



## RESEARCH ARTICLE

# Molecular docking and molecular dynamics simulation study of inositol phosphorylceramide synthase – inhibitor complex in leishmaniasis: Insight into the structure based drug design [version 1; referees: 1 approved with reservations]

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**Abstract**

Inositol phosphorylceramide synthase (IPCS) has emerged as an important, interesting and attractive target in the sphingolipid metabolism of *Leishmania*. IPCS catalyzes the conversion of ceramide to IPC which forms the most predominant sphingolipid in *Leishmania*. IPCS has no mammalian equivalent and also plays an important role in maintaining the infectivity and viability of the parasite. The present study explores the possibility of targeting IPCS; development of suitable inhibitors for the same would serve as a treatment strategy for the infectious disease leishmaniasis. Five coumarin derivatives were developed as inhibitors of IPCS protein. Molecular dynamics simulations of the complexes of IPCS with these inhibitors were performed which provided insights into the binding modes of the inhibitors. *In vitro* screening of the top three compounds has resulted in the identification of one of the compounds (compound 3) which shows little cytotoxic effects. This compound therefore represents a good starting point for further *in vivo* experimentation and could possibly serve as an important drug candidate for the treatment of leishmaniasis.

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## Abbreviations

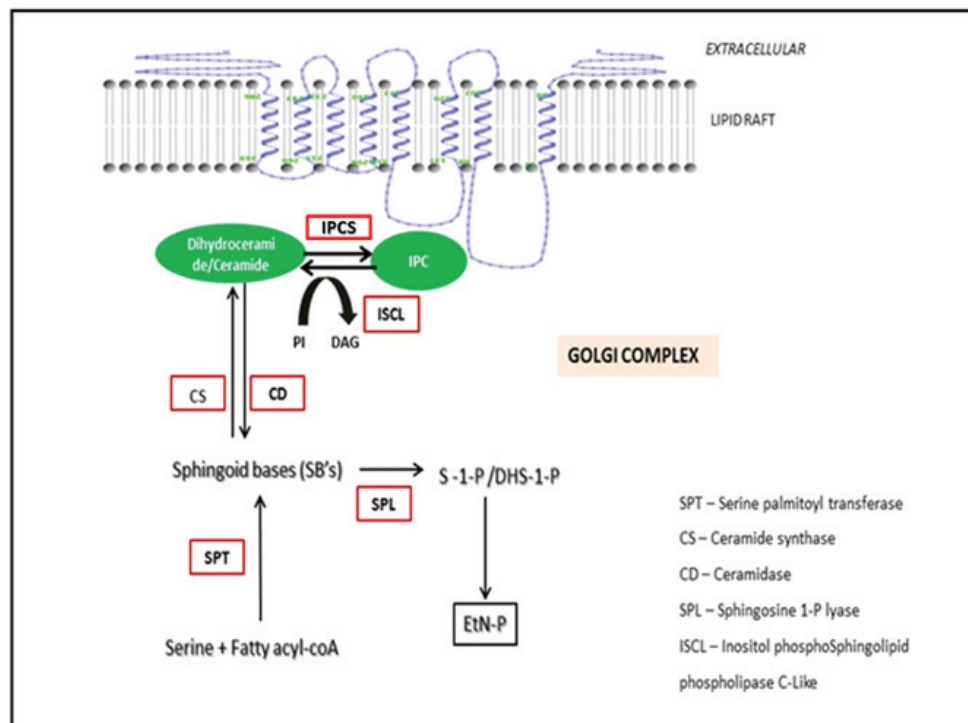
IPCS – Inositol phosphorylceramide synthase, IPC – Inositol phosphorylceramide, AUR1 – Aureobasidin 1, DAG – Diacylglycerol, RMSD – Root Mean Square Deviation, LINCS – Linear constraint solver, PME – Particle Mesh Ewald.

## Introduction

Infectious disease, leishmaniasis, is the major cause of parasitic diseases affecting 12 million people worldwide. Most of the anti-leishmanial compounds do not have well-defined mechanisms. The first line treatment of cutaneous leishmaniasis involves the administration of antimony based compounds. Treatment of *L. major* amastigotes with Sb(V) has been found to induce apoptosis by the induction of oxidative-stress and increase in intracellular calcium<sup>1</sup>. Non-antimony based treatments such as miltefosine, and topical formulations of paromomycin are cost effective, convenient and less toxic than antimony based compounds. Amphotericin B being a liposomal formulation is expensive, has a low therapeutic index and is difficult to administer<sup>2</sup>. Newer formulations for the treatment of this disease include the administration of miltefosine. Miltefosine (hexadecylphosphocholine), originally an anticancer drug has been reported to induce apoptosis of *L. major* amastigotes in the infected macrophages<sup>3</sup>. Development of newer treatment modalities arise from the problem of drug resistance and quick adaptability of the parasite to the host immune response<sup>4-6</sup>.

Sphingolipids like IPC, form an important component of the parasitic membranes<sup>7</sup>. IPCS (inositol phosphorylceramide synthase) is an enzyme involved in the sphingolipid metabolism

of protozoans and other fungal species<sup>8</sup>. The relative importance of IPCS in *Leishmania* has been identified through biochemical network modeling<sup>9</sup>. IPCS catalyzes the conversion of ceramide to IPC which forms the most predominant sphingolipid of the parasite<sup>10</sup> (Figure 1). IPCS also maintains the concentration of DAG and ceramide, both of which serve as secondary messengers in several signal transduction events<sup>11</sup>. IPCS localizes into the lipid rafts of the Golgi complex<sup>12</sup>. Lipid rafts have been proposed to involve in a wide array of events like trafficking of lipid modified proteins in addition to playing an important role in the formation of signal transduction complexes<sup>13</sup>. IPCS has been important for maintaining the viability and the infectivity of several fungal species like *Cryptococcus neoformans*, *Candida albicans* and pathogens like *Leishmania*<sup>14-17</sup>. Interestingly there is no mammalian equivalent of this enzyme and the major sphingolipid in the host is sphingomyelin instead of IPC. Hence IPCS has been considered as a choke point enzyme in the sphingolipid metabolism of *Leishmania* thereby serving as a druggable target for the treatment of several fungal and protozoan diseases like leishmaniasis. *Lmj*IPCS comprises of 338 amino acids and has 6 transmembrane domains and belongs to the PAP2c family<sup>9</sup>. IPCS is encoded by the AUR1 gene. IPCS protein present in fungi exhibits sensitivity to antifungal agents like galbonolide A, aureobasidin A, macrolidegalbonolide and khafrefungin<sup>18,19</sup>. IPCS has been recently discovered in *Leishmania* and to the best of our knowledge there are no reports of inhibitor design against this protein. This paper explores the possibility of targeting IPCS for the development of anti-protozoan compounds. An *in silico* approach for drug design has led to the development of five novel coumarin derivatives. The refinement and validation



**Figure 1. Role of IPCS in the sphingolipid metabolism of *Leishmania*.** IPCS catalyzes the reaction involving the conversion of ceramide to IPC (Inositolphosphorylceramide). IPC forms the most predominant sphingolipid in *Leishmania*. IPCS plays an important role in maintaining the viability of the parasite.

of the docked complexes has been done using molecular dynamics simulations to map the protein ligand interactions. Based on the *in silico* findings, the promising candidates were considered for further experimental evaluation and validation.

## Materials and methods

### Generation of the lead compounds

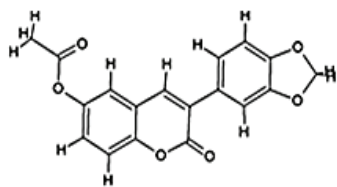
A set of coumarin derivatives were prepared by the assembly of pharmacophoric groups. The 2D structures of the inhibitors were drawn and edited using Chemschetch version 12.01<sup>20</sup> (Figure 2). The SMILES format for all the compounds was generated using Open Babel version 2.3.1<sup>21</sup>. Inhibitors were designed and filtered using the “Lipinski rules of five”<sup>22</sup> and Veber’s rules<sup>23</sup> using the Molinspiration Property Calculation Service ([www.molinspiration.com](http://www.molinspiration.com)).

### Pharmacophore generation

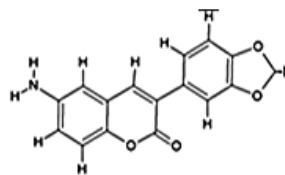
The pharmacophore models describing the inherent chemical features of the inhibitors were generated using the “Feature mapping protocol” available in Discovery Studio version 3.0. ([www.accelrys.com](http://www.accelrys.com)) Pharmacophore models of the inhibitors indicated that the ligand had at least a maximum of 5 pharmacophoric features i.e. Hydrogen bond acceptors (HBA), Hydrogen bond donors (HBD), positive ionizable groups (PI), Ring aromatic groups (RA) and the Hydrophobic groups (HY) present in the ligand.

### Molecular docking

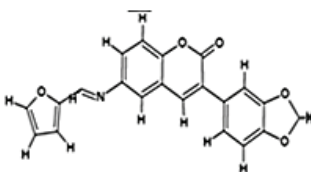
IPCS is one of the emerging drug targets for the treatment of leishmaniasis. The crystal structure of the IPCS protein has not been solved and hence the 3D structure for the IPCS protein developed by our group before has been used for the inhibitor design. The model was developed using the I-TASSER server (<http://zhanglab.ccmb.med.umich.edu/I-TASSER/>). The predicted model has a total of 338 amino acid residues and has 7 transmembrane helices<sup>9</sup>. The 3D structure of IPCS was energy minimized by the steepest gradient method of energy minimization using the GROMACS 4.0 package<sup>24</sup>. Mol2 file format of the inhibitors was converted to PDBQT format using MGL tools prior to docking. All the water and solvent atoms of the protein were removed prior to docking and the polar hydrogens were added. The protein was kept rigid while the ligand was allowed to rotate and explore more flexible binding pockets. Docking of the inhibitors onto the IPCS protein was performed using Auto Dock 4 version 1.5.6 and Auto Dock vina. version 1.1.2. The grid box size dimensions were 40X40X40, the default scoring function was used for docking<sup>25,26</sup>. Binding modes of the docked complexes were obtained and the amino acid residues present at a distance of 5Å were considered as the binding partners of the ligands. The interaction diagrams representing the docked complexes have been generated using Pymol v 1.3.



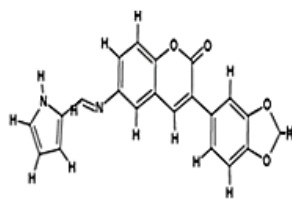
Compound 1  
3-(1,3-Benzodioxol-5-yl)-2-oxo-2H-chromen-6-yl-acetate



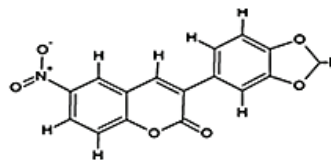
Compound 2  
6-Amino-3-(1,3-benzodioxol-5-yl)-2H-chromen-2-one



Compound 3  
3-(1,3-Benzodioxol-5-yl)-6-[[1E]-2-furylmethylene]amino-2H-chromen-2-one



Compound 4  
3-(1,3-Benzodioxol-5-yl)-6-[[1E]-1H-pyrrol-2-ylmethylene]amino-2H-chromen-2-one



Compound 5  
3-(1,3-Benzodioxol-5-yl)-6-nitro-2H-chromen-2-one

**Figure 2.** 2D representation of the IPCS inhibitors. The designed inhibitors are Coumarin derivatives. Coumarin increases the phagocytic activity of the macrophages.

### Molecular dynamics simulation of the docked complexes

Molecular dynamics simulation is a computational method that provides information regarding the time dependent behavior of any molecular system by integrating Newton's laws of motion. The docked complexes (IPCS-inhibitor complex) were subjected to MD simulation using Desmond version 4.4 (Schodinger Biosuite). MD simulation of both the IPCS protein and IPCS –ligand complexes were performed for a time period of 10ns by using the OPLS force field. The complex was centered in a cubic box and filled with TIP3P water molecules. The system was neutralized and the initial energy minimization for the system was done using the conjugate gradient algorithm. The Martyna-Tobias-Klein scheme was used for pressure coupling. Electrostatic forces were calculated using the PME algorithm<sup>27</sup>. All runs were performed at 300K at constant volume and temperature (NPT ensemble) under certain periodic boundary conditions. RMSD plots for the backbone atoms for both the protein and ligand bound protein were generated to understand the relative stability of the ligand inside its binding pocket and the IPCS-inhibitor complexes were visualized.

### Flow cytometry

Macrophage cell population was collected post 24 h treatment with the compound 3, washed and suspended in 1XPBS. Cells were stained with 10 $\mu$ l of 10 $\mu$ g/mL of propidium iodide (PI) dye (Invitrogen) and acquired on FACS. Total macrophage population was gated based on their forward scatter (FSC) and side scatter (SSC) after excluding the cell debris. A minimum of 10,000 events were acquired for each sample on FACS Canto II (Beckon Dickson, San Jose, California) and analyzed using FACS Diva Software (version 6.2.1) (Beckon Dikson, San Jose, California).

### Results

**Dataset 1. Raw data for 'Molecular docking and molecular dynamics simulation study of inositol phosphorylceramide synthase – inhibitor complex in leishmaniasis: Insight into the structure based drug design'**

<http://dx.doi.org/10.5256/f1000research.9151.d128337>

A description of each file is provided in 'Dataset descriptions'.

A group of coumarin derivatives were prepared as inhibitors of the IPCS protein belonging to *L. major*. Assessment of the drug like properties indicated that all the inhibitors were found to comply with the Lipinski's "Rule of five" (molecular weight ( $M_{wt}$ )  $\leq$  500, clogP  $\leq$  5, H-bond donors (HBD)  $\leq$  5, and acceptors (HBA)  $\leq$  10) and Verber's rules (no. of rotatable bonds  $<$  10, PSA  $\leq$  140 $\text{\AA}^2$ ) (Table 1).

### Molecular docking

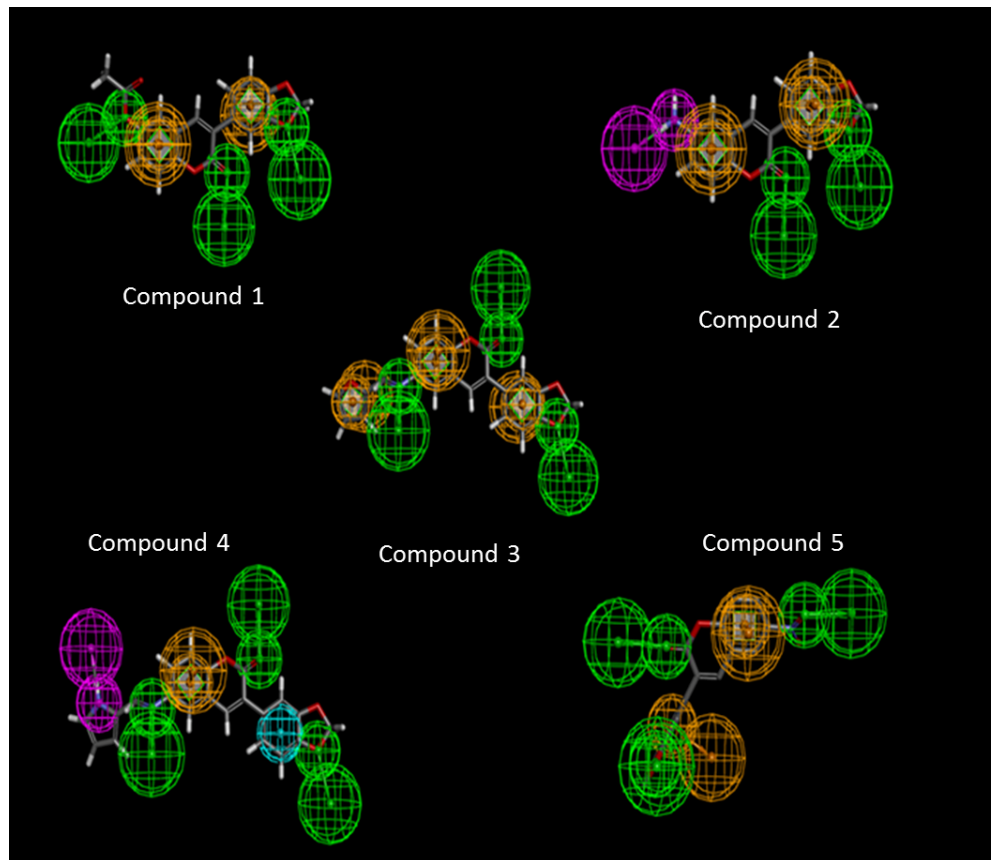
Molecular docking studies reveal the binding modes of the ligand with the IPCS protein giving an insight into the crucial amino acid residues that are involved during the binding. A comparison of the binding energies of all the compounds indicates that compound 3 has the least binding energy among all and hence exhibits maximum affinity towards the IPCS protein (Table 2). The interaction modes of all the IPCS inhibitors post docking along with their pharmacophoric features have been presented [Figure 3]. Binding mode analysis reveals that hydrophilic amino acids like Arg299 and His220 were found to be involved in hydrogen or  $\pi$  bonding with most of the ligands (Table 3). The relative stability of

**Table 1. Molecular descriptors of the lead compounds.** HBA – Hydrogen bond acceptor, HBD – Hydrogen bond donor, HY – Hydrophobic, RA – Ring aromatic, MR – Molar refractivity, NROTB – No. of rotatable bonds, cLogP – log octanol/water partition coefficient, PSA – Polar surface area, NSC – No. of stereo centers.

S.No	Mwt	cLogP	HBA	HBD	HY	RA	MR	NROTB	PSA( $\text{\AA}^2$ )	NSC
1	324	2.8	8	0	2	4	83.82	3	74.98	0
2	281	2.46	5	2	2	4	76.92	1	74.70	0
3	359	4.22	7	0	3	6	78.47	4	73.84	1
4	358	3.86	6	1	3	6	100.70	1	77.36	1
5	311	3.15	9	0	2	4	78.65	2	94.50	0

**Table 2. The approximate free energies of binding ( $\Delta G_b$ ) of the compounds calculated by Autodock vina.**

S.No	Compound Name	Binding energy (Kcal/mol)
1.	(3-(1,3-Benzodioxol-5-yl)-2oxo-2H-chromen-6-yl)-acetate)	-9.0
2.	(6-Amino-3-(1,3-benzodioxol-5-yl)-2H-chromen-2-one)	-8.4
3.	3-(1,3-Benzodioxol-5-yl)-6-([(1E)-2-furylmethylene]amino)-2H-chromen-2-one	-9.8
4.	3-(1,3-Benzodioxol-5-yl)-6-([(1E)-1H-pyrrol-2-ylmethylene]amino)-2H-chromene-2-one	-9.5
5.	(3-(1,3-Benzodioxol-5-yl)-6-nitro-2H-chromen-2-one	-9.0



**Figure 3. Pharmacophore models of the inhibitors.** The pharmacophoric features such as hydrogen bond acceptors (green), hydrogen bond donors (pink), hydrophobic regions (blue) and the aromatic rings in yellow are shown in the figure.

**Table 3. IPCS –inhibitor interactions post docking.**

Compound	Amino acid	Ligand	Type of interaction
1	His220	1,3 benzodioxol group	Hydrogen bonding
1	Asn183	1,3 benzodioxol group	Hydrogen bonding
3	Phe129	1,3 benzodioxol group	Sigma bond formation
4	Arg299	1,3 benzodioxol group	Hydrogen bonding
5	His220	1,3 benzodioxol group	Hydrogen bonding
5	Glu192	Chromene group	Sigma bond formation
5	Arg299	Chromene group	Pi bonding

the compounds within the binding site was maintained due to the van der Waal's interaction between the hydrophobic amino acids of the IPCS protein and the ligand (Table 4).

#### Molecular dynamics simulation of the docked complexes

Protein backbone RMSD plots indicate the stability of the IPCS-inhibitor complex. The drug backbone RMSD plots indicate that compounds 2 and 3 maintained their interactions with the IPCS protein (Figure 4). Binding modes of compounds 1 to 5 post MD simulation have been shown in Figure 5a–e.

#### Cytotoxicity of the proposed inhibitors

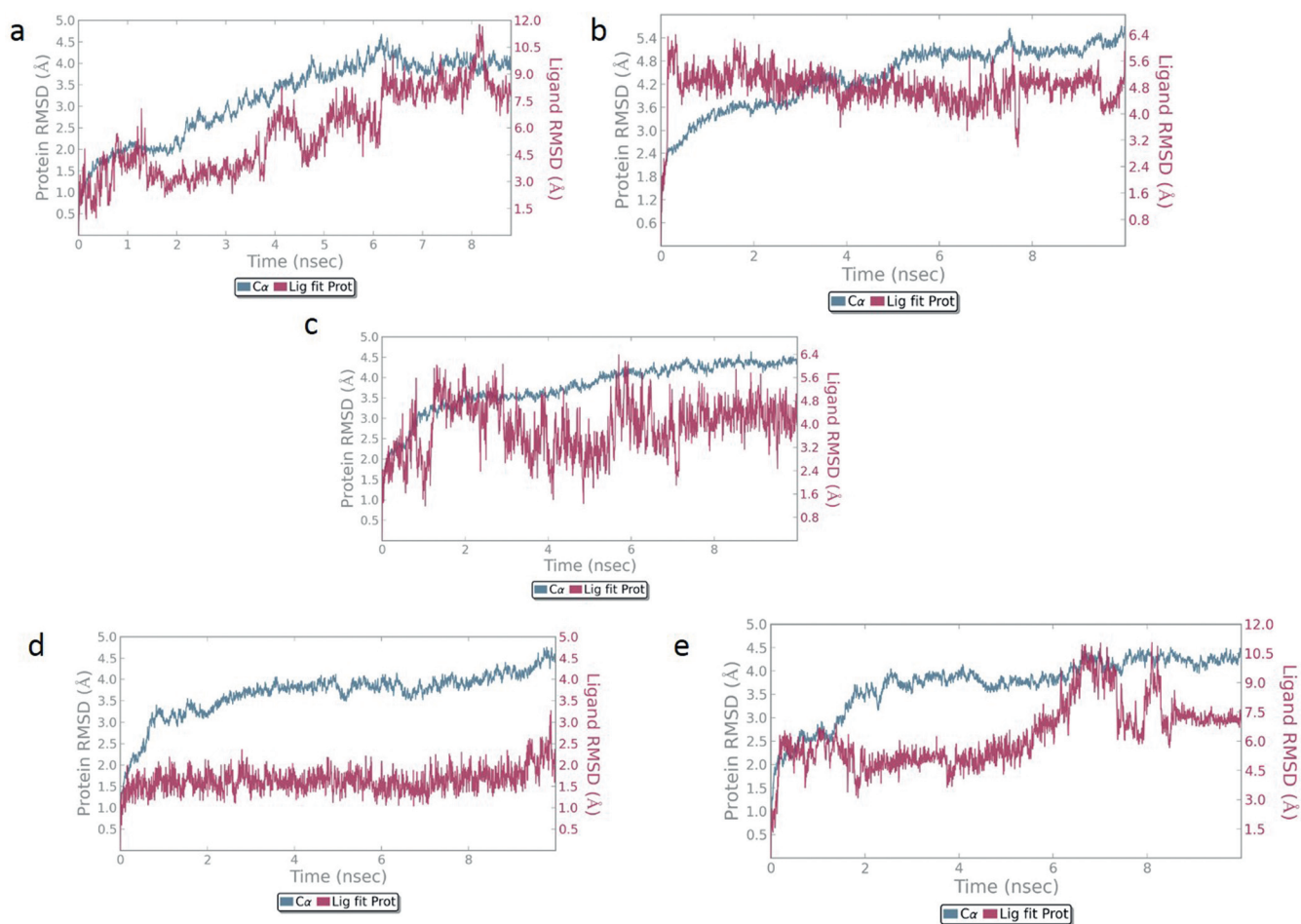
The cytotoxicity profile of compound 3 was checked over the macrophage cell line. Of all five compounds, compound 3 had the highest viability. The viability of C3 treated macrophages (67.3%) was slightly lesser than the control (73.5%).

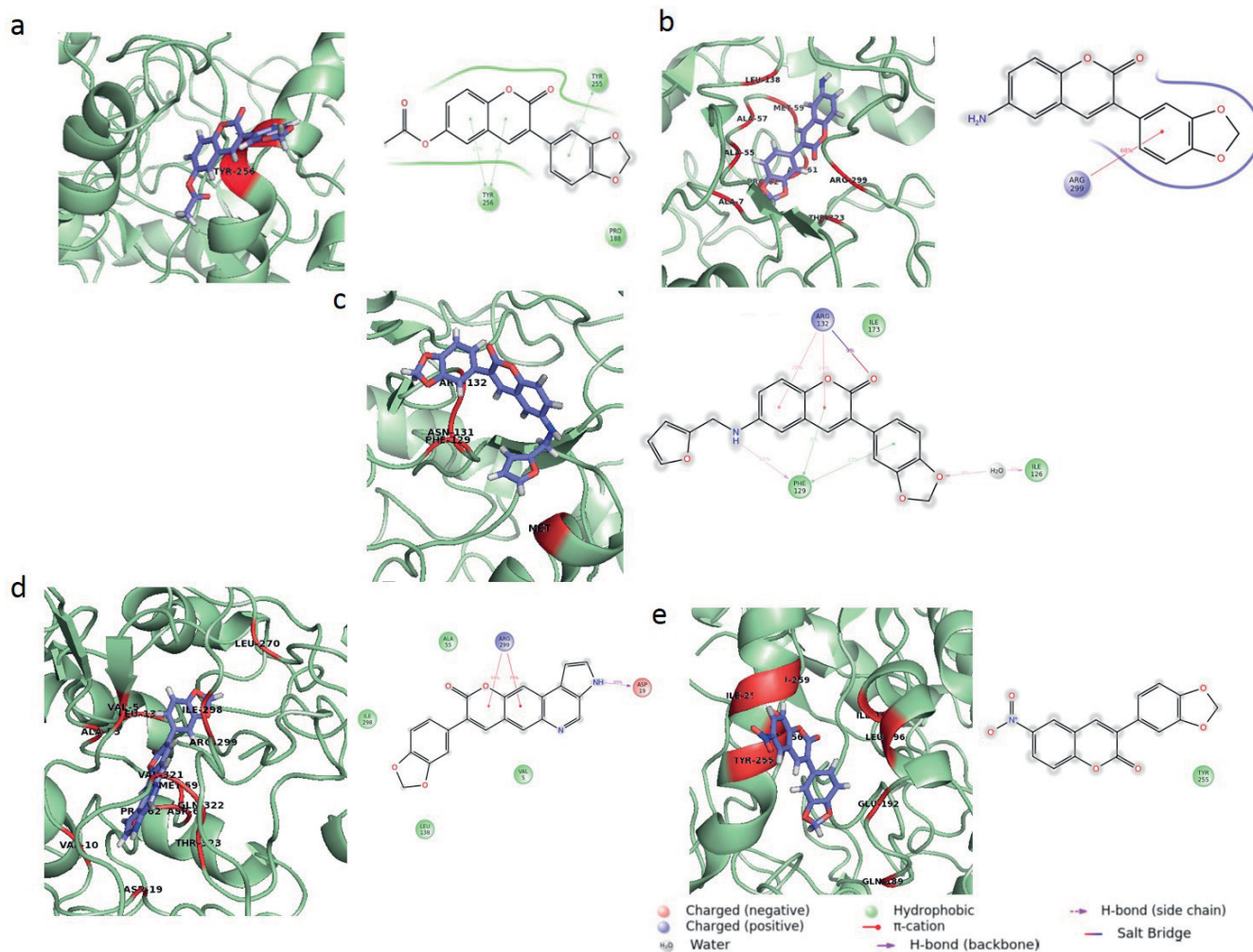
#### Discussion

IPCS (Inositol phosphorylceramide synthase) has been identified as an important drug target in the sphingolipid metabolism of several organisms like fungi, yeast and protozoans like *Leishmania* and

**Table 4.** Comparison of the interacting residues both pre and post MD simulation.

Compound	Binding interactions post docking (pre MD simulation)	Binding interactions post MD simulation
1.	Ile223, Met222, Asn183, Asp182, Pro252, Tyr255, Val195, Pro188, Glu192, Leu196	Tyr256, Prot188, Glue192, Tyr255, Leu196
2.	Arg299, Asp214, Thr6, Ala7, Leu138, Thr323, Ala325, Asp61, Met59, Pro62, Ala57	Arg299, Met59, Ala57, Leu138, Ala7, Pro62, Asp61, Thr323
3.	Arg132, Ala51, Leu130, Val172, Gly49, Met46, Val150	Met46, Phe129, Asn131, Arg132
4.	Pro62, Tyr178, Asp214, Thr6, Ala7, Trp23, Asp19, Ile298, Val5, Leu138, Thr323	Glu63, Val321, Gln322, Arg299, Asp61, Val5, Ile298, Leu270, Ala55, Leu138, Pro62, Met59, Thr323, Asp19, Val10
5.	Tyr255, Asp182, Pro252, Asn187, Gln189	Ile256, Leu259, Leu196, Ile199, Glu192, Asn187, Tyr256, Tyr255

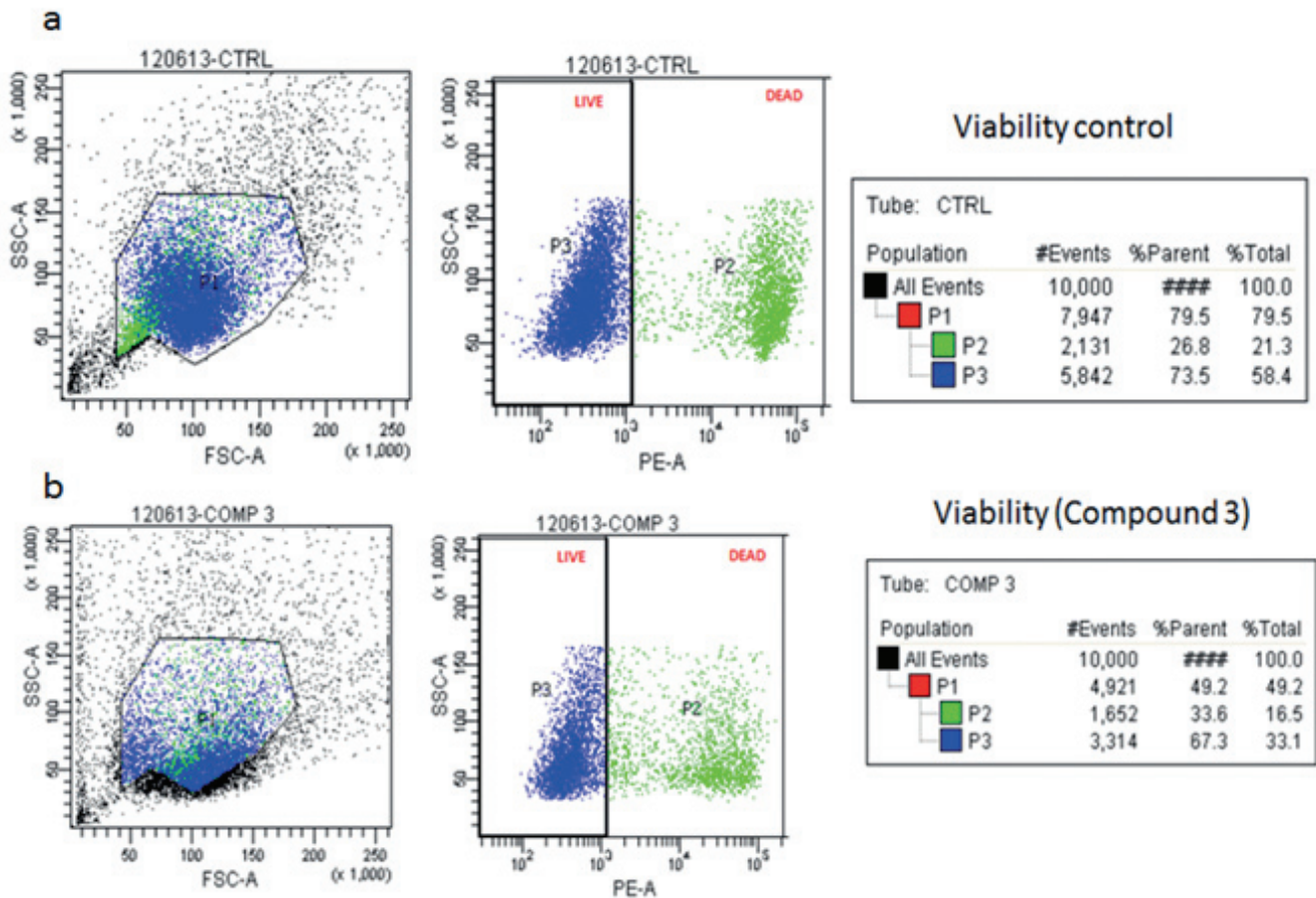
**Figure 4.** RMSD of the IPCS-ligand complexes. Backbone RMSD of **a)** Compound 1 and **b)** Compound 2 **c)** Compound 3 **d)** Compound 4 **e)** Compound 5 is shown in the figure. Compound 1, 2 and 3 appear to maintain their stability within the binding pocket as they show lower RMSD fluctuations.



**Figure 5a–e. Binding modes of IPCS-ligand complexes.** The interaction of the ligand within the IPCS inhibitor complex post MD simulation is shown the figure **a**) IPCS - compound 1 complex **b**) IPCS - compound 2 complex **c**) IPCS - compound 3 complex **d**) IPCS - compound 4 complex and **e**) IPCS - compound 5 complex. MD simulation was performed for a time period of 10ns. Interacting residues are represented in red.

*Trypanosoma*<sup>28</sup>. Systems biology has played a major role in defining the relative importance of IPCS in the sphingolipid metabolism of *Leishmania*, a protozoan responsible for causing an infectious disease leishmaniasis. The quest for developing new inhibitors for any target protein relies mainly on *in silico* approaches like computer based docking which involves the generation of a comprehensive set of ligand conformations that are eventually scored and ranked according to their stability and affinity for the protein. Coumarin has been shown to simulate the macrophages, enhancing their phagocytic ability<sup>29</sup>. A total of five ligands were developed as inhibitors for the IPCS protein. Molecular docking of the inhibitors with the IPCS protein revealed the binding modes of inhibitors. To account for the flexibility of the protein and ligand and to determine the binding affinity of the inhibitors with the IPCS protein, a 10 ns molecular

dynamics simulation of the docked complexes was carried out. Binding mode analysis revealed that the binding modes obtained after MD simulation were more or less similar to that obtained post docking (Table 4). The presence of a large number of H bond acceptors, H bond donors as well as hydrophobic groups in the ligands account for the stability of the ligand inside the binding pocket of IPCS. Based on the RMSD of the ligand-protein complex, it was observed that compounds 1, 2 and 3 maintained their interaction with the protein with lower RMSD fluctuations. Out of these, compound 3 showed the highest binding affinity and its cytotoxicity was assessed using flow cytometry. Cytotoxicity of compound 3 was lesser as compared to other compound. A comparison of compound 3 treated macrophages along with the untreated macrophages has been made in Figure 6.



**Figure 6. FACS analysis for measuring macrophage cell viability.** Macrophages were treated with compound 3 for 24h. **a**) Control cell population displayed a percentage viability of 73.5% **b**) Compound 3 (1mg/ml) treated macrophages displayed a viability of 67.3% post 24hr treatment.

## Conclusion

There is an urgent need to design and develop novel anti-leishmanial compounds due to various problems associated with the current chemotherapeutics for the treatment of this disease. IPCS has been proposed to be a probable drug target in the sphingolipid pathway of *Leishmania*. We have designed a few novel coumarin derivatives using *in silico* approaches. MD simulation post docking studies reveal the interactions between the IPCS protein and ligands. Binding modes obtained after docking and after MD simulation reveal almost identical binding modes which is suggestive of the selectivity and selectivity of the ligand towards the active site of the IPCS protein.

## Data availability

F1000Research: Dataset 1. Raw data for 'Molecular docking and molecular dynamics simulation study of inositol phosphorylceramide synthase – inhibitor complex in leishmaniasis: Insight into the structure based drug design', [10.5256/f1000research.9151.d128337](https://doi.org/10.5256/f1000research.9151.d128337)<sup>30</sup>

## Author contributions

SS designed and conceptualized the experiments. VM carried out the experiments. SS and VM wrote the manuscript. All the authors have read and approved the manuscript.

## Competing interests

No competing interests were disclosed.

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*The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

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# Open Peer Review

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**François Ferron**

UMR 7257, Architecture and Functions of Biological Macromolecules Laboratory, CNRS-Aix-Marseille University, Marseille, France

Mandlik and Singh are presenting a very interesting study against *Leishmania*. Using *in silico* methods they have identified promising compounds.

They chose with great reason IPCS as a target for its uniqueness to the pathogens the study very interesting yet the manuscript needs some clarifications.

1. As there is no structure the authors have done a model, the model should be presented here and I feel a previous reference from 2012 won't do. The reader needs to be introduced to it and at least to have a clear understanding of the catalytic site and docking site.
2. In the material and methods it would be appreciated to have an idea where is the center of the grid (may be with a figure).
3. Can you explain why you chose a grid with large dimensions?
4. The analysis of the viability raises a question. Why the surface p1 is not the same between control and compound? To compare the stats it should be the same size here it is half. Also from fig 6 compound 3 it seems that a lot of data were excluded from P1 area? Can you explain?
5. As perspective are you planning on testing *in vitro* the efficiency of compound 3 and have an idea of binding affinity?

Minor comment on figure 2: structures of compounds are distorted and could you put all the compound in same orientation 1 (and 2 are upside down). It will help to compare the geometry and differences between molecules.

Figure 5: description of interactions could be better represented ma be with LigPlot, as it is it is not clear.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

**Competing Interests:** No competing interests were disclosed.

Author Response 01 Aug 2016

**Shailza Singh**, National Centre for Cell Science, India

1. *As there is no structure the authors have done a model, the model should be presented here and I feel a previous reference from 2012 won't do. The reader needs to be introduced to it and at least to have a clear understanding of the catalytic site and docking site.*

Author's response: The authors have accepted the suggestion made and have now included a figure showing the binding cavity that was predicted for the IPCS protein around which the grid box was centered during docking.

2. *In the material and methods it would be appreciated to have an idea where the center of the grid is (may be with a figure).*

Author's response: Figure 3 has been included keeping in mind the suggestion made.

3. *Can you explain why you chose a grid with large dimensions?*

Author's response: The inhibitors designed have not yet been reported. As there are no studies indicating the exact binding site in the IPCS protein, we have made binding site prediction and the grid box dimensions have been adjusted to incorporate most of the amino acids that fall in the binding site.

4. *The analysis of the viability raises a question. Why the surface p1 is not the same between control and compound? To compare the stats it should be the same size here it is half. Also from fig 6 compound 3 it seems that a lot of data were excluded from P1 area? Can you explain?*

Author's response: The P1 area has been demarcated as per the untreated macrophages (control). As was a decrease in granularity of macrophages post treatment, the cells had lower SSC, however the viability of the cells has not decreased. As per the author's knowledge about flow cytometry, the P1 area has to remain the same both for the control and treated samples.

5. *As perspective are you planning on testing in vitro the efficiency of compound 3 and have an idea of binding affinity?*

Author's response: At present, the authors don't have idea of the binding affinity of compound 3. Such studies can be done in future.

6. *Minor comment on figure 2: structures of compounds are distorted and could you put all the compound in same orientation 1 (and 2 are upside down). It will help to compare the geometry and differences between molecules.*

Author's response: Changes suggested have been made in the Figure 2.

7. *Figure 5: description of interactions could be better represented maybe with LigPlot, as it is not clear.*

Author's response: Changes suggested have been made in the Figure 6.

We thank the reviewer for his valuable suggestions which went a long way in improving the said manuscript.

**Competing Interests:** No competing interests

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