Acetoacetanilide based heterocycles

NOVEL HETEROCYCLIC COMPOUNDS DERIVED FROM ACETOACETANILIDE TOGETHER WITH THEIR ANTITUMOR EVALUATIONS

Keywords: pyrazol, pyridazine, thiophene, 1, 2, 4 triazine, cytotoxic activity.

The reaction of acetoacetanilide (1) with cyclohexanone (2) gave compound 3 which it was reacted with the active methylene reagents 4a, b afforded cyclohexyldiene 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 piperidine 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.

Corresponding Authors
Tel: 00201004712543
Fax: 00238371543
E-mail: karamsyn@yahoo.com
[a] Chemistry Department, Faculty of Biotechnology, October University for Modern Sciences and Arts(MSA), El-Wahat Road, 6 October City, Egypt.
[b] National Organization for Drug Control & Research P.O. 29, Cairo, Egypt

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 antimicrobial5,6 and antiprotozoal activity,7,8 pyridazine derivatives has antimicrobial activity,9 on the other hand 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 1H NMR spectrum of 6a exhibited two multiplets at δ 1.77-1.79 & 2.11-2.16 ppm indicating the five CH2 groups, a singlet at δ 2.37 ppm corresponding to CH3 group, a multiplet at δ 7.25-7.36 ppm for the C6H5 group and a singlet, D2O-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.

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 °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).

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 1H NMR spectrum of compound 3 showed two multiplets at δ 1.77-1.79 & 2.11-2.16 ppm indicating the five CH2 groups, a singlet at δ 2.27 ppm corresponding to CH3 group, a multiplet at δ 7.25-7.36 ppm for the C6H5 group and a singlet, D2O-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.

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.
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 $\delta$ 1.64-1.68 & 2.05-2.12 ppm indicating to the four CH$_2$ groups, a singlet at $\delta$ 2.34 ppm corresponding to the CH$_3$ group, a multiplet at $\delta$ 7.27-7.42 ppm for the C$_6$H$_5$ group and two singlets, D$_2$O-exchangeable at $\delta$ 8.25 and 9.30 ppm for the two NH groups.

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).
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 Fetal bovine serum (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 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.

Results are given in concentrations that were able to cause 50 % of cell growth inhibition (GI50) 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

<table>
<thead>
<tr>
<th>Compound</th>
<th>GI&lt;sub&gt;50&lt;/sub&gt; μmol L&lt;sup&gt;-1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NCI-H460</td>
</tr>
<tr>
<td>3</td>
<td>27.6 ± 2.4</td>
</tr>
<tr>
<td>5a</td>
<td>0.02 ± 0.002</td>
</tr>
<tr>
<td>5b</td>
<td>12.1 ± 0.8</td>
</tr>
<tr>
<td>6a</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>6b</td>
<td>4.2 ± 1.4</td>
</tr>
<tr>
<td>8a</td>
<td>12.6 ± 0.6</td>
</tr>
<tr>
<td>8b</td>
<td>22.4 ± 8.1</td>
</tr>
<tr>
<td>9a</td>
<td>0.01 ± 0.002</td>
</tr>
<tr>
<td>9b</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>9c</td>
<td>6.1 ± 2.4</td>
</tr>
<tr>
<td>9d</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>10a</td>
<td>8.1 ± 2.2</td>
</tr>
<tr>
<td>10b</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>10c</td>
<td>32.0 ± 1.6</td>
</tr>
<tr>
<td>10d</td>
<td>0.01 ± 0.003</td>
</tr>
<tr>
<td>13a</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>13b</td>
<td>10.6 ± 4.6</td>
</tr>
<tr>
<td>15a</td>
<td>20.4 ± 8.1</td>
</tr>
<tr>
<td>15b</td>
<td>0.01 ± 0.008</td>
</tr>
<tr>
<td>15c</td>
<td>2.6 ± 0.6</td>
</tr>
<tr>
<td>15d</td>
<td>0.01 ± 0.2</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.04 ± 0.008</td>
</tr>
</tbody>
</table>

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 instrument in CD\(_2\)SO\(_4\)D as solvent using TMS as internal standard and chemical shifts were expressed as \(\delta\) ppm. Mass spectra were recorded on Kratos (75 eV) MS equipment (Germany).

**2-Cyclohexylidine-3-oxo-N-phenylbutanamide (3)**

To a dry slurry of acetooacetylne (1) (5.31 g, 0.03 mol) containing ammonium acetate (0.50 g) cyclohexanone (2) (2.94 g, 0.03 mol) was added. The reaction mixture was heated 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°C. IR (KBr): \(\nu / cm^{-1} = 3469-3322 (\text{NH}), 3058 (\text{CH}-\text{aromatic}), 2991(\text{CH}_3), 2885(\text{CH}_2), 1695, 1687 (2\text{CO}), 1636 (\text{C=C}).\) \(^1\)H NMR (DMSO-d\(_6\)): \(\delta = 1.73-1.76 (\text{m, 6H, 3CH}_2), 2.11-2.16 (\text{m, 4H, 2CH}_2), 2.27 (\text{s, 3H, CH}_3), 7.25-7.36 (\text{m, 5H, C}_6\text{H}_5), 9.63 (\text{s, 1H, NH, D}_2\text{O-exchangeable}).\) MS (relative intensity) m/z: 352 (M\(^+\), 30.6%). Analysis for C\(_{21}\)H\(_{24}\)N\(_2\)O\(_3\)S: Calcd: C 71.57; H 6.86; N 8.21; S 8.45. Found: C, 71.63; H, 6.86; N, 8.34.  Compound 5a: Yellow crystals from ethanol, yield: 84% (2.958 g); mp: 104°C. IR (KBr): \(\nu / cm^{-1} = 3484-3312 (\text{NH}), 3058 (\text{CH}-\text{aromatic}), 2985 (\text{CH}_3), 2916 (\text{CH}_2), 2222 (\text{CN}), 1690, 1687 (\text{2CO}), 1636 (\text{C=C}).\) \(^1\)H NMR (DMSO-d\(_6\)): \(\delta = 1.13 (\text{t, 3H, } J = 7.44 \text{Hz, CH}_3), 1.72-1.77 (\text{m, 6H, 3CH}_2), 2.12-2.18 (\text{m, 4H, 2CH}_2), 2.35 (\text{s, 3H, CH}_3), 4.22 (\text{q, 2H, } J = 7.44 \text{Hz, CH}_2), 7.28-7.40 (\text{m, 5H, C}_6\text{H}_5), 9.73 (\text{s, 1H, NH, D}_2\text{O-exchangeable}).\) MS (relative intensity) m/z: 352 (M\(^+\), 30.6%). Analysis for C\(_{21}\)H\(_{24}\)N\(_2\)O\(_3\)S: Calcd: C 71.57; H 6.86; N, 8.34.

**2-Dicyano-2-cyclohexylidene-N-phenylacetamide (6b)**

**Compound 6b:** Yellow crystals from ethanol, yield: 84% (0.915 g, 0.003 mol) or compound 5b (1.06 g, 0.003 mol) in 1,4-dioxane (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.

**2-(5-Amino-4-cyanothiophene-3-yl)-2-cyclohexylidene-N-phenylcarbamoylmethyl 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-dioxane (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.

**4,4-Dicyano-2-cyclohexylidine-3-methyl-N-phenylbutanamide-3-enamide (5a) and ethyl-4-(phenylcarbamoyl)-2-cyano-4-cyclohexylidene-3-methyl-2-enamide (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): \(\nu / cm^{-1} = 3466-3322 (\text{NH}), 3054 (\text{CH}-\text{aromatic}), 2980 (\text{CH}_3), 2918 (\text{CH}_2), 2223-2220 (2\text{CN}), 1687 (\text{CO}), 1633 (\text{C=C}).\) \(^1\)H NMR (DMSO-d\(_6\)): \(\delta = 1.75-1.79 (\text{m, 6H, 3CH}_2), 1.87-1.91 (\text{m, 4H, 2CH}_2), 2.28 (\text{s, 3H, CH}_3), 7.29-7.41 (\text{m, 5H, C}_6\text{H}_5), 9.88 (\text{s, 1H, NH, D}_2\text{O-exchangeable}).\) MS (relative intensity) m/z: 305 (M\(^+\), 17.5%). Analysis for C\(_{20}\)H\(_{19}\)N\(_3\)OS: Calcd: C 74.85; H 6.11; N, 13.93 %.
Compound 8a: Orange crystals from DMF, yield: 63 % (6.197 g); mp: 169-171 °C. IR (KBr): v/cm⁻¹ = 3472-3363 (2 NH), 3056 (CH-aromatic), 2974 (CH₃), 2894 (CH₂), 1692, 1684 and 1681 (3CO), 1636 (C=O). ¹H NMR (DMF-d₆): δ = 1.16 (t, 3H, J = 7.62 Hz, CH₃), 1.62-1.73 (m, 4H, 2CH₂), 2.27-2.34 (m, 4H, 2CH₂), 2.42 (s, 3H, CH₃), 4.23 (q, 2H, J = 7.62 Hz, CH₂), 7.28-7.42 (m, 5H, C₆H₅), 8.32, 9.36 (2s, 2H, D₂O-exchangeable 2NH). MS (relative intensity) m/z: 413 (M⁺, 27.4%). Analysis for C₁₉H₁₈N₄O₂S Calcd: C, 61.00; H, 4.39; N, 10.31; S, 7.93 %. Found: C, 61.28; H, 5.42; N, 10.31; S, 7.93 %. 2-(2-Hydrazinyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)ethoxycarbonyl)-2-cyano-3-methylbut-2-enoic acid (9a): Yellow crystals from 1,4 dioxane, yield: 84 % (1.162 g); mp: 266-268 °C. IR (KBr): v/cm⁻¹ = 3489-3322 (2 NH), 3056 (CH-aromatic), 2964 (CH₃), 2883 (CH₂), 1922, 1690, 1667 and 1681 (3CO), 1638 (C=O). ¹H NMR (DMF-d₆): δ = 1.13, 1.16 (2t, 6H, CH₂), 1.60-1.75 (m, 4H, 2CH₂), 1.97-2.05 (m, 4H, 2CH₂), 2.21 (s, 3H, CH₃), 3.50, 4.25 (2q, 4H, 2CH₂), 7.24-7.40 (m, 5H, C₆H₅), 8.30, 9.37 (2s, 2H, D₂O-exchangeable 2NH). MS (relative intensity) m/z: 508 (M⁺, 26.5%). Analysis for C₂₃H₂₃N₅O₄S 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-methylpyrazidine-3-(N-phenyl-carboxamide) (10a): Yellow crystals from ethanol, yield: 50 % (0.50 ml) containing piperidine (0.5 ml) either malononitrile (0.5 g) or ethyl cyanoacetate (4b) (0.34 g, 0.003 mol) was added. The reaction mixture was heated in an oil bath at 120 °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: >290°C IR (KBr): v/cm⁻¹ = 3449-3323 (2NH, 3055 (CH-aromatic), 2955 (CH₃), 2890 (CH₂), 2277-2220 (1693), 1693 (CO), 1636 (C=O). ¹H NMR (DMF-d₆): δ = 1.66-1.69 (m, 4H, 2CH₂), 2.25-2.31 (m, 4H, 2CH₂), 2.40 (s, 3H, CH₃), 7.29-7.40 (m, 5H, C₆H₅), 8.42, 9.29 (2s, 2H, D₂O-exchangeable, 2NH). MS (relative intensity) m/z: 414 (M⁺, 15.7%). Analysis for C₂₃H₂₃N₆O₂S 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 °C. IR (KBr): v/cm⁻¹ = 3480-3323 (2NH), 3053 (CH-aromatic), 2968 (CH₃), 2883 (CH₂), 2223, 2220 (2CN), 1690, 1687 (2CO), 1621 (C=C). ¹H NMR (DMF-d₆): δ = 1.13 (t, 3H, J = 7.31 Hz, CH₃), 1.64-1.70 (m, 4H, 2CH₂), 2.14-2.18 (m, 4H, 2CH₂), 2.28 (s, 3H, CH₃), 4.22 (q, 2H, J = 7.31 Hz, CH₂), 7.24-7.40 (m, 5H, C₆H₅), 8.29, 9.33 (2s, 2H, D₂O-exchangeable 2NH). MS (relative intensity) m/z: 461 (M⁺, 22.5%). Analysis for C₂₃H₂₃N₆O₂S 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 10a: Pale brown crystals from ethanol, yield: 76 % (0.944 g); mp: 150-152 °C. IR (KBr): v/cm⁻¹ = 3548-3321 (2 NH), 3056 (CH-aromatic), 2946 (CH₃), 2862 (CH₂), 2229, 2220 (2CN), 1690 (CO), 1660 (C=N), 1636 (C=O). ¹H NMR (DMF-d₆): δ = 1.64-1.68 (m, 4H, 2CH₂), 2.05-2.12 (m, 4H, 2CH₂),2.34 (s, 3H, CH₃), 7.27-7.42 (m, 5H, C₆H₅), 8.25, 9.30 (2s, 2H, D₂O-exchangeable, 2NH). MS (relative intensity) m/z: 414 (M⁺, 23.3%). Analysis for C₂₃H₂₃N₆O₂S 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 °C. IR (KBr): v/cm⁻¹ = 3469-3340 (NH), 3052 (CH-aromatic), 2978 (CH₃), 2874 (CH₂), 2226, 2220 (2CN), 1693, 1689 (2CO), 1638 (C=C). ¹H NMR (DMF-d₆): δ = 1.62-1.68 (m, 4H, 2CH₂), 2.17-2.22 (m, 4H, 2CH₂), 2.38 (s, 3H, CH₃), 7.28-7.38 (m, 5H, C₆H₅), 9.30 (s, 1H, D₂O-exchangeable, NH). MS (relative intensity) m/z: 415 (M⁺, 37.2%). Analysis for C₂₃H₂₃N₆O₂S 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 °C. IR (KBr): v/cm\(^{-1}\) = 3534-3349 (NH), 3056 (CH-aromatic), 2956 (CH\(_3\)), 2906 (CH\(_2\)), 2222 (CN), 1689, 1682 (C=O), 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\(_6\)H\(_5\)), 8.29, 9.37 (2H, D\(_2\)O-exchangeable, 2NH). MS (relative intensity) m/z: 461 (M\(^+\), 17.6%). Analysis for C\(_{26}\)H\(_{21}\)N\(_5\)O\(_2\)S\(_2\)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 °C. IR (KBr): v/cm\(^{-1}\) = 3476-3336 (NH), 3056 (CH-aromatic), 2980 (CH\(_3\)), 2892 (CH\(_2\)), 2221 (CN), 1693, 1689 and 1684 (C=O), 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\(_6\)H\(_5\)), 9.39 (s, 1H, D\(_2\)O-exchangeable, NH). MS (relative intensity) m/z: 462 (M\(^+\), 21.8%). Analysis for C\(_{28}\)H\(_{26}\)N\(_4\)O\(_3\)S\(_2\)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-(3H)-yl)-4,5,6,7-tetrahydrobenzo[\(d\)]thiophene-3-carbonitrile (13a) and ethyl 2-(6-acetyl-4,5-dihydro-4-phenyl-5-(phenylimino)-3-thio-1,2,4-triazin-2(3H)-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 catalyic 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 °C. IR (KBr): v/cm\(^{-1}\) = 3053 (CH-aromatic), 2973 (CH\(_3\)), 2888 (CH\(_3\)), 2220 (CN), 1660, 1693 and 1684 (C=O), 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\(_6\)H\(_5\)). MS (relative intensity) m/z: 483 (M\(^+\), 14.5%). Analysis for C\(_{30}\)H\(_{21}\)N\(_4\)O\(_2\)S 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 °C. IR (KBr): v/cm\(^{-1}\) = 3056 (CH-aromatic), 2958 (CH\(_3\)), 2893 (CH\(_3\)), 1692, 1687 (C=O), 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\(_6\)H\(_5\)). MS (relative intensity) m/z: 530 (M\(^+\), 26.2%). Analysis for C\(_{32}\)H\(_{23}\)O\(_7\)S 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)diazonyl)-3-methyl-5-phenylamino-1H-pyrazol (15a), 4-((3-cyanoo-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)diazonyl)-3-methyl-5-phenyl-5-phenylamino-1H-pyrazol (15b), 4-(3-ethoxy carbonyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)diazonyl)-3-methyl-5-phenylamino-1H-pyrazol (15c) and 4-(3-ethoxy carbonyl-4,5,6,7-tetrahydrobenzo[b]thiophen-2-yl)diazonyl)-3-methyl-5-phenyl-5-phenylamino-1H-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.
Acknowledgment

The authors would like to thank the research group working at the Medicinal Department at the National Research Center, Dokki, Egypt, for recording the pharmacological data of the synthesized products. Moreover, the effort of Dr. Mohammed Othman, MSA University-Biochemistry Department, is greatly appreciated for his kind revisions for the pharmacological data.

References


Received: 12.04.2014. Accepted: 05.04.2014.