ANSWERING REVIEWERS

August 7, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 12221-review.doc).

Title: Pharmacophore approaches in protein kinase inhibitors design

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Name of Journal: *World Journal of Pharmacology*

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(Reviewer 1)

This review paper summarizes the published data on pharmacophore models for inhibitors of tyrosine protein kinases and serine/threonine kinases from 54 papers. The review focuses on the ligand-based and receptor-based methods in pharmacophore models development and provide useful information to readers.

Several minor changes may improve the paper:

1. It is better to include a table in the beginning to show the major kinases in two families of protein kinase (PTK and Ser/Thr kinases) that will be discussed in this review and the key feature of each kinase (receptor or non receptor).

We have included Table 1 in the Introduction Section to show the protein kinases discussed in this review:
"In the review we discuss the published data on pharmacophore models for inhibitors of several tyrosine protein kinases and serine/threonine protein kinases (Table 1)."

Table 1 Protein kinases discussed in the review

Tyrosine protein kinases	
Epidermal growth factor receptor (EGFR; Erb-1; HER1 in humans)	receptor

Human epidermal growth factor receptor 2 (HER2; erbB2; protooncogene Neu)	receptor
Vascular endothelial growth factor receptor 2 (VEGFR-2)	receptor
Janus kinase 2 (JAK2)	non-receptor
Janus kinase 3 (JAK3)	non-receptor
Spleen tyrosine kinase (Syk)	non-receptor
Zeta-chain-associated protein kinase 70 (ZAP-70)	non-receptor
TEK tyrosine kinase, endothelial (Tie 2)	receptor
Serine/threonine protein kinases	
Cdc2-like kinase (Clk)	non-receptor
Dual-specificity tyrosine-phosphorylation regulated kinase 1A (Dyrk1A)	non-receptor
Checkpoint kinase 1 (Chk1)	non-receptor
Human inhibitor nuclear-factor κB kinase 2 (hIKK-2)	non-receptor
Cyclin-dependent kinase 1 (CDK1)	non-receptor
Cyclin-dependent kinase 2 (CDK2)	non-receptor
Polo-like kinase (PLK)	non-receptor
c-Jun N-terminal kinase 3 (JNK3)	non-receptor
Glycogen synthase kinase 3 (GSK-3)	non-receptor
Mammalian target of rapamycin (mTOR)	non-receptor
p38 mitogen-activated protein kinase (p38 MAPK)	non-receptor
Protein kinase B (PKB; Akt)	non-receptor

2. It is better to include a section about the clinical impact of the pharmacophore approaches in protein kinase inhibitors design. The section could show some clinically successful drugs designed through pharmacophore approaches.

Currently, there are several small-molecular protein kinase inhibitors, which were approved by FDA. The reported drugs target EGFR/ErbB2 (Afatinib, Gefitinib, Lapatinib), VEGFR1/VEGFR2/VEGFR3/PDGFRB/c-KIT (Axitinib, Pazopanib), VEGFR2 (Lenvatinib), ErbB1 (Erlotinib, Lapatinib), JAK (Ruxolitinib), BcrAbl/SRC (Bosutinib, Imatinib, Nilotinib), ALK/Met (Crizotinib), BTK (Ibrutinib), BRAF (Vemurafenib). These inhibitors possess nanomolar activity against protein kinases. Pharmacophore approaches allowed the authors of articles, discussed in this review, to identify only hit compounds which have significantly lower inhibitory activity. Therefore, several steps of optimization are necessary for development of highly active and selective protein kinase inhibitors which can be further used in clinical trials. So, it's very hard to find the

information concerning clinical impact of the pharmacophore approaches in protein kinase inhibitors design.

3. In the manuscript, the names of kinases in the beginning of the first sentence were bold to highlight the kinases. It is better to just list the name of kinase (it could be italic or bold) as a subtitle and then start the description. For example: HER1 (EGFR): HER1 overexpression and ...

We have listed the name of protein kinases as a subtitle and then started the description accordingly to reviewer suggestion.

(Reviewer 2)

This manuscript comprehensively summarizes the cutting-edge knowledge about pharmacophore approaches in protein kinase inhibitors design. Authors briefly defined the concept of pharmacophore modeling as an important tool in drug discovery. In addition, the cases for pharmacophore modeling for protein tyrosine kinases and serine/threonine protein kinase inhibitors are well described. Since many successful stories of pharmacophore approaches are published and facilitates its application to drug discovery, this manuscript will provide researchers with useful insight into emerging technology for further development of pharmaceutical small molecules. Here are some suggestions before publication of this manuscript.

1. In the manuscript, the name of modeling software is sometimes capticalized, but not in other part (HIPHOP vs HipHop, HYPOREFINE vs HypoRefine, CATALYST vs Catalyst). It should be described consistently.

We have written the names for modeling software consistently (Catalyst, HipHop, HypoRefine).

2. In figure 2, amino acids of protein kinases (ACK1 and cAMP-DPK) which are not described in the text are included in multiple amino acids alignment.

We have deleted protein kinases (ACK1 and cAMP-DPK) from multiple amino acids alignment in figure 2

3. In figure 2, hydrophobic pockets II are denoted in yellow color (LG in 1st row, GX in 2nd row and XX in 5th row). However, XX in 5th row are not conserved between protein kinases. Therefore, it is better to erase yellow color in XX of 5th row.

We have erased yellow color in XX of 5th row in figure 2.

(Reviewer 3)

The review by Starosyla et al. is a timely review. The use of pharmacophore approaches is an important tool in protein kinase inhibitor drug design and a review of the literature on this topic is appropriate. The review is well-written and the figures are illustrative.

Minor comments:

1. Abstract, 2nd line: the human kinome is estimated to encode 518 protein kinases, so it is better to replace "more than 800" by "more than 500".

We have replaced "more than 800" by "more than 500" in the second line of Abstract

2. Page 3, line 1: Add (JAK) after Janus kinase since this abbreviation is used throughout the text and in the abstract.

We have added (JAK) after Janus kinase

3. Page 3, heading EGFR inhibitors pharmacophore models: references are lacking.

We have added the references after the sentences:

"The epidermal growth factor receptor (EGFR) family includes four cell surface receptors: HER1 (EGFR/erbB1), HER2/neu (erbB2), HER3 (erbB3) and HER4 (erbB4)[7]."
[7] Nair P. Epidermal growth factor receptor family and its role in cancer progression. *Current Science* 2005: 890-898.

"Binding of specific ligands to three of these receptors causes their dimerization and activation. HER2 is called an "orphan receptor" because it does not interact with any ligand, but it dimerizes with other ligand-bound members of EGFR family[8].
[8] Brennan PJ, Kumogai T, Berezov A, Murali R, Greene MI. HER2/Neu: mechanisms od dimerization/oligomerization. *Oncogene* 2000; 19: 6093-6101 [PMID: 11840330 DOI: 10.1038/sj/onc1205119].

4. Page 3, first paragraph in the section HER2. The authors should mention here Herceptin (trastuzumab) as an inhibitor for HER2. Although not a small molecule type inhibitor, a few words on Herceptin are justified.

We have added the sentence "Herceptin (trastuzumab), a humanized IgG1 against the ectodomain of the HER2 receptor, in combination with chemotherapy, induces regression of HER2-overexpressing metastatic breast tumors and prolongs patient survival[15]" to the first paragraph in the section HER2.
[15] Arteaga CL. Trastuzumab, an appropriate first-line single-agent therapy for HER2-overexpressing metastatic breast cancer. *Breast Cancer Res* 2003; 5: 96-100 [PMID: 12631388 DOI: 10.1186/bcr574].

5. Page 4, section on JAK2 and JAK3. A reference should be added after the first sentence describing that JAK2 and JAK3 are implicated in B- and T-cell-mediated diseases.

We have added the reference after the first sentence in the section on JAK2 and JAK3:
[20] Jasuja H, Chadha N, Kaur M, Silakari O. Dual inhibitors of Janus kinase 2 and 3 (JAK2/3): designing by pharmacophore- and docking-based virtual screening approach. Mol Divers 2014, 18: 253-267 [PMID: 24415188 DOI: 10.1007/s11030-013-9497-z]

6. Page 5: line 12: HIPHOP and HYPOREFINE are written in capital letters, while later on these words are written as HipHop and HydroRefine.

We have written the names for modeling software consistently (Catalyst, HipHop, HypoRefine).

7. Can the authors be more specific on the six compounds that inhibit Syk (e.g. their IC_{50}, their specificity)?

The authors Xie et al. in the paper "Pharmacophore modeling study based on known Spleen tyrosine kinase inhibitors together with virtual screening for identifying novel inhibitors" reported only inhibitory rates for two compounds: AK-968/1209687 and AK-968/15361771 which were equal to 51% and 63%, respectively. We didn't found any information concerning IC_{50} and specificity toward Syk kinase for six inhibitors identified by using pharmacophore screening.

8. Section on Tie2: the space between Tie 2 should be removed.

We have removed the space between Tie2

9. Page 6: first paragraph of Clk and Dyrk: references should be added.

We have added the references after the sentences:

"Clk and Dyrk both belong to CMGC family of protein kinases. They are responsible for phosphorylation of serine-arginine-rich proteins and are important for regulation of basic cellular processes [29]."

[29] Aranda S, Laguna A, de la Luna S. DYRK family of protein kinases: evolutionary relationships, biochemical properties, and functional roles. *FASEB J* 2011, 25:449-462 [PMID: 21048044 DOI: 10.1096/fj.10-165837].

"Specifically, the cdc2-like kinases promote phosphorylation within spliceosome, therefore regulating alternative splicing of mRNA isoforms [30]. Because abnormal gene splicing is the cause of many pathological conditions including cancers,[31, 32] modulation of Clk may represent a promising approach for treatment of such diseases."

[30] Mott BT, Tanega C, Shen M, Maloney DJ, Shinn P, Leister W, Marugan JJ, Inglese J, Austin CP, Misteli T, Auld DS, Thomas CJ. Evaluation of substituted 6-arylquinazolin-4-amines as potent and selective inhibitors of cdc2-like kinases (Clk). *Bioorg Med Chem Lett* 2009, 23: 6700-6705 [PMID: 19837585 DOI: 10.1016/j.bmcl.2009.09.121].

[31] He C, Zhou F, Zuo Z, Cheng H, Zhou R. A global view of cancer-specific transcript variants by subtractive transcriptome-wide analysis. *PloS One* 2009, 4:e4732 [PMID: 19266097 DOI: 10.1371/journal.pone.0004732].

[32] Matlin AJ, Clark F, Smith CW. Understanding alternative splicing: towards a cellular code. *Nat Rev Mol Cell Biol* 2005, 6: 386-398 [PMID: 15956978].

"Dyrk1A has increased expression in Down Syndrown patients [33], and has shown involvement in growth, mental retardation and neurodegeneration [29, 33]."

[33] Park J, Song WJ, Chung KC. Function and regulation of Dyrk1A: towards understanding Down

syndrome. *Cell. Mol. Life Sci* **2009, 66: 3235-3240 PMID: 19685005 DOI: 10.1007/s00018-009-0123-2].**

10. Page 7: the greek letter kappa (κ) should be used for NF- κB.

We have used kappa "κ" instead of "k" for NF-κB

11. Page 7: HYPOGEN and Hypogen (e.g. p. 12) is used in the text. The authors should be consequent.

We have used the name of software "HypoGen" through the manuscript

12. Page 7, section on CDK1 and CDK2, first paragraph describing the biological function of these kinases: a reference should be added.

We have added the references after the sentences describing biological functions of CDK1 and CDK2:

"CDK1 is a serine/threonine protein kinase which plays a key role in cell cycle progression through mitosis [38]."
[38] Chow JP, Poon RY, Ma HT. Inhibitory phosphorylation of cyclin-dependent kinase 1 as a compensatory mechanism for mitosis exit. *Mol Cell Biol* **2011, 31: 1478-1491 [PMID: 21262764 DOI: 10.1128/MCB.00891-10].**

"In fact, CDK1 inhibitors effectively arrested tumor cell growth prompting great recent interest in the discovery and development of new CDK1 inhibitors [39]."
[39] Chen S, Chen L, Le NT, Zhao C, Sidduri A, Lou JP, Michoud C, Portland L, Jackson N, Liu JJ, Konzelmann F, Chi F, Tovar C, Xiang Q, Chen Y, Wen Y, Vassilevet LT. Synthesis and activity of quinolinyl-methylene-thiazolinones as potent and selective cyclin-dependent kinase 1 inhibitors. *Bioorg Med Chem Lett* **2007, 17: 2134-2138 [PMID: 17303421 DOI: 10.1016/j.bmcl.2007.01.081].**

"CDK2 in complex with cyclin E plays a paramount role during the G1/S transition of the cell cycle while in complex with cyclin A, it facilitates the progression of the S phase of the cell cycle. Recent evidence also suggests that CDK2 may have a crucial role in the G2 phase of the cell cycle [41]."
[41] Hu B, Mitra J, van den Heuvel S, Enders GH. S and G2 phase roles for Cdk2 revealed by inducible expression of a dominant-negative mutant in human cells. *Mol Cell Biol* **2001, 21: 2755-2766 [PMID: 11283255 DOI: 10.1128/MCB.21.8.27-55-2766.2001].**

13. It would be interesting for the reader to know if any of the compounds identified as potential inhibitors for the protein kinases discussed in this review are in clinical trials. E.g. inhibitors against Syk (described in reference [20]), CDK1 (identified in reference [30]), PLK1 (described in reference [37]).

To date, several compounds with Syk inhibitory activity in clinical development were reported. For example, fostamatinib (formerly called R788) was until recently furthest along in development for the treatment of rheumatoid arthritis. But we didn't found the information concerning clinical trials of Syk inhibitors (AK-968/1209687 and AK-968/15361771) reported in the article Xie et al, 2009.

The most active CDK1 inhibitor which was found by using pharmacophore screening approach

illustrated IC_{50} value of 0,83 μM (Al-Sha'er et al., 2010). Accordingly to the information at the web-site: http://www.nature.com/nrd/journal/v8/n7/fig_tab/nrd2907_T2.html, the inhibitory activities of CDK inhibitors which are in clinical trials are significantly less.

The authors of the article "Pharmacophore modeling and virtual screening for designing potential PLK1 inhibitors" didn't describe chemical structures of the PLK1 inhibitors which were found by using their pharmacophore model. They only noted that "these refined hit compounds have been shifted to the subsequent in vitro and in vivo studies, the results of which will be reported in the near future". So, it's hard to find whether these compounds are in clinical trials.

14. Section on Akt: the authors should maybe mention that Akt is a family consisting of the members Akt1, Akt2 and Akt3.

We have added the sentence: "The Akt family of serine-threonine kinases consists of three members: Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ [62] to the Section on Akt.

[62] Dillon RL, Muller WJ. Distinct biological roles for the akt family in mammary tumor progression. *Cancer Res* 2010, 70: 4260-4264 [PMID: 20424120 DOI: 10.1158/0008-5472].

15. Conclusion, last line: the authors should add the word "possible" so that the text reads: "the identification of possible protein kinase inhibitors can be…"

We have added the word "possible" to the last sentence of Conclusion section

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Pharmacology*.

Sincerely yours,

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