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Anticancer Evaluation of Some Newly Synthesized Oxadiazol-2-Yl-Pyrazole Derivatives Attached To 4-Benzothiazol-2-Yl Moiety,

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ABSTRACT

Some 1,3,4-oxadiazol-2-yl-pyrazole derivatives were synthesized starting from 5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1H-pyrazole-4-carboxylic acid ethyl ester (**1**) by reacting with different electrophilic and nucleophilic reagents. All of the newly synthesized compounds have been evaluated for their potential cytotoxicity against breast cancer cell line (MCF7), compound **7c** is more potent than Tamoxifen also compounds **3** and **6b** are equipotent to Tamoxifen. These results were consistent with percentage of inhibition values against human VEGF compared with control untreated cells.

Keywords: 4-Benzothiazol-2-yl-phenyl, 1,3,4-oxadiazole, pyrazole, MCF7, VEGFR-2.

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INTRODUCTION

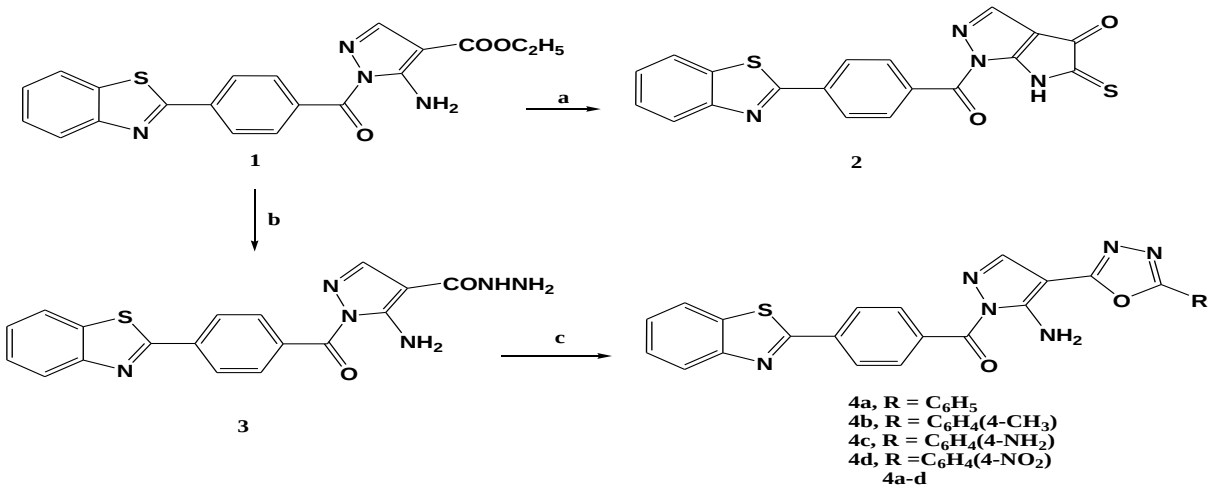
Compounds containing 1,3,4-oxadiazole nucleus are associated with diverse pharmacological activities which have made them important chemotherapeutic agents [1-4]. Many of these compounds have been reported to exhibit analgesic, diuretic, antihypertensive, anti-inflammatory [5], anticonvulsive [6], antibacterial and antifungal [7] properties. Also, pyrazole derivatives attract organic chemists very much due to their biological and chemotherapeutic importance. They are known to exhibit biological activities such as antitumor [8], antileukemic [9], anti-inflammatory [10], analgesic [11], anticoagulant [12] and antimicrobial [13]. The small and simple benzothiazole moiety possesses interesting biological and industrial activities [14]. Many of these compounds showed very intensive antitumor [15-19], antiviral [20], antibacterial [21], antioxidant [22], anticonvulsive [23] and antidepressant [24] activities. Among the most efficient compounds reported are riluzole [25], sulfathiazole, 2-mercapto benzothiazole and 4-fluoro-2-(4-amino-3-methylphenyl)benzothiazole which revealed neuroprotective, anticonvulsive, antiallergic and antibreast cancer activities [16], respectively.

Vascular endothelial growth factor (VEGF) is the most important signaling protein among the other growth factors that plays a vital role in stimulation of angiogenesis [26]. 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 (VEGFR1, VEGFR2 and VEGFR3). These receptors are belonging to receptor tyrosine kinases (RTK). VEGFR2 is considered as important receptor mediating of all the cellular responses to VEGF [27]. Binding of VEGF to its family of receptors (VEGFR), is the key mediator that promotes the proliferation and survival of endothelial cells and consequently cancer progression [28]. Therefore, looking for an effective anti-VEGF/VEGFR drug became the main interest for many research groups aiming to discover a new cancer therapy via angiogenesis inhibition.

Based on the above observations, we report here the synthesis and antibreast cancer activity of some substituted 1,3,4-oxadiazol-2-yl-pyrazole derivatives attached to 4-benzothiazol-2-yl starting from o-aminothiophenol.

Chemistry

5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carboxylic acid ethyl ester **1** was prepared according to the reported procedure [29]. Refluxing compound **1** with carbon disulphide in alkaline medium, yielded thioxo derivative **2**. Reacting compound **1** with hydrazine hydrate to form the corresponding hydrazide **3**. Reacting **3** with the appropriate aromatic acid (benzoic, 4-methyl benzoic, 4-amino benzoic or 4-nitro benzoic acid) in the presence of phosphoryl chloride to give the corresponding [5-amino-4-(5-substituted-phenyl-[1,3,4]-oxadiazol-2-yl)pyrazol-1-yl]-(4-benzothiazol-2-yl-phenyl)-methanone **4a-d** as illustrated in ***Scheme 1***.



Scheme 1: Reagents: (a) CS_2, KOH, EtOH; (b) $NH_2NH_2.H_2O$, EtOH; (c) benzoic, 4-methyl benzoic, 4-amino benzoic or 4-nitro benzoic acid, $POCl_3$

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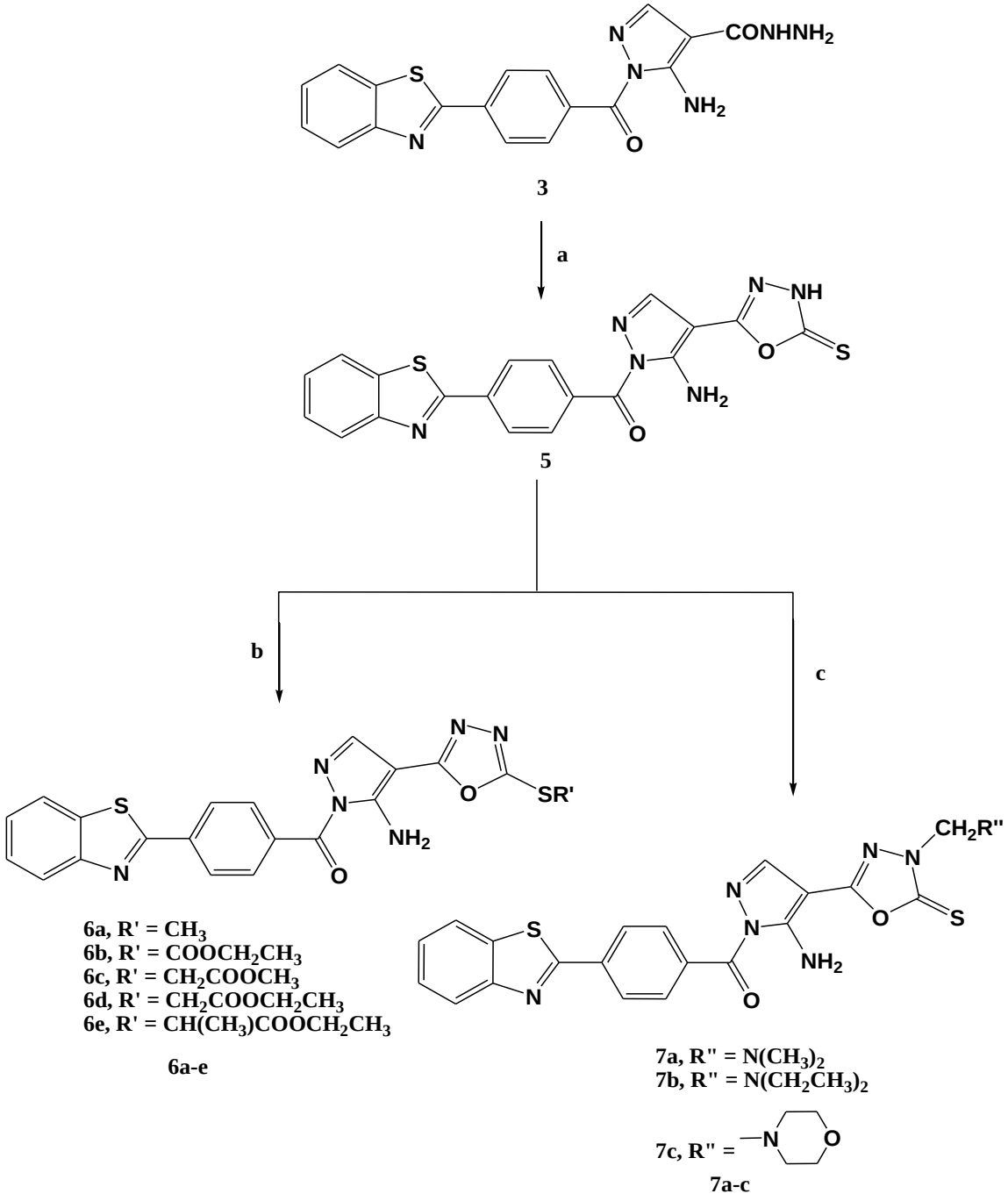
Refluxing hydrazide **3** with carbon disulphide in alkaline medium, gave the corresponding 5-thioxo-1,3,4-oxadiazole **5**. Condensing compound **5** with the required halide (methyliodide, ethylchloroformate, methyl-2-bromoacetate, ethyl-2-bromoacetate or ethyl-2-bromopropanoate) in the presence of sodium carbonate as acid halide abstract and acetone as solvent. The products **6a-e** was obtained mostly in pure state. Heating under reflux compound **5** with formaldehyde and dimethylamine, diethylamine or morpholine gave the Mannich bases **7a-c**, respectively (***Scheme 2***).



Scheme 2: Reagents: (a) CS_2, KOH, EtOH; (b) methyliodide, ethylchloroformate, methyl-2-bromoacetate, ethyl-2-bromoacetate or ethyl-2-bromopropanoate, Na_2CO_3, CH_3COCH_3; (c) dimethylamine, diethylamine or morpholine, formaldehyde, EtOH, HCl

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RESULTS AND DISCUSSION

Biological evaluation

Cytotoxicity against human breast cancer cell line MCF-7

Cytotoxicity of the synthesized compounds was tested using Skehan et al method [30] in human breast cancer cell line MCF-7. The Cytotoxicity results were compared to that of standard drug, Tamoxifen. The benzohydrazide **3** is equipotent to Tamoxifen cyclization to prepare compounds **4a**, **4b**, **4c** and **4d** exhibited no anticancer effect against MCF-7 cell line, but cyclization to form oxadioazole-2-thione **5** gave moderate activity. Reacting compound **5** with different alkyl halides to prepare compounds **6a-e** leads to increase in activity, **6b** are equipotent to Tamoxifen. While preparing mannich's bases, compound **7c** is more potent than Tamoxifen (IC_{50} = 0.01 µmol/ml) with IC_{50} = 0.02µmol/ml. The rest of the compounds were of moderate to weak activity against MCF-7 cell line as shown in (Table 1).

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

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 **7c** were found to be more potent than positive drug, Tamoxifen (98%) against human VEGF with percentage of inhibition values 99% as compared with control untreated cells, while compounds **3** and **6b** are selectively similar to Tamoxifen. These results were consistent with cell cytotoxicity activity against MCF-7 cell line where this compound exhibited excellent activity with IC_{50} ranging from 0.01-0.02µmol/ml.

CONCLUSION

Our main goal throughout this manuscript was the synthesis of new 1,3,4-oxadiazol-2-yl-pyrazole derivatives attached to 4-(benzothiazol-2-yl) moiety as antiangiogenic agents working via inhibiting VEGF-VEGFR2 complex formation, thus suppressing proliferation and survival of endothelial cells and consequently preventing cancer progression.

The bioactivity of the compounds **1-7c** showed that six compounds (**3**, **6a**, **6b**, **6c**, **6d** and **7c**) have shown promising cytotoxic activity against breast cancer cell line MCF-7 with (IC_{50} = 0.01-0.04 µmol/ml) and potential inhibition of human VEGF in MCF-7 cancer cell line with percentages of inhibition (81-99%) in comparison to the positive drug, Tamoxifen (IC_{50} = 0.02µmol/ml, % inhibition = 98%) as compared with control untreated cells.

EXPERIMENTAL

Chemistry

Melting points (˚C) were taken in open capillary tubes using silicon oil on Gallen Kamp apparatus. ^{1}H-NMR Spectra were measured in DMSO-d_6 on JEOL-270 MHz Spectrometer with TMS as an internal standard. Mass Spectra were obtained with a Schimadzu GCS-QP1000EX Spectrometer at 70 eV. The IR Spectra were recorded with a Philips Infra cord Spectrophotometer Model PU 9712 in KBr discs. Elemental analysis was performed at the Micro analytical Laboratory of the National Research Center. The antitumor activity of the synthesized compounds was carried out at the National Research Centre, Cairo, Egypt.

1-(4-Benzothiazol-2-yl-benzoyl)-5-thioxo-5,6-dihydro-1*H*-pyrrolo[2,3-c]pyrazol-4-one (2)

To a mixture consisting of the ester compound **1** (1g; 0.017 mol), potassium hydroxide aqueous solution (0.2g/20 ml) in ethanol, carbon disulphide (3.5 ml) was gently added and the mixture was heated under reflux till no odor of H_2S is detected (12 h). The reaction mixture was then poured on ice-water and rendered acidic with 2N hydrochloric acid. The precipitated solid was filtered, washed with water, dried and crystallized from DMF/ethanol. Yield = 0.9g (91%); m.p. = 282-6˚C. Analysis for $C_{19}H_{10}N_4O_2S_2$ (390.4): Calcd.: C, 58.5; H, 2.6; N, 14.4; S, 16.4; Fd.: C, 58.7; H, 2.8; N, 14.3; S, 16.3. IR (cm^{-1}): 3221 (NH), 1683 (CO), 1671 (CO).

MS: m/z (%): 390 (M^+, 60). ^{1}H-NMR: δ, ppm (DMSO-d_6); 4.05 (s, 1H, NH); 7.96-8.21 (m, 8H, Ar-H); 7.61 (s, 1H, CH).

5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazole-4-carboxylic acid hydrazide (3)

To a solution of ester compound **1** (1g; 0.033 mol) in ethanol, hydrazine hydrate (98%; 2 ml) was added and heated for 5h on a water-bath. The reaction mixture was cooled. The crude product was filtered, washed with water and dried. It was crystallized from ethanol. Yield = 0.8g (83%), m.p. = 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), 1680 (CO), 1668 (CO). MS: m/z (%): 378 (M^+, 32). ^{1}H-NMR: δ, ppm (DMSO-d_6); 3.96 (s, 2H, NH_2); 4.94 (s, 2H, NH_2); 7.50-8.24 (m, 8H, Ar-H); 7.61 (s, 1H, CH); 10.73 (s, 1H, NH).

General procedure for the preparation of [5-amino-4-(5-substituted-phenyl-[1,3,4] oxadiazol-2-yl)-pyrazol-1-yl]-(4-benzothiazol-2-yl-phenyl)-methanone (4a-d)

A mixture of hydrazide **3** (1g, 0.01 mol), appropriate aromatic acid (benzoic, 4- methyl benzoic, 4-amino benzoic or 4-nitro benzoic acid) (0.02 mol) and phosphoryl chloride (10 ml) was heated under reflux on a water-bath for 6-8h. After cooling to room temperature, it was poured onto crushed ice with stirring. The solid thus obtained was filtered, washed with water and crystallized from ethanol.

[5-Amino-4-(5-phenyl-[1,3,4]oxadiazol-2-yl)-pyrazol-1-yl]-(4-benzothiazol-2-yl-phenyl)-methanone (4a)

Yield = 1.1g (90%), m.p. = 210-4˚C. Analysis for $C_{25}H_{16}N_6O_2S$ (464.5): Calcd.: C, 64.6; H, 3.5; N, 18.1; S, 6.9; Fd.: C, 64.7; H, 3.4; N, 18.3; S, 6.8. IR (cm^{-1}): 3110 (NH_2), 1692 (CO). MS: m/z (%): 464 (M^+, 32). ^{1}H-NMR: δ, ppm (DMSO-d_6); 4.94 (s, 2H, NH_2); 7.50-8.24 (m, 13H, Ar-H); 7.61 (s, 1H, CH).

[5-Amino-4-(5-p-tolyl-[1,3,4]oxadiazol-2-yl)-pyrazol-1-yl]-(4-benzothiazol-2-yl-phenyl)-methanone (4b)

Yield = 1.1g (79%), m.p. = 200-4˚C. Analysis for $C_{26}H_{18}N_6O_2S$ (478.5): Calcd.: C, 65.3; H, 3.8; N, 17.6; S, 6.7; Fd.: C, 65.5; H, 3.9; N, 17.5; S, 6.7. IR (cm^{-1}): 3210 (NH_2), 1692 (CO). MS: m/z (%): 478 (M^+, 12). ^{1}H-NMR: δ, ppm (DMSO-d_6); 2.24 (s, 3H, CH_3); 4.14 (s, 2H, NH_2); 7.50-8.24 (m, 12H, Ar-H); 7.61 (s, 1H, CH).

{5-Amino-4-[5-(4-amino-phenyl)-[1,3,4]oxadiazol-2-yl]-pyrazol-1-yl}-(4-benzothiazol-2-yl-phenyl)-methanone (4c)

Yield = 1.2g (95%), m.p. = 262-6˚C. Analysis for $C_{25}H_{17}N_7O_2S$ (479.5): Calcd.: C, 62.6; H, 3.6; N, 20.5; S, 6.7; Fd.: C, 62.6; H, 3.8; N, 20.4; S, 6.8. IR (cm^{-1}): 3428 (NH_2), 3110 (NH_2), 1692 (CO). MS: m/z (%): 479 (M^+, 32). ^{1}H-NMR: δ, ppm (DMSO-d_6); 4.03 (s, 2H, NH_2); 4.94 (s, 2H, NH_2); 7.50-8.24 (m, 12H, Ar-H); 7.61 (s, 1H, CH).

{5-Amino-4-[5-(4-nitro-phenyl)-[1,3,4]oxadiazol-2-yl]-pyrazol-1-yl}-(4-benzothiazol-2-yl-phenyl)-methanone (4d)

Yield = 1.2g (89%), m.p. = 197-9˚C. Analysis for $C_{25}H_{15}N_7O_4S$ (509.5): Calcd.: C, 58.9; H, 3.0; N, 19.2; S, 6.3; Fd.: C, 58.9; H, 3.2; N, 19.1; S, 6.4. IR (cm^{-1}): 3110 (NH_2), 1692 (CO). MS: m/z (%): 509 (M^+, 32). ^{1}H-NMR: δ, ppm (DMSO-d_6); 4.14 (s, 2H, NH_2); 7.50-8.24 (m, 12H, Ar-H); 7.61 (s, 1H, CH).

[5-Amino-4-(5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-yl)-pyrazol-1-yl]-(4-benzothiazol-2-yl-phenyl)-methanone (5)

To a mixture consisting of the hydrazide compound **3** (1g; 0.017 mol), potassium hydroxide aqueous solution (0.2g/20 ml) in ethanol, carbon disulphide (3.5 ml) was gently added and the mixture was heated under reflux till no odor of H_2S is detected (12 h). The reaction mixture was then poured on ice-water and rendered acidic with 2N hydrochloric acid. The precipitated solid was filtered, washed with water, dried and crystallized from DMF/ethanol. Yield = 1g (91%); m.p. = 207-9˚C. Analysis for $C_{19}H_{12}N_6O_2S_2$ (420.5): Calcd.: C, 54.3; H, 2.9; N, 20.0; S, 15.3; Fd.: C, 54.5; H, 2.9; N, 20.1; S, 15.1. IR (cm^{-1}): 3437 (NH), 3110 (NH_2), 1684 (CO). MS: m/z (%): 420 (M^+, 26). ^{1}H-NMR: δ, ppm (DMSO-d_6); 4.14 (s, 2H, NH_2); 7.14 (s, 1H, NH); 7.50-8.24 (m, 8H,

Ar-H); 7.61 (s, 1H, CH). ^{13}C-NMR δ, ppm (DMSO-d_6): 122.4-130.7 (Ar-8CH), 133.2 (C=N), 134.7 (C=C), 137.0 (C-S), 152.1 (C-NH_2), 153.9 (C=N), 154.3 (C-N), 157.9 (C=S), 165.7 (C=C) and 165.7 (C=N).

General procedure for the preparation of [5-amino-4-(5-substituted-thio -[1,3,4]oxadiazol-2-yl)-pyrazol-1-yl]-(4-benzothiazol-2-yl-phenyl)-methanone (6a-e)

A mixture of the compound **5** (1g; 0.006 mol), the required halide (methyliodide, ethylchloroformate, methyl-2-bromoacetate, ethyl-2-bromoacetate or ethyl-2-bromo propanoate) (0.006 mol), anhydrous sodium carbonate (4g.) and acetone (30 ml) was heated under reflux for 8h. Most of the alcohol was distilled off, the residue was diluted with water and the obtained product was collected.

[5-Amino-4-(5-methylthio-[1,3,4]oxadiazol-2-yl)-pyrazol-1-yl]-(4-benzothiazol-2-yl-phenyl)-methanone (6a)

Yield = 0.8g (78%), m.p. = 172-5˚C. Analysis for $C_{20}H_{14}N_6O_2S_2$ (434.5): Calcd.: C, 55.0; H, 3.3; N, 19.3; S, 14.7; Fd.: C, 53.2; H, 3.9; N, 19.3; S, 14.8. IR (cm^{-1}): 3227 (NH_2), 1684 (CO). MS: m/z (%): 434 (M^+, 26). ^{1}H-NMR: δ, ppm (DMSO-d_6); 2.24 (s, 3H, CH_3); 4.14 (s, 2H, NH_2); 7.50-8.24 (m, 8H, Ar-H); 7.61 (s, 1H, CH).

Thiocarbonic acid S-{5-[5-amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*- pyrazol-4-yl]-[1,3,4]oxadiazol-2-yl)-ester O-ethyl ester (6b)

Yield = 0.9g (77%), m.p. = 112-5˚C. Analysis for $C_{22}H_{16}N_6O_4S_2$ (492.5): Calcd.: C, 53.6; H, 3.3; N, 17.1; S, 13.0; Fd.: C, 53.6; H, 3.9; N, 16.8; S, 13.0. IR (cm^{-1}): 3110 (NH_2), 1684 (CO), 1672 (CO). MS: m/z (%): 492 (M^+, 26). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.24 (t, 3H, CH_3); 4.20 (q, 2H, CH_2); 4.94 (s, 2H, NH_2); 7.50-8.24 (m, 8H, Ar-H); 7.61 (s, 1H, CH).

{5-[5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazol-4-yl]-[1,3,4] oxadiazol-2-ylsulfanyl}-acetic acid methyl ester (6c)

Yield = 0.9g (77%), m.p. = 102-5˚C. Analysis for $C_{22}H_{16}N_6O_4S_2$ (492.5): Calcd.: C, 53.6; H, 3.3; N, 17.0; S, 13.0; Fd.: C, 53.6; H, 3.9; N, 16.8; S, 13.0. IR (cm^{-1}): 3110 (NH_2), 1684 (CO), 1672 (CO). MS: m/z (%): 492 (M^+, 29). ^{1}H-NMR: δ, ppm (DMSO-d_6); 3.24 (s, 2H, CH_2); 3.67 (s, 3H, CH_3); 4.04 (s, 2H, NH_2); 7.50-8.24 (m, 8H, Ar-H); 7.61 (s, 1H, CH).

{5-[5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazol-4-yl]-[1,3,4] oxadiazol-2-ylsulfanyl}-acetic acid ethyl ester (6d)

Yield = 1g (83%), m.p. = 92-5˚C. Analysis for $C_{23}H_{18}N_6O_4S_2$ (506.6): Calcd.: C, 54.5; H, 3.6; N, 16.6; S, 12.7; Fd.: C, 54.3; H, 3.8; N, 16.3; S, 12.7. IR (cm^{-1}): 3110 (NH_2), 1687 (CO), 1672 (CO). MS: m/z (%): 506 (M^+, 26). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.67 (t, 3H, CH_3); 3.24 (s, 2H, CH_2); 4.04 (s, 2H, NH_2); 4.24 (q, 2H, CH_2); 7.50-8.24 (m, 8H, Ar-H); 7.61 (s, 1H, CH).

2-{5-[5-Amino-1-(4-benzothiazol-2-yl-benzoyl)-1*H*-pyrazol-4-yl]-[1,3,4] oxadiazol-2-ylsulfanyl}-propionic acid ethyl ester (6e)

Yield = 0.9g (73%), m.p. = 85-9˚C. Analysis for $C_{24}H_{20}N_6O_4S_2$ (520.6): Calcd.: C, 55.4; H, 3.9; N, 16.1; S, 12.3; Fd.: C, 55.4; H, 4.5; N, 16.0; S, 12.3. IR (cm^{-1}): 3110 (NH_2), 1684 (CO), 1672 (CO). MS: m/z (%): 520 (M^+, 24). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.37 (t, 3H, CH_3); 1.49 (d, 3H, CH_3); 3.54 (q, 1H, CH); 4.04 (s, 2H, NH_2); 4.12 (q, 2H, CH_2); 7.50-8.24 (m, 8H, Ar-H); 7.61 (s, 1H, CH).

General procedure for the preparation of [5-amino-4-(4-substituted-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-yl)-pyrazol-1-yl]-(4-benzothiazol-2-yl-phenyl)-methanone (7a-c)

To a solution of compound **5** (2g; 0.006 mol) in absolute ethanol (60 ml), 40% formaldehyde solution (0.18 ml; 0.006 mol) was added and the reaction mixture was heated on a steam-bath till a clear solution was obtained. Dimethylamine, diethylamine or morpholine (0.006 mol) was added followed by few drops of hydrochloric acid. The mixture was heated for further 2h, and then left at room temperature overnight. The

solvent was removed by distillation and the residue was neutralized with sodium carbonate solution. The formed solid was collected and recrystallized from ethanol.

[5-Amino-4-(4-dimethylaminomethyl-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-yl)-pyrazol-1-yl]-(4-benzothiazol-2-yl-phenyl)-methanone (7a)

Yield = 0.9g (79%), m.p. = 180-4˚C. Analysis for $C_{22}H_{19}N_7O_2S_2$ (477.6): Calcd.: C, 55.3; H, 4.0; N, 20.5; S, 13.4; Fd.: C, 55.1; H, 4.0; N, 20.5; S, 13.6. IR (cm^{-1}): 3430 (NH_2), 1682 (CO). MS: m/z (%): 477 (M^+, 24). ^{1}H-NMR: δ, ppm (DMSO-d_6); 2.24 (s, 6H, 2C$\underline{H}_3$); 3.72 (s, 2H, C$\underline{H}_2$); 4.04 (s, 2H, N$\underline{H}_2$); 7.50-8.24 (m, 8H, Ar-H); 7.61 (s, 1H, C$\underline{H}$).

[5-Amino-4-(4-diethylaminomethyl-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-yl)-pyrazol-1-yl]-(4-benzothiazol-2-yl-phenyl)-methanone (7b)

Yield = 1g (83%), m.p. = 210-4˚C. Analysis for $C_{24}H_{23}N_7O_2S_2$ (505.6): Calcd.: C, 57.0; H, 4.6; N, 19.4; S, 12.7; Fd.: C, 56.8; H, 4.6; N, 19.6; S, 12.7. IR (cm^{-1}): 3430 (NH_2), 1678 (CO). MS: m/z (%): 505 (M^+, 24). ^{1}H-NMR: δ, ppm (DMSO-d_6); 1.24 (m, 6H, 2C$\underline{H}_3$); 2.40 (m, 4H, 2C$\underline{H}_2$); 3.72 (s, 2H, C$\underline{H}_2$); 4.04 (s, 2H, N$\underline{H}_2$); 7.50-8.24 (m, 8H, Ar-H); 7.61 (s, 1H, C$\underline{H}$).

[5-Amino-4-(4-morpholin-4-yl-methyl-5-thioxo-4,5-dihydro-[1,3,4]oxadiazol-2-yl)-pyrazol-1-yl]-(4-benzothiazol-2-yl-phenyl)-methanone (7c)

Yield = 1g (81%), m.p. = 160-4˚C. Analysis for $C_{24}H_{21}N_7O_3S_2$ (519.6): Calcd.: C, 55.5; H, 4.1; N, 18.9; S, 12.3; Fd.: C, 55.5; H, 4.1; N, 18.7; S, 12.4. IR (cm^{-1}): 3430 (NH_2), 1682 (CO). MS: m/z (%): 505 (M^+, 24). ^{1}H-NMR: δ, ppm (DMSO-d_6); 2.34 (m, 4H, 2C$\underline{H}_2$); 3.67 (m, 4H, 2C$\underline{H}_2$); 3.72 (s, 2H, C$\underline{H}_2$); 4.04 (s, 2H, N$\underline{H}_2$); 7.50-8.24 (m, 8H, Ar-H); 7.61 (s, 1H, C$\underline{H}$).

Bioactivity materials and methods

Cytotoxicity against human breast cancer cell line MCF-7

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) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) 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). Human Vascular Endothelial Growth Factor (VEGF) ELISA kit was purchase from Glory Science Co., Ltd (Del Rio, TX 78840, USA).

Anticancer activity screening for the tested compounds utilizing human breast MCF-7 cancer cell line 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 (100µg/ml) at 37 ^{o}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.

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

The antiproliferative activity was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the previous reported standard procedure [30]. 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. The relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line 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.

Compound	IC_{50} (µmol/ml)	VEGF (pg/ml)
1	0.13	5160.90±550.00 (1.70%)
2	0.08	4800.00±490.40 (8.57%)
3	0.02	122.98±35.50 (97.85%)
4a	NA	-
4b	NA	-
4c	NA	-
4d	NA	-
5	0.06	4180.60±430.70 (20.37%)
6a	0.04	590.90±50.00 (83.65%)
6b	0.02	130.90±50.00 (96.65%)
6c	0.04	670.60±430.70 (81.37%)
6d	0.03	530.90±50.00 (87.65%)
6e	0.07	4687.30±500.00 (11%)
7a	0.08	4987.30±500.00 (5%)
7b	0.05	4020.60±430.70 (27.37%)
7c	0.01	100.83±22.34 (99.18%)
DMSO	-	5250.00
Tamoxifen	0.02	110.75 (98%)

Data were expressed as M±SE of four independent experiments.
Values between brackets indicated percentage changes as compared with control cancer cells.
NA is no activity

After that, 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, 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 using a tight pestle homogenizer until complete cell disruption, the lysate was used in studying the effect of tested compounds on the level of human vascular endothelial growth factor (VEGF) using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit 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 TRK 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.

Statistical analysis

The results are reported as Mean ± Standard error (S.E.) for at least four times experiments. Statistical differences were analyzed according to one way ANOVA test followed by student's t test wherein the differences were considered to be significant at $p < 0.05$.

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