Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(2):318-327

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis, molecular docking studies and cytotoxic screening of certain novel thiazolidinone derivatives substituted with benzothiazole or benzoxazole

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ABSTRACT

*Substituted thiazolidinone linked to benzothiazoles and benzoxazoles **3a,b** or substituted 5-benzylidene-4-thiazolidinones **4a-h** were synthesized. The antitumor activity of the prepared compounds was evaluated against human breast MCF7 and liver HEPG2 cancer cell lines using Sulphorhodamine-B (SRB) assay method, doxorubicin was used as a reference standard. Most of the tested compounds showed potent antitumor activity especially the p-methoxy-5-benzylidine-4-thiazolidinone derivative of benzoxazole **4c** and benzothiazole **4d**, their IC_{50} against liver HEPG2 cancer cell line are 0.027 nM and 0.026 nM respectively. The IC_{50} of p-chloro-5-benzylidine-4-thiazolidinone linked to benzoxazole **4e** against breast MCF7 cancer cell line, is 19 nM but, p-nitro-5-benzylidine-4-thiazolidinone derivative of benzothiazole **4h** showed a broad spectrum antitumor activity against MCF7 and HEPG2 cell lines, its IC_{50} is 36 and 48 nM respectively. The most active compounds were docked against VEGFR-2 using Moe program and 1Y6A (pdb file) to investigate if these compounds had a similar binding mode to VEGFR-2 inhibitors.*

Keywords: Benzothiazoles, Benzoxazoles, 4-Thiazolidinones, MCF7, HEPG2

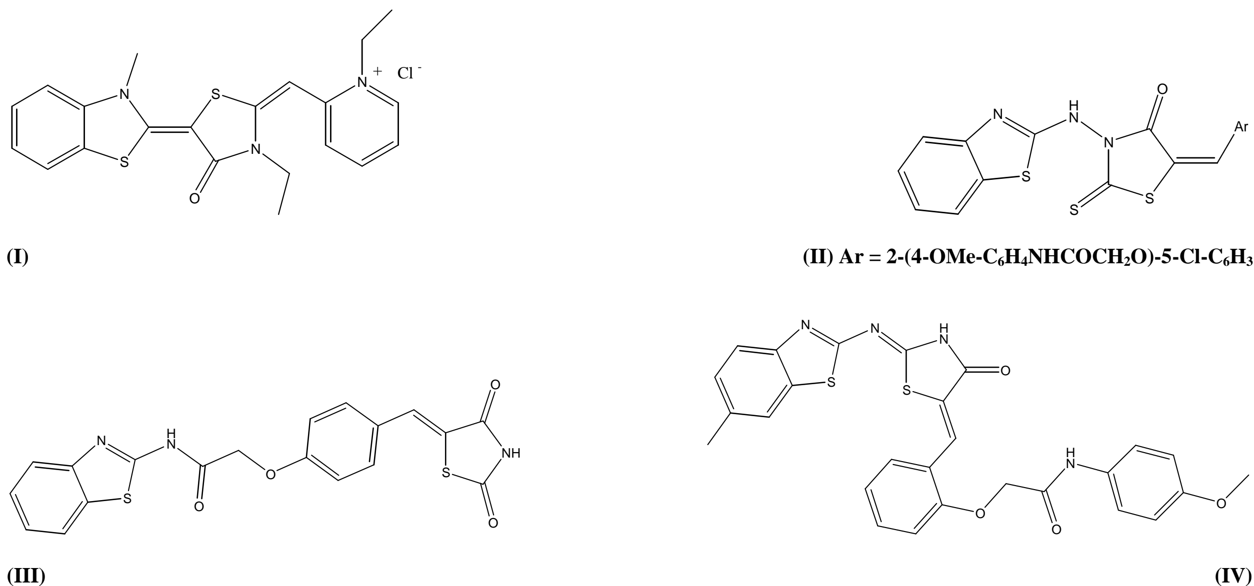
INTRODUCTION

Benzothiazoles [1,2], benzoxazoles [3,4] and thiazolidinones [5,6] are heterocyclic scaffolds in many synthetic compounds, so the chemistry of these derivatives became of increasing interest due to their various biological and pharmacological activities. Benzothiazoles displayed a wide range of biological activities such as antimicrobial [7], antimalarial [8] , antitubercular [9], antiviral [10] and antitumor activity [11,12], the potent inhibitory activity against MCF-7, Mt-1 and MT-3 of some hydroxy-substituted 2-(4-aminophenyl) benzothiazoles was reported [1,13,14]. It is suggested that benzothiazoles act as competitive inhibitors at the ATP binding site of kinases, solving the crystal structure of 5,6-dimethoxy-2-(4-methoxyphenyl)benzothiazole and comparing polyhydroxylated 2-phenylbenzothiazoles with adenine fragment of the ATP supported the suggestion that suitably substituted benzothiazole derivatives may substituted the bioactive natural compounds genistein and quercetin as competitive inhibitors for ATP binding sites at tyrosine kinases [15-17]. Many other benzothiazoles were proposed to act as B-cell lymphoma protein BCL-2 and Raf kinase (Raf-1) inhibitors [18,19]. On the other hand benzoxazole nucleus is a core structure in many synthetic compounds having different biological activities as antimicrobial [20], melatoninergic ligands [21], anti-inflammatory [22] and anticancer [23]. In addition the bis (benzoxazole) natural product UK-1 displayed a potent anticancer activity with IC_{50} value 20 nM against certain solid tumors, leukemia and lymphoma [24,25]. Vascular endothelial growth factor-2 receptor tyrosine kinase (VEGFR-2) inhibitory activity of benzoxazole was also reported by Michele H. Potashman et al [26]. Moreover, 4-thiazolidinones are considered as the privileged heterocyclic system in modern medicinal chemistry especially in the discovery of new anticancer agents due to their great affinity to many anticancer biotargets [27]. Attachment of benzothiazole moiety to thiazolidinone scaffolds **I-IV** (Fig. 1) was observed in the cationic rhodacyanine dye MKT-077**I** that showed selective toxicity to cancer cells [28].

Benzothiazole substituted 4-thiazolidinone **II** exhibited considerable antitumor activity against melanoma, leukemia, lung, colon, ovarian, CNS, renal, prostate and breast cancers [27].

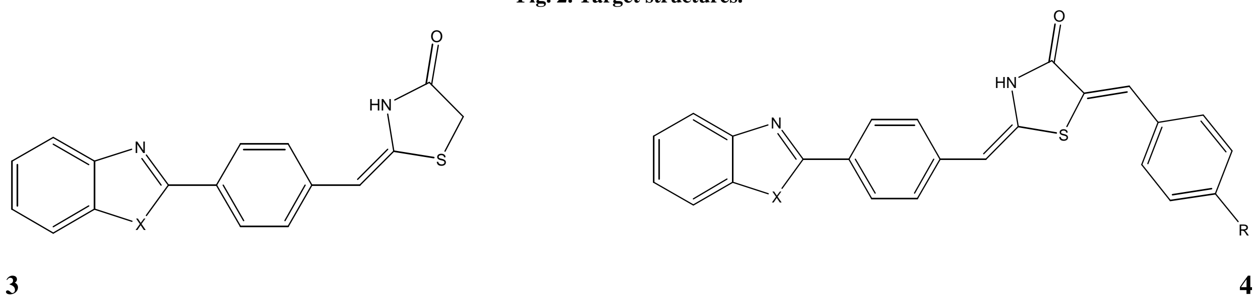
2,4-Thiazolidinedione with benzothiazole moiety **III** showed high activity in the in vitro screening against NSC lung cancer cell line (HOP62) [29]. The anticancer properties of 4-thiazolidinone bearing benzothiazole scaffold **IV** against colon HCT116 and breast MCF7 cancer cell lines was also reported [30].

Fig. 1. Structures of some previously reported thiazolidinones linked to benzothiazole in one molecule as potent antitumor.



All these findings directed us to synthesize a series of substituted benzothaizoles/benzoxazoles to be linked onto 4-thiazolidinone **3** (Fig. 2) or substituted 5-benzylidine-4-thiazolidinones **4** (Fig. 2) in one molecule aiming to obtain more pharmacologically active anticancer compounds.

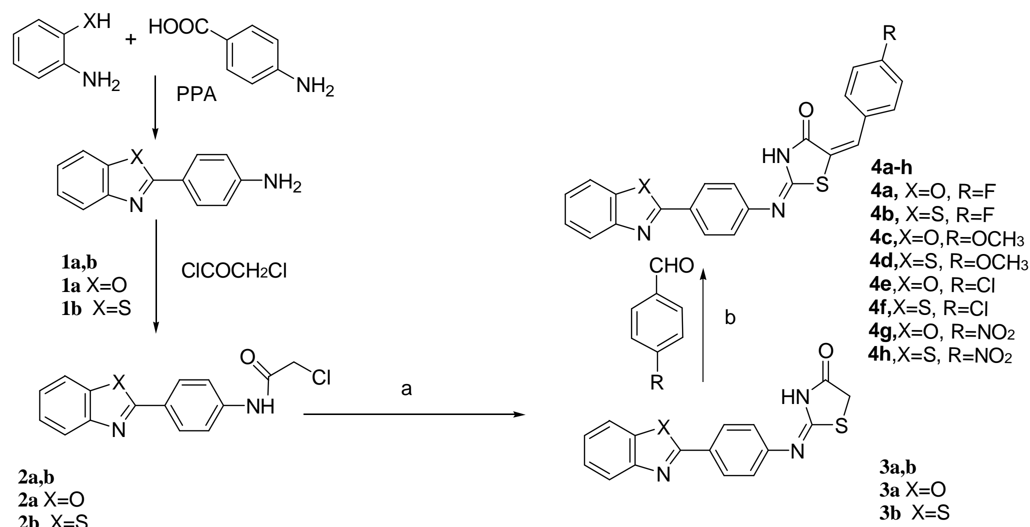
Fig. 2. Target structures.



All the synthesized compounds were screened against breast MCF7 and Liver HEPG2 cancer cell lines and the most active compounds were docked against VEGFR-2 to explore if these compounds have a similar binding mode to VEGFR-2 inhibitors.

Compounds 3,4 were prepared according the following scheme .

Scheme . Synthesis of compounds 3a,b and 4a-h.



Reagent and conditions a) NH_4SCN ,KCO_3, ethanol, reflux for 5h , b) appropriated aldehyde, sod. Acetate, glacial acetic acid, ethanol, reflux for 10 h

EXPERIMENTAL SECTION

2.1. Chemistry

Thin-layer chromatography (TLC) on silica gel sheets that precoated with UV fluorescent silica (MERCK 60 F 254) was used in monitoring the chemical reactions and spots were developed using I2vapour / UV light as visualizing agents. Solvent system was hexane: ethylacetate (in different ratio). Melting points (uncorrected) were measured with an Electrothermal Stuart SMP_3 digital melting point apparatus. IR spectra were recorded on a Shimadzu 435 Spectrometer, using KBr discs. ^{1}H NMR spectra were determined in DMSO-d_6 solvent with Varian Gemini 300 MHZ Spectrometer, peak positions were given in parts per million (δ) downfield the tetramethylsilane as internal standard. All reported products showed ^{1}H NMR spectra in agreement with the assigned structures. GC Mass spectra were recorded on Shimadzu QP-2010 spectrometer and Mass spectra were run on Hewlett Packard 5988 spectrometer at the Microanalytical Center, Cairo University, Egypt. Elemental analyses were performed at the Micro-analytical Center, Cairo University, Egypt. Compound **1a,b** and **2a,b** were prepared according to the reported procedures [31,32].

*2.1.1. General procedure for the preparation of **3a, b.***

A mixture of compound **2a** or **2b** (0.02 mol) , ammonium thiocyanate (0.04 mol) and K_2CO_3 (0.04 mol) was refluxed for 5 hours in absolute ethanol (30 ml). After the reaction was completed, the solvent was removed by distillation under vacuum, the obtained residue washed with water and recrystallized from ethanol/acetone mixture to afford compounds **3a,b**.

*2.1.1.1. 2-(4-Benzoxazol-4-yl-phenyl-imino)-thiazolidin-4-one **3a.***

Yellow crystals, 70% yield, mp 180-182°C. IR: $\upsilon_{max.}$/cm^{-1} 3348 (NH), 3035 (CH aromatic), 2910 (CH aliphatic), 1668 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.18 (s, 2H, CH_2), 7.41 (m, 2H, ArH protons), 7.39-7.52 (d, 2H, Ar H, J = 7.5 Hz) 7.76-7.81 (m, 2H, ArH protons),8.29(d, 2H, Ar H, J = 7.5 Hz) and 10.78 (1H, NH,D_2O exchangeable). MS (m/z): 309 ($M^{+\cdot}$, 9.89%). Anal. Calcd. for $C_{16}H_{11}N_3O_2S$ (309.34): C, 62.12; H, 3.58; N, 13.58. Found: C, 62.10; H, 3.30; N, 13.50.

*2.1.1.2. 2-(4-Benzothiazol-4-yl-phenyl-imino)-thiazolidin-4-one **3b.***

Greenish crystals, 60% yield, m.p. 190-192°C. IR: $\upsilon_{max.}$/cm^{-1} 3250 (NH), 3040 (CH aromatic) 2862 (CH aliphatic), 1669 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.15 (s, 2H, $\underline{CH_2}$), 7.45-7.50 (m, 4H, ArH), 8.05-8.19 (m, 4H, ArH protons), and 10.72 (1H, NH,D_2O exchangeable). MS (m/z): 325 ($M^{+\cdot}$, 12%). Anal. Calcd. for $C_{16}H_{11}N_3OS_2$ (325.03): C, 59.06; H, 3.41,; N, 12.91. Found: C, 59 ; H, 3.30; N, 12.80.

*2.1.2. General procedure for the preparation of **4a–h.***
A well stirred mixture of compound **3a**,**b** (0.01 mol), appropriated aldehyde (0.01 mol), sodium acetate (0.0mol) and (2-3drops) glacial acetic acid in absolute ethanol (50 ml), was heated under reflux for 10 hours. The reaction mixture was cooled, the obtained precipitate was filtered, washed with water, dried and re-crystallized from DMF.

*2.1.2.1. 2-(4-Benzoxazol-2-yl-phenylimino)-5-(4-fluoro-benzylidine)-thiazolidin-4-one **4a**.*
Yellow crystals, 70% yield, m.p. 210-212°C. IR: $\upsilon_{max.}$/cm^{-1} 3260 (NH), 3050 (CH aromatic), 1690 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.33 (s, H, , C=CH), 7.36-7.52 (m, 4H, Ar H,) 7.61-7.82 (m, 4H, ArH), 8.06-8.33 (m, 4H, Ar H) and 9.96 (1H, NH, D_2O exchangeable). MS (m/z): 415 (M$^{+\cdot}$, 62.68%). Anal. Calcd. for $C_{23}H_{14}FN_3O_2S$ (415.44): C, 66.49; H, 3.40,; N, 10.11. Found: C, 66.50; H, 3.50; N, 10.20.

*2.1.2.2. 2-(4-Benzothiazol-2-ylphenylimino)-5-(4-fluorobenzylidine)thiazolidin-4-one **4b**.*
Yellow crystals, 65% yield, m.p. 275-277°C. IR: $\upsilon_{max.}$/cm^{-1} 3379 (NH), 3060 (CH aromatic), 1680 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.32 (s, H, , C=CH), 7.35-7.55 (m, 4H, Ar H,) 7.56-7.08 (m, 4H, ArH protons), 8.10-8.21(m, 4H, Ar H) and 9.97 (1H, NH,D_2O exchangeable). MS (m/z): 431 (M$^{+\cdot}$, 50%) . Anal. Calcd. for $C_{23}H_{14}FN_3OS_2$ (431.06): C, 64,02; H, 3.27,; N, 9.74. Found: C, 64.10 ; H, 3.50; N, 9.80.

*2.1.2.3. 2-(4-Benzoxazol-2-ylphenylimino)-5-(4-methoxybenzylidine)thiazolidin-4-one **4c**.*
Yellow crystals, 65% yield, m.p. 282-282°C. IR: $\upsilon_{max.}$/cm^{-1} 3288 (NH), 3070 (CH aromatic), 2935(CH aliphatic), 1670 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.84(s,3H, OCH_3), 7.12-7.15 (m, 2H, , ArH), 7.41-7.87 (m, 6H, Ar H,) 8.21-8.34 (m, 3H, Ar H) and 9.88 (1H, NH,D_2O exchangeable). MS (m/z): 427 (M$^{+\cdot}$, 55%) . Anal. Calcd. for $C_{24}H_{17}N_3O_3S$ (427.48): C, 67.43; H, 4.01,; N, 9.83. Found: C, 67.40 ; H, 4.10; N, 9.90.

2.1.2.4. 2-(4-Benzothiazol-2-ylphenylimino)-5-(4-methoxybenzylidine)thiazolidin-4-one **(*4d*)**.
Yellowish green crystals, 75% yield, m.p. 260-262°C. IR: $\upsilon_{max.}$/cm^{-1} 3290 (NH), 3052 (CH aromatic), 2936 (CH aliphatic), 1667 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ : 3.84(s,3H, OCH_3), 7.12-7.16 (m, 2H, , C=CH and ArH), 7.47-7.70 (m, 6H, Ar H,),8.09-8.24 (m, 5H, ArH protons) and 9.87 (1H, NH,D_2O exchangeable) , MS (m/z): 443 (M$^{+\cdot}$, 36.56%) . Anal. Calcd. for $C_{24}H_{17}N_3O_2S_2$ (443.54): C, 64.99; H, 3.86,; N, 9.47. Found: C, 64.90; H, 4; N, 9.50.

*2.1.2.5. 2-(4-Benzoxazol-2-yl-phenylimino)-5-(4-chloro-benzylidine)-thiazolidin-4-one **4e.***
Yellowish green crystals, 70% yield, m.p. 180-182°C. IR: $\upsilon_{max.}$/cm^{-1} 3204 (NH), 3050 (CH aromatic), 1685 (C=O). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ7.14-8.19 (m, 13H, , C=CH and ArH), and 10.50 (1H, NH,D_2O exchangeable) , MS (m/z): 431 (M$^{+\cdot}$, 50%) . Anal. Calcd. for $C_{23}H_{14}ClN_3O_2S$ (431.89): C, 63.96; H, 3.27,; N, 9.73**.** Found: C, 64.10; H, 3.50; N, 9.80.

*2.1.2.6. 2-(4-Benzothiazol-2-yl-phenylimino)-5-(4-chloro-benzylidine)-thiazolidin-4-one **4f**.*
Yellowish green crystals, 65% yield, m.p. 270-272°C. IR: $\upsilon_{max.}$/cm^{-1} 3211 (NH), 3060 (CH aromatic), 1660 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.46-7.74 (m, 8H, , C=CH and ArH), 8.04-8.24 (m, 5H, ArH protons) and 10 (1H, NH,D_2O exchangeable) , MS (m/z): 447 (M$^{+\cdot}$, 48.68%) . Anal. Calcd. For $C_{23}H_{14}ClN_3OS_2$ (447.96): Calcd. C, 61.67; H, 3.15; N, 9.38**.** Found: 61.80 ; H, 3.30; N, 9.30.

*2.1.2.7. 2-(4-Benzoxazol-2-yl-phenylimino)-5-(4-nitro-benzylidine)-thiazolidin-4-one **4g**.*
Yellowish green crystals, 65% yield, m.p. 220-222°C. IR: $\upsilon_{max.}$/cm^{-1} 3401 (NH), 3071 (CH aromatic), 1670 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.33-7.96 (m, 8H, , C=CH and ArH), 8.18-8.38 (m, 5H, ArH protons) and 10.70 (1H, NH,D_2O exchangeable). MS (m/z): 442 (M$^{+\cdot}$, 14.27%) . Anal. Calcd. For $C_{23}H_{14}N_4O_4S$ (442.45): Calcd. C, 62,44; H, 3.19,; N, 12.66. Found: C, 62.60; H, 3.20; N, 12.50.

*2.1.2.8. 2-(4-Benzothiazol-2-yl-phenylimino)-5-(4-nitro-benzylidine)-thiazolidin-4-one **4h**.*
Yellowish green crystals, 65% yield, m.p. 180-182°C. IR: $\upsilon_{max.}$/cm^{-1} 3280 (NH), 3065 (CH aromatic), 1680 (C=O). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.40-8.38 (m, 13H, , C=CH and ArH), and 10.65 (1H, NH,D_2O exchangeable). MS (m/z): 458 (M$^{+\cdot}$, 50%). Anal. Calcd. For $C_{23}H_{14}N_4O_3S_2$ (458.51): C, 60.25; H, 3.08; N, 12.22**.** Found: C, 60.10; H, 3.10; N, 12.30.

2.2. Pharmacological studies.
*2.2.1. Cell Culture**.***
Human hepatocarcinoma cell lines (HEPG2) and breast carcinoma cell lines (MCF7) used in this study were obtained from the American type culture collection (ATCC, Minisota, U.S.A.). Dulbecco's modified eagle medium (DMEM), trypan blue, fetal bovine serum, penicillin/ streptomycin antibiotic and trypsin-EDTA were purchased from Sigma Aldrich Chemical Company (St. Louis, Mo, U.S.A.). Applichem, germany was the source of tris buffer.

2.2.2. Evaluation of cellular cytotoxicity

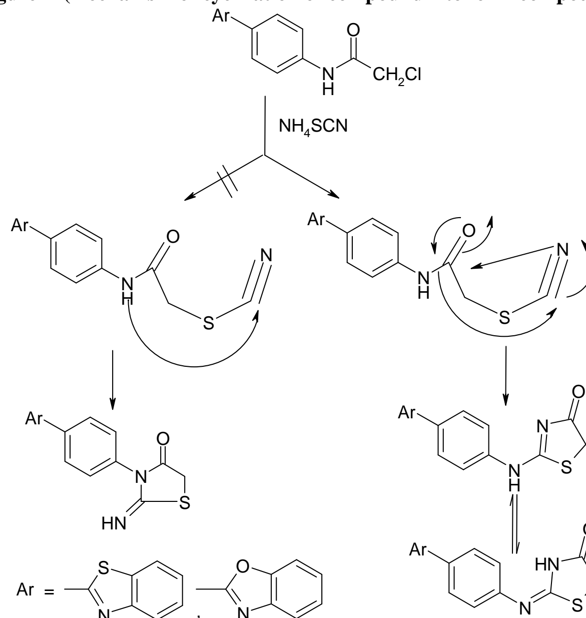
Tumor cells were seeded in 96 well microtiter plates at a concentration of 1000-2000 cells/well (100 µl/well), after 24 hours cells were incubated for 72 hours with various concentrations of the synthesized compounds (0, 0.01, 0.1, 1, 10 and 100µg/ml), 3 wells were used For each derivative concentration and doxorubicin as well, then the medium was discarded, the cells were fixed with 150 µl trichloroacetic acid 10% for 1 hour at 4 ^{0}C. The plates were washed with distilled water using (automatic washer Tecan, Germany) and stained with 50 µl 0.4 % SRB dissolved in 1 % acetic acid for 30 minutes at room temperature in dark. The plates were washed with 1 % acetic acid to remove unbound dye and air-dried (24 hours). The dye was solubilized with 150 µl/well of 10 mMtris base (PH 7.4) for 5 min on a shaker at 1600 rpm. The optical density (OD) of each well will be measured spectrophotometrically at 490 nm with an ELISA microplate reader. The mean background absorbance was automatically subtracted and mean values of each derivative and doxorubicin concentration was calculated. The experiment was repeated 3 times.

RESULTS AND DISCUSSION

3.1. Chemistry

The synthetic pathway for the preparation of substituted benzothiazole and benzoxazole linked to 4-thiazolidinone nucleus **3a,b** and substituted benzothiazole/benzoxazole linked to substituted 5-benzylidine-4-thiazolidinones **4a-h** is outlined in Scheme 1. Compounds **1a,b** and **2a,b** were synthesized according to the previously reported methods [31,32]. Reacting benzothiazole/benzoxazole substituted acyl chloride **2a,b** with ammonium thiocyanate using absolute ethanol as a solvent afforded benzothiazole/benzoxazole substituted with 4-thiazolidinone moiety **3a,b,** the expected mechanism for the reaction is shown in figure A [33].

Figure A (mechanism of cyclization of compound 2 to form compound 3)



^{1}H NMR spectrum of compounds **3a,b** revealed the appearance of a singlet signal each of two protons represent CH_2 of 4-thiazolidinone ring at 4.18 and 4.15 ppm respectively. The mass spectrum showed a molecular ion peak at 309 for compound **3a** and 325 for compound **3b**, structures **3a,b** were confirmed also by the IR spectrum and elemental analysis. Coupling of the active methylene group of the 4-thiazolidinone nucleus with different aldehydes to obtain the substituted 5-benzylidine derivatives **4a-h** was performed by reacting compounds **3a,b** with different p-substituted aldehydes in absolute ethanol as a solvent in presence of glacial acetic acid and sodium acetate. Structures of the obtained compounds **4a-h** were confirmed through different spectroscopic techniques as IR, ^{1}H NMR, Mass and elemental analysis, ^{1} ^{1}H NMR spectrum of compounds **4c,d** revealed the appearance of the methoxy group protons at 3.84 . Mass spectrum and elemental analysis also confirmed the target compounds **4a-h** as presented in the experimental part.

3.2. Antitumor activity

In vitro anticancer screening for the synthesized compounds and doxorubicin as a reference drug control against breast MCF7 and liver HEPG2 cancer cell lines was determined using sulphrodamine-B assay method. 6 concentrations from each compound were used (0-100µg/ml), plotting compound concentration versus the survival fraction was performed and the IC_{50} of the tested compounds was calculated (Table 1).

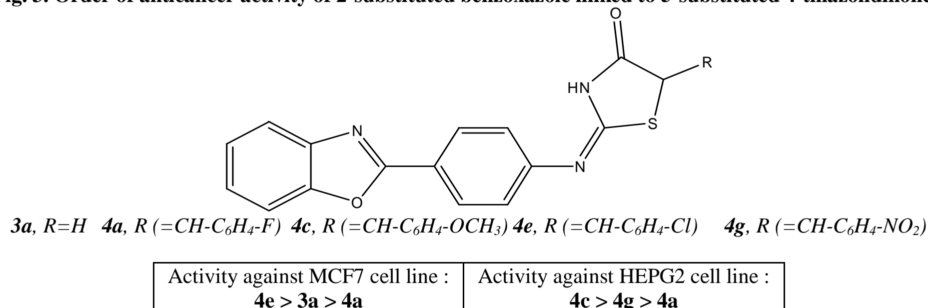
Compound no.	IC_{50} in nM[a] (MCF7)	IC_{50} in nM[a] (HEPG2)
3a	75	> 100
3b	79	83
4a	78	87
4b	> 100	79
4c	> 100	0.027
4d	> 100	0.026
4e	19	> 100
4f	> 100	> 100
4g	> 100	46
4h	36	48
Doxrubicin	29.66	1.036

[a] *The value given are means of three experiments.*

Table.1. IC_{50} of the synthesized compounds against human breast adenocarcinoma cell line (MCF7) and Human hepatocarcinoma cell lines (HEPG2).

IC_{50} is the concentration of the compound that can inhibit the survival of 50% of the incubated cells at the end of incubation period. Most of the tested compounds showed to be of potentially active as antitumor agents against MCF7 and HEPG2 cell lines, their IC_{50} are expressed in nanomole (Table 1) (IC_{50} of doxorubicin in MCF7 (29.66 nM) and HEPG2 (1.036nM). MCF7 human cancer cell line proved to be sensitive toward **3a,b, 4a, 4e** and **4h** compounds with IC_{50} range from 19-79 nM, Moreover, sensitivity against human liver cancer cell line HEPG2 is noticed by compounds **3b, 4a-d and 4g,h** with IC_{50} value ranging from 0.026-87 nM. As for the broad spectrum antitumor activity compounds **3b, 4a** and **4h** that showed activity against both the tested cell lines with IC_{50} range 36-87 nM. but, compound **4f** was found to be the least active one, its IC_{50} exceeds 100 nM against both cell lines. 4-Methoxy-5-benzylidine-4-thiazoidinone either linked to substituted benzoxazole **4c** or benzothiazole **4d** is found to be of high potency against human liver cancer cell line HEPG2 with IC_{50} value 0.027 nM and 0.026 nM respectively. Regarding the activity against human liver cancer cell line HEPG2 (Fig. 3,4), it is observed that substitution at position 5 of the 4-thiazolidinone nucleus with 4-substitutedbenzylidines increase the activity except the 4-chlorobenzylidine derivatives **4e,f**, which is found to be least active one. The activity against liver HEPG2 cancer cell line noticed by the p-substituted 5-benzylidine-4-thiazolidinones linked to substituted benzothiazole or benzoxazole system can be arranged in the following order (according to their IC_{50}), the 4-methoxy derivatives **4c,d** > 4-nitro derivatives **4g,h** > 4-fluoro derivatives **4a,b,** whoever, the 4-chloro substituted derivatives **4e,f** were the least active as their IC_{50} was over 100 nM (Table 1). With regard to the activity against breast MCF7 cancer cell line (Fig. 3,4), compound **4e** was the most active one but less potent against liver HEPG2 cancer cell line. Substituted benzothiazole-4-thiazolidinone **3b**, 4-fluoro-5-benzylidine-4-thiazolidinone linked to substituted benzoxazole **4a** and 4-nitro-5-benzylidine-4-thiazolidinone linked onto substituted benzothiazole **4h** showed potent activity against both cell lines and **4h** was the most active one with IC_{50} of 36 and 48 nM against breast MCF7 and liver HEPG2 cancer cell lines respectively.

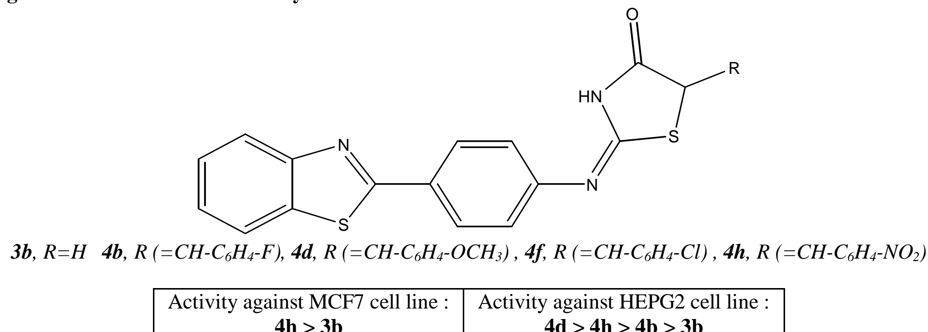
Fig. 3. Order of anticancer activity of 2-substituted benzoxazole linked to 5-substituted 4-thiazolidinones.



***3a**, R=H* ***4a**, R (=CH-C_6H_4-F)* ***4c**, R (=CH-C_6H_4-OCH_3)* ***4e**, R (=CH-C_6H_4-Cl)* ***4g**, R (=CH-C_6H_4-NO_2)*

Activity against MCF7 cell line : **4e > 3a > 4a**	Activity against HEPG2 cell line : **4c > 4g > 4a**

Fig. 4. Order of anticancer activity of 2-substituted benzothiazole linked to 5-substituted 4-thiazolidinones



***3b**, R=H* ***4b**, R (=CH-C_6H_4-F),* ***4d**, R (=CH-C_6H_4-OCH_3) ,* ***4f**, R (=CH-C_6H_4-Cl) ,* ***4h**, R (=CH-C_6H_4-NO_2)*

Activity against MCF7 cell line : **4h > 3b**	Activity against HEPG2 cell line : **4d > 4h > 4b > 3b**

2.3. Molecular docking studies

Vascular endothelial growth factor receptor VEGFR antagonists (inhibitors) play a crucial role in treating various types of cancers [34]. In 2009 Pazopanib was approved for treatment of renal cell carcinoma by FDA and in Sept 2012 Regorafenib was also approved for colorectal carcinoma treatment , so VEGFR-2 is selected to be the biological target for performing the docking studies for active compounds. The most potent compounds with selective toxicity against human liver HEPG2 cancer cell line **4c,d**, the most active compound against breast MCF7 cancer cell line **4e** and the broad spectrum compound that showed good inhibitory activity against both cell lines **4h** were docked against vascular endothelial growth factor receptor 2 (VEGFR-2) to investigate if these compounds have a similar mechanism as VEGFR-2 kinase inhibitors. Docking was performed using MOE 2008.10 software, the crystal structure of VEGFR-2 in complex with N-[5-(ETHYLSULFONYL)-2-METHOXYPHENYL]-5-[3-(2-PYRIDINYL) PHENYL]-1,3-OXAZOL-2-AMINE inhibitor is obtained from protein data bank (PDB ID: 1Y6A), refinement of the crude PDB structure was performed, then saved as moe file to be used for docking simulation. The 2D structure of the selected compounds was converted to their 3D form, and then energy minimized and saved as mol. The observed negative value for the docking score energy (Table 2) indicate the binding affinity of these compounds into VEGFR-2, this may give a reasonable explanation for their high activity.

Compound no.	E	Amino acid
4c	-19.06	Asn-921
4d	-19.28	Asn-921
4e	-19.12	-
4h	-13.37	Asn-921

Table 2. Docking score energy.

It is also noticed (Fig. 5-8) that the most active compounds **4c,d** against liver HEPG2 cell line and the broad spectrum compound **4h** formed a hydrogen bonding with Asn921 amino acid, however, compound **4e** that displayed selective cytotoxic activity against breast MCF7 cancer cell line formed no hydrogen bonding with the receptor (Fig. 7). These results may support the postulation that the active compounds may have analogues binding as the VEGFR-2 inhibitors.

Fig. 5. Ligand interaction of compound 4c with VFER-2, exhibiting interaction with Asn921 amino acid.

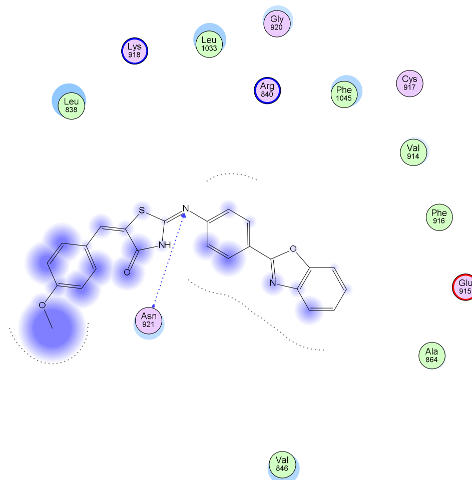


Fig. 6. Ligand interaction of compound 4d with VFER-2, exhibiting interaction with Asn921 amino acid.

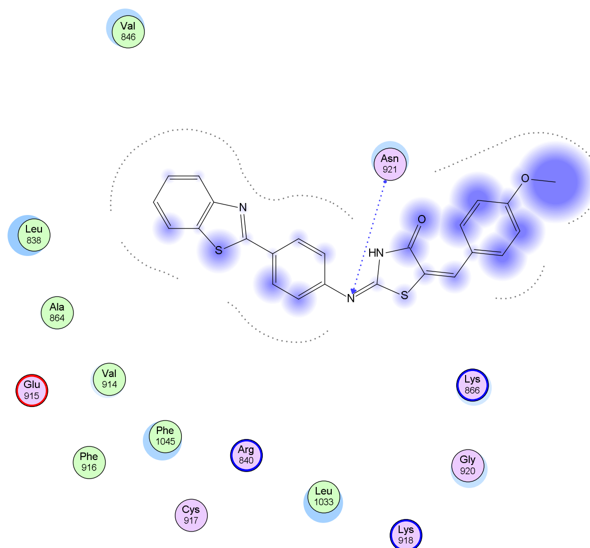


Fig. 7. Ligand interaction of compound 4e with VFER-2.

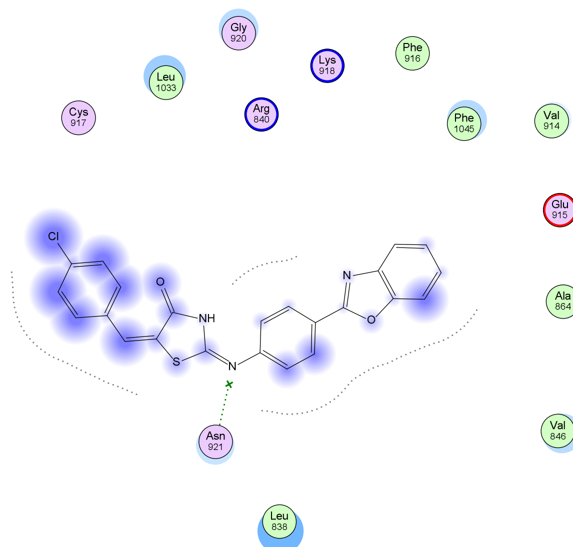
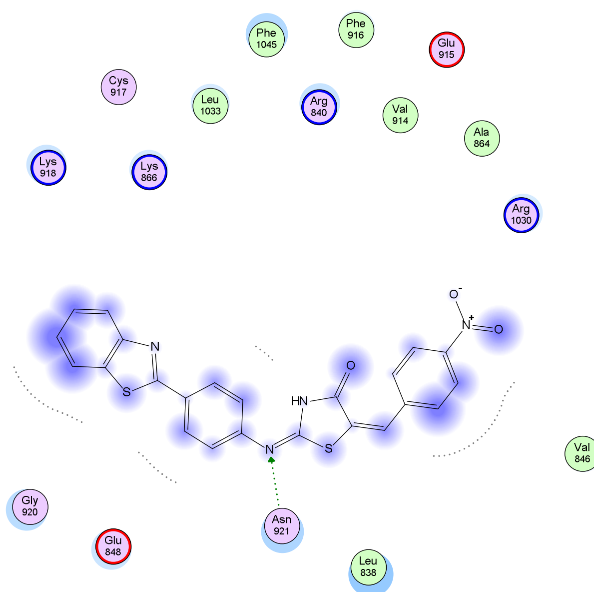


Fig. 8. Ligand interaction of compound 4h with VFER-2, exhibiting interaction with Asn921 amino acid.



CONCLUSION

Synthesis of the target compounds having the formula **3,4** (Fig. 2) were performed, their in vitro antitumor evaluation was carried out against MCF7 and HEPG2 cell lines, the most active compounds **4c,d, 4e, 4h** were docked against VEGFR-2 using 1Y6A PDB file**.**

1-linking 4-thiazolidinone nucleus that have no substitution at position 5 with substituted benzothiazole lead to obtaining a broad spectrum cytotoxic compound **3b** against the two tested cell lines, whoever, linking this nucleus to substituted benzoxazole afforded a selective cytoxic compound **3a** against breast cancer cell line MCF7 (Table 1, Fig. 3,4).
2- Substituted 5-benzylidene-4-thiazolidinone **4** is more active than unsubstituted **3**, whoever, type of substitution is a major limitation.
3- P-methoxy-5-benzylidine-4-thiazolidinone linked to substituted benzoxazole or benzothiazole in one molecule **4c,d** lead to obtaining a highly potent and selective toxicity against liver cancer cell line HEPG2 with IC_{50} value 0.027 and 0.026 nM respectively. Replacement of the methoxy group with nitro **4g,h** or fluoro **4a,b** decreased the activity, whoever, the chloro substituted derivatives **4e,f** are the least active against the same cell line (HEPG2).
4- The p-chloro-5-benzylidine-4-thiazolidinone linked to substituted benzoxazole **4e** showed selective and high activity against breast cancer cell line MCF7 the IC_{50} is 19 nM, on the other hand, attaching this moiety to benzothiazole **4f** diminished this selectivity.
5- Moreover, the p-nitro-5-benzylidine-4-thiazolidinone attached onto substituted benzothiazole give a broad spectrum anticancer agent **4h** against both cell lines**,** whoever linking this moiety to benzoxazole lead to selective toxicity against HEPG2 cell line.
6- Attachement of p-fluoro-5-benzylidine-4-thiazolidinone moiety onto substituted benzoxazole **4a** showed a broad spectrum cytotoxic activity against both cell lines with IC_{50} range 36-83 nM, whoever attaching it to benzothiazole give selective cytotoxic compound against HEPG2 cell line.

The docking studies against VEGFR-2 revealed that: All the active compounds showed a low docking score energy with the receptor. The selective cytotoxic compounds **4c,d** against HEPG2 cell line and the most active broad spectrum cytotoxic compound **4h** showed a hydrogen bonding with Asn921, in addition to their low docking score energy Table 2). On the other hand, the most selective cytotoxic compound **4e** against MCF7 cell line exhibited good binding affinity to VEGFR-2 but no hydrogen bonding formed with the receptor.

Acknowledgments
We are thankful to all Pharmaceutical Organic Chemistry department members, Faculty of Pharmacy , Beni Suef University for helping to complete this thesis.

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