NOVEL HETEROCYCLIC COMPOUNDS DERIVED FROM ACETOACETANILIDE TOGETHER WITH THEIR ANTITUMOR EVALUATIONS

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The reaction of acetoacetanilide **(1)** with cyclohexanone **(2)** gave compound **3** which it was reacted with the active methylene reagents **4a, b** afforded cyclohexylidene derivatives **5a, b**. The latter products were reacted with elemental sulfur in presence of basic catalyst to produce thiophene derivatives **6a, b**. Also compound **1** reacted with diazonium salts **7a, b** then compounds **8a**, **b** were produced respectively, compounds **8a, b** were directed toward the reaction with malononitrile **(4a)**, ethyl cyanoacetate **(4b)** in either ammonium acetate or piperdine to form compounds **9a-d** and **10a-d** respectively**,** also compounds **8a, b** reacted with either phenylisothiocanate **(11)** afforded compounds **13a, b** or hydrazine derivatives **14a, b** to produce compounds **15a-d**. The newly synthesized compounds were evaluated for antitumor activity.

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Introduction

A large number of medicinal compounds which have been discovered belong to a major class of heterocycles containing sulphur and (or) nitrogen. Thiophene and its derivatives constitute one of the major classes in heterocyclic chemistry, thus some of these compounds has interesting biological properties such as cytotoxic, antitumor activity,[1,2] anti-inflammatory and analgesic agents,[3,4,6] antimicrobial[5,6] and antiprotozoal activity,[7,8] pyridazine derivatives has antimicrobial activity,[9] on the other hand the fused 1,2,4 triazine derivatives has Antiproliferative activity,[10] moreover pyrazole derivatives has many biological significant such as Antiproliferative activity.[11]

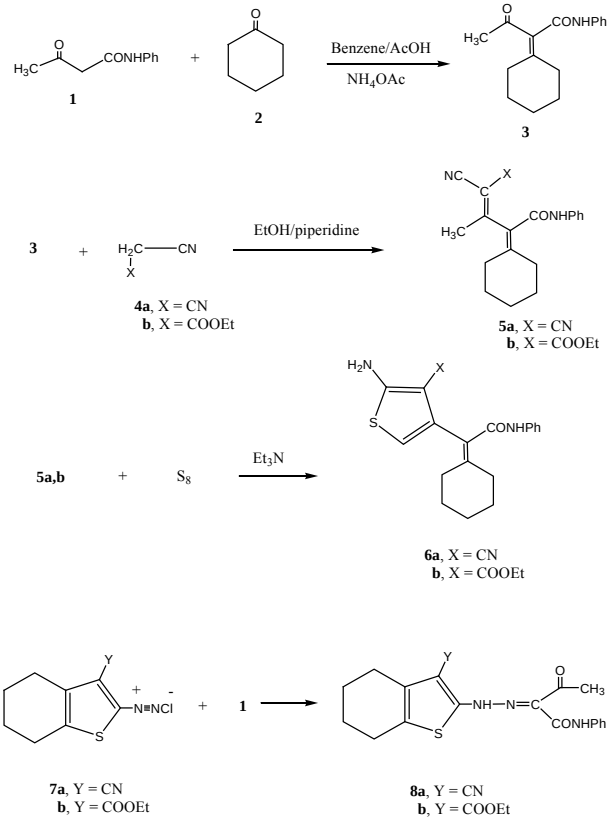
In this article from our view as continuation of such efforts directed towards the synthesis of new heterocyclic compounds based on the presence of acetoacetanilide derivatives, and the screening of their antitumor activity against three different cell lines. The structures of the newly synthesized compounds were established using IR, NMR & Mass spectrometry techniques.

Results and discussion

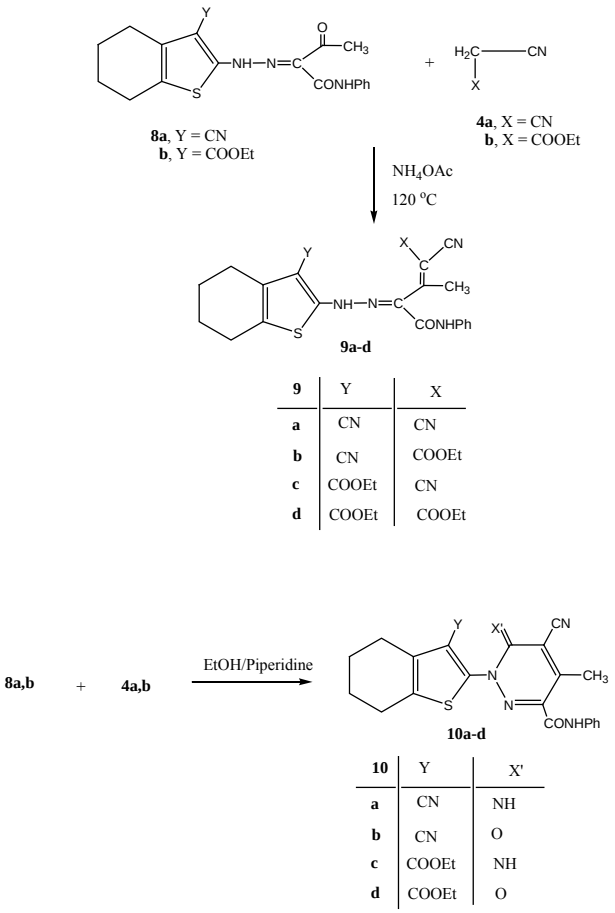
In the present work we report the uses of acetoacetanilide through some heterocyclic synthesis followed by cytotoxic evaluations of the newly obtained compounds. Thus, acetoacetanilide **(1)** reacted with cyclohexanone **(2)** in benzene/AcOH containing ammonium acetate gave the Knoevenagel condensation product **3**. The structure of compound **3** was confirmed based on the analytical and spectral data. Thus, the ^{1}H NMR spectrum of compound **3** showed two multiplets at δ 1.77-1.79 & 2.11-2.16 ppm indicating the five CH_2 groups, a singlet at δ 2.27 ppm corresponding to CH_3 group, a multiplet at δ 7.25-7.36 ppm for the C_6H_5 group and a singlet, D_2O-exchangeable at δ 9.63 ppm for the NH group. Compound **3** reacted with either malononitrile **(4a)** or ethyl cyanoacetate **(4b)** afforded compounds **5a** and **5b** respectively. The existence of the methyl group in conjugation with the cyano group enhances the reactivity of the first.

Thus, the reaction of compounds **5a** and **5b** with elemental sulfur in 1,4-dioxan containing a catalytic amount of triethylamine to give the thiophene derivatives **6a** and **6b** respectively. The analytical and spectral data of compounds **6a** and **6b** were consistence with their respective structures. Thus, the ^{1}H NMR spectrum of **6a** showed two multiplets at δ 1.73-1.76 & 1.91-1.98 ppm indicating the five CH_2 groups, a singlet at δ 4.54 ppm indicating two H of NH_2 group, a singlet at δ 6.73 ppm corresponding to 1H (thiophene ring), a multiplet at δ 7.27-7.38 ppm for the C_6H_5 group and a singlet, D_2O-exchangeable at δ 9.32 ppm for the NH group.

The reaction of compound **1** with either 3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-diazonium chloride **(7a)** or 3-ethoxycarbonyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-diazonium chloride **(7b)** gave the hydrazo derivatives **8a** and **8b** respectively (cf. Scheme 1) . The reaction of the synthesized compounds **8a** or **8b** with either malononitrile **(4a)** or ethyl cyanoacetate **(4b)** in the presence of ammonium acetate at 120 ^{o}C gave the Knoevenagel condensation products **9a-d** respectively. On the other hand carrying the same reaction but in refluxing ethanol containing piperidine gave the pyridazine derivatives **10a-d** respectively (cf. Scheme 2).



Scheme 1. Synthesis of compounds **3-8a,b**



9	Y	X
a	CN	CN
b	CN	COOEt
c	COOEt	CN
d	COOEt	COOEt

10	Y	X'
a	CN	NH
b	CN	O
c	COOEt	NH
d	COOEt	O

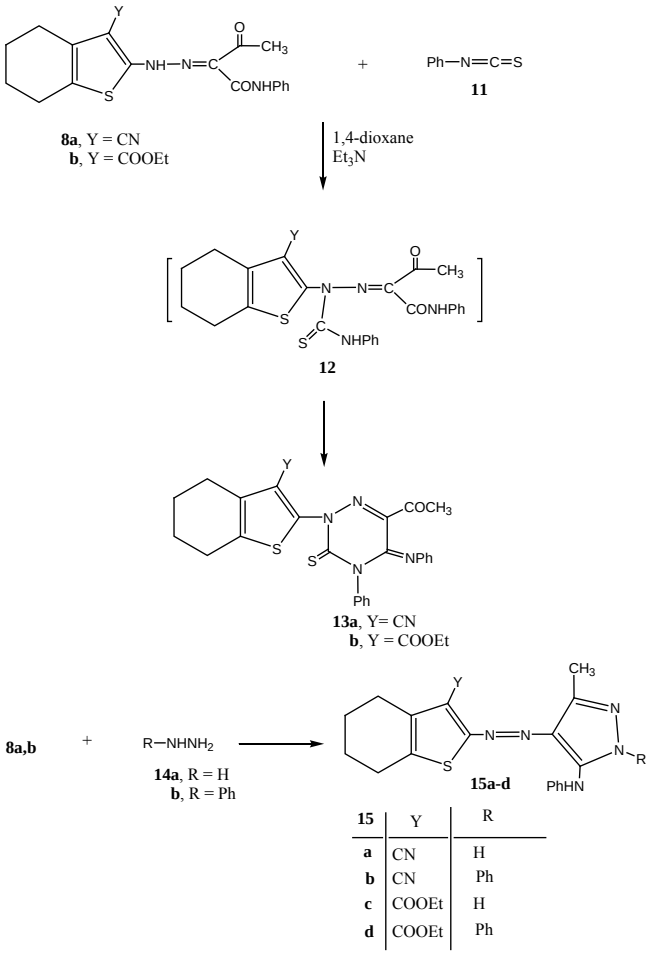
Scheme 2. Synthesis of compound **9a-d, 10a-d**

Formation of the latter products might be explained in terms of first formation of the acyclic intermediates **9a-d** followed by their cyclization.

The structures of compounds **10a-d** were established on their respective analytical and spectral data. Thus, the ^{1}H NMR spectrum of **10a** as specific example showed two multiplets at δ 1.64-1.68 & 2.05-2.12 ppm indicating to the four CH_2 groups, a singlet at δ 2.34 ppm corresponding to the CH_3 group, a multiplet at δ 7.27-7.42 ppm for the C_6H_5 group and two singlets, D_2O-exchangeable at δ 8.25 and 9.30 ppm for the two NH groups.

The reaction of either compound **8a** or **8b** with phenylisothiocyanate **(11)** in presence of 1,4-dioxan containing triethylamine afforded 1,2,4-triazine derivatives **13a** and **13b** respectively. The analytical and spectral data of compound **13a** were in agreement with the assigned structures.

Finally the reaction of either compound **8a** or **8b** with either hydrazine hydrate **(14a)** or phenylhydrazine **(14b)** gave the pyrazole derivatives **15a-d** respectively (cf. Scheme 3).



15	Y	R
a	CN	H
b	CN	Ph
c	COOEt	H
d	COOEt	Ph

Scheme 3. Synthesis of compounds **13a, b-15a-d**

Antitumor activity tests

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

Cell cultures. 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) and NCI-H460 and SF-268 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^{-1}, streptomycin 100 µg mL^{-1}) at 37 ^{o}C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were produced by plating 1.5 x 10^5 cells mL-1 for MCF-7 and SF-268 and 0.75 x 10^4 cells ml^{-1} for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of cell lines which it was evaluated in all tests by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

Tumor cell growth assay: The effects of the newly synthesized compounds **3–15a-d** 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-well plates 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 % (GI_{50}) corresponding to the concentration of the compounds that inhibited 50% of the net cell growth was calculated as described elsewhere[13]. Doxorubicin was used as a positive control and tested in the same manner.

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.

Effect on the Growth of Human Tumor Cell Lines

The effect of the newly synthesized compounds **3-15a-d** was evaluated on the *in vitro* growth of three human tumor cell lines representing different tumor types namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) after a continuous exposure for 48 h. The results were introduced in **Table 1**.

Table 1. Effect of compounds **3-15a-d** on the growth of three human tumor cell lines

Compound	GI_{50}, µmol L^{-1}		
	NCI-H460	**MCF-7**	**SF-268**
3	27.6 ± 2.4	16.9 ± 4.8	16.8 ± 2.6
5a	0.02 ± 0.002	0.01 ± 0.002	0.02 ± 0.001
5b	12.1 ± 0.8	10.3 ± 2.6	6.3 ± 0.8
6a	0.4 ± 0.2	0.2 ± 0.01	0.2 ± 0.06
6b	4.2 ± 1. 4	6.1 ± 2.4	4.0 ± 1.2
8a	12.6 ± 0.6	14.5 ± 0.8	8.7 ± 2.4
8b	22.4 ± 8.1	24.2 ± 2.8	28.3 ± 4.2
9a	0.01 ± 0.002	0.01`± 0.004	0.01 ± 0.001
9b	1.6 ± 0.4	2.2 ± 0. 8	4.0 ± 0.2
9c	6. 1 ± 2.4	8. 1 ± 2.1	4.2 ± 1.3
9d	0.9 ± 0.2	0.1 ± 0.02	0.3 ± 0.05
10a	8. 1 ± 2.2	6.2 ± 1.1	8.20 ± 2.4
10b	0.4 ± 0.2	0.2± 0.06	0.5 ± 0.01
10c	32.0 ± 1.6	40.0 ± 0.4	10.5 ± 1.2
10d	0.01 ± 0.003	0.02 ± 0.001	0.01 ± 0.001
13a	0.2 ± 0.01	0.1 ± 0.02	0.2 ± 0.02
13b	10.6 ± 4.6	8.5 ± 2.8	6.7 ± 1.4
15a	20.4 ± 8.1	22.2 ± 2.8	20.3 ± 4.2
15b	0.01 ± 0.008	0.01`± 0.006	0.01 ± 0.002
15c	2.6 ± 0.6	4.2 ± 0.2	8.0 ± 1.4
15d	0.01 ± 2.4	0.1 ± 0.01	0.2 ± 0.03
Doxorubicin	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007

The all compounds were able to inhibit the growth of the tested human tumor cell lines in a dose-dependent manner. The results indicated through **Table 1** revealed that compounds **5a**, **9a**, **10d** and **15b** showed the highest inhibitory effect against all three tumor cell lines, such activity is higher than the reference doxorubicin. While compounds **6a** and **13a** showed high inhibitory effects against three different cell lines, which are less than the corresponding reference doxorubicin. Compounds **3**, **5b**, **8a**, **8b**, **9c**, **10a**, **10c**, **13b** and **15a** showed the lowest inhibitory effect. The remaining compounds showed a moderate growth inhibitory effect. Comparing compound **5a** and **5b** it is obvious that the presence of the CN group in compound **5a** is responsible for their reactivity over **5b**. Similarly comparing of **6a** with **6b**, **8a** with **8b** and **13a** with **13b** it is obvious that the introduction of the CN group in **6a**, **8a** and **13a** showed higher inhibitory effect towards the three cell lines than that of **6b**, **8b** and **13b**. On the other hand comparison of inhibitory effect of compounds **9a-d**, one can say that compound **9a** with the X = Y = CN showed the highest inhibitory effect among the four compounds such reactivity is higher than that of the reference doxorubicin. Comparison of compounds **10a-d** showed that the effect of X^- = O and Y= COOEt like in **10d** the maximum inhibitory result among the four compounds was obtained. However, when X^- = O and Y = CN as in case of **10b** the inhibitory effect was lowered but it hasn't large amount as the compound is still of the most active compounds among the all test compounds. On the other hand the introduction of NH group like in **10a** decreases the reactivity and such observation was shifted towards lower reactivity in case of **10c** where X = NH and Y = COOEt. Similarly, comparison of compounds **15a-d** showed that when R = Ph and Y= CN like in **15b** the maximum inhibitory effect among the four compounds was obtained.

However, when R = Ph and Y = COOEt as in case of **15d** the inhibitory effect was lowered but it hasn't large amount as the compound is still of the most active compounds among all test compounds. On the other hand introduction of un-substituted compound like in **15a** decreases the reactivity and such observation was shifted towards lower reactivity in case of **15c** where R = H and Y = COOEt.

Experimental

All melting points were determined in open capillaries and are uncorrected. Elemental analyses were performed on a Yanaco CHNS Corder elemental analyzer (Japan). IR spectra were measured using KBr discs on a Pye Unicam SP-1000 spectrophotometer. ^{1}H NMR spectra were measured on a Varian EM 390-200 M*Hz* instrument in CD_3SOCD_3 as solvent using TMS as internal standard and chemical shifts were expressed as δ ppm. Mass spectra were recorded on Kratos (75 eV) MS equipment (Germany).

2-Cyclohexylidene-3-oxo-N-phenylbutanamide (3)

To a dray solid of acetoacetanilide **(1)** (5.31g, 0.03 mol) containing ammonium acetate (0.50 g) cyclohexanone **(2)** (2.94 g, 0.03 mol) was added. The reaction mixture was heated in an oil bath at 120°C for 1h then left to cool then triturated with ethanol and the formed solid product was collected by filtration.

Compound **3:** Pale brown crystals from ethanol, yield: 96 % (7.406 g); mp: 120 ºC. IR (KBr): υ/cm^{-1} = 3448-3320 (NH), 3050 (CH-aromatic), 2991(CH_3), 2885(CH_2) 1695, 1687 (2CO). ^{1}H NMR (DMSO-d_6): δ = 1.77-1.79 (m, 6H, $3CH_2$), 2.11-2.16 (m, 4H, $2CH_2$), 2.27 (s, 3H, CH_3), 7.25-7.36 (m, 5H, C_6H_5), 9.63 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 257 (M^+, 21%). Analysis for $C_{16}H_{19}NO_2$ Calcd: C, 74.68; H, 7.44; N, 5.44. Found: C, 74.93; H, 7.29; N, 5.83 %.

4,4-Dicyano-2-cyclohexylidene-3-methyl-N-phenylbut-3-enamide (5a) and ethyl-4-(phenylcarbamoyl)-2-cyano-4-cyclohexylidene-3-methylbut-2-enoate (5b)

General procedure: To a solution of compound **3** (2.57 g, 0.01 mol) in ethanol (50 ml) containing piperidine (0.5 ml), either malononitrile **(4a)** (0.66 g, 0.01 mol) or ethylcyanoacetate **(4b)** (1.13 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then left to cool and the solid product was formed in each case upon pouring onto ice/water containing few drops of hydrochloric acid, the solid product was collected by filtration.

Compound **5a**: Yellow crystals from ethanol, yield: 86 % (2.624 g); mp: 134 °C. IR (KBr): υ/cm^{-1} = 3466-3323 (NH), 3054 (CH-aromatic), 2980 (CH_3), 2918 (CH_2), 2223-2220 (2 CN), 1687 (CO), 1633 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.75-1.79 (m, 6H, $3CH_2$), 1.87-1.91 (m, 4H, $2CH_2$), 2.28 (s, 3H, CH_3), 7.29-7.41 (m, 5H, C_6H_5), 9.88 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 305 (M^+, 17.5%). Analysis for $C_{19}H_{19}N_3O$ Calcd: C, 74.73; H, 6.27; N, 13.76. Found: C, 74.85; H, 6.11; N, 13.93 %.

Compound **5b**: Yellow crystals from ethanol, yield: 84 % (2.958 g); mp: 104 °C. IR (KBr): υ/cm^{-1} = 3484-3312 (NH), 3058 (CH-aromatic), 2985 (CH_3), 2916 (CH_2), 2222 (CN), 1690, 1687 (2CO), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.13 (t, 3H, J = 7.44 Hz, CH_3), 1.72-1.77 (m, 6H, $3CH_2$), 2.12-2.18 (m, 4H, $2CH_2$), 2.35 (s, 3H, CH_3), 4.22 (q, 2H, J = 7.44 Hz, CH_2), 7.28-7.40 (m, 5H, C_6H_5), 9.73 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 352 (M^+, 30.6%). Analysis for $C_{21}H_{24}N_2O_3$ Calcd: C, 71.57; H, 6.86; N, 7.95. Found: C, 71.73; H, 6.68; N, 8.21 %.

2 - (5 - Amino-4 - cyanothiophen - 3 - yl)- 2- cyclohexylidene-N-phenylacetamide (6a) and 2-amino-4-(cyclohexylidene-phenyl-carbamoyl-methyl) thiophene-3-carboxylic acid ethyl ester (6b)

General procedure: To a solution of each compound **5a** (0.915 g, 0.003 mol) or compound **5b** (1.06 g, 0.003 mol) in 1,4-dioxan (40 ml) containing triethylamine (0.5 ml), elemental sulfur (0.1 g, 0.003 mol) was added. The reaction mixture was heated under reflux for 1.5 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

Compound **6a**: Buff crystals from acetic acid, yield: 72 % (0.728g); mp: 180-182 ºC. IR (KBr): υ/cm^{-1} = 3473-3318 (NH_2, NH), 3058 (CH-aromatic), 2882 (CH_2), 2224 (CN), 1686 (CO), 1633 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.73-1.76 (m, 6H, $3CH_2$), 1.91-1.98 (m, 4H, $2CH_2$), 4.54 (s, 2H, NH_2, D_2O exchangeable), 6.73 (s, 1H, thiophene ring), 7.27-7.38 (m, 5H, C_6H_5), 9.32 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 337 (M^+, 21.2%). Analysis for $C_{19}H_{19}N_3OS$ Calcd: C, 67.63; H, 5.68; N, 12.45; S, 9.50. Found: C, 67.85; H, 5.82; N, 12.62; S, 9.29 %.

Compound **6b**: Pale yellow crystals from acetic acid, yield: 81 % (0.933g); mp: 215-217 ºC. IR (KBr): υ/cm^{-1} = 3469-3322 (NH_2, NH), 3058 (CH-aromatic), 2970 (CH_3), 2892 (CH_2), 1693, 1685 (2CO), 1634 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.12 (t, 3H, J = 7.09 Hz, CH_3), 1.65-1.73 (m, 6H, $3CH_2$), 2.62-2.67 (m, 4H, $2CH_2$), 4.26 (q, 2H, J = 7.09 Hz, CH_2), 4.71 (s, 2H, NH_2, D_2O exchangeable), 6.65 (s, 1H, thiophene ring), 7.27-7.38 (m, 5H, C_6H_5), 9.39 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 384 (M^+, 12.4%). Analysis for $C_{21}H_{24}N_2O_3S$ Calcd: C, 65.60; H, 6.29; N, 7.29; S, 8.34. Found: C, 65.88; H, 5.98; N, 7.52; S, 8.02 %.

2-(2-Hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-cyano)-3-oxo-N-phenyl-butanamide (8a) and 2-(2-hydrazinyl -4,5,6,7-tetrahydrobezo [*b*] thiophene-3-ethoxy-carbonyl)-3-oxo-N-phenylbutanamide (8b)

General procedure: A cold solution of either of 3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-diazonium chloride **(7a)** or 3-ethoxy carbonyl -4,5,6,7-tetrahydrobenzo [*b*] thiophene-2-diazonium chloride **(7b)** [obtained by adding sodium nitrite (1.49 g, 0.02 mol) solution to a cold solution of either 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[*b*] thiophene (3.56 g, 0.02 mol) or ethyl 2-amino-4,5,6,7-tetrahydrobenzo [*b*] thiophene-3-carboxylate (4.51 g, 0.02 mol) in acetic/hydrochloric acid (10:3) with continuous stirring] was added to a cold solution (0-5 °C) of acetoacetanilide **(1)** (3.56 g, 0.02 mol) in ethanol (50 ml) containing sodium hydroxide (10 ml, 10 %). The reaction mixture was stirred at room temperature for 1 h and the solid product was formed, collected by filtration.

Compound **8a**: Orange crystals from DMF, yield: 63 % (4.617 g); mp=108-110 ºC. IR (KBr): υ/cm^{-1} = 3482-3341 (2 NH), 3053 (CH-aromatic), 2981 (CH_3), 2887 (CH_2), 2220 (CN), 1688, 1684 (2CO), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.71-1.75 (m, 4H, 2CH_2), 2.16-2.22 (m, 4H, 2CH_2), 2.37 (s, 3H, CH_3), 7.32-7.41 (m, 5H, C_6H_5), 8.72, 9.15 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 366 (M^+, 11.8%). Analysis for $C_{19}H_{18}N_4O_2S$ Calcd: C, 62.28; H, 4.95; N, 15.29; S, 8.75. Found: C, 62.44; H, 5.22; N, 15.39; S, 8.82 %.

Compound **8b**: Orange crystals from DMF, yield: 75 % (6.197 g); mp: 169-171 ºC. IR (KBr): υ/cm^{-1} = 3472-3363 (2 NH), 3056 (CH-aromatic), 2974 (CH_3), 2894 (CH_2), 1692, 1684 and 1681 (3CO), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.16 (t, 3H, J = 7.62 Hz, CH_3), 1.62-1.73 (m, 4H, 2CH_2), 2.27-2.34 (m, 4H, 2CH_2), 2.42 (s, 3H, CH_3), 4.23 (q, 2H, J = 7.62 Hz, CH_2), 7.28-7.42 (m, 5H, C_6H_5), 8.32, 9.36 (2s, 2H, D_2O-exchangeable 2NH). MS (relative intensity) m/z: 413 (M^+, 27.4%). Analysis for $C_{21}H_{23}N_3O_4S$ Calcd: C, 61.00; H, 5.61; N, 10.16; S, 7.75. Found: C, 61.28 ; H, 5.42; N, 10.31; S, 7.93 %.

2-(2-Hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-cyano)-4,4-dicyano-3-methyl-N-phenyl-but-3-enamide (9a), ethyl 4-(phenylcarbamoyl)-4-(2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-cyano)-2-cyano-3-methyl-but-2-enoate (9b), 2-(2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-ethoxycarbonyl)-4,4-dicyano-3-methyl-N-phenyl-but-3-enami-de (9c) and ethyl 4-(phenylcarbamoyl)-4-(2-hydrazinyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-ethoxycarbonyl)-2-cyano-3-methylbut-2-enoate (9d)

To a dray solid of either compound **8a** (1.01 g, 0.003 mol) or **8b** (1.24 g, 0.003 mol) containing ammonium acetate (0.50 g), either malononitrile **(4a)** (0.2 g, 0.003 mol) or ethylcyanoacetate **(4b)** (0.34 g, 0.003 mol) was added. The reaction mixture was heated in an oil bath at 120 ^{o}C for 1h then left to cool, triturated with ethanol and the solid product was formed and collected by filtration.

Compound **9a**: Pale yellow crystals, yield: 68 % (0.845 g); mp: >290ºC IR (KBr): υ/cm^{-1} = 3449-3323 (2NH), 3055 (CH-aromatic), 2955 (CH_3), 2890 (CH_2), 2227-2220 (3CN), 1693 (CO), 1633 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.66-1.69 (m, 4H, 2CH_2), 2.25-2.31 (m, 4H, 2CH_2), 2.40 (s, 3H, CH_3), 7.29-7.40 (m, 5H, C_6H_5), 8.42, 9.29 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 414 (M^+, 15.7%). Analysis for $C_{22}H_{18}N_6OS$ Calcd: C, 63.75; H, 4.38; N, 20.28; S, 7.74. Found: C, 63.92; H, 4.66; N, 20.32; S, 7.49 %.

Compound **9b**: Pale yellow crystals from ethanol, yield: 78 % (1.08 g); mp: 238-240 ºC. IR (KBr): υ/cm^{-1} = 3480-3323 (2NH), 3053 (CH-aromatic), 2968 (CH_3), 2883 (CH_2), 2223, 2220 (2CN), 1690, 1687 (2CO), 1621 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.13 (t, 3H, J = 7.31 Hz, CH_3), 1.64-1.70 (m, 4H, 2CH_2), 2.14-2.18 (m, 4H, 2CH_2), 2.28 (s, 3H, CH_3), 4.22 (q, 2H, J = 7.31 Hz, CH_2), 7.24-7.40 (m, 5H, C_6H_5), 8.29, 9.33 (2s, 2H, D_2O-exchangeable 2NH). MS (relative intensity) m/z: 461 (M^+, 22.5%). Analysis for $C_{24}H_{23}N_5O_3S$ Calcd: C, 62.46; H, 5.02; N, 15.17; S, 6.95. Found: C, 62.39; H, 4.91; N, 14.92; S, 7.04 %.

Compound **9c**: Yellow crystals from ethanol, yield: 84 % (1.162 g); mp: 266-268 ºC. IR (KBr): υ/cm^{-1} = 3478-3320 (2 NH), 3058 (CH-aromatic), 2972 (CH_3), 2888 (CH_2), 2226, 2221 (2CN), 1692, 1689 (2CO), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.16 (t, 3H, J = 7.48 Hz, CH_3), 1.62-1.73 (m, 4H, 2CH_2), 2.17-2.21 (m, 4H, 2CH_2), 2.32 (s, 3H, CH_3), 4.31 (q, 2H, J = 7.48 Hz, CH_2), 7.30-7.43 (m, 5H, C_6H_5), 8.27, 9.39 (2s, 2H, D_2O-exchangeable 2NH). MS (relative intensity) m/z: 461 (M^+, 34.5%). Analysis for $C_{24}H_{23}N_5O_3S$ Calcd: C, 62.46; H, 5.02; N, 15.17; S, 6.95. Found: C, 62.62; H, 5.29; N, 15.33; S, 7.22 %.

Compound **9d**: Yellow crystals from 1,4 dioxane, yield: 66 % (1.006 g); mp: 188-190 ^{o}C. IR (KBr): υ/cm^{-1} = 3489-3322 (2 NH), 3056 (CH-aromatic), 2964 (CH_3), 2883 (CH_2), 2220 (CN), 1690, 1687 and 1681 (3CO), 1638 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.13, 1.16 (2t, 6H, CH_3), 1.60-1.75 (m, 4H, 2CH_2), 1.97-2.05 (m, 4H, 2CH_2), 2.21 (s, 3H, CH_3), 4.20, 4.25 (2q, 4H, 2CH_2), 7.24-7.40 (m, 5H, C_6H_5), 8.30, 9.37 (2s, 2H, D_2O-exchangeable 2NH). MS (relative intensity) m/z: 508 (M^+, 26.5%). Analysis for $C_{26}H_{28}N_4O_5S$ Calcd: C, 61.40; H, 5.55; N, 11.02; S, 6.30. Found: C, 61.39 ; H, 5.69; N, 11.29; S, 6.49 %.

5-Cyano-1-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)-1,6-dihydro-6-imino-4-methylpyridazine-3-(N-phenyl-carboxamide) (10a), 5-cyano-1-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]-thiophen-2-yl)-1,6-dihydro-4-methyl-6-oxo-pyridazine-3-(N-phenylcarboxamide) (10b), ethyl 2-(3-phenylcarbamoyl) -5-cyano-6-imino-4-methyl-pyridazin-1(6*H*)-yl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (10c) and ethyl 2-(3-phenylcarbamoyl)-5-cyano-4-methyl-6-oxo-pyridazin-1(6*H*)-yl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (10d)

General procedure: To a solution of either compound **8a** (1.01 g, 0.003 mol) or **8b** (1.24 g, 0.003 mol) in ethanol (50 ml) containing piperidine (0.5 ml) either malononitrile **(4a)** (0.2 g, 0.003 mol) or ethyl cyanoacetate **(4b)** (0.34 g, 0.003 mol) was added. The reaction mixture was heated under reflux for 6 h then left to cool and the formed solid product in each case, upon pouring onto ice/water containing few drops of hydrochloric acid was collected by filtration.

Compound **10a**: Pale brown crystals from ethanol, yield: 76 % (0.944 g); mp: 150-152 ºC. IR (KBr): υ/cm^{-1} = 3458-3321 (2 NH), 3056 (CH-aromatic), 2946 (CH_3), 2862 (CH_2), 2229, 2220 (2CN), 1690 (CO), 1660 (C=N), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.64-1.68 (m, 4H, 2CH_2), 2.05-2.12 (m, 4H, 2CH_2), 2.34 (s, 3H, CH_3), 7.27-7.42 (m, 5H, C_6H_5), 8.25, 9.30 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 414 (M^+, 23.3%). Analysis for $C_{22}H_{18}N_6OS$ Calcd: C, 63.75; H, 4.38; N, 20.28; S, 7.74. Found: C, 63.56; H, 4.39; N, 20.19; S, 7.93 %.

Compound **10b**: Yellow crystals from ethanol, yield: 83 % (1.034 g); mp: 140-141 ºC. IR (KBr): υ/cm^{-1} = 3469-3340 (NH), 3052 (CH-aromatic), 2978 (CH_3), 2874 (CH_2), 2226, 2220 (2CN), 1693, 1688 (2CO), 1638 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.62-1.68 (m, 4H, 2CH_2), 2.17-2.22 (m, 4H, 2CH_2), 2.38 (s, 3H, CH_3), 7.28-7.38 (m, 5H, C_6H_5), 9.30 (s, 1H, D_2O-exchangeable, NH). MS (relative intensity) m/z: 415 (M^+, 37.2%). Analysis for $C_{22}H_{17}N_5O_2S$ Calcd: C, 63.60; H, 4.12; N, 16.86; S, 7.72. Found: C, 63.89; H, 4.32; N, 16.95; S, 7.51 %.

Compound **10c**: Yellow crystals from ethanol, yield: 76 % (1.051 g); mp: 164-166 ºC. IR (KBr): υ/cm^{-1} = 3534-3349 (2 NH), 3056 (CH-aromatic), 2956 (CH_3), 2906 (CH_2), 2222 (CN), 1689, 1686 (2CO), 1665 (C=N), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.12 (t, 3H, J = 6.89 Hz, CH_3), 1.64-1.72 (m, 4H, 2CH_2), 2.13-2.17 (m, 4H, 2CH_2), 2.23 (s, 3H, CH_3), 4.20 (q, 2H, J = 6.89 Hz, CH_2), 7.26-7.40 (m, 5H, C_6H_5), 8.29, 9.37 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 461 (M^+, 17.6%). Analysis for $C_{24}H_{23}N_5O_3S$ Calcd: C, 62.46; H, 5.02; N, 15.17; S, 6.95. Found: C, 62.72; H, 5.32; N, 15.49; S, 7.21 %.

Compound **10d**: Yellow crystals from ethanol, yield: 73 % (1.012 g); mp:152-154 ºC. IR (KBr): υ/cm^{-1} = 3476-3336 (NH), 3056 (CH-aromatic), 2980 (CH_3), 2892 (CH_2), 2221 (CN), 1693, 1689 and 1684 (3CO), 1638 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.11 (t, 3H, CH_3), 1.62-1.73 (m, 4H, 2CH_2), 2.12-2.15 (m, 4H, 2CH_2), 2.27 (s, 3H, CH_3), 4.23 (q, 2H, CH_2), 7.28-7.43 (m, 5H, C_6H_5), 9.39 (s, 1H, D_2O-exchangeable, NH). MS (relative intensity) m/z: 462 (M^+, 21.8%). Analysis for $C_{24}H_{22}N_4O_4S$ Calcd: C, 62.32; H, 4.79; N, 12.11; S, 6.93. Found: C, 62.46 ; H, 5.09; N, 12.39; S, 6.69 %.

2-(6-Acetyl-4,5-dihydro-4-phenyl-5-(phenylimino)-3-thio-1,2,4-triazin-2-(3*H*)-yl)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carbonitrile (13a) and ethyl 2-(6-acetyl-4,5-dihydro-4-phenyl-5-(phenylimino)-3-thio-1,2,4-triazin-2(3*H*)-yl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (13b)

General procedure: To a solution of either compound **8a** (1.01 g, 0.003 mol) or **8b** (1.24 g, 0.003 mol) in 1,4-dioxan (40 ml) containing catalytic base "triethylamine" (0.5 ml), phenylisothiocyanate **(11)** (0.41 g, 0.003 mol) was added. The reaction mixture was heated under reflux for 3 h then left to cool and the formed solid product in each case, upon pouring onto ice/water containing few drops of hydrochloric acid was collected by filtration.

Compound **13a**: Orange crystals from DMF, yield: 77 % (1.116 g); mp: 185-187 ºC. IR (KBr): υ/cm^{-1} = 3053 (CH-aromatic), 2973 (CH_3), 2888 (CH_2), 2220 (CN), 1689 (CO), 1634 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.73-1.75 (m, 4H, 2CH_2), 1.98-2.06 (m, 4H, 2CH_2), 3.01 (s, 3H, CH_3), 7.34-7.46 (m, 10H, 2C_6H_5). MS (relative intensity) m/z: 483 (M^+, 14.5%). Analysis for $C_{26}H_{21}N_5OS_2$ Calcd: C, 64.57; H, 4.38; N, 14.48; S, 13.26. Found: C, 64.41; H, 4.08; N, 14.32; S, 13.44 %.

Compound **13b**: Orange crystals from DMF, yield: 75 % (1.193 g); mp: 233-235 ºC. IR (KBr): υ/cm^{-1} = 3056 (CH-aromatic), 2958 (CH_3), 2893 (CH_2), 1692, 1687 (2CO), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.14 (t, 3H, J = 7.42 Hz, CH_3), 1.61-1.74 (m, 4H, 2CH_2), 2.19-2.24 (m, 4H, 2CH_2), 3.05 (s, 3H, CH_3), 4.21 (q, 2H, J = 7.42 Hz, CH_2), 7.28-7.41 (m, 10H, 2C_6H_5). MS (relative intensity) m/z: 530 (M^+, 26.2%). Analysis for $C_{28}H_{26}N_4O_3S_2$ Calcd: C, 63.37; H, 4.94; N, 10.56; S, 12.08. Found: 63.42; H, 5.19; N, 10.36; S, 11.84%.

4-((3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)diazenyl)-3-methyl-5-phenylamino-1*H*-pyrazol (15a), 4-((3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)diazenyl)-3-methyl-1-phenyl-5-phenylamino-1*H*-pyrazol (15b), 4-((3-ethoxycarbonyl-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)diazenyl)-3-methyl-5-phenylamino-1*H*-pyrazol (15c) and 4-((3-ethoxycarbonyl-4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)di-azenyl)-3-methyl-1-phenyl-5-phenylamino-1*H*-pyrazol (15d)

General procedure: To a solution of either compound **8a** (1.01 g, 0.003 mol) or **8b** (1.24 g, 0.003 mol) in ethanol (50 ml) either hydrazine hydrate **(14a)** (0.15 ml, 0.003 mol) or phenyl hydrazine **(14b)** (0.33 g, 0.003 mol) was added. The reaction mixture in each case was heated under reflux for 3 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

Compound **15a**: Pale brown crystals from ethanol, yield: 76 % (0.826 g); mp: 205-207 ºC. IR (KBr): υ/cm^{-1} = 3458-3321 (2NH), 3056 (CH-aromatic), 2966 (CH_3), 2884 (CH_2), 2229 (CN), 1660 (C=N), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.62-1.67(m, 4H, 2CH_2), 1.88-1.93 (m, 4H, 2CH_2), 2.13 (s, 3H, CH_3), 7.26-7.37 (m, 5H, C_6H_5), 8.78, 9.61 (2s, 2H, D_2O-exchangeable, 2NH,). MS (relative intensity) m/z: 362 (M^+, 16.8%). Analysis for $C_{19}H_{18}N_6S$ Calcd: C, 62.96; H, 5.01; N, 23.19; S, 8.85. Found: C, 63.14; H, 4.99; N, 22.94; S, 8.61 %.

Compound **15b**: Yellow crystals from ethanol, yield: 75 % (0.986 g); mp: 245-247 ºC. IR (KBr): υ/cm^{-1} = 3439-3321 (NH), 3056 (CH-aromatic), 2952 (CH_3), 2858 (CH_2), 2223 (CN), 1633 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.61-1.66 (m, 4H, 2CH_2), 2.03-2.07 (m, 4H, 2CH_2), 2.24 (s, 3H, CH_3), 7.24-7.47 (m, 10H, 2C_6H_5), 9.22 (s, 1H, D_2O-exchangeable, NH). MS (relative intensity) m/z: 438 (M^+, 19.2%). Analysis for $C_{25}H_{22}N_6S$ Calcd: C, 68.47; H, 5.06; N, 19.16; S, 7.31. Found: C, 68.73; H, 4.83; N, 19.12; S, 7.22%.

Compound **15 c**: Yellow crystals from ethanol, yield: 76 % (0.934 g); mp: 169-171 ºC. IR (KBr): υ/cm^{-1} = 3573-3329 (2 NH), 3052 (CH-aromatic), 2947 (CH_3), 2868 (CH_2), 1690 (CO), 1649 (C=N), 1632 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.14 (t, 3H, J = 7.48 Hz, CH_3), 1.62-1.70 (m, 4H, 2CH_2), 2.17-2.24 (m, 4H, 2CH_2), 2.38 (s, 3H, CH_3), 4.21 (q, 2H, J = 7.48 Hz, CH_2), 7.29-7.39 (m, 5H, C_6H_5), 8.30, 9.33 (2s, 2H, D_2O-exchangeable, 2NH). MS (relative intensity) m/z: 409 (M^+, 33.4%). Analysis for $C_{21}H_{23}N_5O_2S$ Calcd: C, 61.59; H, 5.66; N, 17.10; S, 7.83. Found: C, 61.48; H, 5.42; N, 17.22; S, 7.89 %.

Compound **15d**: Yellow crystals from ethanol, yield: 68 % (0.99 g); mp: 175-177 ºC. IR (KBr): υ/cm^{-1} = 3482-3329 (NH), 3053 (CH-aromatic), 2971 (CH_3), 2880 (CH_2), 1688 (CO), 1632 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.12 (t, 3H, CH_3), 1.60-1.67 (m, 4H, 2CH_2), 2.18-2.25 (m, 4H, 2CH_2), 2.35 (s, 3H, CH_3), 4.22 (q, 2H, CH_2), 7.26-7.38 (m, 10H, 2C_6H_5), 9.46 (s, 1H, D_2O-exchangeable, NH). MS (relative intensity) m/z: 485 (M^+, 27.1%). Analysis for $C_{27}H_{27}N_5O_2S$ Calcd: C, 66.78; H, 5.60; N, 14.42; S, 6.60. Found: C, 66.52 ; H, 5.32; N, 14.15; S, 6.78 %.

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