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Synthesis, anticancer activity and molecular docking studies of newer quinoline analogues

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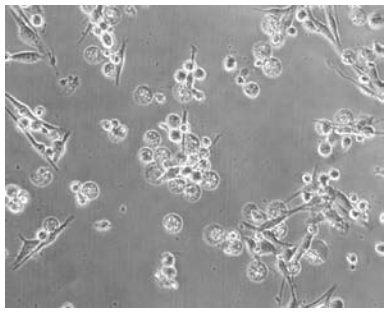
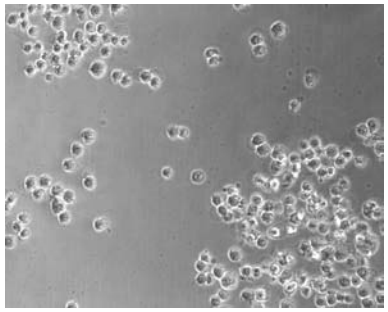
Synthesis, anticancer activity and molecular docking studies of newer quinoline analogues

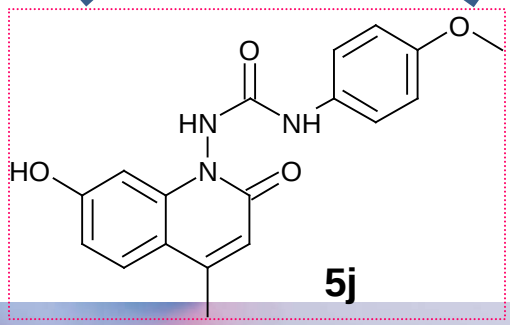
Graphical Abstract



MDA-MB-435; **GI_{50} =** 60.1 µM



HeLa; **GI_{50}** = 35.1 µM



Abstract

A series of new quinoline analogues was prepared in two steps. All the synthesized compounds were characterized by IR, NMR and mass spectral data. The anticancer activity was carried out as per the standard protocol and LC_{50}, TGI and GI_{50} were calculated. 1-(7-Hydroxy-4-methyl-2-oxoquinolin-1(2*H*)-yl)-3-(4-methoxylphenyl)-urea (**5j**) showed maximum anticancer activity with GI_{50} of 35.1 µM against HeLa (cervix cancer cell line) and 60.4 µM against MDA-MB-435 (breast cancer cell line) respectively. A molecular docking study implying epidermal growth factor receptor tyrosine kinase (EGFR-TK) was carried out to observe the binding mode of new quinoline analogues on the active site of EGFR-TK. The compound **5j** showed maximum docking score among the series of compounds. The amino acid residues Met793 showed backbone H-bonding with the hydroxyl group, while Asp855 showed side chain H-bonding with aryl NH group.

Keywords: anticancer activity; EGFR tyrosine kinase; HeLa; MDA-MB-435; quinoline

Introduction

A total of 1,658,370 new cancer cases and 589,430 cancer deaths are projected to occur in the United States in 2015. Despite the availability of improved drugs and targeted cancer therapies, it is expected that the new cases of cancer will jump to 19.3 million worldwide by 2025. The therapeutic applications of antiproliferative drugs are restricted owing to their toxic potentials, resistance, and genotoxicity. The demand for relatively more effective and safer agents for cancer therapy has been a great surge today. Several EGFR-TKIs have been clinically validated for the treatment of cancer patients, yet the search for new active molecules against EGFR-TK is still continuing. It is well known that quinoline analogues are inhibitors of EGFR-TK.

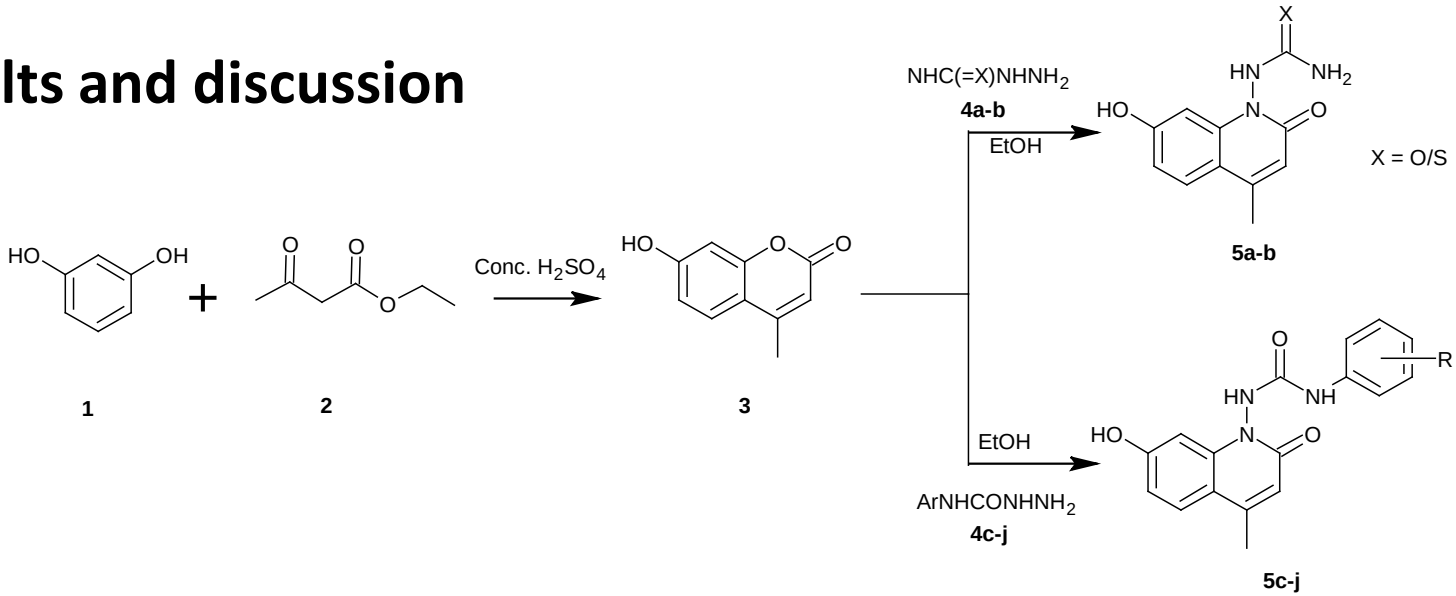
Quinoline nucleus occurs in natural and biologically active substances displaying broad therapeutic applications. Several quinoline analogues were reported having anticancer activity. In the present study, we reported herein the synthesis of a new series of quinoline analogues and their *in vitro* anticancer activity against HeLa (human cervix cancer cell line) and MDA-MB-435 (human breast cancer cell line). A molecular docking study implying EGFR-TK was carried out to observe the binding mode of new quinoline analogues on the active site of EGFR-TK.

Results and discussion

Chemistry

The quinoline analogues (**5a-j**) described in this study are shown in **Table 1** and the reaction sequence for their synthesis is summarized in **Scheme 1**. In the initial step solution of resorcinol (**1**) (0.1 mol; 11.01 g) in ethyl acetoacetate (**2**) (0.1 mol; 13.01 g ~13 mL) was added slowly into the concentrated H_2SO_4 (previously cooled to 5 °C), stirred and the temperature was maintained below 10 °C for 0.5 h to obtain the intermediate7-hydroxy-4-methyl-2*H*-chromen-2-one (**3**). In the subsequent step equimolar quantity of 7-hydroxy-4-methyl-2*H*-chromen-2-one (**3**) (0.005 mol; 0.88 g) and semicarbazide/ thiosemicarbazide/ substituted phenyl semicarbazide (0.005 mol) in ethanol (20 mL) was refluxed for 4-8 h at 200 °C to obtain 1-(7-hydroxy-4-methyl-2-oxoquinolin-1(2*H*)-yl)urea/thiourea (**5a-b**) and 1-(7-hydroxy-4-methyl-2-oxoquinolin-1(2*H*)-yl)-3-substituted phenyl urea (**5c-j**). The reaction was monitored throughout by thin layer chromatography (TLC) using benzene/acetone (1:4) as mobile phase. The yields of the final compounds (**5a-j**) were ranging from 59% to 80% after recrystallization with methylated spirit. Both the analytical and spectral data (IR, ^{1}H NMR and mass spectra) of all the synthesized compounds were in full accordance with the proposed structures.

Results and discussion

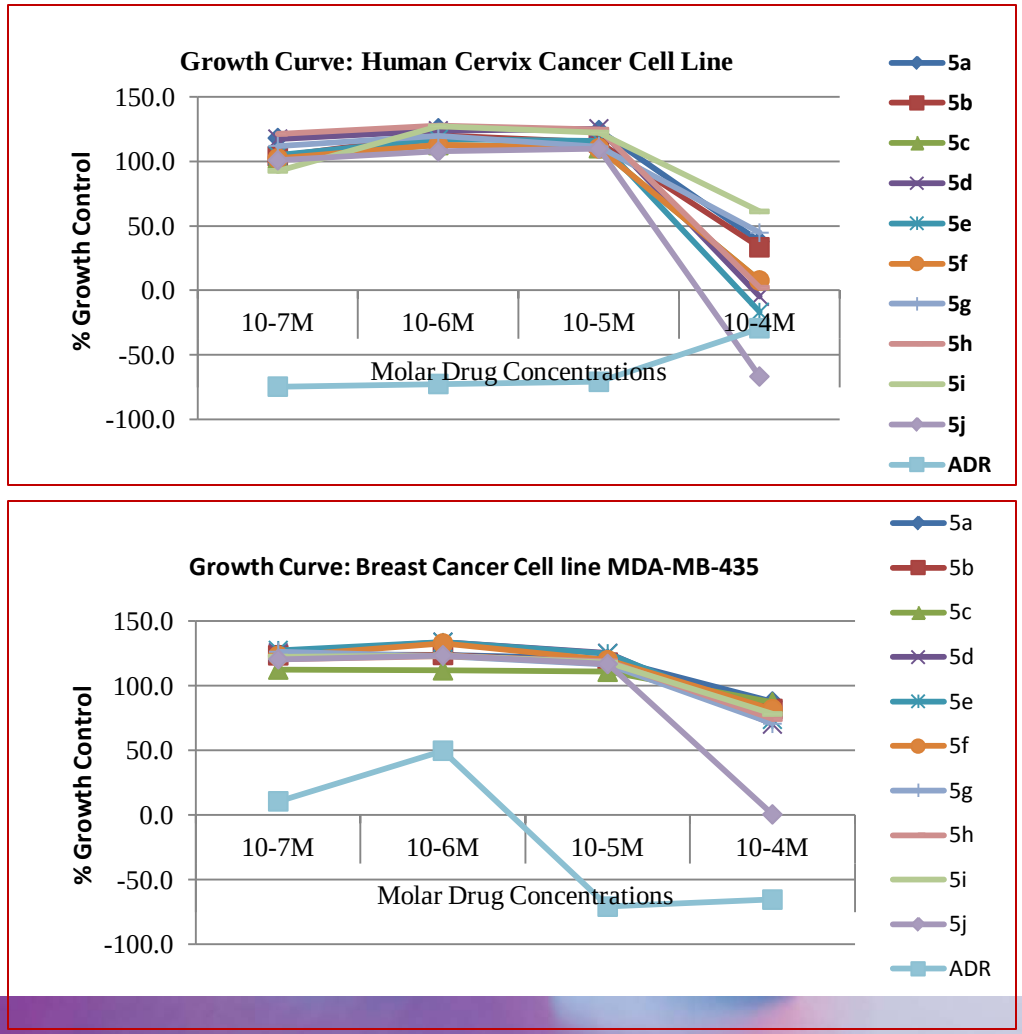


Scheme 1. Protocol for the synthesis of quinoline analogues (**5a-j**)

Table 1. Physical constant of quinoline analogues (**5a-j**)

S. No.	Compounds	X/R	% Yield	Mp (° C)
1	**5a**	O	70	140-142
2	**5b**	S	68	112-114
3	**5c**	H	80	150-152
4	**5d**	2,4-Dimethyl-	70	130-132
5	**5e**	2-Chloro-	65	118-120
6	**5f**	4-Methyl-	59	134-136
7	**5g**	2-Methyl-	73	140-142
8	**5h**	4-Fluoro-	64	136-138
9	**5i**	4-Bromo-	66	126-128
10	**5j**	4-Methoxy-	72	166-168

Results and discussion



Anticancer activity

The cytotoxic result was less at first three concentrations (10^{-7}, 10^{-6} and 10^{-5} M) but 10^{-4} M concentration produced strong cytotoxicity ranging between -66.9 and 61.2 percent growth against HeLa and between 0.6 and 87.8 percent growth against MDA-MB-435. The compound **5j** showed maximum cytotoxicity with -66.9 and 0.6 percent growths against HeLa and MDA-MB-435 respectively. The cytotoxicity of compound **5j** was found to be higher than the standard drug, adriamycin at 10^{-4} M concentration against HeLa.

Results and discussion

Further three parameters (GI_{50}, TGI and LC_{50}) were calculated for all the synthesized compounds. The GI_{50} recorded were ranging between 35.1 and >100 µM against HeLa, while only the compound **5j** registered GI_{50} of 60.4 µM against MDA-MB-435 and rest of the compounds showed GI_{50} of >100 µM. The LC_{50} recorded was found to be >100 µM for both the cell lines, except for the compound **5j** which showed LC_{50} of 91.33 µM against HeLa. The compounds **5j**, **5e** and **5d** showed TGI of 63.19, 88.17 and 97.28 µM respectively against HeLa, while compounds **5e** and **5d** showed TGI of 63.19, and 88.17 µM respectively against MDA-MB-435. The GI_{50}, TGI and LC_{50} were recorded for the quinoline analogues (**5a-j**) and are shown in **Table 2**.

The value of GI_{50} was taken into consideration to establish the structure activity relationship (SAR) of the synthesized compounds. The quinoline having 2,4-dimethyl substitution in phenyl ring was found to be favorable than 4-methyl and 2-methyl substitution, while 2-chloro substitution was found to be more favorable than 4-fluoro and 4-bromo substitutions. The 4-methoxy substitution showed maximum anticancer activity. The images of growth control of MDA-MB-435 and HeLa cancer cell lines by compound **5j** is shown in **Fig. 1**.

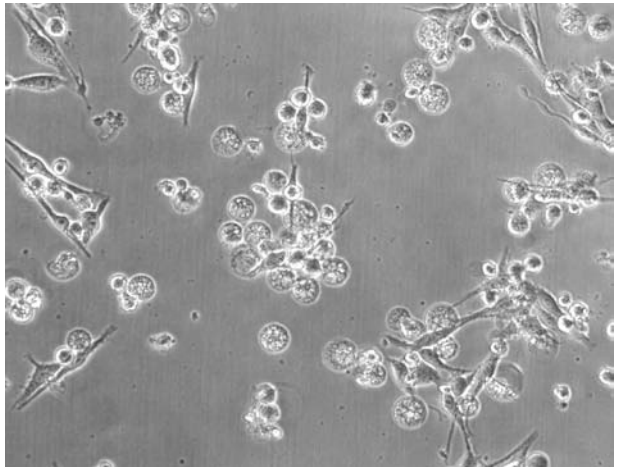
Results and discussion

Table 2. LC_{50}, TGI, and GI_{50} of quinoline analogues (**5a-j**) against HeLa and MDA-MB-435 cancer cell lines

Compound	Drug concentrations calculated from graph (μM)					
	Human Cervix Cancer Cell Line HeLa			Human Breast Cancer Cell Line MDA-MB-435		
	LC_{50}	TGI	GI_{50}	LC_{50}	TGI	GI_{50}
5a	>100	>100	87.0	>100	>100	>100
5b	>100	>100	80.6	>100	>100	>100
5c	>100	>100	73.20	>100	>100	>100
5d	>100	97.28	58.9	>100	97.28	>100
5e	>100	88.17	50.6	>100	88.17	>100
5f	>100	>100	59.9	>100	>100	>100
5g	>100	>100	93.0	>100	>100	>100
5h	>100	>100	62.7	>100	>100	>100
5i	>100	>100	>100	>100	>100	>100
5j	91.33	63.19	35.1	>100	>100	60.4
ADR	54.42	<0.1	<0.1	70.6	1.7	<0.1

ADR = Adriamycin

Results and discussion



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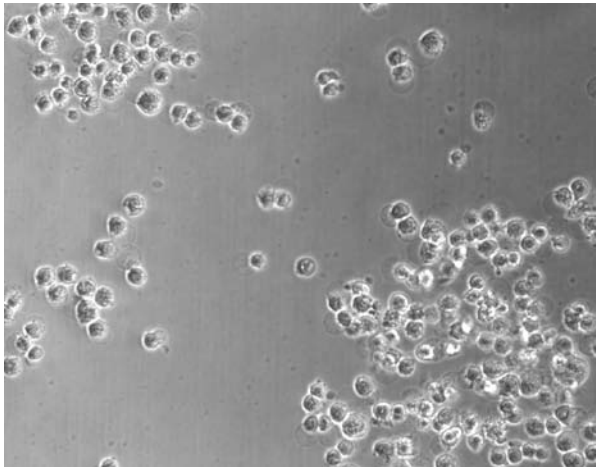

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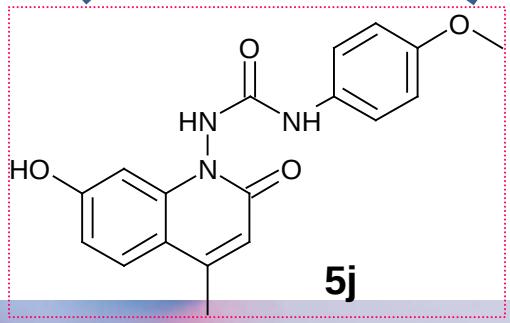

Fig. 1. Images of growth control of MDA-MB-435 and HeLa cancer cell lines by compound **5j**

Results and discussion

Molecular docking study

A molecular docking study implying epidermal growth factor receptor tyrosine kinase (EGFR-TK) was carried out to observe the binding mode of new quinoline analogues (**5a-j**) on the active site of EGFR-TK. Three different binding modes (green, yellow and grey) were observed by ligands (**5a-j**) as shown in the **Fig. 1** and the molecular docking scores are given in **Table 3**. The binding mode of compounds **5c**, **5d**, **5f**, **5h**, **5i** and **5j** (green ligands) with the active site of EGFR-TK showed interaction with backbone H-bonding of hydroxyl group with Met793 and side chain H-bonding of NH with Asp855 (**5f**, **5i** and **5j**). The binding mode of compounds **5b** (yellow ligands) with the active site of EGFR-TK showed backbone H-bonding of hydroxy group with Met793 and side chain H-bonding of terminal amine with Thr854. The binding mode of compounds **5a**, **5e**, and **5g** (grey ligands) with the active site of EGFR-TK showed backbone H-bonding of NH group with Arg841, side chain H-bonding of hydroxyl and aryl NH group with Asp855 and Asn842 respectively while staking with Phe723 (compound **5e**), -cationic interaction of substituted phenyl ring with Arg841 (compound **5g**).

Results and discussion

Molecular docking study

Table 4. The Glide score and E-model Score of the quinoline analogues (**5a-j**)

Compounds	Glide score	E-model score
5a	-4.575	-49.099
5b	-3.056	-46.094
5c	-6.670	-55.600
5d	-5.747	-59.748
5e	-4.385	-60.063
5f	-6.394	-64.002
5g	-4.377	-60.834
5h	-6.088	-57.672
5i	-5.723	-65.779
5j	-7.031	-63.567
Reference [Blair et al., 2007]	-8.288	-68.491

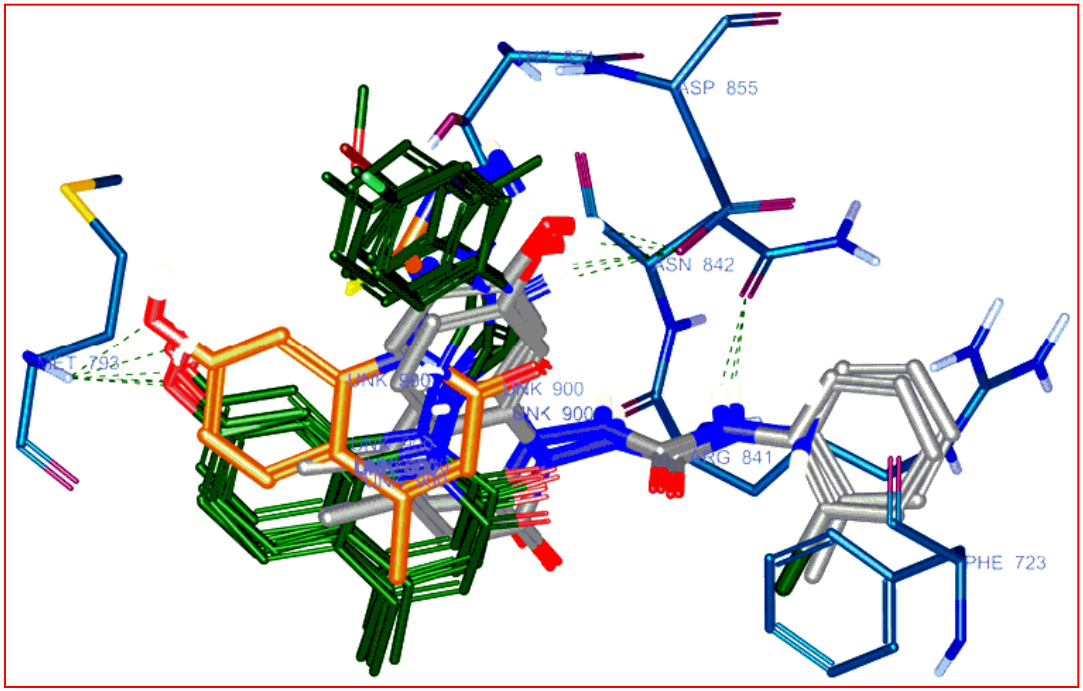

Fig. 1. The binding mode of quinoline analogues (**5a-j**) with EGFR tyrosine kinase active site

Results and discussion

Molecular docking study

The docking score of compound **5j** was found to be maximum showed comparatively higher anticancer activity among the series of quinoline compounds showed hydrophobic interaction with Met 793, Leu792, Ala743, Gly796, Met766, Leu788, Leu777 and Lys745, backbone H-bonding of hydroxyl group with Met793 and side chain H-bonding of NH with Asp855. The 2D binding mode of interaction with EGFR-TK is given in **Fig. 2**.

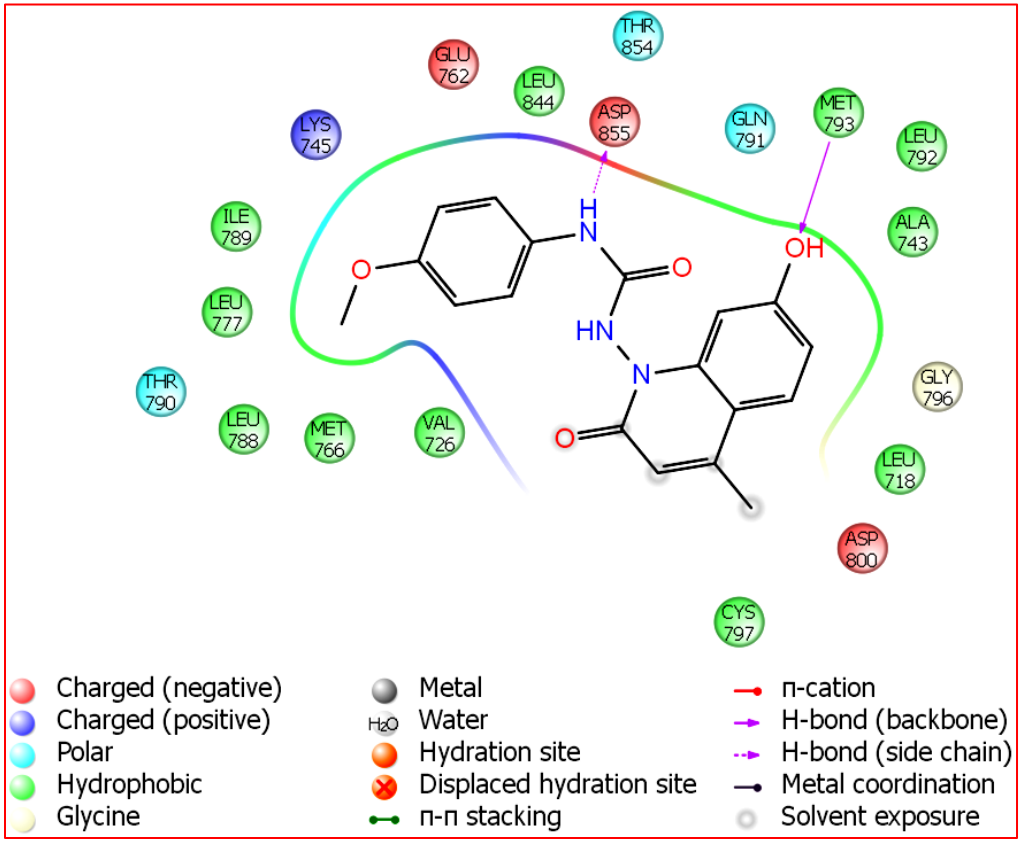

Fig. 2. 2D-Binding mode of interaction of ligand **5j** with EGFR-TK

Conclusions

All the quinoline analogues are synthesized in satisfactory yields. The compound **5j** showed maximum anticancer activity. The structure activity relationship established showed that 4-methoxy substitution was found to be more favorable than 2-chloro and 2,4-dimethyl substitution in the phenyl ring. The molecular docking study implying EGFR-TK showed maximum docking score for the compound **5j**. All these derivatives can be further modified to exhibit more potency. The compound **5j** could be considered as lead for further optimization and drug discovery. The quinoline derivatives discovered in this study may provide valuable information in the field of drug design and cancer therapy.

Acknowledgments

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