Design, Synthesis and in Vitro Cytotoxic Activity of New 4-Anilino-7-Chloro Quinoline Derivatives Targeting EGFR Tyrosine Kinase

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Abstract: Four new 4-anilino-7-chloroquinoline derivatives substituted at position 4 of the anilino moiety with various bioisosteric heterocycles have been designed and synthesized. Virtual screening was carried through docking the designed compounds into the ATP binding site of the epidermal growth factor receptor (EGFR) to predict if these compounds have similar binding mode to the EGFR inhibitors. The newly synthesized compounds were also tested in vitro on human breast carcinoma cell line (MCF-7) in which EGFR is highly expressed. Most of the tested compounds exploited potent cytotoxic activity with IC_{50} values in the nanomolar range in particular compounds II, IVd, Va, Vc, VIa and VII which displayed the highest activity among the tested compounds with IC_{50} equal to 5.70, 5.37, 0.87, 5.10, 1.41 and 2.75 nM respectively. [Journal of American Science. 2010;6(10):73-83]. (ISSN: 1545-1003).

Keywords: Anilinoquinoline, EGFR tyrosine kinase inhibitor-, cytotoxic activity, Docking study.

Introduction

Protein tyrosine kinases are enzymes that provide a central switch mechanism in cellular signal transduction pathways. They are involved in many cellular processes such as cell proliferation, metabolism, survival and apoptosis. Several protein tyrosine kinases are known to be activated in cancer cells and to drive tumor growth and progression. Blocking tyrosine kinase activity therefore represents a rational approach to cancer therapy[1]. Protein kinases (PTK_s) catalyze the phosphorylation of tyrosine and serine / threonine residues in various proteins involved in the regulation of all functions they can be broadly classified as receptor such as EGFR, or non receptor kinases. Inappropriate or uncontrolled activation of many of these kinases, by over expression, constitutive activation, or mutation, has been shown to result in uncontrolled cell growth[2]. The epidermal cell growth factor receptor belongs to the family of transmembrane growth factor receptor PTK_s. The EGFR and c-erb B_2 PTK_s have been identified as interesting targets for medicinal chemistry programs especially in cancer therapy[3-6].

Over expression of these receptors is found in a number of cancers (e.g. breast, ovarian, colon and prostate), their expression levels often correlate with vascularity and has been associated with poor prognosis in patients[7,8]. Inhibition of the EGFR PTK are therefore expected to have great therapeutic potential in the treatment of malignant and non malignant epithelial diseases. Drug discovery efforts have targeted this aberrant kinase activity in cancer and many other diseases[9].Advances in the identification of kinase inhibitors have created hope for the modulation of uncontrolled cell growth in cancer therapy for solid tumor[10]. This strongly suggests that these targets represent drug intervention opportunities due to pivotal role in governing cellular proliferation, survival and metastasis.

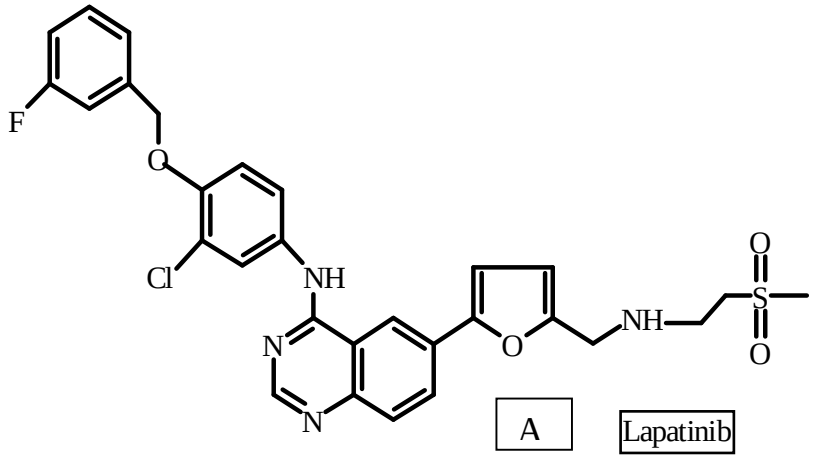
A great number of different structural classes of tyrosine kinase inhibitions which have been reported and reviewed[3-5]. The most promising molecule selective EGFR-TK inhibition includes 4-anilinoquinazoline and its bioisosteric 4-anilinoquinolines.

Several researches including synthesis and biological evaluation on 4- anilinoquinolines as potent inhibitors of epidermal growth factor receptor (EGFR)[11]

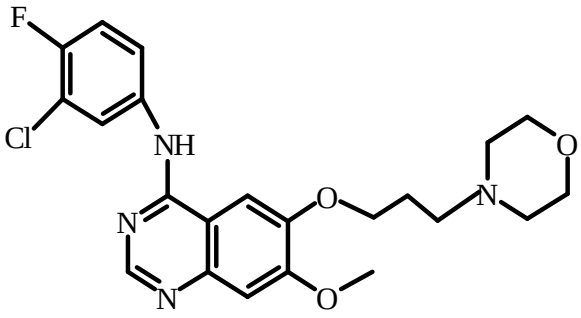
as well as 3D QSAR and docking studies on 4-anilinoquinazoline and 4- anilinoquinoline epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor[12.]

Fig 1 includes some examples that are currently approved drugs or in clinical trials[8].

Figure 1: EGFRTK inhibitors



A Lapatinib



B Gefitinib (Iressa)

In recent years research has led to the discovery of highly selective compounds that target EGFR. These compounds act via competing with ATP for tyrosine kinase. Later on a great structural variety of compounds of structurally diverse classes have proved to be highly potent and selective ATP competitive inhibitors. 4-anilino-quinoline derivatives provide the necessary binding properties for inhibition of the E and B family of tyrosine as they mimic the adenine portion of ATP[13-14]. Intensive research in the area of tyrosine kinase inhibitors led to development of enormous number of active compounds[15-20]. In the same direction and in continuing effort to find more potent selective lead compound, herein, we describe the design and synthesis of four series of 4-anilino-quinoline derivatives as possible antitumor agents that may act through EGFR inhibition.

Thus a new sub-family of compounds containing 4-anilino-quinoline core as promising potent and selective EGFR inhibitors were synthisized Our strategy is directed toward designing a variety of ligands with diverse chemical properties hypothesized that the potency of the molecule might be enhanced by adding alternative binding group such as arylidene hydrazinocarbonyl, oxadiazoline or carbamoyl and thiocarbamoyl in the anilino moiety. In this way such substitution pattern could largets different regions of the protein kinase domain to create differentially selective molecules. Also after discovery of lapatinib, it was claimed that 4-position of the aniline can tolerate a lot of bulky substituents this lead to fundamental change in the pharmacophore in such a way that the bulky group could be oriented deep in the back of the ATP binding site and make predominantly hydrophobic interaction with the protein. The binding mode and docking energy of the designed compounds in EGFR homology model could be helpful tool for prediction their mechanism of antitumor activity.

Chemistry:

General synthesis for target compounds are depicted in scheme I. The acid hydrazide III was synthesized from the corresponding 4-(p-carbethoxyanilino)-7-chloroquinoline obtained by reacting 4,7-dichloroquinoline I with p-aminoethylbenzoate, in absolute ethanol to yield II, reacting II with hydrazine hydrate in absolute ethanol gives the target key compound III. The hydrazone derivatives IV (a-f) were obtained via the reaction of III with different aromatic aldehydes. The oxadiazoline V (a-c) were obtained by cyclizing IV (a,b and c) with acetic anhydride sodium acetate mixture. The thiocarbamoyl VI (a-d), the carbamoyl VIe as well as the 2-mercaptooxadiazol VII were obtained by reaction between III and isothiocyanate or isocyanate and CS_2/KOH respectively.

The structure of the synthesized compounds were confirmed by elemental analysis, IR, ^{1}HNMR and mass spectroscopy.

Experimental part:

Melting points were determined with a Gallenkamp (London, U.K.) melting point apparatus and are uncorrected.IR spectra (KBr,cm^{-1}) were recoreded on Bruker Vector, 22 FT-IR (Fourier Transform Infrared (FTIR)) (Germany) spectrometer. ^{1}HNMR spectra were recorded on a Varian Gemini-200 (200-MHz, Foster City Calif., USA) and Varian Mercury-300 (300-MHz, City: Palo Alto, State: Calif.,USA spectrometers using dimethylsulphoxide (DMSO)-d_6 as a solvent and tetramethylsilane *(TMS)* as an internal standard (Chemical shift in δ, ppm). Mass spectra were determined using Mass spectrometers GC/MS Shimadzu QP 1000 EX (Shimadzu Corporation, Tokyo, Japan) with ionization energy 70 ev. Elemental analyses were determined using Automatic Elemental Analyzer CHN Model 2400 Perkin Elmer (USA) at Microanalytical Center, Faculty of Science, Cairo University, Egypt. All the results of elemental analyses corresponded to the calculated values within experimental error. Progress of the reaction was monitored by thin-layer chromatography (TLC) using precoated TLC sheets with Ultraviolet (UV) fluorescent silica gel (Merck 60F254) and spots were visualized by iodine vapors or irradiation with UV ligh (254 nm). All the chemicals were purchased from *Sigma-Aldrich*.

4-(p-Carbethoxyanilino)-7-chloroquinoline II:

4,7-dichloroquinoline I (0.01 mol) was dissolved in absolute ethanol and poured on to a solution of p-aminoethylbenzoate (0.01 mol) in 10% HCl while stirring at room temperature, set aside for 30 minutes render alkaline with ammonia solution and diluted with water.

The obtained precipitate was filtered and crystallized from ethanol yield 73%, m.p. 230°C ^{1}HNMR (DMSO-d_6): 1.21 – 1.31 (t, 3H, CH_3), 4.19 – 4.28 (q, 2H, $C\underline{H}_2$ – CH_3), 9.2 (s, 1H, NH), 7-8.6 (m, 9H aromatic). Mass m/z. 326 Anal. Calcd for $C_{18}H_{15}N_2ClO_2$:C, 59.5; H, 4.1; N, 8.5. Found: C, 60; H, 4.5; N, 8.2.

4-(p-hydrazinocarbonylanilino)-7-chloroquinoline III.

4-(p-carbethoxyanilino)-7-chloroquinoline II (0.01 mol) was dissolved in absolute ethanol. Hydrazine hydrate (0.01 mol) was added and the mixture was refluxed for 4 hours (T.L.C)

concentrated in vacuo, few drops of water were added and the crarny while precipitate obtained was filtered and crystallized from ethanol-H_2O. Yield: 85%, m.p. 180°C, ^{1}HNMR (DMSO – d_6) 6.5 – 8.6 (m, 11H, aromatic + NH_2) 9.2, 10.2 (s, 2H, 2NH). IR (KBr, cm^{-1}) 1690 (C=O) 3300, 3600 (NH, NH_2). MS m/z: 314 (M^{+2}) Anal. Calcd for $C_{16}H_{13}N_4ClO$: C, 61.4; H, 4.1; N, 17.9. Found C,61.5, H, 4.00, N, 17.37.

4-[4-(Bromobenzylidenehydrazinocarbonyl)anilino]-7-chloro-quinoline IVa.

Procedure:

A mixture of III (0.01 mol) and p-bromobenzaldehyde (0.01 mol) in absolute EtOH and 2 drops of glacial acetic acid was refluxed for 3 hours. The solvent was removed under reduced pressure and the residue was crystallized from DMF/H_2O. to give IVa. Yield 75%, m.p. 145°C, ^{1}HNMR (DMSO – d_6) (300 MH_z) : 7.2 – 8.9 (m, 13 H aromatic + H ald), 10.2, 10.7 (2s, 2H, 2NH). IR (KBr) cm^{-1} 3300 (NH), 1690 (C=O). MS m/z 480 Anal. Calcd for $C_{23}H_{16}N_4Br\,ClO$: C, 57.5; H, 3.5; N, 11.6. Found: C, 57.2; H, 3.2, N, 11.17.

4-[4(Chlorobenzylidenehydrazinocarbonyl)anilino]-7-chloro-quinoline IVb.

Procedure:

The same procedure used for IVa using p-chlorobenzaldehyde

Yield 85%, m.p. 125°C, ^{1}HNMR (DMSO – d_6) (300 MH_Z) 7.4 – 9.1 (m, 13 H, aromatic + H ald), 10.5, 11.2 (2s, 2H, 2NH). IR (KBr) cm^{-1} 3310 (NH), 1695 (C=O). Anal. Calcd for $C_{23}H_{16}N_4Cl_2O$: C, 63.5; H, 3.6; N, 12.9 Found: C, 63.3; H, 3.9; N, 12.71.

4-[4-(4-Methoxy-3-hydroxybenzylidenehydrazinovarbonyl) anilino] -7-chloroquinoline IVc

Procedure:

The same procedure used for IVa using vanilline Yield 80%, m.p. 215°C, ^{1}HNMR (DMSO – d_6) (300 MH_z): 3.9 (s, 3H, OCH_3), 7.2 – 8.6 (m, 13H aromatic + H ald), 10, 11.9, 12 (3s, 2H, 2NH H, OH). IR (KBr) cm^{-1} 3450, 3300 (OH, NH), 1690 (C=O). Anal. Calcd for $C_{24}H_{19}N_4ClO_3$: C, 64.5; H, 4.2; N, 12.5 Found: C, 64.7; H, 4.9; N, 12.2.

4-[4-(4-Methoxybenzylidenehydrazinocarbonyl)-anilino]-7- chloroquinoline IVd:

Procedure:

The same procedure used for IVa using annisaldehyde Yield 75%, m.p. 203°C, ^{1}HNMR (DMSO – d_6) (300 MH_z): 4 (2s, 3H, OCH_3), 7.4 – 8.6 (m, 13H aromatic + H ald) 9.1 (s, 1H ald), 10, 11.2, (s, 2H, 2NH). IR (KBr) cm^{-1} 3290 (NH), 1670 (C=O). MS m/z 430.15. Anal. Calcd for $C_{24}H_{19}N_4ClO_2$: C, 66.8; H, 4.4; N, 13 Found: C, 66.5; H, 4.1; N, 13.61.

4-[4-(4,4-dimethylaminobenzylidenehydrazinocarbonyl) anilino]-7-chloroquinoline IVe:

Procedure**:**

The same procedure used for IVa using 4,4-dimethylaminobenzaldehyde.

Yield 70%, m.p. 230°C, ^{1}HNMR (DMSO – d_6) (300 MH_z) 3.9 (s, 6H, $2CH_3$), 6.7 – 8.5 (m, 13H aromatic + H ald), 9.3, 11.4 (2s, 2H, 2NH). IR (KBr) cm^{-1} 3320, 3300 (2NH), 1650 (C=O). Anal. Calcd for $C_{25}H_{22}N_5ClO$: C, 67.3; H, 4.9; N, 15.7 Found: C, 67.6; H, 4.5; N, 15.1.

4-[4-(4,4-Hydroxybenzylidenehydrazinocarbonyl)anilino]-7-chloroquinoline IVf:

Procedure:

The same procedure used for IVa using p-hydroxybenzaldehyde

Yield 60%, m.p. 166°C, ^{1}HNMR (DMSO – d_6) (300 MH_z)7.2 – 9 (m, 13H aromatic + H ald), 10.5, 11.8, 12 (3s, 2H, NH, H, OH). IR: 3450, 3300 (OH, NH), 1690 (C=O). Anal. Calcd for $C_{23}H_{17}N_4ClO_2$: C, 66.2; H, 4.0; N, 13.4 Found: C, 65.9; H, 4.2; N, 13.19.

]4-[4-(4-Acetyl-5-(4-bromophenyl)–4,5-dihydro,3,4-oxadiazol-2-yl)-anilino]-7-chloroquinoline Va:

A mixture of IVa (0.01 mol) acetic anhydride (5 ml) and anhydrous sodium acetate (0.1 mol) was refluxed for 1 hour. The solution was poured onto ice cold H_2O and the solid separated was cryslallized from ethanol. Yield 55%, m.p. 290°C, ^{1}HNMR (DMSO – d_6) 2.2 (s, 3H, $COC\underline{H}_3$) 6.6 (s, H, CH) 7.5 – 8.4 (m, 13H aromatic), 8.8 (s, 1H, NH). IR: (KBr) cm^{-1} 3360 (NH), 1760 (C=O) 1600 (C = N). MS m/z 520. Anal. Calcd for $C_{25}H_{18}N_4BrClO_2$: C, 57.5; H, 3.4; N, 10.7 Found: C, 57.2; H, 3.1; N, 10.2.

4-[4-(4-Acetyl-5-(4-chlorophenyl)–4,5-dihydro,3,4-oxadiazol-2-yl)-anilino]-7-chloroquinoline Vb:

The same procedure as Va using IVb. Yield 50%, m.p. 245°C, 1HNMR (DMSO – d6) (300 MHz) 2.3 (s, 3H, CO<u>CH3</u>), 6.8, (s, H, CH) 7.6 – 8.5 (m, 13H aromatic), 8.8 (s, 1H, NH). IR (KBr) cm-1: 3360 (NH), 1760 (C=O), 1605 (C = N). MS m/z. Anal. Calcd for C25H18N4Cl2O2: C, 63.1, H, 3.7; N, 11.7 Found: C, 63; H, 3.2; N, 11.5.

4-[4-(4-Acetyl-5-(4-N,N-dimethylaminophenyl)–4,5-dihydro,3,4-oxadiazol-2-yl)-anilino]-7-chloroquinoline Vc:

The same procedure as Va using IVe. Yield 60%, m.p. > 300°C, ^{1}HNMR (DMSO – d_6) (300 MH_z) 2.3 (s, 3H, $COCH_3$), 3.7, (s, 6H, $2CH_3$), 6.6 (s, H, CH), 6.9 – 8.6 (m, 13H aromatic), 9.9 (s, H, NH). IR (KBr) cm^{-1}: 3350 (NH), 1730 (C=O), 1590 (C = N). MS m/z. 484.5, Anal. Calcd for $C_{27}H_{24}N_5ClO_2$: C, 66.3, H, 4.9; N, 14.4. Found: C, 66.8; H, 4.3; N, 14.1.

4-[4-N-Ethylthiocarbamoylhydrazinocarbonyl)-anilino]-
7- chloroquinoline VIa: chloroquinoline VIa:

General procedure:

To a solution of III (0.01 mol) in abs EtOH was added ethylisothiocyanate (0.01 mol) and the mixture was left over night at room temperature. The solvent was removed under reduced pressure and the residue was crystallized from DMF / H_2O. Yield 83%, m.p. 115°C, ^{1}HNMR (DMSO – d_6) (300 MH_z) 1.2 (t, CH_2CH_3), 4.2 (q, CH_2CH_3), 7.6 – 9.3 (m, 9H, aromatic + 1H NH), 9.2 (s, 1H, NH) 9.8 (s, 1H, NH). 10.2 (s, 1H, NH). IR (KBr cm^{-1}) 3400 (NH), 1678 (C=O), Anal. Calcd for $C_{19}H_{18}N_5ClOS$: C, 57.1, H, 4.5; N, 17.5 Found: C, 57.4; H, 4.1; N.,17.2

4-[4-N-Phenylthiocarbamoylhydrazinocarbonyl)-anilino]-
7-chloroquinoline VIb:

Procedure:

The same used for VIa using phenylisothiocyanat.Yield 78%,m.p.122°C,^{1}HNMR (DMSO-d_6) (300 MH_z) 7.2 – 8.6 (m, 14H aromatic + 1H NH), 9.8 (s, 1H, NH) 10.6 (s,1H, NH). 11.2 (s, 1H, NH). IR (KBr cm^{-1}) 3310 (NH), 1672 (C=O), Anal. Calcd for $C_{23}H_{18}N_5ClOS$: C, 61.6, H, 4.0; N, 17.6 Found: C, 61.3; H, 3.97; N, 17.2.

4-[4-N-Allylthiocarbamoylhydrazinocarbonyl)-anilino]-
7-chloroquinoline VIc:

Procedure:

The same used for VIa using allylisothiocyanate Yield 70%, m.p. 118°C, ^{1}HNMR (DMSO – d_6) (300 MH_z) 5.2 (m, 5H allyl), 7.2 -8.6 (m, 9H, aromatic + 1H NH), 9.2, 9.4, 10.2 (3s, 3H, NH). IR (KBr cm^{-1}) 3267 (NH), 1678 (C=O), Anal. Calcd for $C_{20}H_{18}N_5ClOS$: C, 58.3, H, 4.3; N, 17 Found: C, 58.1; H, 4.6; N, 17.2.

4-[4-N-Cyclohexylthiocarbamoylhydrazinocarbonyl)-anilino]-
7-chloroquinoline VId:

Procedure:

The same used for VIa using cyclohexylisothiocyanate instead of ethylisothiocyanate.Yield65%,m.p.210°C,^{1}HNMR (DMSO–d_6) (300 MH_z) 1.2 – 1.7 (m, 10H cyclohexyl), 4 – 4.2 (m, 1H, cyclohexyl) 7.6 – 8.6 (m, 9H aromatic + 1H NH), 9.8, 10.6, 11 (3s, 3H, NH). IR (KBr cm^{-1}) 3260 (NH), 1672 (C=O), Anal. Calcd for $C_{23}H_{24}N_5ClOS$: C, 60.9; H, .5.0, N, 15.4. Found: C, 60.6; H, 5.3; N, 15.1.

4-[4-N-Cyclohexylcarbamoylhydrazinocarbonyl)-anilino]-
7-chloroquinoline VIe:

Procedure:

The same used for VId using cyclohexylisocyanate. Yield 70%, m.p. 220°C, ^{1}HNMR (DMSO – d_6) (300 MH_z) 1.16 – 1.18 (m, 8H cyclohexyl), 4.0 – 4.1 (m, 1H, cyclohexyl) 7.2 - 8 (m, 9H aromatic +1HNH), 8.2, 8.6, 10 (3s, 3H, NH). IR (KBr cm^{-1}) 3280 (NH), 1670 (C=O), Anal. Calcd for $C_{23}H_{24}N_5ClO_2$: C, 63.00; H, 5.2; N, 16. Found: C, 63.2; H, 5.1; N, 15.79.

4-[4,5-Mercapro-1,3,4-oxadiazol-2-ylanilino]-7-chloroquinoline VII:

Procedure:

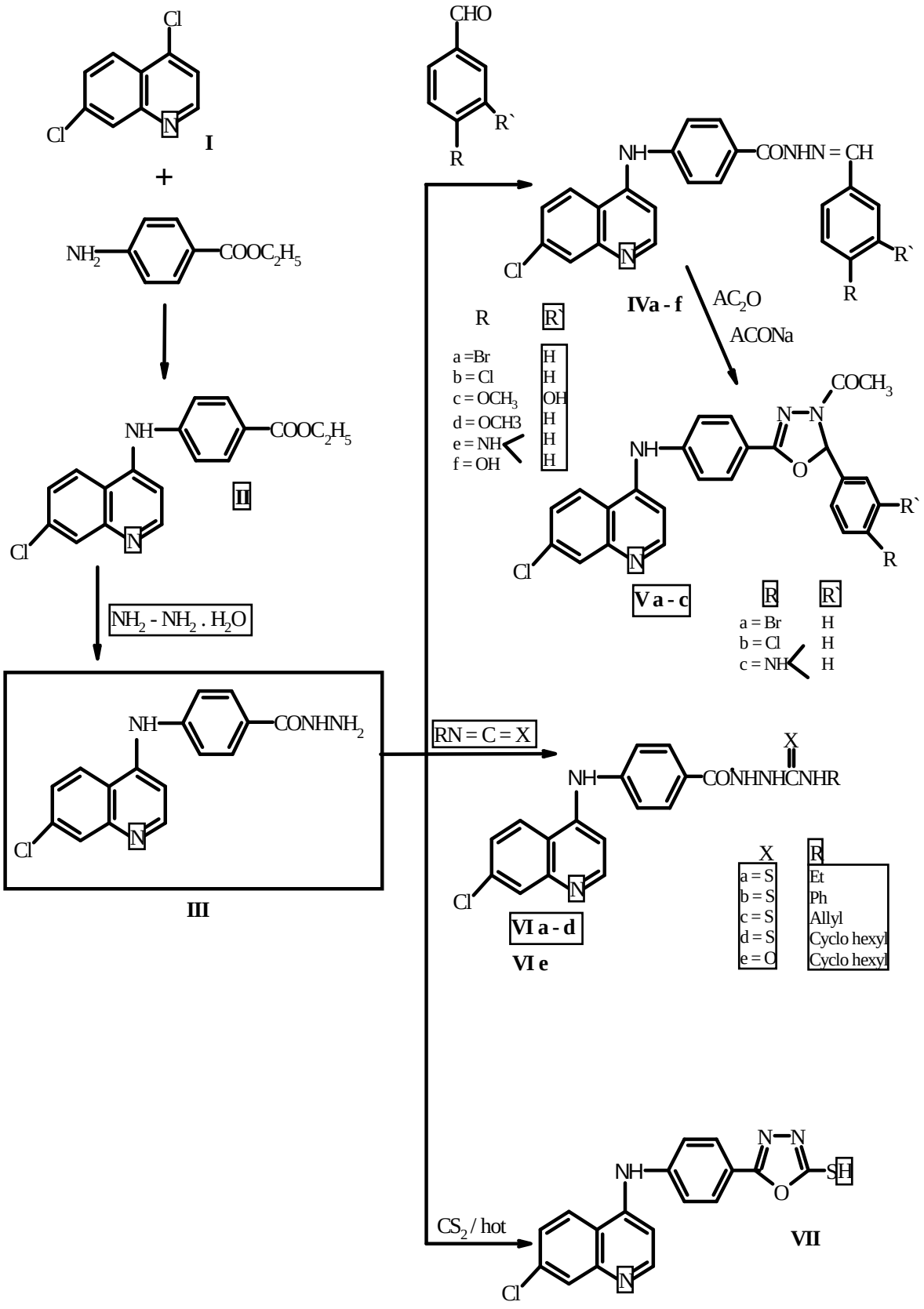
To a solution of KOH (0.01 mol) in EtOH (50 ml) was added III (0.015 mol) and CS_2 (0.01 mol). The mixture was refluxed for 48 hours with stirring. The solvent was removed under reduced pressure and the residue was dissolved in H_2O and acidified with dil HCl. The precipitate was filtered, washed with H_2O and crystallized from EtOH. Yield 65%, m.p. 235°C, ^{1}HNMR (DMSO – d_6) (300 MH_z) 3.2 (s, 1H, SH), 7.0 – 8.2 (m, 9H, aromatic), 8.8 (s, 1H, NH). IR (KBr cm^{-1}) 3427 (NH), 1610 (C = N), Anal. Calcd for $C_{17}H_{11}N_4ClOS$: C, 57.4; H, 3.1; N, 15.7 Found: C, 57.2; H, 3.3; N, 15.35.

Molecular modeling study:

Docking study was carried out for the target compounds into EGFR using SYBYL version 7.3. Tripos Inc with Malegro virtual docker program version 2007. The crystal structure of the enzyme and lapatinib (IXKK) was obtained from protein data bank PDB[21] since it was found that lapatinib which is an anilinoquinazoline derivative mimic ATP and bind to the ATP binding region of the kinase active site.

Therefore our compounds which are bioiosteres of anilino quinazoline derivatives were modeled by positioning them in the lapitinib binding site in accordance with the published crystal structures of quinazoline derivatives bound to kinase[22]. The entire complex was then subjected to alternate cycles of minimization and dynamics the intent was to get a

Scheme I: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d = OCH3
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a = S
b = S
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d = S
e = O
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Et
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Cyclo hexyl
Cyclo hexyl
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VII

satisfactory structure for the complex that was consistent with the published crystal structure[23,24].

From the comparative docking study of our compounds with many structurally related lead compounds, such as lapatinib and gefitinib we could observe how our compounds might bind to the kinase binding site. Based on a knowledge of the structure of similar active sites, we docked Lapatinib into the active site of the enzyme (fig. 2). Docking studies have revealed that Lapatinib ring bind to a narrow hydrophobic pocket in the EGFR TK domain with three hydrogen bond interaction with amino acids in vicinity while the aniline moiety lies in a deep and hydrophobic pocket. The bulky sulfamoyl group at C-4 of aniline moiety lies at the same position of the 3' chloro-4'-(3-fluorobenzyl) oxy moiety of Lapatinib ΣE recorded was equal to – 71 and RMSD equal 0.004 indicating that the ligand chosen interact with the enzyme at the same sites as do the main ligand. Compound Va was then docked into the active site of the enzyme fig. 3 revealing ΣE equal to – 93.4 and hydrogen bond with ASP 855. Compound VIa was also docked in the ATP binding site of EGER TK result of docking revealed ΣE equal to – 79.2 and showing hydrogen bond with Thr 854 we can observe that the quinoline ring as well as the aniline moiety lie in a deep and hydrophobic pocket in the EGFR as in case of the choosen lead compounds with ΣE indicating more stability compared with the lead compound Lapatinib.

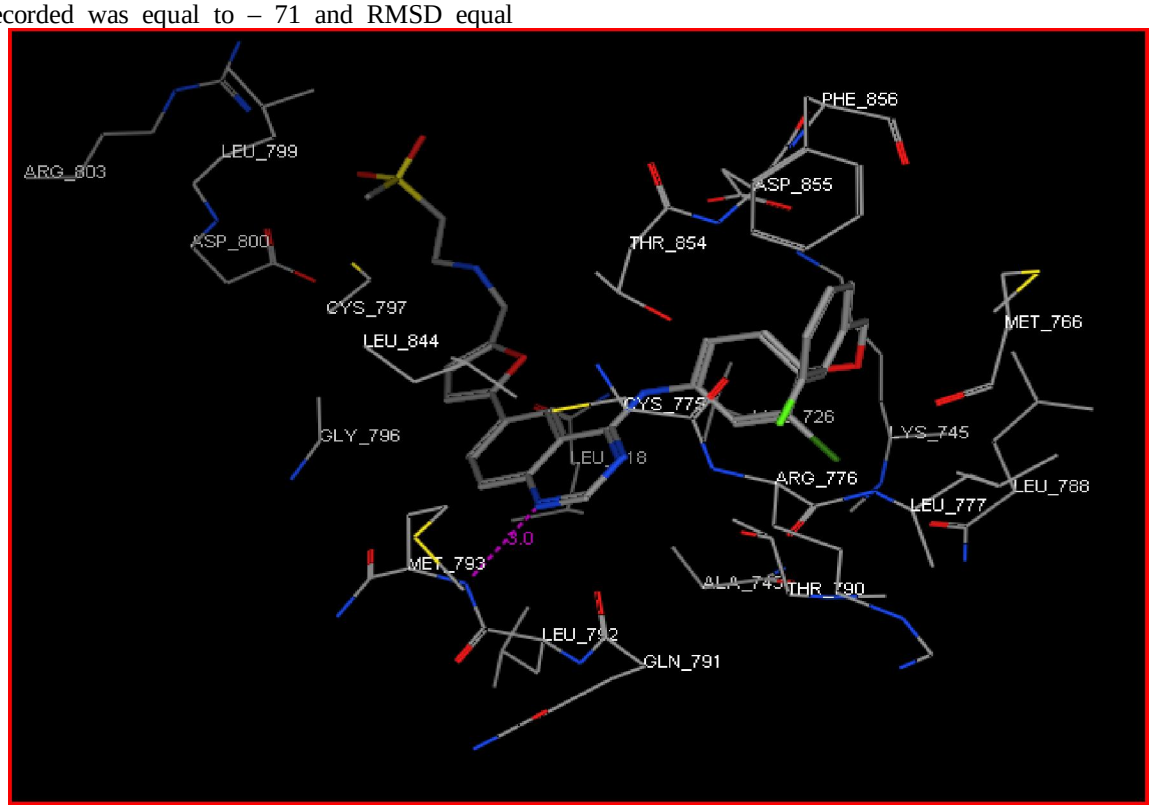

Fig. 2. Lapatinib in the ATP binding site of EGFR – TK. With ΣE equal to -71, RMSD = 0.004 with 3 HB. This picture was created with SYBYL version 7.3.

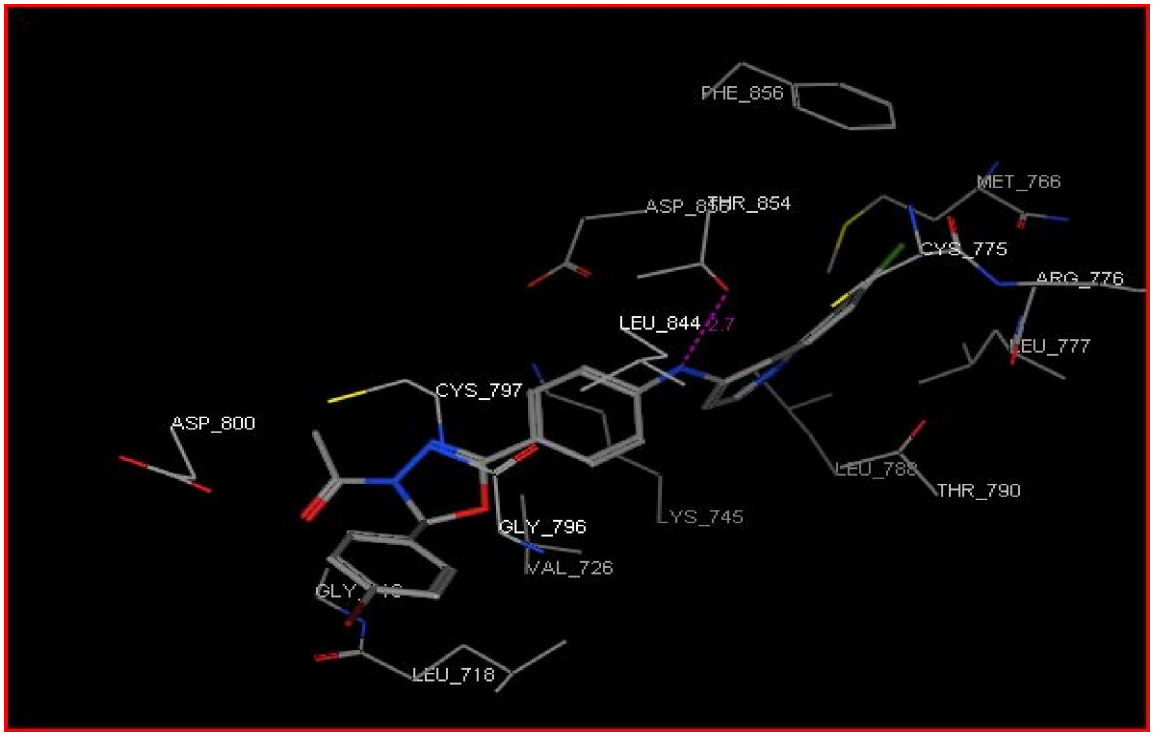

Fig. 3. Compound Va docked at the ATP binding of EGFR – TK with ΣE equal to – 93.4 the fig shows HB with ASP 855

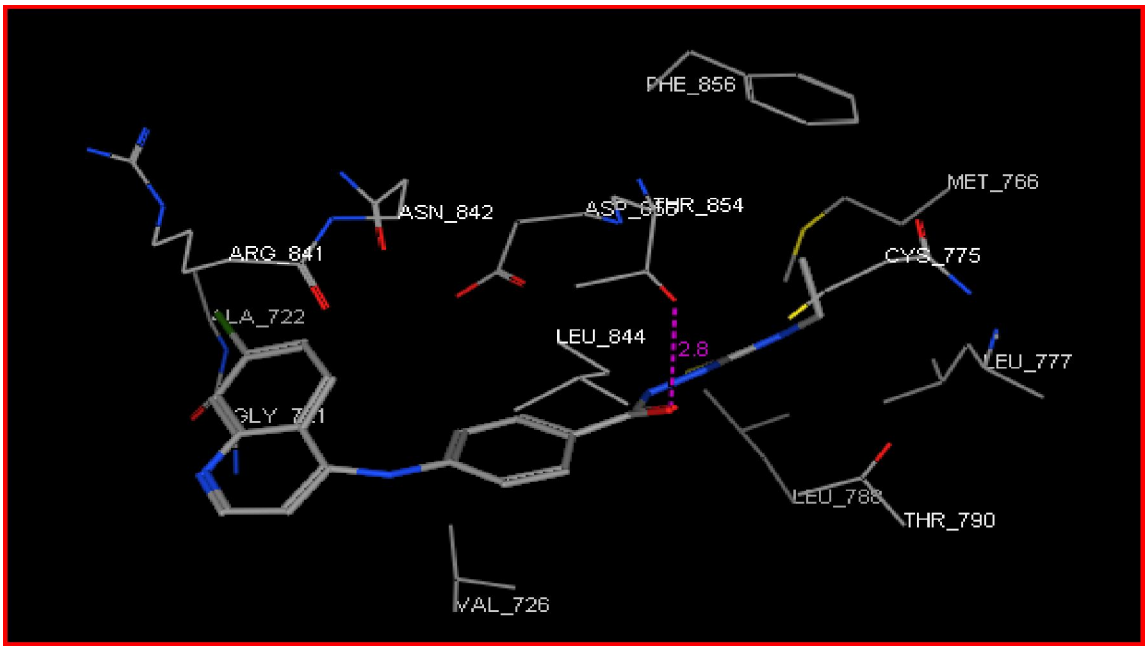

Fig. 4. Binding mode of compound VIa in the ATP binding site of EGFR – TK with ΣE equal to – 79.2 and showing 1HB with Thr 854.

Biological testing:

Material and methods:

The human tumor cell lines (MCF -7) were obtained as a gift from NCI, Merryland, USA. All chemical and solvents were purchased from Sigma – Aldrich.

Measurement of potential cytotoxicity:

The cytotixic activity was measured in vitro for the newly synthesized compounds using the sulfo-Rhodamine – B stain (SRB) assay using the method of Skehan[25] cells were plated in 96 – multiwall microtiter plate (10^4 cells / well) for 24h before treatment with the compound (s) to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volumes. Different concentration of the compound under test (0.1, 2.5, 5 and 10 µg / ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48h at 37°C and in atmosphere of 5% CO_2. After 48h, cells were fixed, washed and stained for 30 minutes with 0.4% (wt / vol) with SRB dissolved in 1% acetic acid unbound dye was removed by four washes with 1% acetic acid and attached slain 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.

Table I: In vitro cytotoxic activities of examples of the synthesized compounds against human breast cancer cell (MCF-7):

Compounds	Cytotoxicity (IC_{50})[a,b] In nM
II	5.7
III	9.87
IV d	5.37
Va	0.87
Vc	5.1
VI a	1.41
VII	2.75

[a]IC_{50}, Compounds concentration required to inhibit tumor cell proliferation by 50%.
[b]Values are means of three experiments

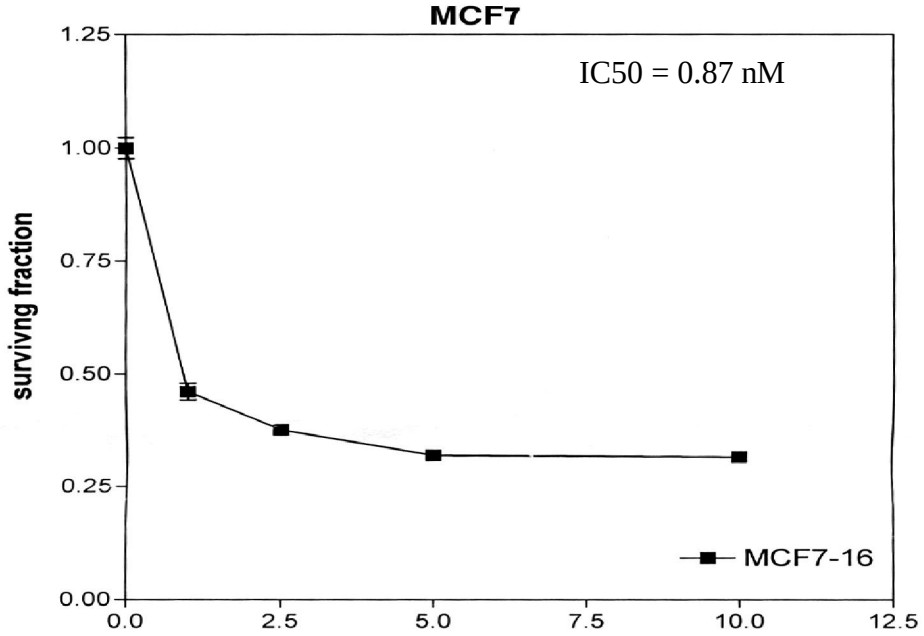

Fig. 5: Effect of different concentrations of Va on the viability of MCF-7 cell line

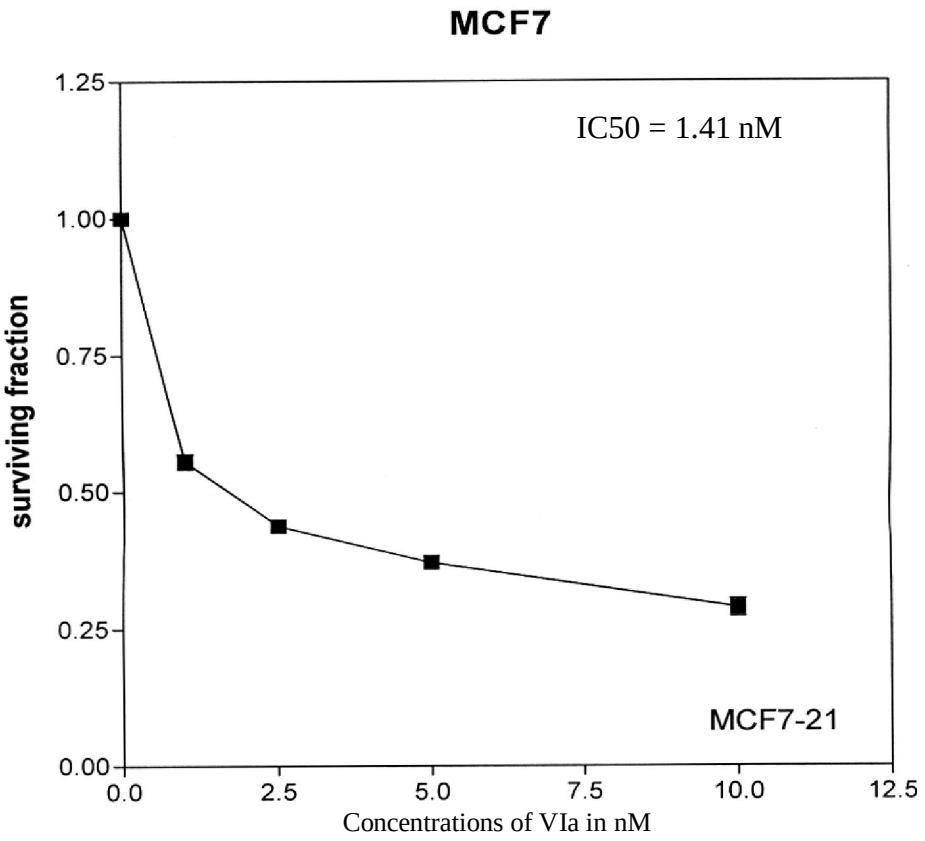

Fig. 6: Effect of different concentrations of VI a on the viability of MCF-7 cell line

Conclusion

Since breast cell are known to overexpress EGFR, which leads to continuous activation of the EGFR pathway involved in cell proliferation, therefore we measured antitumor activity of the compounds in vitro on human breast carcinoma cell line (MCF-7). Most of the tested compounds exhibited potent inhibitory activity against MCF-7 cell line.

In the anilinoqunoline series, compounds Va and VIa are the most potent ones with IC_{50} equal to 0.87 and 1.41 respectively from the biological testing results we conclude that anilinoquinoline with five membered heterocyl oxadiazoline substituted at position-2- with p-bromo phenyl moiety compound Va as well as the ethyl thiocarbamoyl anilinoquioline derivative compound VIa are the most active compounds.

These results indicate a parallel correlation between Molecular Modeling study and measurement of potential cytotoxicity as shown in compound Va & VIa ΣE equal – 93.4 and – 79.2 while IC_{50} equal 0.87 and 1.41 respectively.

These preliminary encouraging results of biological screening of the tested compounds could offer an excellent framework in this field that may lead to discovery of potent antitumor agent.

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4. References

1. Jordan J. D.; Landau E. M.; Iyengar R. Cell 2000, 103, 193.
2. Blume-Jensen P.; Hunter T. Nature 2001, 411, 355.
3. Adams, J. A. Chem. Rev. 2001, 101, 2271.

4. Yarden, Y.; Sliwkowski, M. X. Nat. Rev. 2001, 2, 127.
5. Dumas, J. Curr. Opin. Drug Discov. Develop. 2001, 4, 378.
6. Bridges, A. J. Chem. Rev. 2001, 101, 2541.
7. Slichenmeyer, W. J.; Elliott, W. L.; Fry, D. W. Semin. Oncol. 2001, 28 (Suppl. 16), 67.
8. Rowinsky, E. K. Horizons in Cancer Therapeutics: From Bench to Bedside 2001, 2, 2.
9. Cohen, P. Nat. Rev. Drug Disc. 2002, 1, 309.
10. Gschwind, A.; Fischer, O.M.; Ullrich, A. Nat. Rev. Cancer 2004, 4, 361.
11. Vijaykumar G. Pawar†, Martin L. Sos‡, Haridas B. Rode, Matthias Rabiller, Stefanie Heynck, Willem A. L. van Otterlo, Roman K. Thomas and Daniel Rauh, *J. Med. Chem.*, **2010**, *53* (7), pp 2892–2901.
12. Haregewein Assefa, Shantaram Kamath and John K. Buolamwini, Journal of Computer-Aided Molecular Design 2003, 17 (8) pp 475 – 93.
13. Allan, W.; Brawner, F.; Sridhar, K.; Rabindran, R.; Nilakantan, L. M.; Greenberger, R.; Yu-Fen, W.; Hwei-Ru, T. Bioorg. Med. Chem. Lett. 2002, 12, 2893.
14. Palmer, B. D.; Trumpp-Kallmeyer, S.; Fry, D. W.; Nelson, J. M.; Showalter, H. D. H.; Denny, W. A. J. Med. Chem. 1997, 40, 1519.
15. Lu, A.; th, W.; We, L. Eur. J. Med. Chem. 2007, in press doi: 10.1016/j. ejmech. 2007.09.018.
16. Klutchko, S. R.; Zhou, H; Winters, R. T.; Tran, T. P; Bridges, A. J.; Althaus, I. W.; Amato, D. M.; Elliott, W. L.; Ellis, P. A.; Meade, M. A.; Roberts, B. J.; Fry, D. W.; Gonzales, A. J.; Harvery, P. J.; Nelson, J. M.; Sherwood, V.; Han, H. K.; Pace G.; Smaill, J. B.; Denny, W. A.; Showalter, H. D. J. Med. Chem. 2006, 49, 1475.
17. Yue-Mei, Z.; Stuart, C.; Stephen, B. G.; David, R.; Kathryn, S.; Dana, V.; Edgar, W.; Karen, L. Bioorg. Med. Chem. Lett. 2004, 14, 111.
18. Hennequin, L. F. A.; Ballard, P. Boyle, F. T., Delouvrie, B.; Ellston, R. P. A.; Halsall, C. T.; Craig, H. S.; Kevin, H.; Jane, K.; Elizabeth, P.; Ross, H. S.; Smithb, P.; Vincenth, J. L. Bioorg. Med. Chem. Lett. 2006, 16, 2672.
19. Peter, B.; Bradbury, R. H.; Craig, S. H.; Laurent, F. A.; H.; Mark, H.; Paul, D. J.; Jason, G. K.; Teresa, K.; Andrew, G. L.; Remy, M., Martin, P.; Donald, J. O.; Annie, O.; Nicolas, W.; Emma, J. W. Bioorg. Med. Chem Lett. 2006, 16, 1633.
20. Alessandra, A.; Andrea, T., Fabiana, M.; Andrea, C.; Michela, R., Patrizia, H.; Maria, L. B.; Carlo, M. J. Med. Chem. 2006, 49, 6642.
21. Madhavi, P.; Sunil, K.; Norma, W.; John, G.; Shao-Hui, Z.; Alexei, B.; Guang, Y.; Phoebe, Y.; Frank, G.; Naoki, S.; Miguel, B.; Jie-Fei, C. Bioorg. Med. Chem. Lett. 2007, 17, 5978.
22. Yi, J.; Hui-Yuan, L.; Ki-Ping, L.; Jinzhi, T.; Jian, D.; Xiaomin, L.; Ya-Qiu, L. Bioorg. Med. Chem. 2005, 13, 5613.
23. Website:http://www.rcsb.org/pdb/explore.do ? Structure Id = Ixkk.
24. Shewchuk, L.; Hassell, A.; Wisely, B.; Rocque, W.; Holmes, W.; Veal, J.; Kuyper, L. F. J. Med. Chem. 2000, 43, 133.
25. Edgar, R. W.; Anne, T. T.; Octerloney, B. M.; Derek, Y.; Anne, H.; Scott, H. D.; Byron, E.; Christopher, P.; Earnest, H.; Karen, L.; Krystal, J. A.; David, W. R.; Tona, M. G.; Lisa, S. Cancer research 2004, 64, 6652.
26. Assefa, H.; Kamath, S.; Buolamwini, John K. Journal of Computer-Aided Molecular Design 2003, 17, 475.
27. Skehan, P.; Storeng, R., Scudiero, D., Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Body, M. R. J. Nath Cancer Inst, 1990, 82, 1107.

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