Research Article

Design, One Pot Synthesis and Insilco Studies of Substituted-1H-Pyrido [2, 1-b] Quinazolines as Anti-Angiogenic Agents

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ABSTRACT

A three component, one-pot synthesis of Substituted-1H-Pyrido[2,1-b] quinazoline derivatives by condensation of 2-aminopyrimidines, substituted aromatic aldehydes and ketones in the presence of ethanol by conventional reflux or by microwave synthesis using sulphamic acid as the catalyst was described. These synthesized compounds were screened for anti-angiogenic activity using by zebra fish model assay and CAM assay. The molecular docking studies on EGFR kinase results indicated that two compounds 4i and 4j of binding interactions strongly correlated with crystal ligand. Overall, these findings could suggest that these compounds would be an ideal motif's as an anti-angiogenic agents. Among all the 4a-4j compounds 4e, 4g and 4i shown good Angiogenesis inhibition activity when we done by Zebra Fish assay and Chick Chorioallantoic Membrane Assay.

Keywords: One-pot synthesis, Molecular Docking, EGFR Kinase, Anti-Angiogenic activity.

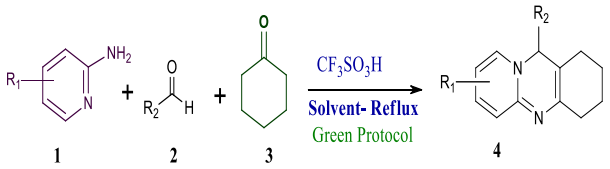
INTRODUCTION

Cancer is one of the leading causes of death worldwide[1] and accounts for almost 13% deaths than any other infectious diseases[2]. According to World Health Organization (WHO), projections of cancer prevalence is expected to raise by 21.7 million cases of oncological patients and 13 million deaths by 2030[3, 4]. With the increase in prevalence of cancer and thereby rapidly escalating costs, there are still types of cancer with massive unmet medical needs [5]. Therefore, the development of novel chemotherapeutic agents to fight against this deadly disease is needed urgently[6]. Nitrogen containing heterocyclic compounds like quinolines and pyridines core rings plays a very important role in drug discovery and development on cancer[7,8]. Molecular docking was performed on new chemical entities and erlotinib to predict the binding mode toward the EGFR kinase (PDB code: 1M17) and have showed all the compounds have shown similar interactions and compared with erlotinib.

MATERIALS AND METHODS

Chemistry

All chemicals and reagents were obtained from Aldrich Lancaster (Alfa Aeser, Johnson Matthey Company, Ward Hill, MA, USA), or Spectrochem Pvt. Ltd. (Mumbai, India) and were used without further purification. Reactions were performed by TLC on silica gel glass plate containing 60 GF-254, and visualization was achieved by UV light or iodine indicator. ^{1}H and ^{13}C NMR spectra were determined in $CDCl_3$ by using Varian and Avance instruments. Chemical shifts are expressed in parts per million (δ in ppm) downfield from internal TMS and coupling constants are expressed in Hz. ^{1}H NMR spectroscopic data coupling constants in Hz, number of protons. ESI mass spectra were recorded on a Micro mass Quattro LC using ESI+ software with capillary voltage 3.98 kV and an ESI mode positive ion trap detector. Melting points were determined with an Electro thermal melting point apparatus.



Scheme-I

General procedure

Preparation of Pyrido [2,1-b]quinazoline (4):

A One-pot and three component reaction were employed for the preparation of Pyrido[2,1-b]quinazoline **(4)** briefly, 2-aminopyridine (**10,** 1.0 mmol), aldehydes (**11,** 1.0 mmol) and ketones (2.0 mmol) was dissolved in EtOH in presence of CF_3SO_3H as a catalyst under nitrogen condition the reaction mixture was stirred with reflux for 5 hours by conventional or 35 minutes under microwave irradiation, the completion of reaction was monitored by the TLC. Then solvent was removed from the reaction by rotavaccum pressure. Resultant reaction mixture extracted with ethyl acetate and water, the organic layer washed with sodium sulphate then it is evaporated to obtain solid compound later purified by the column chromatography by using Ethyl acetate and Hexane as mobile phase to afford a final desired product.

Table 1: Physical data

S.No	Comp.	Name of the compound	Convent. Yield %	MWI Yield %	M.P °C
1	4 A.	11-Phenyl-2, 3, 4, 11-tetrahydro-1*H*-pyrido [2, 1-*b*] quinazoline	50	72	154-156
2	4B.	11-(4-Chlorophenyl)-2, 3, 4, 11-tetrahydro-IH-pyrido [2, 1-*b*] quinazoline	62	74	88-93
3	4C.	11-(4-Nitrophenyl)-2, 3, 4, 11-tetrahydro-1*H*-pyrido[2,1-*b*]quinazoline	67	76	150-151
4	4D.	11-(4-Methoxyphenyl)-2, 3, 4, 11-tetrahydro-1*H*-pyrido[2,1-*b*]quinazoline	68	76	88-89
5	4E.	(p-Tolyl)-2, 3, 4,11-tetrahydro-1*H*-pyrido[2,1-*b*]quinazoline	65	73	125–126
6	4F.	8-Chloro-11-(4-chlorophenyl)-2, 3, 4, 11-tetrahydro-1*H*-pyrido [2, 1-*b*] quinazoline	60	71	131–132
7	4G.	8-chloro-11-(4-fluorophenyl)-2,3,4,11-tetrahydro-1H-pyrido[2,1-b]quinazoline	65	76	88-93
8	4H.	11-(4-trimethoxyphenyl)-2, 3, 4, 11-tetrahydro-1*H*-pyrido[2,1-*b*] quinazoline	68	82	89-92
9	4I.	11-(1-benzyl-1H-indol-3-yl)-2, 3, 4, 11-tetrahydro-1H-pyrido [2, 1-b] quinazoline	89	94	185-186
10	4J	11-(1*H*-indol-3-yl)-2, 3, 4, 11-tetrahydro-1*H*-pyrido [2,1-*b*]quinazoline	71	87	180-182

Spectral Data:

11-Phenyl-2, 3, 4, 11-tetrahydro-1*H*-pyrido [2, 1-*b*] quinazoline (4a)

m.p: 154–156 °C; ^{1}H NMR: *δ* 7.36–7.28 (m, 5H), 6.89 (t, 1H, *J* = 7.8), 6.73–6.69 (m, 2H), 5.98 (t, 1H, *J* = 6.6), 5.37 (s, 1H), 2.38–2.24 (m, 2H), 1.74–1.59 (m, 6H); ^{13}C NMR: *δ* 148.7, 142.6, 136.8, 135.3, 133.1, 128.7, 128.2, 126.8, 123.6, 108.1, 106.6, 68.3, 30.3, 26.6, 23.1, 22.6; MS: *m/z* 263.0 [M + H].

11-(4-Chlorophenyl)-2, 3, 4, 11-tetrahydro-IH-pyrido [2, 1-*b*] quinazoline (4b)

mp: 88–93 ^{0}C; ^{1}H NMR: *δ* 7.50–7.21 (m, 7H), 6.62 (t, 1H, *J* = 6.0), 5.64 (s, 1H), 2.47–2.36 (m, 2H), 1.88–1.61 (m, 6H); ^{13}C NMR: *δ* 148.8, 141.9, 137.5, 135.2, 134.5, 133.0, 129.4, 128.3, 123.6, 108.3, 106.8, 67.8, 29.6, 26.4, 23.0, 22.6; MS: *m/z* 297.0 [M + H].

11-(4-Nitrophenyl)-2, 3, 4, 11-tetrahydro-1*H*-pyrido[2,1-*b*]quinazoline (4c)

mp: 150–151 °C; ^{1}H NMR: *δ* 8.25–8.22 (m, 2H), 7.54–7.45 (m, 3H), 7.34–7.29 (m, 1H), 7.07 (d, 1H, *J* = 6.3), 6.46 (t, 1H, *J* = 6.3), 5.72 (s, 1H), 2.53–2.37 (m, 2H), 1.91–1.61 (m, 6H); MS: *m/z* 308.12 [M + H].

11-(4-Methoxyphenyl)-2, 3, 4, 11-tetrahydro-1*H*-pyrido[2,1-*b*]quinazoline (4d)

mp: 88–89 °C; ^{1}H NMR: *δ* 7.27–7.21 (m, 2H), 6.88–6.84 (m, 3H), 6.76–6.68 (m, 2H), 5.99 (t, 1H, *J* = 6.6), 5.32 (s, 1H), 3.79 (s, 3H), 2.39–2.29 (m, 2H), 1.77–1.57 (m, 6H); ^{13}C NMR: *δ* 159.7, 148.9, 136.6, 135.4, 135.1, 133.4, 128.3,123.5, 114.2, 108.6, 107.0, 67.9, 55.3, 30.2, 26.7, 23.2, 22.8; MS: *m/z* 295.0 [M+ H].

(p-Tolyl)-2, 3, 4,11-tetrahydro-1*H*-pyrido[2,1-*b*] quinazoline (4e)

mp: 125–126 °C; ^{1}H NMR: *δ* 7.24–7.11 (m, 4H), 6.87 (t, 1H, *J* = 7.8), 6.73–6.65 (m, 2H), 5.96 (t, 1H, *J* = 6.6), 5.32 (s, 1H), 2.32–2.30 (m, 5H), 1.79–1.58 (m, 6H); ^{13}C NMR: *δ* 148.9, 139.8, 138.2, 136.5, 135.4, 133.4, 129.6, 126.9, 123.5, 108.5, 106.9, 66.2, 30.2, 26.7, 23.2, 22.7, 21.2; MS: *m/z* 276.9 [M + H].

8-Chloro-11-(4-chlorophenyl)-2, 3, 4, 11-tetrahydro-1*H*-pyrido [2, 1-*b*] quinazoline (4f)

mp: 131–132 °C; ^{1}H NMR: *δ* 7.35–7.32 (m, 2H), 7.27–7.22 (m, 2H), 6.89–6.85 (m, 1H), 6.78 (s, 1H), 6.75 (d, 1H, *J* = 2.1), 5.32 (s, 1H), 2.34–2.23 (m, 2H), 1.77–1.59 (m, 6H); ^{13}C NMR: *δ* 146.8, 140.4, 136.6, 134.9,134.8, 132.5, 129.4, 128.3, 124.6, 115.3, 107.4, 68.0, 29.9, 26.6, 22.9, 22.5; MS: *m/z* 330.8 [M + H].

8-chloro-11-(4-fluorophenyl)-2,3,4,11-tetrahydro-1H-pyrido[2,1-b]quinazoline (4g)

mp: 88–93 ^{0}C; ^{1}H NMR: *δ* 7.50–7.21 (m, 7H), 6.62 (t, 1H, *J* = 6.0), 5.64 (s, 1H), 2.47–2.36 (m, 2H), 1.88–1.61 (m, 6H); ^{13}C NMR: *δ* 148.8, 141.9, 137.5, 135.2, 134.5, 133.0, 129.4, 128.3, 123.6, 108.3, 106.8, 67.8, 29.6, 26.4, 23.0, 22.6; MS: *m/z* 314.0 [M + H].

11-(4-trimethoxyphenyl)-2, 3, 4, 11-tetrahydro-1*H*-pyrido[2,1-*b*] quinazoline (4h):

mp: 89–92 °C; ^{1}H NMR: *δ* 7.32–7.28 (m, 2H), 7.10–6.85 (m, 3H), 6.75–6.65 (m, 2H), 6.10 (s, 1H), 5.75 (d, 1H, *J* = 2.1), 3.85 (s, 3H), 2.34–2.23 (m, 2H), 1.77–1.59 (m, 6H); ^{13}C NMR: *δ* 160.0, 147.2, 135.7, 134.6, 134.1, 132.5, 127.5, 124.5, 115.2, 107.6, 106.8, 66.8, 56., 30.6, 27.0, 24.0, 23.6; MS: m/z 295.5 [M + H].

11-(1-benzyl-1H-indol-3-yl)-2, 3, 4, 11-tetrahydro-1H-pyrido [2, 1-b] quinazoline (4i)

mp: 185–186 °C; ^{1}H NMR: δ 8.20- 7.80(m, 3H), 7.50-7.35 (m, 4H), 7.20-7.05 (m, 5H), 6.65 (s, 1H), 6.47 (1H, dd, *J* = 8.3, 1.9 Hz), 5.99 (1H, d, *J* = 10.1 Hz), 5.55-5.25 (s, 2H), 2.21-2.94 (m, 3H), 2.00 (m, 1H), δ 1.56-1.85 (m,4H). ^{13}C NMR: δ 156.8, 147.8, 137.4, 134.9,134.8, 132.5, 129.4, 128.3, 124.6, 115.3, 107.4, 68.0, 29.9, 26.6, 22.9, 22.5; MS: *m/z* 392.46 [M + H].

11-(1*H*-indol-3-yl)-2, 3, 4, 11-tetrahydro-1*H*-pyrido [2,1-*b*]quinazoline (4j)

mp: 180–182 °C; ^{1}H NMR: δ 7.89–7.32 (m, 4H), 7.27–7.22 (m, 3H), 6.89–6.85 (m, 2H), 6.78 (s, 1H), 6.75 (d, 1H, *J* = 2.1), 2.34–2.23 (m, 2H), 1.77–1.59 (m, 6H); ^{13}C NMR: δ 146.8, 140.4, 136.6, 134.9,134.8, 132.5, 129.4, 128.3, 124.6, 115.3, 107.4, 68.0, 29.9, 26.6, 22.9, 22.5; MS: *m/z* 301.25 [M + H].

Zebra fish model assay

Male and female strains of zebra fishes were taken in 2:1 ratio

↓

Separate - male and female into two different(A&B) tanks with continues air supply(oxygen).

↓

Fishes for 3 days without feed, after 3rd day, start feeding for 7days.

↓

On 11th day take both AB strains (male and female) into one tank

↓ Leave them one day for Mating

Separate the fishes into their respective tank

↓

Collect the embryos of zebrafish by using embryo collector of mesh size 0.9mm

↓ Washed with water and maintained for 24hrs at 28.5˚C

Treated with control, test and standard samples,
blood vessels were counted by using a trinocular microscopeat magnification of **40X**

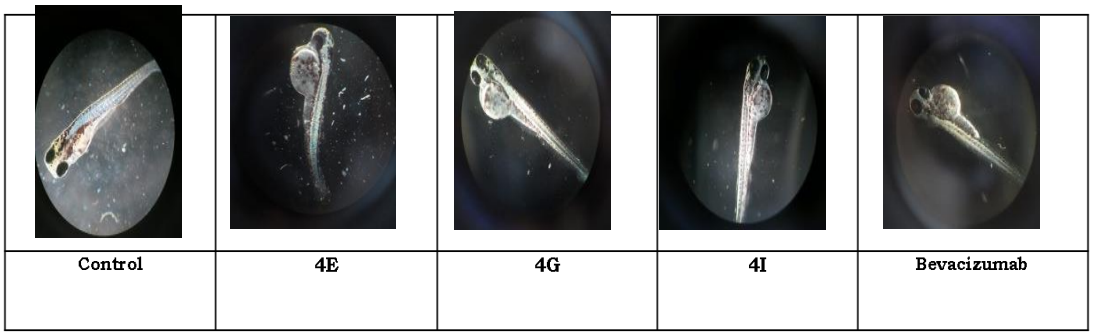


Figure 1: The angiogenic vessels in zibra fish

Pharmacological Studies

Zebra fish model assay

Male and female strains of zebra fishes were taken in 2:1 ratio, Separate - male and female into two different (A&B) tanks with continues air supply (oxygen). Fishes kept for 3 days without feed, after 3rd day, start feeding for 7days. On 11th day take both AB strains (male and female) into one tank Leave them one day for Mating, Separate the fishes into their respective tank, later Collect the embryos of zebrafish by using embryo collector of mesh size 0.9mm. then Washed with water and maintained for 24hrs at 28.5˚C. Treated with control, test and standard samples and blood vessels were counted by using a trinocular microscopeat magnification of 40X [9].

Chick Chorioallantoic Membrane Assay.

Eggs were collected from hatchery at day 0' and checked for any damage than they were randomly grouped, each group containing 6 eggs. Eggs were disinfected using ethanol then incubated at 37 degree Celsius. On the 3 rd day a hole was drilled at the narrow end and 2-3 ml of albumin was withdrawn with 18 gauge hypodermic needle. The hole was sealed with sterile tape and put back for incubation. On the 7 th day a window was opened on the shell, sterile gel foam or Sponge piece was placed on top of the membrane. The

control group was given with saline; the test and standard groups were impregnated with their respective doses.

Eggs were incubated till day 14. On the 14th day the CAM tissues directly beneath the sponge was removed from control and treated CAM samples and then examined under trinocular microscope [10].

Table 2: Inhibition percentage angiogenic vessels in Zebra Fish observed with effect of samples

S.No	Compounds	Percentage angiogenic vessels
1	4a	50.2± 0.125
2	4b	58.3± 0.258
3	4c	53.2± 0.368
4	4d	55.2± 0.412
5	**4e**	**35.8± 1.54**
6	4f	57.6± 0.436
7	**4g**	**32.7± 0.494**
8	4h	58.4± 0.339
9	**4i**	**25.8± 2.125**
	4j	55.7± 0.421
11	Control	95.3±3.50

Molecular Docking Studies

In this molecular docking study were carried out to examine the possible interactions with target enzyme using Dock Methodology of Molecular Operating Environment (MOE) software. The docking methodology consists of many parameters such as target selection and preparation, isolation of binding cavity with site finder, preparation of ligands, and finally docking to its receptor. The crystal structure of a-amylase was retrieved from Protein Data Bank (PDB ID: 4GQR) having a co-crystal ligand MYC and water molecules are removed and protein structure were energy minimized using default settings. The ligands are built using builder in MOE and energy minimized using MMFF94x force field. The docking protocol was carried out with ligand mbd file, Triangle Matcher as Placement, Rescoring using London dG scoring and finally optimized poses are ranked using GBVI/WSA DG score. The docking poses were browsed visually and best interactions were isolated and computed with ligand interactions.

Table 3: Inhibition percentage angiogenic vessels were observed with effect of samples

S.No	Compounds	Angiogenesis score
1	4a	37.1± 0.625
2	4b	35.4± 0.340
3	4c	38.6± 0.494
4	4d	37.2± 0.114
5	**4e**	**35.8± 1.54**
6	4f	38.2± 0.461
7	4g	**22.7± 0.494**
8	4h	36.0± 0.243
9	4i	**11.3± 0.548**
10	4j	34.6± 0.487
10	Control	38.0±1.24
11	Bevacizumab	13.3±1.41

ADME Predictions:

The *in silico* ADME propertities of these synthesized compounds were calculated by using the online server preADMET (http://preadmet.bmdrc.org/). The ADMET properties, human intestinal absorption (HIA), Caco-2 cell permeability, Maden Darby Canine Kidney (MDCK) cell permeability, plasma protein binding and blood brain barrier penetration (BBB) were predicted using this program.

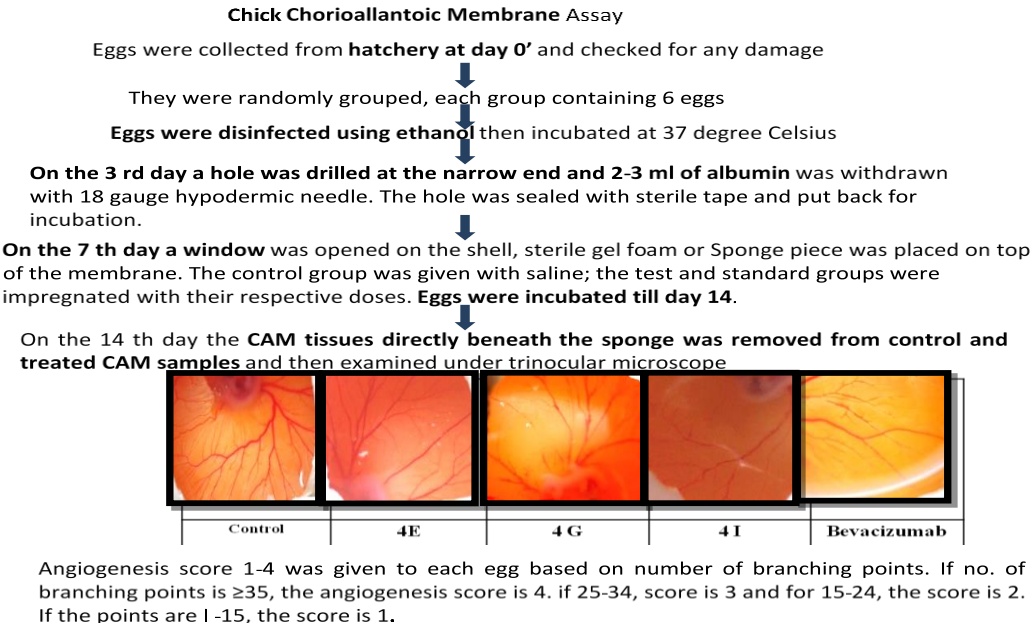


Figure 2: The inhibition of angiogenic vessels

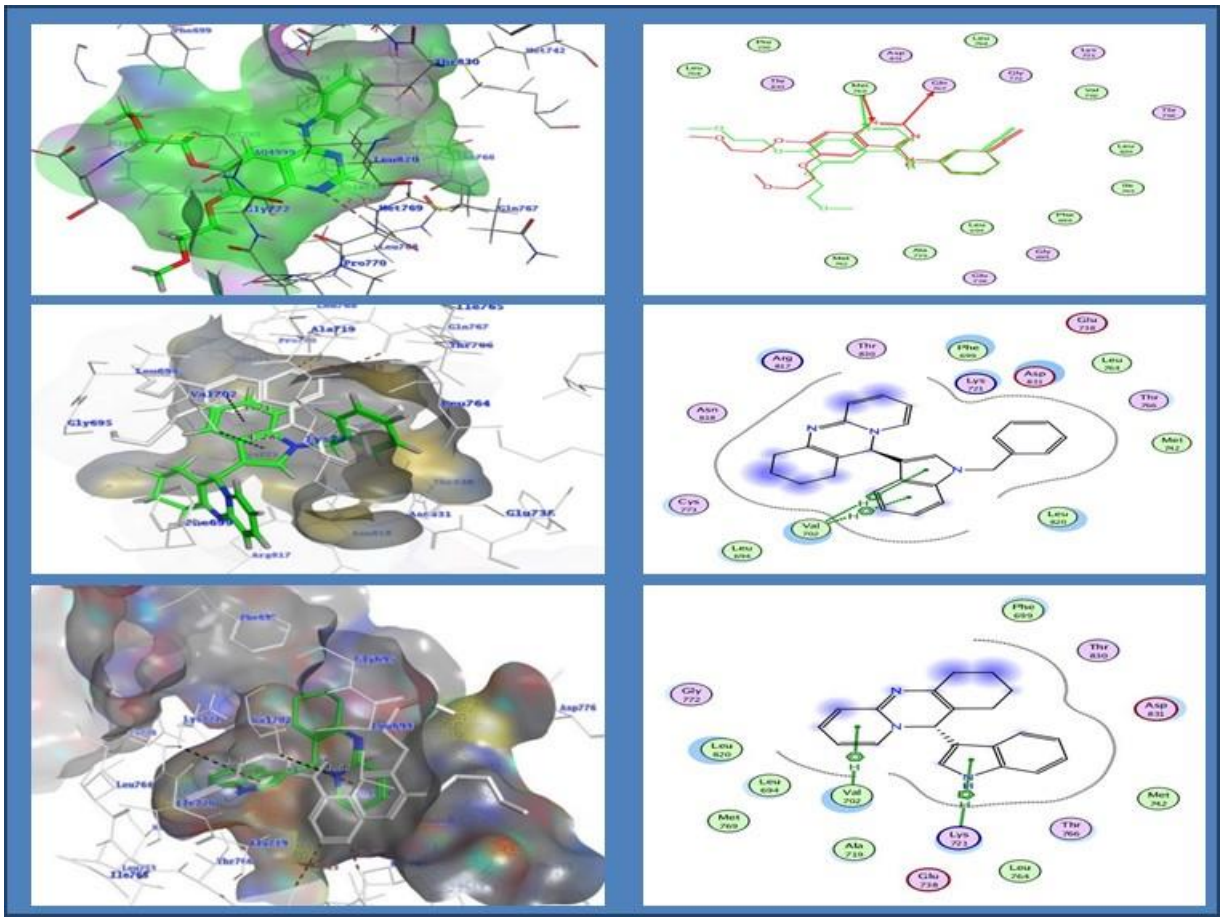


Figure 3: 3D and 2D interactions of erlotinib with the EGFR kinase and overlay complex with crystal ligand; 3D and 2D interactions of compound **4i** with the EGFR kinase with pi-H interactions with Val 702: 3D and 2D interactions of compound **4j** with the EGFR kinase with pi-H interactions with Val 702 and Lys 721.

Table 4: In silico ADME properties predicted for 10 novel compounds.

Compounds	Human intestinal absorption (%)	*in vitro* Caco-2 cell permeability (nm/sec)	*in vitro* MDCK cell permeability (nm/sec)	*in vitro* Plasma protein Binding (%)	*in vivo* blood-brain barrier penetration (C.brain/C.blood)	Skin Permeability
4a	100.000	47.5481	22.3603	90.0252	1.6503	-2.9472
4b	100.000	54.4603	12.3627	99.2229	2.7290	-3.0266
4c	98.4505	1.68389	7.34923	91.2597	0.1858	-3.2588
4d	100.000	57.9616	25.0452	92.4342	0.5698	-3.3084
4e	100.000	35.8399	19.1633	89.6007	2.2799	-2.9323
4f	100.000	47.9087	8.34951	99.9952	3.6766	-3.0692
4g	100.000	55.0284	26.9218	100.000	2.5289	-3.4225
4h	97.5993	56.8273	220.117	88.7345	0.0985	3.70086
4i	100.000	32.3409	13.754	100.000	2.1716	-2.4149
4j	94.3852	27.2204	17.4513	93.5175	9.3757	-3.5947

RESULTS AND DISCUSSION

The molecular docking studies on EGFR kinase results indicated that two compounds **4i** and **4j** of binding interactions strongly correlated with crystal ligand. Overall, these findings could suggest that these compounds would be an ideal motif's as an anticancer agent.

Among all the 4a-4j compounds 4e, 4g and 4i shown good Angiogenesis activity when we done Zebra Fish assay and Chick Chorioallantoic Membrane Assay.

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