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RESEARCH ARTICLE

SYNTHESIS, CHARACTERIZATION AND MOLECULAR DOCKING STUDIES OF ANILINOQUINAZOLINE DERIVATIVES

[1]Preethi Saligrama Devegowda, [2]Vivek Hamse Kameshwar, [3]Kyathegowdanadoddi Srinivasa Balaji, [4]Doddakunche Shivaramu Prasanna, [5]Toreshettahally Ramesh Swaroop, [3]Shankar Jayarama, [1,*]Lokesh Siddalingaiah and [6]Kanchugarakoppal Subbegowda Rangappa

[1]Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore-570 006, India
[2]Department of Biotechnology, Sri Jayachamarajendra College of Engineering, Manasagangotri, Mysore-570 006, India
[3]Department of Biotechnology, Teresian College, Siddarthanagar, Mysore – 570011, India
[4]Department of Nanotechnology, VisvesvarayaTechnological University, Center for Postgraduate Studies, Bengaluru Region, Muddenahalli, Chikkaballapur District -562 101, India
[5]Department of Studies in Organic Chemistry, University of Mysore, Manasagangotri, Mysore-570 006, India
[6]Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore-570 006, India

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ABSTRACT

The current investigation was on synthesis, characterization and molecular docking studies of novel 4-anilinoquinazoline derivatives for their anti-tumor effects.The synthesized novel 4-anilinoquinazoline derivatives were characterized using ^{1}H and ^{13}C Nuclear magnetic resonance (NMR), Infrared (IR) and mass spectroscopic analysis. Compounds PR (1-6) were screened for cytotoxicity assays against various cell lines such as HCT116, K562, SKBR3 and EAC cell lines using MTT assay, trypan blue dye exclusion and LDH release assay. The molecular docking studies were drawn using Maestro 2D sketcher and energy minimize was computed by OPLS 2005. Proteins were prepared by retrieving into Maestro platform Schrödinger, Inc.PR-6 enhanced cytotoxic activity in the range of 10.09±0.92 to 11.24±0.81 µM on all four cell lines, which is comparable to that of standard drugs cisplatin (6.4±1.45 to 8.48±0.63 µM) and doxorubicin (7.89±091 to 8.48±1.02 µM). Furthermore, molecular docking studies of synthesized compounds PR (1-6) as vascular endothelial growth factor receptor-2 (VEGFR-2) and epidermal growth factor receptor (EGFR) inhibitors were performed on crystal structure of VEGFR-2 and EGFR and amongst them PR-6 has shown maximum docking score (-11.13) against VEGFR-2. This finding strongly suggested that PR-6 is effective cytotoxic agent against all the four cancer cell lines *in-vitro* and also significant angiogenic inhibitor as ascertained by its potential interaction with VEGFR-2 and EGFR.

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INTRODUCTION

Angiogenesis is an intricate process of formation of new blood vessels from preexisting blood vessel (Laschke *et al*., 2006; Roopashree *et al.,* 2015). It plays a key role in normal growth and development of tissue. On the other hand, deregulated angiogenesis lead to various disorders such as cancer. Various growth factors that regulate angiogenesis are vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), angiostatin and interferons. Amongst, VEGF is the most prominent inducer of angiogenesis (Polverini, 1995). VEGF plays a key role in migration, proliferation of endothelia cells and leads to formation of capillary like structure. VEGF carry out its action by binding to its receptors (VEGFR1 and VEGFR2) on the endothelial cells, activate the downstream signaling pathway finally leading to tumor vascularization (Balaji *et al*.,2016). Binding of small molecule inhibitors to the VEGFR2 kinase domain leads to blockage of VEGFR-2 mediated signaling pathway. The has shown to inhibit angiogenesis and tumor progression. Epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase belonging to ErbB family that consist of four cell surface receptors: Her1 (ErbB1,

****Corresponding author: Lokesh Siddalingaiah***
Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore-570 006, India

EGFR), Her2 (ErbB2), Her3 (ErbB3) and Her4 (ErbB4) (Bazley and Gullick, 2005). Binding of specific ligands/growth factor to its receptor induces receptor dimerization and autophosporylation, resulting in activation of intracellular signaling pathway, which regulates cell differentiation, growth, migration and apoptosis (Citri and Yarden, 2006). Mutation or over expression of EGFR in many tumors lead to abnormal cell proliferation, invasion of surrounding tissues, and increased angiogenesis (Takeuchi and Ito, 2010). EGFR signaling promotes up-regulation of VEGF, blocking of EGFR by tyrosine kinase inhibitors that down-regulate pro-angiogenic factors leading to a reduction in micro vessel density and metastasis (Tabernero, 2007; Jayarama *et al*., 2017). Both VEGFR2 and EGFR are closely related, they share familiar downstream signal transduction pathways and play important role in tumor growth and angiogenesis (Tabernero, 2007). Blocking of VEGFR2 signaling pathway leads to the antitumor effect of EGFR inhibitors, whereas over activation of VEGF expression is independent of EGFR signaling and thought to be one of the resistance mechanism to anti-EGFR therapy (Garofalo *et al*., 2011).

Quinazoline compounds have been well documented for their anti-cancer (Merck Index, 2001; Wang *et al*., 2015; Mahdavi *et al*., 2015; Zayed *et al*., 2015; Malinowski *et al*., 2015), anti-convulsant activity (Ahmed and Belal, 2015; Al-Salem *et al*., 2015; Ibrahim *et al*., 2015), anti-histaminic activity (Zhang *et al*., 2015), anti-hyperlipidemic activity (Gobinath *et al*., 2015), anti-influenza activity (Mokale *et al*., 2016), anti-inflammatory activity (Liu *et al*., 2015), cox-2 inhibitors (Tutuk *et al*., 2016), anti-microbial activity (Hu *et al.,* 2015) and anti-malarial activity (Patel *et al*., 2015). Quniazoline derivatives also act as cathepsin (Birhan *et al*., 2015), α-glucosidase (Singh and Raghav, 2015; Javaid *et al*., 2015), monoamine oxidase (Gurram *et al*., 2015), poly (ADP-ribose) polymerase (Khattab *et al*., 2015), thymidine phosphorylase (Yao *et al*., 2015) and topoisomerase inhibitors (Javaid *et al*., 2015). As protein tyrosine kinases play a key role in signal transduction pathways that regulate numerous cellular functions including proliferation, differentiation, migration, and angiogenesis, the key targets to fight against cancer and EGFR, VEGFR2 inhibitors have been considered as promising agents to treat cancer (Lemmon and Schlessinger, 2010; Liao, 2007; Visentin *et al*., 2010; Pallis and Syrigos, 2013). Various quinazoline derivatives are reported to show EGFR and VEGFR2 inhibition activities.

Various substituted quinazolines, 4-anilinoquinazoline derivatives, which have shown competitive binding to the adenosine tri-phosphate (ATP) site of EGFR are considered as the most effective tyrosine kinase inhibitors (Li and Li, 2014; Chandregowda *et al*., 2007). These compounds have attracted many researchers who are working to develop antitumor drugs. Already few compounds with 4-anilinoquinazoline moiety such as gefitinib, lapatinib, vandetanib, afatinib, erlotinib and icotinib have been launched as tyrosine kinase inhibitors. In our present work, we employed substituted phenyl urea derivatives instead of substituted aniline group in the structure of erlotinib, gefitinib and vandetanib and methoxy groups in the position 6 and 7 of the quinazoline moiety. A series of novel 6,7-dimethoxy-4-aminoquinazoline with substituted phenylurea derivatives were designed and synthesized based on this scaffold and evaluated for their antitumor activity.

MATERIALS AND METHODS

Experimental Section

All the experimental reagents and solvents were purchased from Sigma Aldrich Chemicals Pvt Ltd. HCT116, K562, SKBR3 and EAC cell lines were purchased from National center for cell science (NCCS), Pune.^{1}H and ^{13}C nuclear magnetic resonance spectra were documented on Shimadzu AMX 400-Bruker, 400 MHz spectrometer using DMSO-$d_6$as solvent and TMS as internal standard. IR spectra were documented on a Shimadzu FT-IR model 8300 Spectrometer. Purity and mass of the compounds were documented by LC/MSD-Trap-XCT.

Chemistry

The key intermediate compound 6, 7-dimethoxy-quinazolin-4(3*H*)-one 1 was synthesized by using the earlier reported procedure (Zabiulla *et al*., 2016).

Synthesis of 6, 7-dimethoxy-4-chloro quinazoline 2

The solution of 6, 7-dimethoxy-quinazolin-4(3*H*)-one 1 (10 mmol) in thionyl chloride (3 mL) with dimethylformamide (DM) (2-3 drops) was refluxed for about 2h. The thionyl chloride was distilled off and the reaction mixture was dumped into crushed ice. The precipitate was filtered and washed with ice cold water. The precipitate was dissolved in chloroform and filtered to remove insoluble impurities. Organic layer was concentrated under reduced pressure to get compound **2**.

Synthesis of *N*-(3-aminophenyl)-6, 7-dimethoxy-quinazolin-4-amine3

The solution of **2** (10 mmol) and *m*-phenylenediamine (10 mmol) in isopropyl alcohol (25 mL) was heated at 60 °C with stirring for about 3-4 h. Reaction was monitored by thin layer chromatography (TLC). After the completion, the reaction mass was dumped into ice-cold sodium bicarbonate solution and extracted with ethyl acetate (50 mL × 3). Organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain 3, which was purified by column chromatography to get pure compound 3.

General procedure for preparation of compounds PR (1-6)

To a stirred solution of compound **3** (1 mmol) and triethyl amine (1 mmol) in dichloromethane (10 mL), aryl isocyanate (1 mmol) was added at 0 °C. The reaction mixture was allowed to stir at room temperature for about 4-5 h. Completion of the reaction was monitored by TLC. The reaction mass was quenched with water and extracted with dichloromethane (25 mL × 2). The organic layer was dried over sodium sulphate and concentrated to get crude product, which was purified by column chromatography to get pure compounds PR (1-6).

1-(3-((6, 7-Dimethoxyquinazolin-4-yl) amino) phenyl)-3-(3-methoxyphenyl)urea (PR-1)

White solid. Yield 75% (Purity 98%). M.P. 160-162°C.IR (KBr, cm^{-1}): 3431, 3222, 1680, 1602, 1315. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.39 (s, 1H, -NH), 9.57 (s, 1H, -NH), 9.11 (s, 1H, -NH), 8.49 (s, 1H, Ar-H), 7.30-7.41 (m, 4H, Ar-H), 7.15-

7.24 (m, 3H, Ar-H), 6.95-6.99 (m, 2H, Ar-H), 6.73 (d, J=7.8 Hz, 1H, Ar-H), 3.97 (s, 3H, -OCH_3), 3.94 (s, 3H, -OCH_3), 3.91 (s, 3H,-OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.4, 160.8, 158.5, 154.6, 152.9, 151.3, 146.6, 142.6, 136.9, 136.7, 129.9, 129.7, 116.4, 113.9, 113.4, 112.9, 111.6, 110.2, 108.2, 108.0, 99.4, 55.8, 56.2, 56.1. MS (ESI + ion): m/z = 446.2. Anal. Calcd for $C_{24}H_{23}N_5O_4$: C 64.71, H 5.20, N 15.72. Found: C 64.75, H 5.24, N 15.76. Yield : 75%.

1-(3-((6,7-Dimethoxyquinazolin-4-yl)amino)phenyl)-3-(4-methoxyphenyl)urea(PR-2)

White solid. Yield 70% (Purity 96%) M.P. 168-170°C.IR (KBr, cm^{-1}): 3439, 3214, 1686, 1606, 1321. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.38 (s, 1H, -NH), 9.55 (s, 1H, -NH), 9.10 (s, 1H, -NH), 8.49 (s, 1H, Ar-H), 7.51 (d, J=8.0Hz, 2H, Ar-H), 7.35-7.40 (m, 2H, Ar-H), 7.18-7.24 (m, 2H, Ar-H), 6.90-7.01 (m, 4H, Ar-H), 3.97 (s, 3H, -OCH_3), 3.94 (s, 3H, -OCH_3), 3.91 (s, 3H,-OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.4, 158.9, 158.5, 154.6, 153.0, 151.3, 146.6, 142.6, 136.7, 131.7, 129.7, 119.8, 114.5, 113.4, 112.9, 111.6, 108.2, 108.0, 99.4, 55.8, 56.3, 56.1. MS (ESI + ion): m/z = 446.2. Anal. Calcd for $C_{24}H_{23}N_5O_4$: C 64.71, H 5.20, N 15.72. Found: C 64.76, H 5.25, N 15.77. Yield: 70%.

1-(3-((6,7-Dimethoxyquinazolin-4-yl)amino)phenyl)-3-(m-tolyl)urea (PR-3)

White solid. Yield 73% (Purity 97%). M.P. 154-156°C.IR (KBr, cm^{-1}): 3450, 3208, 1671, 1596, 1316. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.38 (s, 1H, -NH), 9.55 (s, 1H, -NH), 9.10 (s, 1H, -NH), 8.47 (s, 1H, Ar-H), 7.31-7.41 (m, 4H, Ar-H), 7.18-7.24 (m, 3H, Ar-H), 6.95-6.98 (m, 3H, Ar-H), 3.97 (s, 3H, -OCH_3), 3.94 (s, 3H, -OCH_3), 2.34 (s, 3H, Ar-CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.3, 158.5, 154.6, 152.9, 151.3, 146.6, 142.6, 138.6, 136.7, 135.8, 129.7, 128.8, 125.4, 124.6, 118.6, 113.4, 112.9, 111.6, 108.2, 108.0, 99.4, 55.8, 55.3, 21.3. MS (ESI + ion): m/z = 430.2. Anal. Calcd for $C_{24}H_{23}N_5O_3$: C 67.12, H 5.40, N 16.31. Found: C 67.15, H 5.44, N 16.35. Yield: 73%.

1-(3-((6,7-Dimethoxyquinazolin-4-yl)amino)phenyl)-3-(p-tolyl)urea (PR-4)

White solid. Yield 78% (Purity 96%) M.P. 148-150°C.IR (KBr, cm^{-1}): 3431, 3219, 1662, 1602, 1306. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.40 (s, 1H, -NH), 9.58 (s, 1H, -NH), 9.11 (s, 1H, -NH), 8.49 (s, 1H, Ar-H), 7.56 (d, J=8.0 Hz, 2H, Ar-H), 7.37-7.40 (m, 2H, Ar-H), 7.18-7.25 (m, 4H, Ar-H), 6.94-9.98 (m, 2H, Ar-H), 3.97 (s, 3H, -OCH_3), 3.94 (s, 3H, -OCH_3), 2.34 (s, 3H, Ar-CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.4, 158.5, 154.6, 152.9, 151.3, 146.6, 142.6, 136.8, 136.7, 136.4, 129.7, 129.2, 121.5, 113.4, 112.9, 111.6, 108.3, 108.0, 99.4, 55.8, 55.3, 21.3. MS (ESI + ion): m/z = 430.2. Anal. Calcd for $C_{24}H_{23}N_5O_3$: C 67.12, H 5.40, N 16.31. Found: C 67.16, H 5.45, N 16.36. Yield: 78%.

1-(3,4-Dichlorophenyl)-3-(3-((6,7-dimethoxyquinazolin-4-yl)amino)phenyl) urea (PR-5)

White solid. Yield 82% (Purity 97%). M.P. 178-180°C.IR (KBr, cm^{-1}): 3443, 3225, 1660, 1608, 1319. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.42 (s, 1H, -NH), 9.54 (s, 1H, -NH), 9.13 (s, 1H, -NH), 8.51 (s, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 7.53 (d, J=8.0 Hz, 1H, Ar-H), 7.37-7.42 (m, 3H, Ar-H), 7.18-7.24 (m, 2H, Ar-H), 6.93-6.96 (m, 2H, Ar-H), 3.97 (s, 3H, -OCH_3), 3.94 (s, 3H, -OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.5, 158.5, 154.6, 153.0, 151.3, 146.6, 142.6, 136.7, 136.5, 131.2, 130.5, 129.7, 129.0, 124.3, 121.0, 113.4, 112.9, 111.6, 108.8, 108.4, 99.3, 55.9, 55.5. MS (ESI + ion): m/z = 483.1. Anal. Calcd for $C_{23}H_{19}Cl_2N_5O_3$: C 57.04, H 3.95, N 14.46. Found: C 57.08, H 3.98, N 14.50. Yield :82%.

1-(4-Bromophenyl)-3-(3-((6,7-dimethoxyquinazolin-4-yl) amino)phenyl)urea (PR-6)

White solid. Yield 80% (Purity 99%). M.P. 188-190°C.IR (KBr, cm^{-1}): 3443, 3225, 1660, 1608, 1319. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.42 (s, 1H, -NH), 9.54 (s, 1H, -NH), 9.13 (s, 1H, -NH), 8.52 (s, 1H, Ar-H), 7.71 (d, J=8.0 Hz, 2H, Ar-H), 7.58 (d, J=8.0 Hz, 2H, Ar-H), 7.37-7.41 (m, 2H, Ar-H), 7.17-7.23 (m, 2H, Ar-H), 6.91-6.93 (m, 2H, Ar-H), 3.97 (s, 3H, -OCH_3), 3.94 (s, 3H, -OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 168.6, 158.5, 154.5, 153.1, 151.2, 146.5, 142.6, 138.4, 136.7, 131.8, 129.7, 122.3, 122.0, 113.4, 112.9, 111.6, 108.4, 108.1, 99.4, 55.9, 55.5. MS (ESI + ion): m/z = 494.1 and 496.1. Anal. Calcd for $C_{23}H_{20}BrN_5O_3$: C 55.88, H 4.08, N 14.17. Found: C 55.92, H 4.12, N 14.21. Yield: 80%.

Cytotoxic Assay

A series of newly synthesized compounds PR (1-6) were preliminarily evaluated for their cytotoxic effect on various cell lines using MTT, trypan blue dye exclusion and LDH leak assay (McTigue *et al*., 2012).

Molecular Docking of PR (1-6)

The co-ordinates of VEGFR-2 and EGFR were obtained from the Brookhaven Protein Data Bank (PDB), whose PDB IDs are 4ASE (Yun *et al*., 2007) and 2ITY (Raghavendra *et al*., 2007) respectively. Ligands were drawn using Maestro 2D sketcher and energy minimize was computed by OPLS 2005. Proteins were prepared by retrieving into Maestro platform (Schrödinger, Inc.). Protein structure was corrected, by using Prime software module of Schrödinger to correct the missing loops and in the protein. Water molecules from VEGFR2 and EGFR were removed beyond 5 A° from the hetero atom respectively. Water molecules which were thought to be important in aiding the interaction between the receptor were optimized during protein pepwizard. Automated, necessary bonds, bond orders, hybridization, explicit hydrogens and charges were assigned. OPLS 2005 force field was applied to the protein to restrain minimization and RMSD of 0.30 A° was set to converge heavy atoms during the pre-processing of protein before starting docking. Using Extra-precision (XP) docking and scoring each compound was docked into the receptor grid of radii 20 A° × 20 A° × 20 A° and the docking calculations were judged based on the Glide score, adsorption, distribution, metabolism and excretion (ADME) results and Glide energy. QikProp, the prediction program was used to calculate ADME properties of all the ligand and molecular visualization was done under Maestro (Raghavendra *et al*., 2007; Tri *et al*., 2016).

RESULTS AND DISSCUSION

Chemistry

The key intermediate 6,7-dimethoxy-quinazolin-4(3*H*)-one **1** was synthesised according to the earlier reported protocol.

Compound 1 was heated with thionyl chloride with few drops of DMF to get 4-chloro-6,7-dimethoxyquinazoline **2** which was then reacted with *m*-phenylenediamine in isopropyl alcohol at 80 °C, resulting in N'-(6,7-dimethoxyquinazolin-4-yl)benzene-1,3-diamine 3. Compound 3 was then treated with different isocyanates in dichloromethane, in presence of triethylamine to get final quinazoline-urea derivatives 4a-f titled as PR(1-6) as shown in Figure 1, which were further purified by using silica gel column chromatography. All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. Structures and yields of the compounds are presented in Figure 1.

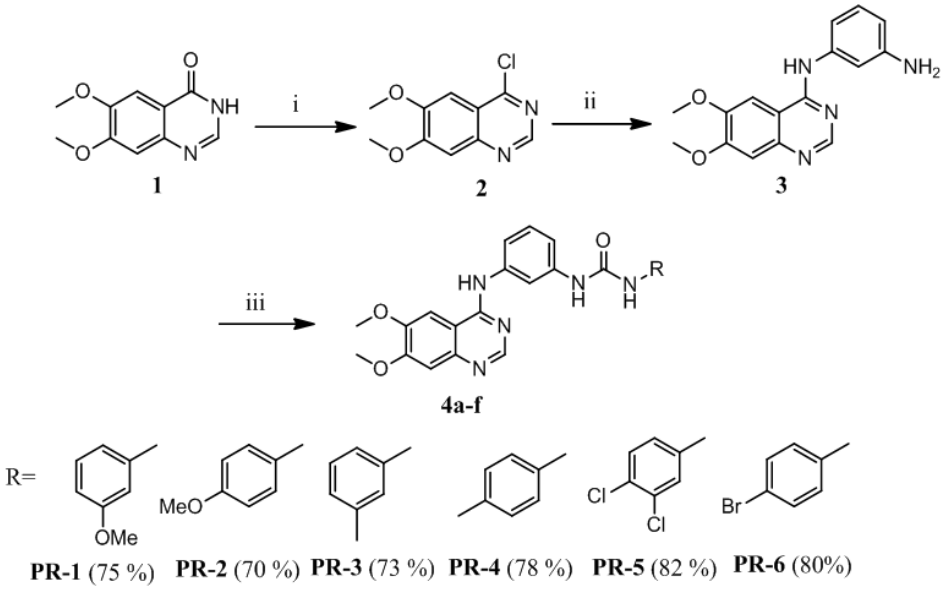

Figure 1. Synthesis of compounds PR (1-6): Reagents and reaction conditions: i) Thionyl chloride, DMF, reflux, 2h; ii) *m*-phenylenediamine, isopropyl alcohol, reflux, 3-4 h; iii) Substituted isocyanates, triethyl amine, dichloromethane, 0°C to RT, 4-5 h

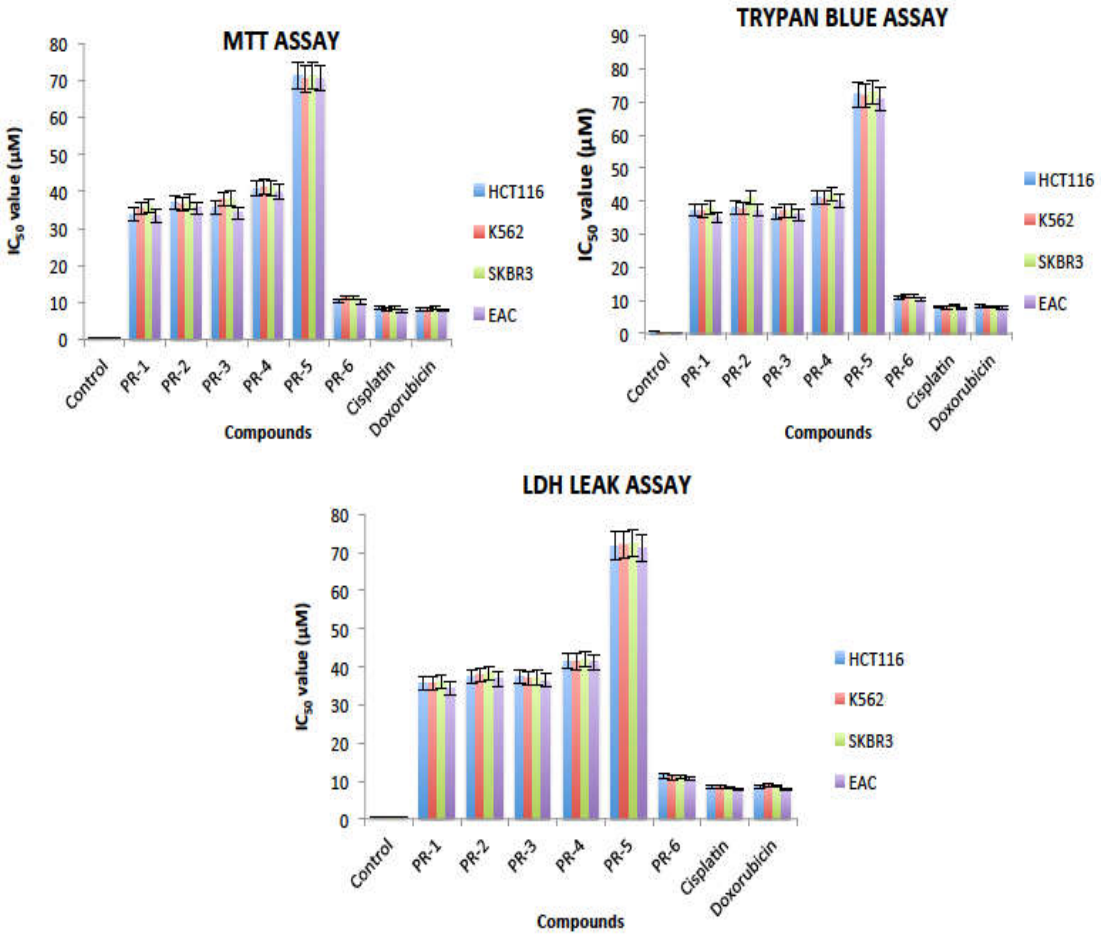

Figure 2. *In-vitro* screening of cytotoxic effect of compounds PR (1-6) : IC_{50} values of compounds PR (1-6) were evaluated using various cancer cell lines such as HCT116, K562 , SKRB3 and EAC cells by (A) MTT assay (B) Trypan blue assay (C) LDH leak assay

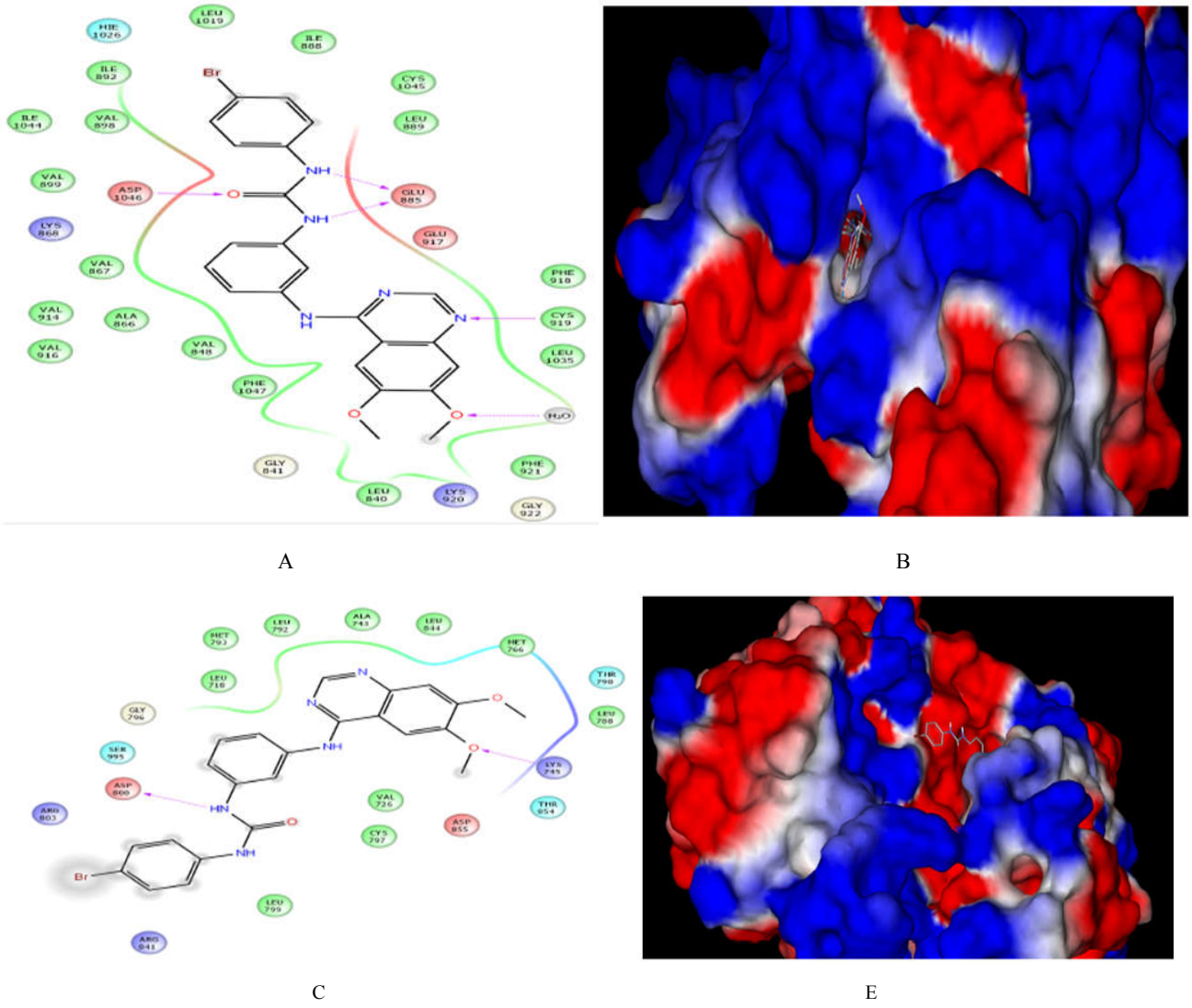

Figure 3. Putative binding pose of PR-6 showing molecular interaction of vascular endothelial growth factor (A), electrostatic binding interaction (B). Molecular interaction of epidermal growth factor receptor with PR-6 (C), electrostatic binding interaction (D)

Table 1. Molecular docking scores of all synthesized compounds against VEGFR and EGFR as obtained through glide docking

Protein	VEGFR-2 (PDB 4ASE)				EGFR (PDB 2ITY)			
Ligands	Docking Score	Glide Ligand Efficiency	Glide Hbond	Glide Evdw	Docking Score	Glide Ligand Efficiency	Glide Hbond	Glide Evdw
PR-1	-8.39	-0.26	-0.60	-18.92	-7.27	-0.214	-0.454	-51.29
PR-2	-7.28	-0.24	-0.15	-25.55	-5.76	-0.175	-0.341	-40.55
PR-3	-8.01	-0.25	-0.01	-34.32	-5.64	-0.182	-0.480	-38.28
PR-4	-9.05	-0.27	-0.57	-22.27	-5.70	-0.178	-0.250	-41.67
PR-5	0.00	0.00	0.00	0.00	-6.00	-0.182	-0.217	-40.46
PR-6	-11.13	-0.36	-0.57	-50.94	-5.49	-0.172	-0.277	-37.12

Table 2. Computer aided ADME screening of the synthesized compounds

Ligands	Mol. wt (Da)	a*	b*	c*	d*	e*	f*	g*	h*	i*
PR-1	445.5	4.05	-6.47	-6.19	1039	-0.94	666	0.30	100	0
PR-2	445.5	4.05	-6.48	-6.20	1039	-0.94	666	0.30	100	0
PR-3	429.5	4.25	-6.78	-6.21	1040	-0.88	666	0.43	100	0
PR-4	429.5	4.25	-6.78	-6.20	1040	-0.88	666	0.43	100	0
PR-5	484.3	4.86	-7.55	-6.11	1039	-0.57	3417	0.50	100	0
PR-6	494.3	4.51	-7.06	-6.24	1040	-0.69	1769	0.42	100	0
Range 95% of Drugs	>500 Da	-6.5/ 0.5	-6.5/ 0.5	< -5	<25poor, >500great	-3.0 / 1.2	<25poor,> 500great	-1.5 / 1.5	<25% is poor	>4

a*-QP Polarizability (A $^{O-3}$) of for octanol/water; b*-QP logS for aqueous solubility, c*-QPlog HERG K^+ Channel Blockage: log IC_{50}; d*- Apparent Caco-2 Permeability (nm/sec); e*- QP log BB for brain/blood; f*- Apparent MDCK Permeability (nm/sec) ; g*- QP log Khsa Serum Protein Binding; h*-%Human Oral Absorption; l*-Volition of Lipinski's rule.

Cytotoxic effects of Compounds PR (1-6) against various cancer cell lines

The synthesized compounds PR (1-6) were evaluated for their cytotoxic effects against different cancer cell lines such as HCT116, K562, SKBR3 and EAC cell lines using MTT assay, trypan blue dye exclusion assay and LDH leak assay (Figure 2). All the synthesized compounds significantly inhibited the growth of cancer cells in a dose-dependent manner (0-100 µM) after 24hrs of incubation. The obtained data indicate that the substituents methoxy and methyl at position 3- and 4- of phenyl ring in PR (1-4) showed less activity 33.43±1.90 to 41.90±1.52 µM on HCT116, K562, SKBR3 and EAC cell lines indicating that these substituents are less effective. Introduction of two chloro groups at position 3- and 4- of phenyl ring (PR-5) reduced the activity 70.46±2.59 to 72.80±1.84 µM on the before mentioned cell lines. Surprisingly, 4-bromo group in PR-6 enhanced the activity in the range of 10.09±0.92 to 11.24±0.81 µM on all four cell lines, which is comparable to that of standard drugs cisplatin 6.4±1.45 to 8.48±0.63 µM and doxorubicin 7.89±091 to 8.48±1.02 µM. Thus, our studies indicate that the compound PR-6 induces cytotoxicity and inhibits cell proliferation against all four cell lines of different origin and it was considered for further detailed analysis.

Docking studies

PR-6 showed better anticancer activity against HCT116, SKBR3, K562 and EAC cell lines. Molecular docking was performed to dissect the probable binding of PR-6 against the proteins which could have showed better down-regulating activity in all tested cell lines. To study the binding mode of new class of ligands against the tyrosine kinase domain of EGFR (2ITY) and VEGFR2 (4ASE) docking was performed (Table 1). Molecular docking results obtained were in concordance with *in-vitro* data and the optimal geometrical pose of PR-6 was same as the reference drug binding site, which is known to acquire optimum in the target proteins EGFR and VEGFR2. Hence it shows that PR-6 could be a better molecule for targeting these two proteins. PR-6 (IC_{50}=10.09µM) showed promising docking score of -11.13 and coordinate hydrogen bonds of PR-6 formed a strong binding with Asp1046 , Cys919 and Glu885 of VEGFR (Fig 3). PR-6 binds in a similar way as the reference ligand (tivozanib) binding to coordinate in VEGFR, as these amino acids resided near reference ligand were thought to be more potent inhibitor (44).

Whereas when compared to EGFR, newly synthesized ligands bind as similar to reference ligand (gefitinib) (Barbosa *et al*., 2014). PR-6 form hydrogen bond (C-N) with Lys745 and Asp800, C-O-O with Ser995 backbone (Fig 4). Merely based on docking score, it suggests that PR-6 is more potent against VEGFR2 than EGFR (Table 1). ADME result indicates that all these molecules possess pharmaceutical properties in the range of 95 % of drugs. The ligand obey the Lipinski's rules: molecular weight below 500 Da, hydrogen bond donor (less than five) and acceptor (less than ten). QPlogPo/w (octanol/water partition coe□cient) for the ligand is less than five. The ligand satisfy the values of partition coe□cient of octanol/gas (QPlogPoct), water/gas (QPlogPw) and brain/blood (QPlogBB), Skin permeability (QPlogKp), aqueous solubility (QPlogS) and Volition of Lipinski's rule are in predicted for ligand within the permissible range. It is a concluded from docking and ADME studies that PR-6 might act as a good anticancer compound with satisfactory ADME properties (Table 2) (Dileep *et al*., 2016). These results shows that newly synthesized ligand PR-6 displays good docking scoring consonant values with *in-vitro* data.

Conclusion

In conclusion, 4-anilino-6,7-dimethoxyquinazoline derivatives were designed and molecular docking was carried out for the designed molecules into the target VEGFR-2 and EGFR. It may be concluded from molecular docking and ADME studies that PR-6 might act as a good anti-cancer agent against vascular endothelial growth factor receptor with satisfactory ADME properties.

Conflict of interests

The authors have no conflict of interest to declare.

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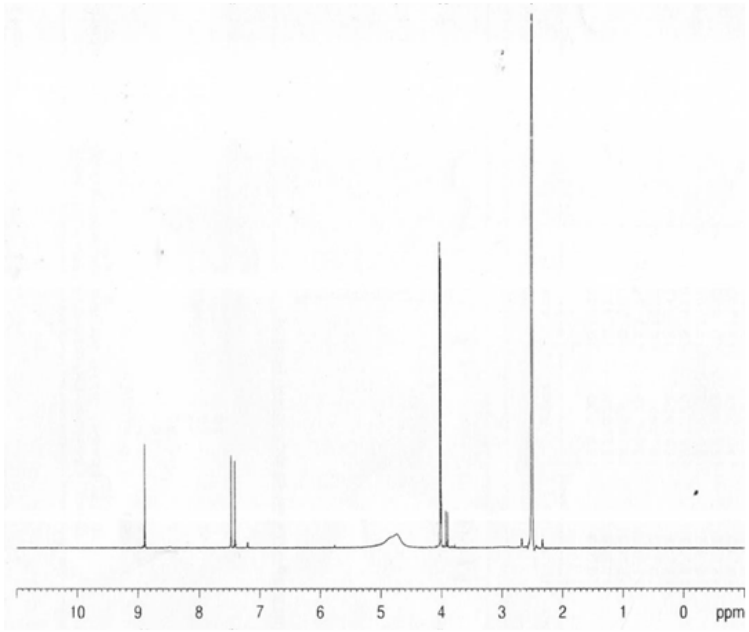
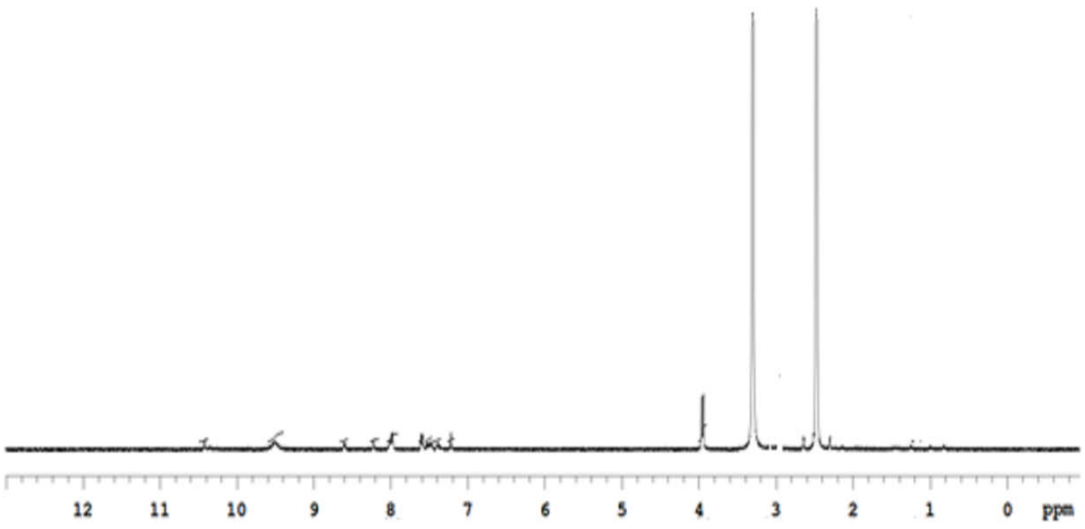
SUPPLEMENTARY DATA

Spectral data of selected compound

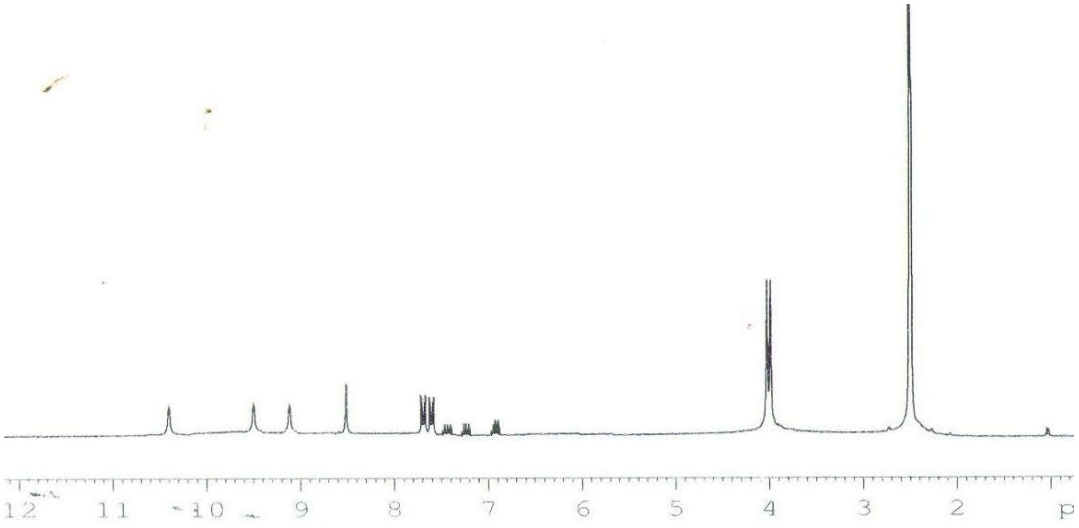
^{1}H NMR spectra of6,7-dimethoxy-4-chloroquinazoline **2**



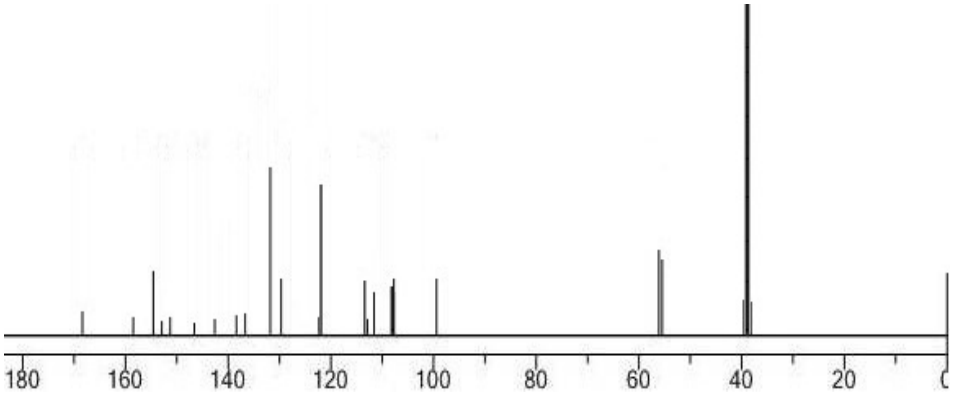
^{1}H NMR spectra of N-(3-aminophenyl)-6,7-dimethoxy-quinazolin-4-amine **3**



^{1}H NMR of 1-(4-Bromophenyl)-3-(3-((6,7-dimethoxyquinazolin-4-yl)amino)phenyl)urea (PR-6)



^{13}C NMR of 1-(4-Bromophenyl)-3-(3-((6,7-dimethoxyquinazolin-4-yl)amino)phenyl)urea (PR-6)



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