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Synthesis and Cytotoxicity of Heterocyclic Compounds Derived from Cyclohexane-1,3-Dione

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Abstract

Cyclohexane-1,3-dione (1) was used as a template to develop new anticancer compounds. The ring modification of compound 1occurred through its reaction with either aromaticaldehydes or benzenediazonium chloride to produce the corresponding products. The latter compounds underwenttheterocyclization reactions through the reaction with elemental sulfur and some active methylene reagents to produce tetrahydrobenzo[*b*]thiophene derivatives. The reaction of compound 1 with elemental sulfur and phenylisothiocyanategave the tetrahydrobenzo[*d*]thiazole derivative. The cytotoxicity of the newly synthesized products against human cancer and normal cell lines was evaluated. Some compounds showed high cytotoxicity against cancer cell lines. The results showed that compounds 3b, 5c, 7b, 10b, 12, 14b, 16, 18b, 19b, 20b, 21 and 24 showed the highest cytotoxicity. Moreover, the toxicity of twelve active compounds were measured.

Keywords

Cyclohexane-1,3-Dione, Thiophene, Thiazole, Pyridazine, Cytotoxicity

Subject Areas: **Biochemistry, Biotechnology**

1. Introduction

As typical reactive 1,3-dicarbonyl compounds, cyclohexane-1,3-dione and its analogy 5,5-dimethyl cyclohexane-1,3-dione(dimedone) have been widely used in versatile synthetic reactions [1]-[5]. Cyclohexan-1,3-dione is not only a typical reagent for Knoevenagel condensation, but also adds easily to electron-deficient alkenes *via* Michael addition. On the other hand, its one or two carbonyl groups could take part in substitution and cyclization reactions through the tautomerizedenolate form. Thus, the cascade reactions of addition, elimination and

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substitution could be achieved in many reactions involving cyclohexan-1,3-dione moiety. On the other hand, 1,3-dicarbonyl derivatives constitute important synthetic intermediates, incorporating multiple functionalities that can be involved either as nucleophilic or electrophilic species in a large variety of synthetic transformations [6]-[8]. Moreover, cyclohexane-1,3-dione derivatives play an important role in organic synthesis due to their usefulness in the preparation of many biologically important compounds [9]. Many herbicides having cyclohexane-1,3-dione backbone such as tralkoxydim, sethoxydim orclethodim are well known [10] [11]. In previous work we were interested in the design, screening, synthesis and biological evaluation of tetrahydrobenzo[*b*]thiophene as anti-cancer agents [12]. In addition, it has been reported that some heterocyclic derivatives with different substituents showed high potency [13] [14]. In an attempt to obtain an antitumor agent with high activity, the substitution pattern at positions 1 and 2 of the cyclohexan-1,3-dione to produce 2-cyclohexene-1-one pharmacophore [15] [16] was selected in order to alter the electronic environment and thus affect the lipophilicity of the target molecules. Thus, in the present work we are demonstrating the uses of cyclohexan-1,3-dione to synthesis fused thiophene, pyran and pyridazine derivatives incorporated 2-cyclohexene-1-one moiety with varieties of functional groups followed by the evaluation of the newly synthesized products towards human cancer and normal cell lines.

2. Results and Discussion

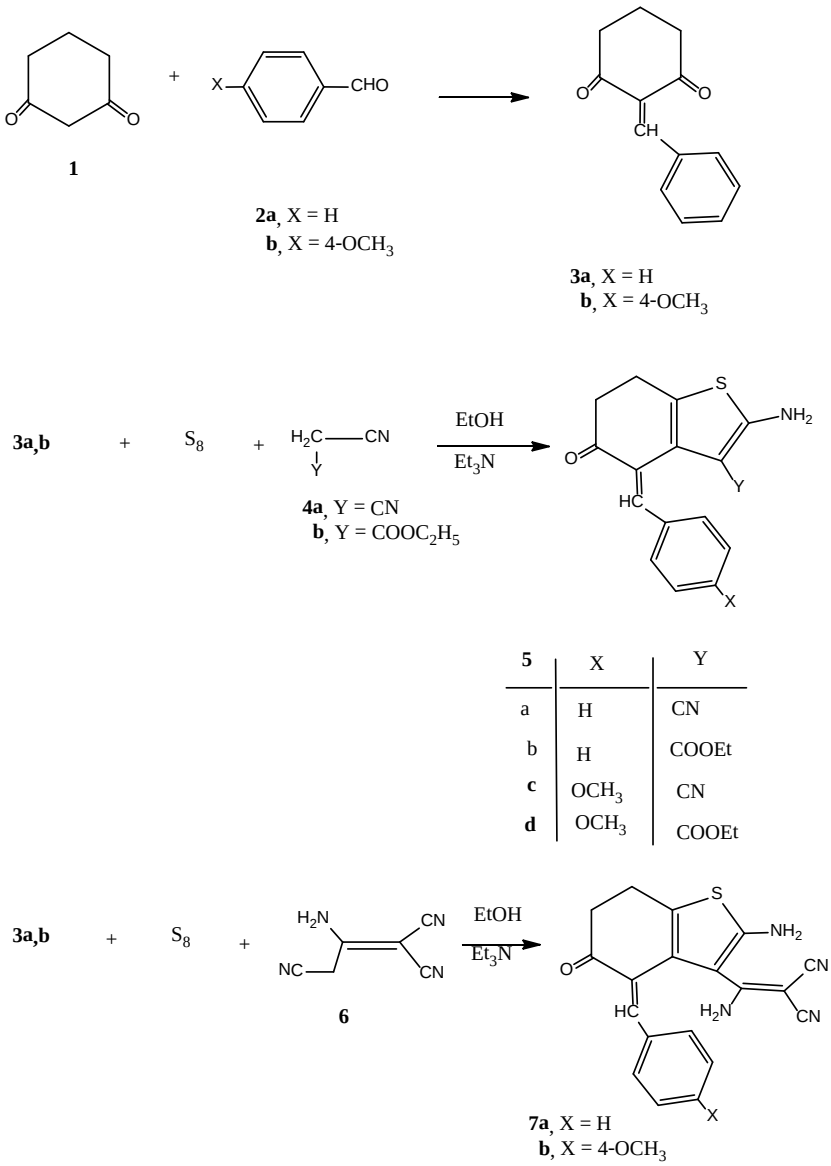
Herein, in order to extend our research on anticancer heterocyclic derivatives with high inhibitory effects toward some cancer cell lines, we report the synthesis of new fused thiophene, thiazole, pyran, pyridazineand 1,2,4-triazine derivatives derived from cyclohexane-1,3-dione (**1**). Moreover, some the newly synthesized products were good candidates as anticancer drugs through their screening towards cancer and normal cell lines. Thus, the reaction of compound **1** with either benzaldehyde or 4-methoxybenzaldehyde gave the corresponding arylidene derivatives **3a**, **b**. Structures of the latter products were established based on analytical and spectral data. Compounds **3a** and **3b** were good candidates for many heterocyclization reactions. Thus, the reaction of eithercompounds **3a** and **3b** with elemental sulfur and either malononitrile or ethyl cyanoacetate gave the tetrahydrobenzo [*b*]thiophene derivatives **5a-d**, respectively. Formation of the latter products was explained in terms of the well known Gewald's thiophene synthesis [17]. The ^{1}H NMR and ^{13}C NMR were the basis for the structure elucidation of compounds **5a-d**.

Similarly the reaction of either **3a** or **3b** with elemental sulfur and the 2-aminoprop-1-ene-1,1,3-tricarbonitrile (**6**) gave the tetrahydrobenzo[*b*]thiophene derivatives **7a** and **7b**, respectively (**Scheme 1**). The analytical and spectral data of **7a** and **7b** were in agreement with their respective structures (see experimental section).

On the other hand, the reaction of cyclohexan-1,3-dione with elemental sulfur and the 2-aminoprop-1-ene-1, 1,3-tricarbonitrile(**6**) gave the tetrahydrobenzo-[4,5]thieno[2,3-*b*]pyridine derivative **9**. Compound **9** was formed via the intermediate formation of **8** followed the Michael addition of the NH_2 group to one of the two CN groups.

4*H*-Benzo[*b*]pyran derivatives form an important class of heterocyclic compounds having remarkable pharmaceutical and biological activities. Therefore, the *4H*-benzo[*b*]pyrans received significant amount of attention from pharmaceutical and organic chemistry communities. The commonly used method for the synthesis of 4*H*-benzo[*b*]pyrans is the condensation of aldehyde, cinnamonitrile derivatives and carbonyl compounds in the presence of acidic or basic catalysts. With optimized reaction condition in hand, our trials to synthesis pyran derivatives using another reaction route starting from compounds **3a** and either of malononitrile (**4a**) or ethyl cyanoacetate (**4b**) gave the tetrahydrobenzo[*b*]pyran derivatives **10a**, **10b**; respectively (**Scheme 2**). The analytical and spectral data of compounds **10a**, **10b** were in analogy with their respective structures. Further confirmations for structures of **10a**, **10b** were obtained through their synthesis via another reaction route. Thus, the reaction of cyclohexan-1,3-dione with either *α*-cyanocinnamonitrile (**11a**) or ethyl *α*-cyanocinnamate (**11b**) gave the same products **10a**, **10b** (same m.p., mixed m.p. and fingerprint IR).

Cyclohexan-1,3-dione (**1**) reacted with 2-aminoprop-1-ene-1,1,3-tricarbonitrile (**6**) in absolute ethanol solution containing triethylamineto give the tetrahydronaphalene-2-one derivative **12**. Formation of the latter product is explained in terms of the first water elimination followed by cyclization. On the other hand, the reaction of either compound **3a** or **3b** with the 2-aminoprop-1-ene-1,1,3-tricarbonitrile (**6**) in ethanol solution containing triethylamine gave tetrahydronaphthalene derivatives **14a** and **14b**, respectively. The most important feature to confirm the structure of **14a** (as an example) through the ^{1}H NMR spectrum that showed, beside the cyclohex-

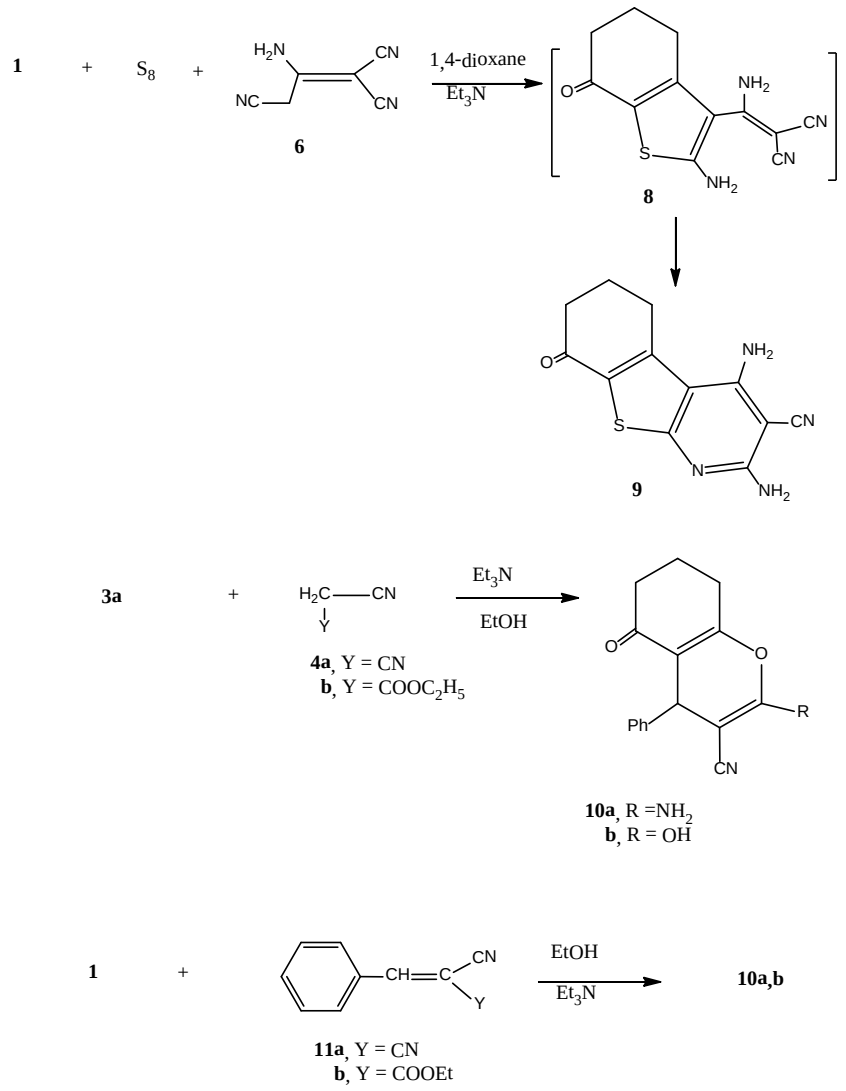


Scheme 1. Synthesis of compounds **3**a,b; **5**a-d and **7**a,b.

ene and the phenyl moieties signals, two singlets (D_2O exchangeable) at δ4.33, 5.01 ppm for the two NH_2 groups and the ^{13}C NMR spectrum showed δ: 20.6, 36.8 (2 CH_2), 95.4, 95.3 (CH=C), 110.2 (CH=C), 116.8, 117.3 (2 CN), 127.7, 128.8, 128.9, 129.0, 129.2, 129.8, 140.7 (two benzene C), 196.4 (C=O).

The reaction of the cyclohexane-1,3-dione with elemental sulfur and phenylisothiocyanate (**15**) in 1,4-dioxane solution containing triethylamine give the 3-phenyl-2-thioxo-2,3,5,6-tetrahydrobenzo[*d*]thiazol-7(4*H*)-one (**16**) (**Scheme 3**). The structure of the latter product was confirmed on the basis of ^1H NMR and ^{13}C NMR (see experimental section).

Next we moved through studying the reactivity of the cyclohexan-1,3-dione (**1**) towards arylhydrazone derivatives followed heterocyclization of the reaction products in the aim of producing new anticancer agents. Thus, the reaction of compound **1** with either benzenediazonium chloride (**17a)** or 4-chlorobenzenediazonium chloride (**17b**) gave the arylhydrazone derivatives **18a** and **18b**, respectively. The high yield of compound **18a** encouraged us to use it for further chemical reactions. In fact compound **18b** was synthesized in order to study the effect of the 4-chloro group through its cytotoxicity compared with **18a**. The reaction of compound **18a** with either malononitrile (**4a**) or ethyl cyanoacetate (**4b**) in the presence of ammonium acetate in an oil bath 120˚C gave the

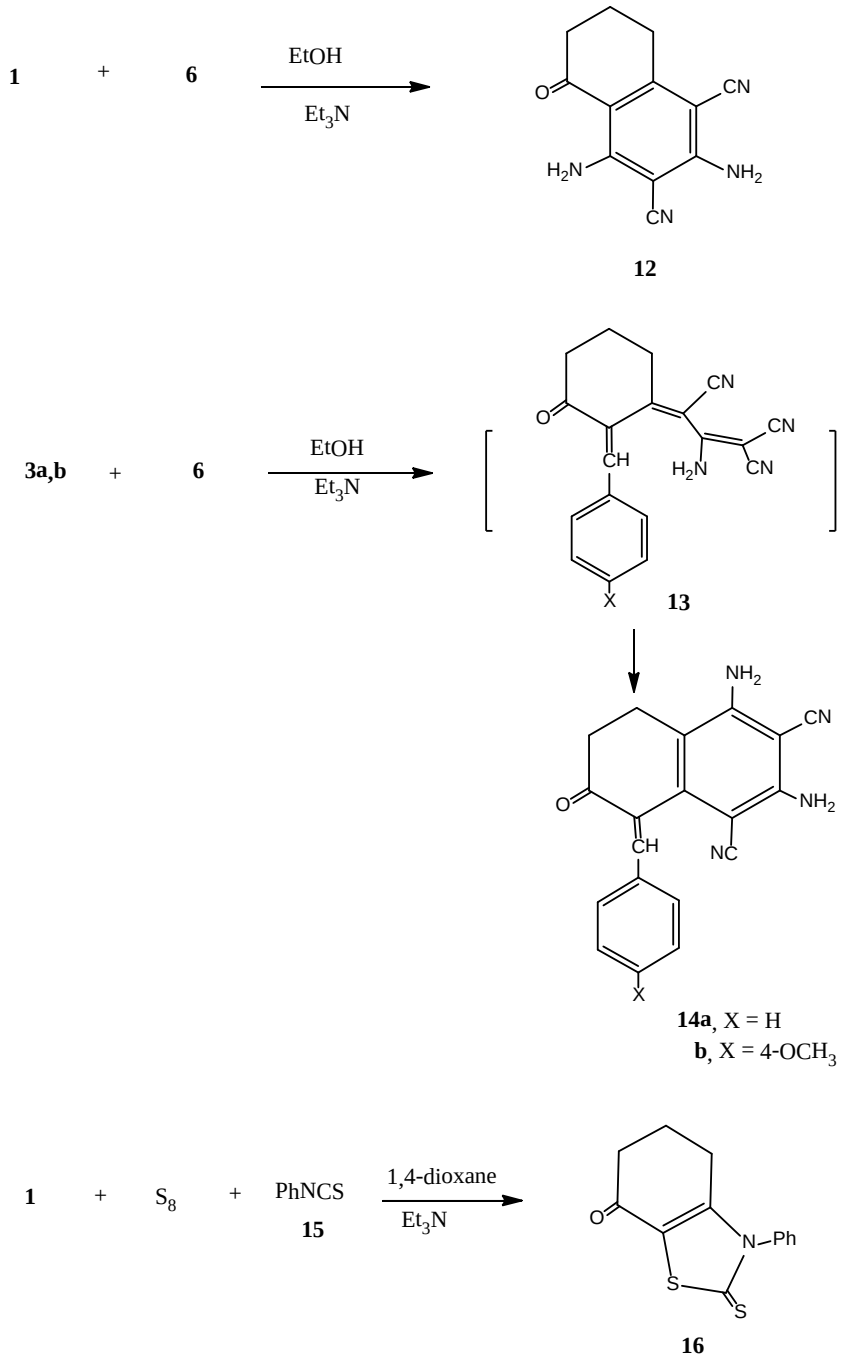


Scheme 2. Synthesis of compounds **9**, **10**b.

Knoevenegal condensation products **19a**, **19b**, respectively. On the other hand, carrying the same reaction, but using 1,4-dioxane and triethylamine gave the 2,3,5,6,7,8-hexahydrocinnoline derivatives **20a**, **20b**; respectively (**Scheme 4**). Formation of the latter products were explained in terms of the intermediate formation of **19a**, **19b** followed by cyclization.

The reactivity of **18a** towards thiophene synthesis applying the method of Gewald's thiophene was studied. Thus, the reaction of compound **18a** with malononitrile (**4a**) and elemental sulfur in 1,4-dioxane containing a catalytic amount of triethylamine gave the cyclohexene[*b*]thiophenederivarive **21**. On the other hand, the reaction of **18a** with ethyl cyanoacetate (**4b**) and elemental sulfur gave the annulated derivative **22**. The structure of the latter product was assigned based on the elemental analysis and other spectroscopic data (see experimental section).

The reaction of compound **18a** with phenylisothiocyanate (**13**) gave cyclohexene[*e*]triazine derivative **23**. Finally we studied the reactivity of compound **18a** with 2-aminoprop-1-ene-1,1,3-tricarbonitrile (**6**) using different reaction conditions. Thus, the reaction of **18a** with compound **6** in the presence of ammonium acetate at 120˚C gave the cyclohexene[*b*]pyridazine **24** derivative. On the other hand, carrying the same reaction but in 1,4-dioxane containing a catalytic amount of triethylamine gave the 5,6,7,8-tetrahydronaphthalene derivative **25** (**Scheme 5**).

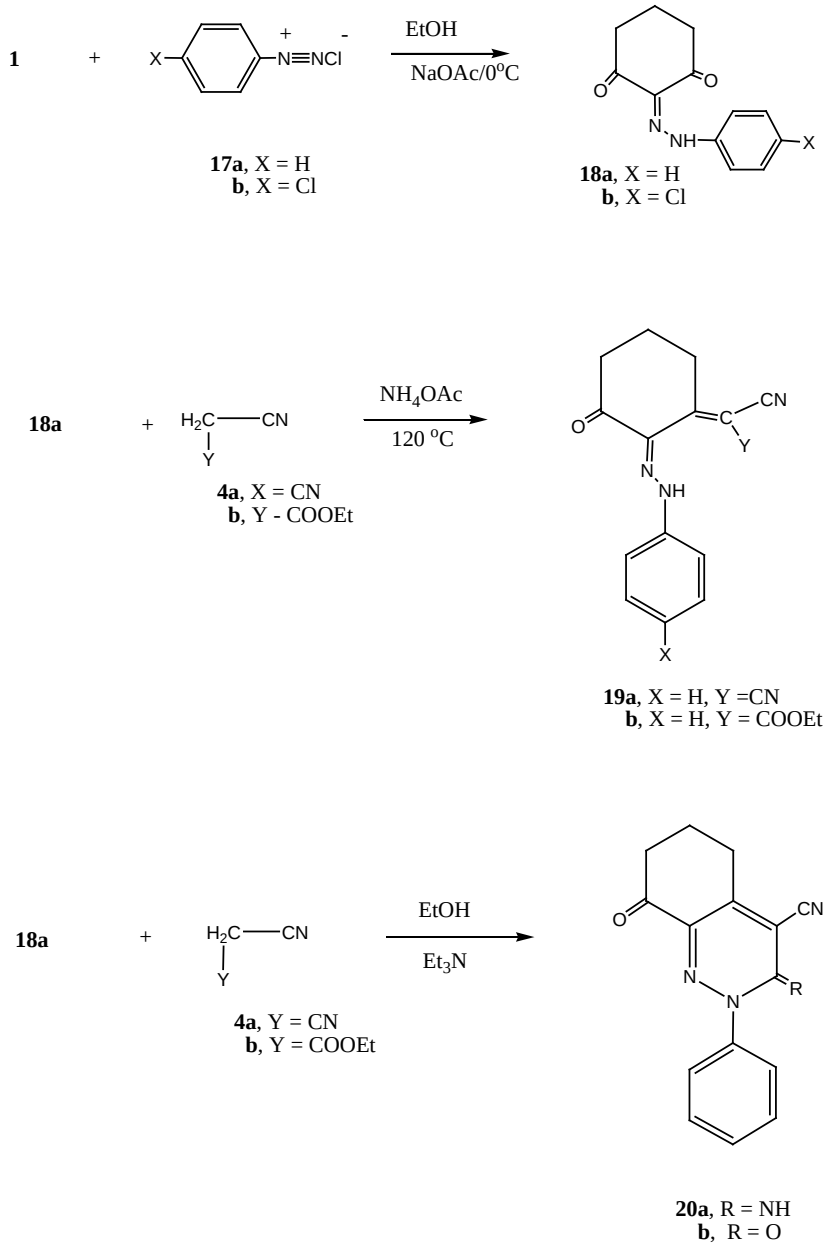


Scheme 3. Synthesis of compounds **12**, **14**a,b and **16**.

3. *In Vitro* Cytotoxic Assay

3.1. Chemicals

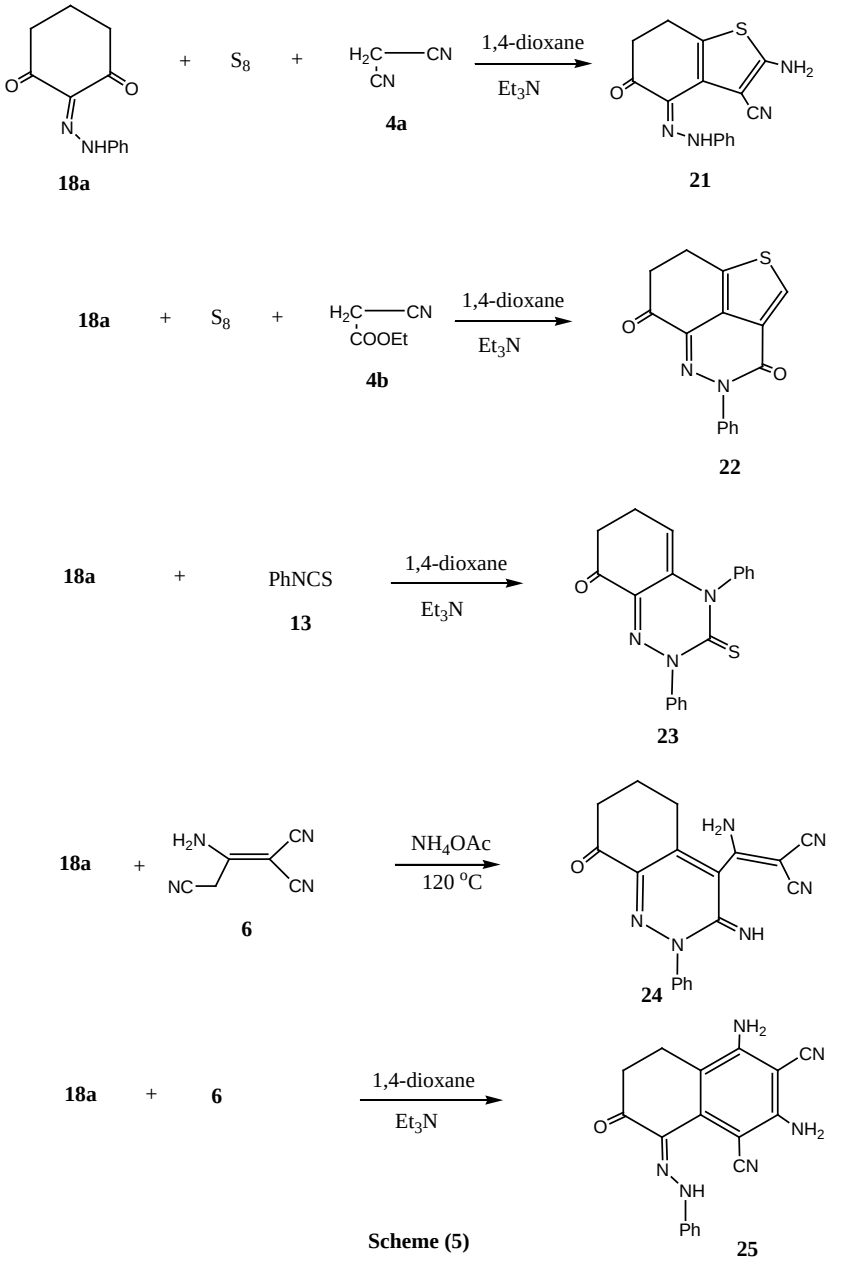
Fetal bovine serum (FBS) and L-glutamine, were purchased from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was purchased from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (Saint Louis, USA).



Scheme 4. Synthesis of compounds **18**a,b; **19**a,b and **20**a,b.

3.2. Cell Cultures

The cell cultures were obtained from the European collection of cell cultures (ECACC, Salisbury, UK) and human gastric cancer (NUGC and HR), 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 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.



Scheme (5)

Scheme 5. Synthesis of compounds **21-25**.

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 colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) 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 (IC_{50} > 10,000 nM). The reference compound used is the CHS-828 which is a pyridylcyanoguanidine antitumor agent.

3.3. Structure Activity Relationship

From **Table 1** it is clear that the cyclohexene moiety was found to be crucial for the cytotoxic effect of the cyclic compounds **3a**, **3b-25**. Compounds **3b**, **5c**, **7b**, **10b**, **12**, **14b**, **16**, **18b**, **19b**, **20b**, **21** and **24** exhibited optimal

Table 1. Cytotoxicity of the newly synthesized products against a variety of cancer cell lines [IC_{50}[b] (nM)].

	Compd Cytotoxocity (IC_{50} in nM)						
	NUGC	DLDI	HA22T	HEPG2	HONE1	MCF	WI38
3a	3342	4220	3310	1328	2824	3243	380
3b	84	46	233	250	220	338	422
5a	2101	1380	2258	2166	2180	2330	512
5b	1135	1240	1278	2359	1266	2555	128
5c	38	46	120	337	441	180	320
5d	1222	3390	2063	2440	2177	3230	882
7a	2228	2342	2100	655	2528	2260	640
7b	122	30	59	270	1140	1160	260
9	2322	1159	2253	3370	2326	2270	380
10a	2265	1220	2257	3228	2250	2220	466
10b	338	232	228	28	84	2224	589
12	1180	3268	2560	2128	3330	1180	280
14a	1235	3160	2061	3218	1186	1693	380
14b	2120	2240	1120	2130	2348	2254	120
16	22	38	48	20	33	73	320
18a	3270	1690	1155	2320	440	2657	540
18b	1355	160	290	221	2229	2332	631
19a	2280	2455	1884	2562	2310	3148	128
19b	1480	1150	1140	1328	1260	1140	220
20a	1148	2163	3063	2232	1480	3860	890
20b	620	255	760	520	2088	1264	634
21	120	441	262	350	472	325	552
22	1145	3210	3218	2276	2672	2711	493
23	3320	2366	2781	3744	1589	1130	650
24	38	46	122	320	480	226	380
25	1153	2439	2670	1266	3200	3266	283
CHS 828	25	2315	2067	1245	15	18	378

[a]NUGC, gastric cancer; DLDI, colon cancer; HA22T, liver cancer; HEPG2, liver cancer; HONEI, nasopharyngeal carcinoma; HR, gastric cancer; MCF, breast cancer; WI38, normal fibroblast cells. [b]The sample concentration produces a 50% reduction in cell growth. CHS-828 is a pyridylcyanoguanidine anti-tumor agent. [c]IC_{50} against the normal fibroblast cells (WI38) are indicated as multiples of 10^4 nM.

cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range. Comparing the cytotoxicity of the benzylidenecyclohexane **3a** and **3b**, it is obvious that the cytotoxicity of **3b** is higher than that of **3a**. The presence of the 4-OCH_3 group in **3b** is responsible for its high potency. Considering the 4,5,6,7-tetrahydrobenzo[*b*]thiophene derivatives **5a-d**, it is clear that the cytotoxicity of **5c** is higher than those of **5a**, **5b** and **5d**. Such high cytotoxicity of **5c** is attributed to the presence of the 4-OCH_3 aryl moiety together with the 3-cyanothiophene moiety. The high cytotoxicity of compound **7b** relative to compound **7a** is also explained in terms of the presence of the 4-OCH_3 aryl moiety. On the other hand, by considering the 5,6,7,8-tetrahydro-4*H*-chromene derivatives **10a**, **10b** it is clear that the presence of the 2-hydroxy group present in **10b** is responsible for its high potency. The 5,6,7,8-tetrahydronaphthalene derivatives **12** and **14a**, **14b** showed low cytotoxicity effect towards the six cancer cell lines.

The 3-phenyl-2-thioxo-2,3,5,6-tetrahydrobenzo[*d*]thiazol-7(4H)-one(**16**) showed the maximum cytotoxicity among the tested compounds, this is attributed to the presence of the thiazole, thioxo moieties. Considering

arylhydrazone derivatives **18a** and **18b**, it is noticed that **18a** showed high cytotoxicity against HONE1 with IC_{50} 440 nM. On the other hand the 4-chlorophenyl derivative **18b** showed high cytotoxicity against the three cancer cell lines DLDI, HA22T and HEPG2 with IG_{50}'s of 160, 290 and 212 nM, respectively. The high oxygen content present in compounds **19b** and **20b** is responsible for the high cytotoxicity of such compounds over the low oxygen content compounds **19a** and **20a** (**Scheme 4**). Finally considering the two isomeric compounds **24** and **25** it is clear that the hexahydrocinnnolin-4-yl **24** has higher cytotoxicity against the six cancer cell lines than the tetrahydronaphthalene derivative **25**. Such high cytotoxicity of **24** is attributed to the presence nitrogen rich pyridazine moiety. From **Table 1** it is noticed that all tested compounds showed very low cytotoxicity towards the normal cell line WI38 and such cytotoxicity levels are multiples of 10^{-4} nM.

4. Toxicity

Bioactive compounds are often toxic to shrimp larvae. Thus, in order to monitor these chemicals' *in vivo* lethality to shrimp larvae (*Artemiasalina*), Brine-Shrimp Lethality Assay [18] [19] was used. Results were analyzed with LC_{50} program to determine LC_{50} values and 95% confidence intervals [20]. Results are given in **Table 2** for the compounds which exhibited optimal cytotoxic effect against cancer cell lines which are the twelve compounds **3b**, **5c**, **7b**, **10b**, **12**, **14b**, **16**, **18b**, **19b**, **20b**, **21** and **24**. The shrimp lethality assay is considered as a useful tool for preliminary assessment of toxicity, and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacteria toxins, pesticides, and cytotoxicity testing of dental materials [21], natural and syntheticorganic compounds. It has also been shown that, *A. salina* toxicity test results have a correlation with rodent and human acute oral toxicity data. Generally, a good correlation was obtained between *A. salina* toxicity test and the rodent data. Likewise, the predictive screening potential of the aquatic invertebrate tests for acute oral toxicity in man, including *A. salina* toxicity test, was slightly better than the rat test for test compounds [22].

In order to prevent the toxicity results from possible false effects originated from solubility of compounds and DMSO's possible toxicity effect, compounds were prepared by dissolving in DMSO in the suggested DMSO volume ranges. It is clear from **Table 2** that the 3-phenyl-2-thioxo-2,3,5,6-tetrahydrobenzo[d]thiazol-7(4H)-one (**16**) and the 2-amino-5-oxo-4-(2-phenylhydrazono)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carbonitrile (**21**) showed non toxicity against the tested organisms.

5. Experimental

^{13}C NMR and ^{1}H NMR spectra were recorded on Bruker DPX400 instrument in DMSO with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (ppm). Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were carried out by the Microanalytical Data Unit Ludwig-Maximilians-Universitat-Munchen, Germany. The progress of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck).

2-*Benzylidenecyclohexane*-1,3-*dione* (**3a**) *and* 2-(4-*methoxybenzy-lidene*)-*cyclohexane*-1,3-*dione* (**3b**)

General procedure: To a solution of cyclohexan-1,3-dione (1.12 g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine (0.50 mL) either benzaldehyde (1.06 g, 0.01 mol) or p-methoxybenzaldehyde (1.36 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 2 h then poured onto ice water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

Compound **3a**: White crystals (EtOH), yield 86% (1.60 g), mp 205˚C - 207˚C. IR (KBr) cm^{-1}: 3067, 1722, 1664, 1605. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.82 - 1.88 (m, 4H, $2CH_2$), 2.24 - 2.26 (m, 2H, CH_2), 6.68 (s, 1H, CH), 7.29 - 7.38 (m, 5H, C_6H_5). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 22.1, 25.7, 39.6 ($3CH_2$), 110.0 (CH), 124.1, 124.9, 128.7, 139.5 (C_6H_5), 139.9 (C=CH), 181.1, 189.5 (2C=O). EIMS m/z 200 $[M]^+$ (15); Analysis Calcd for $C_{13}H_{12}O_2$ (200.23): C, 77.98; H, 6.04. Found: C, 78.21; H, 6.33.

Compound **3b**: Pale yellow crystals (EtOH), yield 82% (1.90 g), mp 218˚C - 220˚C. IR (KBr) cm^{-1}: 3023, 2954, 2889, 1722, 1644, 1605. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.62 - 1.93 (m, 4H, $2CH_2$), 2.24 - 2.26 (m, 2H, CH_2), 3.68 (s, 3H, CH_3), 6.68 (s, 1H, CH), 7.24 - 7.36 (2d, 4H, J = 4.68 Hz, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 22.2, 25.9, 39.4 (3 CH_2), 36.6 (CH_3), 110.1 (CH), 124.0, 124.9, 128.8, 139.6 (C_6H_5), 139.6 (C=CH), 181.0, 189.3 (2C=O). EIMSm/z 230 $[M]^+$ (20); Analysis Calcd for $C_{14}H_{14}O_3$ (230.26): C, 73.03; H, 6.13%. Found: C, 73.31; H, 6.47%.

Table 2. Toxicity assay.

Compound No.	Cons. (µg/ml)	Mortality[a]	Toxicity	LC_{50}	Upper 95% lim.	Lower 95% lim.
3a	10 100 1000	0 3 10	Harmful	316.23	255.25	111.43
5c	10 100 1000	0 4 8	Harmful	17.78	860.12	180.30
7b	10 100 1000	0 5 10	Harmful	251.19	70.22	4.25
10b	10 100 1000	0 2 10	Harmful	115.85	250.37	88.20
12	10 100 1000	0 0 5	Harmful	100.00	104.2	157.62
14b	10 100 1000	0 1 10	Harmful	87.78	320.40	-
16	10 100 1000	0 0 3	Non-toxic	960.27	-	-
18b	10 100 1000	5 10 10	Very toxic	10.00	-	-
19b	10 100 1000	4 10 10	Very toxic	15.19	-	-
20b	10 100 1000	0 2 8	Harmful	622.45	130.20	157.40
21	10 100 1000	0 0 5	Non-toxic	1000.0	-	-
24	10 100 1000	0 5 8	Harmful	614.68	650.37	166.20

[a]Ten organisms (*A. salina*) tested for each concentration.

2-*Amino*-4-*benzylidene*-5-*oxo*-4,5,6,7-*tetrahydrobenzo*[*b*]*thiophene*-3-*carbonitrile* (**5a**), *ethyl* 2-*amino*-4-*benzylidene*-5-*oxo*-4,5,6,7-*tetrahydrobenzo*[*b*]*thiophene*-3-*carboxylate* (**5b**), 2-*amino*-4-(*p-methoxybenzylidene*)-5-*oxo*-4,5,6,7-*tetrahydrobenzo*[*b*]*thiophene*-3-*carbonitrile* (**5c**) *and ethyl* 2-*amino*-4-(*p-methoxybenzylidene*)-5-*oxo*-4,5,6,7-*tetrahydrobenzo*[*b*]*thiophene*-3-*carboxylate* (**5d**)

General procedure: To a solution of either **3a** (2.00 g, 0.01 mol) or **3b** (2.30 g, 0.01 mol) in absolute ethanol (50 mL) containing triethylamine (0.50 mL) and elemental sulfur (0.32 g, 0.01 mol) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture, in each case was heated under reflux for 2 h then was left to cool and the formed solid product, in each case, was collected by filtration.

Compound **5a**: Yellow crystals (EtOH), yield 77% (2.16 g), mp 138˚C - 140˚C. IR (KBr) cm^{-1}: 3466, 3365, 3055, 2948, 2222, 1720, 1657. ^1H NMR (DMSO, 400 MHz): δ = 1.84 - 1.93 (m, 2H, CH_2), 2.26 - 2.28 (m, 2H, CH_2), 4.55 (s, 2H, D_2O exchangeable, NH_2), 7.16 (s, 1H, CH), 7.19 - 7.27 (m, 5H, C_6H_5). ^{13}C NMR (DMSO, 75

MHz) δ: 23.6, 28.8 (CH_2), 110.2 (CH=C), 121.8 (CN), 124.1, 125.8, 128.4, 129.2, 130.3, 137.3, 140.2 (C_6H_5, thiophene C), 147.5 (C=CH), 163.8 (C=O). EIMS m/z280 $[M]^+$ (22); Analysis Calcd for $C_{16}H_{12}N_2OS$ (280.34): C, 68.55; H, 4.31; N, 9.99; S, 11.44. Found: C, 68.77; H, 4.29; N, 10.32; S, 11.61.

Compound **5b**: Yellow crystals (EtOH), yield 84% (2.74 g), mp 228˚C - 230˚C. IR (KBr) cm^{-1}: 3478, 3332, 3050, 2950, 1720, 1688, 1653. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.27 (t, 3H, J = 7.02 Hz, CH_3), 1.92 - 1.94 (m, 2H, CH_2), 2.25 - 2.28 (m, 2H, CH_2), 4.25 (q, 2H, J = 7.02 Hz, CH_2), 4.52 (s, 2H, D_2O exchangeable, NH_2), 6.74 (s, 1H, CH), 7.14 - 8.07 (m, 5H, C_6H_5). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 13.6 (ester CH_3), 24.7, 28.8 (CH_2), 55.4 (ester CH_2), 110.0 (CH=C), 124.3, 125.8, 128.6, 129.0, 130.8, 137.3, 140.0 (C_6H_5, thiophene C), 147.8 (C=CH), 164.3, 180.2 (2C=O). EIMSm/z 327 $[M]^+$(18); Analysis Calcd for $C_{18}H_{17}NO_3S$ (327.40): C, 66.03; H, 5.23; N, 4.28; S, 9.79. Found: C, 65.83; H, 5.08; N, 4.47; S, 7.63.

Compound **5c**: Orange crystals from (EtOH), yield 73% (2.26 g), mp 222˚C - 225˚C. IR (KBr) cm^{-1}: 3466, 3368, 3058, 2938, 2893, 2220, 1720, 1644. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.83 - 1.24 (m, 2H, CH_2), 2.58 - 2.60 (m, 2H, CH_2), 3.24 (s, 3H, CH_3), 4.52 (s, 2H, D_2O exchangeable, NH_2), 6.74 (s, 1H, CH), 7.03 - 7.35 (2dd, 4H, J = 5.26 Hz, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 23.8, 28.6 (3 CH_2), 36.9 (CH_3), 110.0 (CH=C), 120.2 (CN), 124.3, 125.9, 128.4, 129.0, 130.3, 137.5, 140.6 (C_6H_5, thiophene C), 147.2 (C=CH), 164.2 (C=O). EIMS m/z 310 $[M]^+$ (38); Analysis Calcd for $C_{17}H_{14}N_2O_2S$ (310.37): C, 65.79; H, 4.55; N, 9.03; S, 10.33. Found: C, 65.88; H, 4.72; N, 8.79; S, 10.06.

Compound **5d**: Yellow crystals (EtOH), yield 76% (2.71 g), mp 140 - 142˚C. IR (KBr) cm^{-1}: 3478, 3328, 3053, 2949, 2838, 1718, 1687, 1593. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.22 (t, 3H, J = 6.99 Hz, CH_3), 1.66 - 1.74 (m, 2H, CH_2), 2.23 - 2.32 (m, 2H, CH_2), 4.24 (q, 2H, J = 6.99 Hz, CH_2), 4.50 (s, 2H, D_2O exchangeable, NH_2), 6.79 (s, 1H, CH), 7.21 - 7.24 (2dd, 4H, J = 4.86 Hz, C_6H_4). ^{13}C NMR (DMSO-d_6, 75Mz): δ = 13.8 (ester CH_3), 24.6, 28.8, 60.0 (3 CH_2), 55.8 (ester CH_2), 110.2 (CH=C), 124.6, 125.8, 128.8, 129.6, 130.6, 137.6, 140.2 (C_6H_5, thiophene C), 147.6 (C=CH), 164.6 (C=O). EIMSm/z 357 $[M]^+$ (12); Analysis Calcd for $C_{19}H_{19}NO_4S$ (357.42): C, 63.85; H, 5.36; N, 3.92; S, 8.97. Found: C, 63.01; H, 5.22; N, 4.21; S, 9.27.

2-(*Amino*(2-*amino*-4-*benzylidene*-5-*oxo*-4,5,6,7-*tetrahydrobenzo*-[*b*]*thiophen*-3-*yl*)*methylene*)*malononitrile* (**7*a***) *and* 2-(*Amino*(2-*amino*-4-(4-*methoxybenzylidene*)-5-*oxo*-4,5,6,7-*tetrahydrobenzo*[*b*]*thiophen*-3-*yl*) *methylene*) *malononitrile* (**7*b***)

General procedure: To a solution of either **3a** (2.00 g, 0.01 mol) or **3b** (2.30 g, 0.01 mol) in 1,4-dioxane (50 mL) containing triethylamine (0.50 mL) and elemental sulfur (0.32 g, 0.01 mol), compound **6** (1.33 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water mixture containing few drops of hydrochloric acid (till pH 6) was collected by filtration.

Compound **7a**: Yellow crystals (AcOH), yield 80% (2.77 g), mp 180˚C - 183˚C. IR (KBr) cm^{-1}: 3488 - 3328, 3053, 2960, 2222, 2203, 1721, 1635. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.84 - 1.87 (m, 2H, CH_2), 2.42 - 2.48 (m, 2H, CH_2), 3.90, 4.58 (2s, 4H, D_2O exchangeable, $2NH_2$), 7.07 (s, 1H, CH), 7.13 - 7.29 (m, 5H, C_6H_5). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 24.6, 28.8 (2 CH_2), 110.2 (CH=C), 117.5, 118.3 (2 CN), 119.2, 120.4, 124.8, 140.3, 144.8, 145.0 (C_6H_5, thiophene C), 153.5 (C=CH), 167.3 (C=O). EIMSm/z 346 $[M]^+$ (28); Analysis Calcd for $C_{19}H_{14}N_4OS$ (346.41): C, 65.88; H, 4.07; N, 16.17; S, 9.26. Found: C, 65.79; H, 3.89; N, 16.09; S, 9.22.

Compound **7b**: Orange crystals (AcOH), yield 84% (3.16 g), mp 201˚C - 203˚C. IR cm^{-1}: 3745 - 3390, 3066, 2951, 2840, 2220, 2212, 1722, 1660. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.80 - 1.88 (m, 2H, CH_2), 2.44 - 2.52 (m, 2H, CH_2), 3.21 (s, 3H, CH_3), 3.84, 4.52 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.88 (s, 1H, CH), 7.18 - 7.35 (2dd, 4H, J = 6.01 Hz, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 24.9, 28.6 (CH_2), 55.8 (CH_3), 110.2 (CH=C), 117.8, 118.8 (2CN), 119.0, 120.8, 124.6, 140.8, 144.9, 145.2 (C_6H_5, thiophene C), 153.8 (C=CH), 168.0 (C=O). EIMSm/z 376 $[M]^+$ (20); Analysis Calcd for $C_{20}H_{16}N_4O_2S$ (376.43): C, 63.81; H, 4.28; N, 14.88; S, 8.52. Found: C, 63.56; H, 4.48; N, 15.04.

2,4-*diamino*-8-*oxo*-5,6,7,8-*tetrahydrobenzo*[4,5]*thieno*[2,3-*b*]*pyridine*-3-*carboxylate* (**9**)

To a solution of compound **1** (1.12 g, 0.01 mol) in 1,4-dioxane (50 mL) containing triethylamine (0.50 mL) and elemental sulfur (0.32 g, 0.01 mol) compound **6** (1.33 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2h then poured onto ice/water mixture containing few drops of hydrochloric acid (till pH 6) was collected by filtration.

Compound **9**: Yellow crystals (AcOH), yield 65% (1.67 g), mp 166˚C - 168˚C. IR (KBr) cm^{-1}: 3588 - 3329, 3034, 2925, 2185, 1720, 1633. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.81 - 1.88 (m, 4H, $2CH_2$), 2.49 - 2.51 (m, 2H, CH_2), 3.56, 4.71 (2s, 4H, D_2O exchangeable, $2NH_2$). ^{13}C NMR (DMSO-d_6, 75 MHz): δ =20.1, 32.2, 32.3 (3 CH_2), 116.0 (CN), 129.6, 133.8, 138.9, 140.3, 144.6, 146.0 (thiophene, pyridine C), 168.2 (C=O), 174.4 (C=N).

EIMS m/z 258 $[M]^+$ (20); Analysis Calcd for $C_{12}H_{10}N_4OS$ (258.30): C, 55.80; H, 3.90; N, 21.69; S, 12.41. Found: C, 55.69; H, 4.18; N, 21.82; S, 12.29.

2-*Amino*-5,6,7,8-*tetrahydro*-5-*oxo*-4-*phenyl*-4*H*-*chromene*-3-*carbonitrile* (**10a**) *and* 2-*hydroxy*-5,6,7,8-*tetrahydro*-5-*oxo*-4-*phenyl*-4*H*-*chromene*-3-*carbonitrile* (**10b**)

General procedure: Method (A): To a solution of either compound **3a** (2.00 g, 0.01 mol) or **3b** (2.30 g, 0.01 mol) in 1,4-dioxane (50 mL) containingtriethylamine (0.50 mL) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then poured onto ice/water and the formed solid product was collected by filtration.

Method B: To a solution of compound **1** (1.12 g, 0.01 mol) in ethanol (50 mL) containing triethylamine (0.50 mL) either α-cyanocinnamonitrile (1.54 g, 0.01 mol), ethyl α-cyanocinnamate (2.02 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 4 h then poured onto ice/water and the formed solid product was collected by filtration.

Compound **10a**: Pall yellow crystals (EtOH), yield 73% (1.94 g), mp 233˚C - 235˚C. IR (KBr) cm^{-1}: 3583 - 3322, 3052, 2925, 2190, 1680, 1635. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.90 - 1.96 (m, 4H, $2CH_2$), 2.24 - 2.51 (m, 2H, CH_2), 4.19 (s, 2H, D_2O exchangeable, NH_2), 7.01 (s, 1H, pyran H-4), 7.17 - 7.31 (m, 5H, C_6H_5). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 20.3, 27.0, 36.0 ($3CH_2$), 114.3 (pyran C-4), 120.3 (CN), 120.3, 127.7, 128.8, 128.9, 129.0, 140.7, 159.0 (C_6H_5, pyran C), 196.4 (C=O). EIMS m/z 266 $[M]^+$ (26); Analysis Calcd for $C_{16}H_{14}N_2O_2$ (266.29): C, 72.16; H, 5.30; N, 10.52. Found: C, 72.38; H, 5.26; N, 10.47.

Compound **10b**: Yellow crystals (EtOH), yield 60% (1.60 g), mp 122 - 114˚C. IR (KBr) cm^{-1}: 3583 - 3322, 3052, 2925, 2220, 1689, 1638. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.91 - 1.96 (m, 4H, $2CH_2$), 2.23 - 2.50 (m, 2H, CH_2), 6.99 (s, 1H, pyran H-4), 7.26 - 7.36 (m, 5H, C_6H_5), 10.22 (s, 1H, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 20.1, 27.2, 36.8 ($3CH_2$), 114.0 (pyran C-4), 120.1 (CN), 120.2, 126.9, 128.4, 128.5, 129.2, 140.3, 159.5 (C_6H_5, pyran C), 194.2 (C=O). EIMSm/z 267 $[M]^+$ (18); Analysis Calcd for $C_{16}H_{13}NO_3$ (267.28): C, 71.90; H, 4.90; N, 5.24. Found: C, 72.16; H, 5.21; N, 5.29.

2,4-*Diamino*-5-*oxo*-5,6,7,8-*tetrahydronaphthalene*-1,3-*dicarbonitrile* (**12**)

To a solution of compound **1** (1.12 g, 0.01 mol) in absolute ethanol (40 mL) containing triethylamine, compound **6** (1.33 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h and the formed solid product upon cooling was collected by filtration.

Compound **12**: Yellow crystals (EtOH), yield 80% (1.80 g), mp 220˚C - 228˚C. IR (KBr) cm^{-1}: 3339 - 3197, 2920, 2222, 2190, 1683, 1630. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.68 - 1.71 (m, 4H, $2CH_2$), 2.34 - 2.46 (m, 2H, CH_2), 5.21, 5.79 (2s, 4H, D_2O exchangeable, $2NH_2$). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 20.3, 28.5, 50.20 ($3CH_2$), 118.2, 118.6 (2CN), 119.8, 120.6, 124.4, 129.6 (benzene C), 196.8 (C=O). EIMSm/z 226 $[M]^+$ (30); Analysis Calcd for $C_{12}H_{10}N_4O$ (226.23): C, 63.71; H, 4.46; N, 24.76. Found: C, 63.58; H, 4.62; N, 24.41.

2,4-*Diamino*-8-*benzylidene*-7-*oxo*-5,6,7,8-*tetrahydronaphthalene*-1,3-*dicarbonitrile* (**14a**) *and* 2,4-*diamino*-8-(4-*methoxybenzylidene*)-7-*oxo*-5,6,7,8-*tetrahydronaphthalene*-1,3-*dicarbonitrile* (**14a**)

General procedure: To a solution of either compound **3a** (2.00 g, 0.01 mol) or **3b** (2.30 g, 0.01 mol) in ethanol (50 mL) containing triethylamine (0.50 mL), compound **6** (1.33 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 6 h. The solid product, formed in each case, upon pouring onto ice/water mixture containing few drops of hydrochloric acid.

Compound **14a**: Yellow crystals (1,4-dioxane), yield 77% (2.42 g), mp 199˚C - 201˚C. IR (KBr) cm^{-1}: 3742 - 3343, 3055, 2948, 2220, 2208, 1683, 1632. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.84 - 1.96 (m, 2H, CH_2), 2.23 - 2.50 (m, 2H, CH_2), 4.33, 5.01 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.87 (s, 1H, CH=C), 7.27 - 7.37 (m, 5H, C_6H_5). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 20.6, 36.8 ($2CH_2$), 95.4, 95.3 (<u>CH</u>=C), 110.2 (CH=<u>C</u>), 116.8, 117.3 (2CN), 127.7, 128.8, 128.9, 129.0, 129.2, 129.8, 140.7 (two benzene C), 196.4 (C=O). EIMSm/z 314 $[M]^+$ (38); Analysis Calcd for $C_{19}H_{14}N_4O$ (314.34): C, 72.60; H, 4.49; N, 17.82. Found: C, 72.88; H, 4.69; N, 17.59.

Compound **14b**: White crystals (EtOH), yield 68% (2.34 g), mp 180˚C - 183˚C. IR (KBr) cm^{-1}: 3577 - 3320, 3050, 2920, 2218, 2201, 1677, 1636. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.83 - 1.94 (m, 2H, CH_2), 2.28 - 2.51 (m, 2H, CH_2), 3.65 (s, 3H, CH_3), 3.51, 4.65 (2s, 4H, D_2O exchangeable 2 NH_2), 6.45 (s, 1H, CH=C), 7.28 - 7.38 (2dd, 4H, J = 5.69 Hz, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 20.1, 37.8 (2 CH_2), 55.9 (OCH_3), 95.2 (<u>CH</u>=C), 110.2 (CH=<u>C</u>), 118.0, 118.3 (2 CN), 127.5, 128.4, 128.9, 129.0, 129.2, 129.9, 136.8, 155.0 (two benzene C), 194.3(C=O). EIMS m/z 344 $[M]^+$ (22); Analysis Calcd for $C_{20}H_{16}N_4O_2$ (344.37): C, 69.76; H, 4.68; N, 16.27. Found: C, 70.03; H, 4.88; N, 16.17.

3-*Phenyl*-2-*thioxo*-2,3,5,6-*tetrahydrobenzo*[*d*]*thiazol*-7(4*H*)-*one* (**16**)

To a solution of compound **1** (1.12 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine, elemental sulfur (0.32 g, 0.01 mol) and phenylisothiocyanate (1.30 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 2 h then left to cool. The formed solid product was collected by filtration.

Compound **16**: Yellow crystals from 1,4-dioxane, yield 90% (2.35 g), mp 240˚C - 244˚C. IR (KBr) cm^{-1}: 3035, 2944, 1662, 1582. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.00 - 2.03 (m, 4H, CH_2), 2.45 - 2.47 (m, 2H, CH_2), 7.30 - 7.56 (m, 5H, C_6H_5). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 20.6, 24.6, 36.9 ($3CH_2$), 127.7, 128.9, 129.2, 130.5, 136.8, 139.9, 157.8 (benzene, thiophene C), 180.1 (C=S), 191.34 (C=O). EIMSm/z 264 $[M]^+$ (22); Analysis Calcd for $C_{13}H_{11}NOS_2$ (261.36): C, 59.74; H, 4.24; N, 5.36; S, 24.54. Found: C, 59.93; H, 4.29; N, 5.41; S, 24.36.

2-(2-*Phenylhydrazono*)*cyclohexane*-1,3-*dione*(**18a**) *and* 2-(2-4-*chlorophenylhydrazono*)*cyclohexane*-1,3-*dione* (**18b**)

General procedure: To a cold solution (0˚C - 5˚C) of compound **1** (1.12 g, 0.01 mol) in ethanol (50 mL) containing sodium acetate (3.50 g, 0.50 mol) either benzenediazonium chloride (0.01 mol) or 4-chlorobenzene-diazonium chloride (0.01 mol [prepared by adding a cold solution of sodium nitrite (0.70 g, in water (10 mL)) to a cold solution (0˚C - 5˚C) of either aniline oil (0.93 g, 0.01 mol) or 4-chloroaniline (1.27 g, 0.01 mol) in concentrated hydrochloric acid (12 mL) with continuous stirring] was added with continuous stirring. The whole reaction mixture was left at room temperature for 1 h then the formed solid product was collected by filtration.

Compound **18a**: Orange crystals from 1,4-dioxane, yield 82% (1.72 g), mp 138˚C - 140˚C. IR (KBr) cm^{-1}: 3540 - 3426, 3050, 2947, 1740, 1668, 1619. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.93 - 1.96 (m, 2H, CH_2), 2.45 - 2.47 (m, 4H, $2CH_2$), 7.42 - 7.61 (m, 5H, C_6H_5), 14.87 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 18.9, 26.2 (3 CH_2), 117.5, 126.9, 130.3, 131.6 (benzene C), 163.1 (C=N), 196.4 (2 C=O). EIMS m/z 216 $[M]^+$ (20); Analysis Calcd for $C_{12}H_{12}N_2O_2$ (216.24): C, 66.65; H, 5.59; N, 12.96. Found: C, 66.84; H, 5.60; N, 13.28.

Compound **18b**: Orange crystals (EtOH), yield 90% (2.25 g), mp 212˚C - 215˚C. IR (KBr) cm^{-1}: 3540 - 3447, 3049, 2946, 1720, 1663, 1619. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.93 - 1.98 (m, 2H, CH_2), 2.45 - 2.68 (m, 4H, $2CH_2$), 7.48 - 7.62 (2dd, 4H, j = 6.02 Hz, C_6H_4), 14.70 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 Mz): δ = 18.3, 26.1 ($3CH_2$), 119.3, 130.1, 130.6 141.5 (benzene C), 198.2 (C=N), 193.7 (C=O). EIMS: m/z 250 $[M]^+$ (17). Analysis Calcd for $C_{12}H_{11}ClN_2O_2$ (250.68): C, 57.49; H, 4.42; N, 11.17. Found: C, 57.27; H, 4.77; N, 10.93.

2-(3-*Oxo*-2-(2-*phenylhydrazono*)*cyclohexylidene*)*malononitrile* (**19a**) *and ethyl* 2-*cyano*-2-(3-*oxo*-2-(2-*phenyylhydrazono*)*cyclohexylidene*)*acetate* (**19b**)

General procedure: To the dry solid of compound **18a** (2.16 g, 0.01 mol) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) and ammonium acetate (0.50 g) were added. The reaction mixture, in each case was heated in an oil bath at 120˚C for 15 min. The solid product produced after boiling with ethanol was collected by filtration.

Compound **19a**: Yellowcrystals (EtOH), yield 78% (2.06 g), mp 210˚C - 213˚C. IR (KBr) cm^{-1}: 3544 - 3373, 3033, 2934, 2222, 2220, 1722, 1632. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.93 - 1.98 (m, 2H, CH_2), 2.45 - 2.67 (m, 4H, CH_2), 7.42 - 7.59 (m, 5H, C_6H_5), 14.86 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 18.29, 33.2, 39.2 ($3CH_2$), 103.6, 104.4 (C=C), 114.5, 117.6 (2CN), 119.3, 130.0, 130.6 141.6 (benzene C), 151.4 (C=N), 162.8 (C=O). EIMSm/z 264 $[M]^+$ (23); Analysis Calcd for $C_{15}H_{12}N_4O$ (264.28): C, 68.17; H, 4.58; N, 21.20. Found: C, 68.37; H, 4.72; N, 21.09.

Compound **19b**: Yellow crystals (EtOH), yield 70% (2.17 g), mp 150˚C - 152˚C. IR (KBr) cm^{-1}: 3522 - 3329, 3038, 2936, 2212, 1720, 1687, 1630. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.19 (t, 3H, J = 7.33 Hz, CH_3), 1.87 - 1.93 (m, 2H, CH_2), 2.43 - 2.65 (m, 4H, $2CH_2$), 4,22 (q, 2H, J = 7.33 Hz, CH_2), 7.38 - 7.54 (m, 5H, C_6H_5), 14.82 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 13.6 (CH_3), 18.29, 33.0, 39.4 ($3CH_2$), 59.5 (CH_2), 103.8, 104.9 (C=C), 116.5 (CN), 119.0, 120.5, 130.6, 141.6 (benzene C), 150.2 (C=N), 160.8 (C=N), 163.1, 164.4 (2C=O). EIMSm/z 311 $[M]^+$ (20); Analysis Calcd for $C_{17}H_{17}N_3O_3$ (311.34): C, 65.58; H, 5.50; N, 13.50. Found: C, 65.88; H, 5.71; N, 13.69.

3-*Imino*-8-*oxo*-2-*phenyl*-2,3,5,6,7,8-*hexahydrocinnoline*-4-*carbonitrile* (**20a**) *and* 3,8-*dioxo*-2-*phenyl*-2,3,5,6,7,8-*hexahydrocinnoline*-4-*carbonitrile* (**20b**)

General procedure: To a solution of compound **18a** (2.16 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture, in each case was heated under reflux for 3 h then evaporated under vacuum. The solid product produced after triturating the remaining product with diethyl ether was collected by filtration.

Compound **20a**: Orange crystals (EtOH), yield 80% (2.11 g), mp 150˚C - 153˚C. IR (KBr) cm^{-1}: 3741 - 3376,

3055, 2933, 2193, 1737,1655, 1631. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.94 - 1.98 (m, 2H, CH_2), 2.48 - 2.50 (m, 4H, CH_2), 7.45 - 7.67 (m, 5H, C_6H_5), 8.09 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 18.2, 18.3, 39.2 ($3CH_2$), 103.6, 104.4 (C=C), 118.6 (CN), 119.0, 130.3, 130.8, 133.4, 138.3, 141.9, 142.0 (benzene, pyridazine C), 173.2 (C=N), 196.5 (CO). EIMS m/z 264 $[M]^+$ (20%); Analysis Calcd for $C_{15}H_{12}N_4O$ (264.28): C, 68.17; H, 4.58; N, 21.20. Found: C, 68.28; H, 4.77; N, 21.08.

Compound **20b**: Yellow crystals (EtOH), yield 69 % (1.82 g), mp 139˚C - 141˚C. IR (KBr) cm^{-1}: 3055, 2945, 2212, 1693, 1684, 1630. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.94 - 1.98 (m, 2H, CH_2), 2.49 - 2.68 (m, 4H, CH_2), 7.45 - 7.61 (m, 5H, C_6H_5). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 18.3, 33.0, 39.1 ($3CH_2$), 117.5 (CN), 118.6, 119.0, 120.3, 126.9, 130.6, 131.9, 141.6, 142.1 (benzene, pyridazine C), 168.3 (C=N), 198.1 (C=O). EIMSm/z 265 $[M]^+$ (28%); Analysis Calcd for $C_{15}H_{11}N_3O_2$ (265.27): C, 67.92; H, 4.18; N, 15.84. Found: C, 67.77; H, 4.30; N, 15.64.

2-*Amino*-5-*oxo*-4-(2-*phenylhydrazono*)-4,5,6,7-*tetrahydrobenzo*[*b*]-*thiophene*-3-*carbonitrile* (**21**) *and* 4-*amino*-2-*phenyl*-6,7-*dihydro*-2*H*-*thieno*[4,3,2-*de*]*cinnoline*-3,8-*dione* (**22**)

General procedure: To a solution of compound **18a** (2.16 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol). The reaction mixture was heated under reflux for 2 h then poured onto ice/water and the formed solid product was collected by filtration.

Compound **21**: Orange crystals (EtOH), yield 70% (2.07 g), mp 138˚C - 140˚C. IR (KBr) cm^{-1}: 3417 - 3320, 3053, 2947, 2197, 1742, 1634. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.93 - 1.96 (m, 2H, CH_2), 2.46 - 2.50 (m, 2H, CH_2), 6.42 (s, 2H, D_2O exchangeable, NH_2), 7.22 - 7.57 (m, 5H, C_6H_5), 14.84 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 18.4, 39.8 ($2CH_2$), 117.6 (CN), 119.0, 126.9, 130.2, 132.0, 137.8, 142.4, 143.7 (benzene, thiophene C), 193.7 (C=N), 198.1 (CO). EIMS m/z 296 $[M]^+$ (12); Analysis Calcd for $C_{15}H_{12}N_4OS$ (296.35): C, 60.79; H, 4.08; N, 18.91; S, 10.82. Found: C, 60.84; H, 3.79; N, 18.69; 10.63.

Compound **22**: Yellow crystals (EtOH), yield 76% (2.17 g), mp 170˚C - 173˚C. IR (KBr) cm^{-1}: 3450 - 3328, 3056, 2945, 1738, 1671, 1615. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.45 - 2.47 (m, 2H, CH_2), 2.56 - 2.64 (m, 2H, CH_2), 4.82 (s, 2H, D_2O exchangeable, NH_2), 7.40 - 7.57 (m, 5H, C_6H_5). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 18.4, 39.2 ($2CH_2$), 117.6, 119.0, 126.9, 130.0, 130.2, 131.9, 140.9, 142.0 (benzene, pyridazine, thiophene C), 193.7 (C=N), 198.1 (C=O). EIMS m/z 282 $[M]^+$ (20); 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, 60.85; H, 3.72; N, 14.28; S, 11.09.

2,4-*Diphenyl*-3-*thioxo*-3,4,6,7-*tetrahydrobenzo*[*e*][1,2,4]*triazin*-8(2*H*)-*one* (**23**)

To a solution of compound **18a** (2.16 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL), phenylisothiocyanate (1.30 g, 0.01 mol) was added. The reaction mixture 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.

Compound **23**: Pale yellow crystals (EtOH), yield 62% (2.06 g), mp 220˚C - 222˚C. IR (KBr) cm^{-1}: 3046, 2944, 1720, 1680, 1624. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.85 - 1.92 (m, 2H, CH_2), 2.40 - 2.62 (m, 2H, CH_2), 7.12 (m 1H, cyclohexene CH), 7.33 - 7.61 (m, 10H, $2C_6H_5$). ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 33.0, 39.6 ($2CH_2$), 117.5 (CH), 117.9, 124.1, 124.9, 126.9, 128.9, 130.3, 140.0 (two benzene, pyridazine C), 193.7193.8 (C=N, C=S), 198.1 (CO). EIMS m/z 333 $[M]^+$ (28); Analysis Calcd for $C_{19}H_{15}N_3OS$ (333.41): C, 68.45; H, 4.53; N, 12.60; S, 9.62. Found: C, 68.29; H, 4.71; N, 12.49; S, 9.77.

2-(*Amino*(3-*imino*-8-*oxo*-2-*phenyl*-2,3,5,6,7,8-*hexahydrocinnolin*-4-*yl*)*methylene*)*malononitrile* (**24**)

To a dry solid of compound **18a** (2.16 g, 0.01 mol), compound **6** (1.33 g, 0.01 mol) and ammonium acetate (0.50 g) were added. The reaction mixture, in each case was heated in an oil bath at 120˚C for 15 min. The solid product produced after boiling with ethanol was collected by filtration.

Compound **24**: Yellowcrystals (EtOH), yield 80% (2.31 g), m.p. 210˚C - 213˚C. IR (KBr) cm^{-1}: 3545 - 3373, 2934, 2220 - 2190, 1680, 1655, 1630. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.48 - 2.50 (m, 2H, CH_2), 2.60 - 2.68 (m, 4H, $2CH_2$), 3.31 (s, 2H, D_2O exchangeable, NH_2), 7.48 - 7.62 (m, 5H, C_6H_5), 14.70 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 18.3, 39.7, 40.1 ($3CH_2$), 118.2, 118.6 (2CN), 117.2, 118.0 (2CN), 119.2, 120.6 (C=C), 121.7, 124.4, 129.6 132.1, 141.5, 144.6(benzene, pyridazine C), 193.7 (C=N), 198.2,196.8 (C=O). EIMSm/z330 $[M]^+$ (28); Analysis Calcd for $C_{18}H_{14}N_6O$ (330.34): C, 65.44; H, 4.27; N, 25.44. Found: C, 65.51; H, 4.29; N, 25.63.

2,4-*Diamino*-7-*oxo*-8-(2-*phenylhydrazono*)-5,6,7,8-*tetrahydronaphthalene*-1,3-*dicarbonitrile* (**25**)

To a solution of compound **18a** (2.61 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL),

compound **6** (1.33 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 6 h. The solid product, formed in each case, upon pouring onto ice/water mixture containing few drops of hydrochloric acid.

Compound **25**: White crystals (EtOH), yield 73% (2.40 g), mp 212˚C - 215˚C. IR (KBr) cm^{-1}: 3642 - 3340, 3055, 2944, 2220, 2206, 1680, 1630. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.82 - 1.96 (m, 2H, CH_2), 2.20 - 2.50 (m, 2H, CH_2), 3.06, 3.86 (2s, 4H, D_2O exchangeable, $2NH_2$), 7.25 - 7.61 (m, 5H, C_6H_5), 10.62 (s, 1H, D_2O exchangeable NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 20.6, 36.9 ($2CH_2$), 116.8, 117.3 (2CN), 127.4, 128.8, 128.6, 129.0, 129.2, 129.8, 140.5 (two benzene C), 193.7 (C=N), 196.2 (C=O). EIMSm/z 330 $[M]^+$ (38); Analysis Calcd for $C_{18}H_{14}N_6O$ (330.34): C, 65.44; H, 4.27; N, 25.44. Found: C, 65.73; H, 4.39; N, 25.68.

6. Conclusion

In summary, we have shown herein that our strategy is compatible with the synthesis of a wide range of cyclohexane-1,3-dione derivatives and particularly when being corporate to heterocyclic and fused derivatives. The cytotoxicity of the newly synthesized products were evaluated against human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38). The results showed that compounds **3b**, **5c**, **7b**, **10b**, **12**, **14b**, **16**, **18b**, **19b**, **20b**, **21** and **24** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range. In addition, compounds **16** and **21** showed non toxicity when tested *in vivo* lethality toshrimp larvae (*Artemiasalina*).

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