Dear editor Dr. Olgica Nedić and anonymous reviewers,

Thank you so much for your useful comments to help improving our manuscript **No. JSCS8813**. Here, according to your suggestions we have performed careful and systematic revisions through the manuscript. In addition, the style, format, and references of the paper have been prepared consistently with the "Instructions for Authors" as well as the recent publications in "***Journal of the Serbian Chemical Society***". We therefore hope the current form of manuscript can meet your requirement. Thanks!

To Reviewer G

In this article authors analysed by computational studies all clinically observed EGFR missense mutants in NSCLC in order to identify those substantial mutations that may significantly increase or decrease ATP affinity causing also generic resistance for ATP competitive TKIs. They found already known "generic" resistance mutation T790M that according to this study increases ATP affinity by establishing additional S...Pi interaction. They also found two new generic"resistance mutations A839T and E758G that improve ATP affinity by forming favorable hydrogen bond and eliminating electrostatic effect between kinase and ATP. Although, this looks like article for computational/theoretical chemistry section it could be published but after following major revision.

Reply: Dear reviewer, thank you for your positive attitude towards our work! In this revision, in order to give a more comprehensive/systematic profile of ATP response to kinase mutations, we have further extended the investigated kinase mutations from NSCLC-related EGFR mutations to cancer-related drug-resistant mutations of four EGFR kinase family members (*i.e.* EGFR/ErbB1, ErbB2, ErbB3 and ErbB4). In addition, we also employed binding assays to measure the direct binding affinities of ATP to few representative wild-type and mutant kinases. We think

these extended efforts in the revision could make this work more solid and valuable. Thanks!

(1) What is missing in this article is how these findings and given theoretical explanations of mechanism how resistance mutations in EGRF are influencing ATP affinity can help in the treatment of NSCLC. There is no conclusion at the end of Results and Discussion section, but only general discussion thought section about mechanism for changing ATP affinity and it should be added.

Authors should emphasize what is the novelty in this article compared to the previous studies and how it can help in the treatment of NSCLC.

Reply: Thank you for your comments! For this we would like to give an explanation.

As you know, EGFR has been well established as a therapeutic target of lung cancer and many other tumors. For example, a number of small-molecule EGFR inhibitors such as Erlotinib, Gefitinib and Lapatinib have been approved by US FDA to treat diverse cancers. However, many patients treated with EGFR inhibitors have been clinically observed to cause acquired drug resistance, largely limiting the application of EGFR inhibitors. The acquired resistance is commonly associated with kinase mutations during the cancer therapy process. Traditionally, the mutations are thought to directly influence the binding behavior of kinase inhibitors to EGFR, for example, impairing inhibitor binding capability by inducing steric hindrance or breaking favorable interactions upon the mutation. Therefore, over the past two decades a large number of computational and experimental investigations have been performed to explore how to influence inhibitor binding by acquired-resistant kinase mutations.

Nevertheless, in a highly cited paper Yun and co-workers proposed that, instead of influencing inhibitor binding, the EGFR T790M gatekeeper mutation can cause acquired drug resistance by increasing ATP affinity [Yun CH, et al. *Proc Natl Acad Sci USA* 2008, 105: 2070–2075]. This is a very valuable discover that provides a new insight into the molecular mechanism of mutation-caused acquired drug resistance

(because kinase inhibitors need to compete with kinase substrate ATP to inhibit the kinase activity). Although the new finding is very interesting, ***since then only very few kinase mutations have been considered to influence drug sensitivity by changing ATP affinity, but no systematic investigation has been addressed on the effect of clinically observed diverse drug-resistant mutations on ATP binding. Therefore, it is still unclear whether kinase mutation-induced ATP affinity change is or not a common phenomenon to cause generic drug resistance.*** We think this is because experimental analysis of all kinase mutation–ATP pairs is too time-consuming and expensive. Instead, we in this study combined *in silico* analysis and *in vitro* assay to systematically investigate the effect of clinically reported drug-resistant kinase mutations on ATP binding, which would obtain a comprehensive profile of ATP response to all clinically observed drug-resistant kinase mutations with an acceptable cost. We think this is valuable for both theoretical study and clinical practice. *For example, the obtained profile can be used to explain the new molecular mechanism of drug resistance established by mutation-caused ATP affinity increase and, more significantly, to guide rational design and optimization of new kinase inhibitors to combat the generic drug resistance by overcoming the increased ATP affinity.*

In this revision, according to your suggestion we have added a Conclusion Section to give a brief description about the clinical significance of this work in the treatment of cancers. Thanks!

(2) Authors should also connect more their results with similar in the literature especially results and discussion at the last two pages where they explained hydrogen-bond forming mutations and electrostatics-reshaping mutations (10 and 11).

Reply: Thank you for your suggestion!

In fact, besides the pioneer work of Yun et al. [*Proc Natl Acad Sci USA* 2008, 105: 2070–2075], there were no systematic study regarding the drug resistance caused by ATP affinity change upon kinase mutations. Most previous works focused on the effect of hydrogen-bond forming mutations and electrostatics-reshaping mutations on

inhibitor binding (but not ATP binding). Therefore, we did not connect our results with literatures.

Here, according to your suggestion we have added discussion and comparison about how kinase mutations influence nonbonded interactions with ATP and inhibitors, and cited relevant literatures in the discussion and comparison. Thanks!

To Reviewer I

Revision regarding the Introduction part is needed. It is necessary to consult a clinician in order to make appropriate corrections in the first part of the Introduction.

Reply: Dear reviewer, thank you for pointing out this issue; we are so sorry! In fact, we are all chemical researchers so that the medicinal and clinical descriptions in Introduction section may not be very good. In this revision, we have reduced/rewritten such descriptions in Introduction Section and primarily focused on the chemical and biological aspects of ATP response to drug-resistant kinase mutations. In addition, we have discussed with a medicinal professor in our university to avoid the inaccurate descriptions regarding clinical aspect in Introduction. In this way, we think the revised Introduction can better meet the theme of the Journal of the Serbian Chemical Society. We hope you can agree it. Thank you so much!