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Synthesis of some novel 4-benzothiazol-2-yl-benzoyl-1*H*-pyrazoles, and evaluation as antiangiogenic agents

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Abstract Some 4-(1,3-benzothiazol-2-yl)-benzoyl-1*H*-pyrazole derivatives have been synthesized by reaction of *o*-aminothiophenol (**1**) with different electrophilic and nucleophilic reagents. All of the newly synthesized compounds were evaluated for cytotoxicity against breast cancer cell line MCF-7. Two of the compounds were more potent than tamoxifen and three were as potent as tamoxifen. These results were consistent with percentage inhibition of human VEGF compared with control untreated cells.

Keywords 4-Benzothiazol-2-yl-benzoyl · Pyrazole · MCF-7 · VEGFR

Introduction

Angiogenesis, formation of new blood vessels, is crucially important to cancer cell survival and tumor growth, and is regarded as necessary for metastasis of all solid tumors [1–3]. This process consists of formation of new capillaries by splitting of pre-existing blood vessels. Among the other growth factors that are vital to stimulation of angiogenesis, vascular endothelial growth factor (VEGF) is the most important signaling protein [4]. The VEGF protein family consists of six members (VEGF-A, B, C, D, E and placenta growth factor). These proteins can bind to their VEGF receptors, VEGFR1, VEGFR2, and VEGFR3, which are receptor tyrosine kinases (RTK). VEGFR2 is regarded as an important receptor mediating all cellular

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responses to VEGF [5]. Because binding of VEGF to its family of receptors (VEGFR) is a crucial step that promotes the proliferation and survival of endothelial cells and, consequently, cancer progression [6], the search for an effective anti-VEGF/VEGFR drug has become a major interest of many research groups seeking to new cancer therapy via inhibition of angiogenesis.

The benzothiazole ring system is a core structure of a variety of natural and synthetic compounds with a broad range of biological activity [7–13] including anticancer activity [14–19]. During the past few years, several attempts have been made to modify their benzothiazole nucleus to improve their antitumor activity. Such modification has resulted in identification of a variety of promising benzothiazole derivatives with remarkable anticancer activity against malignant cell lines. Pyrazole derivatives have also attracted the attention of organic chemists because of their biological and chemotherapeutic importance. They are known to have such biological activity as antitumor [20], antileukemic [21], anti-inflammatory [22], analgesic [23], anticoagulant [24], and antimicrobial [25] activity.

Urea derivative **A** (Fig. 1) has good inhibitory activity against liver, breast, and gastric cell lines and acts as an inhibitor of Raf-1 kinase [26]. Thiourea derivative **B** (Fig. 1) has antiproliferative activity against two human monocytic cell lines (U 937 and THP-1) [27].

Semi(thiosemi)carbazide groups are present in many anti-tumor agents [28–30] and there have been several reports [31–35] suggesting use of the 1,2,4-triazole nucleus as a potential source of new anticancer agents. For example, the

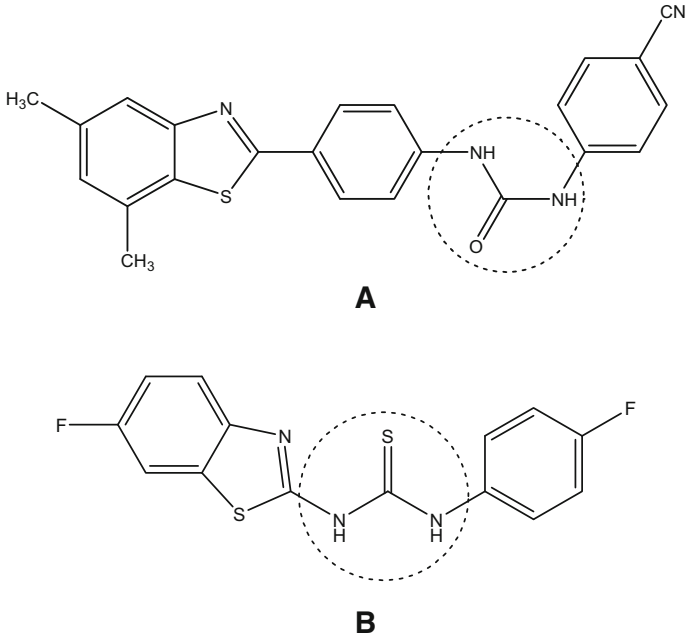


Fig. 1 The structures of compounds **a** and **b**

benzothiazole-1,2,4-triazole conjugate R115866 [35] has been reported to be a potent anticancer agent against the MCF-7 cell line.

Stimulated by these observations, we report here the synthesis and anti-breast cancer activity of some substituted 4-benzothiazol-2-yl-benzoyl-1*H*-pyrazoles prepared from *o*-aminothiophenol.

Results and discussion

Chemistry

4-(1,3-Benzothiazol-2-yl)benzonitrile (**2**) was prepared by reaction of *o*-aminothiophenol (**1**) with 4-cyanobenzaldehyde in absolute ethanol, as reported elsewhere [36]. Acid hydrolysis of the carbonitrile group by stirring with 70 % sulfuric acid gave 4-(1,3-benzothiazol-2-yl)benzoic acid (**3**). This was followed by esterification and reaction with hydrazine hydrate to form the corresponding 4-(1,3-benzothiazol-2-yl)benzohydrazide (**5**). Heating of compound **5** with ethoxymethylenemalononitrile or ethyl (ethoxymethylene)cyanoacetate, under reflux in ethanol, yielded the corresponding 5-amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1*H*-pyrazole-4-carbonitrile (**6**) or ethyl 5-amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1*H*-pyrazole-4-carboxylate (**7**), respectively. Heating of **7** under reflux with hydrazine hydrate furnished the corresponding carbohydrazide **8**, as illustrated in Scheme 1.

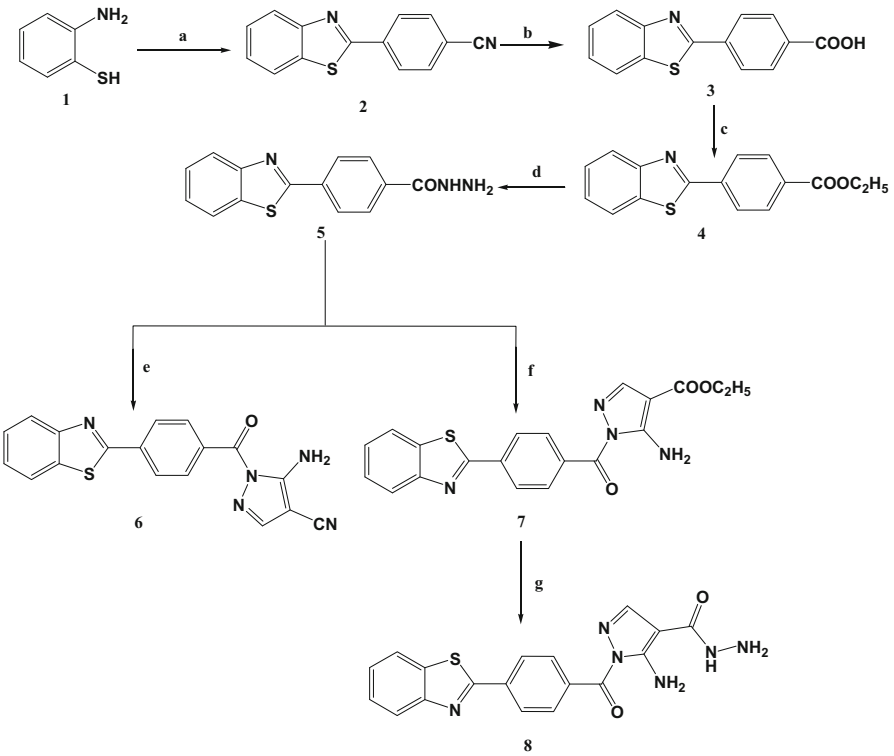
Compound **9** was prepared by reaction of **6** with 5-nitroisatin in absolute ethanol containing a few drops of glacial acetic acid. Compounds **10a** and **10b** were synthesized by heating carbonitrile **6** with 4′-chlorophenyl isocyanate or phenyl isothiocyanate under reflux in dry benzene. Stirring with 70 % sulfuric acid gave the corresponding ureido/thioureido derivatives **11a** and **11b**, as illustrated in Scheme 2.

Indole derivative **12** was prepared by reaction of carbohydrazide **8** with 5-nitroisatin in absolute ethanol containing a few drops of glacial acetic acid. Compounds **13a** and **13b** were synthesized by heating **8** with 4′-chlorophenyl isocyanate or phenyl isothiocyanate under reflux in dry benzene. Heating of **13a** or **13b** with sodium hydroxide under reflux for 4 h afforded the triazoles **14a** or **14b**, respectively. Condensation of triazole **14a** with the appropriate organohalogen compound (methyl iodide, ethyl chloroformate, methyl 2-bromoacetate, ethyl 2-bromoacetate or ethyl 2-bromopropanoate) in the presence of anhydrous sodium carbonate, to abstract the halogen atom, and acetone as solvent afforded compounds **15a–e**, usually in the pure state (Scheme 3).

Biological evaluation

Cytotoxicity against human breast cancer cell line MCF-7

The cytotoxicity of compounds **6–15e** against the human breast cancer cell line MCF-7 was tested by use of the method of Skehan et al. [37]. The cytotoxicity results were compared with that of the standard drug tamoxifen. The activity of the ureido and thioureido compounds **11a** and **11b** was better than or equal to that of



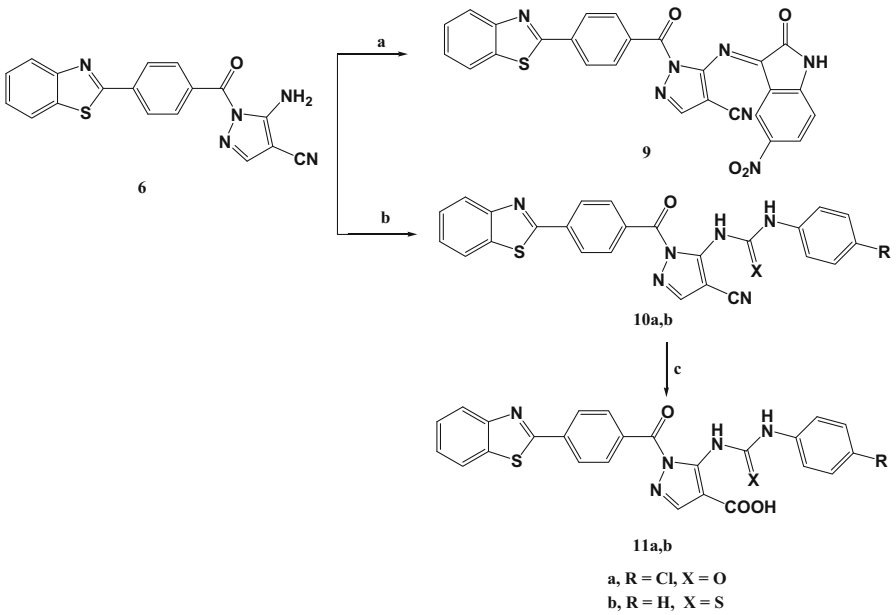
Scheme 1 Reagents: **a** 4-cyanobenzaldehyde, EtOH; **b** 70 % H_2SO_4; **c** EtOH, H_2SO_4; **d** $NH_2NH_2{\cdot}H_2O$, EtOH; **e** ethoxymethylenemalononitrile, EtOH; **f** ethyl (ethoxymethylene)cyanoacetate, EtOH; **g** $NH_2NH_2{\cdot}H_2O$, EtOH

tamoxifen (IC_{50} = 0.01–0.02 μmol/ml). The activity of carbohydrazide **8** was equal to that of tamoxifen, and indole derivative **12** was more potent than the reference drug. Cyclization of semi(thio)carbazide derivatives **13a** and **13b** to prepare the triazole derivatives **14a** and **14b**, respectively led to a slight increase of anticancer activity against the MCF-7 cell line. Condensation of compound **14a** with different alkyl halides to prepare compounds **15a–e** led to increased activity for compound **15a** only, which was equipotent with tamoxifen; the other derivatives had no activity. The other compounds had moderate to weak activity against the MCF-7 cell line (Table 1).

The promising anticancer compounds were not toxic to a normal cell line (human normal melanocyte, HFB4).

In-vitro VEGF inhibition in human breast cancer cell line MCF-7

Angiogenesis is essential for tumor progression because tumor masses cannot grow larger than 2 mm^3 without nourishment from blood vessels. Vascularization is, moreover, required for the process of extravasation in metastasis [38]. Hence, establishment of a chemotherapeutic strategy to block angiogenesis has attracted



Scheme 2 Reagents: **a** 5-nitroisatin, EtOH, glacial acetic acid; **b** 4′-chlorophenyl isocyanate or phenyl isothiocyanate, benzene; **c** 70 % H_2SO_4

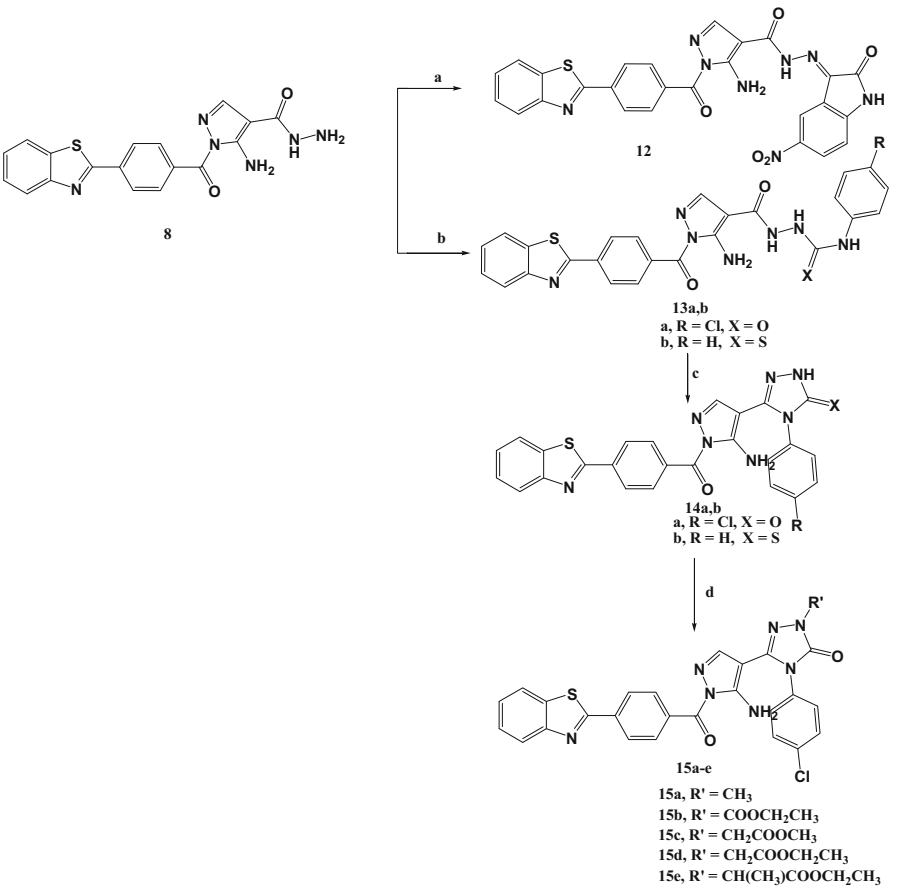
much attention in recent years. Alteration of cellular adaptation to hypoxia is also fundamental to cancer treatment, because angiogenesis or other metabolic modifications will be stimulated to maintain tumor cell survival [39]. Vascular endothelial growth factor (VEGF) has been identified as the most important angiogenic factor for tumor progression; it is released by a variety of tumor cells and is overexpressed in a variety of human cancers. Drugs that inhibit production of VEGF or block its receptor signaling significantly inhibit tumor growth [40, 41].

In this biological in-vitro study double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used to determine the level of human VEGF in human breast cancer cell line MCF-7 treated with compounds **6–15e** and in untreated cells. The results (Table 1) showed that compound **12** was more potent than tamoxifen (98 %) against human VEGF, with 99 % inhibition compared with control untreated cells. The potency of compounds **8**, **11a**, **11b**, and **15a** was similar to that of tamoxifen (91.67–97.32 %). These results were consistent with the compounds' cytotoxicity against MCF-7 cell line (the compounds had excellent activity with IC_{50} ranging from 0.01 to 0.02 μmol/ml).

Experimental

General

Melting points (°C) were determined in open capillary tubes, with use of silicone oil and Gallenkamp equipment (Ultraporter, Walsall, UK). ^{1}H NMR spectra were



Scheme 3 Reagents: **a** 5-nitroisatin, EtOH, glacial acetic acid; **b** 4′-chlorophenyl isocyanate or phenyl isothiocyanate, benzene; **c** NaOH; **d** methyl iodide, ethyl chloroformate, methyl 2-bromoacetate, ethyl 2-bromoacetate, or ethyl 2-bromopropanoate, Na_2CO_3, acetone

acquired with a Jeol (Canada) 270-MHz spectrometer, with DMSO-d_6 as solvent and tetramethylsilane as an internal standard. Mass Spectra were obtained with a GCS-QP1000EX spectrometer (Schimadzu Scientific Instruments, Italy) at 70 eV. IR spectra were recorded with a Philips model PU 9712 Infracord spectrophotometer (PerkinElmer, Waltham, Masssachusetts 02451, USA) as KBr discs. Elemental analysis was performed at the Microanalytical Laboratory of the National Research Center. All the reactions were monitored by thin-layer chromatography (TLC) on silica gel with chloroform as mobile phase.

*4-(1,3-Benzothiazol-2-yl)benzonitrile (**2**)* 4-Cyanobenzaldehye (1.05 g, 0.21 mol) and *o*-aminothiophenol **1** (1 ml, 0.21 mol) were dissolved in ethanol. This mixture was heated under reflux for 5 h then cooled to room temperature. Water was then added slowly to the mixture with stirring. The suspension was maintained at −5 °C

Table 1 Effect of the synthesized compounds on the human breast cancer cell line MCF-7 IC_{50} (μmol/ml) and the VEGF level (pg/ml) in breast cancer cell line MCF-7

Compound	IC_{50}	VEGF
6	0.06	4500.80 ± 460.60 (14.27 %)
7	0.08	5160.90 ± 550.00 (1.70 %)
8	0.02	322.98 ± 35.50 (93.85 %)
9	NA	–
10a	0.08	5060.90 ± 550.00 (3.70 %)
10b	0.09	5200.30 ± 535.90 (0.95 %)
11a	0.02	224.98 ± 35.50 (95.98 %)
11b	0.01	120.90 ± 38.00 (97.32 %)
12	0.01	106.75 ± 36.00 (99 %)
13a	0.07	4914.83 ± 500.00 (6.38 %)
13b	0.09	5220.70 ± 540.80 (0.60 %)
14a	0.05	4170.82 ± 460.90 (19.13 %)
14b	0.07	4938.83 ± 500.00 (6.88 %)
15a	0.02	395.70 ± 210.80 (91.67 %)
15b	NA	–
15c	NA	–
15d	NA	–
15e	NA	–
DMSO	–	5250.00
Tamoxifen	0.02	110.75 (98 %)

Data are expressed as means from four independent experiments

Values in parentheses indicated percentage changes compared with control cancer cells

NA no activity

overnight. The product was washed repeatedly with ethanol–water (1:1) then recrystallized from acetone. Yield = 1.2 g (64 %), mp = 150–2 °C. Analysis for $C_{14}H_8N_2S$ (236.2): Calcd.: C, 69.0; H, 4.5; N, 12.4; S, 14.2; Fd.: C, 69.2; H, 4.6; N, 12.5; S, 14.3.

*4-(1,3-Benzothiazol-2-yl)benzoic acid (**3**)* A mixture of **2** (2 g, 0.01 mol) and 30 ml 70 % sulfuric acid was stirred in a 100-ml three-necked flask at 140 °C for 5 h, then poured into 150 ml water. The resulting precipitate was isolated by filtration. Recrystallization from dilute ethanol afforded white crystals. Yield = 0.8 g (74 %), mp = 250–3 °C. Analysis for $C_{14}H_9NO_2S$ (255.3): Calcd.: C, 65.9; H, 3.6; N, 5.5; S, 12.6; Fd.: C, 66.0; H, 3.6; N, 5.6; S, 12.8. MS: *m*/*z* (%): 255 (M^+, 100).

*Ethyl 4-(1,3-benzothiazol-2-yl)benzoate (**4**)* A few drops of concentrated sulfuric acid were added to a solution of compound **3** (2 g; 0.073 mol) in absolute ethanol and the mixture was heated under reflux for 4 h. The crude product was filtered, air-dried, and crystallized from ethanol. Yield = 2.1 g (94 %), mp = 102–6 °C. Analysis for $C_{16}H_{13}NO_2S$ (283.3): Calcd.: C, 67.8; H, 4.6; N, 4.9; S, 11.3; Fd.: C, 68.0; H, 4.6; N, 5.0; S, 11.5. MS: *m*/*z* (%): 283 (M^+, 15). ^{1}H NMR: δ, ppm (DMSO-d_6); 1.32 (t, 3H, $C\underline{H}_3$); 4.32 (q, 2H, $C\underline{H}_2CH_3$); 7.55 (mm, 2H, $H_5 + H_6$

benzothiazole); 7.59 (dd, 2H, $H_2 + H_6$ benzene); 8.03 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole).

*4-(1,3-Benzothiazol-2-yl)benzohydrazide (**5**)* Hydrazine hydrate (98 %; 2 ml) was added to a solution of ester **4** (1 g; 0.033 mol) in ethanol and the mixture was heated for 5 h on a water-bath then cooled. The crude product was isolated by filtration, washed with water, dried, and crystallized from ethanol. Yield = 0.8 g (84 %), mp = 234–8 °C. Analysis for $C_{14}H_{11}N_3OS$ (269.3): Calcd.: C, 62.4; H, 4.1; N, 15.6; S, 11.9; Fd.: C, 62.5; H, 4.2; N, 15.7; S, 12.0. IR (cm^{-1}): 3453 (NH), 3314 (NH_2), 1684 (CO). MS: *m/z* (%): 269 (M^+, 16). ^{1}H NMR: δ, ppm (DMSO-d_6); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.66 (dd, 2H, $H_2 + H_6$ benzene); 8.01 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 9.96 (s, 2H, N$\underline{H}_2$, exchangeable with D_2O); 10.45 (s, 1H, N$\underline{H}$, exchangeable with D_2O).

*General procedure for preparation of 5-amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole-4-substituted (**6, 7**)* Ethoxymethylenemalononitrile or ethyl ethoxymethylenecyanoacetate (0.01 mol) was added to a solution of hydrazide **5** (1 g, 0.01 mol) in 30 ml ethanol and the mixture was heated under reflux for 6–8 h. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The solid product was recrystallized from ethanol.

*5-Amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole-4-carbonitrile (**6**)* Yield = 0.7 g (55 %); mp = 266–8 °C. Analysis for $C_{18}H_{11}N_5OS$ (345.4): Calcd.: C, 62.6; H, 3.2; N, 20.3; S, 9.3; Fd.: C, 62.5; H, 3.3; N, 20.2; S, 9.4. IR (cm^{-1}): 3424 (NH_2), 2217 (CN), 1677 (CO). MS: *m/z* (%): 345 (M^+, 57). ^{1}H NMR: δ, ppm (DMSO-d_6); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 10.76 (s, 2H, N$\underline{H}_2$).

*Ethyl-5-amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole-4-carboxylate (**7**)* Yield = 0.9 g (62 %); mp = >300 °C. Analysis for $C_{20}H_{16}N_4O_3S$ (392.4): Calcd.: C, 61.2; H, 4.1; N, 14.3; S, 8.2; Fd.: C, 61.3; H, 4.5; N, 14.6; S, 8.4. IR (cm^{-1}): 3224 (NH_2), 1677 (CO), 1662 (CO). MS: *m/z* (%): 392 (M^+, 57). ^{1}H NMR: δ, ppm (DMSO-d_6); 1.19 (t, 3H, C$\underline{H}_3$); 3.30 (q, 2H, C$\underline{H}_2$); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 10.77 (s, 2H, N$\underline{H}_2$).

*5-Amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole-4-carbohydrazide (**8**)* Hydrazine hydrate (98 %; 2 ml) was added to a solution of ester compound **7** (1 g; 0.033 mol) in ethanol, and the mixture was heated for 5 h on a water-bath. The reaction mixture was cooled. The crude product was filtered, washed with water, dried, then crystallized from ethanol. Yield = 0.8 g (83 %), mp = 182–5 °C. Analysis for $C_{18}H_{14}N_6O_2S$ (378.4): Calcd.: C, 57.1; H, 3.7; N, 22.2; S, 8.5; Fd.: C, 57.2; H, 3.9; N, 22.1; S, 8.6. IR (cm^{-1}): 3343 (NH), 3165 (NH_2), 1677 (CO), 1662

(CO). MS: *m/z* (%): 379 ($M^{+\cdot}$, 16); 378 (M^+, 32). ^{1}H NMR: δ, ppm (DMSO-d_6); 3.96 (s, 2H, N$\underline{H}_2$, exchangeable with D_2O); 4.94 (s, 2H, N$\underline{H}_2$, exchangeable with D_2O); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 10.73 (s, 1H, N$\underline{H}$, exchangeable with D_2O).

*1-[4-(1,3-Benzothiazol-2-yl)benzoyl]-5-(5-nitro-2-oxo-1,2-dihydroindol-3-ylideneamino)-1H-pyrazole-4-carbonitrile (**9**)* 5-Nitroisatin (0.9 g, 0.018 mol) and few drops of glacial acetic acid were added to a solution of compound **6** (1 g; 0.018 mol) in absolute ethanol (30 ml) and the mixture was heated under reflux for 6 h. The solvent was removed by distillation and the solid product was crystallized from ethyl acetate–petroleum ether (95:5). Yield = 1.4 g (93 %); mp = 272–5 °C. Analysis for $C_{26}H_{13}N_7O_4S$ (519.5): Calcd.: C, 60.1; H, 2.5; N, 18.9; S, 6.2; Fd.: C, 60.0; H, 2.5; N, 19.0; S, 6.2. IR (cm^{-1}): 3335 (NH), 2217 (CN), 1705 (CO), 1673 (CO). MS: *m/z* (%): 520 ($M^{+\cdot}$, 62); 519 (M^+, 69). ^{1}H NMR: δ, ppm (DMSO-d_6); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 7.91 (d, 1H, H_7 indole); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 8.27 (d, 1H, H_6 indole); 8.50 (s, 1H, H_4 indole); 10.79 (s, 1H, N$\underline{H}$, exchangeable with D_2O).

*General procedure for preparation of 1-{1-[4-(1,3-benzothiazol-2-yl)benzoyl]-4-cyano-1H-pyrazol-5-yl}-3-(un)substituted-phenylurea/thiourea (**10a, b**)* A mixture of **6** (1 g, 0.01 mol) and 4′-chlorophenyl isocyanate or phenyl isothiocyanate (0.01 mol) in dry benzene was heated under reflux for 6 h. The solid material obtained on cooling was isolated by filtration and recrystallized from methanol.

*1-{1-[4-(1,3-Benzothiazol-2-yl)benzoyl]-4-cyano-1H-pyrazol-5-yl}-3-(4-chlorophenyl)urea (**10a**)* Yield = 1.2 g (83 %); mp = >300 °C. Analysis for $C_{25}H_{15}ClN_6O_2S$ (498.9): Calcd.: C, 60.2; H, 3.0; N, 16.8; S, 6.4; Fd.: C, 60.1; H, 2.9; N, 16.9; S, 6.4. IR (cm^{-1}): 3395 (NH), 3228 (NH), 2217 (CN), 1689 (CO), 1662 (CO). MS: *m/z* (%): 500 (M^+, 3.3); 498 (M^+, 10). ^{1}H NMR: δ, ppm (DMSO-d_6); 6.91 (s, 2H, 2N$\underline{H}$, exchangeable with D_2O); 7.25 (dd, 2H, $H_3 + H_5$ chlorophenyl); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.58 (dd, 2H, $H_2 + H_6$ chlorophenyl); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole).

*1-{1-[4-(1,3-Benzothiazol-2-yl)benzoyl]-4-cyano-1H-pyrazol-5-yl}-3-phenylthiourea (**10b**)* Yield = 1.2 g (86 %); mp = >300 °C. Analysis for $C_{25}H_{16}N_6OS_2$ (480.6): Calcd.: C, 62.5; H, 3.4; N, 17.5; S, 13.3; Fd.: C, 62.4; H, 3.3; N, 17.4; S, 13.4. IR (cm^{-1}): 3401 (NH), 3296 (NH), 2215 (CN), 1667 (CO). MS: *m/z* (%): 481 ($M^{+\cdot}$, 16); 480 (M^+, 30). ^{1}H NMR: δ, ppm (DMSO-d_6); 5.02 (s, 2H, 2N$\underline{H}$, exchangeable with D_2O); 6.45 (dd, 2H, $H_2 + H_6$ phenyl); 6.68 (m, 1H, H_4 phenyl); 7.08 (mm, 2H, $H_3 + H_5$ phenyl); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole). ^{13}C-NMR δ, ppm (DMSO-d_6): showed

the presence of 25 signals correspond to the 25 different carbon groups, signals appeared at δ 117.2 (CN), 122.4–130.7 (Ar-13CH), 133.2 (C=N), 134.7 (C=C), 137.0 (CS), 152.1 (C-NH_2), 153.9 (C=N), 154.3 (C-N), 165.7 (C=C), 165.7 (C=N), 166.2 (C=O) and 179.9 (C=S).

*General procedure for preparation of 1-[4-(1,3-benzothiazol-2-yl)benzoyl]-5-[3-(un)substituted-phenylureido/thioureido]-1H-pyrazole-4-carboxylic acid (**11a, b**)* A mixture of **10a** or **10b** (1 g, 0.01 mol) and 30 ml 70 % sulfuric acid was stirred in a 100-ml three-necked flask at 140 °C for 5 h then poured into 150 ml water. The resulting precipitate was isolated by filtration then recrystallized from diluted ethanol.

*1-[4-(1,3-Benzothiazol-2-yl)benzoyl]-5-[3-(4-chlorophenyl)ureido)]-1H-pyrazole-4-carboxylic acid (**11a**)* Yield = 0.8 g (77 %); mp = >300 °C. Analysis for $C_{25}H_{16}ClN_5O_4S$ (517.9): Calcd.: C, 58.0; H, 3.1; N, 13.5; S, 6.2; Fd.: C, 58.1; H, 3.1; N, 13.4; S, 6.2. IR (cm^{-1}): 3441 (NH), 3270 (NH), 3188 (OH), 1702 (CO), 1692 (CO), 1665 (CO). MS: *m/z* (%): 519 (M^+, 3.3); 517 (M^+, 10). ^{1}H NMR: δ, ppm (DMSO-d_6); 6.91 (s, 2H, 2NH, exchangeable with D_2O); 7.25 (dd, 2H, $H_3 + H_5$ chlorophenyl); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.58 (dd, 2H, $H_2 + H_6$ chlorophenyl); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 11.01 (s, H, OH, exchangeable with D_2O).

*1-[4-(1,3-Benzothiazol-2-yl)benzoyl]-5-(3-phenylthioureido)-1H-pyrazole-4-carboxylic acid (**11b**)* Yield = 0.9 g (87 %); mp = >300 °C. Analysis for $C_{25}H_{17}N_5O_3S_2$ (499.6): Calcd.: C, 60.1; H, 3.4; N, 14.0; S, 12.8; Fd.: C, 60.1; H, 3.2; N, 13.8; S, 12.9. IR (cm^{-1}): 3441 (NH), 3270 (NH), 3188 (OH), 2215 (CN), 1667 (CO). MS: *m/z* (%): 500 ($M^{+\cdot}$, 16); 499 (M^+, 30). ^{1}H NMR: δ, ppm (DMSO-d_6); 4.13 (s, 2H, 2NH, exchangeable with D_2O); 6.45 (dd, 2H, $H_2 + H_6$ phenyl); 6.68 (m, 1H, H_4 phenyl); 7.08 (mm, 2H, $H_3 + H_5$ phenyl); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 10.73 (s, 1H, OH, exchangeable with D_2O).

*5-Amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole-(5-nitro-2-oxo-1,2-dihydroindol-3-ylidene)-4-carbohydrazide (**12**)* The method is the same as described for preparation of indole **9** but with use of compound **8** instead of **6**. Yield = 1.2 g (82 %); mp = 220–5 °C. Analysis for $C_{26}H_{16}N_8O_5S$ (552.5): Calcd.: C, 56.5; H, 2.9; N, 20.3; S, 5.8; Fd.: C, 56.4; H, 2.8; N, 20.4; S, 5.8. IR (cm^{-1}): 3335 (NH), 3206 (NH), 3195 (NH_2), 1702 (CO), 1692 (CO), 1665 (CO). MS: *m/z* (%): 553 ($M^{+\cdot}$, 12); 552 (M^+, 39). ^{1}H NMR: δ, ppm (DMSO-d_6); 2.94 (s, 2H, NH_2, exchangeable with D_2O); 3.27 (s, 1H, NH, exchangeable with D_2O); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 7.91 (d, 1H, H_7 indole); 8.12–8.23 (dd, 2H,

$H_4 + H_7$ benzothiazole); 8.27 (d, 1H, H_6 indole); 8.50 (s, 1H, H_4 indole); 10.00 (s, 1H, N<u>H</u>, exchangeable with D_2O).

*General procedure for preparation of {5-amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole}-4-(un)substituted phenylsemicarbazide/thiosemicarbazide (**13a, b**)* The method is the same as described for preparation of urea and thiourea **10a** and **10b** but with use of compound **8** instead of **6**.

*{5-Amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole}-4-(4-chlorophenyl) semicarbazide (**13a**)* Yield = 1.3 g (93 %); mp = 245–8 °C. Analysis for $C_{25}H_{18}ClN_7O_3S$ (531.9): Calcd.: C, 56.4; H, 3.4; N, 18.4; S, 6.0; Fd.: C, 56.4; H, 3.6; N, 18.4; S, 6.0. IR (cm^{-1}): 3395 (NH), 3228 (NH_2), 1720 (CO), 1692 (CO), 1674 (CO). MS: *m/z* (%): 533 (M^+, 3.3); 531 (M^+, 10). ^1H NMR: δ, ppm (DMSO-d_6); 6.53 (s, 2H, N<u>H</u>$_2$, exchangeable with D_2O); 7.25 (dd, 2H, $H_3 + H_5$ chlorophenyl); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.58 (dd, 2H, $H_2 + H_6$ chlorophenyl); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 8.87 (s, 1H, N<u>H</u>, exchangeable with D_2O); 9.02 (s, 1H, N<u>H</u>, exchangeable with D_2O); 10.46 (s, 1H, N<u>H</u>, exchangeable with D_2O).

*{5-Amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole}-4-phenyl thiosemicarbazide (**13b**)* Yield = 1.2 g (88 %); mp = 270–4 °C. Analysis for $C_{25}H_{19}N_7O_2S_2$ (513.6): Calcd.: C, 58.5; H, 3.7; N, 19.1; S, 12.5; Fd.: C, 58.4; H, 3.7; N, 19.0; S, 12.6. IR (cm^{-1}): 3395 (NH), 3228 (NH_2), 1683 (CO), 1667 (CO). MS: *m/z* (%): 514 ($M^{+\cdot}$, 22); 513 (M^+, 35). ^1H NMR: δ, ppm (DMSO-d_6); 6.30 (s, 2H, N<u>H</u>$_2$, exchangeable with D_2O); 6.45 (dd, 2H, $H_2 + H_6$ phenyl); 6.68 (m, 1H, H_4 phenyl); 7.08 (mm, 2H, $H_3 + H_5$ phenyl); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 8.57 (s, 1H, N<u>H</u>, exchangeable with D_2O); 8.92 (s, 1H, N<u>H</u>, exchangeable with D_2O); 10.00 (s, 1H, N<u>H</u>, exchangeable with D_2O).

*General procedure for preparation of 5-{5-amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole}-4-[4-(un)substituted phenyl]-[1,2,4]-triazol-3-one/thione (**14a,b**)* A mixture of compound **13a** or **13b** (0.01 mol) and sodium hydroxide (40 mg, 2 M solution) was heated under reflux for 4 h. After cooling, the solution was acidified with hydrochloric acid and the precipitate was isolated by filtration. The product was then crystallized from methanol.

*5-{5-Amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole}-4-(4-chlorophenyl)-2,4-dihydro-[1,2,4]-triazol-3-one (**14a**)* Yield = 0.8 g (82 %), mp = 220–2 °C. Analysis for $C_{25}H_{16}ClN_7O_2S$ (513.9): Calcd.: C, 58.4; H, 3.1; N, 19.1; S, 6.2; Fd.: C, 58.5; H, 3.3; N, 19.0; S, 6.2. IR (cm^{-1}): 3395 (NH), 3228 (NH_2), 1685 (CO), 1669 (CO). MS: *m/z* (%): 515 (M^+, 3.3); 513 (M^+, 10). ^1H NMR: δ, ppm (DMSO-d_6); 7.25 (dd, 2H, $H_3 + H_5$ chlorophenyl); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole);

7.58 (dd, 2H, $H_2 + H_6$ chlorophenyl); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 8.84 (s, 2H, $N\underline{H}_2$); 10.46 (s, 1H, N<u>H</u>).

5-{5-Amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole}-4-phenyl-2,4-dihydro-[1,2,4]-triazol-3-thione ***(14b)*** Yield = 0.7 g (72 %), mp = 257–9 °C. Analysis for $C_{25}H_{17}N_7OS_2$ (495.6): Calcd.: C, 60.6; H, 3.5; N, 19.8; S, 12.9; Fd.: C, 60.6; H, 3.4; N, 19.6; S, 13.0. IR (cm^{-1}): 3395 (NH), 3228 (NH_2), 1667 (CO). MS: *m/z* (%): 496 ($M^{+\cdot}$, 9.8); 495 (M^+, 22). ^{1}H NMR: δ, ppm (DMSO-d_6); 4.30 (s, 2H, $N\underline{H}_2$); 6.45 (dd, 2H, $H_2 + H_6$ phenyl); 6.68 (m, 1H, H_4 phenyl); 7.08 (mm, 2H, $H_3 + H_5$ phenyl); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 8.57 (s, 1H, N<u>H</u>).

General procedure for preparation of 5-{5-amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole}-4-(4-chlorophenyl)-2-substituted-2,4-dihydro-[1,2,4]-triazol-3-one ***(15a–e)*** A mixture of compound **14a** (2 g; 0.067 mol), the appropriate organohalogen compound (methyl iodide, ethyl chloroformate, methyl 2-bromoacetate, ethyl 2-bromoacetate, or ethyl 2-bromopropanoate) (0.067 mol), anhydrous sodium carbonate (4 g), and acetone (30 ml) was heated under reflux for 8 h. Most of the solvent was removed by distillation, the residue was diluted with water, and the product obtained was collected.

5-{5-Amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole}-4-(4-chlorophenyl)-2-methyl-2,4-dihydro-[1,2,4]-triazol-3-one ***(15a)*** Yield = 0.8 g (78 %), mp = 142–5 °C. Analysis for $C_{26}H_{18}ClN_7O_2S$ (527.9): Calcd.: C, 59.2; H, 3.4; N, 18.6; S, 6.1; Fd.: C, 59.3; H, 3.6; N, 18.5; S, 6.1. IR (cm^{-1}): 3290 (NH_2), 1685 (CO), 1666 (CO). MS: *m/z* (%): 529 (M^+, 33); 527 (M^+, 100). ^{1}H NMR: δ, ppm (DMSO-d_6); 2.71 (s, 3H, $C\underline{H}_3$); 7.25 (dd, 2H, $H_3 + H_5$ chlorophenyl); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.58 (dd, 2H, $H_2 + H_6$ chlorophenyl); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 9.72 (s, 2H, $N\underline{H}_2$).

3-{5-Amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole}-4-(4-chlorophenyl)-5-oxo-4,5-dihydro-[1,2,4]-triazole-1-carboxylic acid ethyl ester ***(15b)*** Yield = 0.9 g (79 %), mp = 92–5 °C. Analysis for $C_{28}H_{20}ClN_7O_4S$ (585.5): Calcd.: C, 57.4; H, 3.4; N, 16.7; S, 5.5; Fd.: C, 57.5; H, 3.6; N, 16.6; S, 5.5. IR (cm^{-1}): 3220 (NH_2), 1710 (CO), 1685 (CO), 1662 (CO). MS: *m/z* (%): 587 (M^+, 3.3); 585 (M^+, 10). ^{1}H NMR: δ, ppm (DMSO-d_6); 1.31 (t, 3H, $C\underline{H}_3$); 4.11 (q, 2H, $C\underline{H}_2$); 7.25 (dd, 2H, $H_3 + H_5$ chlorophenyl); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.58 (dd, 2H, $H_2 + H_6$ chlorophenyl); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 9.72 (s, 2H, $N\underline{H}_2$).

*3-{5-Amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole}-4-(4-chlorophenyl)-5-oxo-4,5-dihydro-[1,2,4]-triazol-1-yl-acetic acid methyl ester (**15c**)* Yield = 0.9 g (79 %), mp = 102–5 °C. Analysis for $C_{28}H_{20}ClN_7O_4S$ (586.0): Calcd.: C, 57.4; H, 3.4; N, 16.7; S, 5.5; Fd.: C, 57.5; H, 3.6; N, 16.6; S, 5.5. IR (cm^{-1}): 3246 (NH_2), 1708 (CO), 1685 (CO), 1662 (CO). MS: *m/z* (%): 588 (M^+, 3.3); 586 (M^+, 10). ^{1}H NMR: δ, ppm (DMSO-d_6); 3.71 (s, 3H, C$\underline{H}_3$); 4.11 (s, 2H, C$\underline{H}_2$); 7.25 (dd, 2H, $H_3 + H_5$ chlorophenyl); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.58 (dd, 2H, $H_2 + H_6$ chlorophenyl); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 9.72 (s, 2H, N$\underline{H}_2$).

*3-{5-Amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole}-4-(4-chlorophenyl)-5-oxo-4,5-dihydro-[1,2,4]-triazol-1-yl-acetic acid ethyl ester (**15d**)* Yield = 0.8 g (69 %), mp = 83–5 °C. Analysis for $C_{29}H_{22}ClN_7O_4S$ (600.1): Calcd.: C, 58.1; H, 3.7; N, 16.3; S, 5.3; Fd.: C, 58.0; H, 3.6; N, 16.4; S, 5.3. IR (cm^{-1}): 3246 (NH_2), 1710 (CO), 1685 (CO), 1662 (CO). MS: *m/z* (%): 602 (M^+, 3.3); 600 (M^+, 10). ^{1}H NMR: δ, ppm (DMSO-d_6); 1.31 (t, 3H, C$\underline{H}_3$); 4.11 (s, 2H, C$\underline{H}_2$); 4.15 (q, 2H, C$\underline{H}_2$); 7.25 (dd, 2H, $H_3 + H_5$ chlorophenyl); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.58 (dd, 2H, $H_2 + H_6$ chlorophenyl); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 9.72 (s, 2H, N$\underline{H}_2$).

*2-{3-{5-Amino-1-[4-(1,3-benzothiazol-2-yl)benzoyl]-1H-pyrazole}-4-(4-chlorophenyl)-5-oxo-4,5-dihydro-[1,2,4]-triazol-1-yl}-propionic acid ethyl ester (**15e**)* Yield = 0.85 g (71 %), mp = 95–9 °C. Analysis for $C_{30}H_{24}ClN_7O_4S$ (614.1): Calcd.: C, 58.7; H, 3.9; N, 16.0; S, 5.2; Fd.: C, 58.7; H, 3.9; N, 16.0; S, 5.2. IR (cm^{-1}): 3446 (NH_2), 1710 (CO), 1685 (CO), 1662 (CO). MS: *m/z* (%): 616 (M^+, 3.3); 614 (M^+, 10). ^{1}H NMR: δ, ppm (DMSO-d_6); 1.31 (t, 3H, C$\underline{H}_3$); 1.73 (d, 3H, C$\underline{H}_3$); 4.15 (q, 2H, C$\underline{H}_2$); 4.61 (q, 1H, C$\underline{H}$); 7.25 (dd, 2H, $H_3 + H_5$ chlorophenyl); 7.55 (mm, 2H, $H_5 + H_6$ benzothiazole); 7.58 (dd, 2H, $H_2 + H_6$ chlorophenyl); 7.61 (s, 1H, pyrazole); 7.67 (dd, 2H, $H_2 + H_6$ benzene); 7.87 (dd, 2H, $H_3 + H_5$ benzene); 8.12–8.23 (dd, 2H, $H_4 + H_7$ benzothiazole); 9.72 (s, 2H, N$\underline{H}_2$).

Bioactivity materials and methods

Cytotoxicity against human breast cancer cell line MCF-7

Antitumor activity against MCF-7 was determined in the National Research Center, Division of Genetic Engineering and Biotechnology, Department of Biochemistry, Cairo, Egypt. Fetal bovine serum (FBS) and L-glutamine, were obtained from Gibco Invitrogen (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), tamoxifen, penicillin, streptomycin, and sulfo-rhodamine-B stain (SRB) were obtained from

Sigma (St Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade and purchased from Sigma–Aldrich (St Louis, MO, USA).

Antitumor activity was measured in vitro by use of the sulfo-rhodamine-B stain (SRB) assay and the standard procedure reported elsewhere [37]. Ninety-six-well microtiter plates were inoculated with cells (10^4 cells/well) 24 h before treatment with the tested compounds, to enable attachment of cells to the walls of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compounds under test (0–100 μg/ml) were added to the cells. Triplicate wells were prepared for each dose. Monolayers of cells were incubated with the compounds for 48 h at 37 °C in an atmosphere of 5 % CO_2. After 48 h the cells were fixed, washed, and stained for 30 min with 0.4 % (*w*/*v*) SRB dissolved in 1 % acetic acid. Unbound dye was removed by four washes with 1 % acetic acid, and attached stain was recovered with Tris–EDTA buffer. Color intensity was measured in an ELISA reader and optical density was determined at 492 nm. After the specified time the relationship between surviving fraction and drug concentration was plotted to obtain the survival curve. The concentration required for 50 % inhibition of cell viability (IC_{50}) was calculated; the results are given in Table 1.

In-vitro VEGF inhibition of human breast cancer cell line MCF-7

The effect of the compounds on the level of human vascular endothelial growth factor (VEGF) was determined in human breast cancer cell line MCF-7 obtained from the American Type Culture Collection (Rockville, MD, USA). The tumor cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % heat-inactivated fetal calf serum (GIBCO), penicillin (100 U/ml), and streptomycin (100 μg/ml), at 37 °C, in humidified atmosphere containing 5 % CO_2. Cells at a concentration of 0.50×10^6 were grown in a 25 cm^2 flask in 5 ml complete culture medium.

The cells in the culture medium were treated with 20 μl of one-tenth of the IC_{50} values of the compounds and the standard reference drug, tamoxifen, dissolved in DMSO, then incubated for 24 h at 37 °C, in a humidified 5 % CO_2 atmosphere. The cells were harvested and homogenates were prepared in saline by use of a tight pestle homogenizer until complete cell disruption.

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) for determination of the level of human VEGF in samples. The VEGF is added to a monoclonal antibody enzyme well pre-coated with human VEGF monoclonal antibody and incubated. VEGF antibodies labeled with biotin and combined with Streptavidin-HRP to form an immune complex are then added and the mixture is incubated then washed to remove uncombined enzyme. Chromogen solutions A and B are then added and the color of the liquid changes to blue. Acid is then added and the color finally becomes yellow. Optical density was determined at 450 nm. Color intensity and concentration of human VEGF in the sample are positively correlated. Levels (pg/ml) of human VEGF in the samples were calculated from the standard curve as means from triplicate determinations.

Conclusion

Our main objective in this study was the synthesis of new urea, indole, and triazole derivatives of 4-(1,3-benzothiazol-2-yl)-benzoyl-1*H*-pyrazole and testing of the compounds as anticancer agents working by inhibiting VEGF–VEGFR2 complex formation, thus suppressing proliferation and survival of endothelial cells and consequently preventing cancer progression.

Five of the compounds (**8**, **11a**, **11b**, **12**, and **15a**) had promising cytotoxic activity against breast cancer cell line MCF-7; inhibition of human VEGF in MCF-7 cancer cell line was comparable with that of tamoxifen.

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Conflict of interest The authors have declared no conflicts of interest.

References

1. J. Folkman, Nat. Med. **1**, 27 (1995)
2. R.S. Kerbel, Carcinogenesis **21**, 505 (2005)
3. A.F. Karamysheva, Biochemistry **73**, 751 (2008)
4. P. Carmeliet, Oncology **69**, 4 (2005)
5. N. Ferrara, Oncology **69**, 11 (2005)
6. M.R. Raspollini, G. Amunni, A. Villanucci, G. Baroni et al., Int. J. Gynecol. Cancer **14**, 815 (2004)
7. R.D.R. Reis, E.C. Azevedo et al., Eur. J. Med. Chem. **46**, 1448 (2011)
8. I. Caleta, M. Kralj, M. Marjanovic, B. Bertosa, S. Tomic, G. Pavlovic, K. Pavelic, G.K. Zamola, J. Med. Chem. **52**(6), 1744 (2009)
9. J. Shukla, K. Hazra, P. Rashmi, L.V.G. Nargund, Der Chem. Sinici **2**(3), 4 (2011)
10. C. Jimenez, Bioactive Nat. Prod. **25**, 811 (2001)
11. G. Turan-Zitouni, S. Demirayak, A. Ozdemir, Z.A. Kaplancikli, M.T. Yildiz, Eur. J. Med. Chem. **39**(3), 267 (2003)
12. Y.J. Cao, J.C. Dreixler, J.J. Couey, K.M. Houamed, Eur. J. Pharmacol. **449**, 47 (2002)
13. A.D. Ramirez, S.K.F. Wong, F.S. Menniti, Eur. J. Pharmacol. **475**, 29 (2003)
14. S.W. Gangadhar, B.C. Anil, N.B. Vijay, R.S. Giridhar, V.K. Sharad, J. Pharm. Res. **7**, 823 (2013)
15. S. Sohail, R. Naghmana, G.J. Peter, A. Muhammad, H. Rizwan, Eur. J. Med. Chem. **45**(4), 1323 (2010)
16. I. Hutchinson, S.A. Jennings, B.R. Vishnuvajjala, A.D. Westwell, M.F.G. Stevenes, J. Med. Chem. **45**(3), 744 (2002)
17. C.O. Leong, M. Gaskell, E.A. Martin, R.T. Heydon, P.B. Farmer, M.C. Bibby, P.A. Cooper, J.A. Double, T.D. Bradshaw, M.F.G. Stevens, Br. J. Cancer **88**, 470 (2003)
18. B.L. Eric, A.d-B. Monique, R.A.V. Thatyana, O.d-M. Manuel, C.M. Raquel, D.Y. Jwliane, Z.L. Kátia, Eur. J. Med. Chem. **86**, 12 (2014)
19. D.F. Shi, T.D. Bradshaw, M.S. Chua, A.D. Westwell, M.F.G. Stevens, Bioorg. Med. Chem. Lett. **11**, 1093 (2001)
20. R. Lin, G. Chiu, Y. Yu, P.J. Connolly et al., Bioorg. Med. Chem. Lett. **17**(16), 4557 (2007)
21. G. Daidone, B. Maggio, D. Raffa, S. Plescia et al., IL Farmaco **59**, 413 (2004)
22. A.A. Bekhit, T.A. El-Azim, Bioorg. Med. Chem. **12**(8), 1935 (2004)
23. G. Menozzi, L. Merello, P. Fossa, L. Mosti et al., IL Farmaco **58**(9), 795 (2003)
24. J.G. Varnes, D.A. Wacker, I.C. Jacobson, M.L. Quan et al., Bioorg. Med. Chem. Lett. **17**, 6481 (2007)

25. T. Tanitame, Y. Oyamada, K. Ofuji, H. Terauchi et al., Bioorg. Med. Chem. Lett. **15**(19), 4299 (2005)
26. E.Y. Song, N. Kaur, M.Y. Park, Y. Jin, K. Lee, G. Kim, K.Y. Lee, J.S. Yang, J.H. Shin, K.Y. Nam, K.T. No, G. Han, Eur. J. Med. Chem. **43**, 1519 (2008)
27. R.M. Kumbhare, T. Dadmal, U. Kosurkar, V. Sridhar, J.V. Rao, Bioorg. Med. Chem. Lett. **22**, 453 (2012)
28. K.M. Amin, M. El-Zahar, M.M. Anwar, M.M. Kamel, M.H. Mohamed, Acta Poloniae Pharm. Drug Res. **66**(3), 279 (2009)
29. S. Shubhanjali, S. Radhey, K.S. Sushant, S. Ajit, K. Pankaj, Asian Pac. J. Trop. Biomed. **2**(2), S1040 (2012)
30. I. Perkovic, I. Butula, M. Kralj, I. Martin-Kleiner, J. Balzarini, D. Hadjipavlou-Litina, A.-M. Katson, B. Zorc, Eur. J. Med. Chem. **51**, 227 (2012)
31. I.F. Nassar, S.A. El-Assaly, Der Pharm. Chem. **3**(1), 229 (2011)
32. A. Kumar, I. Ahmad, B.S. Chhikara, R. Tiwari, D. Mandal, K. Parang, Bioorg. Med. Chem. Lett. **21**, 1342 (2011)
33. B.S. Holla, B. Veerendra, M.K. Shivananda, B. Poojary, Eur. J. Med. Chem. **38**, 759 (2003)
34. S. Pautus, S.W. Yee, M. Jayne, M.P. Coogan, C. Simons, Bioorg. Med. Chem. **14**, 3643 (2006)
35. P. Stoppie, M. Borgers, P. Borghgraef, L. Dillen et al., J. Pharmacol. Exp. Ther. **293**(1), 304 (2000)
36. S.E.H. Wageeh, M.A. Kamelia, A.E.A. Samy, A.A.M. Eman, Der Pharma Chemica **3**(6), 282 (2011)
37. P. Skehan, R. Storeng, D. Scudiero, A. Monks et al., J. Natl Cancer Inst. **82**, 1107 (1990)
38. H.M. Verheul, E.E. Voest, R.O. Schlingemann, J. Pathol. **202**, 5 (2004)
39. N. Ferrara, Nat. Rev. Cancer **2**, 795 (2002)
40. J. Folkman, Nat. Rev. Drug Discov. **6**, 273 (2007)
41. K.J. Gotink, H.M.W. Verheul, Angiogenesis **13**, 1 (2010)