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Design, Synthesis and Evaluation of Antioxidant Activity of Some Coumarin Derivatives

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ABSTRACT

Coumarin derivatives are an important class of heterocyclic compounds, specifically 4-amino substituted coumarins with antioxidant, anticancer activities. The above observations prompted us to synthesize new coumarins with various substitutions. The starting material 4-chloro-2*H*-chromen-2-one was synthesized by refluxing a mixture of 4-hydroxy-2*H*-chromen-2-one in phosphoryl chloride. The 4-substituted amine derivatives of coumarin were synthesized by refluxing 4-chloro-2*H*-chromen-2-one with 4-substituted amines and anhydrous K_2CO_3 and methanol. All the eleven 4-substituted amine derivatives of coumarin were synthesized by replacing chloro group with different amines. These coumarin derivatives were evaluated for *in vitro* antioxidant activity using quercetin as standard.

KEYWORDS

Coumarins, Antioxidant activity, Molecular docking.

INTRODUCTION

Free radicals and other reactive oxygen species (ROS) generated during metabolic pathways in human body are highly active to chemical reactions with other molecules. Usually these are derived from oxygen, nitrogen and sulphur containing molecules such as superoxide anion, perhydroxyl radical, hydroxyl radical, nitric oxide and other species such as hydrogen peroxide, singlet oxygen, hypochlorous acid and peroxynitrite. Excessive amounts of reactive oxygen species have deleterious effects on molecules including proteins, lipids, RNA, DNA and carbohydrates and can result in cell death [1]. Thus, reactive oxygen species contributes to the pathogenesis of inflammatory, cardiovascular, cancer, diabetes, Alzheimer's, cataracts, autism and aging [2,3]. Coumarins are heterocyclic molecules that have been associated with beneficial effects on human health, such as reducing the risk of cancer, diabetes, cardiovascular and brain diseases. These effects are thought to be related to the radical scavenging effect, due to their antioxidant activities [4]. Coumarin ring containing compounds have proven to be active as antibacterial [5-7], antifungal [8], anti-inflammatory [9], anticoagulant [10], anti-HIV [11] and antitumor agents [12]. Naturally occurring coumarin deriva-

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tives like daphnetin, esculetin, esculin, mendiaxon, fraxetin, scopoletin, auraptene have been reported to possess potential antioxidant activity. In continuation to our earlier works on synthetic coumarin derivatives [13-24] as potential bioactive molecules, the present work describes the synthesis of 4-substituted coumarin derivatives. The epidermal growth factor receptor inhibitors like erlotinib, gefitinib, lapatinib are quinazolin-4-amine derivatives. In order to investigate the role of amino substitution at 4th position of coumarin on the antioxidant potential and subsequently the potential of such compounds as possible epedermal growth factor receptor (EGFR) inhibitors, 4-substituted coumarin derivatives were synthesized and evaluated for their antioxidant activity.

EXPERIMENTAL

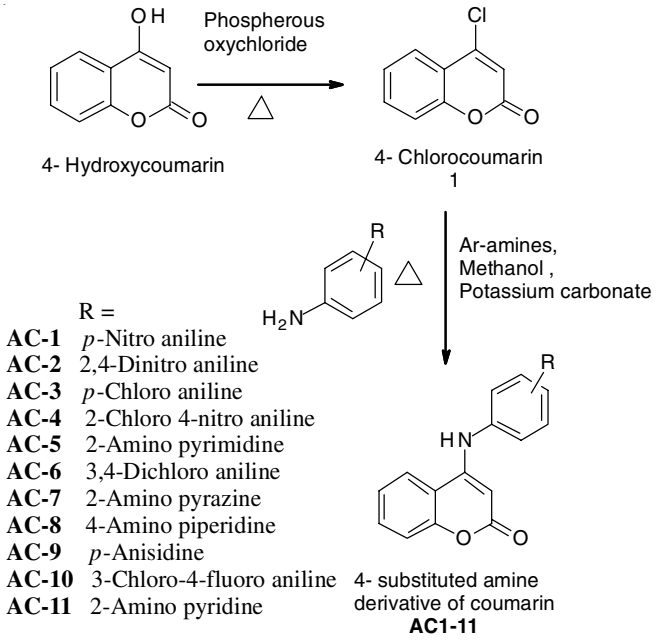
Commercial grade solvents were used without their purifications. The melting points of the synthesized compound were determined in open capillary tubes by using VMP-D melting point apparatus (Veego Instrument Corporation, Mumbai, India) and are uncorrected. The FT-IR spectrum were recorded on Shimadzu IR Affinity-1 instrument. The ^{1}H NMR spectra were recorded on Bruker 500 MHz by using TMS as an internal standard and $CDCl_3$ as solvent and chemical values are given in δ scales. The follow-up of reactions was monitored by thin-layer chromatography (TLC) on silica gel-precoated aluminum sheets (Type 60, F_{254}, Merck, Germany) and the spots were detected by exposer to UV lamp at λ 254 nm for 20-30 s.

Docking study: The molecular docking studies were carried out using the crystal structure of protein tyrosine kinase, epedermal growth factor receptor (EGFR) [25]. The crystal structure available in RCSB protein data bank (PDB ID: 1M17) was retrived and used for docking simulation. Prior to the simulations, all bound ligands, cofactors and water molecules were removed from the proteins. The macromolecule was further refined by relaxing the close contacts between the residues and polar hydrogen atoms were added. The structures of designed inhibitor molecules were drawn and subsequently converted to 3D in chemdraw and energy minimized with the combination of steepest descent and conjugate gradient in chimera. Gasteiger charges were computed and the Auto Dock atom types were defined using Auto Dock version 4.2, the graphical user interface of Auto Dock MGL Tools [26]. The Lamarckian genetic algorithm (LGA), which is considered one of the best docking algoritham available in Auto Dock, was employed [27,28]. This algorithm yields superior docking performance compared to simulated annealing or the simple genetic algorithm and the other search algorithms. Then, the 3D grid box were chosen to define the binding site for the ligands. The grid maps representing the intact ligand in the actual docking target site were generated with Auto Grid tool (part of the Auto Dock MGL Tools). The docking simulation was carried out employing the rigid docking protocol to calculate the binding free energy for the designed inhibitor at the predefined binding site of the macromolecule. The analysis of binding free energy, how the ligand bind at the binding site, the type of interactions it produces and analysis of best docked conformer was carried out using Discovery Studio Visualizer.

General method for the synthesis of 4-substituted amine derivatives of coumarin

Synthesis of 4-chlorocoumarin: Equimolar quantities of 4-hydroxycoumarin (0.01 mol) and phosphoryl oxychloride (0.01 mol) was refluxed for 6 h. The progress of reaction was monitored by preparative TLC. The resultant mixture was poured over crushed ice and the solid obtained was filtered. The resultant 4-chlorocoumarin was recrystallized from methanol.

Synthesis of 4-substituted amine derivatives of coumarin: Equimolar mixture of 4-chlorocoumarin (0.01 mol) and 4 substituted amines (0.01 mol) with catalytic amount of potassium carbonate in methanol (15 mL) as a solvent was refluxed for 3-4 h. The progress of reaction was monitored by TLC. Upon completion of reaction the reaction mixture was cooled to obtain a solid which was filtered and recrystallized from methanol to obtain 4-substituted amine derivatives of coumarin (**Scheme-I**).



Scheme-I

4-(4-Nitrophenyl amino)-2*H*-chromen-2-one (AC-1): m.f.: $C_{15}H_{10}N_2O_4$; Yield: 66 %; m.w.: 282.25; m.p.: 180-182 °C; TLC mobile phase *n*-hexane: ethyl acetate (8:2); R_f value 0.55; FT-IR (KBr, ν_{max}, cm^{-1}): 3481 (N-H *str.*); 3073 (C-H *str.*); 1704 (C=O *str.*); 1012 (C-O *str.*); 1426 (C-N *str.*); ^{1}H NMR: (500 MHz, $CDCl_3$) δ ppm: 7.55-8.27 (m, 4H, Ar-H); 7.26-7.39 (d, 2H, Ar-H); 7.04 (s, 1H, Ar-CH); 4.04 (s, 1H, N-H); 6.89-6.87 (d, 2H, Ar-CH).

4-(2,4-Dinitrophenyl amino)-2*H*-chromen-2-one (AC-2): m.f.: $C_{15}H_9N_3O_6$; %Yield 72 %, m.w.: 227.25; m.p.: 220-222 °C, TLC mobile phase *n*-hexane: ethyl acetate (8:2); R_f value 0.63; FT-IR (KBr, ν_{max}, cm^{-1}): 3481 (N-H *str.*); 3073 (C-H *str.*); 1704 (C=O *str.*); 1012 (C-O *str.*); 1426 (C-N *str.*); ^{1}H NMR: (500 MHz, $CDCl_3$) δ ppm: 7.55-8.27 (m, 4H, Ar-H); 7.26-7.39 (d, 2H Ar-H); 7.26-7.39 (s,1H, Ar-H); 7.04 (s, 1H, N-H); 6.89-6.87 (s, 1H, Ar-H).

4-(4-Chlorophenyl amino)-2*H*-chromen-2-one] (AC-3): m.f.: $C_{15}H_{10}NO_2Cl$; %Yield 60 %, m.w.: 271.7; m.p.: 142-144 °C, TLC mobile phase *n*-hexane: ethyl acetate (8:2); R_f

value 0.56; FT-IR (KBr, ν_{max}, cm^{-1}): 3481 (N-H *str.*); 3073 (C-H *str.*); 1704 (C=O *str.*); 1012 (C-O *str.*); 605 (C-Cl *str.*) ^{1}H NMR: (500 MHz, $CDCl_3$) δ ppm: 7.55-8.27 (m, 4H Ar-H); 7.26-7.39 (d, 2H Ar-H); 7.04 (s, 1H Ar-H); 4.04 (s, 1H N-H); 6.89-6.87 (d,2H, Ar-H).

4-(2-Chloro-4-nitrophenyl amino)-2*H*-chromen-2-one (AC-4): m.f.: $C_{15}H_9N_2O_4Cl$; %Yield 55 %, m.w.: 316.7; m.p.: 230-232 °C, TLC mpbile phase *n*-hexane: ethyl acetate (8:2); R_f value 0.59; FT-IR (KBr, ν_{max}, cm^{-1}): ^{1}H NMR: (500 MHz, $CDCl_3$) δ ppm: 7.55-8.27 (m, 4H Ar-H); 7.26-7.39 (d, 2H Ar-H); 7.04 (s, 1H Ar-H.); 4.04 (s, 1H N-H); 6.89-6.87(d, 2H Ar-H); H_g, 6.89 (s, 1H Ar-H).

4-(Pyrimidine-2-yl-amino)-2*H*-chromen-2-one (AC-5): m.f.: $C_{13}H_9N_3O_2$; %Yield 74 %, m.w.: 239.23; m.p.: 135-137 °C, TLC mobile phase *n*-hexane: ethyl acetate (8:2) R_f value 0.66; FT-IR (KBr, ν_{max}, cm^{-1}): 3481 (N-H *str.*); 3073 (C-H *str.*); 1704 (C=O *str.*); 1012 (C-O *str.*); 2263 (C=N *str.*); ^{1}H NMR: (500 MHz, $CDCl_3$) δ ppm: 7.55-8.27 (m, 4H Ar-H); 7.26-7.39 (m, 3H Ar-H); 7.04 (s, 1H Ar-H); 4.04 (s, 1H N-H).

4-(3,4-Dichlorophenyl amino)-2*H*-chromen-2-one (AC-6): m.f.: $C_{15}H_9NO_2Cl_2$; Yield: 66 % m.w.: 306.14; m.p.: 170-172 °C, TLC mobile phase *n*-hexane: ethyl acetate (8:2) R_f value 0.64; FT-IR (KBr, ν_{max}, cm^{-1}): 3481 (N-H *str.*); 3073 (C-H *str.*); 1704 (C=O *str.*); 1012 (C-O *str.*); 1426 (C-N *str.*); 605 (C-Cl *str.*); ^{1}H NMR: (500 MHz, $CDCl_3$) δ ppm: 7.55-8.27 (m, 4H Ar-H); 7.26-7.39 (d, 2H Ar-H); 7.04 (s, 1H Ar-H); 4.04 (s, 1H N-H); 6.89-6.87 (d, 2H Ar-H); 6.89 (s, 1H Ar-H).

4-(Pyrazin-2-yl amino)-2*H*-chromen-2-one (AC-7): m.f.: $C_{13}H_9N_3O_2$; Yield: 46 % m.w.: 239.23 m.p.: 145-147 °C, TLC mobile phase *n*-hexane: ethyl acetate (8:2); R_f value 0.59 FT-IR (KBr, ν_{max}, cm^{-1}): 3481 (N-H *str.*); 3073 (C-H *str.*); 1704 (C=O *str.*); 1012 (C-O *str.*); 2263 (C=N *str.*); ^{1}H NMR: (500 MHz, $CDCl_3$) δ ppm: 7.55-8.27 (m, 4H Ar-H); 7.26-7.39 (d, 2H Ar-H); 7.04 (s, 1H Ar-H); 4.04 (s, 1H N-H); 6.89-6.87 (d, 2H Ar-H).

4-(Piperidine-4-yl amino)-2*H*-chromen-2-one (AC-8): m.f.: $C_{14}H_{16}N_2O_2$; Yield: 68 %; m.w.: 244.29; m.p.: 130-132 °C, TLC mobile phase *n*-hexane: ethyl acetate (8:2); R_f value 0.55; FT-IR (KBr, ν_{max}, cm^{-1}): 3481 (N-H *str.*); 3073 (C-H *str.*); 1704 (C=O *str.*); 1012 (C-O *str.*); 2223 (C-N *str.*); ^{1}H NMR: (500 MHz, $CDCl_3$) δ ppm: 7.55-8.27 (m, 4H Ar-H); 6.50-6.96 (m, 8H Ar-H); 7.04 (s, 1H, Ar-H); 4.04 (s, 1H N-H); 3.50-3.08 (s, 2H Ar-N-H);.

4-(4-Methoxy phenyl amino)-2*H*-chromen-2-one (AC-9): m.f.: $C_{16}H_{13}NO_3$; Yield: 70 % m.w.: 267.28; m.p.: 165-167 °C, TLC mobile phase *n*-hexane: ethyl acetate (8:2); R_f value 0.58; FT-IR (KBr, ν_{max}, cm^{-1}): 3481 (N-H *str.*); 3073 (C-H *str.*); 1704 (C=O *str.*); 1012 (C-O *str.*); ^{1}H NMR: (500 MHz, $CDCl_3$) δ ppm: 7.55-8.27 (m, 4H Ar-H); 7.26-7.39 (m, 3H Ar-H); 7.04 (s, 1H Ar-H); 4.04 (s, 1H N-H).

4-(3-Chloro-4-fluoro phenyl amino)-2*H*-chromen-2-one (AC-10): m.f.: $C_{15}H_9NO_2ClF$; Yield: 60 % m.w.: 289.69 m.p.: 147-149 °C, TLC mobile phase *n*-hexane: ethyl acetate (8:2); R_f value 0.45; FT-IR (KBr, ν_{max}, cm^{-1}): 3481 (N-H *str.*); 3073 (C-H *str.*); 1704 (C=O *str.*); 1012 (C-O *str.*); 1057 (C-F *str.*); 605 (C-Cl *str.*); ^{1}H NMR: (500 MHz, $CDCl_3$) δ ppm: 7.55-8.27; (m, 4H Ar-H); 7.26-7.39 (m, 3H Ar-H); 7.04 (s, 1H Ar-H); 4.04 (s,1H N-H).

4-(Pyridine-2-ylamino)-2*H*-chromen-2-one (AC-11): m.f.: $C_{14}H_{10}N_2O_2$; Yield: 66 % m.w.: 238.24 m.p.: 180-182 °C, TLC mobile phase *n*-hexane: ethyl acetate (8:2); R_f value 0.55; FT-IR (KBr, ν_{max}, cm^{-1}): 3481 (N-H *str.*); 3073 (C-H *str.*); 1704 (C=O *str.*); 1012 (C-O *str.*); 2263 (C=N *str.*); ^{1}H NMR: (500 MHz, $CDCl_3$) δ ppm: 7.55-8.27 (m, 4H Ar-H); 7.26-7.39 (m, 3H Ar-H.); 7.04 (s, 1H Ar-H.); 4.04 (s, 1H N-H).

Antioxidant activity by DPPH method: All the synthesized compounds were evaluated for antioxidant activity by DPPH assay [29]. DPPH is 1,1-diphenyl-2-picryl hydrazyl, a stable free radical and contains a delocalized spare electron. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in methanol solution at about 517 nm. When a solution of DPPH is mixed with a solution of substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour (a residual pale yellow colour from the picryl group and requires control reading). Thus DPPH represent the free radicals produced in a system and antioxidants suppresses their formation.

General method of antioxidant DPPH assay: The standard solution was prepared by dissolving 100 mg of quercetin in methanol to give the concentration of 10, 20, 30, 40 and 50 µg/mL. The tests solutions were prepared by dissolving 100 mg of compounds (**AC-1** to **AC-11**) in 10 mL of methanol to give 1000 µg/mL of stock solution of each compound. The concentrations 10, 20, 30, 40 and 50 µg/mL were prepared using this stock solution. To each dilution 150 µL of DPPH was added and kept in dark for 30 min. The 150 µL DPPH solution was added to 10 mL methanol and absorbance was taken immediately at 517 nm as control reading. 10 mL of different concentrations of test sample (10, 20, 30, 40, 50 µL) prepared with methanol were taken and 150 µL DPPH solution was added to each test tube. Absorbance was taken at 517 nm in UV-visible spectrophotometer (Jasco, UV-700) after 15 min using methanol as a blank. The free radical scavenging activity (FRSA) (% inhibition) was calculated using the following equation. The % inhibition for different log concentrations were plotted to obtain a concentration *vs*. % inhibition graph from which IC_{50} value was calculated:

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

RESULTS AND DISCUSSION

Docking study: The preceding results encouraged us to study the molecular docking of the most active compounds against EGFR, which is over expressed in numerous tumours such as prostate, breast, hepatocellular carcinoma. All docking calculations were performed using Autodock software. The molecular docking results of all the compounds demonstrated an approximate orientation of the molecule in comparison with erlotinib inside the putative binding site of EGFR pocket with some additional interactions such as hydrogen bond interactions, hydrophobic interactions with surrounding amino acids. These docking results showed three classical and five non-classical hydrogen bonds, where the distinctive residue Thr766 formed bifurcated hydrogen bonds with oxygen and

carbon atoms of 4-substituted amine derivatives of coumarin ring system. In addition, the amino acid residue Thr830 formed also found to for such hydrogen bond interaction. Table-1 and Fig. 1 show the keys interactions with various amino acid residues at the binding site of EGFR.

Antioxidant DPPH assay: Antioxidant behaviour of these coumarins derivatives (**AC-1** to **AC-11**) is measured *in vitro* by the inhibition of generated stable 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical. Table-2 shows the antioxidant activity at different test concentrations and IC_{50} value in µg/mL. From the results it is found that the compound **AC-4** possess highest antioxidant potential as compared to other compounds and reference quercetin.

Conclusion

From the results of *in vitro* antioxidant activity it was found that the synthesized 4-amino substituted coumarin derivatives possess moderate antioxidant property. The DPPH radical scavenging activity was undertaken to evaluate the effect of substituent on the antioxidant activities of the all synthesized

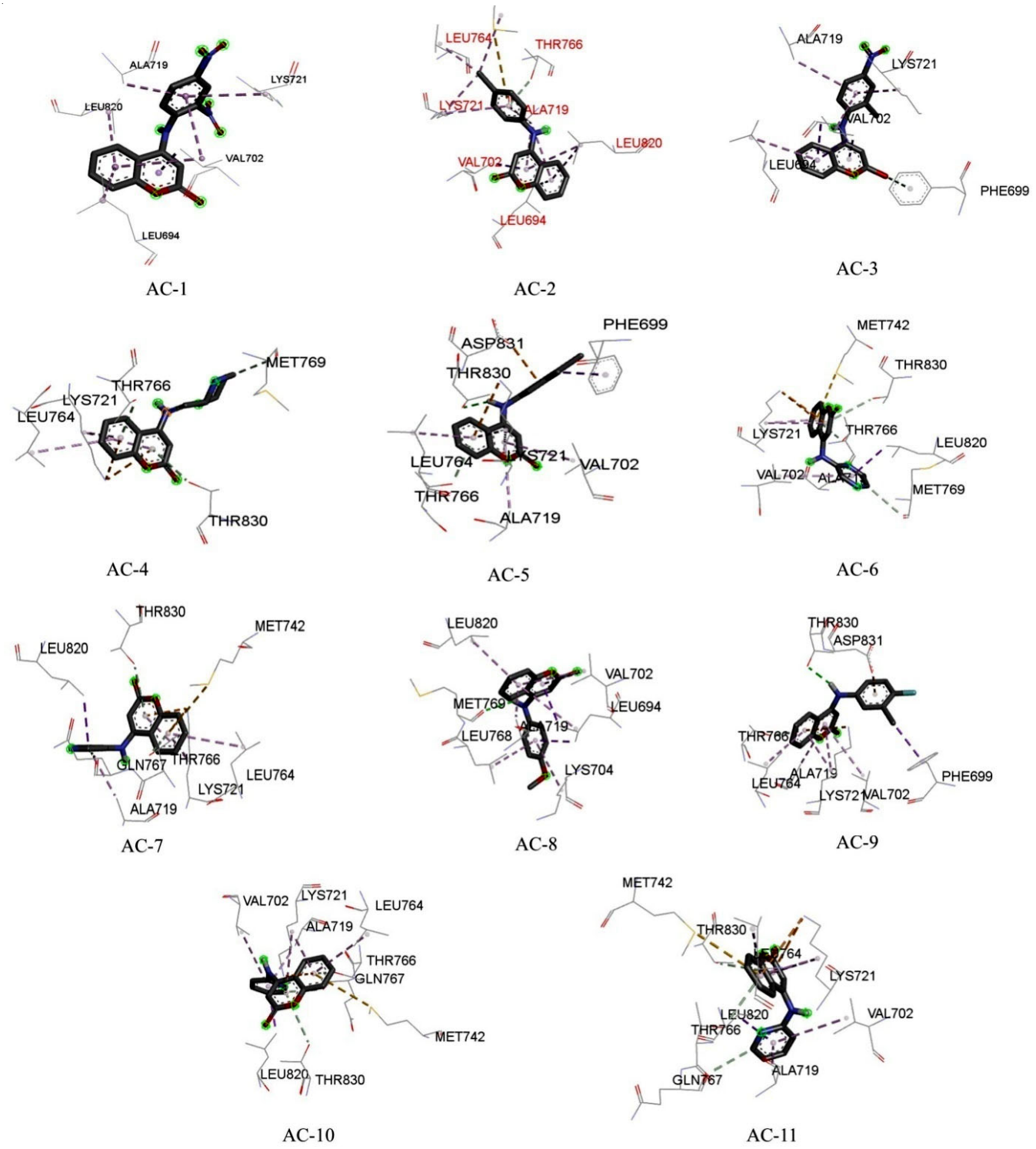


Fig. 1. Ligand interaction with protein (**AC-1** to **AC-11**)

TABLE-1
BINDING FREE ENERGY AND KEY INTERACTIONS AT THE BINDING SITE

Compounds	Binding free energy (Kcal/mol)	Interactions
AC-1	-7.3	LEU694, VAL702, ALA719, LYS721
AC-2	-7.7	LEU694, VAL702, ALA719, LYS721, LEU764, THR766, LEU820
AC-3	-7.5	PHE699, LEU694, VAL702, ALA719, LYS721
AC-4	-6.8	THR830, LEU764, LYS721, THR766, MET769
AC-5	-7.6	ALA719, THR766, LEU764, LYS721, VAL702, THR830, ASP831, PHE699
AC-6	-6.7	VAL702, ALA719, MET769, LEU820, LYS721, THR766, THR830, MET742
AC-7	-7.0	ALA719, LYS721, LEU764, GLN767, THR766, LEU820, THR830, MET742
AC-8	-7.1	LYS704, LEU768, ALA719, MET769, LEU694, VAL702, LEU820
AC-9	-7.6	LYS721, VAL72, LEU764, ALA719, THR766, PHE699, THR830, ASP831
AC-10	-7.2	LEU820, THR830, MET742, GLN767, THR766, ALA719, VAL702, LYS721, LEU764
AC-11	-7.2	GLN767, ALA719, THR766, LEU820, VAL702, LYS721, LEU764, THR830, MET742
Erlotinib	-6.6	LEU820, MET796, THR830, GLN767, LEU694, ALA719, LYS721, LEU764

TABLE-2
ANTIOXIDANT ACTIVITY

Compounds	% Inhibition					IC_{50} (µg/mL)
	Concentration (µg/mL)					
	10	20	30	40	50	
AC-1	10.00	18.4	22.23	21.43	28.71	475.33
AC-2	12.26	20.25	27.88	30.34	33.78	166.26
AC-3	14.35	14.70	16.50	13.39	13.51	1539.00
AC-4	29.46	30.03	34.98	50.80	57.08	45.28
AC-5	17.30	20.13	34.69	40.58	78.31	37.72
AC-6	7.98	11.40	20.72	21.86	24.52	150.31
AC-7	12.76	34.69	38.21	39.92	40.68	69.32
AC-8	10.45	10.47	18.46	23.28	13.29	20384.49
AC-9	4.84	10.36	15.30	16.73	19.96	1345.86
AC-10	2.75	13.40	15.68	18.63	18.82	937.56
AC-11	6.36	8.67	10.22	15.30	17.20	8044.51
Quercetin	15.36	24.68	34.82	46.23	59.53	44.23

compound. Among all synthesized compounds, **AC-4**, **AC-5** and **AC-7** exhibited good radical scavenging activities compared to Quercetin. These compounds with antioxidant potential can be potential EGFR tyrosine kinase inhibitors and can be promising antiproliferative agents. The reason for higher antioxidant activity of compound **AC-4** may be due to the chloro and nitro substituents on the phenyl ring; where as in compounds **AC-5** and **AC-7** the heterocyclic substitutions pyrimidine and pyrazine may be contributing to the antioxidant activity. Therefor, these molecules could be developed for antioxidant agent and antiproliferative activity.

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