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The Design of Novel Protein Kinase Inhibitors Using the Naturally Occurring Isojacareubin Scaffold As A Lead

Jeanelle Caruana*, Claire Shoemake

ABSTRACT

Therapeutic areas for Protein Kinase inhibitors include cancer. A study has indicated that isojacareubin, a plant-derived natural product, inhibits Protein Kinase C. This study aimed to design *in silico* protein kinase inhibitors using isojacareubin as a molecular template. Virtual screening and *de novo* design were carried out during the study. A total of three hundred hits were produced from virtual screening using the best binding conformer of isojacareubin. Two hundred molecules were generated *de novo* from each seed structure of isojacareubin. Lipinski Rules compliant molecules were chosen from each study, and thus, were orally bioavailable. The binding affinities (p*K*d) of the Lipinski Rules compliant molecules produced ranged from 7.16 to 10.00. The molecules require further validation through *in silco* molecular dynamics and confirmed through *in vitro* assays.

Keywords: Protein Kinase C; Isojacareubin; Bisindolylmaleimide inhibitor; Conformational Analysis; Virtual Screening; *De novo*.

*Corresponding Author Email: jeanelle.x.caruana.14@um.edu.mt
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INTRODUCTION

Protein Kinases (PKs) are a group of enzymes which phosphorylate cellular proteins. Phosphorylation results in the reversible alteration of structure and function of the substrate proteins.[1] Dysregulation of Protein Kinase C (PKc) isoenzymes is associated with the promotion and propagation of cancer. For these reasons, PKc inhibitors are attractive anti-cancer drugs.[2] Isojacareubin shows PKc inhibition.[3] This study aimed to design and optimize PKc inhibitors which exhibit a high affinity for PKc and oral bioavailability, using isojacareubin as a molecular template.

MATERIALS AND METHOD

Study 1: Conformational Analysis

X-ray crystallographic deposition 2I0E[4] was selected from the Protein Data Bank (PDB)[5]. The deposition described the bound coordinates of the Bisindolylmaleimide (BIM) inhibitor with PKc. The deposition 2I0E[4] was inputted into SYBYL®-X version 1.1[6]. This crystallographic deposition was simplified computationally through the removal of chemical moieties not considered critical to ligand binding, to decrease computer intensiveness. The dimeric deposition was reduced to a single monomer and co-crystallized water molecules were also removed. The result was a monomer of PKc bound to the BIM inhibitor (Ref. Figure 1).

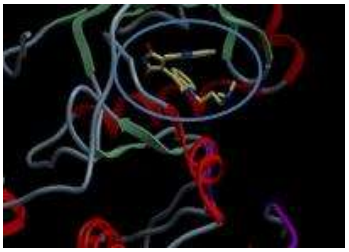


Figure 1: Bisindolylmaleimide inhibitor bound in catalytic site of Protein Kinase C, generated using MolSoft® version 3.5[7]. The Bisindolylmaleimide inhibitor is encircled in blue.

The BIM inhibitor was extracted from the ligand binding pocket (LBP) of the PKc monomer using SYBYL®-X version 1.1[6]. The BIM inhibitor and Chain A were inputted into X-Score® version 1.3[8] and the Ligand Binding Affinity (LBA) (p*K*d) of the BIM inhibitor for the LBP of PKc was measured. Isojacareubin was constructed *de novo* in SYBY®-X version 1.1[6]. and sketched using Accelrys Draw® v.4.1[9]

Isojacareubin was docked into the PKc LBP in SYBYL®-X version 1.1[6]. The BIM inhibitor was selected as a template and isojacareubin was selected as the ligand. This resulted in the

production of twenty one binding conformers. This was done in order for the LBA (p*K*d) and ligand binding energy (LBE) (kcal mol^{-1}) of each binding conformer to be quantified.

The different conformations could adopt a total of five orientations; thus the conformations were separated according to the orientation they adopted.

Each binding conformer was inputted into X-Score® version 1.3[8] and their LBA (p*K*d) for the LBP of PKc was computed. Each binding conformer was also imported to SYBYL®-X version 1.1[6] and their LBE (kcal mol^{-1}) was calculated.

A graph presenting the LBA (p*K*d) and LBE (kcal mol^{-1}) (y-axis) against conformer number (x-axis) was plotted. The conformer with the optimal combination of high LBA (p*K*d) and low LBE (kcal mol^{-1}) was identified as the best binding conformer.

Study 2- Virtual Screening

A consensus pharmacophore was generated in LigandScout v3.12[10] and used to screen the online database ZINCPharmer[11], with the search being filtered to identify only Rule of 3 compliant hits for lead molecules. A protomol was modelled using SYBYL®-X version 1.1[6]. The hits were then uploaded into the protomol and their affinity calculated in terms of a total score generated in SYBYL®-X version 1.1[6]. The optimal structures with the highest total score were identified.

Study 3- *de novo* Design

LigBuilder® v.1.28[12] was used to carry out the structure-based drug design. The POCKET module within LigBuilder® v.1.28[12] was run using the PKc monomer and isojacareubin in order to produce key interaction sites and a pharmacophore model. The binding conformers having the optimal LBA and LBE combination identified for each different orientation were edited in SYBYL® -X v.1.1[6]. The resultant seeds were provided with growing sites via an atom change to H.spc atom. Each seed structure was exported to LigBuilder® v.1.28[12]. Molecular growth at the assigned growing site for each seed structure was allowed via the GROW module within LigBuilder®v.1.28[12]. The ligand collection file produced in the GROW module was then used in the PROCESS module within LigBuilder®v.1.28[12] to generate 200 molecules for each seed structure. Lipinski Rules[13] compliant molecules were selected and those with the highest p*K*d values were chosen from each family.

RESULTS

Study 1- Conformational Analysis

Isojacareubin was guided into the active site of PKc and this gave rise to the generation of twenty one binding conformers for isojacareubin to the active site of Protein Kinase C (Ref. Figure 2).

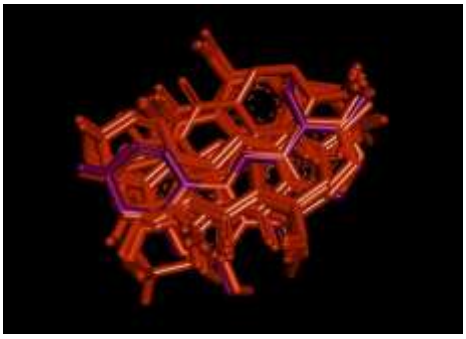


Figure 2: The twenty one superimposed conformers of isojacareubin, generated using Accelrys *Discovery Studio*® v.4.0[14]. The optimal conformer is highlighted in purple.

The graph plotted from the LBA (p*K*d) and LBE (kcal mol^{-1}) values of the twenty one isojacareubin conformations is represented in Figure 3. The best binding conformer would have the lowest energy required for docking in the LBP, combined with high molecular affinity.

Study 2- Virtual Screening

The consensus pharmacophore is represented in figure 4. The consensus pharmaphore represented the average critical points of the optimal isojacareubin conformation and the bioactive BIM inhibitor with the PKc_LBP.

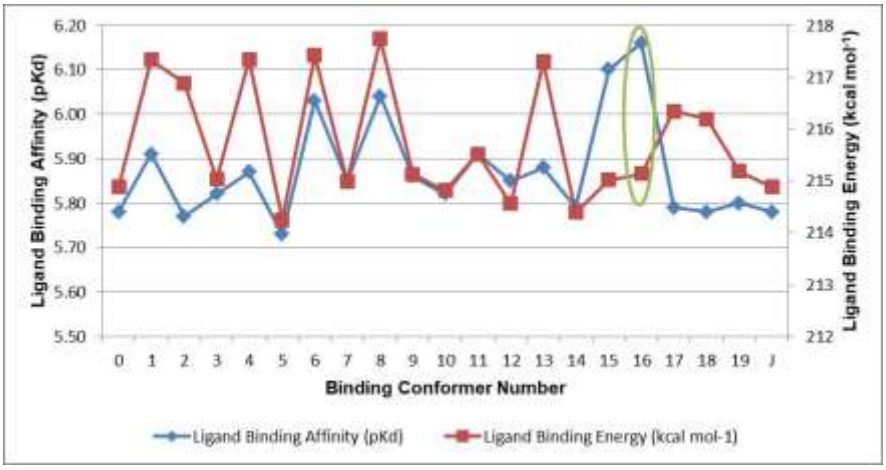


Figure 3: Graph of ligand binding affinity (p*K*d) and ligand binding energy (kcal mol^{-1}) against Conformer Number for isojacareubin. The best binding conformer is encircled in green.

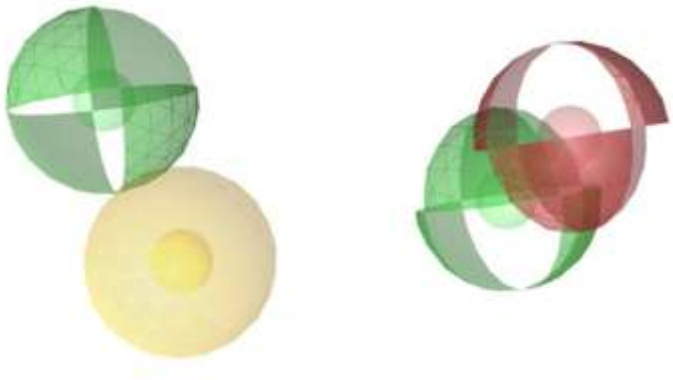


Figure 4: Consensus pharmacophore, generated using LigandScout v3.12[10]. The pharmacophore model consisted of one hydrophobic feature (yellow sphere), two hydrogen bond donor features (green spheres) and one hydrogen bond acceptor feature (red sphere).

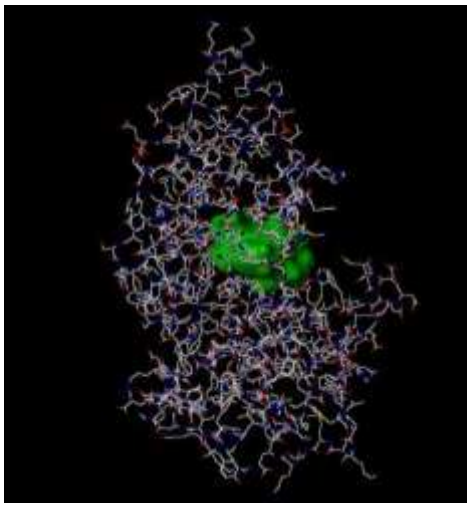
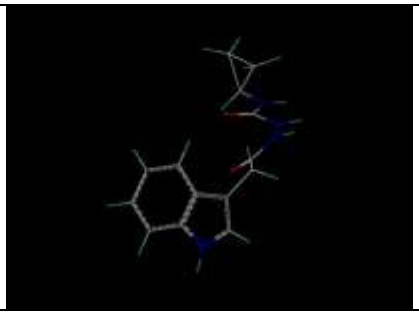
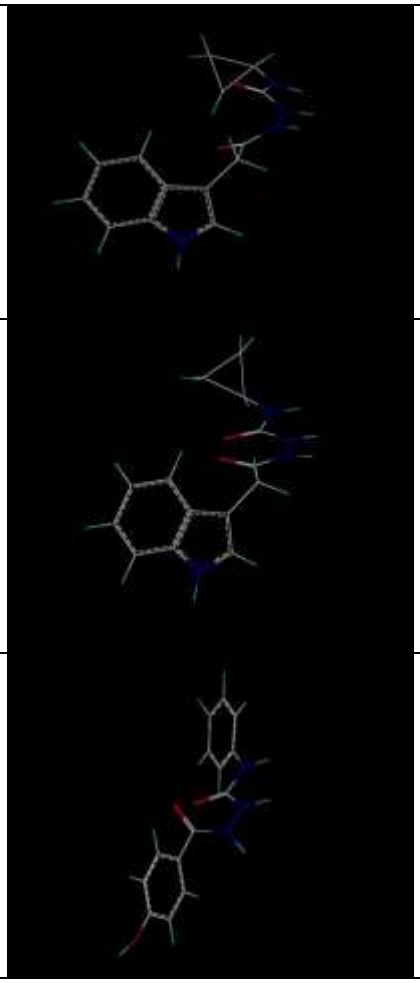


Figure 5: Protomol of 2I0E[4], generated using SYBYL®-X version 1.1[6].

A protomol (Ref. Figure 5) that defines the surface morphology of the active site was modelled. The optimal structures established through virtual screening were identified and was shown in table 1.

Table 1: Top four hits identified from ZINCPharmer[11]. The structures were produced using SYBYL®-X version 1.1[6].

Molecule ID	Structure	Total Score
ZINC49127037		7.16

ZINC49127037_002		7.09
ZINC49127037_003		6.74
ZINC02143630		6.13

Study 3- *de novo* Design

When the POCKET module within LigBuilder® version 1.2[12] was run, a pharmacophore model and key interaction sites (Ref. Figure 6) within the LBP of PKc were derived.

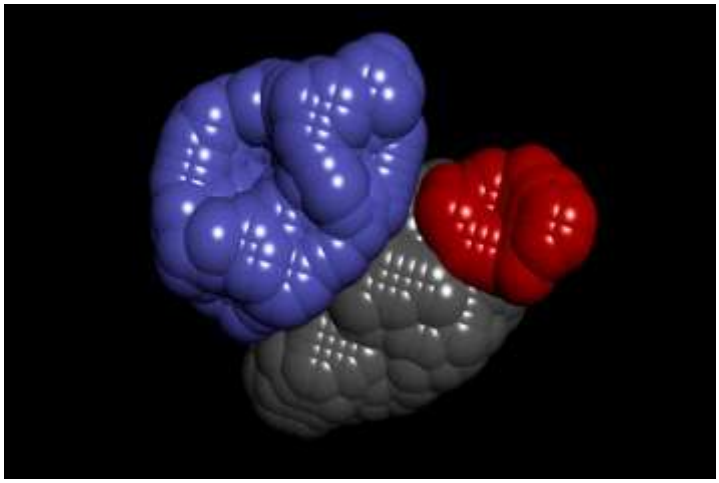
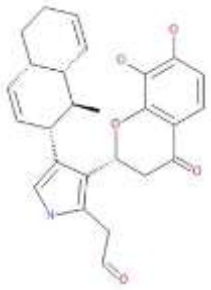
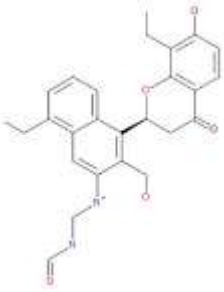
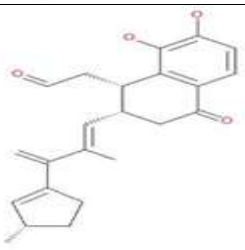


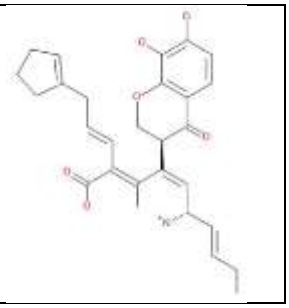
Figure 6: Key interaction sites within the ligand binding pocket of Protein Kinase C, generated using Accelrys Discovery Studio® v.4.0[14]. Hydrogen bond donor grids are displayed in blue, hydrogen bond acceptor grids in red and hydrophobic grids in grey.

The best binding isojacareubin conformers with different orientations are conformers three, eight, eleven, fourteen and sixteen and two hundred molecules were produced *de novo* for each seed from the different conformers. Conformer three did not produce viable seeds. Lipinski Rules[13] state that poor absorption or permeation is more likely when the number of hydrogen bond donors and acceptors is greater than 5 and greater than 10 respectively, molecular weight is greater than 500 and logP is greater than 5.

The structures and properties of the novel molecules created from the seed structures were presented in table 2, those molecules with the highest p*K*d values were chosen from each seed.

Table 2: Properties and structures of the Lipinski Rules[13] compliant molecules generated from seed structures. The structures were generated using Accelrys *Draw*® v.4.1[9].

Molecule from a Seed of a Specific Conformer	Molecular Weight	LogP	LBA (p*K*d)	Number of Hydrogen Bond Donors	Number of Hydrogen Bond Acceptors	Structure
Conformer 8	428	4.71	9.94	3	6	
Conformer 11	373	4.63	9.99	4	5	
Conformer 14	361	4.38	10	2	4	

Conformer 16	475	4.76	9.89	4	6	

DISCUSSION

Virtual Screening

The higher the total score, the higher the probability of binding to the LBP. ZINC49127037, ZINC49127037_002 and ZINC49127037_003 had a higher total score than the best binding conformer of isojacareubin. This may be attributed due to unfavourable interactions as a result of steric bumps between the LBP of PKc and the molecule (Ref. Figure 7). ZINC49127037, ZINC49127037_002 and ZINC49127037_003 showed no such interactions.

Whilst ZINC02143630 exhibited a slightly lower binding affinity (p*K*d) than that of conformer 16 of isojacareubin, with a difference of 0.03. This may possibly due to additional sulphur bonding interactions, alongside unfavourable donor-donor interactions, that ZINC02143630 exhibited when binding to the LBP of PKc (Ref. Figure 8). ZINC49127037, ZINC49127037_002 and ZINC49127037_003 showed no sulphur binding interactions.

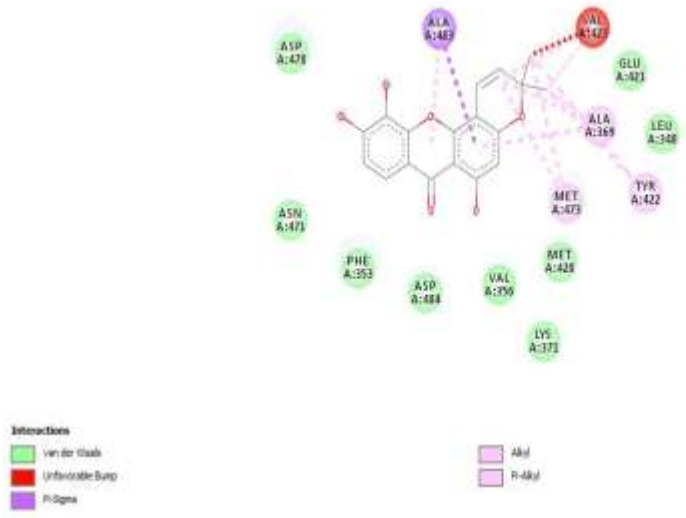


Figure 7: Depiction of interactions between conformer 16 of isojacareubin and the amino acids in the ligand binding pocket of Protein Kinase C, generated using Accelrys Discovery Studio® version 4.0[14].

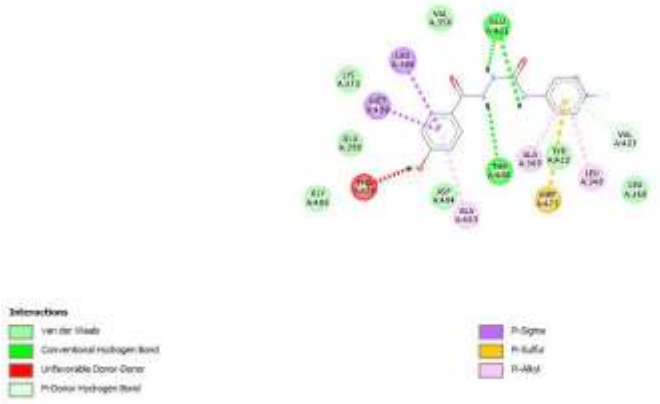


Figure 8: Depiction of interactions between ZINC02143630 and the amino acids in the ligand binding pocket of Protein Kinase C, generated using Accelrys Discovery Studio® version 4.014.

de novo

The molecule with the highest binding affinity (pKd) was chosen from each seed for interpretation of interactions between the LBP of the PKc and the molecule.

The interactions between the LBP of PKc and the molecules were displayed in the figures 9-12.

All of the molecules have a much higher Binding Affinity (p*K*d) than the best binding conformer of isojacareubin, which was 6.16. This was not only because these molecules did not display any unfavourable interactions like conformer 16 of isojacareubin (Ref Figure 7), but also due to the numerous hydrogen bonding and hydrophobic interactions which strengthened the ligand-receptor complex.

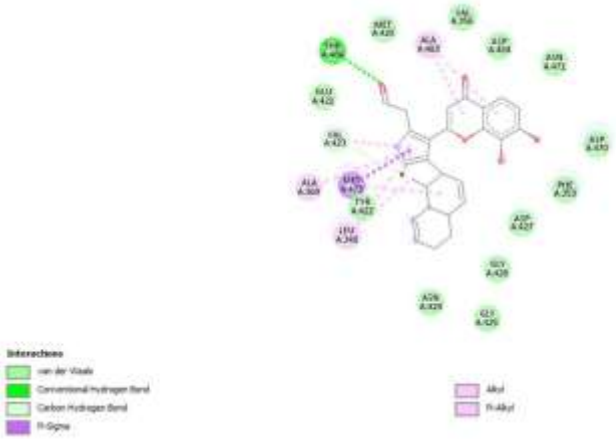


Figure 9: Depiction of interactions between the molecule of the seed of conformer 8 of isojacareubin and the amino acids in the ligand binding pocket of Protein Kinase C, generated using Accelrys Discovery Studio® version 4.0[14].

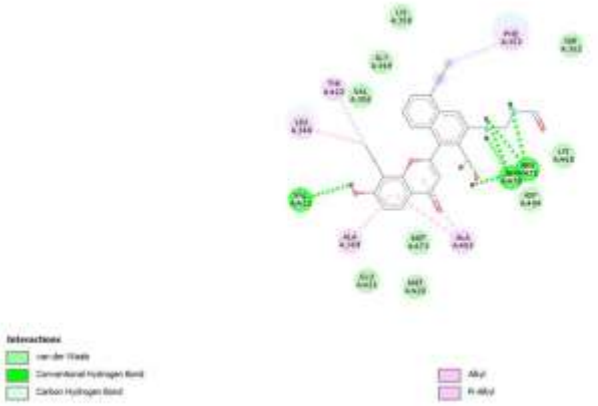


Figure 10: Depiction of interactions between the molecule of the seed of conformer 11 of isojacareubin and the amino acids in the ligand binding pocket of Protein Kinase C, generated using Accelrys Discovery Studio® version 4.0[14].

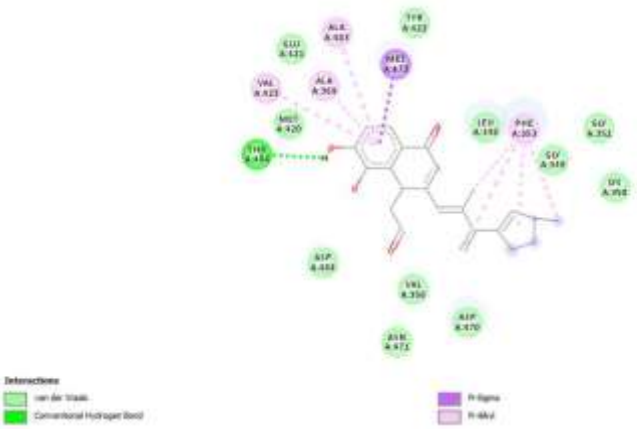


Figure 11: Depiction of interactions between the molecule of the seed of conformer 14 of isojacareubin and the amino acids in the ligand binding pocket of Protein Kinase C, generated using Accelrys Discovery Studio® version 4.0[14].

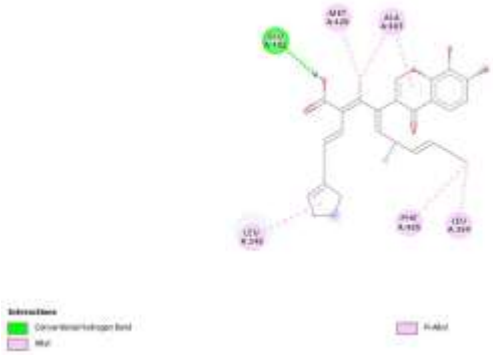


Figure 12: Depiction of interactions between the molecule of the seed of conformer 16 of isojacareubin and the amino acids in the ligand binding pocket of Protein Kinase C, generated using Accelrys Discovery Studio® version 4.0[14].

CONCLUSION

This study identified novel PK inhibitors which have high LBA (p*K*d) and are Lipinski Rules[13] compliant, consequently making them bioavailable. The molecules require further validation through *in silco* molecular dynamics and confirmed through *in vitro* assays.

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