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Design and synthesis of certain novel arylidene thiazolidinone derivatives as anticancer agents

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ABSTRACT

*New 5-subistituted-4-thiazolidinone derivatives **4a-g** were synthesized through chloroacetylation of 2-amino-benzothiazole derivatives and further cyclyised by the use of ammonium thiocyanate then subjected to different aryl and heteroaryl aldehydes. All the prepared compounds were evaluated for their antitumor activity against human breast (MCF-7) and non-small cell lung (A549) cancer cell lines. Most of compounds showed moderate antitumor activity especially the 5-(2-thiophen-arylidene)-4-thiazolidinone derivative **4a** with IC_{50} 13.25 µM against human breast MCF-7 cell line and 12.08 µM against non-small cell lung A549 cell line. Also, 5-(2-methoxy-arylidene)-4-thiazolidinone derivative **4b** have the antitumor activity with IC_{50} 14.51 µM against MCF-7 cell line and 13.25 µM against A549 cell line. All the prepared compounds were docked against EGFR using 4-anilinoquinazoline inhibitor (4AQ) (PDB ID: IM17) in away to explore their binding mode.*

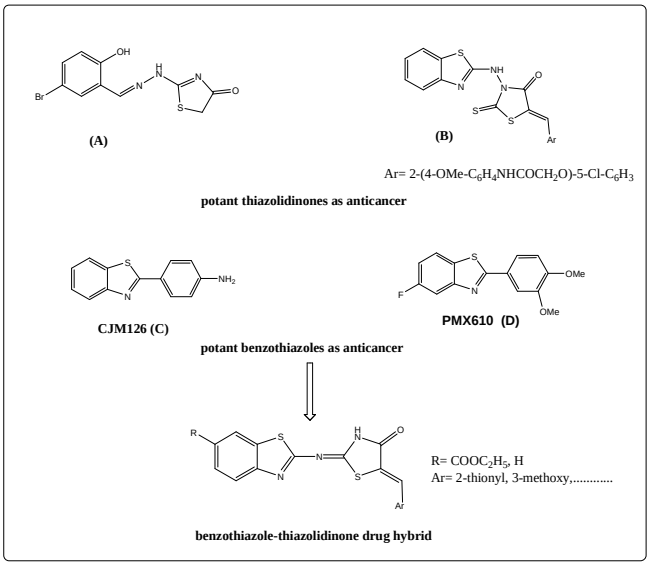
Keywords: Benzothiazole, 4-Thiazolidinone, MCF-7, A549, EGFR.

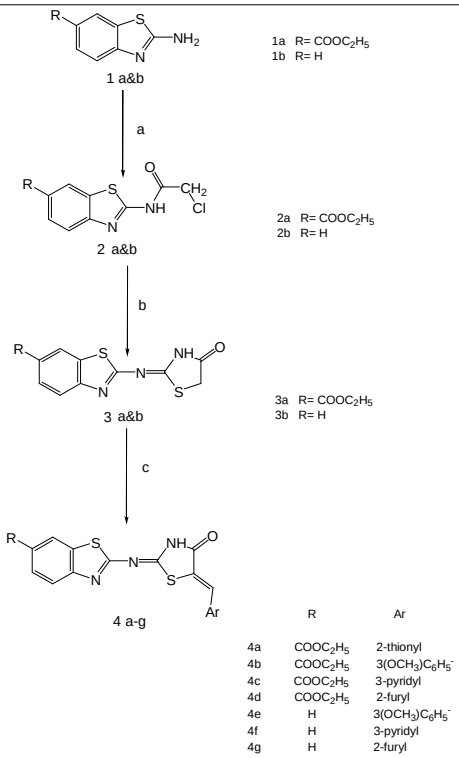
INTRODUCTION

Cytotoxic drugs and chemotherapy still remain the most important area of research. Enhancement and approaches to anticancer drugs seemed to be a major area of investigation, despite the continuous progress of anticancer agents, overall control of cancer is still a dream [1]. Great effort was exerted to develop new anticancer agents with high toxicity toward cancer cells and with a minimal toxicity against normal cells [2]. Hence several benzothiazoles and thiazolidinones were known for their anticancer activity (Figure1) as shown in compound (**A**). The preliminary results indicated that this compound exhibited more potent antitumor activity than doxorubicin [3]. Also, compound (**B**) exhibited considerable antitumor activity against melanoma, leukemia, lung, colon, ovarian, CNS, renal, prostate and breast cancers [4]. In addition, 2-(4-aminophenyl)-benzothiazole (**C**) and their analogues are a novel class of potent and selective antitumor agents [5,6]. Modifications on the benzothiazole nucleus had resulted in a large number of compounds having diverse pharmacological activities, among them, imidazo benzothiazoles, polymerized benzothiazoles and other substituted benzothiazoles such as 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (**D**) had been shown to exhibit exquisitely potent and selective *in vitro* antitumor properties in human cancer cell lines (e.g., colon, non-small cell lung and breast) and also antitumor activity against malignant cell lines [7,8]. In addition, both thiazolidinone and benzothiazole derivatives have a wide range of biological activities such as antitumor [3,9], anti-

inflammatory [10,11], antimicrobial [12,13], antiviral [14,15], and antifungal [16,17]. In view of these reported data and in continuation of our previous work on the synthesis of new anticancer agents [18-21], we now report synthesis of a benzothiazole-thiadiazolidinone hybrid derivatives **4a-g** and their cytotoxic activity against human breast (MCF-7) and non-small cell lung (A549) cancer cell lines.

Fig.1: chemical structures of some potent thiazolidinones, benzothiazoles (A-D), and the new benzothiazole-thiazolidinone hybrid drug





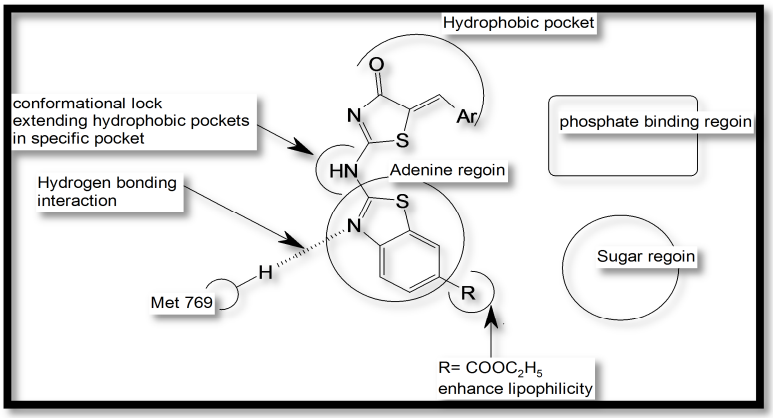
Scheme 1: Reagents and conditions; (a) $ClCOCH_2Cl$, DMF , rt; (b) NH_4SCN, EtOH, reflux 3 h.;(c) MeCOOH, MeCOONa, ArCHO, reflux 10 h.

Rationale and design

From literature survey, benzothiazole assumed to compete with ATP for binding at the catalytic domain of tyrosine kinase [22]. The ATP binding site has the following features; Adenine region contains two key hydrogen bonds

formed by the interaction of N-1 and N-6 amino group of the adenine ring. Many potent inhibitors used one of these Hydrogen bonds. Also, a sugar region which is a hydrophilic region, except a few e.g EGFR. In addition to a phosphate binding region that is used for improving inhibitor selectivity. Finally a Hydrophobic pocket though not used by ATP but plays an important role in inhibitor selectivity [9]. In this study, we presented a new sub-family of compounds containing 2-amino-benzothiazole core as EGFR inhibitors. Our strategy was directed toward the design of variety of ligands which were structurally similar with basic skeleton, 4-anilino quinazoline of tinibs (erlotinib, lapatinib, gefitinib and canaratinib) with diverse chemical properties (Figure 2).

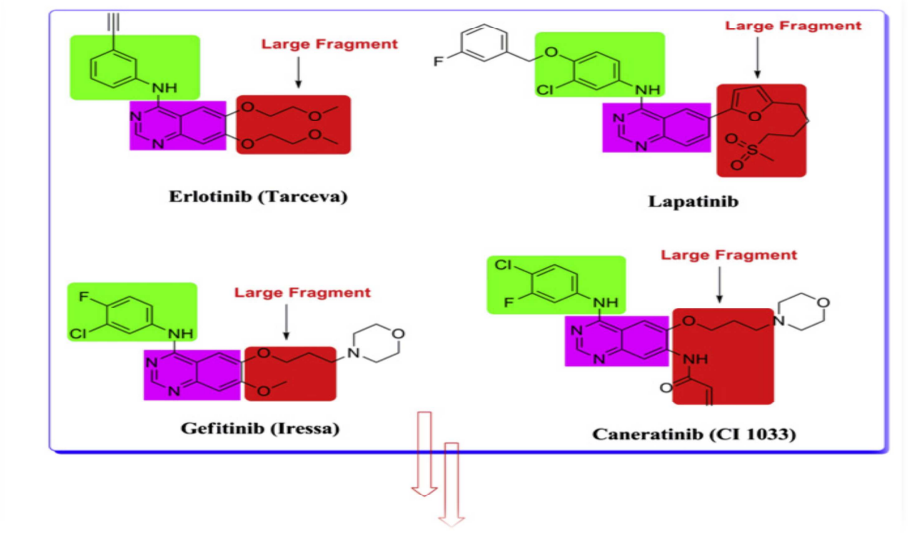
Fig. 2 Proposed hypothetical model of compounds 4a-g bound to ATP binding site of EGFR protein tyrosine kinase



The presence of ester group in compounds **4a-d** enhanced lipophilicity which explained why these derivatives were biologically active than others.

We replaced quinazoline ring with benzothiazole core since both are isosteric with adenine portion of ATP and can mimic the ATP competitive binding regions of EGFR tyrosine kinase. Like 4-aniline group in tinibs (erlotinib, lapatinib, gefitinib and canaratinib) we introduced thiazolidinone NH group such secondary amino group is acting as conformational lock similar to the extended substituted aniline portion of erlotinib. This thiazolidinone NH portion would fit to the hydrophobic pockets of EGFR -tyrosine kinase, making predominantly hydrophobic interactions with the protein (Figure 3).

Fig. 3 Rational designing of proposed compounds based upon known EGFR-Tyrosine kinase inhibitor



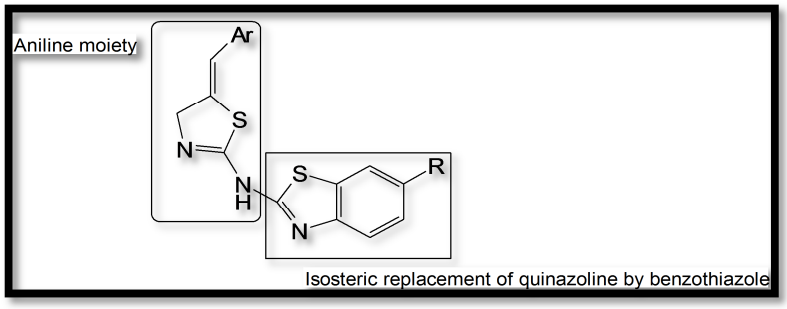
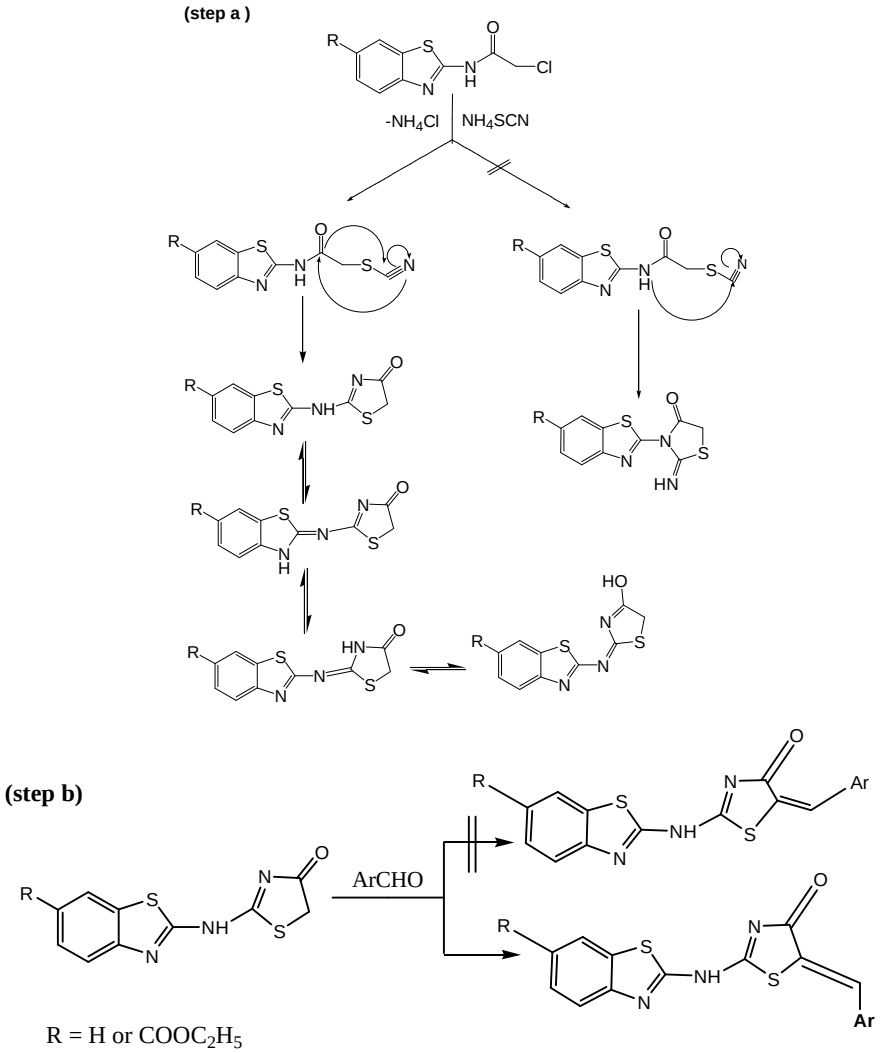


Fig.4 (a) Mechanism of synthesis for compounds 3a,b; (b) the E/Z isomerism of compounds 4a-g



RESULTS AND DISCUSSION

Chemistry

The synthetic pathway for the preparation of thiazolidinone compounds **3a,b** and 5-arylidene-4-thiazolidinones **4a-g** is outlined in Scheme 1. The synthetic procedures were carried out by reacting substituted benzothiazoles **1a,b** with chloroacetyl chloride in DMF at room temperature to give *N*-(6-subistituted benzothiazole)-2-yl-2-chloro-acetamide (**2a,b)** then cyclization in the presence of ammonium thiocyanate, with excellent yield of substituted thiazolidinones

(3a,b). The final compounds substituted thiazolidinones with arylidene moiety **(4a-g),** were obtained by reaction of compounds **(3a,b)** with different aryl and heteroaryl aldehydes in presence of anhydrous sodium acetate reagent.
In the ^{1}H NMR spectra of thiazolidinones (**3a,b)** revealed the appearance of a single peak of two protons represent **CH_2** of 4-thiazolidinone ring at δ 3.99 ppm and a NH proton appeared at δ 12.18–13.06 ppm, accounting for a lactam proton but not for an imine proton which was expected around δ 9.70 ppm. IR spectral data of these compounds showed a NH group as a multiple band near 3100 cm^{-1} and a strong band of the C=O group in the 1725–1687 cm^{-1} region,[12,23,24] which supported the feature of a γ-lactam heterocycle. The mass spectrum of compound **(3a)** showed a molecular ion peak at 321($M^{+\cdot}$, 100%), 323 (M^+**2**, 11.23%). This was considered to be a strong confirmation for the ring closure shown in (Figure 4 (step a)).

The Z configuration of the exocyclic C=C bond, in the 5-arylidene derivatives (**4a-g)**, was attributed on the basis of ^{1}H NMR spectral analysis, since the methine proton resonated, as expected, at higher chemical shift values (δ 7.5-7.8 ppm region as a single peak of one proton) due to the deshielding effect of the adjacent C=O, than it would do in E isomers, because of the lower deshielding effect of 1-S (Figure 4 (step b)). IR spectrum of compounds **(4a-g)** showed a NH group as a multiple band in the 3100-3177 cm^{-1} region, a band of CH aromatic in the 3043-3053 cm^{-1} region, a band of CH aliphatic in the 2918-2986 cm^{-1} region, a strong band of the C=O group in the 1686–1719 cm^{-1} region and a band of C=N in the 1568-1583 cm^{-1} region. The mass spectrum showed a molecular ion peak at 415 ($M^{+\cdot}$, 16.51%) & 417 (M^+**2**, 2.79%) for compound (**4a)** and 439 ($M^{+\cdot}$, 24.47%) & 441 (M^+**2**, 3.32%) for compound **(4b)**.

Biological evaluation
All the synthesized benzothiazole derivatives, compound **C** and doxorubicin [as reference drugs control] were evaluated *in vitro* for their cytotoxic activity against human breast (MCF-7) and non-small cell lung (A549) cancer cell lines, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay[25].

The half maximal inhibitory concentration (IC_{50}) was calculated. Doxorubicin and compound **(C)** were 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 and shown in table 1. IC_{50} of doxorubicine showed that, it was more active than all prepared compounds against the two cell lines, but $IC_{50}$of compound **(C)** showed that, it was less active than all prepared compounds against the two cell lines.

Table 1.IC_{50} of the synthesized compounds against MCF-7 and A549 cancer cell lines

Compounds	IC_{50}(μM)[a)]	
	MCF-7	A549
4a	13.25	12.08
4b	14.51	13.25
4c	16.25	14.23
4d	15.02	14.30
4e	23.98	21.60
4f	16.89	14.25
4g	17.60	18.03
C	98.67	38.08
doxorubicin	1.83	0.93

The obtained data revealed that, most of the newly synthesized compounds showed moderate antitumor activity in the micro molar range and were showed in (figure 5,6,7). Among the tested compounds, the most potent cytotoxic effect was obtained by compound **(4a)** 2-(4-oxo-5-thiophen-2-ylmethylene-4,5-dihydro-thiazol-2-ylamino) benzothiazole-6-carboxylic acid ethyl ester against MCF-7 and A549 cell lines with IC_{50} value of 13.25 µM and 12.08 µM respectively followed by **(4b)** which show IC_{50} value of 14.51 µM and 13.25 µM respectively whilst compound **(4e)** exhibited the least cytotoxic activity with IC_{50} value of 23.98 µM and 21.6 µM respectively.

Fig.5 A graph representing IC_{50} (µM) against (MCF-7) and (A549) cell lines at compounds (4a-g), compound (C) and Dox

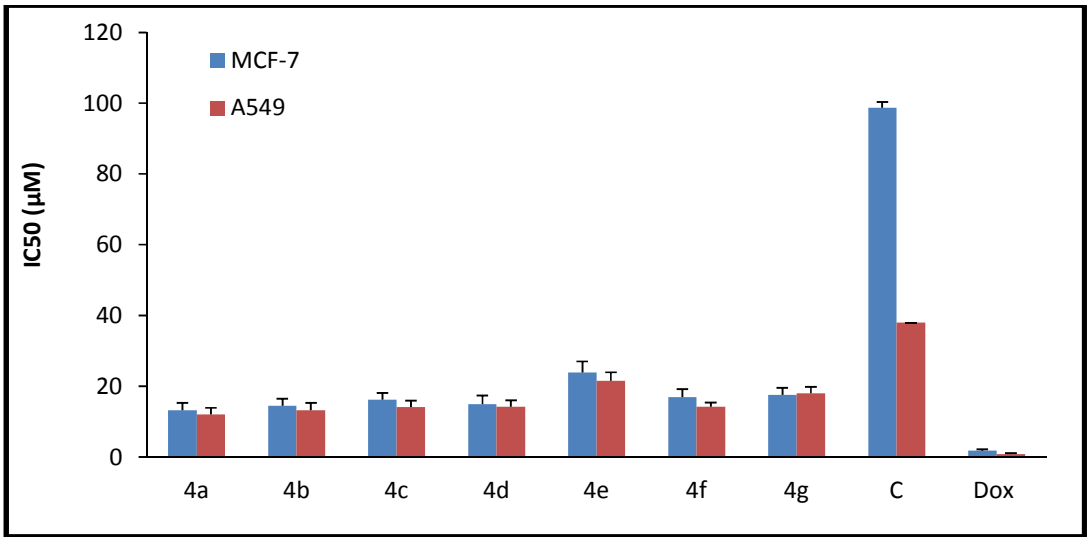


Fig.6 A graph representing IC_{50} (µM) against (MCF-7) cell line at compounds 4a-g, compound C and Dox

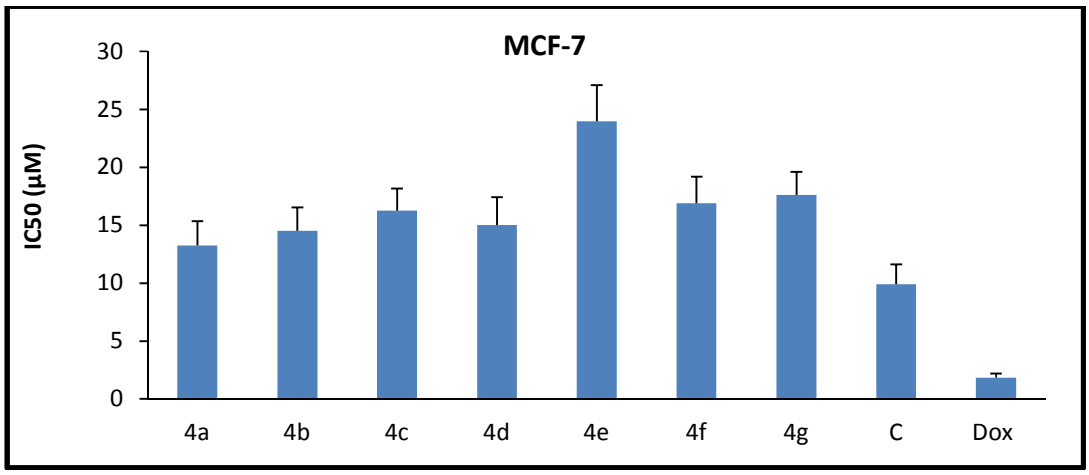
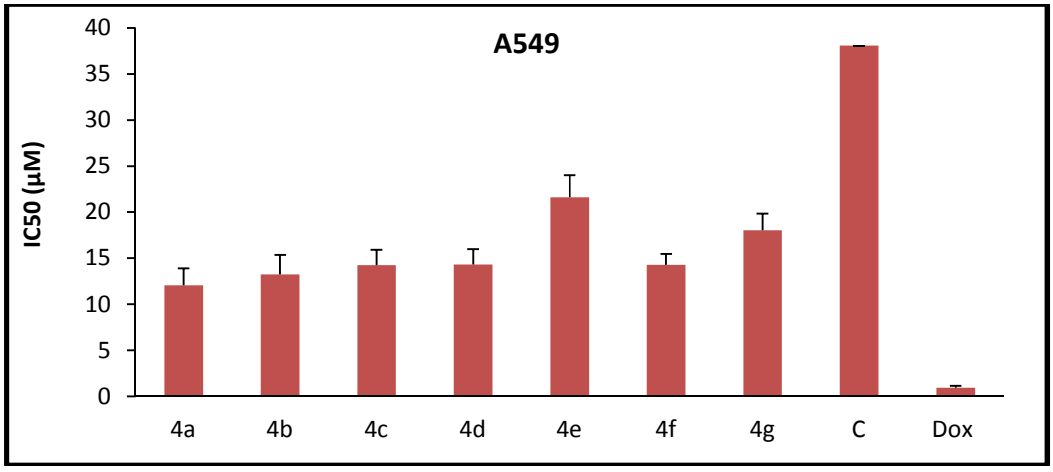


Fig.7 A graph representing IC_{50} (µM) against (A549) cell line at compounds (4a-g), compound (C) and Dox



From the results of the antitumor screening against MCF-7 and A549 cell lines, some structure activity relationship could be suggested. As a general scaffold, compounds (**4a-d)** were more potent than compounds (**4e-g)** for the same substituents due to presence of ester group in compounds **(4a-d)** enhanced lipophilicity so increased their biological activity.

Molecular modeling

Carcinogenesis is usually accompanied by over activation of receptor tyrosine kinase (RTK) signaling pathways, so inhibitors which block these receptors have a significant therapeutic potential in cancer treatment [26]. 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) [27] was used.

The most potent compounds **(4a,b)** and the least active compound (**4e)** were docked against epidermal growth factor receptor (EGFR) kinase to investigate if these compounds have a similar mechanism as EGFR kinase inhibitors. Docking was performed using MOE 2008.10 software, First of all, the erlotinib ligand was flexibly docked to the binding site, and the docking conformation corresponding to the lowest energy score value was selected as the most probable binding conformation, then we docked the prepared compounds **(4a,b&e)**. Docking studies showed that these compounds occupied the EGFR binding site as compared with erlotinib (Figure 8,9,10).

Fig.8 ligand interaction of compound (4a) with the active site of EGFR

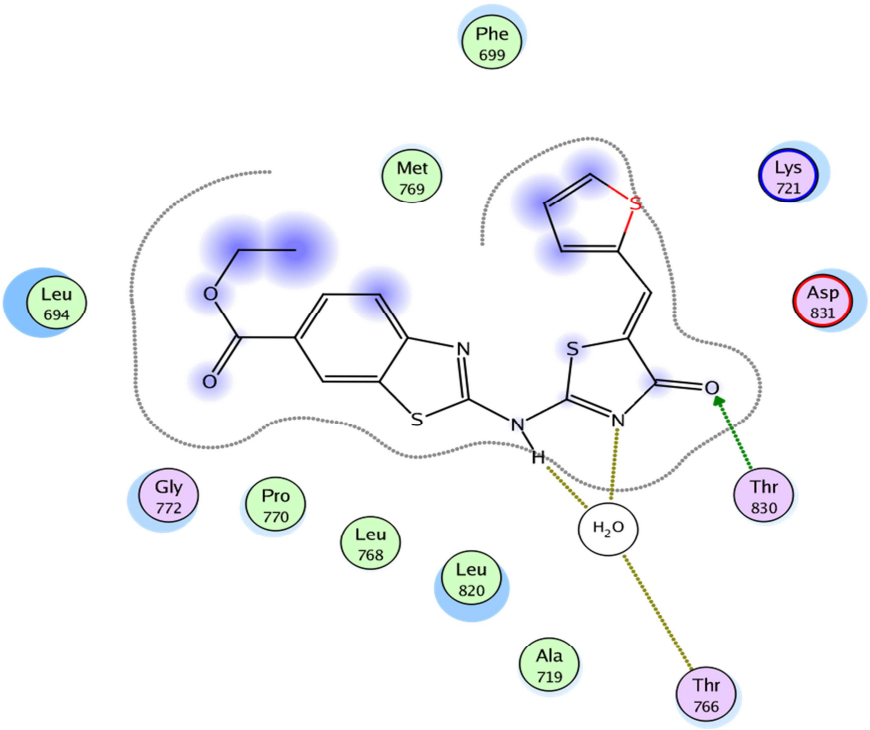


Fig.9 ligand interaction of compound (4b) with the active site of EGFR

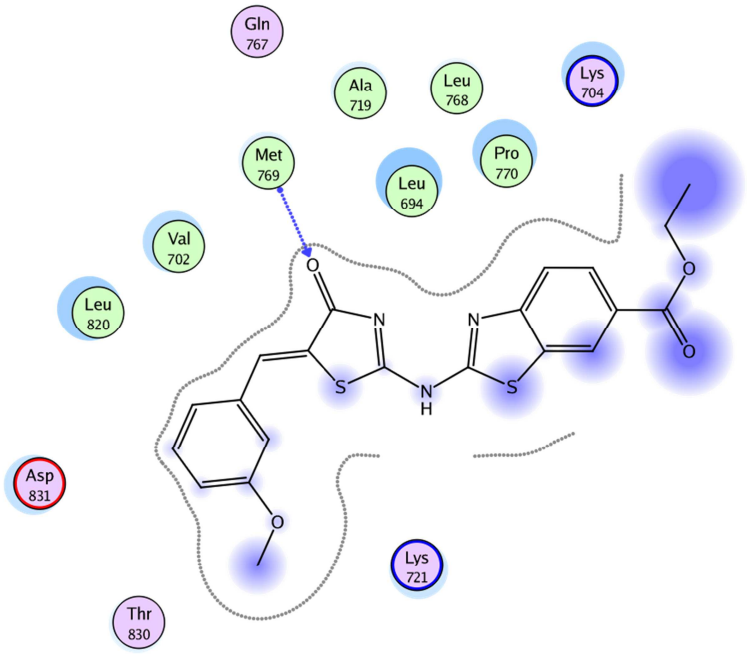


Fig.10 ligand interaction of compound (4e) with the active site of EGFR

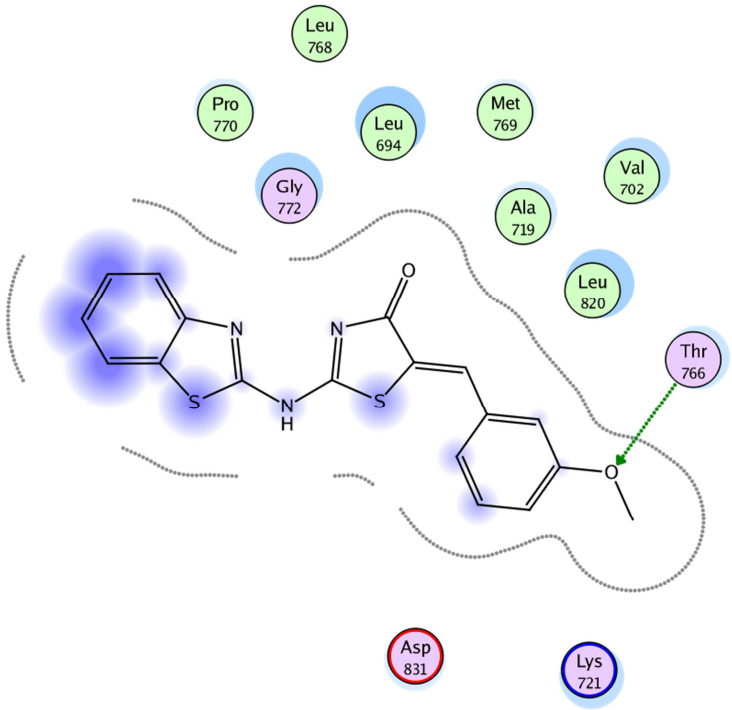
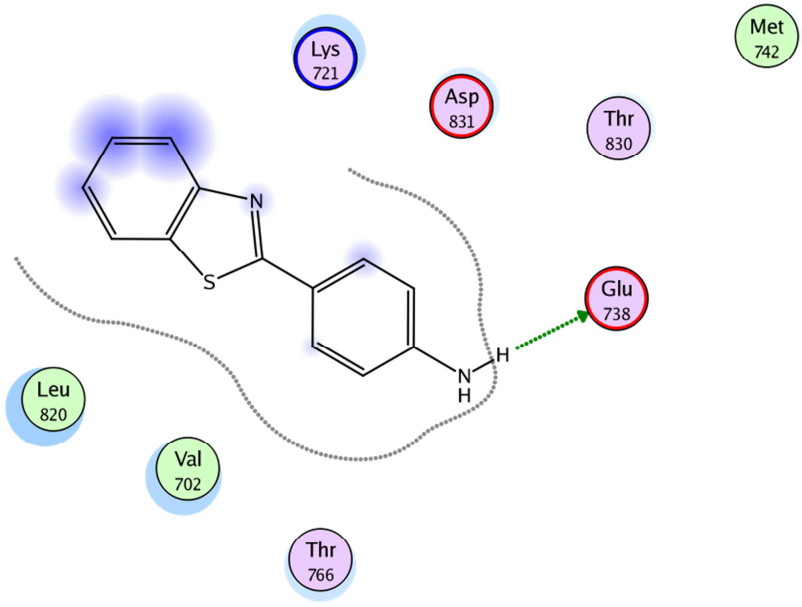


Fig.11 ligand interaction of compound (C) with the active site of EGFR



The binding energies for the most active derivatives (**4a**,**b)** were found to be -11.33, -11.33 kcal/mol compared to -11.37 kcal/mol observed for erlotinib. Also these compounds exhibited three and one hydrogen bonding interactions respectively in comparison to three hydrogen bonding interactions exhibited by erlotinib. Similarly, compound **(4e)**, showed the highest binding energy scores (-8.5 kcal/mol) and one hydrogen bonding interactions in comparison to erlotinib. The docking scores, amino acid interactions and the hydrogen bond lengths were summarized in Table 2.

Table2. Docking score energy

Compound no.	Affinity (Kcal/mol)	Amino acid	Functional group	No. of hydrogen bonds
ligand	-11.37	Thr-766 and Met-769	-N=C	3
4a	-11.33	Thr-766 and Thr-830	-CO, -N=C, -NH	3
4b	-11.33	Met-769	-CO	1
4e	-8.5	Thr-766	-OCH3	1

The surface of EGFR binding pocket is mainly hydrophobic and the known inhibitor 4AQ forms mostly hydrophobic contacts and only one hydrogen bond with the backbone of Met-769. The docked inhibitors (compounds **4a,b,e & C**) made 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. The hydrogen bonding interaction with Thr-766, Thr-830 or Met-769 made by compounds **(4a-d)** may have increased their binding specificity and hence increased their activity over compounds **(4e-g**) which forming hydrogen bond only with Thr-830 or Asp-831. The predicted binding pose of compound **(C)** form a hydrogen bonding interaction with the carboxyl group of Glu-738 (Figure 11) which may also increased binding specificity in the active site.

CONCLUSION

New 5-arylidene thiazolidinone derivatives had been synthesized and evaluated for their potential as antitumor lead compounds, depending on the previous reported antitumor activity for numerous compounds having benzothiazole scaffolds. These compounds were tested in vitro on human breast MCF-7 and non-small cell lung A549 cancer cell lines which showed potent antitumor activity in the micro molar level. Doxorubicin and 2-(4-aminophenyl)-benzothiazole were used as a reference standards.

The antitumor screening revealed that benzothiazole-6-carboxylic acid ethyl ester derivatives **(4a-d)** were more potent than benzothiazole ones (**4e-g)** due to the ester group enhance lipophilicity. Compound (**4a)** was found to be

the most active compound due to the presence of ester group in position 6 of benzothiazole ring. Molecular docking studies for the synthesized compounds were performed and confirmed their biological activity. The docked inhibitors (compounds **4a,b,e** & **C**) made hydrophobic interactions with the side chains of hydrophobic residues embedded in the active site of EGFR which is consistent with the hydrophobic interactions made by the known inhibitor 4AQ. The docking studies against 1M17 revealed that: all the active compounds showed a low docking score energy with the receptor.

Experimental part

Chemistry

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded as films on NaCl plates using a Nicolet 550 Series II Magna FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker Avance III 400 MHz for ^{1}H and 100 MHz for ^{13}C (Bruker AG, Switzerland) with BBFO Smart Probe and Bruker 400 AEON Nitrogen-Free Magnet, Faculty of Pharmacy, Beni Suef University, Egypt in DMSO-d_6 with TMS as the internal standard, where *J* (coupling constant) values are estimated in Hertz (Hz) and chemical shifts were recorded in ppm on δ scale. Mass spectra (MS) were recorded on Hewlett Packard 5988 spectrometer. Microanalyses for C, H and N were carried out on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the Micro analytical unit of Cairo University, Egypt and all compounds were within ± 0.4% of the theoretical values. All other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification**.** Compounds **(1a,b)**, (**2a,b)** and (**3b)** were prepared according to the reported procedures [24,28-31].

General procedure for synthesis of 2-(4-oxo-4, 5-dihydro-thiazol-2-ylamino)-benzothiazole derivatives 3a,b: A solution of compound (**2a** or **2b**, 5 mmol) and ammonium thiocyanate (0.7 gm; 10 mmol) in 20 mL of ethanol was heated under reflux for 3 h. and allowed to stand for 24 h. The light brown precipitate was filtered, washed with water and then crystallized from the appropriate solvent.

2-(4-oxo-4, 5-dihydro-thiazol-2-ylamino)-benzothiazole-6-carboxylic acid ethyl ester (3a)

65% yield; brown crystals; mp 228-230 ^{o}C (crystallized from chloroform); IR (KBr disc) 3130 (NH), 3050 (C-H aromatic), 2930 (C-H aliphatic), 1704 (C=O), 1556 (C=N) cm^{-1}; ^{1}HNMR (CDCl$_3$-d_6): δ 1.44 (t, *J* = 7.2 Hz, 3H, C*H*$_3$), 3.98 (s, 2H, C*H*$_2$), 4.43 (q, *J* = 7.2 Hz, 2H, C*H*$_2$CH$_3$), 7.88 (d, *J* = 8 Hz, 1H, benzothiazole H-6), 8.15 (d, *J* = 8 Hz, 1H, benzothiazole H-7), 8.52 (s, 1H, benzothiazole H-4), NH not observed; EIMS (m/z): 321 (M$^{+\cdot}$, 100%), 323 (M^{+}2, 11.23%); Anal.calcd. for $C_{13}H_{11}N_3O_3S_2$: C, 48.58; H, 3.45; N, 13.08. found: C, 48.88; H, 3.74; N, 12.98.

General procedure for synthesis of compounds 4a-g: To a solution of compound (**3a** or **3b**, 4 mmol) and anhydrous sodium acetate (0.7 gm; 8 mmol) in glacial acetic acid (35 mL), was added the respective aromatic aldehydes (6 mmol). The mixture was heated under reflux for 10 h. and then poured into ice-cold water. The precipitate was filtered and crystallized from acetic acid. Physical and spectral data are listed below.

2-(4-Oxo-5-thiophen-2-ylmethylene-4,5-dihydro-thiazol-2-ylamino)-benzothiazole-6-carboxylic acid ethyl ester (4a): 65% yield; orange red crystals; mp 226-228 ^{o}C; IR (KBr disc) 3114 (NH), 3043 (C-H aromatic), 2978 (C-H aliphatic), 1714 (C=O), 1580 (C=N) cm^{-1}; ^{1}HNMR (DMSO-d_6); δ 1.36 (t, *J* = 6.4 Hz , 3H, C*H*$_3$), 4.34 (q, *J* = 6.4 Hz, 2H, C*H*$_2$), 7.31-7.35 (m, 1H, thiophen C4-H), 7.72 (s, 1H, arylidene-H), 7.83-7.85 (m, 1H, thiophen H-3), 7.94-7.96 (m, 1H, thiophen H-5), 7.99-8.01 (m, 1H, benzothiazole H-6), 8.02-8.05 (m, 1H, benzothiazole H-7), 8.63 (s, 1H, benzothiazole H-4), 12.96 (s, 1H, NH (D_2O exchangeable)); EIMS (m/z): 415 (M$^{+\cdot}$, 16.51%), 417 (M^{+}**2**, 2.79%), 140 (100%); Anal.calcd. for $C_{18}H_{13}N_3O_3S_3$: C, 52.03; H, 3.15; N, 10.11. found: C, 51.83; H, 3.33; N, 9.95.

2-[5-(3-Methoxy-benzylidene)-4-oxo-4,5-dihydro-thiazol-2-ylamino]-benzothiazole-6-carboxylic acid ethyl ester (4b) :50% yield; yellow crystals; mp 213-215 ^{o}C; IR (KBr disc) 3121 (NH), 3050 (C-H aromatic), 2967 (C-H aliphatic), 1710 (C=O), 1576.52 (C=N) cm^{-1}; ^{1}HNMR (400 MHz, DMSO): δ 1.34 (t, *J* = 6.8Hz , 3H, CH$_2$C*H*$_3$), 3.82 (s,1H, C*H*$_3$), 4.32 (q, *J* = 6.8 Hz, 2H, C*H*$_2$CH$_3$), 7.01-7.04 (m, 1H, phenyl H-4), 7.18 (s, 1H, phenyl H-2), 7.21-7.23 (m, 1H, phenyl H-6), 7.44-7.46 (m, 1H, phenyl H-5), 7.61 (s, 1H, arylidene H), 7.79-7.80 (m, 1H, benzothiazole H-6), 7.95-7.97 (m, 1H, benzothiazole H-7), 8.51 (s, 1H, benzothiazole H-4), NH not observed; EIMS (m/z): 439 (M$^{+\cdot}$, 24.47%), 441 (M^{+}**2**, 3.32%), 164 (100%); Anal.calcd. for $C_{21}H_{17}N_3O_4S_2$: C, 57.39; H, 3.90; N, 9.56. found: C, 57.09; H, 4.05; N, 9.45.

2-(4-Oxo-5-pyridin-3-ylmethylene-4,5-dihydro-thiazol-2-ylamino)-benzothiazole-6-carboxylic acid ethyl ester (4c): 65% yield; reddish yellow crystals; mp 265-267 ^{o}C; IR (KBr disc) 3177 (NH), 3043 (C-H aromatic), 2976 (C-H aliphatic), 1709 (C=O), 1574 (C=N) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 1.35 (t, J = 6.8 Hz , 3H, CH_3), 4.34 (q, J = 6.8 Hz, 2H, CH_2) 7.59-7.62 (m, 1H, pyridine H-5), 7.76 (s,1H, arylidene H), 7.92-7.93 (m, 1H, pyridine H-6), 8.00-8.02 (m, 1H, benzothiazole H-6), 8.04-8.06 (m, 1H, benzothiazole H-7), 8.60-8.62 (m, 1H, pyridine H-4), 8.64 (s, 1H, pyridine H-2), 8.89 (s, 1H, benzothiazole H-4) ,NH not observed; EIMS (m/z): 410 (M$^{+\cdot}$, 14.62%), 80 (100%); Anal.calcd. for $C_{19}H_{14}N_4O_3S_2$: C, 55.60; H, 3.44; N, 13.65. found: C, 55.30; H, 3.74; N, 13.93.

2-(5-Furan-2-ylmethylene-4-oxo-4,5-dihydro-thiazol-2-ylamino)-benzothiazole-6-carboxylic acid ethyl ester (4d): 65% yield; black crystals; mp 279-280 ^{o}C; IR (KBr disc) 3100 (NH), 3050 (C-H aromatic), 2979 (C-H aliphatic), 1708 (C=O), 1568 (C=N) cm^{-1}; ^{1}HNMR (400 MHz, DMSO): δ 1.34 (t, J = 6.8 Hz , 3H, CH_3), 4.35 (q, J = 6.8 Hz, 2H, CH_2), 6.78-6.80 (m, 1H, furan H-4), 7.13-7.18 (m, 1H, furan H-5), 7.59 (s, 1H, arylidene H), 7.91-7.92 (m, 1H, furan H-3), 8.00-8.02 (m, 1H, benzothiazole H-6), 8.12-8.15 (m, 1H, benzothiazole H-7), 8.61 (s, 1H, benzothiazole H-4), 13.01 (s, 1H, NH (D_2O exchangeable)); EIMS (m/z): 399 (M$^{+\cdot}$, 52.73%), 53 (100%); Anal.calcd. for $C_{18}H_{13}N_3O_4S_2$: C, 54.12; H, 3.28; N, 10.52. found: C, 54.22; H, 3.55; N, 10.30.

2-(Benzothiazol-2-ylamino)-5-(3-methoxy-benzylidene)-thiazole-4-one (4e): 60% yield; yellow crystals; mp 223-225 ^{o}C; IR (KBr disc) 3126 (NH), 3050 (C-H aromatic), 2986 (C-H aliphatic), 1719 (C=O), 1578 (C=N) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 3.85 (s, 3H, CH_3), 7.09-7.10 (m, 1H, phenyl H-4), 7.27 (s, 1H, phenyl H-2), 7.28-7.29 (m, 1H, phenyl H-6), 7.36-7.39 (m, 1H, phenyl H-5), 7.46-7.49 (m, 1H, benzothiazole H-5), 7.50-7.54 (m, 1H, benzothiazole H-6), 7.76 (s, 1H, arylidene H), 7.88-7.89 (m, 1H, benzothiazole H-4), 8.00-8.02 (m, 1H, benzothiazole H-7), 12.99 (s, 1H, NH (D_2O exchangeable)); ^{13}CNMR (DMSO-d_6): 55.74, 116.00, 116.80, 122.20, 122.66, 124.98, 125.14, 127.04, 130.97, 133.13, 133.69, 135.10, 151.22, 159.09, 160.11, 167.49, 168.00, 193.00; EIMS (m/z): 367 (M$^{+\cdot}$, 90.41%), 369 (M$^{+2\cdot}$, 78.08%), 56 (100%); Anal.calcd. for $C_{18}H_{13}N_3O_2S_2$: C, 58.84; H, 3.57; N, 11.44; found: C, 58.78; H, 3.76; N, 11.22.

2-(Benzothiazol-2-ylamino)-5-pyridin-3-ylmethylene-thiazole-4-one(4f): 50% yield; yellow crystals; mp 222-224 ^{o}C; IR (KBr disc) 3150 (NH), 3053 (C-H aromatic), 2918 (C-H aliphatic), 1709 (C=O), 1583 (C=N) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 7.36-7.40 (m, 1H, benzothiazole H-5), 7.49-7.52 (m, 1H, benzothiazole H-6), 7.61-7.64 (m, 1H, pyridine H-5), 7.82 (s, 1H, arylidene H), 7.94-7.98 (m, 1H, pyridine H-6), 8.01-8.03 (m, 1H, benzothiazole H-4), 8.07-8.09 (m, 1H, benzothiazole H-7), 8.65-8.66 (m, 1H, pyridine H-4), 8.91 (s, 1H, pyridine H-2), 13.04 (s, 1H, NH (D_2O exchangeable)); EIMS (m/z): 338 (M$^{+\cdot}$, 25.89%), 340 (M^{+}**2**$^{\cdot}$, 2.56%), 135 (100%); Anal.calcd. for $C_{16}H_{10}N_4OS_2$; C, 56.79; H, 2.98; N, 16.56. found: C, 56.49; H, 2.70; N, 16.50.

2-(Benzothiazol-2-ylamino)-5-furan-2-ylmethylene-thiazole-4-one (4g): 55% yield; black crystals; mp 253-255 ^{o}C; IR (KBr disc) 3123 (NH), 3045 (C-H aromatic), 2954 (C-H aliphatic), 1686 (C=O), 1577 (C=N) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 6.77-6.80 (m, 1H, furan H-4), 7.12-7.14 (m, 1H, furan H-5), 7.33-7.37 (m, 1H, benzothiazole H-5), 7.46-7.50 (m, 1H, benzothiazole H-6), 7.59 (s, 1H, arylidene H), 7.87-7.89 (m, 1H, furan H-3), 7.97-7.99 (m, 1H, benzothiazole H-4), 8.12-8.14 (m, 1H, benzothiazole H-7), 12.81 (s, 1H, NH (D_2O exchangeable)); ^{13}CNMR (DMSO-d_6): 114.10, 119.44, 121.54, 122.07, 122.53, 124.83, 126.93, 133.70, 148.13, 150.09, 151.36, 160.12, 162.76, 167.34, 168.81; EIMS (m/z): 327 (M$^{+\cdot}$, 63.95%), 329 (M$^{+2\cdot}$, 69.77%), 176(100%); Anal.calcd. for $C_{15}H_9N_3O_2S_2$: C, 55.03; H, 2.77; N, 12.84. found: C, 55.03; H, 2.77; N, 12.56.

Pharmacological studies

Cell Culture

The two human cancer cell lines used in this study breast carcinoma (MCF-7) and Non Small-Cell Lung Cancer (A549) were purchased from American Type Culture Collection. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM: Gibco, USA) supplemented with 10% fetal bovine serum (Gibco), penicillin/streptomycin (Gibco).4',6-Diamidino-2-phenylindole (DAPI) nuclear staining indicated that these cells were devoid of mycoplasma contaminations. All incubations were done at 37°C in a humidified incubator containing 5% CO_2. Cells in log-phase growth were harvested by trypsinization for use in various assays.

Cell Viability Analysis

Cell viability was assessed using MTT assay (Sigma-Aldrich, St. Louis, MO, USA) in 6 replicates as described previously [32].In brief, a total of 1×10^4 cells per well were seeded into 96-well tissue culture plates in DMEM containing 10% FBS to a final volume of 0.2 ml. The cells were subjected to different treatments 24 h post seeding.

Following the incubation for 48 h with doxorubicin (positive control), test drugs or vehicle (DMSO), the media were removed, replaced by 200 µL DMEM containing 0.5 mg/mL of MTT and cells were incubated for 2 h. Next, the supernatants were removed and the precipitated formazan was dissolved by adding 200 µl of DMSO. Absorbance at 570 nm was determined using a microplate reader (Model 450 Mioroplate Reader; Bio-Rad).Results were calculated by subtracting blank readings.

Molecular modeling

Preparation for docking

The crystal structures of erlotinib bound at EGFR (PDB: ID 1M17) (21) active site (obtained from protein data bank at research collaboration for structural Bioinformatics (RSCB) protein database [PDB].

Validation of docking procedure

Docking of the co-crystallized ligand should be carried out to study the scoring energy (s), root mean standard deviation (rmsd) and amino acid interactions. The root mean square deviation (RMSD) which is measure of superposing was 0.50Aº for the lead compound. Docking was performed using London dG force and refinement of the results was done using Force field energy.

Preparing compounds for docking

Preparation of the synthesized compounds for docking was achieved *via* their 3D structure built by MOE. Certain procedures should be taken before docking which include: 3D protonation of the structures, running conformational analysis using systemic search, selecting the least energetic conformer and applying the same docking protocol used with ligand.

The previous measures were taken and docking for the synthesized compounds was applied. Amino acid interactions and the hydrogen bond lengths were summarized in Table 2.

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