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Protein Kinase A-like Kinase and Cell Division Related Protein Kinase 2 as Drug Target for African Sleeping Sickness

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Abstract—*Human African trypanosomiasis (HAT), also known as sleeping sickness, has been considered one of the most important human diseases in Africa. It is caused by infection with protozoan parasite, Trypanosoma,which is transmitted to humans by tsetse fly (Glossina genus). This disease takes two forms, depending on the involvement of parasite: Trypanosoma brucei gambiense (accounts for >98% of cases) and Trypanosoma brucei rhodesiense. This endemic disease with high rate of mortality has caused major epidemics in the past and had impacted the development of the rural populations at a large scale. The drugs currently available are not sufficient enough to combat the disease. Therefore, newer, more effective, non-toxic and affordable drugs are badly needed. Protein kinases represent promising drug targets for a number of human diseases. In the present project two kinases i.e. Protein Kinase A-like kinase (PKAC1) and cell division related protein kinases 2 were modelled to study their prospects as potential target against HAT. In the absence of PDB structures, the modeled structures of the two kinases were used for the analysis of the binding pockets and were then compared to their respective human homologues.*

1. INTRODUCTION

Human African trypanosomiasis (HAT), also known as sleeping sickness, has been afflicting populations in Africa in areas where its specific vector, the tsetse fly, thrives [1]. It is endemic in 36 countries of sub-Saharan Africa infecting an estimated 50,000 people, predominantly in rural populations strongly associated with agriculture. This endemic disease has a very high level of mortality and has been responsible for large epidemics in the past. This disease had a major impact on the development of rural populations. It is caused by infection with protozoan parasite, *Trypanosoma*, which is transmitted to humans by the bite of tsetse fly (*Glossina* genus). It is transmitted congenitally from mother to unborn child, but can also occur through contaminated blood transfusions. Cattle and other wild mammals act as reservoir hosts of the parasites.

This disease exists in two clinical forms affecting distinct parts of Africa: a chronic form in West and Central Africa caused by *Trypanosoma brucei gambiense* (>95% of reported cases) and an acute form in East and Southern Africa caused by *Trypanosoma brucei rhodesiense* (<5% of cases reported) [2]. The first phase of the infection i.e. haemolymphatic phase is characterized by the multiplication of the trypanosomes in subcutaneous tissues, blood and lymph which manifests in the form of bouts of fever, headaches, joint pains and itching [3]. The second stage i.e. the neurological phase is marked by the infection of the central nervous system by the parasite. This phase is characterized by symptoms such as behavioral changes, disturbance of sleep cycle, confusion, sensory disturbances and poor coordination [4, 5].

The drugs currently available are not sufficient enough to combat the disease for several reasons. Certain drugs such as Pentamidine and Suramin have undesirable side-effects whereas Melarsoprol, in addition, is toxic at its therapeutic dose [6]. It is one of the "neglected tropical diseases" due to lack of profitable market for pharmaceutical industries. Therefore, newer, more effective, non-toxic and affordable drugs are badly needed.

Protein kinases represent promising drug targets for a number of human diseases. Some of them have been shown to be essential for proliferation and/or viability of parasite life-cycle stages that are clinically relevant [7]. In the present research article, two kinases: Tb09.211.2410 i.e. Protein Kinase A-like kinase (PKAC1) and ref|XP_822746.1| i.e. cell division related protein kinase 2 are modeled as target proteins against *T. brucei*. In *T. brucei*, PKA-like kinase regulates cell cycle progression and basal body segregation. Cell division related protein kinase 2 has been implicated in the control of cell cycle.

2. METHODOLOGY

Three dimensional structures for both the proteins; Protein Kinase A-like Kinase and Cell Division Related Protein Kinase 2 of *Trypanosoma brucei* are not available till date. The nucleotide sequence of Protein Kinase A-like Kinase was obtained from the NCBI database using the gene identifier Tb09.211.2410; which was then used to retrieve the amino acid sequence from UniProtKB_Nucleotide database. Further, the available three dimensional structures of the sequences, homologous to the TbrPKAC1, were obtained from UniProtKB_PDB through blastx. For Cell Division Related Protein Kinase 2, the amino acid sequence was obtained from the NCBI database by using NCBI Reference Sequence XP_822746.1. The available three dimensional structures of the sequences, which were homologous to the Cell Division Related Protein Kinase 2, were obtained from UniProtKB_PDB through blastx.

The three template sequences and the respective *Tbr* kinase sequences were aligned using T-Coffee [8] multiple sequence alignment software and the observed alignment accuracy confirmed the quality of the homology model. Two different models of both the kinases were built using python based programme MODELLER. The two models thus produced were evaluated using MolProbity [9, 10].

A high throughput in-silicon screening program LIDAEUS (**Li**gand **D**iscovery **A**t **E**dinburgh **U**niver**S**ity) was used for ligand screening. It selects molecules from the small molecule database, EDULISS (EDinburgh University LIgand Selection System); dock it into the grid of site points, created in the binding pocket of the target protein. It matches atoms of the molecule to site points, explores the binding pocket thoroughly, identifies appropriate poses, and screens the 100 hits [11]. Top 20 hits of the LIDAEUS result for both the *Tbr* kinases were screened and 10 ligands were selected on the basis of best surface-fit in the binding pocket of the model. On the basis of their ligand efficiency top 4 ligands were selected and studied in detail for their binding characteristics (Table 1 and 2).

Table 1. Screening of LIDAEUS Hits for TbrPKAC1

Hit No.	Ligand	Molecular formula	ΔH	Non H atoms	Ligand efficiency	Rank
1	SPH1-000-211	C10 H9 N O2	-17.3752	13	1.34	2
2	SPH1-000-156	C7 H5 F3 O3	-14.3726	13	1.11	4
3	SPH1-000-023	C6 H6 O3	-13.6886	9	1.52	1
4	SPH1-000-601	C15 H16 F3 N3	-12.1438	21	0.58	10
5	SPH1-000-161	C7 H8 O3	-11.7916	10	1.18	3
6	SPH1-000-752	C14 H17 N3	-11.2754	17	0.66	9
7	SPH1-001-048	C11 H14 N3	-10.4969	14	0.75	7
8	SPH1-000-898	C8 H11 N O2	-8.89084	11	0.81	5
9	SPH1-000-142	C9 H14 O2	-8.1824	11	0.74	8
10	SPH1-000-799	C7 H8 O3	-7.92404	10	0.79	6

Table 2. Screening of LIDAEUS Hits for TbrCRK

Hit No.	Ligand	Molecular formula	ΔH	Non H atoms	Ligand efficiency	Rank
1	SPH1-000-078	C9 H10 O3	-18.5364	12	1.54	1
2	SPH1-000-223	C12 H17 N O2	-15.851	15	1.06	4
3	SPH1-000-617	C10 H11 N O2	-14.365	13	1.1	2
4	SPH1-000-416	C11 H8 O3	-14.2247	14	1.02	5
5	SPH1-000-055	C11 H8 Cl2 N2 O	-13.8611	16	0.87	8
6	SPH1-000-748	C10 H9 N O2	-13.8091	13	1.06	3
7	SPH1-000-367	C10 H8 O3	-12.5701	13	0.97	6
8	SPH1-000-428	C10 H11 N O S	-12.2505	13	0.94	7
9	SPH1-000-435	C12 H13 N S	-12.0758	14	0.86	9
10	SPH1-000-224	C12 H18 N2 O	-11.9264	15	0.8	10

The binding sites of the modelled proteins were analysed using an open-source, user-sponsored, molecular visualization system PyMOL [12] and then compared to the binding sites of their respective Human Homologs to further explore them as potential drug target.

3. SELECTION OF THE TEMPLATE

The selection of the template was done on the basis of e-value, R-value, resolution, b-factor, Ramachandran plot, number of water molecules and sequence completeness. Two models were built for each target protein. For PKAC1, first model was built using template 1APM_E (52% sequence identity) and 2ERZ_E 52% sequence identity) both from Mus musculus. The Ramachandran plot showed 97% amino acids in favoured and 99.7% in allowed region with 1 outlier (Figure 1A). The second model was also built using both templates from Mus musculus namely 1APM_E (52% sequence similarity) and 1ATP_E (52% sequence similarity). The Ramachandran plot showed 97.3% amino acids in favoured and 99.4% in allowed region region with 2 outliers (Figure 1B). Therefore, for PKAC1 model 1 was selected for binding pocket analysis and subsequent ligand docking studies.

For ref|XP_822746.1|, first model was built using template 1HO1_A from *Escherichia coli* and 1OIT_A (59% sequence identity) from *Homo sapiens*. The Ramachandran plot showed 77% amino acids in favoured and 88.7% in allowed region with 35 outliers (Figure 1C). The second model was built using both templates from *Homo sapiens* namely 3PXF_A (58% sequence similarity) and 3PXQ_A (58% sequence similarity). The Ramachandran plot showed 93.2% amino acids in favoured and 98.4% in allowed region with 5 outliers (Figure 1A). Therefore, for ref|XP_822746.1| model 2 was selected for binding pocket and subsequent ligand docking analysis.

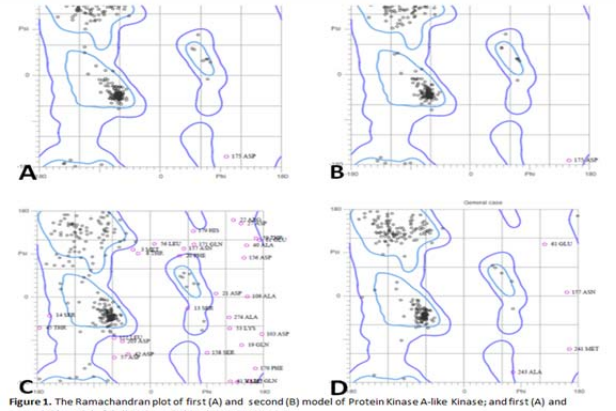

Figure 1. The Ramachandran plot of first (A) and second (B) model of Protein Kinase A-like Kinase; and first (A) and second (B) model of Cell Division Related Protein Kinase 2.

4. ANALYSIS OF BINDING POCKETS

Based on the distribution and binding of ligand in the binding pocket, three different binding sites were identified in both the target proteins, and then compared with the respective human homologue to establish the specificity of binding sites in parasite protein. The x-ray crystallography structure of 2GU8 and 1GZ8 were used as human homologue for PKAC1 and ref|XP_822746.1| respectively (Figure 2). When aligned with PKAC1, 2GU8 showed 49.26% sequence identity and RMS value of 0.035 Å. In case of ref|XP_822746.1|, 1GZ8 showed 52.03% sequence identity and RMS value of 0.871 Å. These values indicated that the human homologues selected are good enough to study the binding specificity of drug with parasite proteins.

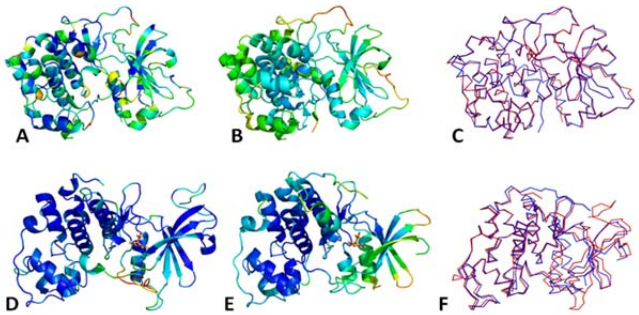

Figure 2. Complete monomer model of Protein kinase A-like kinase (A) Tbr; (B) human; (C) Alignment of Tbr model (red) with human homolog (blue) and complete monomer model of Cell Division Related Protein Kinase 2 (A) Tbr; (B) human; (C) Alignment of Tbr model (red) with human homolog (blue).

The binding pocket analysis of modeled PKAC1 showed no significant differences when compared with its human homologue 2GU8. The three potential binding sites identified in the modeled protein were exactly similar. The binding sites 1 in both have H-bond donor residues (Glu and Thr) whereas binding site 2 has both H-bond donor (Asp and Lys) and acceptor (Asp) residues. A hydrophobic zone was constituted by Val and Ala residues in binding site 3.

The binding pocket analysis of modeled ref|XP_822746.1| showed significant differences when compared with its human homologue 1GZ8. The binding pocket was more exposed in the modeled protein (Figure 3, A) whereas there was steric hindrance on the upper side at the opening of binding pocket of 1GZ8 [Figure 3, B (arrow)]. Thus, render 1GZ8 difficult to access by the potential drug molecules. Of the three binding sites identified based on the distribution of ligand in binding pocket, binding site 1 and 2 showed significant differences in modeled parasite protein and its human homologue 1GZ8. In binding site 1 of modeled protein, Asp-105 and Glu-100 were present as H-bond donor and H-bond acceptor and a strong hydrophobic zone was formed by Leu-152, Val-102 and Phe-99 residues as compared to 1GZ8 (Figure 4, A-B). In binding site 2 of 1GZ8, H-bond acceptor (Gln-131) was present making it polar whereas a strong hydrophobic zone was constituted by Ala-149, Ala-162 and Tyr-34 in the modeled parasite protein (Figure 4, C-D). The binding site 3 in both was characterised by the one H-bond donor (Lys residue) and a hydrophobic zone.

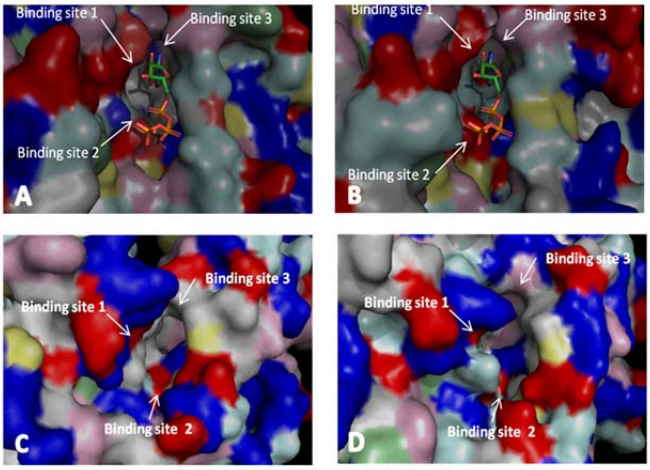

Figure 3. Potential binding sites in binding pocket of modeled PKAC1 (A) and its human homologue, 2GU8 (B) showing binding of ATP; and binding pockets of modeled cell division related protein kinase 2 (C) and its human homologue, 1GZ8 (D).

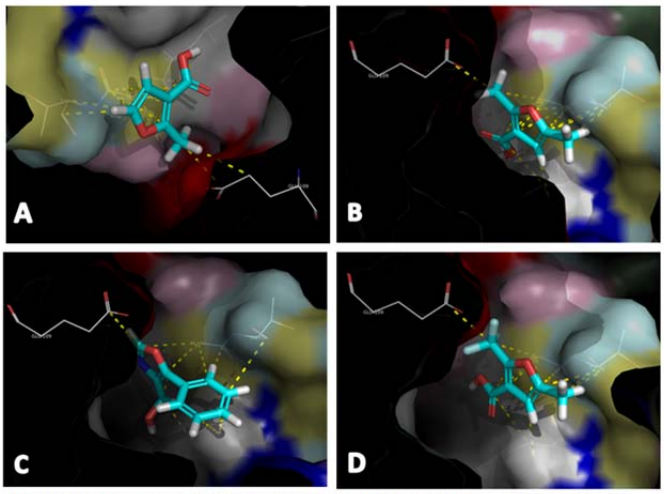

Figure 4. Binding interaction model of Protein kinase A-like kinase with (A) ligand 3, (B) ligand 5, (C) ligand 1, (D) ligand 2.

5. ANALYSIS OF BINDING MODE

Ligand discovery and virtual screening was done using Lidaeus program. For both modeled protein top 50 Lidaeus hits were screened. Out of these, 10 were selected on the basis of best surface-fit in the binding pocket. Subsequently, top 4 ligands were selected on the basis of their ligand efficiency and studied in detail for their binding characteristics in terms of presence of number of hydrogen bonds, H-bond donors, H-bond acceptors, rotational bonds present. The possibility of Van der Waals and hydrophobic interactions was also studied for the top 4 ligands in both modeled proteins (Table 3 and 4).

Table 3: Summary of the study of binding characteristics of top 4 ligands for Protein Kinase A-like Kinase.

Ligand No.	Ligand name	HB	LE	HBD	HBA	RB	Hydrophobic interactions	Van der Waals interaction
3	2-methyl-3 furoic acid	0	1.52	1	3	1	Leu-31	Glu-109,Gly-32, Thr-33
1	5-phenyl-1,3-oxazol-4-yl)methanol	0	1.33	1	3	2	Val-39, Leu-31	Thr-33, Gly-32, Glu-139
5	2,5-dimethyl-3-furoic acid	0	1.17	1	3	1	Val-39, Leu-31	Thr-33, Gly-32, Glu-139
2	5-methyl-2-(trifluoromethyl)-3-furoic acid	0	1.11	1	6	1	Val-39, Leu-31	Thr-33, Gly-32, Glu-109

Table 4: Summary of the study of binding characteristics of top 4 ligands for cell division related protein kinase 2.

Ligand No.	Ligand name	HB	LE	HBD	HBA	RB	Hydrophobic interactions	Van der Waals interaction
1	3-(2-hydroxy phenyl) propanoic acid	0	1.54	2	3	3	Absent	Leu-29, Gly-30, Lys-52, Gly-32, Asp-163, Asn-150, Ala-149
3	1, 2, 3, 4-tetrahydro-6-quinolinecarboxylic acid	0	1.105	2	3	1	Leu-29, Leu-152	His-104, Asp-105, Asp-103, Gly-30, Gly-32, Glu-31
6	2-(1H-indol-3-yl) acetic acid	0	1.06	2	2	2	Leu-29, Gly-30, Val-37	Glu-31
2	(4-benzyl-1,4-oxazinan-2-yl) methanol	0	1.06	1	3	3	Leu-29, Val-102, Leu-152	Lys-108, Asp-105

Abbreviations: HB = hydrogen bond(s), LE = ligand efficiency, HBD = hydrogen bond donor(s), HBA = hydrogen bond acceptor(s), RB = rotational bond(s)

For PKAC1, none of the four selected ligands showed hydrogen bond formation but were good in terms of other properties as listed in table 1. Ligand 1 i.e. (5-phenyl-1,3-oxazol-4-yl) methanol with ligand efficiency lower than ligand 3 was analysed as the best ligand in terms of its binding characteristics. It was forming hydrophobic interaction with Leu-31& Val-39 and Van der Waals interactions with Glu-139, Gly-32, Thr-33. Nevertheless, other ligands were also as good in terms of binding properties but having slightly lower ligand efficiency.

The modeled cell division related protein kinase 2 by virtue of having significant differences in binding sites 1 and 2 from its human homologue was interesting to analyse for binding characteristics of the screened ligands. Amongst four ligand studied in detail for their binding mode, ligand 3 i.e. 1, 2, 3, 4-tetrahydro-6-quinolinecarboxylic acid emerged as the best ligand. It was fitting very nicely in the binding pocket. It was forming two hydrogen bonds with Asp-105 residue (figure 5, A, C). This was an important interaction as human homologue lacks Asp residue in this binding site. In addition, it was forming strong hydrophobic interactions with Leu-29, Leu-152 and Van der Waals interactions with His-104, Asp-105, Asp-103, Gly-30, Gly-32, Glu-31 residues (figure 5, A & B).

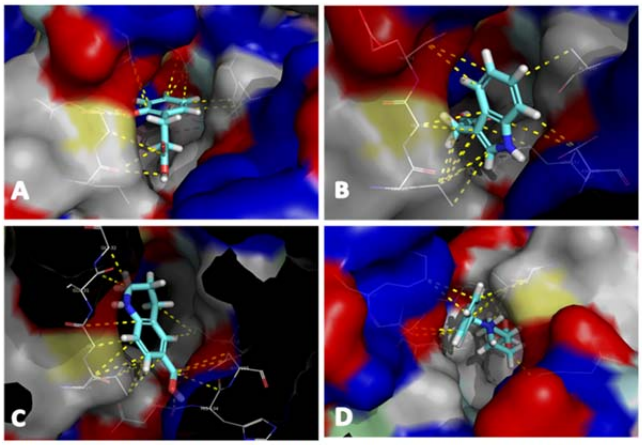

Figure 5. Binding interaction of Cell division related protein kinase 2 with (A) ligand 1, (B) ligand 6, (C) ligand 3, (D) ligand 2.

Ligand 1 i.e. 3-(2-hydroxy phenyl) propanoic acid was also a good candidate with highest ligand efficiency but not as good as ligand 3, as it lacks potential hydrophobic interactions that ligand 3 was capable of forming within the binding pocket. Furthermore, most of the interactions of ligand 3 with the protein were observed in the binding site 1 and 2 which are quite different from its human homologue. Furthermore, there can be a possibility of hydrogen bond formation between ligand 3 and Glu-100 residue if the benzene ring of ligand 3 can be engineered to increase its proximity to Glu-100 (figure 5, C). Thus, strengthening the candidature of ligand 3 as the potential drug molecule.

6. SUMMARY

Tb09.211.2410 i.e. Protein kinase A-like kinase (PKAC1) and ref|XP_822746.1 i.e. Cell division related protein kinase 2 of *Trypanosoma brucei* were modelled to study their prospects as potential target for drug discovery. Protein kinase A-like kinase (PKAC1) has not been found as a good drug target attributing to its binding pocket similarity to its human homologue. This can be attributed to the fact that the model building was accomplished using x-ray crystallographic structures of the mammalian proteins. This could have been better had there been x-ray crystallography structures of related parasite present available in the PDB. However, drug designing can be done targeting PKAC1 but it is going to be quite challenging for the companies.

Cell-division related protein kinase 2 appears to be a good target for drug designing owing to its binding pocket differences from its human homologue. Binding site 1 and 2 of the modelled protein can be effectively exploited to come up with a potential drug molecule which will be specific to the parasite protein. The ligand 3 i.e. 2-(1H-indol-3-yl) acetic acid has been analysed as the best ligand because of its binding properties. Furthermore, it has been suggested that ligand 3 can be engineered to introduce methyl group in the benzene ring to increase its proximity to the Glu-100 residue to establish hydrogen bond formation effectively.

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