Dear Editor,

**Article ID: 4810**

**Docking Studies Reveal Zerumbone Targets -catenin of the Wnt--catenin Pathway in Breast Cancer**

Thank you for considering our manuscript for your journal. The revised manuscript is being submitted along with this letter. We have incorporated the reviewer’s comments to the best of our abilities. The changes have been highlighted in the manuscript. All figures have been resized according to the recommended artwork. However, some figures appear smaller due to page number constraints.

I hope you are satisfied with our efforts. Please let me know if you need any other information.

Thank you

Yours sincerely

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**Reviewer’s Comments**

Reviewer A:

ADDITIONAL COMMENTS
Please indicate the page numbers for suggested corrections.
Please, be as specific as possible if major correction by the author(s) is
recommended! :
        Figure 1 is very low in quality and some text is unreadable.

**ANS: Figure 1 on Page 3 has been redrawn and resized to 90mmX 52.2mm and 500dpi**

Figure 2 has very low resolution, and atom labels are unreadable. Giving an
actual chemical formula (2D) would also be beneficial.

**ANS: As recommended by the reviewer, the 2D chemical structure of zerumbone is included is appropriate resized and referenced. The structure appears on Page 4**

Equation 2 has a missing bracket.
**ANS: The bracket has been added**.

Figure 3 has very low resolution.

**ANS: The resolution has been improved.**

Figure 4 has low resolution. Panel labels are not of consistent size. Panel
C has unreadable text.

**ANS: The figures with low resolutions have been removed due to space constraints. Only Panel C has been retained. Its resolution is improved according to instructions.**Figure 5 has low resolution.

**ANS: The resolution has been improved.**
Authors should use the consistent fonts and sizes to label different panels
on figure 6. The same is valid for figures 7, 8 and 9.

**ANS: The resolutions of all figures have been improved.**

Figure 10 contains some unreadable text. Furthermore, it introduces many new
abbreviations that were never introduced before and are confusing. This
figure should be simplified to carry a stronger and better message.

**ANS: The figure has been deleted to make the script more concise.**

The authors should consider condensing the manuscript.

**ANS: The Script has been made as concise as possible.**

REPORT:
        Many docking programs are not able to deal with the conformational
flexibility of the macrocycles directly. The authors should explain how
CDOCKER performs flexible ligand docking with zerumbone.

On lines 155-168, authors describe a well-known theory on how protein force
fields work. It is not necessary to describe this in details. It would be
more interesting to describe how CDOCKER handles small molecules, having in
mind that common CHARMM force fields (22, 22\*, 27, 36, ...) are not
parameterized for small molecules.

**ANS: The methodology has been included under the DOCKING WITH CDOCKER heading in the EXPERIMENTAL SECTION. The change is highlighted.**
Figure 3 represents interaction/binding energies between PKF118-310 or
zerumbone with several proteins. The binding is on the level of -20 to -80
kcal/mol. Is this covalent binding? Namely, biotin-avidin is among the
strongest known non-covalent interactions, and its energy is ~ 20 kcal/mol.
Drug non-covalent binding is usually weaker.

**ANS: The PKF 118-310 and zerumbone show non-covalent and electrostatic interactions with the -catenin, which is responsible for the high binding energies of the order of -80 kcal /mol. Detailed answer regarding the different types of interactions contributing to the binding energy has been provided in the manuscript below Figure 3.**
Figure 4A represents the sequence alignment of the murine and human FZD8,
which was mentioned to have 98% sequence identity. These two proteins are
virtually identical, and there is absolutely no valid reason to perform
homology modeling to obtain the human protein. Since the only difference is
in the two C-terminal residues, they could be ignored if they are not in the
binding sites. If they are, Modeller has several tools to model terminal
residues.

**ANS: The reviewer’s comments are accepted and only the final model is presented as a reference point.**
Authors claim that zerumbone has a weak affinity for FZD (line 226). The
binding energy of 44.35 kcal/mol (line 234) is far from weak.

**ANS: The word “weak” has been removed from the line.**
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Reviewer B:

ADDITIONAL COMMENTS
Please indicate the page numbers for suggested corrections.
Please, be as specific as possible if major correction by the author(s) is
recommended! :
        page 3 line 81 "sequiterpene" - there is a missing letter "s"

**ANS: The correction has been made**

Fig 2 should be slightly improved

**ANS: The figure has been improved**

Fig 3 should be also improved

**ANS: The figure has been improved and resized**

page 13, line 297, "The results shown in Fig. 7 (A, B) indicated that the
β-catenin-TCF4 complex is very strong"  - if you used rigid protein-small
molecule docking procedure, how can you estimate the strength of
protein-protein interaction? Be careful with such considerations. That is
the question for molecular dynamics simulations, but, however, you stated
that on page 17, lines 394-396.

**ANS: The statement has been modified.**