyclopentanone* (**3b**)

Yellow crystals, m.p. 70˚C, yield 62% (1.35 g) IR(KBr) (υ-cm^{-1}): 3104, 3062 (CH aromatic), 2988 (CH_2), 1669 (C=O), 1620 (C=C). ^{1}HNMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.52 - 2.24 (m, 6H, $3CH_2$), 7.33 - 7.46 (m, 4H, C_6H_4), 7.50 (s, 1H, CH=C). Analysis Calcd for $C_{12}H_{11}NO_3$ (217.22): C, 66.35; H, 5.10; N, 6.45. Found: C, 66.82; H, 5.29; N, 6.27.

2-(4-*Methylbenzylidene*)*cyclopentanone* (**3c**)

Yellow crystals, m.p. 58˚C, yield 77% (1.43 g) IR (KBr) (υ-cm^{-1}): 3058 (CH aromatic), 2878 (CH_2), 1690 (C=O), 1629 (C=C). ^{1}HNMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.58 - 2.78 (m, 6H, $3CH_2$), 3.130 (s, 3H, CH_3), 7.28 - 7.39 (m, 4H, C_6H_4), 7.61 (s, 1H, CH=C). Analysis Calcd for $C_{13}H_{14}O$ (186.25): C, 83.83; H, 7.58. Found: C, 83.62; H, 7.80.

General procedure for synthesis of 2-*amino*-5,6,7,8-*tetrahydro*-4-*phenyl chromene*-3-*carbonitrile derivatives* **5*a-c***

Equimolar amounts of malononitrile (0.66 g, 0.01 mol) and **3a** (1.72 g, 0.01 mol), **3b** (2.17 g, 0.01 mol) or **3c** (1.86 g, 0.01 mol) were dissolved in ethanol (28 mL) containing a catalytic amount of triethylamine and heated under reflux at 120˚C for 4 hours. The reaction mixture allowed to cool to room temperature and then poured onto ice/water mixture. The mixture was neutralized by adding a few drops of concentrated HCl. The solid product formed in each case was collected by filtration and crystallized from ethanol.

2-*Amino*-4-*phenyl*-4,5,6,7-*tetrahydrocyclopenta*[*b*]*pyran*-3-*carbonitrile* (**5*a***)

Yellow crystals, m.p. 133˚C - 136˚C, yield 80% (1.90 g). IR (KBr) (υ-cm^{-1}): 3459 - 3323 (NH_2), 3055 (CH aromatic), 2978 (CH_2), 2220 (CN). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.29 - 2.55 (m, 6H, $3CH_2$), 4.48 (s, 2H, NH_2, D_2O exchangeable), 6.28 (s, 1H, pyran H-4), 7.26 - 7.39 (m, 5H, C_6H_5). Analysis Calcd for $C_{15}H_{14}N_2O$ (238.28): C, 75.61; H, 5.92; N, 11.76. Found: C, 75.83; H, 6.29; N, 11.84.

2-*Amino*-4-(4-*nitrophenyl*)-4,5,6,7-*tetrahydrocyclopenta*[*b*]*pyran*-3-*carbonitrile* (**5*b***)

Brown crystals, m.p. 135˚C - 137˚C, yield 79% (2.24 g) IR (KBr) (υ-cm^{-1}): 3373 - 3329 (NH_2), 3060 (CH aromatic), 2921 (CH_2), 2222 (CN), 1638 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.78 - 2.29 (m, 6H, $3CH_2$), 4.68 (s, 2H, NH_2), 6.78 (s, 1H, pyran H-4), 7.25 - 7.42 (m, 4H, C_6H_4). Analysis Calcd for: $C_{15}H_{13}N_3O_3$ (283.28) Calcd: C, 63.60; H, 4.63; N, 14.83. Found: C. 63.88; H, 4.92; N, 14.68.

2-*Amino*-4-(*p-tolyl*)-4,5,6,7-*tetrahydrocyclopenta*[*b*]*pyran*-3-*carbonitrile* (**5*c***)

Orange crystals, m.p. 168˚C - 170˚C, yield 88% (2.22 g). IR (KBr) (υ-cm^{-1}): 3449 - 3432 (NH_2), 3054 (CHaromatic), 2210 (CN), 1630 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.18 - 2.39 (m, 6H, $3CH_2$), 4.79 (s, 2H, NH_2, D_2O exchangeable), 3.16 (s, 3H, CH_3), 6.27 (s, 1H, pyran H-4), 7.26 - 7.43 (m, 4H, C_6H_4). Analysis Calcdfor $C_{16}H_{16}N_2O$ (252.31): C, 76.16; H, 6.39; N, 11.10. Found: C, 76.29; H, 6.42; N, 10.98.

General procedure for synthesis of cyclopenta[*b*]*pyridinederivatives* (**6*a-c***)

Equimolar amount of malononitrile (0.66 g, 0.01 mol) and ammonium acetate (0.77 g, 0.01 mol) in ethanol (20 mL) was added to either **3a** (1.72 g, 0.01 mol), **3b** (2.17 g, 0.01 mol) or **3c** (1.86 g, 0.01 mol). The reaction mixture was heated under reflux at 120˚C for 4 hours, then allowed to cool to room temperature and poured onto ice/water mixture. The mixture was neutralized by adding few drops of concentrated HCl. The solid products formed was collected by filtration and crystallized from ethanol.

2-*Amino*-4-*phenyl*-4,5,6,7-*tetrahydro*-1*H*-*cyclopenta*[*b*]*pyridine*-3-*carbonitrile* (**6*a***)

Reddish brown crystals, m.p. 186˚C - 189˚C, yield 72% (1.71 g). IR(KBr) (υ-cm^{-1}): 3468, 3328 (NH_2, NH), 3055 (CHaromatic), 2986 (CH_2), 2220 (CN), 1633 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.38 - 2.59 (m, 6H, $3CH_2$), 4.28 (s, 2H, D_2O exchangeable, NH_2), 7.19 (s, 1H, pyridine H-4), 7.23 - 7.48 (m, 5H, C_6H_5), 8.32 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHZ) (δ-ppm): 28.2, 38.9, 44.05 ($4CH_2$), 116.4 (CN), 119.5, 120.6, 123.6, 124.4, 124.6, 129.2, 133.1, 134.4, 142.6 (C_6H_5, pyridine C). Analysis Calcd for $C_{15}H_{15}N_3$ (237.30): C, 75.92; H, 6.37; N, 17.71. Found: C, 76.22; H, 6.28; N, 17.72.

2-*Amino*-4-(4-*nitrophenyl*)-4,5,6,7-*tetrahydro*-1*H*-*cyclopenta*[*b*]*pyridine*-3-*carbonitrile* (**6*b***)

Yellow crystals, m.p. 166˚C - 168˚C, yield 79% (2.23 g). IR (KBr) (υ-cm^{-1}): 3429 - 3329 (NH_2, NH), 3060 (CH aromatic), 2979 (CH_2), 2220 (CN), 1634 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.39 - 2.70 (m, 6H, $3CH_2$), 4.70 (s, 2H, D_2O exchangeable, NH_2), 7.19 (s, 1H, pyridine H-4), 7.26 - 7.45 (m, 4H, C_6H_4), 8.42 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHZ (δ-ppm): 28.4, 43.6, 45.8 ($4CH_2$), 116.3 (CN),

120.8, 122.3, 122.9, 123.8, 125.9, 126.7, 129.2, 130.6, 131.6 (C_6H_5, pyridine).Analysis Calcd for $C_{15}H_{14}N_4O_2$ (282.30): C, 63.82; H, 5.00; N, 19.85. Found: C, 64.29; H, 5.26; N, 20.16.

2-*Amino*-4-(*p-tolyl*)-4,5,6,7-*tetrahydro*-1*H*-*cyclopenta*[*b*]*pyridine*-3-*carbonitrile* (**6*c***)

Yellow crystals, m.p. 155˚C - 158˚C, yield 80% (2.01 g). IR (KBr) (υ-cm^{-1}): 3488 - 3329 (NH_2, NH), 3055 (CH aromatic), 2978 (CH_2), 2220 (CN), 1629 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.49 - 2.80 (m, 6H, $3CH_2$), 3.11 (s, 3H, CH_3), 4.72 (s, 2H, D_2O exchangeable, NH_2), 7.11 (s, 1H, pyridine H-4), 7.24 - 7.43 (m, 4H, C_6H_4), 9.4 (s, 1H, D_2O exchangeable, NH). Analysis Calcd for $C_{16}H_{17}N_3$ (251.33): C, 76.46; H, 6.82; N, 16.72. Found: C, 76.59; H, 7.04; N, 16.93.

General procedure for synthesis of cyclopenta[*b*]*thiophene*-3-*carbonitrile derivative* (**7*a-c***)

Equimolar amount of malononitrile (0.66 g, 0.01 mol) and elemental sulfur (0.3 g, 0.01 mol) and either **3a** (1.72 g, 0.01 mol), **3b** (2.17 g, 0.01 mol) or **3c** (1.86 g, 0.01 mol) were dissolved in 1.4 dioxane (40 mL) containing a catalytic amount of triethylamine (0.50 mL). The whole reaction mixture, in each case was heated under reflux for 2 h. The reaction mixture allowed to cool to room temperature and then poured onto ice/water. The mixture was neutralized by adding a few drops of concentrated HCl. Solid products formed was collected by filtration and crystallized from 1,4dioxane.

2-*Amino*-4-*benzylidene*-5,6-*dihydro*-4*H*-*cyclopenta*[*b*]*thiophene*-3-*carbonitrile* (**7*a***)

Pale yellow crystals, m.p. 120˚C - 122˚C, yield 80% (2.02 g). IR (KBr) (υ-cm^{-1}): 3465 - 3312 (NH_2), 3058 (CH aromatic), 2974 (CH_2), 2220 (CN), 1633 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.52 - 2.83 (m, 4H, $2CH_2$), 4.76 (s, 2H, D_2O exchangeable, NH_2), 7.21 (s, 1H, CH=C), 7.24 - 7.39 (m, 5H, C_6H_5). Analysis Calcd for $C_{15}H_{12}N_2S$ (252.33): C, 71.40; H, 4.79; N, 11.10; S, 12.71. Found: C, 71.53; H, 4.93; N, 11.52; S, 12.89.

2-*Amino*-4-(4-*nitrobenzylidene*)-5,6-*dihydro*-4*H*-*cyclopenta*[*b*]*thiophene*-3-*carbonitrile* (**7*b***)

Pale yellow crystals, m.p. 177˚C - 179˚C, yield: 80% (2.38 g). IR (KBr) (υ-cm^{-1}): 3482 - 3320 (NH_2), 3054 (CH aromatic), 2991 (CH_2), 2220 (CN), 1632 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.43 - 2.87 (m, 4H, $2CH_2$), 4.53 (s, 2H, D_2O exchangeable, NH_2), 7.18 (s, 1H, C=CH), 7.26 - 7.39 (m, 4H, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHZ (δ-ppm): 26.2, 35.8 ($2CH_2$), 116.3 (CN), 91.3, 92.6 (CH=C), 120.8, 122.4, 123.1, 123.9, 125.3, 128.4, 129.4, 130.8 (C_6H_5, thiophene). Analysis Calcd for $C_{15}H_{11}N_3O_2S$ (297.33): C, 60.59; H, 3.73; N, 14.13; S, 10.78. Found: C, 60.72; H, 3.94; N, 14.06; S, 10.88.

2-*Amino*-4-(4-*methylbenzylidene*)-5,6-*dihydro*-4*H*-*cyclopenta*[*b*]*thiophene*-3-*carbonitrile* (**7*c***)

Orange crystals, m.p. 188˚C - 191˚C, yield: 77% (2.05 g). IR (KBr) (υ-cm^{-1}): 3429 - 3313 (NH_2), 3054 (CH aromatic), 2979 (CH_2), 2220 (CN), 1629 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.39 - 2.87 (m, 4H, $2CH_2$), 3.13 (s, 3H, CH_3), 4.84 (s, 2H, D_2O exchangeable, NH_2), 7.21 (s, 1H, C=CH), 7.27 - 7.39 (m, 4H, C_6H_4). Analysis Calcd for $C_{16}H_{14}N_2S$ (266.36) C, 72.15; H, 5.30; N, 10.52; S, 12.04. Found: C, 71.88; H, 5.42; N, 10.31; S, 11.93.

General procedure for synthesis of 2-*cyclopentylidene*) *thiourea derivatives* (**9*a-c***)

Equimolar amount of thiourea (0.76 g, 0.01 mol), either of **3a** (1.72 g, 0.01 mol), **3b** (2.17 g, 0.01 mol) or **3c** (1.86 g, 0.01 mol) were dissolved in ethanol (25 mL) containing a catalytic amount of triethylamine and heated under reflux for 2 hours. The reaction mixture allowed to cool to room temperature and then poured onto ice/water mixture. The mixture was neutralized by adding few drops of concentrated HCl. The solid product formed was collected by filtration, crystallized from ethanol.

2-*Benzylidene cyclopentylidenethiourea* (**9*a***)

Yellow crystals, m.p. 137˚C - 139˚C, yield 73% (1.60 g). IR (KBr) (υ-cm^{-1}): 3467 - 3324 (NH_2), 3055 (CH aromatic), 2983 (CH_2), 1630 (C=C). ^{1}H NMR (δ-ppm): 1.44 - 2.73 (m, 6H, $3CH_2$), 4.49 (s, 2H, D_2O exchangeable, NH_2), 7.05 (s, 1H, C=CH), 7.25 - 7.41 (m, 5H, C_6H_5). ^{13}C NMR (DMSO-d_6, 75 MHZ (δ-ppm): 28.2, 45.8, 46.2 ($4CH_2$), 89.3, 90.6 (CH=C), 120.8, 121.3, 125.2, 127.8 (C_6H_5), 167.2 (C=S), 173.1 (C=N). Analysis Calcd for $C_{13}H_{14}N_2S$ (230.33): C, 67.79; H, 6.13; N, 12.16; S, 13.92. Found: C, 67.84; H, 5.83; N, 11.92; S, 14.11.

4-*Nitrobenzylidene*)*cyclopentylidenethiourea* (**9*b***)

Orange crystals, m.p. 244˚C - 248˚C, yield: 66% (1.82 g). IR (KBr) (υ-cm^{-1}): 3480 - 3322 (NH_2), 3060 (CH aromatic), 2979 (CH_2), 1620 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.39 - 2.88 (m, 6H, $3CH_2$), 4.72 (s, 2H, NH_2), 7.16 (s, 1H, CH=C), 7.22 - 7.67 (m, 4H, C_6H_4). Analysis Calcd for $C_{13}H_{13}N_3O_2S$ (275.33):C, 56.71; H, 4.76; N, 15.26; S, 11.65. Found: C, 56.53; H, 4.93; N, 15.42; S, 11.82.

2-(4-*Methylbenzylidene*)*cyclohexylidenethiourea* (**9*c***)

Brown crystals, m.p. 220˚C - 223˚C, yield: 67% (1.63 g). IR (KBr) (υ-cm^{-1}): 3480 - 3329 (NH_2), 3045 (CH

Aromatic), 2975 (CH_2), 1629 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.40 - 2.69 (m, 6H, $3CH_2$), 3.10 (s, 3H, CH_3), 4.60 (s, 2H, D_2O exchangeable, NH_2), 7.18 (s, 1H, CH=C), 7.23 - 7.41 (m, 4H, C_6H_4), Analysis Calcd for $C_{14}H_{16}N_2S$ (244.36): C, 68.81; H, 6.60; N, 11.46; S, 13.12. Found: C, 68.93; H, 6.73; N, 11.58; S, 13.49.

General procedure for synthesis of 3-(2-*mercapto*-2,5,6,7-*tetrahydro*-1*H*-*cyclopenta*[*d*]*pyrimidin*-1-*yl*)-3-*oxo-propane nitrile derivatives* (**10*a-c***)

Equimolar amount of thiourea (0.76 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol) and any of **3a** (1.72 g, 0.01 mol), **3b** (2.17 g, 0.01 mol), or **3c** (1.86 g, 0.01 mol) were dissolved in ethanol (25 mL) containing a catalytic amount of triethylamine and heated under reflux for 5 h. The reaction mixture allowed to cool to room temperature and then poured onto ice/water mixture. The mixture was neutralized by adding few drops of concentrated HCl. The solid product formed upon cooling was collected by filtration and crystallized from ethanol.

3-(2-*Mercapto*-4-*phenyl*-2,5,6,7-*tetrahydro*-1*H*-*cyclopenta*[*d*]*pyrimidin*-1-*yl*)-3-*oxopropanenitrile* (**10*a***)

Yellow crystals, m.p. 180˚C - 183˚C, yield: 69% (2.05 g). IR (KBr) (υ-cm^{-1}): 3058 (CH aromatic), 2984 (CH_2), 2222 (CN), 1697 (C=O), 1645 (C=N), 1630 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.39 - 2.84 (m, 6H, $3CH_2$), 3.84 (s, 2H, CH_2), 5.62 (s, 1H, SH), 6.70 (s, 1H, pyrimidine H-2), 7.28 - 7.40 (m, 5H, C_6H_5). Analysis Calcd for $C_{16}H_{15}N_3OS$ (297.37): C, 64.62; H, 5.08; N, 14.13; S, 10.78. Found: C, 64.91; H, 5.26; N, 14.37; S, 10.94.

3-(2-*Mercapto*-4-(4-*nitrophenyl*)-2,5,6,7-*tetrahydro*-1*H*-*cyclopenta*[*d*]-*pyrimidin*-1-*yl*)-3-*oxopropanenitrile* (**10*b***)

Yellow crystals, m.p. 194˚C - 196˚C, yield: 93% (3.18 g). IR (KBr) (υ-cm^{-1}): 3056 (CH aromatic), 2893 (CH_2), 2220 (CN), 1690 (C=O), 1644 (C=N), 1631 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.49 - 2.83 (m, 6H, $3CH_2$), 3.85 (s, 2H, CH_2), 4.62 (s, 1H, SH), 6.03 (s, 1H, pyrimidine H-2), 7.21 - 7.44 (m, 4H, C_6H_4). Analysis Calcd for $C_{16}H_{14}N_4O_3S$ (342.37): C, 56.13; H, 4.12; N, 16.36; S, 9.37. Found: C, 56.22; H, 4.32; N, 16.08; S, 9.28.

3-(2-*Mercapto*-4-(*p-tolyl*)-2,5,6,7-*tetrahydro*-1*H*-*cyclopenta*[*d*]*pyrimidin*-1-*yl*)-3-*oxopropanenitrile* (**10*c***)

Orange brown crystals, m.p.177˚C - 179˚C, yield: 85% (2.64 g). IR (KBr) (υ-cm^{-1}): 3060 (CH aromatic), 2987 (CH_2), 2221 (CN), 1669 (C=O), 1645 (C=N), 1630 (C=C). ^{1}H NMR (δ-ppm): 1.39 - 2.82 (m, 6H, $3CH_2$), 3.14 (s, 3H, CH_3), 3.48 (s, 2H, CH_2), 4.62 (s, 1H, SH), 6.30 (s, 1H, pyrimidine H-2), 7.24 - 7.49 (m, 4H, C_6H_4). Analysis Calcd for: $C_{17}H_{17}N_3OS$ (311.40): C, 65.57; H, 5.50; N, 13.49; S, 10.30. Found: C, 65.77; H, 5.39; N, 13.72; S, 10.26.

General procedure for synthesis of 2-*hydroxy*-4,5,6,7-*tetrahydro*-1*H*-*cyclopenta*[*b*]*pyran*-3-*carbonitrile derivatives* (**12*a,b***)

Equimolar amount of ethyl 2-cyanoacetate (1.13 mL, 0.01 mol) **3b** (2.17 g, 0.01 mol), or **3c** (1.86 g, 0.01 mol) were dissolved in ethanol (25 mL) containing a catalytic amount of triethylamine and heated under reflux for 45 min in first case and for 3 hours in second case. The reaction mixture allowed to cool to room temperature and then poured onto ice/water mixture. The mixture was neutralized by adding concentrated HCl. The solid product formed was collected by filtration, crystallized from ethanol.

2-*Hydroxy*-4-(4-*nitrophenyl*)-4,5,6,7-*tetrahydrocyclopenta*[*b*]*pyran*-3-*carbonitrile* (**12*a***)

Yellow crystal, m.p. 180˚C - 183˚C. 69% (1.96g). IR (KBr) (υ-cm^{-1}): 3328 (OH), 3055 (CH aromatic), 2977 (CH_2), 2220 (CN), 1632 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.51 - 2.83 (m, 6H, $3CH_2$), 5.80 (s, 1H, pyran H-4), 7.25 - 7.39 (m, 4H, C_6H_4), 10.29 (s, 1H, D_2O exchangeable, OH). Analysis Calcd for $C_{15}H_{12}N_2O_4$ (284.27): C, 63.38; H; 4.25; N, 9.85. Found: C, 63.49; H, 4.33; N, 9.59.

2-*Hydroxy*-4-(*p-tolyl*)-4,5,6,7-*tetrahydro*-1*H*-*cyclopenta*[*b*]*pyran*-3-*carbonitrile* (**12*b***)

Yellow crystals, m.p. 111˚C - 113˚C. yield 80% (2.02 g). IR (KBr) (υ-cm^{-1}): 3544 - 3329 (OH), 3055 (CH aromatic), 2980 (CH_2), 2210 (CN). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.41 - 2.65 (m, 6H, $3CH_2$), 3.11 (s, 3H, CH_3), 7.03 (s, 1H, pyran H-4), 7.26-7.58 (m, 4H, C_6H_4), 10.22 (s, 1H, OH). Analysis Calcd for $C_{16}H_{15}NO_2$ (253.30): C, 75.87; H, 5.97; N, 5.53. Found: C, 75.58; H, 6.21; N, 5.80.

General procedure for synthesis of 4-1,4,5,6,7,8-*hexahydroquinoline*-3-*carbonitrile* (**13*a,b***)

Equimolar amounts of ethyl 2-cyanoacetate (1.13 mL, 0.01 mol) and any of 2-(4-*nitrobenzylidene*) *cyclopentanone* (2.17 g, 0.01 mol) or 2-(4-*Methylbenzylidene*) *cyclopentanone* (1.86 g, 0.01 mol) were dissolved in ethanol (25 mL) containing catalytic amount of ammonium acetate (0.77 gm, 0.01 mol) and heated under reflux at 100˚C for 2 hours. The reaction mixture allowed to cool to room temperature and then poured onto ice/water mixture. The mixture was neutralized by adding few drops of concentrated HCl. The solid product formed was

collected by filtration, crystallized from ethanol.

2-*Hydroxy*-4-(4-*nitrophenyl*)-4,5,6,7-*tetrahydro*-1*H*-*cyclopenta*[*b*]*pyridine*-3-*carbonitrile* (**13*a***)

Yellow crystals, m.p. 210˚C - 214˚C. yield: 70% (1.98 g). IR(KBr) (υ-cm^{-1}): 3522 - 3312 (OH, NH), 3051 (CH aromatic), 2986 (CH_2), 2220 (CN), 1634 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.49 - 2.80 (m, 6H, 3CH_2), 5.99 (s, 1H, pyridineH-4), 7.30-7.39 (m, 4H, C_6H_4), 8.29 (s, 1H, D_2O exchangeable, NH), 10.22 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHZ (δ-ppm): 26.9, 41.4, 44.3 (3CH_2), 116.7 (CN), 120.2, 121.4, 123.1, 125.3, 125.8, 126.2, 129.4, 155.8, 157.3 (C_6H_4, pyran).Analysis Calcdfor $C_{15}H_{13}N_3O_3$ (283.28): C, 63.60; H; 4.63; N, 14.83. Found: C, 63.49; H, 4.74; N, 15.02.

2-*Hydroxy*-4-(*p*-*tolyl*)-4,5,6,7-*tetrahydro*-1*H*-*cyclopenta*[*b*]*pyridine*-3-*carbonitrile* (**13*b***)

Yellow light crystals, m.p. 160˚C. yield: 85% (2.14 g). IR (KBr) (υ-cm^{-1}): 3442 - 3315 (OH, NH), 3003 (CH aromatic), 2937 - 2830 (CH_2), 2210 (CN). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.23 - 1.57 (m, 6H, 3CH_2), 3.81 (s, 3H, CH_3), 7.00 (s, 1H, pyridine H-4), 7.00 - 7.58 (m, 4H, C_6H_4), 8.29 (s, 1H, D_2O exchangeable, NH), 10.22 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHZ) (δ-ppm): 30.1, 45.6, 49.5, 50.5 (4CH_2), 96.07 (OCH_3), 117.6 (CN), 120.5, 120.6, 124.6, 129.2, 129.8, 133.1, 134.5, 140.3, 145.2 (C_6H_4, pyridine C). Analysis Calcd for $C_{16}H_{16}N_2O$(252.31): C, 76.16; H, 6.39; N, 11.10. Found: C, 76.26; H, 6.42; N, 10.84.

*Synthesis of ethyl*4-(*methoylbenzylidene*)-2-*amino*-*octa*-*hydrobenzo*-[*b*]*thiophene*-3-*carboxylate* (**14**)

Equimolar amount of 2-(4-methylbenzylidene) cyclopentanone **3c** (1.86 g, 0.01 mol), elementals sulfur (0.32 g, 0.01 mol) and ethyl 2-cyanoacetate (1.16 mL, 0.01 mol) were dissolved in ethanol (20 mL) containing catalytic amount of triethylamine and heated under reflux for 2 h. The reaction mixture allowed to cool to room temperature and then poured onto ice/water mixture. The mixture was neutralized by adding few drops of concentrated HCl. The solid product formed was collected by filtration, crystallized from ethanol.

Yellow crystals, m.p. 222˚C - 225˚C, yield: 77% (2.53 g). IR (KBr) (υ-cm^{-1}): 3469 - 3319 (NH_2), 3059 (CH aromatic), 2986 (CH_2), 1703 (C=O), 1610 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHZ) (δ-ppm): 1.13 (t, 3H, J = 7.22 Hz, CH_3), 2.49 - 2.59 (m, 4H, 2CH_2), 3.09 (s, 3H, CH_3), 4.21 (q, 2H, J = 7.22 Hz, CH_2), 4.29 (s, 2H, D_2O exchangeable, NH_2), 7.21 - 7.38 (m, 5H, CH=C, C_6H_4). Analysis Calcd for $C_{18}H_{19}NO_2S$ (313.41): C, 68.98; H, 6.11; N, 4.47; S, 10.23. Found: C, 68.57; H, 6.04; N, 4.49; S, 9.89.

5. Conclusions

Our results showed that the electronegative NO_2 and CN hydrophobic groups in the Compounds might play a very important role in enhancing the cytotoxic effect.

In summary, we have developed a convenient synthetic approach to 26 samples. The regioselective attack by different reagents on the active center moiety led to the diversity of the produced systems, CHNS Elemental analyses, IR, ^{1}H NMR spectral data. The cytotoxicity of the newly synthesized products showed that the thiophene derivative **14** showed the maximum cytotoxicity among the tested compounds.

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