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Synthesis and Cytotoxicity Screening of Some Novel Benzofuranoyl-pyrazole Derivatives against Liver and Cervix Carcinoma Cell Lines

Magdy I. El-Zahar*, Somaia S. Abd El-Karim and Manal M. Anwar

Therapeutical Chemistry Department, National Research Centre, Dokki, Cairo, Egypt.

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ABSTRACT

A series of new pyrazole, thiazole and thiazolinone derivatives incorporated into benzofuran were synthesized by using 3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-carboxaldehyde as starting material. A total of 41 novel compounds were synthesized. Some of these synthesized compounds were evaluated for cytotoxicity activity against HEPG2 (liver carcinoma cell line) and HELA (cervix carcinoma cell line). The tested compounds (1, 2c, 7c, 8b, 9b, 13b and 14b) showed better activities at low concentration against the commonly used human carcinoma cell lines. A detailed synthesis, spectroscopic data and cytotoxicity screening for the new compounds are described.

KEY WORDS

Pyrazole, thiazolinone, benzofuran, benzofuranoyl-pyrazoles, cytotoxic screening, HEPG2, HELA.

1. Introduction

The benzofuran ring system in conjugation with aromatic or heterocyclic moieties has attracted considerable attention in recent studies due to their wide spectrum of biological activities such as oxytocin antagonism,[1] neuroprotection,[2] antioxidant,[3] anti-inflammatory,[3] antibacterial,[3] cholinesterase inhibition,[4] beta-aggregate specific effect for Alzheimer's disease treatment,[5] H-3 receptor antagonism with further cognition dysfunction improvement,[6] antidepressant,[7] anticonvulsant[8,9] and anticancer against different types of carcinoma.[10–13] Despite significant progress achieved in anticancer therapy, the management of malignancies in humans still constitutes a major challenge for contemporary medicine.[14] Various methods and therapies have been developed to treat this disease, but all methods have limitations.[15] As such, there is an urgent need to develop effective new anticancer drugs. Taking all of the above evidence into account, the aim of this research is to synthesize novel derivatives for anticancer evaluation as a trial to obtain new antitumour agents of higher activity and lower side effects.

In view of the antineoplastic activity of pyrazole derivatives,[16] it was aimed to synthesize a benzofuran ring system attached to a pyrazole moiety as such skeleton appeared of highly promising antitumour activity. Moreover, due to the anticancer activity ascribed to different heterocyclic ring systems such as thiazoles,[17] thiazolidinones[18–20] and pyrimidines[21–23] against different types of carcinoma cell lines, it was decided to design a series of known heterocyclic moieties attached to a benzofuran-pyrazole backbone.

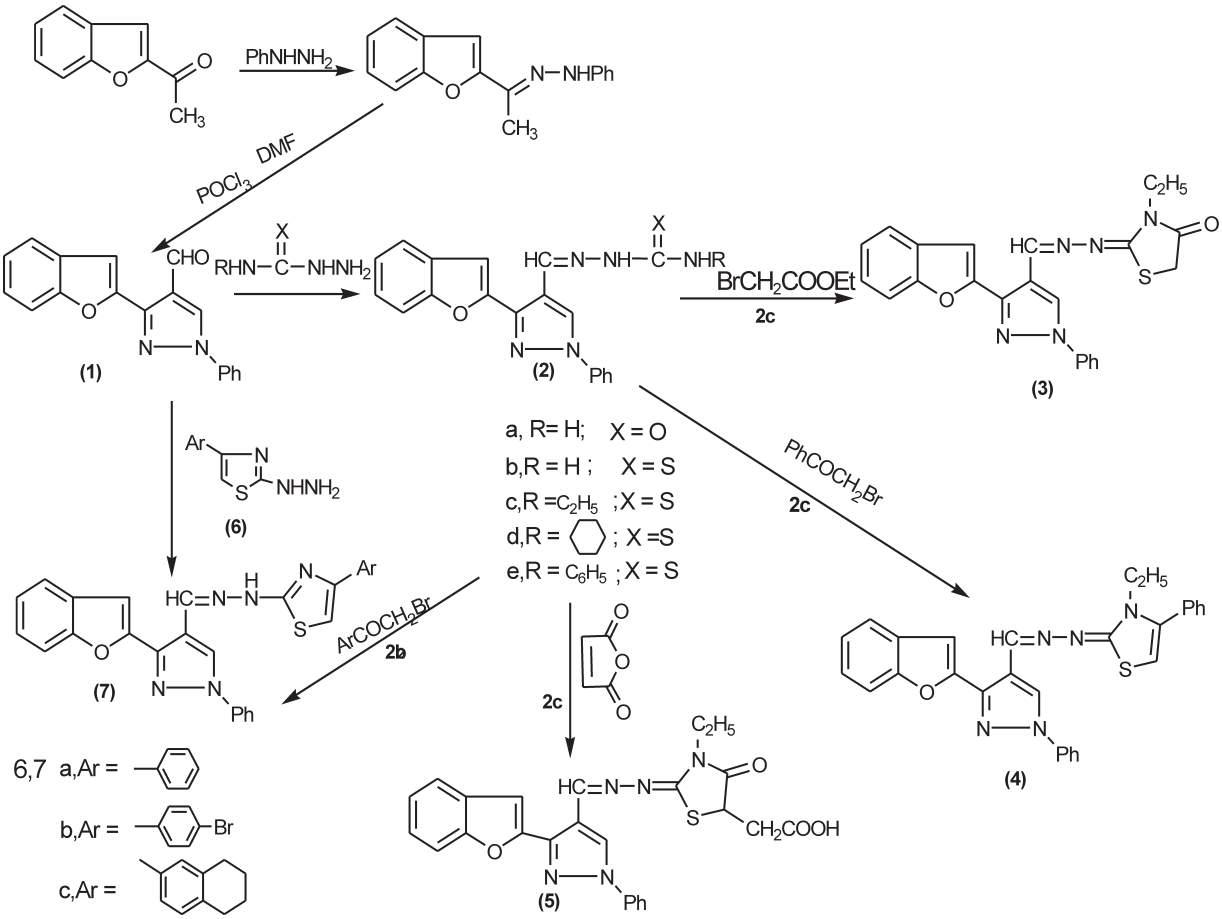
2. Results and Discussion

2.1. Chemistry

2-Acetylbenzofuran was prepared according to the reported method,[24] and condensed with phenyl hydrazine in absolute ethanol to obtain 1-(benzofuran-2-yl)ethylidene-2-phenylhydrazone.[25] Treatment of this hydrazone with phosphorus oxychloride and dimethyl formamide (1:2) at 0–5 °C (Vilsmeier-Haack reaction) gave the desired 3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-carboxaldehyde (**1**) according to the reported method,[26,27] but with slight modifications. The structure of the novel compound **1** was confirmed by the spectral data. In the IR spectrum a band at 1669 cm^{-1} characteristic of the CHO group was observed, while the ^1H NMR spectrum showed a singlet signal for one proton at δ 8.50 ppm due to the pyrazole fragment and a singlet signal for one proton at δ 10.40 ppm due to the CHO fragment.

The carboxaldehyde **1** is considered to be a useful starting material for further synthesis, thus **1** was treated with substituted thiosemicarbazide and/or semicarbazide in absolute ethanol to give the corresponding thiosemicarbazone and/or semicarbazone novel derivatives **2a–e**. The structures of compounds **2a–e** were confirmed by the spectral data. In the IR spectra, there are bands in the 3467–3122 cm^{-1} (-NH) and 1599–1554 cm^{-1} (C=N) regions. The ^1H NMR spectrum of **2c** shows a triplet signal for three protons at δ 1.0 ppm and a quartet signal for two protons at δ 3.40 ppm due to the CH_3CH_2- fragment. The mass spectra of compounds **2a–e** show molecular ion peaks for each corresponding compound at m/z 345, 362, 389, 443 and 435, respectively. Cyclization of **2c** either by ethyl bromoacetate, phenacyl bromide and/or maleic acid anhydride according to a reported method[28] furnished the novel thiazole derivatives **3**, **4** and **5**, respectively. The structures of compounds **3, 4** and **5** were confirmed by the spectral data. In the IR spectrum of compound **3** a band at 1710 cm^{-1} (C=O) is observed. The ^1H NMR spectrum of compound **3** shows a singlet signal for two protons at δ 3.45 ppm due to CH_2 of the thiazolidinone fragment. The mass spectrum of compound **3** shows a molecular ion peak at m/z 429 while the mass spectrum of compound **4** shows the presence of a molecular ion peak at m/z 489. In the IR spectrum of compound **5** there are bands at 3397 cm^{-1} (OH) for the carboxylic acid group and 1706 cm^{-1} (cyclic ketone). On the other hand, the cyclization of **2b** by phenacylbromide, *p*-bromophenacylbromide and/or 6-bromoacetyl-1,2,3,4-tetrahydronaphthalene[17] gave the novel thiazole derivatives[29] **7a–c**, respectively. Alternative synthesis of compounds **7a–c** was investi-

* To whom correspondence should be addressed. E-mail: melzahar@hotmail.com



Scheme 1

gated by refluxing compound **1** with 1-(4-phenylthiazol-2-yl) hydrazine,[30] 1-(4-*p*-bromophenylthiazol-2-yl)hydrazine[31] and/or 4-(1,2,3,4-tetrahydronaphthalen-6-yl)thiazol-2-hydrazine (**6a–c)** respectively; only **6c** is novel. The structures of the novel compounds **7a–c** were confirmed by the spectral data. In the IR spectra a band in the 3342–3292 cm^{-1} (-NH) region is present, while the ^1H NMR spectrum of compound **7c** shows a singlet signal for one proton at δ 6.6 ppm due to the thiazole fragment. The mass spectra of compounds **7a–c** show the corresponding molecular ion peaks at m/z 461, 539 and 515, respectively (Scheme 1).

It is known that α,β-unsaturated ketones are considered to be active starting materials for the preparation of different heterocyclic compounds[32] and accordingly the carboxaldehyde **1** was condensed with different ketones (*p*-methylacetophenone, *p*-bromoacetophenone, 6-acetyl-1,2,3,4-tetrahydronaphthalene and/or 2-acetylthiophene) to give the corresponding novel chalcone derivatives **8a–d**, respectively. The structures of compounds **8a–d** were confirmed by the spectral data. In the IR spectrum of compound **8b** a band at 1633 cm^{-1} (α,β-unsaturated ketone) is observed. The ^1H NMR spectrum of **8a** shows a singlet signal for three protons at δ 2.46 ppm due to the CH_3 fragment, and that of **8c** shows a multiplet signal for four protons at δ 1.93 ppm due to two CH_2 groups of the tetrahydronaphthalene fragment and a multiplet signal for four protons at δ 2.44 ppm due to two CH_2 groups of the tetrahydronaphthalene fragment. The mass spectra of compounds **8a–d** show the corresponding molecular ion peaks at m/z 404, 468, 444 and 396, respectively. Further cyclo-condensation of **8a–d** by hydrazine hydrate (99 %) in absolute ethanol[33] furnished the corresponding novel 1*H*-pyrazoline derivatives **9a–d**, while cyclo-condensation with hydrazine hydrate in acetic acid[34] afforded the novel N-acetylpyrazoline derivatives **10a–d**. On the other hand when the cyclo-condensation reaction was carried out by using N-substituted hydrazine[33] in absolute ethanol, it gave the corresponding N-substituted pyrazoline derivatives **11a,b,** respectively. The structure of the pyrazoline derivatives **9a–d, 10a–d** and **11a,b** were confirmed by the spectral data. In the IR spectra of compounds **9a–d** absorption bands in the 3313–3238 cm^{-1} region, characteristic of a NH group, are observed. Mass spectra of compounds **9a–d** show the corresponding molecular ion peaks at m/z 418, 482, 458 and 410, respectively. The ^1H NMR spectra of **10a–c** show a singlet signal for three protons at δ 2.49 ppm due to the $COCH_3$ fragment, two multiplet signals for two protons at δ 3.26 and δ 3.88 ppm due to CH_2 of the pyrazoline fragment and a multiplet signal for one proton at δ 6.16 ppm due to the pyrazoline fragment. The mass spectra of **10a–d** show molecular ion peaks at m/z 460, 524, 500 and 452, respectively. The mass spectra of compounds **11a,b** show the corresponding molecular ion peaks at m/z 496 and 558, respectively. Moreover the cyclo-condensation of **8a** with thiourea in 1 % alcoholic potassium hydroxide[35] gave the corresponding novel thiopyrimidine derivative **12**. The structure of compound **12** was confirmed by spectral data. Its mass spectrum shows a molecular ion peak at m/z 462.

It has been suggested that Schiff's bases could be hydrolyzed selectively by tumour cells to serve as alkylating agents at the same time as the active amine is freed to act as an antimetabolite.[36] Thus, the condensation of the carboxaldehyde derivative **1** with different aromatic amines (aniline, *p*-toluidine, *p*-bromoaniline, *m*-fluoroaniline and/or *p*-aminobenzoic acid) was performed to yield the corresponding novel Schiff's bases

13a–e, respectively. The structures of compounds **13a–e** were confirmed by their spectral data. The ^{1}H NMR spectrum of **13b** shows a singlet signal for three protons at δ 2.40 ppm due to the CH_3 fragment and another singlet signal for one proton at δ 8.7 ppm due to the CH=N fragment. The mass spectra of compounds **13a–e** show the corresponding molecular ion peaks at m/z 363, 377, 441, 381 and 407, respectively.

Compounds **13a–c,e** were cyclized with thioglycolic acid in dry benzene to obtain the corresponding novel 4-oxothiazolidin-2-pyrazole derivatives[37] **14a–d**. The structures of compounds **14a–d** were confirmed by the spectral data. The ^{1}H NMR spectrum of **14b** shows a multiplet signal for two protons at δ 3.50–3.70 ppm due to CH_2 of the thiazolidinone fragment and a singlet signal for one proton at δ 6.41 ppm due to the thiazolidinone fragment. The mass spectra of compounds **14b–d** show the corresponding molecular ion peaks at m/z 451, 515 and 481, respectively.

Further condensation of the carboxaldehyde derivative **1** was carried out with compounds having an active methylene group such as malononitrile, ethyl cyanoacetate, diethylmalonate and malonic acid to afford the corresponding condensation products **15a–d**, respectively. The structures of the novel compounds **15a–d** were confirmed by their spectral data. In the IR spectrum of **15a** an absorption band at 2225 cm^{-1} (CN) appears while the IR spectrum of **15c** shows absorption bands at 2219 cm^{-1} (CN) and at 1730 cm^{-1} (CO, ester). The ^{1}H NMR spectrum of **15b** shows a multiplet signal for six protons at δ 1.58–1.65 ppm due to two CH_3 groups of two ester fragments and a multiplet signal for four protons at δ 4.56–4.67 ppm due to two CH_2 groups of two ester fragments. The mass spectra of **15b,d** show the corresponding molecular ion peaks at m/z 430 and 330, respectively (Scheme 2).

2.2. Structure Activity Relationship

The cytotoxic effect of some of the newly-prepared compounds was evaluated in assays applying a human liver carcinoma cell line (HEPG2) and a human cervix carcinoma cell line (HELA), respectively.

The IC_{50} values are summarized in Table 1. It is clear that the benzofuranoyl-pyrazole backbone is crucial for the cytotoxic activity against the two types of carcinoma cell lines (compounds **1, 2c, 7c, 8b, 9b, 13b, 14b**). The replacement of the aldehydic group of compound **1** (IC_{50}: HEPG2 3.5 μg mL^{-1} and HELA 5.6 μg mL^{-1}) by ethyl thiosemicarbazide side chain (compound **2c**) exhibits significant enhancement of the cytotoxic activity against both HEPG2 (IC_{50} 1.9 μg mL^{-1}) and HELA (IC_{50} 3.1 μg mL^{-1}). By contrast, the heterocyclic compound containing a tetrahydronaphthalenylthiazole moiety (compound **7c**) shows less cytotoxic activity against HEPG2 (IC_{50} 5.0 μg mL^{-1}) and complete loss of activity against HELA. This observation indicates that the side chain containing S and N atoms plays a more important role in the improvement of the potency of the cytotoxic activity of the compounds. Also, the conversion of the aldehyde group of the starting derivative **1** into an α,β-unsaturated ketone side chain as in compound **8b**, provides a remarkable increase in the cytotoxic activity of **8b** against both HEPG2 (IC_{50} 1.0 μg mL^{-1}) and HELA (IC_{50} 2.2 μg mL^{-1}). However, the substituted pyrazoline moiety synthesis (compound **9b**) produces a slight decrease in cytotoxicity against HEPG2 (IC_{50} 2.6 μg mL^{-1}) and loss of activity against HELA. However, the replacement of the aldehyde group of **1** with an azomethine group (compound **13b**) improved the cytotoxic activity against HELA (IC_{50} 3.0 μg mL^{-1}) and slightly decreased the activity against HEPG2 (IC_{50} 4.6 μg mL^{-1}). At the same time, cyclization of the azomethine group to the thiazolidinone moiety (compound **14b**) led to a loss of cytotoxic activity against the liver carcinoma cell line but fortunately intensified its cytotoxic activity against HELA (IC_{50} 1.2 μg mL^{-1}).

Table 1 Effect of the selected compounds on liver carcinoma cell line (HEPG2) and cervix carcinoma cell line (HELA) compared with 5-fluorouracil (the highest concentration of each compound used was 10 μg mL^{-1}).

Compound no.	Cell Line [a]	
	HEPG2 IC_{50}/μg mL^{-1})	HEL A IC_{50}/μg mL^{-1})
5-Fluorouracil	5.0	1.0
1	3.5	5.6
2c	1.9	3.1
7c	5.0	Not active up to 10 μg mL^{-1}
8b	1.0	2.2
9b	2.6	Not active up to 10 μg mL^{-1}
13b	4.6	3.0
14b	Not active up to 10 μg mL^{-1}	1.2

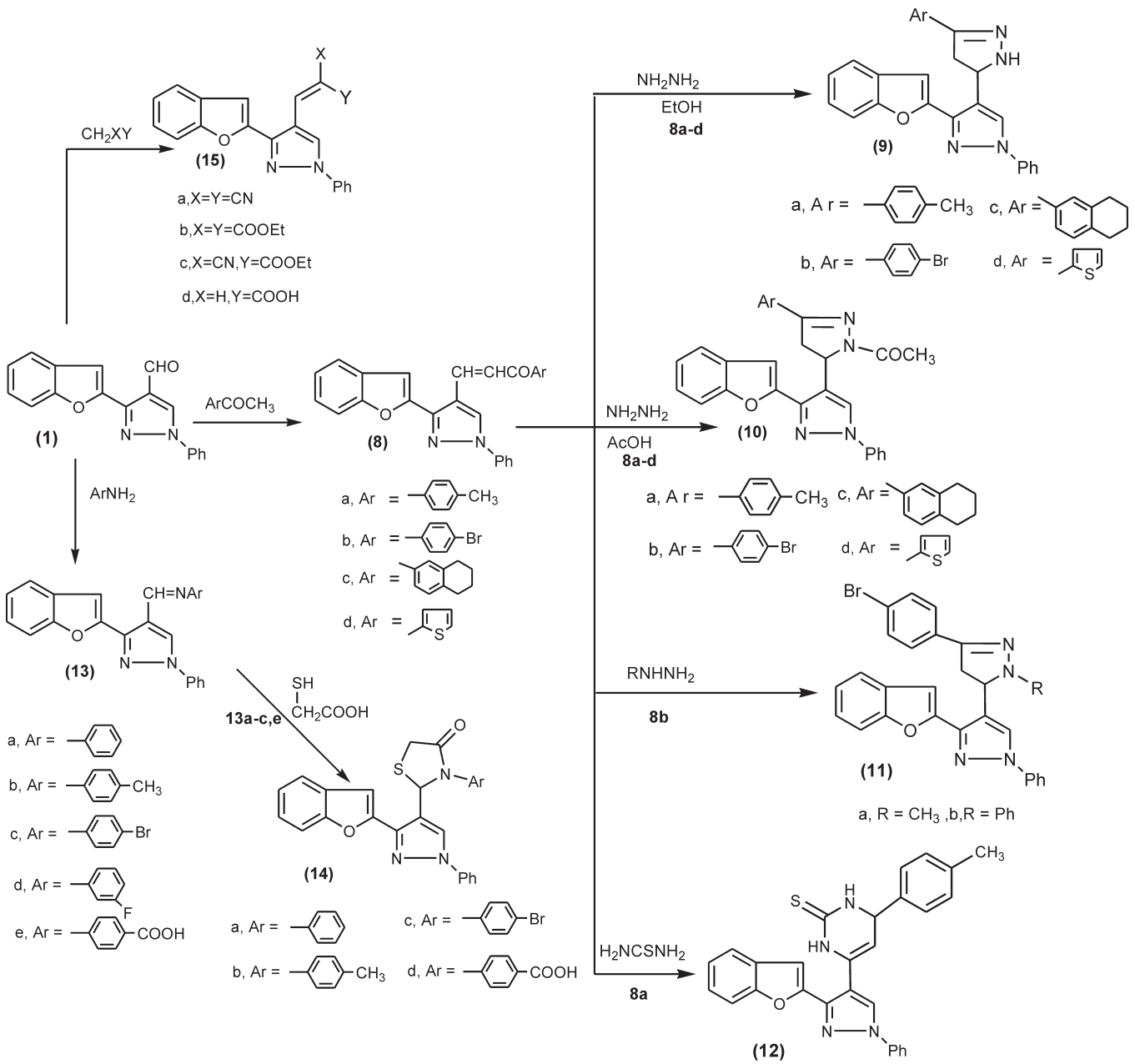
[a] IC_{50}: dose of the compound which reduces survival to 50 % (±5 % error).

It can be concluded, according to the SAR investigation of some novel derivatives, that the benzofuranoyl-pyrazole ring system, the thiosemicarbazide side chain, the azomethine group and the α,β-unsaturated ketone moiety are key structural requirements for the cytotoxic activity against both HEPG2 and HELA. The presence of a thiazolidinone ring produces the most potent cytotoxic activity against HELA. The presence of an α,β-unsaturated ketone side chain produces the most significant cytotoxic activity against both HEPG2 and HELA. The difference in the potency of the antitumour effectiveness of the compounds may depend upon the difference in their hydrophobicity, conformational flexibility and charge formation. All these factors play very important roles in alteration of the replication and repair functions of DNA and/or in the interaction with some cellular constituents.[38]

3. Experimental

3.1. Chemistry

All melting points are uncorrected and were taken in open capillary tubes using Electrothermal apparatus 9100 (London, UK). Elemental microanalyses were carried out at the Microanalytical Unit, Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt, using Vario Elementar (Berlin, Germany) and were found to be within ±0.5 % of the theoretical values. Infrared spectra were recorded on a Jasco FT/IR-6100 Fourier transform infrared spectrometer (Tokyo, Japan) using the KBr disc technique at the Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. ^{1}H NMR spectra were determined by using a Jeol EX-270 NMR spectrometer (Tokyo, Japan) at the Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt, while ^{13}C NMR spectra were recorded using a Varian-300 NMR spectrometer (Madison, WI, USA) at Marquette University, Milwaukee, WI, USA, and measured using TMS as an internal standard. The mass spectra were measured with a Finnigan MAT SSQ-7000 mass spectrometer (Olympia, WA, USA) at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. Follow-up of the reactions and checking of the purity of the compounds were made by TLC on silica gel-precoated aluminium sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected by exposure to a UV lamp at 254 nm for a few seconds. Colour intensity in



Scheme 2

cytotoxic screening was measured using a Cecan ELIZA reader (Vienna, Austria).

The chemical names for the prepared compounds are given according to the IUPAC system. 2-Acetylbenzofuran,[24] 1-(benzofuran-2-yl)ethylidene-2-phenylhydrazone,[25] 3-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-4-carboxaldehyde[26,27] (**1**), 1-(4-phenylthiazol-2-yl)hydrazine[30] **(6a),** 1-(4-*p*-bromophenylthiazol-2-yl)hydrazine[31] **(6b)** and 6-bromoacetyl-1,2,3,4-tetrahydronaphthalene[17] were prepared according to methods reported in the literature.

3.1.1. *Synthesis of 3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazole-4-carboxyaldehyde* (**1**)

Phosphorus oxychloride (20 mL, 0.2 mol) was added dropwise with stirring to dimethyl formamide (150 mL) at 0–5 °C. Then (1-benzofuran-2-yl)ethylidene-2-phenyl hydrazine (23.5 g, 0.094 mol) was added portion-wise with continuous stirring, left overnight at room temperature, poured onto ice-cold water and neutralized with ammonium hydroxide solution (5 %). The formed precipitate was filtered, dried and recrystallized from acetic acid to give the title compound **1**, in 90 % yield, m.p. 160–162 °C, IR (KBr): 1669 cm^{-1} (CO, aldehyde); ^{1}H NMR ($CDCl_3$): δ 7.20–7.80 (10H, m, Ar-H), 8.50 (1H, s, pyrazole ring), 10.40 ppm (1H, s, CHO); ^{13}C NMR ($CDCl_3$): δ 76.80, 77.23, 77.65, 107.30, 111.68, 120.04, 121.90, 123.07, 123.52, 125.57, 128.38, 129.84, 131.78, 138.90, 144.69, 148.73, 155.25, 185.11 ppm; MS, m/z (%): 288.1 [M^+] (37), 289.2 [M^{+1}] (9), 260.3 [M^+-CO] (8), 77.2 [due to C_6H_5] (100). Anal. calcd. for $C_{18}H_{12}N_2O_2$ (288.30): C, 74.99; H, 4.20; N, 9.72 %; found: C, 74.67; H, 4.33; N, 9.38 %.

3.1.2. *Synthesis of 1-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)semicarbazide* (**2a**)

A mixture of **1** (2.88 g, 0.01 mol), semicarbazide hydrochloride (1.2 g, 0.01 mol) and sodium acetate (1.7 g, 0.02 mol) in 20 mL ethanol was stirred for 1 h at room temperature then refluxed for 3 h. The formed precipitate was filtered, washed several times with water, dried and recrystallized from absolute ethanol to give the title compound **2a,** in 86 % yield, m.p. 216 °C. IR (KBr):

3467, 3270, 3149 (NH_2, NH), 1697 cm^{-1} (C=O amide); ^1H NMR ($CDCl_3$): δ 7.10–7.73 (10H, m, Ar-H), 8.19 (1H, s, CH=N), 8.35 (1H, s, pyrazole ring), 8.56 (2H, s, NH_2 exchangeable with D_2O), 10.21 ppm (1H, s, NH, exchangeable with D_2O); MS, m/z (%): 345 [M^+] (50), 286 [M^+-CON_2H_3] (46), 285 [286-1] (100). Anal. calcd. for $C_{19}H_{15}N_5O_2$ (345.35): C, 66.08; H, 4.38; N: 20.28 %; found: C, 66.32; H, 4.51; N, 20.42 %.

3.1.3. *Synthesis of 1-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)methylene) thiosemicarbazide* (**2b**)

A mixture of **1** (2.88 g, 0.01 mol) thiosemicarbazide (0.91 g, 0.01 mol) in 20 mL ethanol containing a few drops of glacial acetic acid was refluxed for 3 h. The formed precipitate was filtered, dried and recrystallized from absolute ethanol to give the title compound **2b**, in 98 % yield, m.p. 222 °C. IR (KBr): 3344, 3292, 3176 (NH_2, NH), 1599 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 6.98–7.68 (10H, m, Ar-H), 8.21 (1H, s, CH=N), 8.37 (1H, s, pyrazole ring), 8.46 (2H, s, NH_2, exchangeable with D_2O), 10.01 ppm (1H, s, NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$): δ 76.79, 77.28, 77.55, 104.91, 111.93, 117.24, 119.76, 121.50, 123.38, 125.16, 126.28, 127.74, 128.44, 129.71, 139.34, 143.52, 149.61, 155.23, 177.88 ppm; MS, m/z (%): 362 [M^++1] (10), 344 [M^+-NH_3] (18), 302 [M^+-$CSNH_2$ + 1] (23), 170 [due to $C_{10}H_8N_3$] (100). Anal. calcd. for $C_{19}H_{15}N_5OS$ (361.42): C, 63.14; H, 4.18; N, 19.38; S, 8.86 %; found: C, 63.36; H, 4.42; N, 19.22; S, 8.71 %.

3.1.4. *Synthesis of 1-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-4-substituted thiosemicarbazide* (**2c–e**)

General Procedure

A mixture of compound **1** (0.58 g, 0.02 mol) and substituted thiosemicarbazide, namely, ethylthiosemicarbazide, cyclohexylthiosemicarbazide and/or phenylthiosemicarbazide (0.002 mol) in 15 mL absolute ethanol containing a few drops of concentrated hydrochloric acid was refluxed for 3 h. The formed precipitate was filtered, dried and recrystallized from absolute ethanol (99 %) to give the title compounds **2c–e**.

3.1.4.1. *1-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-4-ethylthio-semicarbazide* (**2c**)

Yield 80 %, m.p. 221 °C, recrystallized from absolute ethanol; IR (KBr): 3356, 3162 (NH, NH), 1596 (C=N), 1545 cm^{-1} (C=S). ^1H NMR ($CDCl_3$): δ 1.0 (3H, t, CH_3), 3.40 (2H, q, CH_2), 6.90–7.50 (10H, m, Ar-H), 8.15 (1H, s, CH=N), 8.25 (1H, s, pyrazole ring), 10.10 ppm (1H, s, NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$): δ 14.78, 39.45, 76.89, 77.21, 77.53, 104.94, 111.97, 117.18, 119.64, 121.43, 123.44, 125.00, 126.23, 127.74, 128.39, 129.71, 139.28, 143.64, 149.65, 155.14, 176.76 ppm; MS, m/z (%): 389 [M^++1] (6), 358 [M^+-C_2H_6] (9), 357 [358-1] (35), 302 [M^+-$CSNHC_2H_5$] (90), 285 [M^+-$NHCSNHC_2H_5$] (76), 55 [C_4H_7] (100). Anal. calcd. for $C_{21}H_{19}N_5OS$ (389.47): C, 64.76; H, 4.92; N, 17.98; S, 8.23 %; found: C, 64.42; H, 4.72; N, 17.65; S, 8.56 %.

3.1.4.2. *1-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-4-cyclohexyl-thiosemicarbazide* (**2d**)

Yield 89 %, m.p. 256 °C, recrystallized from methanol; IR (KBr): 3342, 3130 (NH, NH), 1554 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 1.1–1.75 (10H, m, cyclohexyl ring), 2.21 (1H, m, cyclohexyl ring), 7.02–7.66 (10H, m, Ar-H), 8.19 (1H, s, CH=N), 8.28 (1H, s, pyrazole ring), 10.15 ppm (1H, s, NH, exchangeable with D_2O); MS, m/z (%): 443 [M^+] (7), 345 [M^+-$C_8H_{12}N$] (8), 344 [M^+-$C_8H_{13}N$] (32), 286 [M^+-$C_7H_{13}N_2S$] (100), 302 [M^+-$C_7H_{11}NS$] (14), 285 [286-1] (90). Anal. calcd. for $C_{25}H_{25}N_5OS$ (443.18): C, 67.69; H, 5.68; N, 15.79; S, 7.23 %; found: C, 67.29; H, 5.97; N, 15.91; S, 7.33 %.

3.1.4.3. *1-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-4-phenylthiosemicarbazide* (**2e**)

Yield 78 %, m.p. 140 °C, recrystallized from methanol; IR (KBr): 3325, 3122 (NH, NH), 1595 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 6.94–7.76 (15H, m, Ar-H), 8.13 (1H, s, CH=N), 8.22 (1H, s, pyrazole ring), 10.11 ppm (1H, s, NH, exchangeable with D_2O); MS, m/z (%): 435 [M^+-2] (6), 362 [M^+-C_6H_3] (85), 344 [M^+-C_6H_7N] (40), 286 [M^+-$C_7H_7N_2S$] (72), 285 [286-1] (100). Anal. calcd. for $C_{25}H_{19}N_5OS$ (437.52): C, 68.63; H, 4.38; N, 16.01; S, 7.33 %; found: C, 68.25; H, 4.44; N, 16.32; S, 7.55 %.

3.1.5. *Synthesis of 1-((3-Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl) methylene)-2-(3-ethyl-4-oxothiazolidin-2-ylidinene)hydrazine* (**3**)

A mixture of compound **2c** (0.78 g, 0.002 mol) and ethyl bromoacetate (0.23 mL, 0.002 mol) in 15 mL absolute ethanol containing a few drops of piperidine was refluxed for 3 h. The formed precipitate was filtered, dried and recrystallized from absolute ethanol to give the title compound **3**.

Yield 85 %, m.p. 196 °C, IR (KBr): 1710 (C=O), 1617 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 1.0 (3H, t, CH_3), 3.45 (2H, s, thiazolidinone ring), 3.6 (2H, q, CH_2), 6.92–7.55 (10H, m, Ar-H), 8.15 (1H, s, CH=N), 8.60 ppm (1H, s, pyrazole ring); ^{13}C NMR ($CDCl_3$): δ 12.82, 32.75, 38.83, 76.79, 77.21, 77.63, 105.71, 111.75, 118.04, 119.87, 121.52, 123.37, 125.02, 127.63, 127.77, 128.60, 129.73, 139.41, 149.68, 151.28, 155.17, 162.45, 171.98 ppm; MS, m/z (%): 429 [M^+] (100), 430 [M^++1] (23), 286 [M^+-$C_4H_7ON_2S$] (59), 387 [M^+-$COCH_2$] (8). Anal. calcd. for $C_{23}H_{19}N_5O_2S$ (429.48): C, 64.31; H, 4.46; N, 16.30; S, 7.46 %; found: C, 64.52; H, 4.64; N, 16.41; S, 7.73 %.

3.1.6. *Synthesis of 1-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl) methylene)-2-(3-ethyl-4-phenylthiazol-2(3H)-ylidene)hydrazine* (**4**)

A mixture of compound **2c** (0.78 g, 0.002 mol) and phenacyl bromide (0.4 g, 0.002 mol) in 15 mL absolute ethanol containing a few drops of piperidine was refluxed for 10 h. The formed precipitate was filtered, dried and recrystallized from chloroform to give the title compound **4**. Yield 87 %, m.p. 239 °C, IR (KBr): 1590 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ 1.11 (3H, t, CH_3), 3.58 (2H, q, CH_2), 6.93–7.98 (16H, m, Ar-H, thiazole ring), 8.17 (1H, s, CH=N), 8.55 ppm (1H, s, pyrazole ring); ^{13}C NMR ($CDCl_3$): δ 13.91, 39.13, 76.81, 77.23, 77.59, 104.34, 105.51, 111.88, 118.13, 119.82, 121.45, 123.30, 125.12, 127.59, 127.66, 128.65, 128.75, 129.24, 129.68, 135.76, 139.44, 142.32, 149.71, 151.31, 155.20, 161.55 ppm; MS, m/z (%): 489 [M^+] (100), 460 [M^+-C_2H_5] (4), 286 [M^+-$C_{11}H_{11}N_2S$] (13), 203 [$C_{11}H_{11}N_2S$] (13). Anal. calcd. for $C_{29}H_{23}N_5OS$ (489.59): C, 71.14; H, 4.74; N, 14.30; S, 6.55 %; found: C, 71.34; H, 4.44; N, 14.72; S, 6.77 %.

3.1.7. *Synthesis of 2-[((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl) methylene)hydrazono)-3-ethyl-4-oxothiazolidin-5-yl] acetic acid* (**5**)

A mixture of compound **2c** (0.78 g, 0.002 mol) and maleic anhydride (0.2 g, 0.002 mol) in 15 mL glacial acetic acid was refluxed for 10 h. The formed precipitate was filtered, dried and recrystallized from acetic acid to give the title compound **5**. Yield 64 %, m.p. 239 °C; IR (KBr): broad band centered at 3397 (OH, acid), 1706 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 1.06 (3H, t, CH_3), 3.11–3.33 (2H, m, CH_2COOH), 3.62 (2H, q, CH_2), 3.70 (1H, m, oxothiazolidine ring), 8.14 (1H, s, CH=N), 8.59 (1H, s, pyrazole ring), 10.87 ppm (1H, s, COOH); ^{13}C NMR ($CDCl_3$): δ 13.24, 33.51, 36.74, 39.19, 76.77, 77.25, 77.60, 105.61, 111.82, 118.16, 119.83, 121.49, 123.29, 125.19, 127.64, 127.68, 128.72, 129.67, 139.46, 149.73, 151.33, 155.24, 161.66, 172.35, 175.24 ppm; MS, m/z (%): 487 [M^+] (100), 488 [M^++1] (30), 286 [M^+-$C_7H_9N_2O_3S$] (75). Anal.

calcd. for $C_{25}H_{21}N_5O_4S$ (487.52): C, 61.58; H, 4.34; N, 14.36; S, 6.57 %; found: C, 61.59; H, 4.51; N, 14.77; S, 6.38 %.

3.1.8. *Synthesis of 4-(1,2,3,4-Tetrahydronaphthalen-6-yl)thiazol-2-hydrazine* (**6c**)

A mixture of 6-bromoacetyl-1,2,3,4-tetrahydronaphthalene[17] (2.5 g, 0.01 mol) and thiosemicarbazide (0.91 g, 0.01 mol) in absolute ethanol (30 mL) was refluxed for 3 h. The reaction mixture was made alkaline with 1 mL 10 % sodium hydroxide solution.

It was poured into ice-cold water and the precipitate formed was filtered, dried and recrystallized from cyclohexane to give the title compound in 90 % yield, m.p. 160–162 °C. IR (KBr): 3365, 3219, 3090 (NH, NH_2), 2929 (CH_2 of tetrahydronaphthalene), 1634 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 1.7, 2.9 (4H, 4H, m, m, tetrahydronaphthalene), 6.8 (1H, s, thiazole ring), 7.3–7.7 ppm (3H, m, Ar-H); MS, m/z %: 245 [M^+] (80). Anal. calcd. for $C_{13}H_{15}N_3S$ (245.35): C, 63.63; H, 6.16; N, 17.12 %; found: C, 63.97; H, 6.66; N, 17.45 %.

3.1.9. *Synthesis of 1-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-2-(4-substituted thiazol-2-yl)hydrazine* (**7a–c**)

Method A

A mixture of compound **2b** (0.73 g, 0.002 mol), appropriate bromoacetyl derivatives, namely phenacylbromide, *p*-bromophenacylbromide and/or 6-bromoacetyltetrahydronaphthalene[27] (0.002 mol) in 15 mL absolute ethanol was refluxed for 3 h. Sodium hydroxide solution (10 %) was added to the reaction mixture then poured onto ice-cold water; the formed precipitate was filtered, dried and recrystallized from isopropyl alcohol, absolute ethanol (99 %) and ethyl acetate respectively as mentioned for each compound in the series below to give the title compounds **7a–c**, respectively.

Method B

A mixture of compound **1** (0.58 g, 0.002 mol), 1-(4-phenylthiazol-2-yl)hydrazine, 2-(4-*p*-bromophenylthiazol-2-yl)hydrazine and/or 2-(4-(1,2,3,4-tetrahydronaphthalene-6-yl)thiazol-2-yl) hydrazine **6a–c** (0.002 mol) in 15 mL absolute ethanol was refluxed for 3 h. The formed precipitate was filtered, dried and recrystallized from isopropyl alcohol, absolute ethanol (99 %) and ethyl acetate respectively as mentioned for each compound in the series below to give the title compounds **7a–c**, respectively. The products were identified by their melting points and R_f values in comparison with authentic samples previously obtained by method A and showed similar spectral data.

3.1.9.1. *1-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl) methylene)-2-(4-phenyl-thiazol-2-yl) hydrazine* (**7a**)

Yield 78 % [A], 80 % [B], m.p. 203–205 °C, recrystallized from isopropyl alcohol; IR (KBr): 3293 (NH), 1597 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 6.62 (1H, s, thiazole ring), 7.12–7.85 (16H, m, Ar-H, NH, exchangeable with D_2O), 8.36 (1H, s, CH=N), 8.79 ppm (1H, s, pyrazole ring); ^{13}C NMR ($CDCl_3$): δ 76.66, 77.32, 77.70, 102.43, 106.34, 111.72, 120.10, 121.95, 123.12, 123.59, 125.60, 126.94, 127.41, 128.41, 129.79, 131.82, 132.46, 138.92, 141.24, 144.64, 147.07, 148.68, 155.04, 168.01 ppm; MS, m/z (%): 461 [M^+] (100), 286 [M^+-$C_9H_7N_2S$] (38), 176 [due to $C_9H_8N_2S$] (40). Anal. calcd. for $C_{27}H_{19}N_5OS$ (461.54): C, 70.26; H, 4.15; N, 15.17; S, 6.96 %; found: C, 70.48; H, 4.48; N, 15.38; S, 6.32 %.

3.1.9.2. *1-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl) methylene)-2-(4-(4-bromophenyl) thiazol-2-yl)hydrazine* (**7b**)

Yield 94 % [A], 89 % [B], m.p. 240–241 °C, recrystallized from absolute ethanol (99 %); IR (KBr): 3289 (NH), 1590 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 6.58 (1H, s, thiazole ring), 7.12–7.85 (15H, m, Ar-H, NH, exchangeable with D_2O), 8.44 (1H, s, CH=N), 8.97 ppm (1H, s, pyrazole ring); MS, m/z (%): 539, 541 [M^+] (8.6, 8.4), 286 [M^+-$C_9H_6BrN_2S$] (97), 285 [286-1] (100), 254, 256 [$C_9H_7BrN_2S$] (52, 55). Anal. calcd. for $C_{27}H_{18}BrN_5OS$ (540.43): C, 60.01; H, 3.36; N, 12.96; S, 5.93 %; found: C, 60.28; H, 3.57; N, 12.65; S, 5.72 %.

3.1.9.3. *1-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-2-(4-(1,2,3,4-tetrahydronaphthalene-6-yl)thiazol-2-yl)hydrazine* (**7c**)

Yield 81 % [A], 78 % [B], m.p. 215 °C, recrystallized from ethyl acetate; IR (KBr): 3342 (NH), 3045 (CH-aromatic), 2921 (CH_2-aliphatic), 1620 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 1.8, 2.8 (4H, 4H, m, m, tetrahydronaphthalene), 6.6 (1H, s, thiazole ring), 7.1–7.9 (14H, m, Ar-H, NH, exchangeable with D_2O), 8.4 (1H, s, CH=N), 8.80 ppm (1H, s, pyrazole ring); MS, m/z (%): 515 [M^+] (35), 516 [M^++1] (15), 286 [M^+-$C_{13}H_{13}N_2S$] (100), 287 [286+1] (45), 229 [due to $C_{13}H_{13}N_2S$] (30), 230 [229+1] (65). Anal. calcd. for $C_{31}H_{25}N_5OS$ (515.63): C, 72.21; H, 4.89; N, 13.58; S, 6.22 %; found: C, 72.45; H, 5.11; N, 13.21; S, 6.52 %.

3.1.10. *Synthesis of 3-(3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-1-substituted prop-2-en-1-one* (**8a–d**)

General Procedure

A mixture of compound **1** (0.58 g, 0.002 mol) and appropriate acetyl derivatives, namely *p*-methylacetophenone, *p*-bromoacetophenone, 6-acetyl-(1,2,3,4-tetrahydronaphthalene and/or 2-acetylthiophene (0.002 mol) in 15 mL alcoholic sodium hydroxide (10 %) was stirred for 3 h. The reaction mixture was left overnight at room temperature, filtered, washed several times with water, dried and recrystallized from absolute ethanol (99 %) to give the title compounds **8a–d**, respectively.

3.1.10.1. *3-(3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-1-p-tolylprop-2-en-1-one* (**8a**)

Yield 90 %, m.p. 136 °C, recrystallized from absolute ethanol; IR (KBr): 1640 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.46 (3H, s, CH_3), 7.27–8.38 ppm (17H, m, Ar-H, CH=CH, CH pyrazole ring); ^{13}C NMR ($CDCl_3$): δ 22.42, 76.86, 77.44, 77.68, 107.12, 111.71, 120.09, 121.86, 123.21, 123.66, 125.62, 126.65, 128.42, 129.89, 131.71, 133.57, 138.85, 142.36, 143.46, 144.74, 148.55, 155.31, 182.34 ppm; MS, m/z (%): 404 [M^+] (41), 405 [M^++1] (11), 285 [M^+-C_8H_7O] (90), 286 [285 + 1] (18), 119 [C_8H_7O] (94), 91 [119-CO] (100). Anal. calcd. for $C_{27}H_{20}N_2O_2$ (404.46): C, 80.18; H, 4.98; N, 6.93 %; found: C, 80.27; H, 4.65; N, 6.43 %.

3.1.10.2. *3-(3-Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(4-bromophenyl)prop-2-en-1-one* (**8b**)

Yield 92 %, m.p. 189 °C, recrystallized from absolute ethanol; IR (KBr): 3061 (CH=CH), 1633 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 7.25–8.40 ppm (17H, m, Ar-H, CH=CH, CH-pyrazole ring); MS, m/z (%): 468, 470 [M^+] (5, 4), 285 [M^+-C_7H_4OBr] (100), 286 [285+1] (20), 183, 185 [C_7H_4OBr] (6.4, 6.2), 155, 157 [C_7H_4OBr-CO] (11, 7). Anal. calcd. for $C_{26}H_{17}BrN_2O_2$ (469.33): C, 66.54; H, 3.65; N, 5.97 %; found: C, 66.78; H, 3.99; N, 6.21 %.

3.1.10.3. *3-(3-(Benzfuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(1,2,3,4-tetrahydronaphthalen-6-yl)prop-2-en-1-one* (**8c**)

Yield 87 %, m.p. 122 °C, recrystallized from absolute ethanol; IR (KBr): 1645 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 1.93, 2.44 (4H, 4H, m, m, tetrahydronaphthalene), 7.21–8.39 ppm (16H, m, Ar-H, CH=CH, CH-pyrazole ring); MS, m/z (%): 444 [M^+] (33), 445 [M^++1] (9), 285 [M^+-$C_{11}H_{11}O$] (100), 286 [285+1] (18), 159

[$C_{11}H_{11}O$] (24). Anal. calcd. for $C_{30}H_{24}N_2O_2$ (444.52): C, 81.06; H, 5.44; N, 6.30 %; found: C, 81.44; H, 5.73; N, 6.57 %.

3.1.10.4. *3-(3-Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-1-(thiophene-2-yl)prop-2-en-1-one* (**8d**)

Yield 85 %, m.p. 150 °C, recrystallized from absolute ethanol; IR (KBr): 1642 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 7.17–8.37 ppm (15H, m, Ar-H, CH=CH, thiophene ring, CH pyrazole ring); MS, m/z (%): 396 [M^+] (6), 285 [M^+-C_5H_3OS] (20), 111 [C_5H_3OS] (100). Anal. calcd. for $C_{24}H_{16}N_2O_2S$ (396.46): C, 72.71; H, 4.07; N, 7.07; S, 8.09 %; found: C, 72.93; H, 4.39; N, 7.41; S, 8.28 %.

3.1.11. *Synthesis of 3-(Benzofuran-2-yl)-4-(4,5-dihydro-3-substituted-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole* (**9a–d**)

General Procedure

A mixture of compound **8a–d** (0.002 mol) and hydrazine hydrate 98 % (0.1 mL, 0.002 mol) in absolute ethanol was refluxed for 3 h. The formed precipitate was filtered, dried and recrystallized from absolute ethanol (99 %) to give the title compounds **9a–d**, respectively.

3.1.11.1. *3-(Benzofuran-2-yl)-4-(4,5-dihydro-3-p-tolyl-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole* (**9a**)

Yield 73 %, m.p. 124 °C, recrystallized from absolute ethanol; IR (KBr): 3311 (NH), 1596 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 2.33 (3H, s, CH_3), 3.21, 3.84 (1H, 1H, m, m, CH_2-pyrazoline ring), 5.11 (1H, m, CH-pyrazoline ring), 7.12–7.71 (15H, m, Ar-H, CH-pyrazole ring), 8.91 ppm (1H, NH, exchangeable with D_2O); ^{13}C NMR ($CDCl_3$): δ 24.11, 46.11, 56.21, 80.12, 80.28, 80.61, 107.19, 114.51, 122.78, 124.77, 125.86, 127.61, 127.85, 128.19, 129.35, 130.37, 131.27, 132.64, 133.64, 135.22, 136.41, 142.92, 150.31 ppm; MS, m/z (%): 418 [M^+] (100), 417 [M^+ – 1] (43), 419 [M^++1] (25), 327 [M^+-C_7H_7] (3), 325 [M^+-C_7H_9] (23), 301 [327-CN] (6), 300 [301-1] (14], 285 [301-NH_2] (17). Anal. calcd. for $C_{27}H_{22}N_4O$ (418.49): C, 77.49; H, 5.30; N, 13.39 %; found: C, 78.21; H, 5.52; N, 13.61 %.

3.1.11.2. *3-(Benzofuran-2-yl)-4-(3-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole* (**9b**)

Yield 51 %, m.p. 162–163 °C, recrystallized from absolute ethanol; IR (KBr): 3238 (NH), 1596 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 3.22, 3.83 (1H, 1H, m, m, CH_2-pyrazoline ring), 5.14 (1H, m, CH-pyrazoline ring), 7.12–7.75 (15H, m, Ar-H, CH-pyrazole ring), 8.88 ppm (1H, NH, exchangeable with D_2O); MS, m/z (%): 482, 484 [M^+] (98, 100), 481, 483 [M^+– 1] (48, 69), 301 [M^+-C_7H_4NBr] (14), 285 [301-NH_2] (57). Anal. calcd. for $C_{26}H_{19}BrN_4O$ (483.36): C, 64.61; H, 3.96; N, 11.59 %; found: C, 64.88; H, 4.23; N, 11.97 %.

3.1.11.3. *3-(Benzofuran-2-yl)-4-(4,5-dihydro-3-(1,2,3,4-tetrahydro-naphthalen-6-yl)-1H- pyrazol-5-yl)-1-phenyl-1H-pyrazole* (**9c**)

Yield 62 %, m.p. 151 °C, recrystallized from absolute ethanol; IR (KBr): 3313 (NH), 1596 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 1.76, 2.75 (4H, 4H, m, m, tetrahydronaphthalene), 3.27, 3.83 (1H, 1H, m, m, CH_2-pyrazoline ring), 5.18 (1H, m, CH-pyrazoline ring), 7.11–7.72 (14H, m, Ar-H, CH-pyrazole ring), 8.92 ppm (1H, NH, exchangeable with D_2O); MS, m/z (%): 458 [M^+] (100), 457 [M^+–1] (44), 301 [M^+-$C_{11}H_{11}N$] (4), 285 [301-NH_2] (12). Anal. calcd. for $C_{30}H_{26}N_4O$ (458.55): C, 78.58; H, 5.72; N, 12.22 %; found: C, 78.87; H, 6.23; N, 12.46 %.

3.1.11.4. *3-(Benzofuran-2-yl)-4-(4,5-dihydro-3-(thiophen-2-yl)-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole* (**9d**)

Yield 53 %, m.p. 181 °C, recrystallized from absolute ethanol; IR (KBr): 3238 (NH), 1598 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 3.22, 3.81 (1H, 1H, m, m, CH_2-pyrazoline ring), 5.08 (1H, m, CH-pyrazoline ring), 7.14–7.69 (14H, m, Ar-H, CH-pyrazole ring), 8.89 ppm (1H, NH, exchangeable with D_2O); MS, m/z (%): 410 [M^+] (100), 411 [M^++1] (22). Anal. calcd. for $C_{24}H_{18}N_4OS$ (410.49): C, 70.22; H, 4.42; N, 13.65, S, 7.81 %; found: C, 70.58; H, 4.86; N, 13.98; S, 7.66 %.

3.1.12. *Synthesis of 1-(5-(3-Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydro-3-substituted pyrazol-1-yl)ethanone* (**10a–d**)

General Procedure

A mixture of compound **8a–d** (0.002 mol) and hydrazine hydrate (98 %) (0.1 mL, 0.002 mol) in 15 mL glacial acetic acid was refluxed for 3 h. The formed precipitate was filtered, dried and recrystallized from acetic acid to give the title compounds **10a–d**.

3.1.12.1. *1-(5-(3-Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydro-3-p-tolyl-pyrazol-1-yl) ethanone* (**10a**)

Yield 88 %, m.p. 233 °C, recrystallized from acetic acid; IR (KBr): 1660 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.38 (3H, s, CH_3), 2.49 (3H, s, $COCH_3$), 3.26, 3.88 (1H, 1H, m, m, CH_2-pyrazoline ring), 6.16 (1H, m, CH-pyrazoline ring), 7.12–7.71 ppm (15H, m, Ar-H, CH-pyrazole ring); MS, m/z (%): 460 [M^+] (100), 461 [M^++1] (14), 417 [M^+-$COCH_3$] (23), 326 [417-C_7H_7] (2), 300 [326-CN] (12), 284 [300-NH_2] (12). Anal. calcd. for $C_{29}H_{24}N_4O_2$ (460.53): C, 75.63; H, 5.25; N, 12.17 %; found: C, 75.42; H, 5.36; N, 12.28 %.

3.1.12.2. *1-(5-(3-Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-bromophenyl)-4,5-dihydro pyrazol-1-yl)ethanone* (**10b**)

Yield 90 %, m.p. 224 °C, recrystallized from acetic acid; IR (KBr): 1665 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.48 (3H, s, $COCH_3$), 3.24, 3.85 (1H, 1H, m, m, CH_2-pyrazoline ring), 6.16 (1H, m, CH-pyrazoline ring), 7.22–7.73 ppm (15H, m, Ar-H, CH-pyrazole ring); ^{13}C NMR ($CDCl_3$): δ 25.46, 45.62, 56.24, 80.08, 80.40, 80.72, 107.06, 114.54, 122.80, 124.68, 126.10, 127.83, 127.86, 128.15, 129.35, 130.39, 131.45, 132.76, 133.60, 135.33, 136.35, 142.92, 150.02, 157.12 ppm; MS, m/z (%): 524, 526 [M^+] (100, 81), 481, 483 [M^+-$COCH_3$] (41, 36), 300 [M^+-C_7H_4Br] (10), 284 [300-NH_2] (15). Anal. calcd. for $C_{28}H_{21}BrN_4O_2$ (525.38): C, 64.01; H, 4.03; N, 10.66 %; found: C, 64.32; H, 4.22; N, 10.88 %.

3.1.12.3. *1-(5-(3-Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-4,5- dihydro-3-(1,2,3,4-tetrahydro-naphthalene-6-yl) pyrazol-1-yl)ethanone* (**10c**)

Yield 89 %, m.p. 205–207 °C, recrystallized from acetic acid; IR (KBr): 1655 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 1.79, 2.76 (4H, 4H, m, m, tetrahydronaphthalene), 2.40 (3H, s, $COCH_3$), 3.29, 3.85 (1H, 1H, m, m, CH_2-pyrazoline ring), 6.17 (1H, m, CH-pyrazoline ring), 7.07–7.69 ppm (14H, m, Ar-H, CH-pyrazole ring); MS, m/z (%): 500 [M^+] (100), 501 [M^++1] (24), 457 [M^+-$COCH_3$] (33), 458 [457+1] (27). Anal. calcd. for $C_{32}H_{28}N_4O_2$ (500.59): C, 76.78; H, 5.64; N, 11.19 %; found: C, 76.52; H, 5.23; N, 11.41 %.

3.1.12.4. *1-(5-(3-Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydro-3-(thiophen-2-yl) pyrazol-1-yl)ethanone* (**10d**)

Yield 76 %, m.p. 271 °C, recrystallized from acetic acid; IR (KBr): 1661 cm^{-1} (C=O); ^{1}H NMR ($CDCl_3$): δ 2.39 (3H, s, $COCH_3$), 3.28, 3.82 (1H, 1H, m, m, CH_2-pyrazoline ring), 6.14 (1H, m, CH-pyrazoline ring), 7.13–7.71 ppm (14H, m, Ar-H, CH-pyrazole ring); MS, m/z (%): 452 [M^+] (100), 453 [M^++1] (33), 409 [M^+-$COCH_3$] (27), 410 [409+1] (40), 300 [409-C_5H_3NS] (5), 284 [300-NH_2] (7). Anal. calcd. for $C_{26}H_{20}N_4O_2S$ (452.53): C, 69.01; H, 4.45; N, 12.38; S, 7.09 %; found: C, 69.22; H, 4.71; N, 12.43; S, 7.23 %.

3.1.13. *Synthesis of (Benzofuran-2-yl)-4-(3-(4-bromophenyl)-4,5-dihydro-1-substituted-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole* (**11a,b**)

General Procedure

A mixture of compound **8b** (0.94 g, 0.002 mol) and methylhydrazine and/or phenylhydrazine (0.002 mol) in absolute ethanol was refluxed for 3 h. The formed precipitate was filtered, dried, and recrystallized from absolute ethanol (99 %) to give the title compounds **11a,b**, respectively.

3.1.13.1. *3-(Benzofuran-2-yl)-4-(3-(4-bromophenyl)-4,5-dihydro-1-methyl-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole* (**11a**)

Yield 50 %, m.p. 142 °C, recrystallized from absolute ethanol; IR (KBr): 1590 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 2.24 (3H, s CH_3), 3.11, 3.75 (1H, 1H, m, m, CH_2-pyrazoline ring), 5.04 (1H, m, CH-pyrazoline ring), 7.08–7.67 ppm (15H, m, Ar-H, CH-pyrazole ring); ^{13}C NMR ($CDCl_3$): δ 36.56, 45.64, 56.33, 80.28, 80.51, 80.88, 107.13, 114.61, 122.75, 124.71, 126.22, 127.85, 127.91, 128.31, 129.40, 130.41, 131.39, 132.81, 133.66, 135.41, 136.39, 142.87, 150.11 ppm; MS, m/z (%): 496, 498 [M^+] (11, 12), 210, 212 [$C_8H_7N_2Br$] (10, 8), 286 [M^+-$C_8H_7N_2Br$] (7), 285 [286-1] (23), 182, 184 [C_7H_5NBr] (20, 17), 77 [C_6H_5] (100). Anal. calcd. for $C_{27}H_{21}BrN_4O$ (497.39): C, 65.20; H, 4.26; N, 11.26 %; found: C, 65.37; H, 4.44; N, 11.51 %.

3.1.13.2. *3-(Benzofuran-2-yl)-4-(3-(4-bromophenyl)-4,5-dihydro-1-phenyl-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole* (**11b**)

Yield 53 %, m.p. 232 °C, recrystallized from absolute ethanol; IR (KBr): 1585 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 3.13, 3.69 (1H, 1H, m, m, CH_2-pyrazoline ring), 5.10 (1H, m, CH-pyrazoline ring), 6.98–7.77 ppm (20H, m, Ar-H, CH-pyrazole ring); MS, m/z (%): 558, 560 [M^+] (17, 18), 286 [M^+-$C_{13}H_9N_2Br$] (6), 285 [286-1] (17), 91 [C_6H_5N] (50), 77 [C_6H_5] (100). Anal. calcd. for $C_{32}H_{23}BrN_4O$ (559.46): C, 68.70; H, 4.14; N, 10.01 %; found: C, 69.12; H, 4.32; N, 10.41 %.

3.1.14. *Synthesis of 6-(3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-3,4-dihydro-4-p-tolyl-pyrimidine-2(1H)-thione* (**12**)

A mixture of compound **8a** (0.89 g, 0.002 mol) and thiourea (0.16 g, 0.002 mol) in ethanolic potassium hydroxide (1 %) was refluxed for 24 h. The formed precipitate was filtered, washed several times with water, dried and recrystallized from absolute ethanol to give the title compound (**12**). Yield 48 %, m.p. 255 °C; IR (KBr): 3223 cm^{-1} (NH); ^{1}H NMR ($CDCl_3$): δ 2.12 (3H, s, CH_3), 4.99 (1H, d, CH pyrimidine ring), 7.07–7.89 (15H, m, Ar-H, CH pyrimidine ring), 8.32 ppm (1H, s, pyrazole ring); ^{13}C NMR ($CDCl_3$): δ 22.67, 53.22, 77.12, 77.56, 79.65, 100.45, 107.21, 112.64, 120.12, 121.85, 123.26, 123.49, 125.61, 128.32, 129.78, 131.82, 133.49, 138.84, 139.00, 144.64, 146.83, 148.80, 155.25, 176.34 ppm; MS, m/z (%): 462 [M^+] (100), 463 [M^++1] (34), 402 [M^+-NHSH] (48), 371 [M^+-C_7H_7] (14), 369 [371-2] (21), 285 [371-$C_2H_2N_2S$] (34), 77 [C_6H_5] (47). Anal. calcd. for $C_{28}H_{22}N_4OS$ (462.57): C, 72.70; H, 4.79; N, 12.11; S, 6.93 %; found: C, 72.51; H, 4.87; N, 12.31; S, 6.66 %.

3.1.15. *Synthesis of N-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)-3 or 4-substituted benzenamine* (**13a–d**) *and 4-((3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl) methylene-amino)benzoic acid* (**13e**)

General Procedure

A mixture of compound **1** (0.58 g, 0.002 mol) and aromatic amines, namely aniline, *p*-toluidine, 4-bromoaniline, 3-fluoroaniline and/or 4-aminobenzoic acid (0.002 mol) in 10 mL absolute ethanol was refluxed for 2 h, filtered, dried and recrystallized from absolute ethanol (99 %) to give the title compounds **13a–e**, respectively.

3.1.15.1. *N-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl) methylene)benzenamine* (**13a**)

Yield 79 %, m.p. 146 °C, recrystallized from absolute ethanol; IR (KBr): 1585 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 7.15–7.87 (15H, m, Ar-H), 8.65 (1H, s, CH=N), 8.99 ppm (1H, s, pyrazole ring); MS, m/z (%): 363 [M^+] (60), 362 [M^+– 1] (100), 286 [M^+-C_6H_5] (4). Anal. calcd. for $C_{24}H_{17}N_3O$ (363.41): C, 79.32; H, 4.72; N, 11.56 %; found: C, 79.55; H, 4.96; N, 11.73 %.

3.1.15.2. *N-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl) methylene)-4-methyl-benzenamine* (**13b**)

Yield 93 %, m.p. 139 °C, recrystallized from absolute ethanol; IR (KBr): 1582 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 2.40 (3H, s, CH_3), 7.20–7.90 (14H, m, Ar-H), 8.7 (1H, s, CH=N), 9.0 ppm (1H, s, pyrazole ring); ^{13}C NMR ($CDCl_3$): δ 21.21, 76.80, 77.23, 77.65, 105.59, 111.66, 119.74, 121.03, 121.30, 121.59, 123.44, 125.03, 127.75, 128.02, 128.55, 129.76, 130.06, 135.98, 139.43, 144.28, 149.85, 152.38, 155.18 ppm; MS, m/z (%): 377 [M^+] (72), 376 [M^+– 1] (73), 286 [M^+-C_7H_7] (9), 91 [C_7H_7] (66), 77 [C_6H_5] (100). Anal. calcd. for $C_{25}H_{19}N_3O$ (377.42): C, 79.55; H, 5.07; N, 11.13 %; found: C, 79.32; H, 5.39; N, 11.42 %.

3.1.15.3. *N-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl) methylene)-4-bromo-benzenamine* (**13c**)

Yield 92 %, m.p. 156 °C, recrystallized from absolute ethanol; IR (KBr): 1592 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 7.19–7.79 (14H, m, Ar-H), 8.61 (1H, s, CH=N), 9.11 ppm (1H, s, pyrazole ring); MS, m/z (%): 441, 443 [M^+] (83, 70), 440, 442 [M^+– 1] (100, 89), 286 [M^+-C_6H_4Br] (4). Anal. calcd. for $C_{24}H_{16}N_3OBr$ (442.31): C, 65.17; H, 3.65; N, 9.50 %; found: C, 65.38; H, 3.24; N, 9.86 %.

3.1.15.4. *N-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl) methylene)-3-fluoro-benzenamine* (**13d**)

Yield 54 %, m.p. 99 °C, recrystallized from absolute ethanol; IR (KBr): 1587 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 7.22–7.81 (14H, m, Ar-H), 8.58 (1H, s, CH=N), 9.10 ppm (1H, s, pyrazole ring); MS, m/z (%): 381 [M^+] (100), 380 [M^+– 1] (32). Anal. calcd. for $C_{24}H_{16}FN_3O$ (381.40): C, 75.58; H, 4.23; N, 11.02 %; found: C, 75.91; H, 4.56; N, 11.34 %.

3.1.15.5. *4-(3-benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl) methyleneamino)-benzoic acid* (**13e**)

Yield 98 %, m.p. 260 °C, recrystallized from absolute ethanol; IR (KBr): broad band centered at 3422 (OH, acid), 1709 (C=O), 1593 cm^{-1} (C=N); ^{1}H NMR ($CDCl_3$): δ 7.22–7.81 (14H, m, Ar-H), 8.62 (1H, s, CH=N), 9.12 (1H, s, pyrazole ring), 10.73 ppm (1H, s, COOH); MS, m/z (%): 407 [M^+] (19), 363 [M^+-CO_2] (4), 285 [M^+-$C_7H_6O_2$] (3), 77 [C_6H_5] (100). Anal. calcd. for $C_{25}H_{17}N_3O_3$ (407.42): C, 73.70; H, 4.20; N, 10.31 %; found: C, 73.42; H, 4.32; N, 10.63 %.

3.1.16. *Synthesis of 2-(3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-3-substituted thiazolidin-4-one* (**14a-c**) *and 4-(2-(3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-4-oxothiazolidin-3-yl) benzoic acid* (**14d**)

General Procedure

A mixture of compound **13a–c,e** (0.002 mol) and thioglycolic acid (0.2 mL, 0.002 mol) in 20 mL dry benzene was heated under reflux for 6–10 h. The solvent was evaporated under reduced

pressure and the formed residue was treated with dilute solution of sodium carbonate (10 %). The formed precipitate was filtered, dried and recrystallized from absolute ethanol (99 %) to give the title compounds **14a–d,** respectively.

3.1.16.1. *2-(3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-3-phenylthiazolidin-4-one* (**14a**)

Yield 56 %, m.p. 177 °C, recrystallized from absolute ethanol; IR (KBr): 1685 cm^{-1} (CO); ^1H NMR ($CDCl_3$): δ 3.42–3.69 (2H, m, thiazolidinone ring), 6.43 (1H, s, thiazolidinone ring), 6.81–7.62 ppm (16H, m, Ar-H,CH pyrazole ring,); MS, m/z (%):437 [M^+] (80), 436 [M-1] (100). Anal. calcd. for $C_{26}H_{19}N_3O_2S$ (437.51): C, 71.38; H, 4.38; N, 9.60; S, 7.33 %; found: C, 71.51; H, 4.81; N, 9.72; S, 7.55 %.

3.1.16.2. *2-(3-Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-3-p-tolylthiazolidin-4-one* (**14b**)

Yield 55 %, m.p. 167 °C, recrystallized from absolute ethanol; IR (KBr): 1680 cm^{-1} (CO); ^1H NMR ($CDCl_3$): δ 1.9 (3H, s, CH_3), 3.5–3.7 (2H, m, thiazolidinone ring), 6.41 (1H, s, thiazolidinone ring), 6.7–7.4 (14H, m, Ar-H), 7.6 ppm (1H, s, pyrazole ring); ^{13}C NMR ($CDCl_3$): δ 22.49, 31.11, 52.71, 76.66, 77.33, 77.54, 107.26, 111.71, 120.17, 121.84, 121.95, 123.14, 123.57, 125.61, 128.40, 128.86, 129.91, 131.80, 133.21, 135.62, 138.88, 144.72, 148.69, 155.19, 170.55 ppm; MS, m/z (%): 451 [M^+] (69), 452 [M^++1] (19), 376 [M+-$COCH_2SH$] (24), 285 [376-C_7H_7] (40), 318 [M^+-C_8H_7NO] (8), 314 [318-4] (100). Anal. calcd. for $C_{27}H_{21}N_3O_2S$ (451.54): C, 71.82; H, 4.69; N, 9.31; S, 7.10 %; found: C, 71.52; H, 4.87; N, 9.47; S, 7.34 %.

3.1.16.3. *2-(3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-bromophenyl)thiazolidin-4-one* (**14c**)

Yield 43 %, m.p. 124 °C, recrystallized from absolute ethanol; IR (KBr): 1682 cm^{-1} (CO); ^1H NMR ($CDCl_3$): δ 3.32–3.62 (2H, m, thiazolidinone ring), 6.33 (1H, s, thiazolidinone ring), 6.91–7.72 ppm (15H, m, Ar-H, CH pyrazole ring); MS, m/z (%): 515, 517 [M^+] (71, 77), 440, 442 [M^+-$COCH_2SH$] (13, 20), 285 [440-C_6H_4Br] (37), 77 [C_6H_5] (100). Anal. calcd. for $C_{26}H_{18}BrN_3O_2S$ (516.41): C, 60.47; H, 3.51; N, 8.14; S, 6.21 %; found: C, 60.29; H, 3.86; N, 8.36; S, 6.45 %.

3.1.16.4. *4-(2-(3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-4-oxothiazolidin-3-yl)benzoic acid* (**14d**)

Yield 51 %, m.p. 250 °C, recrystallized from absolute ethanol; IR (KBr): broad band centered at 3129 (OH, acid), 1691 cm^{-1} (CO); ^1H NMR ($CDCl_3$): δ 3.43–3.64 (2H, m, thiazolidinone ring), 6.45 (1H, s, thiazolidinone ring), 6.87–7.92 (15H, m, Ar-H, CH pyrazole ring), 10.57 ppm (1H, s, COOH); MS, m/z (%): 481 [M^+] (7), 406 [M^+-$COCH_2SH$] (14), 285 [406-$C_7H_5O_2$] (28), 77 [C_6H_5] (100). Anal. calcd. for $C_{27}H_{19}N_3O_4S$ (481.52): C, 67.35; H, 3.98; N, 8.73; S, 6.66 %; found: C, 67.82; H, 3.76; N, 8.92; S, 6.94 %.

3.1.17. *Synthesis of 2-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)methylene)malononilrile* (**15a**) *and ethyl 3-(3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-2- cyanoacrylate* (**15c**)

A mixture of compound **1** (0.58 g, 0.002 mol) and malononitrile and/or ethyl cyanoacetate (0.002 mol) in 15 mL absolute ethanol containing a few drops of piperidine was warmed for 15 min and stirred for 1 h at room temperature. The formed precipitate was filtered, dried, and recrystallized from absolute ethanol (99 %) to give the title compounds **15a,c**, respectively.

3.1.17.1. *2-((3-(Benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl) methylene)malononilrile* (**15a**)

Yield 74 %, m.p. 181 °C, recrystallized from absolute ethanol; IR (KBr): 2225 cm^{-1} (CN); ^1H NMR ($CDCl_3$): δ 7.37–8.12 (10H, m, Ar-H), 8.44 (1H, s, pyrazole ring), 8.56 ppm (1H, s, CH=C); MS, m/z (%): 336 [M^+] (100). Anal. calcd. for $C_{21}H_{12}N_4O$ (336.35): C, 74.99; H, 3.60; N, 16.66 %; found: C, 74.62; H, 3.81; N, 16.87 %.

3.1.17.2. *2-Ethyl 3-(3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)-2-cyanoacrylate* (**15c**)

Yield 78 %, m.p. 170 °C, recrystallized from absolute ethanol; IR (KBr): 2219 (CN), 1730 cm^{-1} (CO, ester), disappearance of CHO band; ^1H NMR ($CDCl_3$): δ 1.61 (3H, t, CH_3), 4.48 (2H, q, CH_2), 7.39–8.12 (10H, m, Ar-H), 8.41 (1H, s, pyrazole ring), 8.51 ppm (1H, s, CH=C); ^{13}C NMR ($CDCl_3$): δ 14.60, 62.02, 76.80, 77.23, 77.65, 93.41, 107.30, 111.62, 115.43, 120.08, 121.91, 123.09, 123.55, 125.71, 128.32, 129.79, 131.76, 138.92, 144.72, 148.64, 152.45, 155.22, 167.40 ppm; MS, m/z (%): 383 [M^+] (100). Anal. calcd. for $C_{23}H_{17}N_3O_3$ (383.40): C, 72.05; H, 4.47; N, 10.96 %; found: C, 72.36; H, 4.71; N, 11.21 %.

3.1.18. *Synthesis of Diethyl 2-((3-(benzofuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)methylene) malonate* (**15b**)

A mixture of compound **1** (0.58 g, 0.002 mol) and diethylmalonate (0.32 mL, 0.002 mol) in 15 mL absolute ethanol containing a few drops of piperidine was refluxed for 3 h. The formed precipitate was filtered, dried and recrystallized from absolute ethanol to give the title compound **15b**. Yield 72 %, m.p. 121 °C; IR (KBr): 1735 cm^{-1} (CO, ester), disappearance of CHO band; ^1H NMR ($CDCl_3$): δ 1.58–1.65 (6H, m, 2 CH_3), 4.56–4.67 (4H, m, 2 CH_2), 7.47–8.02 (10H, m, Ar-H), 8.53 (1H, s, pyrazole ring), 8.66 ppm (1H, s, CH=C); ^{13}C NMR ($CDCl_3$): δ 14.60, 14.70, 62.02, 62.30, 77.10, 77.53, 77.95, 106.51, 112.10, 115.74, 120.27, 121.90, 123.77, 125.55, 128.27, 128.74, 129.19, 130.10, 133.56, 139.62, 145.61, 149.39, 155.55, 164.95, 167.40 ppm; MS, m/z (%): 430 [M^+] (100), 431 [M^++1] (22), 357 [M^+-$COOC_2H_5$] (13), 356 [357–1] (33), 284 [357-$COOC_2H_5$] (34), 285 [284+1] (46). Anal. calcd. for $C_{25}H_{22}N_2O_5$ (430.45): C, 69.76; H, 5.15; N, 6.51 %; found: C, 69.43; H, 5.36; N, 6.81 %.

3.1.19. *Synthesis of 3-(3-(Benzofuran-2-yl)-phenyl-1H-pyrazol-4-yl)acrylic acid* (**15d**)

A mixture of compound **1** (0.58 g, 0.002 mol) and malonic acid (0.21 g, 0.002 mol) in 10 mL absolute ethanol containing 1 mL of pyridine was refluxed for 10 h, cooled and poured onto ice-water and acidified by dilute hydrochloric acid. The formed precipitate was filtered, dried and recrystallized from absolute ethanol to give the title compound **15d**, in 93 % yield, m.p. 208–210 °C; IR (KBr): broad band centred at 3124 (OH, acid), 1686 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 6.94–7.91 (12H, m, Ar-H, CH=CH), 8.41 (1H, s, pyrazole ring), 10.93 ppm (1H, s, COOH); MS, m/z (%): 330 [M^+] (53), 285 [M-COOH] (100). Anal. calcd. for $C_{20}H_{14}N_2O_3$ (330.33): C, 72.72; H, 4.27; N, 8.48 %; found: C, 72.44; H, 4.58; N, 8.71 %.

3.2. Cytotoxicity Screening

Materials and Methods

The aim of the present study was to illustrate the effect of some newly synthesized compounds on the human liver carcinoma cell line (HEPG2) and the human cervix carcinoma cell line (HELA) in comparison with 5-fluorouracil (5-FU) in a trial to get more effective and less toxic agents.

Experiments were set up using the two human carcinoma cell lines to identify the potential toxicity of seven selected newly synthesized compounds (**1, 2c, 7c, 8b, 9b, 13b, 14b**) along with 5-fluorouracil as a standard reference compound by SRB using

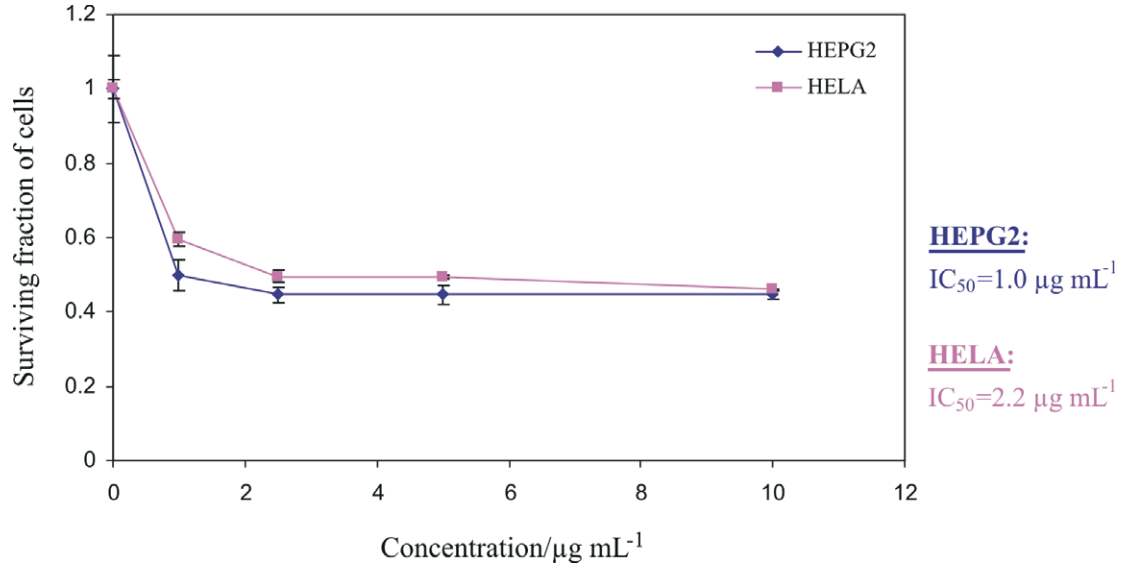


Figure 1 Cytotoxicity screening of compound **8b** against HEPG2 and HELA(±5 % error).

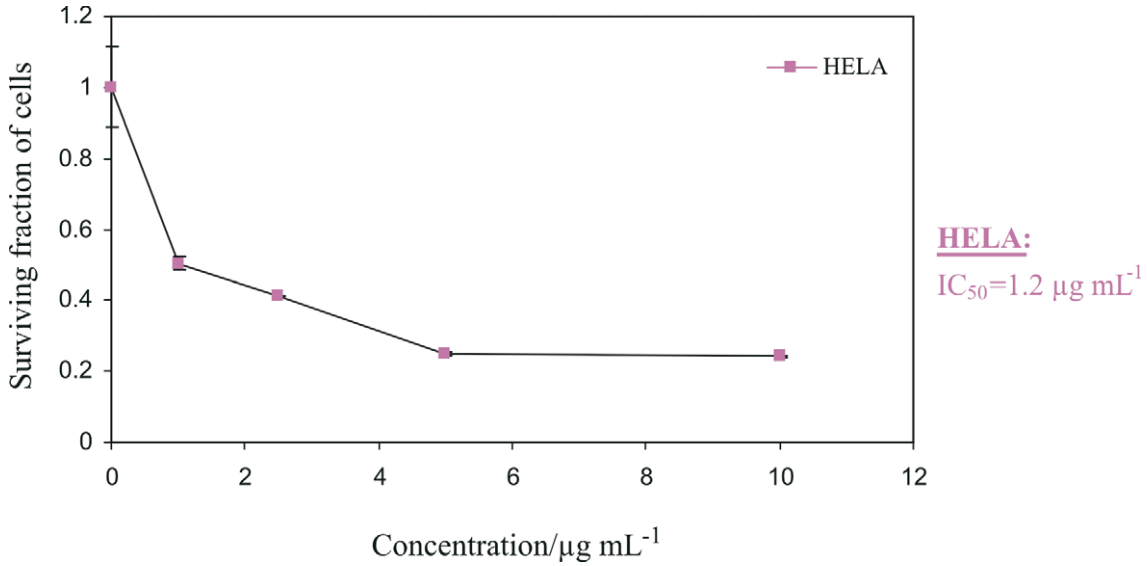


Figure 2 Cytotoxicity screening of compound **14b** against HELA (±5 % error).

the method of Skehan *et al.*[39]

Cells were plated in 96-multiwell plate (10^4 cells well^{-1}) for 24 h before treatment with compounds to allow attachment of cells to the wall of the plate. Different concentrations of the compound under test (0, 1, 2.5, 5 and 10 μg mL^{-1}) were added to the cell monolayer. Each concentration was evaluated three times (each dose was incubated with the cells in three different wells). Monolayer cells were incubated with the compounds for 48 h at 37 °C and in an atmosphere of 5 % CO_2. After 48 h, the cells were fixed, washed and stained with sulpho-rhodamine-B stain. Excess stain was washed with acetic acid and the attached stain was recovered with a tris EDTA buffer. The colour intensity was measured using an ELISA reader. The relation between the surviving fraction and the concentration (μg mL^{-1}) was plotted to obtain the survival curve of each tumour cell line after treatment with the specified compounds (see Figs 1 and 2 for the most active compounds; the figures for other less active compounds are supplied as Supplementary Material).

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Supplementary material to:

M.I. El-Zahar, S.S. Abd El-Karim and M.M. Anwar, *S. Afr. J. Chem.,* 2009, **62**, 189–199.

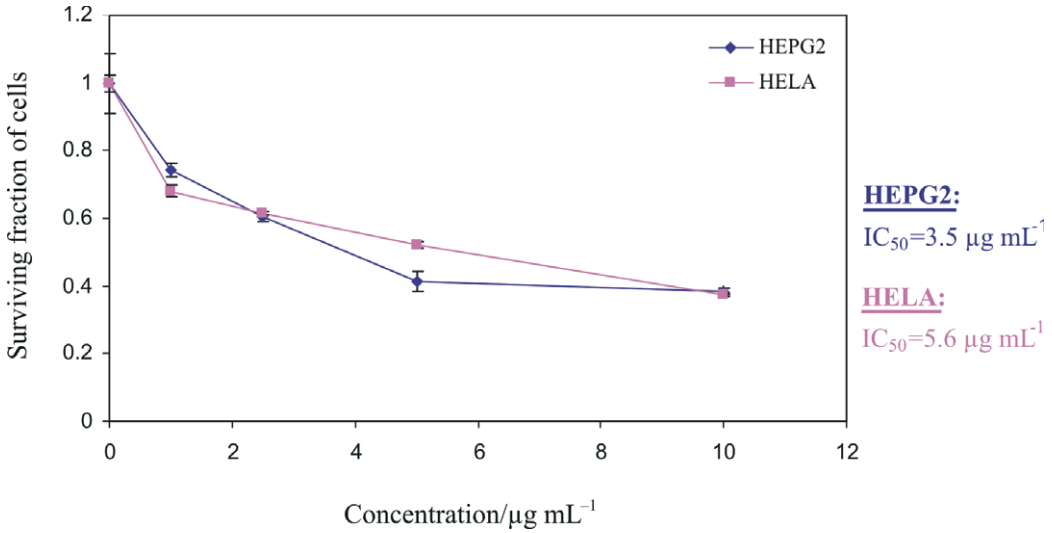

Figure S1 Cytotoxic activity of compound **1** against HEPG2 and HELA.

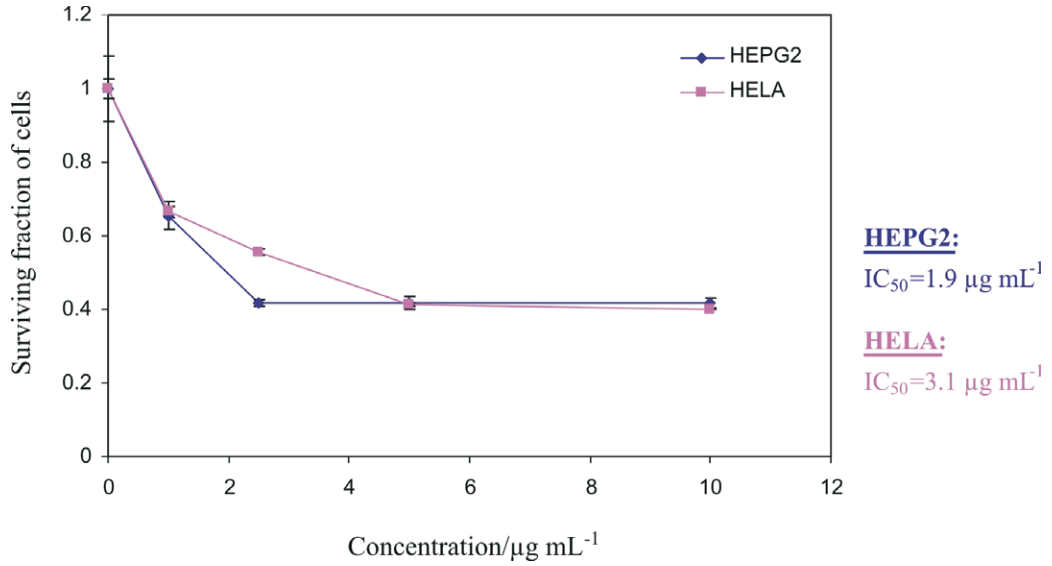

Figure S2 Cytotoxic activity of compound **2c** against HEPG2 and HELA.

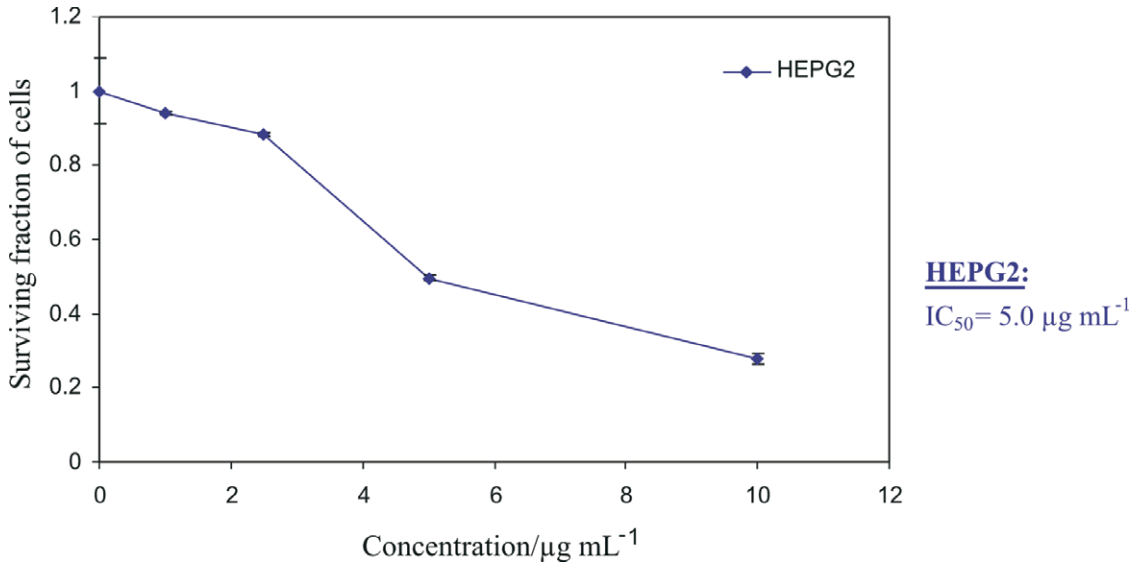

Figure S3 Cytotoxic activity of compound **7c** against HEPG2.

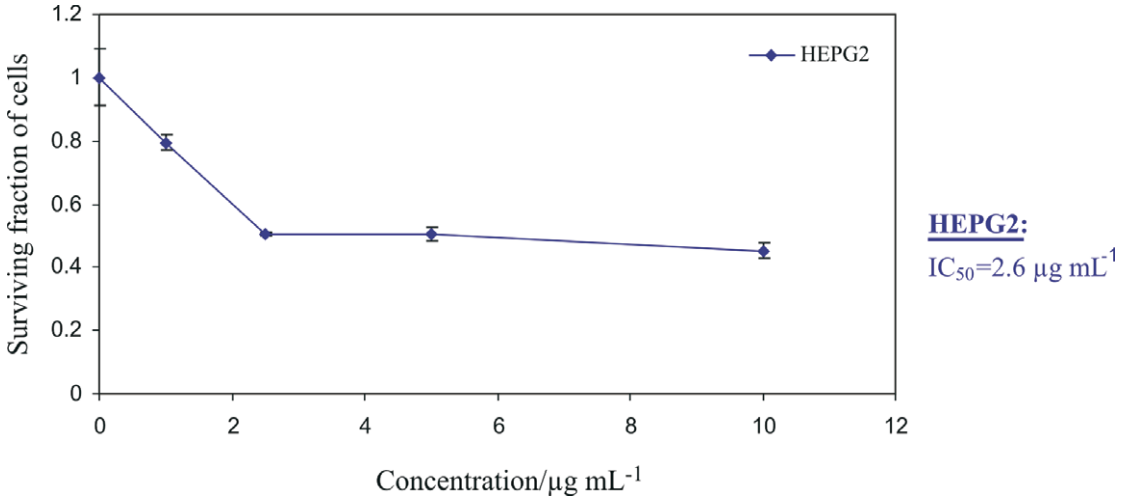

Figure S4 Cytotoxic activity of compound **9b** against HEPG2.

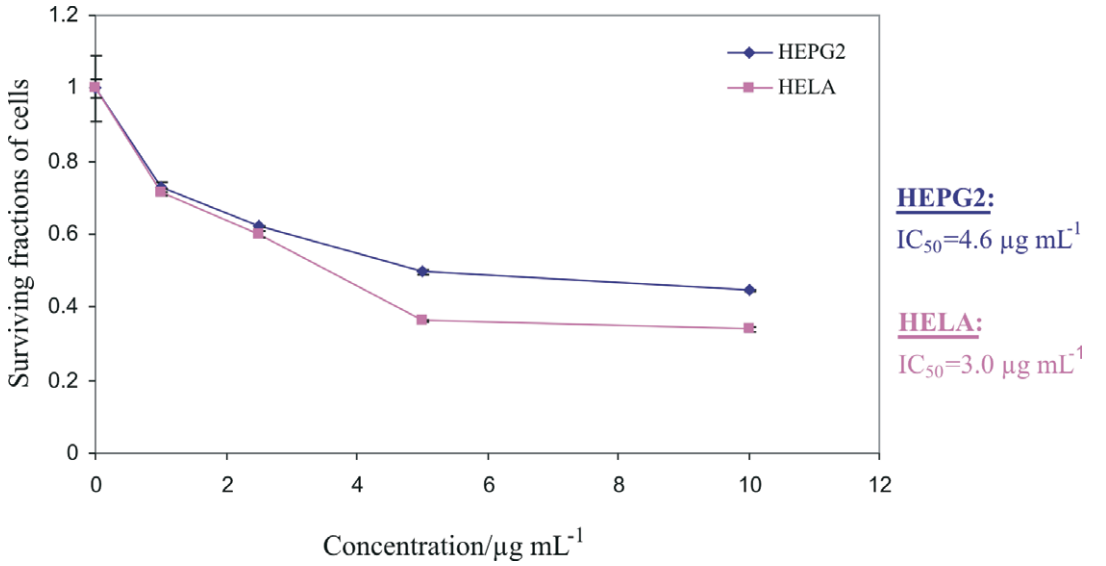

Figure S5 Cytotoxic activity of compound **13b** against HEPG2 and HELA.

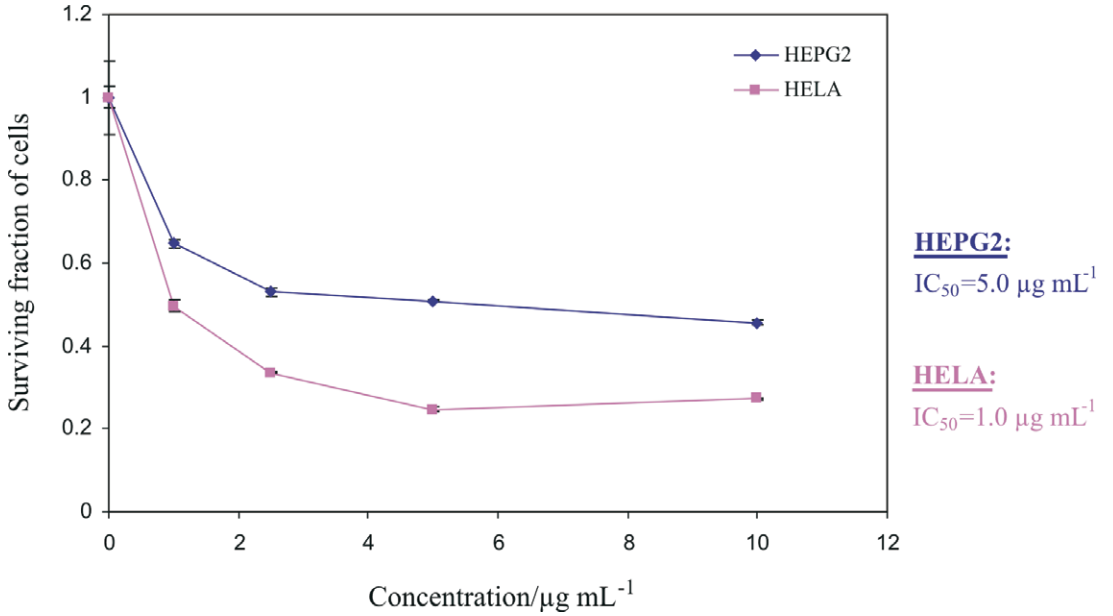

Figure S6. Cytotoxic activity of 5-FU against HEPG2 and HELA.