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DISCOVERY OF SOME NOVEL BENZOTHIAZOLE DERIVATIVES AS VEGFR-2 INHIBITORS

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Abstract

A series of benzothiazole derivatives were prepared by condensation of 5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carbonitrile (**1**) and/or 5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carboxylic acid hydrazide (**6**) with different electrophilic and nucleophilic reagents. All of the newly synthesized compounds have been evaluated for their potential cytotoxicity against breast cancer cell line (MCF-7); compounds **5c**, **8b** and **8c** are more potent than tamoxifen while compounds **5b** and **8a** are equipotent to tamoxifen. These results were consistent with percentage of inhibition values against human VEGF compared with control untreated cells.

Key words: 4-Benzothiazol-2-yl-benzoyl. Benzamide. Sulfonamide. Pyrrole. MCF-7. VEGF-2.

Introduction

Cancer still remains a threat to men's health, representing the second leading cause of death worldwide [1]. It is estimated that 12 million people will die from cancer in 2030 [2]. There is thus an urgent need for the discovery and development of more effective and safer drugs for the treatment of cancer. Benzothiazoles are an important class of heterocycles, which can serve as unique and versatile scaffolds for experimental drug design [3]. This ring system displayed a broad spectrum of biological activities [4-7] including anticancer effects [8-13]. Modifications on the benzothiazole ring have resulted in the identification of various promising benzothiazole scaffold that possess remarkable anticancer activity against malignant cell lines.

The amide derivative displays inhibiting activity against liver, breast and gastric cell lines and act as inhibitor of Raf-1 kinase [14]. The chemistry of sulfonamides has been of increasing interest since many of these derivatives display extensive *in vitro* and/or *in vivo* antitumor activity [15-18]. Indisulam (E7070), an indole sulfonamide derivative, is

clinically used for treatment of colorectal, breast, head and neck cancer [15]. Moreover, pyrrole ring and its related fused heterocycle such as indole are also important building blocks in medicinal chemistry led to the discovery of a number of derivatives endowed with anticancer effects [19-21].

Vascular endothelial growth factor (VEGF) is an important signaling protein involved in both vasculogenesis (the formation of the circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature). *In vitro*, VEGF has been shown to stimulate endothelial cell mitogenesis and cell migration. VEGF also enhances microvascular permeability and is sometimes referred to as vascular permeability factor. The VEGF family consists of six members of proteins (VEGF-A, B, C, D, E and placenta growth factor). These proteins can bind to their VEGF receptors (VEGFR-1, VEGFR-2 and VEGFR-3). These receptors are belonging to receptor tyrosine kinases (RTK) [22-25].

VEGF receptor-2 plays an essential role in angiogenesis during adulthood and is the predominant mediator of VEGF-stimulated endothelial cell migration, proliferation, survival, and enhanced vascular permeability [26]. Further study indicated this receptor was expressed not only in vascular endothelial cells but also in certain tumor cells, and its activation can regulate cell proliferation, apoptosis, invasion, and migration [27]. Recently, it has been reported that VEGFR-2 may be expressed in vascular endothelial cells, endometrial glandular epithelium, and stroma cells [28]. Research using animal models of endometriosis and endometriosis patients also indicated that VEGFR-2 is related to this disease [29, 30]. Furthermore, therapeutic inhibition of VEGFR-2 action is now having an impact in the clinic for the treatment of a number of diseases.

Materials and Methods

Chemistry

Melting points (˚C) were determined in open capillary tubes using silicon oil on Gallen Kamp apparatus (Ultraporter Company, Walsall, United Kingdom). ^{1}H-NMR Spectra were measured in DMSO-d_6 on JEOL-270 MHz Spectrometer (JEOL, Canada) with tetramethylsilane as an internal standard. Mass Spectra were obtained with a Schimadzu GCS-QP1000EX Spectrometer (Schimadzu Scientific Instruments, Italy) at 70 ev. The IR Spectra were recorded with a Philips Infra cord Spectrophotometer Model PU 9712 (PerkinElmer, 940 Winter Street, Waltham, Masssachusetts 02451, USA) in KBr discs. Elemental analysis was performed at the Micro analytical Laboratory of the National Research Center. All the reactions were monitored by thin layer chromatography (TLC) on silica gel with chloroform as mobile phase.

Starting Materials:

5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carbonitrile (**1**) [31] and 5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carboxylic acid hydrazide (**6**) [31] were prepared by the appropriate reported procedure. All other chemicals used in this study were commercially available.

General procedure for the preparation of N-[2-(4-benzothiazol-2-yl-benzoyl)-4-cyano-2*H*-pyrazol-3-yl]-(un)substituted-benzamide 2a-c:

Compound **1** (1g, 0.016 mol) was dissolved in dry acetone (50 mL). Triethylamine (2.5 mL) was added to this solution. Then a solution of benzoyl chloride, 4-methylbenzoyl chloride and/or 4-nitrobenzoyl chloride (0.016 mol) in dry acetone was added and the mixture was stirred for 2h at room temperature.

The solid formed was filtered off and the solvent was removed from the clear solution under reduced pressure. The crude product was purified by crystallization from DMF.

N-[2-(4-Benzothiazol-2-yl-benzoyl)-4-cyano-2*H*-pyrazol-3-yl]-benzamide (2a):

Yield = 1.2g (92%); mp 210-214˚C; IR (cm^{-1}) 3138 (NH), 2217 (CN), 1691 (CO), 1670 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 7.30-8.26 (m, 13H, Ar-H), 7.61 (s, 1H, CH), 8.38 (s, 1H, NH, exchangeable with D_2O); MS *m/z* 449 (M^+, 57). Anal. Calcd for $C_{25}H_{15}N_5O_2S$ (449.5): C, 66.8; H, 3.4; N, 15.6; S, 7.1. Found: C, 66.8; H, 3.6; N, 15.8; S, 7.2.

N-[2-(4-Benzothiazol-2-yl-benzoyl)-4-cyano-2*H*-pyrazol-3-yl]-4-methyl-benzamide (2b):

Yield = 1.1g (82%); mp 192-194˚C; IR (cm^{-1}) 3138 (NH), 2217 (CN), 1691 (CO), 1670 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.35 (s, 3H, CH_3), 7.30-8.26 (m, 12H, Ar-H), 7.61 (s, 1H, CH), 8.38 (s, 1H, NH, exchangeable with D_2O); MS *m/z* 463 (M^+, 57). Anal. Calcd for $C_{26}H_{17}N_5O_2S$ (463.5): C, 67.4; H, 3.7; N, 15.1; S, 6.9. Found: C, 67.5; H, 3.9; N, 15.0; S, 6.9.

N-[2-(4-Benzothiazol-2-yl-benzoyl)-4-cyano-2*H*-pyrazol-3-yl]-4-nitro-benzamide (2c):

Yield = 1.3g (91%); mp 260-264˚C; IR (cm^{-1}) 3138 (NH), 2217 (CN), 1691 (CO), 1670 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 7.30-8.26 (m, 12H, Ar-H), 7.61 (s, 1H, CH), 8.38 (s, 1H, NH, exchangeable with D_2O); MS *m/z* 463 (M^+, 65). Anal. Calcd for $C_{25}H_{14}N_6O_4S$ (494.5): C, 60.7; H, 2.9; N, 17.0; S, 6.5. Found: C, 60.6; H, 2.8; N, 17.1; S, 6.5.

General procedure for the preparation of 1-(4-benzothiazol-2-yl-benzoyl)-5-[4-(un)substituted-benzoylamino]-1*H*-pyrazole-4-carboxylic acid 3a-c: A mixture of **2a-c** (2g, 0.01 mol) and 30 mL 70% sulfuric acid was stirred in 100 mL three-necked flask at 140ºC for 5h, then suspended in 150 mL water and the resulting precipitate was filtered off. The products were recrystallized from EtOH.

1-(4-Benzothiazol-2-yl-benzoyl)-5-benzoylamino-1*H*-pyrazole-4-carboxylic acid (3a):

Yield = 0.9g (87%); mp 242-244˚C; IR (cm^{-1}) 3200 (NH), 3138 (OH), 1691 (CO), 1670 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 7.30-8.26 (m, 13H, Ar-H), 7.61 (s, 1H, CH), 8.38 (s, 1H, NH, exchangeable with D_2O), 11.08 (s, 1H, OH, exchangeable with D_2O); MS *m/z* 468 (M^+, 72). Anal. Calcd for $C_{25}H_{16}N_4O_4S$ (468.5): C, 64.1; H, 3.4; N, 12.0; S, 6.8. Found: C, 64.1; H, 3.2; N, 11.8; S, 6.9.

1-(4-Benzothiazol-2-yl-benzoyl)-5-(4-methyl-benzoylamino)-1*H*-pyrazole-4-carboxylic acid (3b):

Yield = 0.85g (82%); mp 292-294˚C; IR (cm^{-1}) 3200 (NH), 3138 (OH), 1691 (CO), 1670 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.35 (s, 3H, CH_3), 7.30-8.26 (m, 12H, Ar-H), 7.61 (s, 1H, CH), 8.38 (s, 1H, NH, exchangeable with D_2O), 11.08 (s, 1H, OH, exchangeable with D_2O); MS *m/z* 482 (M^+, 57). Anal. Calcd for $C_{26}H_{18}N_4O_4S$ (482.5): C, 64.7; H, 3.8; N, 11.6; S, 6.7. Found: C, 64.8; H, 3.9; N, 11.5; S, 6.7.

1-(4-Benzothiazol-2-yl-benzoyl)-5-(4-nitro-benzoylamino)-1*H*-pyrazole-4-carboxylic acid (3c):

Yield = 0.8g (77%); mp 282-284˚C; IR (cm^{-1}) 3232 (NH), 3138 (OH), 1691 (CO), 1668 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 7.30-8.26 (m, 12H, Ar-H), 7.61 (s, 1H, CH), 8.38 (s, 1H, NH, exchangeable with D_2O), 11.00 (s, 1H, OH, exchangeable with D_2O); MS *m/z* 513 (M^+, 24). Anal. Calcd for $C_{25}H_{15}N_5O_6S$ (513.5): C, 58.5; H, 2.9; N, 13.6; S, 6.2. Found: C, 58.4; H, 3.0; N, 13.7; S, 6.2.

General procedure for the preparation of N-[2-(4-benzothiazol-2-yl-benzoyl)-4-cyano-2*H*-pyrazol-3-yl]-substituted-sulfonamide 4a-c:

Compound **1** (1g, 0.016 mol) was dissolved in dry acetone (50 mL). Triethylamine (2.5 mL) was added to this solution. Then a solution of benzene sulfonyl chloride, toluene sulfonyl chloride and/or camphor-10-sulfonyl chloride (0.016 mol) in dry acetone was added and the mixture was stirred for 8h at room temperature. The solid formed was filtered off and the solvent was removed from the clear solution under reduced pressure. Purification of the product was carried out by preparative thin-layer chromatography using ethyl acetate as eluent. The products were recrystallized from EtOH.

N-[2-(4-Benzothiazol-2-yl-benzoyl)-4-cyano-2*H*-pyrazol-3-yl]-benzene sulfonamide (4a):

Yield = 1.2g (86%); mp 130-134˚C; IR (cm^{-1}) 3347 (NH), 2222 (CN), 1640 (CO), 1375 (SO_2); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 4.38 (s, 1H, NH, exchangeable with D_2O), 7.30-8.36 (m, 13H, Ar-H), 7.61 (s, 1H, CH); MS *m/z* 485 (M^+, 37). Anal. Calcd for $C_{24}H_{15}N_5O_3S_2$ (485.5): C, 59.4; H, 3.1; N, 14.4; S, 13.2. Found: C, 59.3; H, 3.1; N, 14.3; S, 13.3.

N-[2-(4-Benzothiazol-2-yl-benzoyl)-4-cyano-2*H*-pyrazol-3-yl]-4-methyl-benzene sulfonamide (4b): Yield = 1.3g (90%); mp 105-108˚C; IR (cm^{-1}) 3347 (NH), 2219 (CN), 1640 (CO), 1375 (SO_2); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.35 (s, 3H, CH_3), 4.38 (s, 1H, NH, exchangeable with D_2O), 7.30-8.36 (m, 12H, Ar-H), 7.61 (s, 1H, CH); MS *m/z* 499 (M^+, 37). Anal. Calcd for $C_{25}H_{17}N_5O_3S_2$ (499.5): C, 60.1; H, 3.4; N, 14.0; S, 12.9. Found: C, 60.0; H, 3.3; N, 13.9; S, 13.0.

N-[2-(4-Benzothiazol-2-yl-benzoyl)-4-cyano-2*H*-pyrazol-3-yl]-C-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-yl)-methane sulfonamide (4c):

Yield = 1.5g (93%); mp 112-115˚C; IR (cm^{-1}) 3347 (NH), 2222 (CN), 1640 (CO), 1375 (SO_2); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.24 (s, 3H, H_h), 1.27 (s, 3H, H_g), 1.75-2.87 (m, 7H, $H_{c\text{-}f}$), 3.22 (d, J = 6.8 Hz, 1H, H_a); 3.31 (d, J = 6.8 Hz, 1H, H_b), 7.18-8.33 (m, 8H, Ar-H), 7.61 (s, 1H, CH), 10.41 (s, 1H, NH, exchangeable with D_2O) as shown in figure **1**; MS *m/z* 559 (M^+, 76). Anal. Calcd for $C_{28}H_{25}N_5O_4S_2$ (559.6): C, 60.1; H, 4.5; N, 12.5; S, 11.5. Found: C, 60.1; H, 4.4; N, 12.3; S, 11.6.

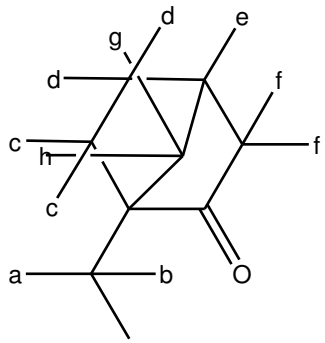


Figure 1

General procedure for the preparation of 1-(4-benzothiazol-2-yl-benzoyl)-5-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)/(2,5-dioxo-pyrrolidin-1-yl)/(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-1*H*-pyrazole-4-carbonitrile 5a-c:

To a stirred solution of compound **1** (1g; 0.017 mol) in glacial acetic acid (10 mL), acid anhydride (maleic anhydride, succinic anhydride and/or phthalic anhydride) (0.0348 mol) was added. The mixture was heated under reflux with stirring for 8h. The precipitate formed was filtered, washed with water and the crude product was crystallized from EtOH.

1-(4-Benzothiazol-2-yl-benzoyl)-5-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-1*H*-pyrazole-4-carbonitrile (5a):

Yield = 1.0g (81%); mp 102-105˚C; IR (cm^{-1}) 2217 (CN), 1719 (CO), 1692 (CO), 1686 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 6.18 (d, J = 6.9 Hz, 2H, 2CH=), 7.21-8.06 (m, 8H, Ar-H), 7.61 (s, 1H, CH); MS *m/z* 425 (M^+, 62). Anal. Calcd for $C_{22}H_{11}N_5O_3S$ (425.4): C, 62.1; H, 2.6; N, 16.5; S, 7.5. Found: C, 62.0; H, 2.5; N, 16.6; S, 7.5.

1-(4-Benzothiazol-2-yl-benzoyl)-5-(2,5-dioxo-pyrrolidin-1-yl)-1*H*-pyrazole-4-carbonitrile (5b):

Yield = 0.9g (73%); mp 122-125˚C; IR (cm^{-1}) 2219 (CN), 1719 (CO), 1692 (CO), 1686 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.34 (t, J = 7.1 Hz, 2H, CH_2), 2.41 (t, J = 7.1 Hz, 2H, CH_2), 7.47-8.23 (m, 8H, Ar-H), 7.61 (s, 1H, CH); MS *m/z* 427 (M^+, 32). Anal. Calcd for $C_{22}H_{13}N_5O_3S$ (427.4): C, 61.8; H, 3.1; N, 16.4; S, 7.5. Found: C, 61.9; H, 3.3; N, 16.3; S, 7.5.

1-(4-Benzothiazol-2-yl-benzoyl)-5-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-1*H*-pyrazole-4-carbonitrile (5c):

Yield = 1.1g (80%); mp 180-184˚C; IR (cm^{-1}) 2218 (CN), 1719 (CO), 1692 (CO), 1686 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 7.47-8.23 (m, 12H, Ar-H), 7.61 (s, 1H, CH); MS *m/z* 475 (M^+, 32). Anal. Calcd for $C_{26}H_{13}N_5O_3S$ (475.5): C, 65.7; H, 2.8; N, 14.7; S, 6.7. Found: C, 65.6; H, 2.9; N, 14.8; S, 6.7.

General procedure for the preparation of (un)substituted-benzoic acid N'-[5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carbonyl]-hydrazide 7a-c:

The foregoing method is the same as described for the preparation of benzamides **2a-c**, using compound **6** instead of **1**.

Benzoic acid N'-[5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carbonyl]-hydrazide (7a):

Yield = 1.1g (86%); mp 190-194˚C; IR (cm^{-1}) 3343 (NH), 3165 (NH_2), 1704 (CO), 1692 (CO), 1686 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 3.96 (s, 2H, NH_2, exchangeable with D_2O), 7.30-8.26 (m, 13H, Ar-H), 7.61 (s, 1H, CH), 11.38 (s, 2H, 2NH, exchangeable with D_2O);^{13}C-NMR (270 MHz, DMSO-d_6) δ 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), 164.8 (C=O), 165.2 (C=O) and 166.2 (C=O); MS *m/z* 482 (M^+, 57). Anal. Calcd for $C_{25}H_{18}N_6O_3S$ (482.5): C, 62.2; H, 3.8; N, 17.4; S, 6.7. Found: C, 62.3; H, 3.9; N, 17.3; S, 6.7.

4-Methyl-benzoic acid N'-[5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carbonyl]-hydrazide (7b):

Yield = 1.2g (92%); mp 172-174˚C; IR (cm^{-1}) 3303 (NH), 3165 (NH_2), 1702 (CO), 1692 (CO), 1686 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.35 (s, 3H, CH_3), 3.96 (s, 2H, NH_2, exchangeable with D_2O), 7.30-8.26 (m, 12H, Ar-H), 7.61 (s, 1H,

CH), 11.38 (s, 2H, 2NH, exchangeable with D_2O); MS *m/z* 496 (M^+, 32). Anal. Calcd for $C_{26}H_{20}N_6O_3S$ (496.5): C, 62.9; H, 4.1; N, 16.9; S, 6.5. Found: C, 62.9; H, 4.3; N, 16.9; S, 6.5.

4-Nitro-benzoic acid N'-[5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carbonyl]-hydrazide (7c):

Yield = 1.1g (79%); mp 225-228˚C; IR (cm^{-1}) 3300 (NH), 3165 (NH_2), 1704 (CO), 1692 (CO), 1686 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 3.96 (s, 2H, NH_2, exchangeable with D_2O), 7.30-8.26 (m, 12H, Ar-H), 7.61 (s, 1H, CH), 11.38 (s, 2H, 2NH, exchangeable with D_2O); MS *m/z* 496 (M^+, 32). Anal. Calcd for $C_{25}H_{17}N_7O_5S$ (527.5): C, 56.9; H, 3.3; N, 18.6; S, 6.1. Found: C, 56.9; H, 3.6; N, 18.8; S, 6.0.

General procedure for the preparation of N'-{[5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carbonyl]-hydrazide}-substituted-sulfonamide 8a-c:

The foregoing method is the same as described for the preparation of sulfonamides **4a-c**, using compound **6** instead of **1**.

N'-{[5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carbonyl]-hydrazide}-benzenesulfonamide (8a):

Yield = 1.2g (88%); mp 116-119˚C; IR (cm^{-1}) 3343 (NH), 3165 (NH_2), 1707 (CO), 1683 (CO), 1375 (SO_2); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.28 (s, 1H, NH, exchangeable with D_2O), 4.12 (s, 2H, NH_2, exchangeable with D_2O), 7.30-8.36 (m, 13H, Ar-H), 7.61 (s, 1H, CH), 8.12 (s, 1H, NH, exchangeable with D_2O); MS *m/z* 518 (M^+, 37). Anal. Calcd for $C_{24}H_{18}N_6O_4S_2$ (518.5): C, 55.6; H, 3.5; N, 16.2; S, 12.4. Found: C, 55.6; H, 3.4; N, 16.0; S, 12.5.

N'-{[5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carbonyl]-hydrazide}-4-methyl-benzenesulfonamide (8b):

Yield = 1.3g (92%); mp 95-98˚C; IR (cm^{-1}) 3343 (NH), 3165 (NH_2), 1707 (CO), 1683 (CO), 1375 (SO_2); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.28 (s, 1H, NH, exchangeable with D_2O), 2.45 (s, 3H, CH_3), 4.12 (s, 2H, NH_2, exchangeable with D_2O), 7.38-8.36 (m, 12H, Ar-H), 7.61 (s, 1H, CH), 8.12 (s, 1H, NH, exchangeable with D_2O); MS *m/z* 532 (M^+, 45). Anal. Calcd for $C_{25}H_{20}N_6O_4S_2$ (532.6): C, 56.4; H, 3.8; N, 15.8; S, 12.0. Found: C, 56.5; H, 3.9; N, 15.7; S, 12.0.

N'-{[5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carbonyl]-hydrazide}-C-(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hept-1-yl)-methane sulfonamide (8c):

Yield = 1.3g (83%); mp 82-85˚C; IR (cm^{-1}) 3343 (NH), 3165 (NH_2), 1707 (CO), 1683 (CO), 1375 (SO_2); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 1.24 (s, 3H, H_h), 1.27 (s, 3H, H_g), 1.75-2.87 (m, 7H, H_{c-f}), 3.22 (d, J = 6.9 Hz,1H, H_a), 3.31 (d, J = 6.9 Hz, 1H, H_b), 3.41 (s, 1H, NH, exchangeable with D_2O), 4.12 (s, 2H, NH_2, exchangeable with D_2O), 7.47-8.16 (m, 8H, Ar-

H), 7.61 (s, 1H, CH), 11.12 (s, 1H, NH, exchangeable with D_2O) as shown in figure **1**; MS *m/z* 592 (M^+, 31). Anal. Calcd for $C_{28}H_{28}N_6O_5S_2$ (592.7): C, 56.7; H, 4.8; N, 14.2; S, 10.9. Found: C, 56.8; H, 4.9; N, 14.1; S, 10.9.

General procedure for the preparation of 5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carboxylic acid-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)/(2,5-dioxo-pyrrolidin-1-yl)/(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-amide 9a-c:

The foregoing method is the same as described for the preparation of pyrrole derivatives **5a-c**, using compound **6** instead of **1**.

5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carboxylic acid-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-amide (9a):

Yield = 1.0g (83%); mp 112-115˚C; IR (cm^{-1}) 3343 (NH), 3165 (NH_2), 1717 (CO), 1692 (CO), 1683 (CO), 1669 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 3.96 (s, 2H, NH_2, exchangeable with D_2O), 6.84 (d, *J* = 6.9 Hz, 2H, 2CH=), 7.21-8.06 (m, 8H, Ar-H), 7.61 (s, 1H, CH), 8.08 (s, 1H, NH, exchangeable with D_2O); MS *m/z* 458 (M^+, 57). Anal. Calcd for $C_{22}H_{14}N_6O_4S$ (458.4): C, 57.6; H, 3.1; N, 18.3; S, 7.0. Found: C, 57.4; H, 3.0; N, 18.5; S, 7.0.

5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carboxylic acid-(2,5-dioxo-pyrrolidin-1-yl)-amide (9b):

Yield = 0.9g (74%); mp 144-147˚C; IR (cm^{-1}) 3343 (NH), 3165 (NH_2), 1717 (CO), 1692 (CO), 1683 (CO), 1669 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 2.34 (t, *J* = 7.1 Hz, 2H, CH_2), 2.41 (t, *J* = 7.1 Hz, 2H, CH_2), 3.96 (s, 2H, NH_2, exchangeable with D_2O), 7.47-8.23 (m, 8H, Ar-H), 7.61 (s, 1H, CH), 8.08 (s, 1H, NH, exchangeable with D_2O); MS *m/z* 460 (M^+, 57). Anal. Calcd for $C_{22}H_{16}N_6O_4S$ (460.5): C, 57.4; H, 3.5; N, 18.3; S, 7.0. Found: C, 57.3; H, 3.3; N, 18.4; S, 7.0.

5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carboxylic acid-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-amide (9c): Yield = 1.0g (75%); mp 195-199˚C; IR (cm^{-1}) 3343 (NH), 3165 (NH_2), 1717 (CO), 1692 (CO), 1683 (CO), 1669 (CO); ^{1}H-NMR (270 MHz, DMSO-d_6) δ 3.96 (s, 2H, NH_2, exchangeable with D_2O), 7.47-8.23 (m, 12H, Ar-H), 7.61 (s, 1H, CH), 8.08 (s, 1H, NH, exchangeable with D_2O); MS *m/z* 508 (M^+, 43). Anal. Calcd for $C_{26}H_{16}N_6O_4S$ (508.5): C, 61.4; H, 3.2; N, 16.5; S, 6.3. Found: C, 61.5; H, 3.2; N, 16.4; S, 6.3.

Bioactivity materials and methods

Cytotoxicity against human breast cancer cell line MCF-7: The antitumor activity against MCF-7 was performed 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 Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was provided from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), Tamoxifen, penicillin, streptomycin and Sulfo-Rhodamine-B stain (SRB) were obtained from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich chemical Co. (St. Louis, MO, USA).

The antitumor activity was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the previous reported standard procedure [32]. Cells were inoculated in 96-well microtiter plate (10^4 cells/well) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall 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 individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5 % CO_2. After 48 h 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 the optical density was determined at 492 nm. The relation between surviving fraction and drug concentration is plotted to get the survival curve after the specified time. The concentration required for 50 % inhibition of cell viability (IC_{50}) was calculated and the results are given in Table **1**.

Table-1: Effect of the synthesized compounds on the human breast cancer cell line MCF-7 and the VEGF level (pg/ml) in breast cancer cell line MCF-7.

Compounds	**IC_{50} (µmol/ml)**	**VEGF (pg/ml)**
1	0.13	4500.80±460.60 (14.27%)
2a	0.07	4600.75±440.35 (11%)
2b	0.07	4800.00±490.40 (8.57%)
2c	0.04	3280.32±350.00 (37.51%)
3a	NA	-
3b	NA	-

3c	0.09	4200.80±460.60 (24.27%)
4a	0.06	3980.80±400.00 (24.17%)
4b	0.06	4180.60±430.70 (20.37%)
4c	0.04	930.33±340.75 (82.57%)
5a	0.03	495.50±340.20 (89.32%)
5b	0.02	355.88±386.34 (92.46%)
5c	0.01	180.45±19.70 (96.56%)
6	0.02	322.98±35.50 (93.95%)
7a	0.05	3567.50±365.60 (32.80%)
7b	0.03	482.50±340.20 (90.32%)
7c	0.04	980.50±365.60 (81.80%)
8a	0.02	331.34±32.80 (93.90%)
8b	0.01	100.34±35.80 (99.45%)
8c	0.01	115.34±35.80 (97.90%)
9a	0.06	4180.60±430.70 (20.37%)
9b	0.05	3580.50±365.60 (31.80%)
9c	0.04	3395.50±340.20 (35.32%)
DMSO	-	5250.00
Tamoxifen	0.02	110.75 (98%)

Data were expressed as M of four independent experiments.

Values between brackets indicated percentage changes as compared with control cancer cells.

NA is no activity

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

The effect of tested compounds on the level of human vascular endothelial growth factor (VEGF) was determined utilizing human tumor cell lines including 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 x 10^6 were grown in a 25 cm^2 flask in 5 ml of complete culture medium.

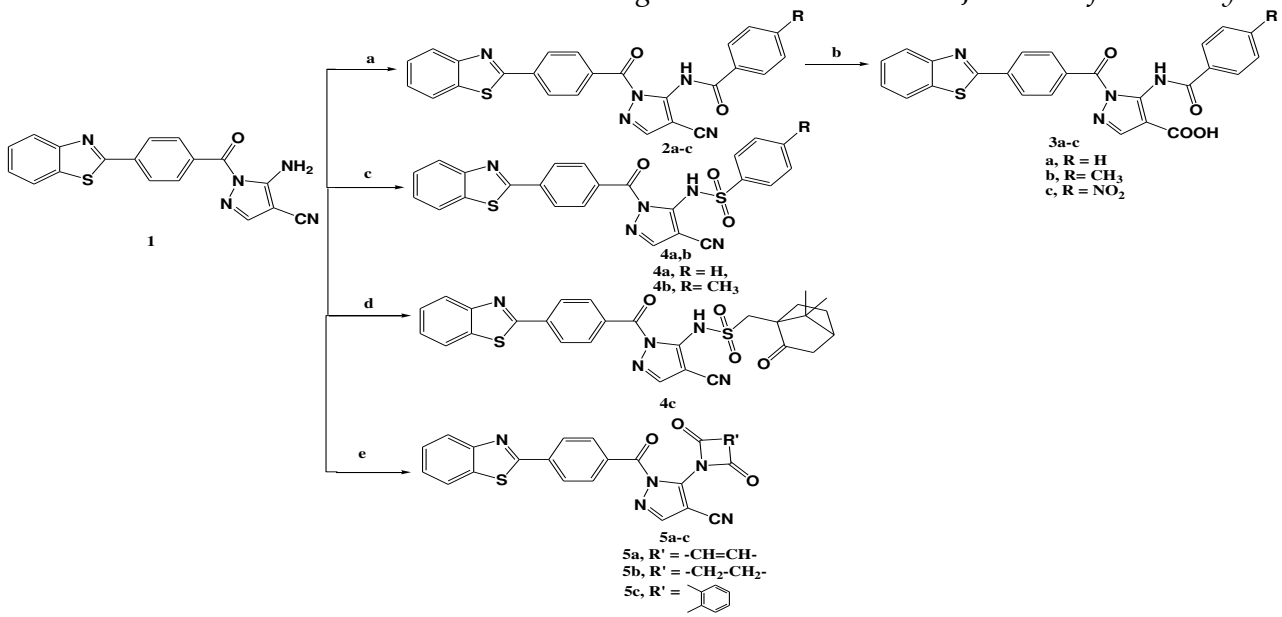
The cells in culture medium were treated with 20 µl of 1/10 of IC_{50} values of the compounds and the standard reference drug, tamoxifen dissolved in DMSO, then incubated for 24 h at 37 ºC, in a humidified 5 % CO_2 atmosphere. The cells were harvested and homogenates were prepared in saline using a tight pestle homogenizer until complete cell disruption.

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of human VEGF in samples. Which depend on the principle that, add VEGF to monoclonal antibody enzyme well which is pre-coated with human VEGF monoclonal antibody, incubation; then, add VEGF antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen solution A, B, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the human VEGF of sample were positively correlated and the optical density was determined at 450 nm. The level of human VEGF in samples was calculated (pg/ml) as triplicate determinations from the standard curve.

Results and discussion

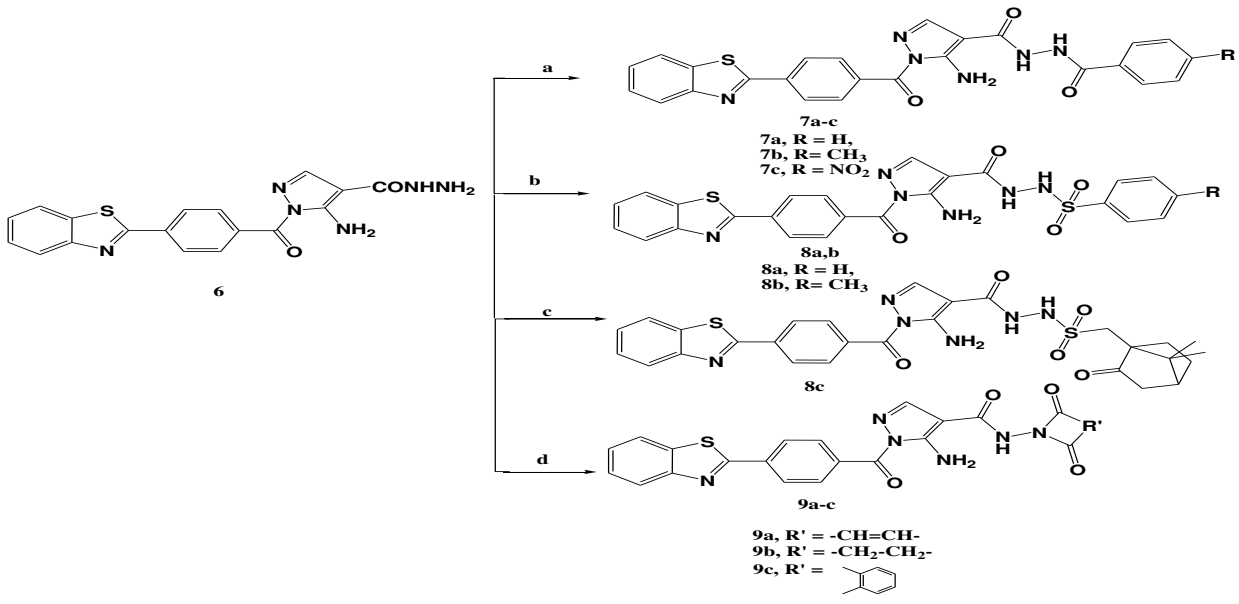
Chemistry

The discovery of benzothiazoles and continuous study of their biological activities [1] led us to construct compounds containing 5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole incorporated into different benzamide, sulfonamide and pyrrole analogues which might be of potential anticancer properties against experimental tumor cell lines. Thus the reaction of 5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carbonitrile [31] (**1**) dissolved in 10 ml dry benzene and few drops of triethylamine with benzoyl chloride, 4-methylbenzoyl chloride and/or 4-nitrobenzoyl chloride, gave the corresponding benzamides **2a-c**, stirring the benzamides **2a-c** with 70% sulfuric acid to give 1-(4-benzothiazol-2-yl-benzoyl)-5-(4-(un)substituted-benzoylamino)-1*H*-pyrazole-4-carboxylic acid derivatives **3a-c**. In addition, stirring compound **1** with sulfonyl chloride, toluene sulfonyl chloride and/or camphor-10-sulfonyl chloride in dry acetone and triethylamine, yielded the corresponding sulfonamides **4a-c** as illustrated in Scheme **1**. Products **5a-c** formed *via* stirring of compound **1** with the appropriate acid anhydride (maleic anhydride, succinic anhydride or phthalic anhydride) in glacial acetic acid.



Scheme-1:Reagents: (a) benzoyl chloride, 4-methylbenzoyl chloride or 4-nitrobenzoyl chloride, TEA, benzene; **(b)** 70% H_2SO_4; **(c)** sulfonyl chloride, toluene sulfonyl chloride, TEA, acetone; **(d)** camphor-10-sulfonyl chloride, TEA, acetone; **(e)** maleic anhydride, succinic anhydride or phthalic anhydride, glacial acetic acid.

Thus the reaction of 5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carboxylic acid hydrazide [31] (**6**) dissolved in 10 ml dry benzene and few drops of triethylamine with benzoyl chloride, 4-methylbenzoyl chloride and/or 4-nitrobenzoyl chloride, gave the corresponding benzoic acid hydrazide derivatives **7a-c**. In addition, stirring compound **6** with sulfonyl chloride, toluene sulfonyl chloride and/or camphor-10-sulfonyl chloride in dry acetone and triethylamine yielded the corresponding sulfonamides **8a-c** as illustrated in Scheme **2**. Products **9a-c** formed *via* stirring of compound **6** with the appropriate acid anhydride (maleic anhydride, succinic anhydride and/or phthalic anhydride) in glacial acetic acid.



Scheme-2: Reagents: (a) benzoyl chloride, 4-methylbenzoyl chloride or 4-nitrobenzoyl chloride, TEA, benzene; **(b)** sulfonyl chloride, toluene sulfonyl chloride, TEA, acetone; **(c)** camphor-10-sulfonyl chloride, TEA, acetone; **(d)** maleic anhydride, succinic anhydride or phthalic anhydride, glacial acetic acid.

Biological evaluation

Cytotoxicity against human breast cancer cell line MCF-7

Cytotoxicity of the synthesized compounds was tested using Skehan *et al* method [32] in human breast cancer cell line MCF-7. The cytotoxicity results were compared to that of standard drug, tamoxifen. Preparing sulfonamides **8a-c** results in increase in activity to be more potent and equipotent (0.01-0.02 µmol/mL) to tamoxifen (0.02 µmol/mL).The isoindole derivative **5c** is more potent (0.01 µmol/mL) than that of the reference drug, while the pyrrolidine derivative **5b** is equipotent. Oxidation of benzamides **2a-c** to prepare carboxylic acid derivatives **3a-c** leads to decrease of anticancer activity against MCF-7 cell line. The rest of the compounds were of moderate to weak activity against MCF-7 cell line as shown in (Table **1**).

The promising anticancer compounds did not show any toxicity on the 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 mass cannot grow bigger than 2 mm^3 without nourishment of blood vessels. Moreover, vascularization is required for the process of extravasation in metastasis [33]. Hence, establishment of chemotherapeutic strategy by blocking angiogenesis attracts much attention in recent years. Besides, alteration of the cellular adaptation to hypoxia is also fundamental in cancer treatment because angiogenesis or other metabolic modifications will be stimulated to maintain tumor cell survival [34]. Vascular endothelial growth factor (VEGF) has been identified as the most important angiogenic factor for tumor progression because it is released by a variety of tumor cells and over expresses in different human cancers. Drugs that can inhibit the production of VEGF or block its receptor signaling show significant inhibition of tumor growth [35, 36].

This biological *in vitro* study was done using double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of human VEGF in human breast cancer cell line MCF-7 samples as compared to the inhibition for the untreated cells. The screening results (Table **1**) showed that compound **8b** were found to be more potent than positive drug, tamoxifen (98 %) against human VEGF with percentage of inhibition values 99.45 % as compared with control

untreated cells, while compounds **5b**, **5c**, **8a** and **8c** are selectively similar to tamoxifen (92.46-97.90 %). These results were consistent with cell cytotoxicity activity against MCF-7 cell line where this compound exhibited excellent activity with IC_{50} ranging from 0.01-0.02 µmol/ml.

Conclusion

Our main goal throughout this study was the synthesis of new benzamide, sulfonamide and pyrrole derivatives attached to 4-(1,3-benzothiazol-2-yl)-benzoyl-1*H*-pyrazole moiety as anticancer agents working *via* inhibiting VEGF-VEGFR-2 complex formation, thus suppressing proliferation and survival of endothelial cells and consequently preventing cancer progression.

The bioactivity of the compounds **2a-9c** showed that five compounds (**5b**, **5c** and **8a-c**) have shown promising cytotoxic activity against breast cancer cell line MCF-7 with (IC_{50} = 0.01-0.02 µmol/mL) and potential inhibition of human VEGF in MCF-7 cancer cell line with percentages of inhibition (92.46-99.45%) in comparison to the positive drug, tamoxifen (IC_{50} = 0.02 µmol/mL, % inhibition = 98%) as compared with control untreated cells. The obtained data is valuable for the further synthesis of the compounds of similar structure and search for the more effective substances possessing antibreast cancer activities.

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Conflicts of interest:

The authors have declared no conflicts of interest.

References

1. R.D.R. Reis, E.C. Azevedo et al., Eur. J. Med. Chem., 2011, Vol 46, pp1448-1452.
2. I. Caleta, M. Kralj, M. Marjanovic, B. Bertosa, S. Tomic, G. Pavlovic, K. Pavelic, G.K. Zamola, J. Med. Chem., 2009, Vol 52(6), pp1744-1756.
3. G. Henriksen, A.I. Hauser, A.D. Westwell, B.H. Yousefi, M. Schwaiger, A. Drzezga, H.-J. Wester, J. Med. Chem., 2007, Vol 50, pp1087-1089.

4. C. Jimenez, Bioactive Nat. Prod., 2001, Vol 25(f), pp811-817.
5. G. Turan-Zitouni, S. Demirayak, A. Ozdemir, Z.A. Kaplancikli, M.T. Yildiz, Eur. J. Med. Chem., 2004, 39(3), pp267-272.
6. Y.J. Cao, J.C. Dreixler, J.J. Couey, K.M. Houamed, Eur. J. Pharmacol., 2002, Vol 449, pp47-54.
7. A D. Ramirez, S.K.F. Wong, F.S. Menniti, Eur. J. Pharmacol., 2003, Vol 475, pp29-35.
8. E. Kashiyama, I. Hutchinson, M.S. Chua, S.F. Stinson, L.R. Phillips, G. Kaur, E.A. Sausville, T.D. Bradshaw, A.D. Westwell, M.F.G. Stevenes, J. Med. Chem., 1999, Vol 42, pp4172-4184.
9. D.F. Shi, T.D. Bradshaw, S. Wrigley, C.J. McCall, P. Lelieveld, I. Fichtner, M.F.G. Stevens, J. Med. Chem., 1996, Vol 39, pp3375-3384.
10. I. Hutchinson, S.A. Jennings, B.R. Vishnuvajjala, A.D. Westwell, M.F.G. Stevenes, J. Med. Chem., 2002, Vol 45(3), pp744-747.
11. 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, British J. Cancer, 2003, Vol 88, pp470-477.
12. L.W. Wattenberg, J.L. Leong, Cancer Res., 1965, Vol 25, pp365-370.
13. D.F. Shi, T.D. Bradshaw, M.S. Chua, A.D. Westwell, M.F.G. Stevens, Bioorg. Med. Chem. Lett., 2001, Vol 11, pp1093-1095.
14. 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., 2008, Vol 43, pp1519-1524.
15. F. Abbate, A. Casini, T. Owa, A. Scozzafava, C.T. Supuran, Bioorg. Med. Chem. Lett., 2004, Vol 14, pp217-223.
16. M.S. Al-Said, M.M. Ghorab, S.I. Al-qasoumi, E.M. El-Hossary, E. Noaman, Eur. J. Med. Chem., 2010, Vol 45, pp3011-3018.
17. A. Casini, A. Scozzafava, A. Mastrolorenzo, C. T. Supuran, Curr. Cancer Drug Tar., 2002, Vol 2, pp55-75.
18. M.M. Ghorab, F.A. Ragab, M.M. Hamed, Eur. J. Med. Chem., 2009, Vol 44, pp4211-4217.
19. B.K. Banik, M. Cardona, Tetra. Lett., 2006, Vol 47(41), pp7385-7389.
20. M.A.A. Radwan, M. El-Sherbiny, Bioorg. Med. Chem., 2007, Vol 15, pp1206-1211.
21. L.L. Wang, J.J. Li, Z.B. Zheng, H.Y. Liu, G.J. Du, S. Li, Eur. J. Pharmacol., 2004, Vol 502(1-2), pp1-10.

22. P. Carmeliet, Oncology, 2005, Vol 69, pp4-10.

23. N. Ferrara, Oncology, 2005, Vol 69, pp11-16.

24. M.R. Raspollini, G. Amunni, A. Villanucci, G. Baroni et al., Int. J. Gynecol. Cancer, 2004, Vol 14, pp815-823.

25. K. Holmes, O.L. Roberts, A.M. Thomas, M.J. Cross, Cellular Signalling, 2007, Vol 19(10), pp2003-2012.

26. R.J. Roskoski, Biochem. Biophys. Res. Commun., 2008, Vol 375, pp287-291.

27. S. Patyna, A.D. Laird, D.B. Mendel et al., Mol. Cancer Ther., 2006, Vol 5, pp1774-1782.

28. D.E. Machado, M.S. Abrao, P.T. Berardo, C.M. Takiya, L.E. Nasciutti, Fertil Steril., 2008, Vol 90, pp148-155.

29. C.H. Kim, J.W. Ahn, S.H. Kim, H.D. Chae, B.M. Kang, J. Obstet. Gynaecol. Res., 2011, Vol 37, pp1631-1635.

30. D.E. Machado, P.T. Berardo, C.Y. Palmero, L.E. Nasciutti, J. Exp. Clin. Cancer Res., 2010, Vol 29, pp4-9.

31. A.A.M. Eman, M.A. Mamdouh, Res. J. Pharma. Bio. Chem. Sci., 2015, Vol 6, pp524-532.

32. P. Skehan, R. Storeng, D. Scudiero, A. Monks et al., J. Natl. Cancer Inst., 1990, Vol 82, pp1107-1112.

33. H.M. Verheul, E.E. Voest, R.O. Schlingemann, J. Pathol., 2004, Vol 202, pp5-13.

34. N. Ferrara, Nat. Rev. Cancer, 2002, Vol 2, pp795-803.

35. J. Folkman, Nat. Rev. Drug Discovery, 2007, Vol 6, pp273-286.

36. K.J. Gotink, H.M.W. Verheul, Angiogenesis, 2010, Vol 13, pp1-14.

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