Research Article

Selected Paper from the 11th Pure and Applied Chemistry International Conference 2017 (PACCON 2017)

Molecular Docking Analysis on Epidermal Growth Factor Receptor Wild Type ($EGFR^{wt}$) with Quinazoline Derivative Compounds as Tyrosine Kinase Inhibitors

Herlina Rasyid and Ria Armunanto*
Austrian-Indonesian Centre (AIC) for Computational Chemistry, Department of Chemistry, Universitas Gadjah Mada, Indonesia

Bambang Purwono
Laboratory of Organic Chemistry, Department of Chemistry, Universitas Gadjah Mada, Indonesia

* Corresponding author. E-mail: ria.armunanto@ugm.ac.id
DOI: 10.14416/j.ijast.2017.12.001
Received: 27 December 2016; Accepted: 16 March 2017; Published online: 1 December 2017

Abstract
Molecular docking analysis for protein $EGFR^{wt}$ with quinazoline derivatives had been carried out. Six quinazoline derivatives obtained from previous experiment and two compounds predicted by QSAR were docked into $EGFR^{wt}$ using AutoDock program. Comparing between six compounds and erlotinib, these compounds have lower binding energies of −6.54, −6.48, −6.22, −6.24, −6.11 and −6.09, respectively than erlotinib (−4.84 kcal/mol). Docking result of two compounds resulted from QSAR exhibited lower binding energies of −4.85 and −5.96 kcal/mol than erlotinib. Binding pose from those compounds took place in amino acid residue Met769 in distance range of hydrogen bond 1.7 until 2.1 Å.

Keywords: Molecular docking, $EGFR^{wt}$ protein, AutoDock, Quinazoline

1 Introduction

Since 1985, lung cancer had been the most leading cancer in the world. There was 17.6% people death in the world caused by lung cancer and approximately 49.9% of this cases happened in developing countries [1]. In 2008, lung cancer became the main cause of cancer death in men with 951.000 deaths [2]. Nowadays, inhibition of cancer growth focused on protein receptor which had an important role in inhibition mechanism, such as EGFR protein [3], [4]. EGFR protein is a member of erbB1 family. This protein could influence the tumor growth including proliferation, angiogenesis, metastasis, and inhibition in apoptosis process [5].

One of strategies to inhibit EGFR protein was by used small molecules tyrosine kinase inhibitors. This type inhibitor bind an H-bond to ATP binding site, thereby blocking signal transduction from the EGFR [6]. Some quinazoline derivative compounds had been used as tyrosine kinase inhibitor, such erlotinib [7] as the first generation of EGFR inhibitiors, afanitib or BIBW2992 [8] the second generation, and Osimertinib as the third generation of EGFR inhibitiors [9].

The Structure of co-crystal of erlotinib [10] with EGFR protein contribute an important information in search of the bioactive compound. From this co-crystal, binding pose of inhibitor and protein could be studied and utilized to design a new bioactive compound which have the same key interaction. Afatinib as analogue compounds which

Please cite this article as: H. Rasyid, B. Purwono, and R. Armunanto, "Molecular docking analysis on Epidermal Growth Factor Receptor wild type ($EGFR^{wt}$) with quinazoline derivative compounds as tyrosine kinase inhibitors," *KMUTNB Int J Appl Sci Technol*, vol. 10, no. 4, pp. 293–299, Oct.–Dec. 2017.

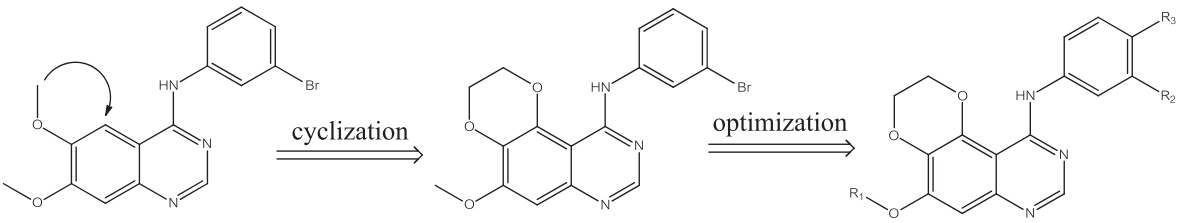


Figure 1: Design strategy of novel EGFR inhibitors [13].

have similar interaction in EGFR protein, due to the same binding mode on amino acid residue Met793 and Cys797 [11]. The compound, PD153035, didn't show any side effect until the doses up to 2.5 μM [12]. Modification through cyclization in compound PD153035 at the position 5- and 6- of quinazoline ring (Figure 1) had been done and complete a novel series of EGFR inhibitors [13].

Due to the novel series of EGFR inhibitor, QSAR analysis had been carry out and successfully design two derivatives compound that had better activities which could be described in a lower IC_{50} value [14]. However, docking analysis of these two predictive compounds had not been analysis. This research will focus on looking for binding site, binding pose, and inhibition mechanism of some quinazoline derivatives compounds.

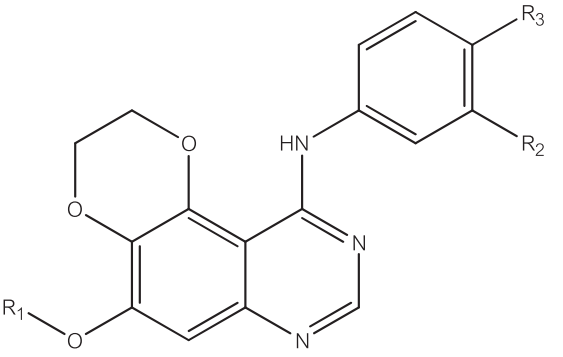
Nowadays, computational chemistry has been considered as an effective way to help in understanding the interaction of drug and protein [15], [16]. Autodock program could help to predict and rank the structures arising from the association between ligand and a target protein in 3D structure [17].

2 Computational Methods

Three dimensional structure of EGFR protein was taken from Protein Data Bank (PDB ID: 1M17). Erlotinib as a ligand standard was extracted from the PDB file. Some of quinazoline derivative compounds were taken from previous study for compound B1, B2, B3, B4, B5, and B6 [13] to know pose of binding site and also two compound (Q1 and Q2) resulted from QSAR analysis [14]. List of compounds can be seen in Table 1. Software packaging used in this study were AutoDock 4.0 software with the help of AutoDock Tools, Pymol, Chimera, Gaussian 09 [18].

Preparation of protein molecule. Complex protein-ligand in the PDB file was cleaned from all

Table 1: Core structure and list of docked compounds



Compound	R_1	R_2	R_3
B1		Cl	F
B2		ethynyl	H
B3		MeO-	H
B4		Cl	Me
B5		NO_2	H
B6		H	Me
Q1		CF_3	NH_2
Q2		ethyl	ethyl

of residue such as water and ligand erlotinib. Then, all of the sequence protein was added with hydrogen and charge using dockprep analysis and saved in pdb format file.

Preparation of ligand standard. Erlotinib as standard ligand was taken from the PDB code 1M17. Ligand erlotinib was selected and choosed inverted

all molecule. Then, delete all of selected molecule. All of hydrogen and charge were added to the ligand and saved in pdb format file.

Redocking analysis. Protein EGFR and erlotinib docked in affinity grid maps size was 30 × 30 × 30 Å with 1.000 Å of spacing. Lamarckian Genetic Algorithm (LGA) was used to search the lowest energy for each docking conformation [19]. Resulted 10 conformations for each automated docking was analysed to know free energy binding, inhibition constant, and pose of binding site. Successful redocking result could be seen in lower RMSD, usually lower than 2 Å [20].

Preparation of ligand and docking analysis. Eight of quinazoline derivative compounds was drew and optimized in Gaussian using DFT (B3LYP/6-31G). All of compounds was docked in binding site which known in redocking analysis. The affinity grid maps size and LGA was set up same with redocking.

3 Results and Discussions

3.1 Redocking analysis

Standard ligand (erlotinib) was docked into EGFR protein to know the binding site of protein. Coordinate of resulted binding site was 22.0732; 0.288; 52.7863. Table 2 served the result of dockingsimulation which produced 10 conformations. There are seven conformations which have RMSD value lower than 2 Å. Successful redocking analysis will be seen from RMSD value and the matching pattern in hydrogen bond interaction

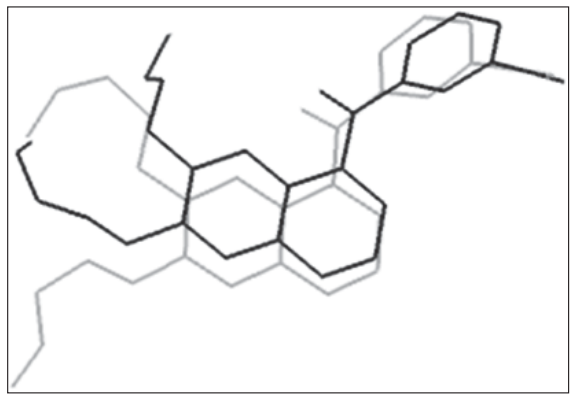


Figure 2: The overlapping structure of erlonotib before docking (green) and conformation 7 after redocking (pink).

compared with experimental result. Conformation 7 exhibited the lowest RMSD value (1.31 Å) between all of the cluster rank and showed an agreement in hydrogen bond interaction, due to the matching structure orientation between both of them. Indicating this conformation was in stable condition when binding with protein receptor. Overlapping structure of erlotinib before docking and structure of conformation 7 served in Figure 2. In this step, we can know that binding pose of erlotinib to the EGFR protein was influenced with amino acid residue of Met769. This result met with previous result which said that inhibitor interaction of erlotinib due to erlotinib accepts an H-bond from Met769 amide nitrogen [10].

Table 2: Redocking result of EGFR protein with erlotinib

Conformation	Δ*G* (kcal/mol)	K_i (μM)	Hydrogen Bond	RMSD (Å)
1	−4.94	240.14	Cys773 (1.915 Å)	1.80
2	−4.83	290.01	Met769 (2.115 Å)	1.58
3	−4.75	309.68	-	1.55
4	−4.99	220	Cys773 (1.926 Å)	1.74
5	−3.87	146	Met769 (2.08 Å)	2.93
6	−4.88	266	-	1.37
7	−4.84	282.66	Met769 (2.117 Å)	1.31
8	−4.93	243.01	Cys773 (2.164 Å)	1.63
9	−3.96	125	Met769 (2.12Å)	3.03
10	−3.63	217	-	4.01

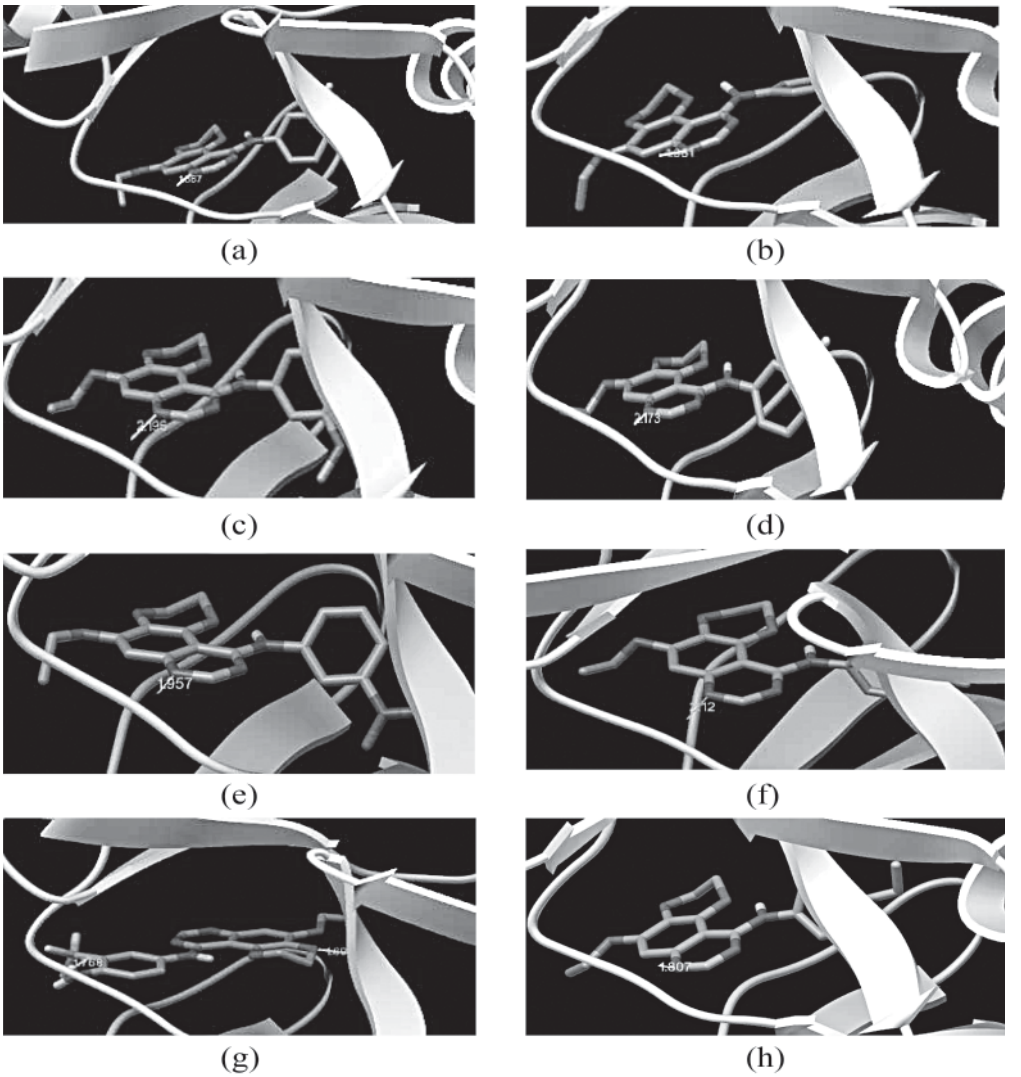


Figure 3: Conformation of binding pose for compound B1−B6 (a:B1, b:B2, c:B3, d:B4, e:B5, f:B6, g:Q1, h:Q2).

3.2 Docking analysis for all compounds

Molecular docking of B1–B6 and Q1–Q2 were carried out using the resulted binding site from redocking analysis. All of compounds were set up to have the same position with erlotinib. Figure 3 displayed the best conformation of binding pose for each compound. Binding pose of compound B1 showed the same binding pattern with erlotinib in amino acid residue of Met769 in distance of hydrogen bond was 1.867 Å. This result appropriate with previous study [21] which docked compound B1 using GOLD and obtained only one hydrogen bond in Met769 in distance of bond was 2.14 Å. Compound B1also execute using AutoDock package and found two hydrogen bond directly through compound between N atom of quinazoline with Met769 and fluoro atom with Thr766 [13].

The interaction of B2–B6 and Q1–Q2 compounds was located in amino acid residue of Met769. This result indicating that these derivative compounds have a correct binding site, due to the same amino acid

interaction in redocking analysis. This result was in good agreement with SAR analysis which could be seen in N11 atom of quinazoline with Met769 [6].

Autodock4 could convert binding energy to the inhibition constant by using equation (1) below.

$$K_i = \exp\left(\frac{\Delta G}{(R \text{ X } T)}\right) \quad (1)$$

Where ΔG is the docking energy, R in calorie is 1.98719, and T is 298.15 K [20].

Table 3 displayed the docking result for each analogues compound. There were 10 conformations cluster rank for each docked compound. Compounds with the best conformation will have the lowest energy binding. The best conformation rank was taken from the lowest energy and lowest inhibition constant for each compound. Comparing with erlotinib (–4.84 kcal/mol), all of these quinazoline derivative compounds had lower binding energy value and inhibition constant, indicating these analogues compound had better anticancer activities. Inhibition constant of all compounds are also lower than erlotinib (282.6 μM). This result suggested that these all compounds have a better stability and stronger interaction with protein receptor.

Table 3: Docking result of all compounds

Conformation of Compound	Binding Energy (kcal/mol)	K_i (μM)	Hydrogen Bond
B1	–6.54	16.06	Met769 (1.867 Å)
B2	–6.48	17.86	Met769 (1.981 Å)
B3	–6.22	27.74	Met769 (2.196 Å)
B4	–6.24	26.74	Met769 (2.173 Å)
B5	–6.11	33.4	Met769 (1.957 Å)
B6	–6.09	34.42	Met769 (2.120 Å)
Q1	–4.85	276.56	Met769 (1.898 Å) Lys721 (1.768 Å)
Q2	–5.96	42.52	Met769 (1.807 Å)

4 Conclusions

Redocking analysis using compound erlotinib as standar ligand had been done and resulted conformation 7 as the best conformation, due to the lowest RMSD value and the matching binding pose. Docking analysis in protein $EGFR^{wt}$ with compound B1−B6 and Q1−Q2 gave a lower binding energy than erlotinib. Inhibition constant of these compounds are also lower than erlotinib. This result indicated that all of compounds have a stronger interaction in protein $EGFR^{wt}$. The binding pose of all compound took place in amino acid residue Met769 with a distance of hydrogen bond in range 1.7 until 2.1 Å.

Acknowledgements

Financial support for this works from Program Pendidikan Magister Menuju Doktor Untuk Sarjana Unggul (PMDSU) scholarship of Ministry of Research, Technology and Higher Education is gratefully acknowledged. Gaussian 09 licenses were provided by Austrian-Indonesian Centre (AIC) for computational program is gratefully acknowledged. The authors would like to convey special appreciation to the academic committee of Pure and Applied Chemistry International Conference (PACCON2017) for providing the opportunity for this work to be published in this journal.

References

[1] D. Behera, "Managing lung cancer in developing countries: Difficulties and solutions," *Indian Journal of Chest Disease and Allied Science*, vol. 48, pp. 243–244, 2006.

[2] American Cancer Society, *Global Cancer Facts & Figure*, 2nd Edition, Atlanta: American Cancer Society, 2011.

[3] N. Zhao, X. Zhang, H. Yang, J. Yang, and Y. Wu, "Efficacy of epidermal growth factor receptor inhibitiors versus chemotherapy as second-line treatment in advanced non-small-cell lung cancer with wild-type EGFR: A meta-analysis of randomized controlled clinical trials," *Lung Cancer*, vol. 85, pp. 66–73, Jul. 2014.

[4] Y. Chen, Y. Luo, C. Wu, Y. Lee, R. Perng, and J. Whang-Peng, "Erlotinib or chemotherapy in second-line or later treatment of tumor EGFR wild-type pulmonary adenocarcinoma patients," *Journal of Cancer Research and Practice*, vol. 2, no.1, pp. 3–11, Mar. 2015.

[5] J. Baselga, "Why the epidermal growth factor receptor? The rationale for cancer therapy," *Oncologist*, vol. 7, Supplement 4, pp. 2–8, Aug. 2002.

[6] R. S. M. Ismail, N. S. M. Ismail, S. Abuserii, and D. A. A. El Ella, "Recent advances in

4-aminoquinazoline based scaffold derivatives targeting EGFR kinases as anticancer agents," *Future Journal of Pharmaceutical Sciences*, vol. 2, pp. 9–19, Jun. 2016.

[7] F. A. Shepherd, J. R. Pereira, T. Ciuleanu, E. H. Tan, V. Hirsh, S. Thongprasert, D. Campos, S. Maoleekoonpiroj, M. Smylie, R. Martins, M. van Kooten, M. Dediu, B. Findlay, D. Tu, D. Johnston, A. Bezjak, G. Clark, P. Santabárbara, and L. Seymour, "Erlotinib in previously treated non–small-cell lung cancer," *The New England Journal of Medicine*, vol. 2, no. 353, pp. 123–132, Jul. 2005.

[8] D. Li, L. Ambrogio, T. Shimamura, S. Kubo, M. Takahashi, L. R. Chirieac, R. F. Padera, G. L. Shapiro, A. Baum, F. Himmelsbach, W. J. Rettig, M. Meyerson, F. Solca, H. Greulich, and K. K. Wong, "BIBW2992; an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models," *Oncogene*, vol. 27, pp. 4702–4711, Aug. 2008.

[9] H. Cheng, S. K Nair, and B. W. Murray, "Recent progress on third generation covalent EGFR inhibitors," Bioorganic *& Medicinal Chemistry Letter*, vol. 26, no. 8, pp. 1861–1868, Apr. 2016.

[10] J. Stamos, M. X. Sliwkowski, and C. Eigenbrot, "Structure of epidermal growth factor receptor kinase domain alone and in complex with a 4-aminoquinazoline inhibitor," *The Journal of Biological Chemistry*, vol. 277, pp. 46265–46272, Nov. 2002.

[11] Y. Tu, Y. OuYang, S. Xu, Y. Zhu, G. Li, C. Sun, P. Zheng, and W. Zhu, "Design, synthesis, and docking studies of afaniib analogs bearing cinnamamide moiety as potent EGFR inhibitors," *Bioorganic & Medicinal Chemistry*, vol. 24, pp. 1495–1503, Apr. 2016.

[12] A. J. Bridges, H. Zhou, D. R. Cody, G. W. Rewcastle, A. McMichael, H. D. H. Showalter, D. W Fry, A. J. Kraker, and W. A. Denny, "Tyrosine kinase inhibitors. 8. an unusually steep structure-activity relationship for analogues of 4-(3-Bromoanilino)-6,7-dimethoxyquinazoline (PD 153035), a potent inhibitor of the epidermal growth factor receptor," *Journal of Medicinal Chemistry*, vol. 39, pp. 267–276, Jan. 1996.

[13] X. Qin, Z. Li, L. Yang, P. Liu, L. Hu, C. Zeng, and Z. Pan, "Discovery of new [1,4]dioxino [2,3-f]quinazoline-based inhibitors of EGFR including the T790M/L858R mutant," *Bioorganic & Medicinal Chemistry*, vol. 24, pp. 2871–2881, Jul. 2016.

[14] H. Rasyid, R. Armunanto, and B. Purwono, "Study of Quinazoline Derivative Compound as Anticancer on $EGFR^{WT}$ Protein using Quantitative Structure-Activity Relationship (QSAR)," *International Journal of Pharmaceutical Sciences Review and Research*, vol. 42, pp. 44–49, Dec. 2016.

[15] Y. Xu, Y. Cao, H. Ma, H. Li, and G. Ao, "Design, synthesis, and molecular docking of α, β-unsaturated cyclohexanone analogous of curcumin as potent EGFR inhibitors with antiproliferative activity," *Bioorganic & Medicinal Chemistry*, vol. 21, pp. 388–394, Jan. 2013.

[16] I. S. Yadav, P. P. Nadekar, S. Shrivastava, A. Sangamwar, A. Chaudhury, and S.M. Agarwal, "Ensemble docking and molecular dynamics identify knoevenagel curcumin derivatives with potent anti-EGFR activity," *Gene*, vol. 539, pp. 82–90, April 2014.

[17] S. F. Sousa, P. A. Fernandes, and M. J. Ramos, "Protein-ligand docking: Current status and future challenges," *Proteins*, vol. 65, no. 1, pp. 15–26, Oct. 2006.

[18] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S.

Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, Gaussian 09, Inc., Wallingford CT, 2016.

[19] G. M. Morris, R. Huet, W. Lindstrom, M. Sanner, R. K. Belew, D. S. Goodsell, and A. J. Olson, "Autodock4 and autoDockTools4: Automated docking with selective reseptor flexibility," *Journal of Computational Chemistry,* vol. 30, no. 16, pp. 2785–2791, Dec. 2009.

[20] R. Huey and G. M. Morris, *Using Autodock with AutoDockTools: A tutorial,* La Jolla, California USA: The Scripps Research Institute, Molecular Graphics Laboratory, 2008, pp. 54−56

[21] G. Verma, M. F. Khan, W. Akhtar, M. W. Alam, M. Akhter, O. Alam, S. M. Hasan, and M. Shaquiquzzaman, "Pharmacophore modeling, 3D-QSAR, docking and ADME prediction of quinazoline based EGFR inhibitors," *Arabian Journal of Chemistry*, Article in Press, Sep. 2016.