Full Paper

Synthesis, Anti-Breast Cancer Activity, and Molecular Modeling of Some Benzothiazole and Benzoxazole Derivatives

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A new series of benzothiazoles and benzoxazoles was synthesized using 4-benzothiazol-2-yl-phenylamine and 4-benzoxazol-2-yl-phenylamine as starting materials. All the prepared compounds were evaluated for their antitumor activities against human breast cancer cell lines, MCF-7 and MDA-231, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability analysis. Almost all the tested compounds revealed potent antitumor activity, especially the *N*-methyl piperazinyl substituted derivatives **6f** and **6c**, which displayed the most potent inhibitory activity with IC_{50} values ranging from 8 to 17 nM. Docking the synthesized compounds into the epidermal growth factor receptor (EGFR), which is highly expressed in breast cancer, was employed to explore the possible interactions of these compounds with the EGFR. The activity of the reported compounds supports its clinical promise as a component of therapeutic strategies for cancer, for which high concentrations of chemotherapeutic agents are always a major limitation.

Keywords: Antitumor / Benzothiazoles / Benzoxazoles / MCF-7 / MDA-231 / Molecular modeling / Tyrosine kinase

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Introduction

Cancer is a complex, prevalent and fatal disease that notably affects almost every tissue in the human body. It is one of the leading causes of death worldwide. Lung, stomach, liver, colon, and breast cancers are the most common causes of cancer death every year. Breast cancer comprises 23% of all cancers (excluding non-melanoma skin cancers) in women, and it is considered as the main and the most frequent cause of cancer death among women all over the world. In 2008, breast cancer caused about half million deaths in women in USA. Awfully, the incidence and mortality rates of breast cancer in developing countries are much higher than those in developed countries with fewer chances for treatment [1–5]. Currently, a lot of anticancer drugs have been clinically used successfully for the treatment of several malignancies. However, solid tumors, such as breast cancer, resist most of the clinically-available anticancer agents probably due to gene mutations or epigenetic modifications during the course of therapy that affect the uptake, metabolism or efflux of drugs from cancer cells [6]. Since the response of solid tumors to available anticancer chemotherapeutic agents is limited, searching for novel easily accessible drugs with low cost and superior efficacy is desired.

Receptor protein tyrosine kinases (RPTKs) have a crucial role in the development and progression of many types of cancer. Over-activation of these receptors is usually accompanied by carcinogenesis [7]. Of the 30 RPTKs that are currently known to be implicated in human cancers, the epidermal growth factor receptor (EGFR) is frequently expressed at high levels in certain carcinomas, especially breast, colon, and bladder

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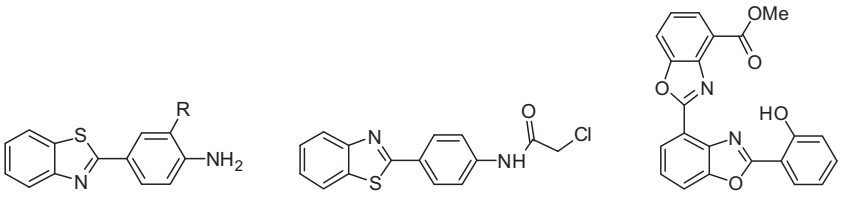


Figure 1. Structures of some previously reported benzothiazoles and benzoxazoles as potent antitumor agents.

cancers [8]. Thus, targeting EGFR represents a potential approach for the design of novel antiproliferative drugs [9].

Benzothiazoles and their related heterocycles benzoxazoles are considered unique and versatile scaffolds used for the design of several analogs of pharmacological interest [10]. A lot of benzothiazole derivatives exhibit a number of interesting biological activities including anticancer activity against several tumors [11–14]. Benzoxazole derivatives also show diverse pharmacological activities as analgesic [15], anti-inflammatory [16], antitubercular [17], anthelmentic [18], antifungal [19], antimalarial [20], antidiabetic [21], anticonvalsant [22], and antitumor [23]. Bradshaw et al. [24, 25] reported the synthesis of a series of 3′-substituted-2-(4′-aminophenyl)-benzothiazoles **I** (Fig. 1), that showed a unique profile of growth inhibition when tested against MCF-7 and MDA-468 cell lines. Moreover, the 2-(4-acylaminophenyl) benzothiazole **II** (Fig. 1) was found to be very active against the MCF-7 cell line [26]. The bis(benzoxazole) *Streptomyces*-derived natural product UK-1 **III** (Fig. 1) and some of its synthesized analogues displayed potent activity against a wide spectrum of human cancer cell lines [27]. Benzoxazole and benzothiazole scaffolds were designed and synthesized as Raf kinase inhibitors [28]. It was also reported that some polyhydroxylated phenyl benzothiazoles have ATP antagonistic effects and act as tyrosine kinase inhibitors by binding to the ATP binding site of protein kinases [29, 30]. This obvious role for benzothiazole derivatives and its related heterocyclic benzoxazoles in the treatment of human cancer inspired us to design some novel derivatives with similar scaffolds having the general formula **A** and **B** (Fig. 2) and aiming to have potentially active compounds against the solid tumors such as breast cancer. Docking studies of these compounds into the ATP binding site of EGFR are also presented to explore the structure-activity relationships between the inhibitors and EGFR.

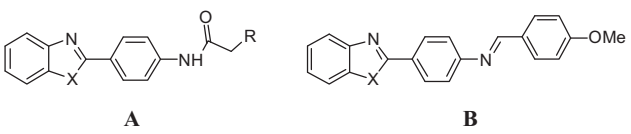
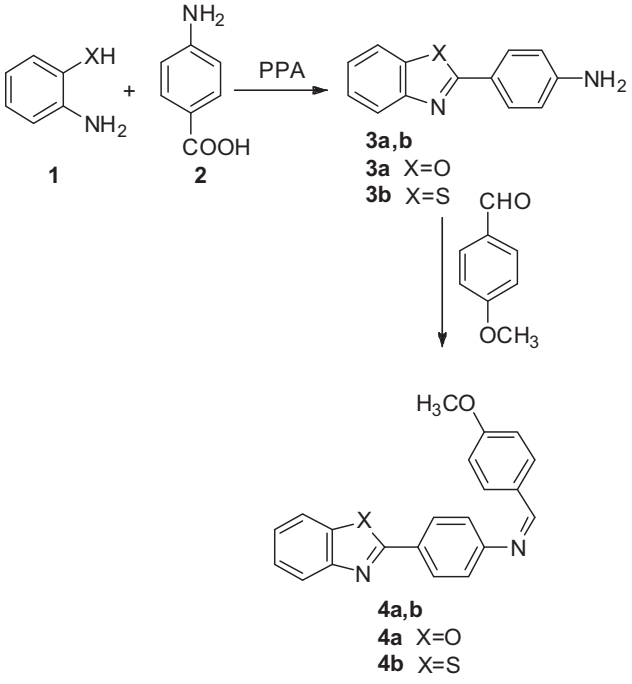


Figure 2. General formula of the synthesized benzothiazole and benzoxazole derivatives.

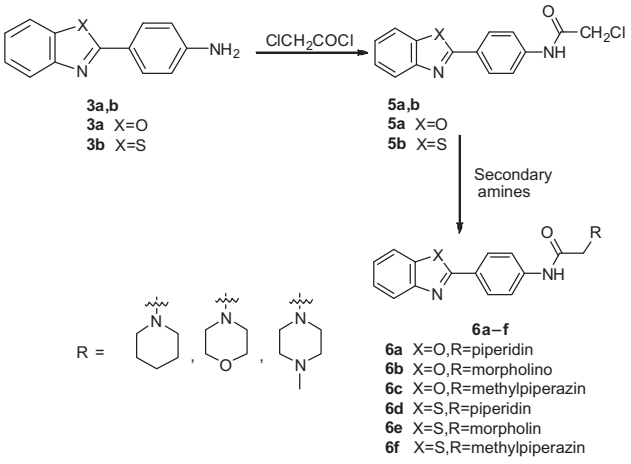
Results and discussion

Chemistry

The synthetic pathways adopted for the preparation of the new compounds are illustrated in Schemes 1 and 2. The starting materials **3a**,**b** and **5a**,**b** were synthesized according to the previously reported methods [26, 31–35]. Compounds **3a**,**b** were then subjected to the reaction with 4-methoxybenzaldehyde in absolute ethanol to afford compounds **4a**,**b** (Scheme 1). The structures of **4a**,**b** were confirmed on the basis of their elemental and spectral data. The ^{1}H NMR spectra indicated the presence of a 1,2-disubstituted benzene ring together with two 1,4-disubstituted benzene rings, a methoxy group at δ 3.85 in addition to a singlet at δ 8.63 corresponding to N=CH proton. Moreover, the prepared structures were further confirmed by ^{13}C NMR spectra. The mass spectrum



Scheme 1. Synthesis of compounds **4a**,**b**.



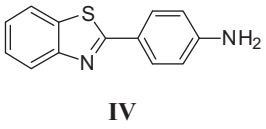
Scheme 2. Synthesis of compounds **6a–f**.

also revealed molecular ion peaks $[M+H]^+$ at 329.1283 and 345.1052 indicating **4a** and **4b**, respectively.

Furthermore, the substituted benzothiazoles and benzoxazoles **6a–f** were obtained by the reaction of compounds **5a**,**b** with different secondary amines in absolute ethanol in the presence of triethylamine (Scheme 2). The structures of compounds **6a–f** were elucidated using ^{1}H NMR spectra, which showed a 1,2-disubstituted benzene ring together with a 1,4-disubstituted benzene ring, and a singlet signal at δ 9.99–10.14 corresponding to NH proton. In addition, the ^{1}H NMR spectra showed the characteristic signals for different secondary amine protons as morpholino, piperidino and methyl piperazyl protons at δ 2.40–3.66. The ^{13}C NMR spectra also showed peaks at aliphatic region (δ 23–66) corresponding to the aliphatic carbons in addition to the aromatic carbons. The accurate mass spectral analysis also confirmed the structures of **6a–f** indicating the exact molecular ion peaks for these compounds.

In vitro anticancer screening

All the synthesized benzothiazole and benzoxazole derivatives were evaluated *in vitro* for their cytotoxic activity against human breast cancer cell lines, MCF-7 and MDA-231, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay [36]. 4-Benzothiazol-2-yl-phenylamine **IV** (Fig. 3) was also tested for its activity against both cell lines; to investigate the effect of structural changes on the activity, the half maximal inhibitory concentration (IC_{50}) was calculated. The IC_{50} of the tested compounds as well as of compound **IV** are shown in Table 1 and the most potent compounds are represented graphically in Fig. 4. The obtained data revealed that most of the newly synthesized compounds showed potent antitumor activity in the nanomolar range. Among the tested compounds, the most potent cytotoxic effect against



IV

Figure 3. 4-Benzothiazol-2-yl-phenylamine **IV**.

Table 1. IC_{50} of the synthesized compounds against human breast cancer cell lines.

Compound	IC_{50} (nM)[a)]	
	MDA-231	MCF-7
4a	>50	>50
4b	>50	35
6a	42	30
6b	37	31
6c	17	12
6d	34	29
6e	16	20
6f	8	10
IV	13	18

[a)] The values given are means of three experiments.

MCF-7 cell line was obtained by compound 4-methylpiperazin-1-yl-acetamide derivative of benzothiazole **6f** with IC_{50} value of 10 nM, followed by **6c** which showed IC_{50} value of 12 nM whilst compound **4a** exhibited the least cytotoxic activity.

From the results of the antitumor screening against MCF-7 and MDA-231 cell lines, some structure activity relationship could be suggested. As a general scaffold, the benzothiazole

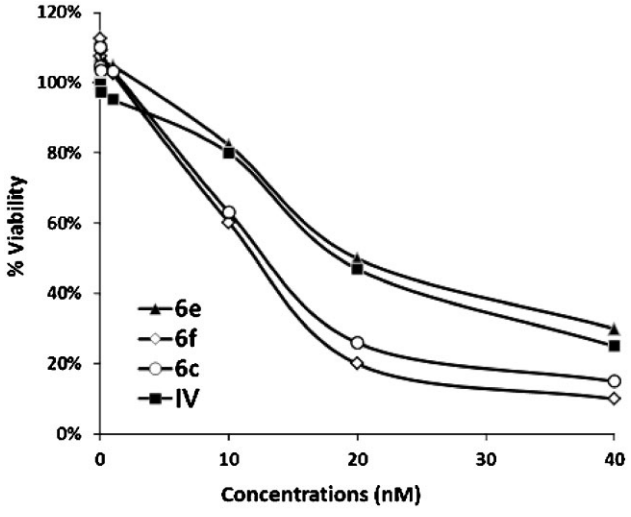


Figure 4. Viability percent at different concentrations of the most potent compounds on MCF-7.

system **4b,6d–f** was more potent than the benzoxazole one **4a,6a–c** for the same substituents. Acetamide substituted benzoxazoles and bezothiazoles **6a–f** (general formula A) showed more potential cytotoxic activity than the 4-methoxy-benzylidine derivatives **4a,b** (general formula B). The methyl piperazine substituted benzoxazole and benzothiazole derivatives **6c,f** were found to be more potent than morpholine substituted derivatives **6b,e** which were more active than piperidine substituted analogues **6a,d**.

Molecular docking studies

Carcinogenesis is usually accompanied by overactivation of receptor tyrosine kinase (RTK) signaling pathways, so inhibitors which block these receptors have a significant therapeutic potential in cancer treatment [9]. On this basis, RTK was selected as the target receptor for docking studies of the synthesized compounds. EGFR kinase domain in complex with 4-anilinoquinazoline inhibitor (4AQ) (PDB ID: IM17) [37] was used.

Docking calculations were carried out using DOCK6.5. The inhibitor 4-anilinoquinazoline was extracted from the X-ray crystal structure, then re-docked in the active site of EGFR and the docking parameters were adjusted accordingly. DOCK6.5 [38] predicted a docking pose similar to the experimental binding pose in the original X-ray crystal structure thereby validating the docking approach (Fig. 25 in Supporting Information). The protein was prepared using Dock Prep tool in UCSF Chimera [39]. Each ligand was docked into the active site using the flexible ligand sampling algorithm of DOCK6.5. Solvent was deleted from the original structure except for one water molecule in the active site. Each inhibitor molecule was oriented, minimized using Amber force filed and screened for Van der Waals and electrostatic interactions complementarity with the EGFR active site generating the best energy scored conformation of each docked molecule. Hydrophobic interactions are principle constituents in the predicted binding modes (Figs. S27–S35, Supporting Information, SI). The predicted binding poses of 4-methoxy-benzylidine derivatives **4a** and **4b** make hydrophobic contacts with the hydrophobic amino acids in the active site. However, the predicted binding poses of the acetamide substituted benzoxazole and benzothiazole derivatives **6a–6f** (Figs. 5–8, S30–S37, SI) make a hydrogen bonding interaction in addition to hydrophobic contacts except in only one case where compound **6b** was predicted to form only hydrophobic contacts. The amide hydrogen of compounds **6c** and **6d** makes hydrogen-bonding interaction with the backbone of Pro-770. The carbonyl oxygens of compounds **6a**, **6e**, and **6f** make hydrogen bonding interaction with the backbone of Cys-773.

The surface of the EGFR binding pocket is mainly hydrophobic (Fig. S26, SI) and the known inhibitor 4AQ forms mostly hydrophobic contacts and only one hydrogen bond with the backbone of Met-769. The docked inhibitors make hydrophobic interactions with the side chains of hydrophobic residues embedded in the active site which is consistent with the hydrophobic interactions made by the known inhibitor 4AQ [37]. The hydrogen bonding interaction to either Pro-770 or Cys-773 made by the acetamide substituted benzoxazole and benzothiazole derivatives **6a–6f** may have increased their binding specificity and hence increased their activity over the 4-methoxy-benzylidine derivatives **4a** and **4b**. The predicted binding pose of compound **IV** forms a hydrogen bonding interaction with the carboxyl group of Glu-738 (Fig. S37, SI) which may also increased binding specificity in the active site.

Conclusion

A series of new benzothiazole and benzoxazole derivatives have been synthesized and evaluated for their potential as antitumor lead compounds, depending on the previously reported antitumor activity for numerous compounds having benzothiazole and benzoxazole scaffolds. The newly synthesized compounds were tested *in vitro* on human breast

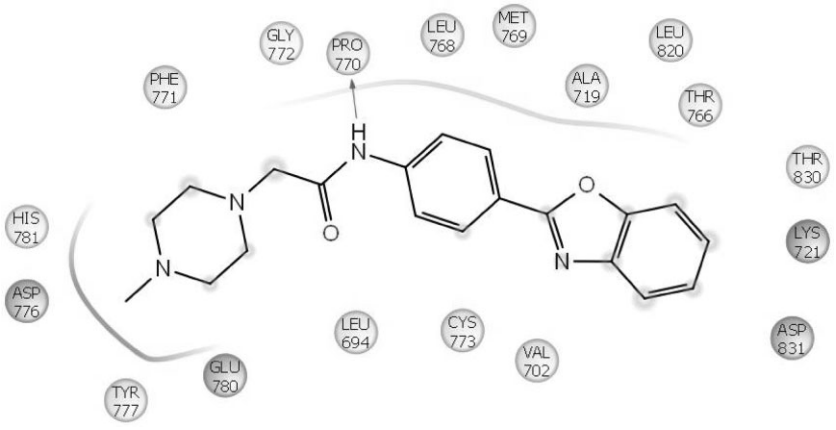


Figure 5. 2D predicted binding pose of compound **6c** in the active site of EGFR.

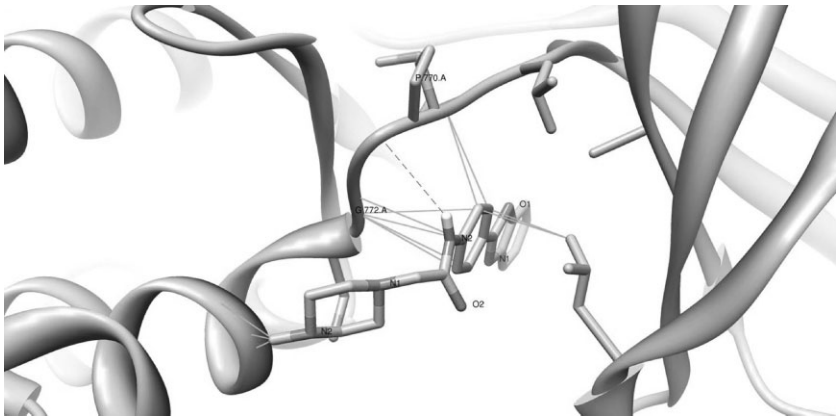


Figure 6. Putative interactions in the EGFR active site with **6c**. Hydrogen bond with the backbone of Pro-770 is shown as dashed line. Hydrophobic contacts are demonstrated as solid lines.

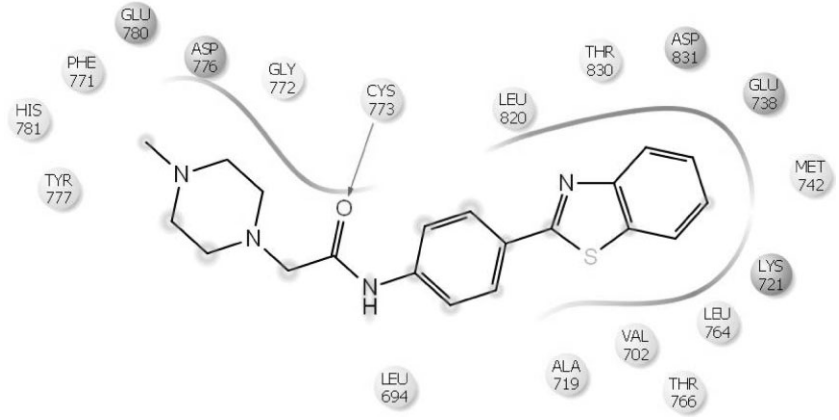


Figure 7. 2D predicted binding poses of compound **6f** in the active site of EGFR.

cancer cell lines, MCF-7 and MDA-231. Most of the tested compounds showed potent antitumor activity in the nanomolar level. The antitumor screening revealed that benzothiazole derivatives were more potent than benzoxazole ones. In addition, the acetamide substituted derivatives of both benzoxazole and benzothiazole **6a–f** were more potent than the 4-methoxy-benzylidine analogues **4a,b**. Moreover, the 4-methylpiperazin-1-yl substituted acetamides of both benzoxazole and benzothiazole **6c,f** are found to be the most potent cytotoxic derivatives. Molecular docking studies for the

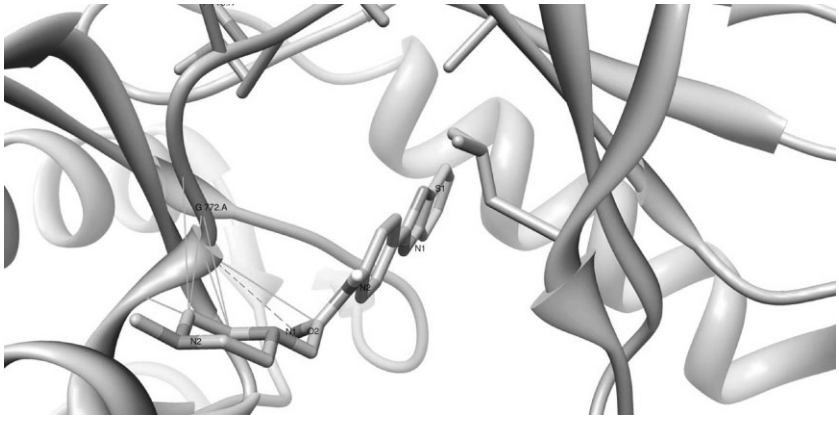


Figure 8. Putative interactions in the EGFR active site with **6f.** Hydrogen bond with the backbone of Cys-773 is shown as dashed line. Hydrophobic contacts are demonstrated as solid lines.

synthesized compounds were performed and molecular interactions were explored. The hydrophobic contacts with the hydrophobic amino acids embedded in the active site of EGFR is the main constituent, consistent with the hydrophobic interactions of the known inhibitor 4-anilinoquinazoline (4AQ) within the active site of EGFR. The amide group of the acetamide substituted benzoxazole and benzothiazole derivatives was predicted to make extra electrostatic interactions which may increase their binding specificity and activity over the 4-methoxy-benzylidine analogues. The methyl piperazinyl substituted derivatives of both benzothiazole **6f** and benzoxazole **6c** could be screened for their selective toxicity against a panel of cancer cell lines as well as their toxicity against normal human cell lines which could be of value for these compounds to be used as anticancer drugs. These results also support the clinical promise of these compounds as a component of therapeutic strategies for cancer, for which high concentrations of chemotherapeutic agents are always a major limitation. Moreover, the uncomplicated methodology used for the preparation of these potent compounds allows for obtaining sufficient amounts for more in-depth clinical studies. The simplicity of the prepared compounds with their potent anti-breast cancer activity highlights the potential of smart organic synthesis for the discovery of new drug leads.

Experimental

Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel sheets pre-coated with UV fluorescent silica (MERCK 60 F 254) and spots were developed using I_2 vapor/UV light as visualizing agents. Solvent system was chloroform/methanol (in different ratios). ^{1}H and ^{13}C NMR spectra were determined in DMSO-d_6 solvent with a Varian Inova 400 MHZ spectrometer, Department of Chemistry, University of Aberdeen. Peak positions were given in parts per million (δ). All NMR data were processed using MestReNova 7.1.0. All reported products showed NMR spectra in agreement with the assigned structures. High resolution mass spectral data was obtained from a Thermo Instruments MS system (LTQ XL/LTQ Orbitrap Discovery) coupled to a Thermo Instruments HPLC system (Accela PDA detector, Accela PDA autosampler and Accela pump), Department of Chemistry, University of Aberdeen. The following conditions were used: capillary voltage 45 V, capillary temperature 320°C, auxiliary gas flow rate 10–20 arbitrary units, sheath gas flow rate 40–50 arbitrary units, spray voltage 4.5 kV, mass range 100–2000 amu (maximum resolution 30,000). Melting points were determined on a Griffin instrument and are uncorrected. IR spectra were recorded on a Shimadzu 435 spectrometer, using KBr discs and values were represented in cm^{-1}. Elemental analyses were performed at the Micro-Analytical Center, Cairo University, Egypt. Compounds **3a**,**b** and **5a**,**b** were prepared according to the previously reported procedures [26, 31–35]. Docking studies were performed using DOCK 6.5 [38]. Crystal structure of protein tyrosine kinase domain (PDB codes: 1M17) is obtained from protein data bank [37]. As for protein preparation for docking studies and docking, procedures were performed following the standard protocol implemented in DOCK 6.5. The protein and ligand 4AQ are prepared in UCSF Chimera [38]. Receptor spheres were placed on the receptor surface using the sphgen program within the DOCK package. Receptor electrostatic and VDW grid points were calculated by the grid program within the DOCK package. The inhibitors coordinate files were prepared using Maestro v9.0 software package (Schrödinger LLC) and UCSF Chimera [38] was used further to add charges using AM1-BCC method. All 2D interaction pictures diagrams were generated using Maestro v9.0 software package (Schrödinger LLC). All 3D interaction models and surfaces were rendered using UCSF Chimera [38].

*General procedure for the preparation of **4a,b***

A mixture of compound **3a** or **3b** (0.005 mol), 4-methoxybenzaldehyde (0.7 g, 0.0055 mol) in absolute ethanol (25 mL) and glacial acetic acid (0.5 mL) was heated under reflux for 3 h. The solid formed on hot was filtered, dried and re-crystallized from ethanol/acetone mixture to give compounds **4a** and **b**.

*(4-Benzoxazo-2-yl-phenyl)(4-methoxy-benzylidine)amine **4a***

Yellow crystals, 70% yield, mp 144–146°C. IR: $\upsilon_{max.}$/cm^{-1} 3050 (CH aromatic), 2950 (CH aliph), 1593 (C=N), 1581 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz at 298 K): δ 3.85 (s, 3H, OCH_3), 7.10 (d, J = 8.7 Hz, 2H, Ar–H), 7.41–7.45 (m, 4H, Ar–H), 7.79 (t, J = 8.6 Hz, 2H, Ar–H), 7.93 (d, J = 8.5 Hz, 2H, Ar–H), 8.23 (d, J = 7.8 Hz, 2H, Ar–H), 8.63 (s, 1H, CH=N); ^{13}C NMR (DMSO-d_6): δ 55.5, 110.8, 114.4 (2C), 119.7, 121.9 (2C), 123.3, 124.8, 125.3, 128.4 (2C), 128.6, 130.9 (2C), 141.6, 150.2, 154.8, 161.4, 162.2, 162.3. HRESIMS m/z 329.1283 $[M+H]^+$ (calcd. for $C_{21}H_{17}N_2O_2^+$ 329.1285); Calcd. C, 76.81; H, 4.91; N, 8.53. Found: C, 76.70; H, 4.80; N, 8.50.

*(4-Benzothiazol-2-yl-phenyl)(4-methoxy-benzylidine)-amine **4b***

Greenish crystals, 60% yield, m.p. 155–157°C. IR: $\upsilon_{max.}$/cm^{-1} 3061, 3050 (CH aromatic), 2949 (CH aliph.), 1596 (C=N), 1556 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz at 298 K): δ 3.85 (s, 3H, OCH_3), 7.10 (d, J = 8.6 Hz, 2H, Ar–H), 7.41 (d, J = 8.2 Hz, 2H, Ar–H), 7.46 (t, J = 7.8 Hz, 1H, Ar–H), 7.54 (t, J = 7.9 Hz, 1H, Ar–H), 7.93 (d, J = 8.6 Hz, 2H, Ar–H), 8.06 (d, J = 8.1 Hz, 1H, Ar–H), 8.12–8.16 (m, 3H, Ar–H), 8.63 (s, 1H, CH=N). ^{13}C NMR (DMSO-d_6): δ 55.5, 114.4 (2C), 121.9 (2C), 122.3, 122.7, 125.4, 126.6, 128.3 (2C), 128.7, 130.0, 130.8 (2C), 134.4, 153.7, 154.3, 161.1, 162.2, 166.9; HRESIMS m/z 345.1052 $[M+H]^+$ (calcd. for $C_{21}H_{17}N_2OS^+$ 345.1056); Calcd. C, 73.23; H, 4.68; N, 8.13; Found: C, 73.30; H, 4.70; N, 8.20.

*General procedure for the preparation of **6a–f***

A well-stirred mixture of compound **5a** or **5b** (0.01 mol), appropriate secondary amine (0.01 mol) and (2–3 drops) triethylamine in absolute ethanol (100 mL), was heated under reflux for 24 h. The mixture was filtered while hot and the solvent was removed by distillation under vacuum. The obtained residue was washed with water, filtered, dried and re-crystallized from DMF.

*N-(4-Benzoxazol-2-ylphenyl)-2-piperidin-1-yl-acetamide **6a***

Yellow crystals, 70% yield, m.p. 185–187°C. IR: $\upsilon_{max.}$/cm^{-1} 3314 (NH), 3051 (CH aromatic), 2931 (CH aliph), 1687 (C=O), 1608 (C=N), 1595 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz at 298 K): δ 1.41

(m, 2H, CH_2), 1.58 (m, 4H, 2 × CH_2), 2.47 (m, 4H, 2 × CH_2), 3.12 (s, 2H, O=C–CH_2), 7.39–7.43 (m, 2H, Ar–H), 7.75–7.78 (m, 2H, Ar–H), 7.90 (d, J = 8.4 Hz, 2H, Ar–H), 8.15 (d, J = 8.4 Hz, 2H, Ar–H), 10.03 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 23.5, 25.4 (2C), 54.0 (2C), 62.7, 110.8, 119.5 (3C), 121.0, 124.8, 125.2. 128.1 (2C), 141.6, 141.9, 150.1, 162.2, 169.2; HRESIMS *m/z* 336.1703 $[M+H]^+$ (calcd. for $C_{20}H_{22}N_3O_2^+$ 336.1707); Calcd. C, 71.62; H, 6.31; N, 12.53. Found: C, 71.60; H, 6.40; N, 12.50.

N-(4-Benzoxazol-2-ylphenyl)-2-morpholin-4-yl-acetamide **6b**

Yellow crystals, 65% yield, m.p. 200–202°C. IR: $\upsilon_{max.}/cm^{-1}$ 3313 (NH), 3050 (CH aromatic), 2965 (CH aliph), 1687 (C=O), 1608 (C=N), 1596(C=C). ^{1}H NMR (DMSO-d_6, 400 MHz at 298 K): δ 2.50 (t, J = 4.4 Hz, 4H, 2 × CH_2), 3.18 (s, 2H, O=C–CH_2), 3.65 (t, J = 4.5 Hz, 4H, 2 × CH_2), 7.37–7.43 (m, 2H, Ar–H), 7.76 (m, 2H, Ar–H), 7.90 (d, J = 8.7 Hz, 2H, Ar–H), 8.15 (d, J = 8.7 Hz, 2H, Ar–H), 10.11 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 53.1 (2C), 62.1, 66.1 (2C), 110.8, 119.5 (2C), 119.6, 121.0, 124.8, 125.2, 128.1 (2C), 141.6, 141.9, 150.1, 162.2, 168.7; HRESIMS *m/z* 338.1497 $[M+H]^+$ (calcd. for $C_{19}H_{20}N_3O_3^+$ 338.1499): Calcd. C, 67.64; H, 5.68; N, 12.46. Found: C, 67.60; H, 5.60; N, 12.50.

N-(4-Benzoxazol-2-ylphenyl)-2-(4-methylpiperazin-1-yl)-acetamide **6c**

Yellow crystals, 65% yield, m.p. 190–192°C. IR: $\upsilon_{max.}/cm^{-1}$ 3324 (NH), 3051 (CH aromatic), 2938 (CH aliph), 1689 (C=O), 1610 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz at 298 K): δ 2.26 (s, 3H, N–CH_3), 2.50–2.59 (m, 8H, aliphatic protons), 3.17 (s, 2H, O=C–CH_2), 7.38–7.42 (m, 2H, Ar–H), 7.74–7.77 (m, 2H, Ar–H), 7.89 (d, J = 8.6 Hz, 2H, Ar–H), 8.15 (d, J = 8.6 Hz, 2H, Ar–H), 10.14 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 45.1, 52.1 (2C), 54.2 (2C), 61.5, 110.8, 119.5 (3C), 121.0, 124.8, 125.2, 128.1 (2C), 141.6, 141.9, 150.1, 162.2, 168.8; HRESIMS *m/z* 351.1813 $[M+H]^+$ (calcd. for $C_{20}H_{23}N_4O_2^+$ 351.1816): Calcd. C, 68.55; H, 6.33; N, 15.99. Found: C, 68.40; H, 6.30; N, 15.90.

N-(4-Benzothiazol-2-ylphenyl)-2-piperidin-1-yl-acetamide **6d**

Yellowish green crystals, 75% yield, m.p. 190–192°C. IR: $\upsilon_{max.}/cm^{-1}$ 3316 (NH), 2933 (CH aliph), 1682 (C=O), 1598 (C=N). ^{1}H NMR (DMSO-d_6, 400 MHz at 298 K): δ 1.40 (m, 2H, CH_2), 1.57 (m, 4H, 2 × CH_2), 2.47 (m, 4H, 2 × CH_2), 3.11 (s, 2H, O=C–CH_2), 7.42–7.55 (m, 2H, Ar–H), 7.86 (d, J = 8.8 Hz, 2H, Ar–H), 8.01–8.06 (m, 3H, Ar–H), 8.12 (d, J = 8.1 Hz, 1H, Ar–H), 9.99 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 23.5, 25.4 (2C), 54.0 (2C), 62.7, 119.6 (2C), 122.2, 122.6, 125.2, 126.6, 127.7, 127.9 (2C), 134.3, 141.4, 153.6, 166.9, 169.1. HRESIMS *m/z* 352.1475 $[M+H]^+$ (calcd. for $C_{20}H_{22}N_3O_2S^+$ 352.1478): Calcd. C, 68.35; H, 6.02; N, 11.96. Found: C, 68.30; H, 6.10; N, 11.90.

N-(4-Benzothiazol-2-ylphenyl)-2-morpholin-4-yl-acetamide **6e**

Yellowish green crystals, 70% yield, m.p. 192–194°C. IR: $\upsilon_{max.}/cm^{-1}$ 3292 (NH), 3051 (CH aromatic), 2925 (CH aliph), 1683 (C=O), 1588 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz at 298 K): δ 2.49 (t, J = 4.5 Hz, 4H, 2 × CH_2), 3.18 (s, 2H, O=C–CH_2), 3.65 (t, J = 4.5 Hz, 4H, 2 × CH_2), 7.41–7.55 (m, 2H, Ar–H), 7.86 (d, J = 8.4 Hz, 2H, Ar–H), 8.01–8.06 (m, 3H, Ar–H), 8.11 (d, J = 8.0 Hz, 1H, Ar–H), 10.07 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 53.1 (2C), 62.0, 66.1 (2C), 119.7 (2C), 122.2, 122.6, 125.2, 126.6, 127.7, 127.9 (2C), 134.3, 141.4, 153.6, 166.9, 168.6. HRESIMS *m/z* 354.1268 $[M+H]^+$ (calcd. for $C_{19}H_{20}N_3O_2S^+$ 354.1271): Calcd. C, 64.57; H, 5.42; N, 11.89. Found: C, 64.60; H, 5.60; N, 11.90.

N-(4-Benzothiazol-2-ylphenyl)-2-(4-methylpiperazin-1-yl)-acetamide **6f**

Yellowish green crystals, 65% yield, m.p. 198–200°C. IR: $\upsilon_{max.}/cm^{-1}$ 3304 (NH), 3052 (CH aromatic), 2933 (CH aliph), 1688 (C=O), 1590 (C=C). ^{1}H NMR (DMSO-d_6, 400 MHz at 298 K): δ 2.19 (s, 3H, N–CH_3), 2.41–2.54 (m, 8H, aliphatic protons), 3.17 (s, 2H, O=C–CH_2), 7.41–7.54 (m, 2H, Ar–H), 7.85 (d, J = 8.6 Hz, 2H, Ar–H), 8.01–8.07 (m, 3H, Ar–H), 8.11 (d, J = 8.1 Hz, 1H, Ar–H), 10.03 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 45.6, 52.5 (2C), 54.4 (2C), 61.7, 119.6 (2C), 122.2, 122.6, 125.2, 126.5, 127.7, 127.9 (2C), 134.3, 141.4, 153.6, 166.9, 168.7. HRESIMS *m/z* 367.1581 $[M+H]^+$ (calcd. for $C_{20}H_{23}N_4OS^+$ 367.1587): Calcd. C, 65.55; H, 6.05; N, 15.29. Found: C, 65.50; H, 6.10; N, 15.20.

Pharmacological studies

Cell culture

Human breast cancer cells, MCF-7 and MDA-231, were obtained from the American Type Culture Collection (Manassas, VA) and cultured in Dulbecco's modified Eagle's medium/F12 medium (DMEM/F-12, Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Gibco). All cells were cultured at 37°C in a humidified incubator containing 5% CO_2.

Cell viability assay

The effect of test compounds on cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay in six replicates as reported before [40]. Briefly, MCF-7 or MDA-231 cells were seeded in 96-well plates for 24 h, and treated with test agents in 5% FBS-supplemented DMEM/F-12 for 72 h. Controls received DMSO vehicle at a concentration equal to that in drug-treated cells. After treatment, cells were incubated in the same medium containing 0.5 mg/mL MTT at 37°C for 2 h. Reduced MTT was solubilized in DMSO (200 μL/well) for determination of absorbance at 570 nm using a microplate reader.

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