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Schiff's base derivatives bearing nitroimidazole and quinoline nuclei: New class of anticancer agents and potential EGFR tyrosine kinase inhibitors

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ABSTRACT

New Schiff's base derivatives **5a–j** have been synthesized by reaction between 2-phenoxyquinoline-3-carbaldehydes **3a–j** and 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide **4** in presence of nickel(II) nitrate as a catalyst in ethanol under reflux in good yield (78–92%). All compounds were tested for anticancer and inhibition of EGFR. Of the compounds studied, majority of the compounds showed effective antiproliferation and inhibition of EGFR and HER-2 activities. Compound **5h** showed most effective inhibition (IC_{50} = 0.12 ± 0.05 μM) by binding in to the active pocket of EGFR receptor with minimum binding energy (ΔG_b = −58.3691 kcal/mol). The binding was stabilized by two hydrogen bonds, two π–cation and one π–sigma interactions. Compound **5d** showed most effective inhibition (IC_{50} = 0.37 ± 0.04 μM).

Epidermal growth factor receptor (EGFR) is a kind of tyrosine kinase firstly reported in the literature.[1,2] It has become one of the targets of anticancer drug research and development because of its widely distribution in the cell and important role in cell life. EGFRs are distributed in mammalian epithelial cell membranes and have relationships with cell proliferation, death, and differentiation. They are junctions to deliver extracellular growth signals intracellular. EGFR family comprise four members, including: EGFR (HER1/ErbB-1), ErbB-2 (HER2/neu), ErbB-3 (HER3), and ErbB-4 (HER4).[3] EGFR tyrosine kinase-mediated cell growth signaling pathway plays an important role in the formation and development of many types of solid tumors, such as nonsmall cell lung cancer,[4] head and neck cancer,[5] and glioblastomas.[6] Over expression of EGFR family receptors have always been observed in these tumors, approximately in 60% of all tumors.[4] EGFR and ErbB-2 are the hottest targets in current research and their over expression or abnormal activation often cause cell malignant transformation. Also they have relationship with postoperative adverse, radiotherapy and chemotherapy resistance and tumor angiogenesis.[7]

Further, Schiff's bases constitute an important class of biologically active drug molecules which has attracted attention of medicinal chemists due to their wide range of pharmacological properties. These compounds are being synthesized as drugs by many researchers in order to combat diseases with minimal toxicity and maximal effects. These predictions has provided therapeutic pathway to develop new effective biologically active Schiff's base derivatives. A number of Schiff's base derivatives have been reported to exert notably antibacterial,[8] antimycobacterial,[9] antitumor,[10] antileishmanial activity,[11] DNA-binding activities, etc.

Encouraged by potential clinical applications of Schiff's base derivatives and our previous investigations on EGFR inhibitory activity of nitroimidazole derivatives[12] and other literature survey on quinoline having the same activity,[13] we report herein, the synthesis and anticancer activity as well as EGFR inhibitory activity of Schiff's base derivatives having nitroimidazole and quinoline moieties in a single scaffold with the goal to develop more effective target molecule for EGFR.

Schiff's base derivatives **5a–j** have been synthesized by reaction between 2-phenoxyquinoline-3-carbaldehydes[14] **3a–j** and 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide[15] **4** in presence of nickel(II) nitrate as a catalyst in ethanol under reflux in good yield (78–92%) (Scheme 1). The reaction was also attempted using AcOH,[16,17] and conc. H_2SO_4,[18] however, some shortcomings was observed such as incompletion of reaction, long reaction time; lose of yield and purification problems. Therefore, we applied $Ni(NO_3)_2 \cdot 6H_2O$ as a catalyst[12a] for this reaction to avoid such drawbacks as well as to develop easy and efficient method for the synthesis of new Schiff's base derivatives **5a–j**.

The structures of all the new synthesized compounds were established by ^1H NMR, ^{13}C NMR, FT-IR, elemental analysis, and

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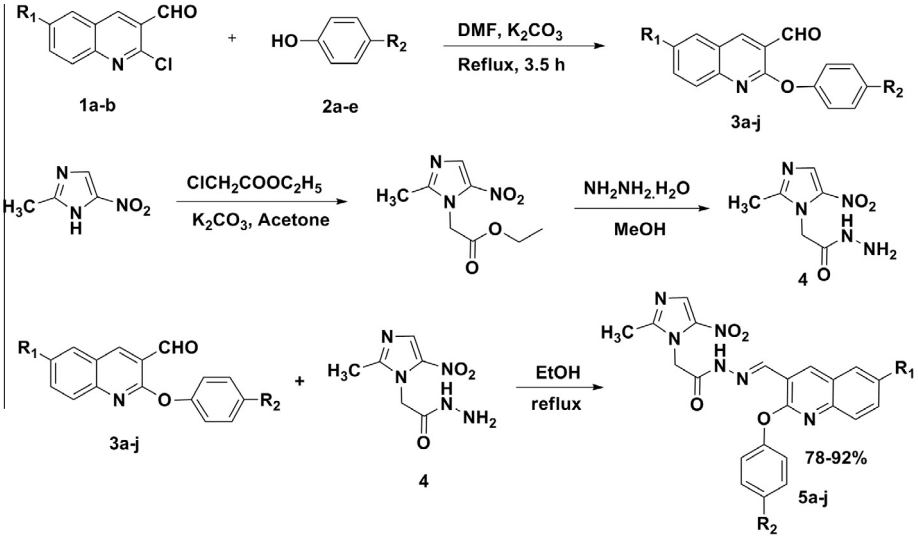

Comp	R_1	R_2	Comp	R_1	R_2
5a	H	H	**5f**	CH_3	H
5b	H	CH_3	**5g**	CH_3	CH_3
5c	H	OCH_3	**5h**	CH_3	OCH_3
5d	H	F	**5i**	CH_3	F
5e	H	Cl	**5j**	CH_3	Cl

Scheme 1. Synthetic pathway for the compounds **5a–j**.

molecular weight of compounds confirmed by mass spectrometry. Mass spectroscopy of compounds showed molecular ion peak $[M+H]^+$ corresponding to the exact mass.

All compounds were tested against EGFR and HER-2 kinases as well as against A549 adenocarcinomic human alveolar basal epithelial cell line and Hep G2 liver cancer cell line (Table 1). Upon investigation of antiproliferative activity of compounds **5a–j**, it has been observed that against A549, compounds **5h** (IC_{50} = 0.16 ± 0.03 μM) and **5i** (IC_{50} = 0.19 ± 0.07 μM) showed most potent as well as compounds **5c** (IC_{50} = 0.92 ± 0.14 μM), **5j** (IC_{50} = 1.23 ± 0.10 μM) and **5g** (IC_{50} = 1.19 ± 0.02 μM) showed good activity as compared to other compounds of the series. Compounds **5d** (IC_{50} = 0.18 ± 0.05 μM), **5e** (IC_{50} = 0.22 ± 0.01 μM) and **5i** (IC_{50} = 1.56 ± 0.16 μM) against Hep G2 showed most effective activity as compared to other compounds. As shown in Table 1, against EGFR, compound **5h** (IC_{50} = 0.12 ± 0.05 μM) displayed the most potent inhibitory activity as well as compounds **5c** (IC_{50} = 0.27 ± 0.10 μM), **5g** (IC_{50} = 0.18 ± 0.03 μM) and **5i** (IC_{50} = 0.42 ± 0.02 μM) showed excellent activity as compared to other compounds and less comparable to the positive control erlotinib (IC_{50} = 0.032 ± 0.02 μM). Compounds **5d** (IC_{50} = 0.37 ± 0.04 μM), (IC_{50} = 0.43 ± 0.09 μM) and **5i** showed excellent activity as compared to other compounds of the series.

Table 1
Inhibition of EGFR and HER-2 kinases as well as antiproliferative activity of compounds **5a–j**

	IC_{50} (μM ± SD)			
Compound	EGFR	HER-2	A549	Hep G2
5a	10.35 ± 0.54	15.3 ± 11.47	9.45 ± 0.23	13.28 ± 0.09
5b	23.13 ± 0.12	7.67 ± 0.36	21.51 ± 0.01	27.12 ± 0.10
5c	0.27 ± 0.10	12.02 ± 0.07	0.92 ± 0.14	4.13 ± 0.07
5d	21.02 ± 0.21	0.37 ± 0.04	3.12 ± 0.09	0.18 ± 0.05
5e	5.12 ± 0.33	4.10 ± 1.07	9.03 ± 0.06	0.22 ± 0.01
5f	3.17 ± 0.02	1.58 ± 0.58	7.18 ± 0.14	15.09 ± 0.08
5g	0.18 ± 0.03	17.0 ± 40.66	1.19 ± 0.02	21.90 ± 0.13
5h	0.12 ± 0.05	2.18 ± 0.08	0.16 ± 0.03	3.15 ± 0.14
5i	0.42 ± 0.02	0.43 ± 0.09	0.19 ± 0.07	1.56 ± 0.16
5j	8.66 ± 0.13	19.0 ± 11.23	1.23 ± 0.10	3.18 ± 0.04
Erlotinib	0.032 ± 0.002	0.16 ± 0.02	0.13 ± 0.01	0.12

Structure activity relationship (SAR) was carried out from EGFR inhibitory and anticancer activities. According to the activity data, it has been observed that the change in R_1 and R_2 substitution may lead to change in the activity against employed cancer cells as well as EGFR. Compounds having R_1 = –CH_3 and/or R_2 = –OCH_3 have proved as most effective members against EGFR and A549, while against HepG2, compounds having electron withdrawing groups such as –F and –Cl have proved as effective members of the series. Moreover, reviewing and comparing the activity data, it is worthy to mention that the anticancer and activity against EGFR of the target compounds depends not only on the bicyclic heteroaromatic pharmacophore, but also on the nature of the substituents.

Molecular docking study: To gain better understanding on the potency of all compounds and guide further SAR studies, we proceeded to examine the interaction of those with EGFR (PDB code: 1M17) by molecular docking, which was performed by simulation of compounds into the ATP binding site in EGFR. The binding energy of all the compounds is mentioned in Table 2. Of the compounds studied, compound **5h** was nicely bound into the active site of EGFR with minimum binding energy ΔG_b = −58.3691 kcal/mol. The binding model of compound **5h** and EGFR is depicted in Figures 1 and 1A. The amino acid residues which had interaction with EGFR were labeled. In the binding mode, compound **5h** was nicely bound to the ATP binding site of EGFR through hydrophobic interaction and the binding was stabilized by two hydrogen bonds, two π–cation and one π–sigma interactions. Among two hydrogen bonds, one formed between O-atom of carbonyl and THR766 with

Table 2
Binding energy of synthesized compounds **5a–j**

Compounds	CDOCKER interaction energy$-\Delta G_b$ (kcal/mol)
5a	51.0943
5b	48.0622
5c	53.7634
5d	48.9263
5e	51.1701
5f	51.2811
5g	58.1884
5h	58.3691
5i	53.3504
5j	51.1685

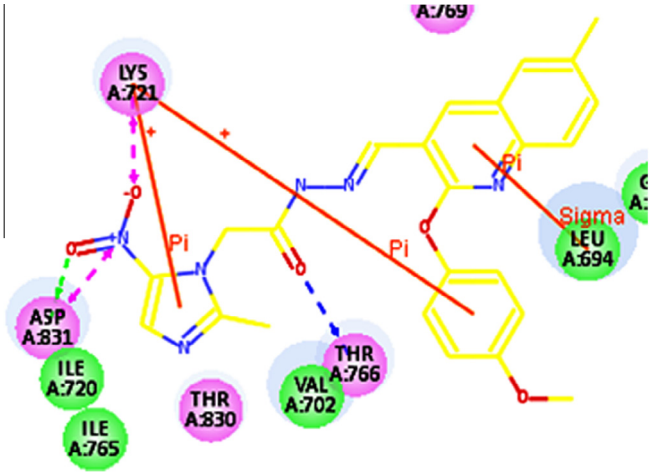

Figure 1. 2D binding model of compound **5h** into the active site of EGFR.

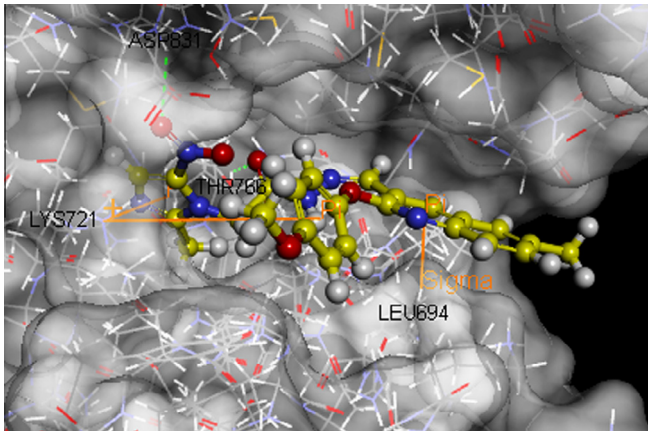

Figure 1A. Surface model of compound **5h** into the active site of EGFR (3D model).

distance = 1.97376 Å, DHA angle = 147.7° and HAY angle = 114.4°, while another H-bond formed between oxygen atom of nitro group and ASP831 with distance = 2.48427 Å, DHA angle = 132.9° and HAY angle = 129.6°. Among two π–cation interactions, one formed between nitroimidazole ring and LYS721 with distance = 4.76674 Å while another one formed between phenoxy ring of quinoline nucleus and LYS721 with distance = 6.76259 Å. One π–sigma interaction formed between quinoline ring and LEU694 with distance = 2.72184 Å. Based on the favorable EGFR inhibitory activity of Schiff's base derivatives having nitroimidazole moiety, it could be concluded that the every important parts of the molecule is taking part in the interaction with EGFR and it might be responsible for the effective activity of compound **5h** against EGFR.

In the conclusion, new series of Schiff's base derivatives having nitroimidazole and quinoline nuclei has been synthesized by reaction between 2-phenoxyquinoline-3-carbaldehydes and 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)acetohydrazide in presence of nickel(II) nitrate as a catalyst in ethanol under reflux in good yield. This synthetic strategy allows the assimilation of two promising bioactive nuclei in a single scaffold through an easy way. Reviewing the biological activity data, it has been concluded that compounds **5c**, **5d**, **5e**, **5g**, **5h** and **5i** are found to be most effective compounds of the series. Compound **5h** showed most effective inhibition by binding in to the active pocket of EGFR receptor with minimum binding energy may be because of substitution nature and position as well as spatial relation. According to this, it is worthy to mention that the Schiff's base derivatives having nitroimidazole and quinoline nuclei have become vital spot of anticancer as well as EGFR and HER-2 inhibition medicine research.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.041.

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