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Design of Novel Protein Kinase Inhibitors Using the Naturally Occurring Staurosporine Scaffold as a Lead

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ABSTRACT

Staurosporine is a naturally occurring alkaloid isolated from the bacterium *Streptomyces staurosporesa*. It inhibits the protein kinases class of enzymes (including protein kinase C) inducing apoptosis and thus resulting in it having potential anti-tumour activity. Recent studies showed that staurosporine had high affinity for the protein kinase C receptor, however lacked selectivity resulting in a wide adverse effect profile. Thus, this study targets the protein kinase C receptor for the development of novel structures capable of its inhibition using staurosporine as template molecule. This study has yielded two molecular cohorts, one from each approach. These were filtered for Lipinski Rule compliance and segregated into families of pharmacophoric similarity and ranked in order of affinity. The molecules with the best ligand binding affinities were generated using the *de novo* approach. The best molecule from the *de novo* approach had an affinity of 10, while the best molecule from the virtual screening approach had an *affinity* of 9.65. This study was valuable in demonstrating that the staurosporine scaffold was suitable for the identification and design of high affinity structures capable of modulating the protein kinase C receptor through two distinct approaches- *de novo* design and virtual screening. The affinities of the optimal molecules exceeded that of staurosporine, and these molecules will be proposed for further study. Specifically, their enhanced molecular interactions will be explained from an atomic perspective, and also through molecular dynamic simulation studies.

Keywords: Staurosporine, Protein Kinase C Inhibitors, Anti-Cancer Activity.

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INTRODUCTION

The protein kinase C (PKc) family is being intensively studied to manage tumours since it was identified as a major receptor for the tumour-promoting phorbol esters¹. Each PKc subtype plays a different role in cancer progression² thus making PKc inhibitors important anticancer drugs. Staurosporine represents the original indolocarbazole isolated from *Streptomyces staurosporeus*³ and is a highly promiscuous, ATP-competitive kinase inhibitor⁴ that potently binds to and inhibits most PKc isozymes *in vitro* inducing cell apoptosis therefore possibly tumour death³. Consequently, staurosporine will be used as a template, for the design of clinically useful high affinity PKc inhibitors.

Method

PDB ID crystallographic deposition 1STC⁵ (Figure 1) was selected as a template for this study. It described the bound co-ordinates of cAMP-dependent protein kinase, alpha-catalytic subunit in complex with staurosporine resolved at 2.3Å. Isolation and extraction of the ligand from the receptor was carried out in Sybyl-X[®] v1.1⁶ such that the *apo* receptor was obtained in different file formats – pdb and mol2 respectively. The binding affinity of staurosporine for its cognate receptor was consequently calculated in X-Score[®] v1.3⁷.

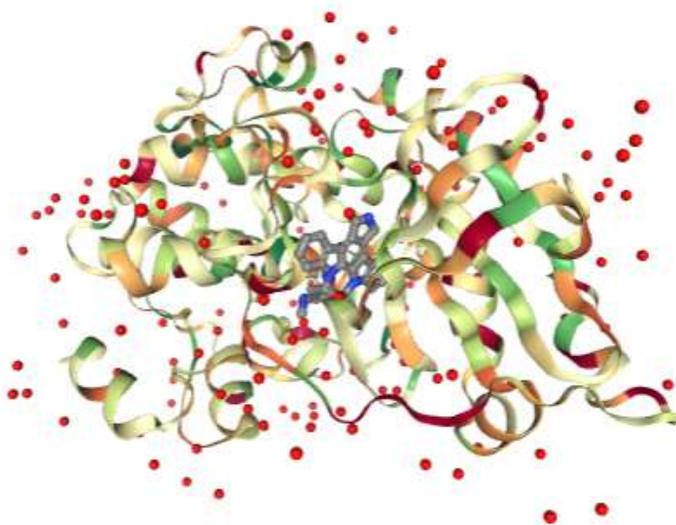


Figure 1. PDB ID 1stc⁵ deposition.

PDB ID 1stc⁵ displaying PKc bound to staurosporine generated in NGL Viewer[®] ¹¹. The reddish colour represents hydrophilic areas while the greenish colour represents hydrophobic areas.

Virtual screening (VS) approach

A consensus pharmacophore (Figure 2), representing the optimal staurosporine conformer, and the bioactive PKc inhibitor (2S)-3-phenyl-N~1~-[2-(pyridin-4-yl)-5,6,7,8-

tetrahydro[1]benzothieno[2,3-d]pyrimidin-4-yl]propane-1,2-diamine as described in PDB ID 3ZH8 was used to query the molecular data base ZINCPharmer^{® 8}. This process identified small molecules present on this database which were morphologically, 3 dimensionally (3D), and electronically similar to the query. A protomol, or an idealized Ligand Binding Pocket (LBP) representing the energetically unsatisfied space at the interior of the PKc was modelled. The Lipinski Rule⁹ compliant molecules that were identified from ZINCPharmer^{® 8} during VS were uploaded into the modeled protomol and their affinity quantified. The optimal structures identified through this approach were earmarked for further optimisation.

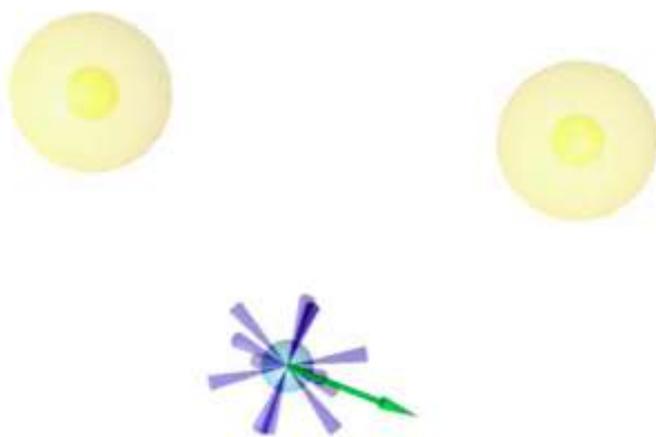


Figure 2. Consensus pharmacophore.

This four-feature pharmacophore model consists of two hydrophobic features (yellow spheres), one hydrogen bond donor feature (green vector) and a positive ionizable area (blue location sphere). It was generated using LigandScout^{® v3.12}¹²

De novo drug design approach

2-dimensional (2D) topology maps describing the critical interactions forged between staurosporine and the PKc receptor were generated in Posview^{® 11}. This data was used to guide the modeling of seed structures. Special Hydrogen atoms (*H.spc*) were designed on these designated fragments such that these would be capable of sustaining user directed growth. A LBP map of the PKc receptor was generated using the pocket algorithm of LigBuilder^{® v.1.2}¹⁰. The designed seed fragments were planted into this restricted pharmacophoric space, and novel molecular growth driven using the GROW module of LigBuilder^{® v.1.2}¹⁰ was carried out. The resultant molecular cohort was assembled into a molecular database, with only the Lipinski Rule⁹ compliant molecules being segregated into pharmacophorically similar families and ranked in order of affinity. The optimal structures emanating from this process were identified.

RESULTS AND DISCUSSION

Virtual screening (VS) approach

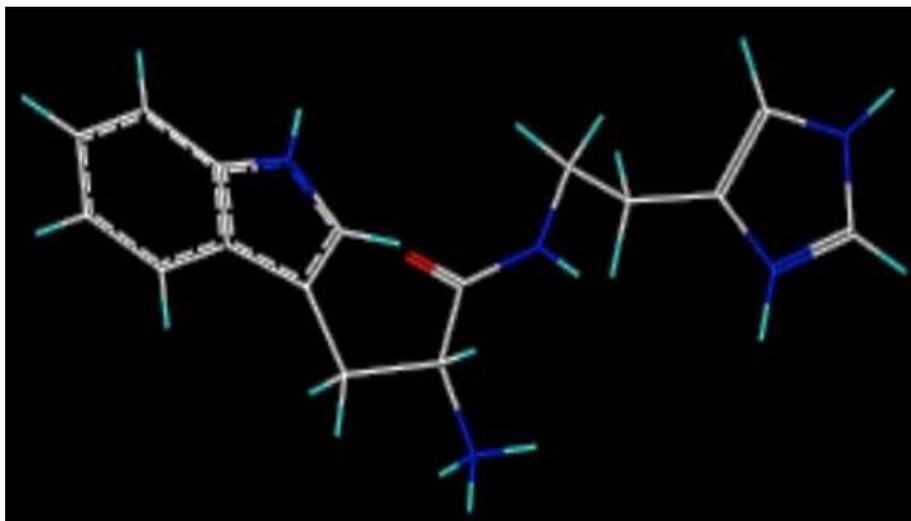


Figure 3: 2D structure of molecule ZINC13554963.

The VS approach resulted in a total of 1282 molecules out of which 195 molecules were found to be Lipinski Rule⁹ compliant. Their total score values ranged from 9.65 to 6.78. The molecule with the highest total score of 9.65 was ZINC13554963 (Figure 3).

De novo drug design approach

The 4 seed fragments that were planted into the PKc_LBP yielded a total of 72 Lipinski Rule⁹ compliant molecules, which were divided for each seed into pharmacophorically similar families and ranked in order of affinity. The molecule with the highest affinity derived from each seed was tabulated in Table 1.

DISCUSSION

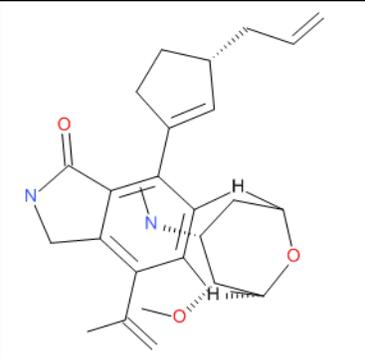
This study was valuable in demonstrating that the staurosporine scaffold was suitable for the identification and design of high affinity structures capable of modulating the PKc receptor through two distinct approaches –VS and *de novo* design. The affinities of the optimal molecules exceeded that of staurosporine, will be proposed for further study. Specifically, their enhanced molecular interactions will be explained from an atomic perspective, and also through Molecular Dynamic simulation studies.

Table 1. Molecules showing highest affinity from each seed.

The Ligand Binding Affinity (LBA), the Molecular Weight in Daltons and the Measure of Lipophilicity (LogP) were generated using LigBuilder[®] v.1.2¹⁰. The Number of Hydrogen Bond

Donors, the number of Hydrogen Bond Acceptors and structures were generated using Accelrys Draw[®] v.4.1¹³.

Seed	LBA	MW	Log P	H-bond Acceptors	H-bond Donors	2D - Structure
1	9.86	494	4.3	6	2	
2	9.47	493	4.98	6	4	
3	9.72	480	4.92	4	3	

4	10	418	4.81	4	2	
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CONCLUSION

In conclusion, this study has shown that staurosporine is a useful lead for ligand generation. A library of drug-like molecules with inhibitory activity on PKc was generated. This library is suitable for in vitro testing. This will evaluate whether the enzyme inhibition is replicated in vitro. If this proves true the generated molecules are viable candidates for further drug development as future anticancer drugs.

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