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Prediction of Medicinal Properties of Marine Biota using Computational Bioinformatics Techniques

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ABSTRACT

Leishmaniasis, being one of the Neglected Diseases, has very insignificant global Research and development attention. There is a need to employ *in silico* bioinformatics tools in support of pharmacological screening to expedite the drug development process with optimal success rate. The present *in silico* studies revealed that punaglandin 3 and kalihinol X isolated from the soft coral *Telesto riisei* and Fijian sponge species of *Acanthella* sp., respectively showed promising antileishmanial activity, thereby, saving a substantial amount of time, energy and resources which would have otherwise been wasted, in random pharmacological screening of marine samples with no surety of targeting a right sample for a right screen.

Key words: Bioinformatics, leishmaniasis, *In silico* prediction, marine bioactive molecule, Soft Coral, *Telesto riisei,* sponge, *Acanthella,* pharmacological screening

INTRODUCTION

Parasitic diseases today are a tremendous threat to mankind around the world. Particularly, malaria, leishmaniasis, sleeping sickness and Chagas disease are the three life-threatening neglected diseases affecting an estimated 21.3 million people among the poorest economic sectors (Hotez *et al*., 2007). The most relevant of them is leishmaniasis, caused by *Leishmania* sp., Leishmaniasis is a disease that has been reported from 88 countries around the world, ranging from the tropics to the subtropics and including southern Europe (Gachet *et al*., 2010). The number of humans infected has reached 12 million with an annual incidence of 2 million and an additional 350 million people are estimated to be at risk. There are two main clinical forms of leishmaniasis: (i) visceral (VL) and (ii) cutaneous (CL) (http: //www.who.int/healthinfo/global_burden_disease/projections/en/index.html). Although therapy of this disease has improved in recent years (Berman, 2005), still it represents an important health problem on a global scale. Furthermore, less than 1% of the 1393 drugs marketed between 1975 and 1999 were registered for tropical diseases, e.g., miltefosine against leishmaniasis (Gachet *et al*., 2010). Currently available drugs in the treatment of the parasitic diseases pose many disadvantages due to their strong side-effects, long treatment cycles, poor efficacy, high costs, limited availability and the occurrence of drug resistance (Hotez *et al*., 2007; Gachet *et al*., 2010).

Approximately, 80% of the world population uses traditional medicine, primarily based on natural products (Marcia *et al*., 2005) and natural products continue to play an important role in drug development programs (Strohl, 2000; Butler, 2004; Butler, 2008). Of the 14 anti-parasitic

drugs approved during 1981-2006, seven are natural products or derived from natural products including artemisinin and three of its derivatives (Newman and Cragg, 2007).

Marine biota can be ideal resource for exploring potent chemical entities to be used in drug discovery as it is the marine environment which due to its hostilities towards almost every class of marine organisms, induces the inhabitants to produce/metabolise a variety of molecules with unique structural features to survive in the adverse physical and chemical conditions in the marine environment (Jain *et al*., 2008). In recent years a significant number of novel metabolites with potent anti-parasitic properties have been discovered from marine and terrestrial plants and many more are yet to be discovered. However, only a few marine derived products are in the market. Unlike terrestrial plants marine biota are not commonly reported to have folklore uses/medicinal properties, but application of bioinformatics can reveal medicinal properties of marine bioresources as well as compounds derived from them thereby minimizing the discovery process and expenses very effectively.

In the present study, a total of 148 molecules derived from marine biota were picked up through extensive online searches with special reference to Chemical Abstracts via SciFinder, for present *in silico* studies on their antileishmanial properties, toxicity, druggability status employing bioinformatics tools and techniques, thereby time/money-saving as compared to the random screening of marine samples.

MATERIALS AND METHODS

Ligand identification: A database on small molecules derived from marine biota was created after extensive and exhaustive searches of a number of databases-Bibliographic as well as Chemical databases through SciFinder, with concept that drugable molecules are small molecules having only two or three aromatics rings. The possible reason is that, with a large number of aromatic rings, the hydrophilicity is reduced, as a result of which the water solubility is decreased, leading to poor drug distribution in the body (Kumar *et al*., 2009).

Druglikeness prediction: All the marine compounds in the database created for the present studies are meant for docking studies, but before that, it is worthwhile to screen all the compounds for their druglikeness. As the compounds taken up for further *In silico* studies should be with improved potency, reduced off target activities and physicochemical/metabolic properties suggestive for reasonable *in vivo* pharmacokinetics. MolSoft (http: //www.molsoft.com/mprop/) software was used for optimizing lead by calculating molecular and physicochemical properties relevant to drug design, including log P, molecular Polar Surface Area (PSA) and the rule of 5 descriptors, for find out potential lead compounds. Lipinski's Rule of Five, a rule of thumb important for drug development where in a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity was attempted to optimize the molecule. As, Lipinski's Rule of Five states that, in general, an orally active drug has:

- Not more than 5 hydrogen bond donors (OH and NH groups)
- Not more than 10 hydrogen bond acceptors (notably N and O)
- A molecular weight under 500 g mol^{-1}
- A partition coefficient log P less than 5

Target identification: A detailed literature survey yielded that the glycolytic pathway has to be considered as a potential drug target against the parasitic protozoan species of *Trypanosoma* and *Leishmania* (Nowickia *et al*., 2008). Further analysis was focused on the identification of inhibitors targeted against *Leishmania mexicana* pyruvate kinase (PyK) using bioinformatics techniques. These inhibitors can be the promising candidates for the development towards the design of anti-leishmanial drugs as therapeutics.

PyK catalyses the conversion of phosphoenolpyruvate (PEP) to pyruvate, producing ATP. The determination of the crystal structure of *Leishmania mexicana* PyK (Lm Pyk) (Rigden *et al*., 1999) and comparisons with human PyKs have highlighted significant differences at the effector site that would, therefore, appear to be the promising target (Nowickia *et al*., 2008). The crystal structure of *Leishmania mexicana* PyK (PDB ID: 3E0W) was downloaded from Protein Data Bank (PDB) (http: //www.pdb.org/pdb/home/home.do).

Further, it was also planned to consider one more target, trypanothione reductase in another species of Leishmania that is *Leishmania infantum*. The trypanothione reductase which has essential role in the parasite thiol metabolism and its absence from the mammalian host render it a highly attractive target for structure based drug development against leishmania (Bishal *et al*., 2008). The crystal structure of *Leishmania infantum* Trypanothione reductase (PDB ID: 2JK6) was downloaded from Protein Data Bank (PDB) (http: //www.pdb.org/pdb/home/home.do) for our *In silico* studies.

Cavity site prediction: The cavity site with highest volume and area was predicted using CASTp server (Computed Atlas of Surface Topography of Protein) (http://cast.engr.uic.edu). Binding sites/active sites of proteins and DNAs are often associated with structural pockets and cavities. CASTp server uses the Weighted Delaunay Triangulation and the alpha complex for shape measurements. It provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities, for proteins and other molecules. It measures analytically the area and volume of each pocket and cavity, both in solvent accessible surface (SA, Richards' surface) and molecular surface (MS, Connolly's surface). It also measures the number of mouth openings, area of the openings and circumference of mouth lips, in both SA and MS surfaces for each pocket. The results are shown on the screen or e-mailed back. The e-mailed results include measured parameters for pockets, cavities and mouth openings, as well as listing of wall atoms and mouth atoms for each pocket. In addition, a downloadable PyMOL plugin help us to visualize the pocket of our interest. In CASTp calculation request the modeled protein was submitted and the suitable cavity selected.

Docking: Docking "a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex". Docking was accomplished employing AUTODOCK4 (http://autodock.scripps.edu/) which is a suite of automated docking tools was used to predict the affinity, activity, binding orientation of marine lead compounds to our target protein PyK. It is comprised of two main programs; AutoDock performs the docking of the ligand to a set of grids describing the target protein; Auto Grid pre-calculates these grids. In addition to using them for docking, the atomic affinity grids can also be visualized, for providing a better guidance to organic synthetic chemists in designing better binders. Also, there was a graphical user interface

called AutoDockTools, or ADT which amongst other things helps to set up which bonds will be treated as rotatable in the ligand as well as to analyze dockings (http://autodock.scripps.edu/).

RESULTS AND DISCUSSION

Various studies on diversified environment explored that the marine organisms are the rich source of unique chemical compounds which hold tremendous pharmaceuticals and these natural chemical entities are the potent leads for treatment of many diseases (Jain *et al*., 2008). The studies aimed at *in silico* target based screening of marine bioactive compounds for the prediction of their antileishmanial properties were conducted systematically, the results are discussed below.

Creation of marine compound database: In a Top down approach, to begin with, a comprehensive database of marine derived compounds was created through a detailed literature survey focused on marine biota derived compounds. A total of 148 compounds isolated from various marine biotas like sponges, algae, fungi, bacteria, coelenterates, corals, echinoderms and fish were selected which were not so far explored for antileishmanial activity.

Druggability and druglikeness: The compound in the database were subjected to druggability constraints i.e., Lipinski's rule of five. Out of 148 compounds only 22 compounds could cross the barrier of rule of five and found to be druggable. The druglikeness prediction of these 22 compounds was also done and druglikeness model score and molecular property prediction are shown in Table 1. The larger the value of the druglikeness model score is the higher

Table 1: Druglikeness and molecular property prediction

Property compound	Molecular formula	Molecular weight	Hydrogen bond donor count	Hydrogen bond acceptor count	Drugikeness score
Punaglandin 3	C25H33ClO8	496.97	1	8	1.01
Kalihinol X	C22H33ClN2O2S	425.02762	1	4	0.75
Membranolide C	C22H32O5	376.48648	1	5	-0.16
Avrainvilleol	C15H14Br2O4	418.07726	3	4	0.91
Bolinaquinone	C22H30O4	358.4712	1	4	0.34
Aspermytin A	C19H32O3	308.24	2	3	0.53
Perybysin A	C16H26O3	294.22	2	3	-0.81
Linckoside C	C20H40O5	360.29	3	5	-0.50
Cavernolide	C21H32O2	316.24	0	2	-0.61
Cadlinolide C	C23H36O3	360.27	0	3	0.05
Dihydroxytetrahydrofuran	C14H20N6O	288.17	4	5	1.36
Ageladine a	C12H17Br2N5	388.99	1	3	-0.08
Homofascaplysin a	C21H19N2O2	331.38776	2	2	0.49
ARA-C	C9H13N3O5	242.09	5	6	0.97
Naamine d	C19H21N3O2	323.38894	2	4	-0.21
Axisonitrile-3	C16H25N	31.3764	0	1	-0.15
Ascididemin	C18H9N3O	283.28356	0	4	0.26
Manoalide	C25H36O5	416.55034	2	5	0.56
Lysophosphatidylcholine	C10H24O7P	287.13	2	7	-0.22
Dysibetaine cpa	C8H14NO4	188.09	2	4	-0.92
Ptilocaulis guanidine	C2H6N2	58.05	0	4	-1.17
Puupehenones	C21H28O3	328.44522	1	3	0.41

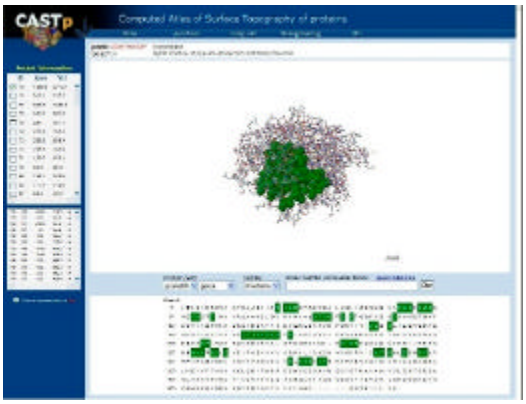

Fig. 1: Cavity site of *Leishmania mexicana* pyruvate kinase (PyK)

will be the probability that the particular molecule will be active (http: //www.molinspiration.com/docu/miscreen/druglikeness.html). In this study punnaglandin 3, kalihinol X, avrainvilleol, bolinaquinone, aspermytin A, cadlinolide C, dihydroxytetrahydrofuran, homofascaplysin a, ARA-C, ascididemin, manoalide and puupehenones are showing positive druglikeness model score so they tend to be more active compounds.

Selection/Identification of target: Pyruvate kinase that we have chosen as drug target is a homotetrameric enzyme that has essential role in the ATP supply of leishmanial protozoan as it catalyze the final reaction of glycolysis in which phosphoenolpyruvate and ADP are converted into pyruvate and ATP (Morgan *et al*., 2010). The determination of the crystal structure of Leishmania mexicana PyK (Lm Pyk) (Rigden *et al*., 1999) and the comparison of structure of human PyKs have highlighted significant differences in the effector site and allosteric control as Lm Pyk are unique in responding to F2,6BP but all mammalian allosterically regulated pyruvate kinases are controlled by F1,6BP (Morgan *et al*., 2010).

For *In silico* target based screening of 22 short listed compounds, we used *Leishmania mexicana* pyruvate kinase (PyK) as our drug target. The protein cavity was identified by using CASTp (Computed Atlas of Surface Topography of Protein). The protein cavity having largest area 1209.9 and volume 2712.7 was chosen (Fig. 1). The residues present in the cavity are summarized in the supplementary Appendix Table 1.

Further to explore the efficacy of marine compounds for their antileishmanial activity we expanded our target domain in another lesihmanial species. We considered Trypanothione Reductase of *L. infantum* as another target. It is the trypanothione system which is necessary for protozoan survival, wherein the dithiol trypanothione is required for the synthesis of DNA precursors, the homeostasis of ascorbate, the detoxification of hydroperoxides and the sequestration/export of thiol conjugates (Bishal *et al*., 2008). Moreover, the majority of peroxidases that eliminate the Reactive Oxygen Species (ROS) generated in the aerobic metabolism are trypanothione dependent (Muller *et al*., 2003). In addition, the absence of this pathway from the mammalian host and the sensitivity of trypanosomatids to oxidative stress make it an attractive target for structure-based drug design. The binding cavity in Trypanothione Reductase was identified by using CASTp (Computed Atlas of Surface Topography of Protein). The protein cavity having largest area 3760.5 and volume 7768.2 was selected (Fig. 2). The residues present in the

Table 2: Docking results of marine compounds against *Leishmania mexicana* pyruvate kinase (PyK) protein target

Inhibitors	Docked energy (kcal mol^{-1})
Punaglandin 3	-14.45
Kalihinol x	-13.02
Membranolide C	-11.78
Avrainvilleol	-11.63
Bolinaquinone	-11.43
Aspermytin A	-11.21
Perybysin A	-10.71
Linckoside C	-10.38
Cavernolide	-10.37
Cadlinolide C	-9.94
Dihydroxytetrahydrofuran	-9.86
Ageladine a	-9.47
Homofascaplysin a	-9.30
ARA-C	-9.16
Naamine d	-8.83
Axisonitrile-3	-8.70
Ascididemin	-8.55
Manoalide	-8.37
Lysophosphatidylcholine	-7.39
Dysibetaine cpa	-5.94
Ptilocaulis guanidine	-4.36
Puupehenones	-4.29

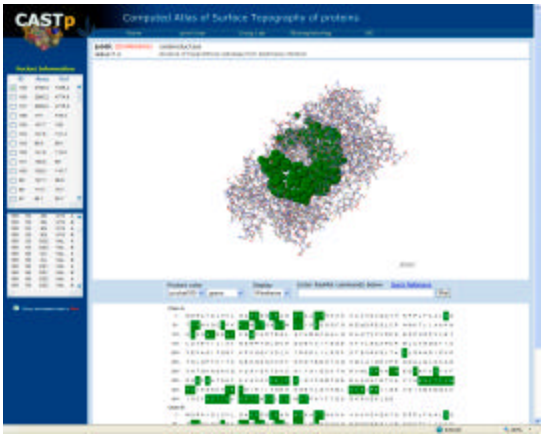

Fig. 2: Cavity site of *Leishmania infantum* trypanothione reductase

cavity are summarized in the Appendix Table 2. The above two targets can play very significant role in antileishmanial drug designing/development (Fairlamb, 1989).

Docking: Twenty two compounds were further considered for docking studies to find out the receptor binding affinity for their antileishmanial activity. We docked these compounds in the binding cavity of the protein. The docking results of all 22 compounds are depicted in Table 2.

As control/standard, we also did docking of *Renieramycin* A. (Nakao *et al*., 2004) against *Leishmania mexicana* Pyruvate Kinase (PyK) that have already been reported for its

Table 3: Comparision of Docking results of compounds against Pyruvate kinase and trypanothione reductase targets

Inhibitors	Docked Energy (kcal mol^{-1}) *Leishmania mexicana* pyruvate kinase (PyK)	Docked Energy (kcal mol^{-1}) *Leishmania infantum* trypanothione reductase (TR)
Marine compounds not reported for antileishmanial property		
Punaglandin 3	-14.45	-16.56
Kalihinol x	-13.02	-12.60
Marine compounds known for antileishmanial property (control)		
Renieramycin	-12.66	-11.25

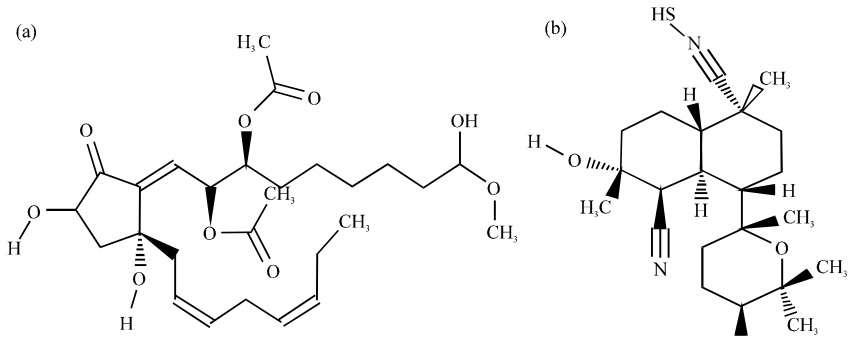

Fig. 3(a-b): The structure of (a) Punaglandin 3 and (b) Kalihinol X

antileishmanial activity to find out its affinity to bind the target for comparative purposes and it was found that *Renieramycin* A. shows docking scores-12.66 which is greater than the docking scores of Punaglandin 3 and Kalihinol X,-14.45 and 13.02, respectively. In another studies on the target trypanothion reductase from *L. infantum* the two compounds, Punaglandin 3 and Kalihinol X showed-16.56, 12.60 docking score, respectively while Renieramycin A. (Nakao *et al.*, 2004) showed-11.25 docking score. Table 3 depicts the comparison of docking results of compounds against Pyruvate kinase and trypanothione reductase targets, indicating that the two compounds are better placed against PyK and TR than the control compound-Renieramycin A.

These results shows that the two compounds Punaglandin 3 and Kalihinol X (Fig. 3) show potent antileishmanial property against both the species of *L. mexicana* and *L. infantum* causing Cutaneous leishmaniasis and Visceral leishmaniasis, respectively, in comparison to already known antileihmanial compound Renieramycin (Nakao *et al.*, 2004), Punaglandin 3 and Kalihinol X are more effective against *L. mexicana* than *L. infantum*.

Based on our present study, Punaglandin 3 is found to be the most potential compound that can be used as drug against leishmania as the docking score is -14.45 and 16.56 in *L. maxicana* and *L. infantum*, respectively which shows possible interaction between the ligand and receptor. *Telesto riisei* and *Acanthella* sp., marine bioresources containing Punaglandin 3 and Kalihinol X, respectively, so far have not been reported for this new activity or medicinal property. The docking results are shown in Fig. 4-7.

It was reported earlier that punaglandins which are highly functional cyclopentadienone and cyclopentenone prostaglandins chlorinated at the endocyclic alpha-carbon position, isolated from the soft coral *Telesto riisei* show inhibition of ubiquitin isopeptidase activity and antineoplastic

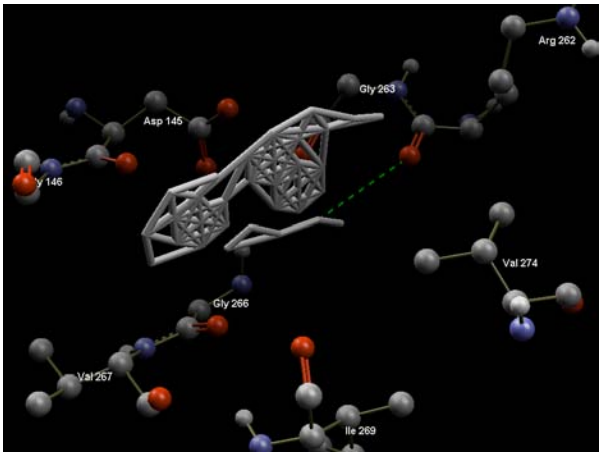

Fig. 4: Docking result showing interaction of Kalihinol X with *Leishmania mexicana* pyruvate kinase (PyK) target

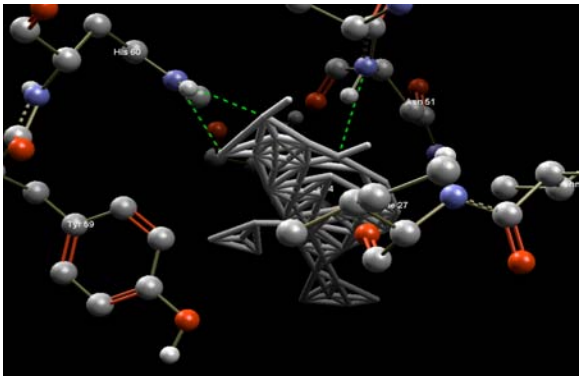

Fig. 5: Docking result showing interaction Punaglandin 3 with *Leishmania mexicana* pyruvate kinase (PyK) target

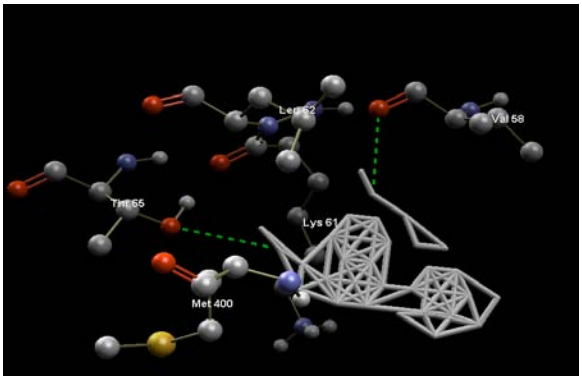

Fig. 6: Docking result showing interaction of Kalihinol X with *Leishmania infantum* trypanothione reductase target

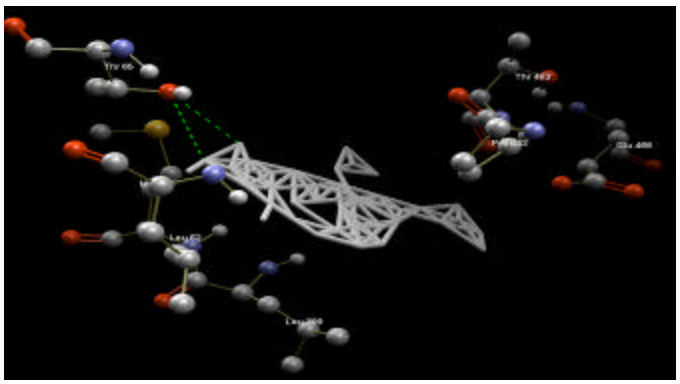

Fig. 7: Docking result showing interaction of Punaglandin 3 with Leishmania infantum trypanothione reductase target

effects (Verbitski *et al*., 2004). Similarly, Kalihinol X, a diterpene isolated from the Hainan sponge *Acanthella* sp. belonging to family Axinellidae , inhibits bacterial folate biosynthesis (Chang *et al*., 1987; Paul *et al*., 2007).

CONCLUSION

Thus, the conclusion appears in escapable that punaglandin 3 and kalihinol X compounds show promising antileishmanial activity and are the probable drug on which further studies aimed at isolation, purification, *In vivo* confirmation and designing of chemical libraries of these two chemical entities from marine biota could be taken up.

However, out of the two i.e., punaglandin 3 and kalihinol X, the former is showing better efficiency in two different species of Leishmania on two different targets (PyK, TR) than the later, therefore the compound (punaglandin 3) could be recommended as potential lead for drug designing and development against leishmaniasis. In this way, *in silico* target based screening studies could help us to arrive at potent bioactive molecules with confirmed medicinal properties from marine biota which is not investigated /reported earlier for antileishmanial properties, in a comparatively shorter time and lesser cost. Thus, it can also be predicted that soft coral T*elesto riisei* shows *in silico* antileishmanial properties in addition to earlier reported inhibition of ubiquitin isopeptidase activity and antineoplastic effects.

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APPENDIX

Table A1: Selected cavity site of *Leishmania mexicana* pyruvate kinase (PyK)

Residue number	Residue	Chain	Residue number	Residue	Chain
26	THR	A	240	ILE	A
27	ILE	A	241	GLU	A
28	ILE	A	259	ASN	A
29	GLY	A	260	MET	A
49	PRO	A	260	VAL	A
50	ARG	A	261	VAL	A
51	MET	A	261	ALA	A
53	ASN	A	263	ALA	A
54	SER	A	263	GLY	A
55	HIS	A	264	GLY	A
60	TYR	A	264	ASP	A
63	HIS	A	266	ASP	A
84	ASP	A	266	GLY	A
85	THR	A	295	GLY	A
86	LYS	A	295	ALA	A
90	GLU	A	296	ALA	A
98	ARG	A	296	THR	A
144	ASP	A	297	THR	A
145	ASP	A	297	GLN	A
147	ASP	A	300	GLN	A
169	ILE	A	300	GLU	A
170	HIS	A	303	GLU	A
171	THR	A	303	THR	A
172	ILE	A	304	THR	A
173	SER	A	304	TYR	A
173	ASP	A	328	TYR	A
174	ASP	A	330	MET	A
175	ARG	A	330	SER	A
176	ARG	A	331	SER	A
178	GLY	A	331	GLY	A
211	ASN	A	332	GLY	A
212	SER	A	334	GLU	A
238	PHE	A	335	ALA	A
239	LYS	A			

Table 2A: Selected cavity site of *Leishmania infantum* Trypanothione Reductase (TR)

Residue number	Residue	Chain	Residue number	Residue	Chain
13	GLY	B	58	VAL	B
13	GLY	A	58	VAL	A
14	SER	B	58	VAL	B
14	SER	A	58	VAL	A
14	SER	B	58	VAL	B
14	SER	A	58	VAL	A
14	SER	B	58	VAL	B
14	SER	A	61	LYS	B
14	SER	B	61	LYS	A

Table 2A: Continued

Residue number	Residue	Chain	Residue number	Residue	Chain
14	SER	A	61	LYS	B
17	LEU	A	61	LYS	A
17	LEU	B	61	LYS	B
17	LEU	A	61	LYS	A
17	LEU	B	61	LYS	B
17	LEU	A	61	LYS	A
17	LEU	B	61	LYS	B
17	LEU	A	61	LYS	A
17	LEU	B	61	LYS	B
18	GLU	A	61	LYS	A
18	GLU	B	62	LEU	B
18	GLU	A	62	LEU	A
18	GLU	B	62	LEU	B
18	GLU	A	62	LEU	A
18	GLU	B	62	LEU	B
18	GLU	A	62	LEU	A
18	GLU	B	62	LEU	B
18	GLU	A	62	LEU	A
18	GLU	B	64	VAL	B
18	GLU	A	64	VAL	A
18	GLU	B	65	THR	B
21	TRP	A	65	THR	A
21	TRP	B	65	THR	B
21	TRP	A	65	THR	A
21	TRP	B	65	THR	B
21	TRP	A	65	THR	A
21	TRP	B	65	THR	B
21	TRP	A	65	THR	A
21	TRP	B	68	GLN	B
21	TRP	A	68	GLN	A
21	TRP	B	68	GLN	B
21	TRP	A	68	GLN	A
21	TRP	B	68	GLN	B
21	TRP	A	68	GLN	A
21	TRP	B	68	GLN	A
21	TRP	A	68	GLN	B
21	TRP	B	68	GLN	A
21	TRP	A	69	TYR	B
21	TRP	B	69	TYR	A
21	TRP	A	69	TYR	B
21	TRP	B	69	TYR	A
22	ASN	A	69	TYR	B
22	ASN	A	69	TYR	A
22	ASN	B	69	TYR	B
25	VAL	A	71	ASP	A
25	VAL	B	72	LEU	B
26	THR	A	72	LEU	A
26	THR	B	72	LEU	B

Table 2A: Continued

Residue number	Residue	Chain	Residue number	Residue	Chain
26	THR	B	72	LEU	A
49	GLY	A	72	LEU	B
49	GLY	B	72	LEU	A
52	CYS	A	75	GLU	B
52	CYS	B	75	GLU	A
52	CYS	A	75	GLU	B
52	CYS	B	75	GLU	A
53	VAL	A	102	VAL	B
53	VAL	B	102	VAL	A
58	VAL	A	102	VAL	B
102	VAL	A	347	GLU	A
102	VAL	B	347	GLU	B
105	SER	B	353	LYS	A
105	SER	A	355	ARG	B
105	SER	B	355	ARG	B
105	SER	A	355	ARG	B
106	ILE	B	355	ARG	B
106	ILE	A	355	ARG	A
106	ILE	B	355	ARG	B
106	ILE	A	358	ASP	B
106	ILE	B	358	ASP	B
106	ILE	A	367	PHE	A
106	ILE	B	367	PHE	B
106	ILE	A	367	PHE	A
109	SER	B	367	PHE	B
109	SER	A	367	PHE	A
109	SER	B	367	PHE	B
109	SER	A	368	SER	A
109	SER	B	368	SER	B
109	SER	A	369	ILE	A
109	SER	B	369	ILE	B
109	SER	A	370	PRO	A
110	TYR	B	370	PRO	B
110	TYR	A	370	PRO	A
110	TYR	B	370	PRO	B
110	TYR	A	371	PRO	A
110	TYR	B	371	PRO	B
110	TYR	A	371	PRO	A
110	TYR	B	371	PRO	B
110	TYR	A	390	ALA	B
110	TYR	B	392	TYR	B
110	TYR	A	392	TYR	B
113	MET	B	394	SER	A
113	MET	A	394	SER	B
113	MET	B	395	SER	A
113	MET	A	395	SER	B
113	MET	B	396	PHE	A
113	MET	A	396	PHE	B

Table 2A: Continued

Residue number	Residue	Chain	Residue number	Residue	Chain
113	MET	B	396	PHE	A
113	MET	A	396	PHE	A
241	GLN	A	396	PHE	B
241	GLN	B	396	PHE	A
335	THR	A	396	PHE	B
335	THR	B	396	PHE	A
336	PRO	A	396	PHE	B
336	PRO	B	396	PHE	A
336	PRO	A	396	PHE	B
336	PRO	B	397	THR	A
336	PRO	A	397	THR	B
336	PRO	B	398	PRO	A
336	PRO	A	398	PRO	B
336	PRO	B	398	PRO	A
339	ILE	A	398	PRO	B
339	ILE	B	398	PRO	A
339	ILE	A	398	PRO	B
339	ILE	B	398	PRO	A
339	ILE	A	398	PRO	B
339	ILE	B	399	LEU	A
340	ASN	A	399	LEU	B
340	ASN	B	399	LEU	A
340	ASN	A	399	LEU	B
340	ASN	B	399	LEU	A
340	ASN	B	399	LEU	B
340	ASN	B	399	LEU	A
340	ASN	A	399	LEU	B
340	ASN	B	399	LEU	A
343	ALA	A	399	LEU	B
343	ALA	B	400	MET	A
347	GLU	A	400	MET	B
347	GLU	B	400	MET	A
400	MET	B	433	SER	B
400	MET	A	435	PRO	A
400	MET	B	435	PRO	B
400	MET	A	435	PRO	A
400	MET	B	435	PRO	B
400	MET	A	435	PRO	A
400	MET	B	435	PRO	B
401	HIS	A	436	GLU	B
401	HIS	B	436	GLU	A
401	HIS	A	436	GLU	B
401	HIS	B	436	GLU	A
402	ASN	A	436	GLU	B
402	ASN	B	446	LYS	B
409	LYS	A	451	ILE	B
409	LYS	B	451	ILE	B
409	LYS	A	452	SER	B

Table 2A: Continued

Residue number	Residue	Chain	Residue number	Residue	Chain
409	LYS	B	452	SER	B
409	LYS	A	455	HIS	B
409	LYS	B	455	HIS	B
409	LYS	A	455	HIS	A
409	LYS	B	455	HIS	B
409	LYS	A	455	HIS	B
409	LYS	B	455	HIS	A
409	LYS	A	455	HIS	B
409	LYS	B	456	SER	A
409	LYS	A	456	SER	B
409	LYS	B	456	SER	A
409	LYS	A	456	SER	B
409	LYS	B	456	SER	A
410	GLU	A	456	SER	A
410	GLU	B	456	SER	B
410	GLU	A	457	THR	A
410	GLU	B	457	THR	B
411	PHE	A	457	THR	A
411	PHE	B	457	THR	B
411	PHE	A	458	ILE	A
411	PHE	B	458	ILE	B
411	PHE	A	458	ILE	A
411	PHE	B	458	ILE	A
411	PHE	A	458	ILE	B
411	PHE	B	458	ILE	A
417	THR	B	458	ILE	B
419	GLU	B	459	GLY	A
419	GLU	B	459	GLY	B
419	GLU	B	459	GLY	A
419	GLU	B	459	GLY	B
422	GLY	B	461	HIS	A
431	GLY	A	461	HIS	B
431	GLY	B	461	HIS	A
432	ASP	A	461	HIS	B
432	ASP	B	461	HIS	A
432	ASP	A	461	HIS	B
432	ASP	B	461	HIS	A
432	ASP	A	461	HIS	B
432	ASP	B	461	HIS	A
432	ASP	A	461	HIS	B
432	ASP	B	462	PRO	A
432	ASP	A	462	PRO	B
432	ASP	B	462	PRO	A
432	ASP	A	462	PRO	B
432	ASP	B	462	PRO	A
432	ASP	A	462	PRO	B
432	ASP	B	463	THR	A
433	SER	A	463	THR	B

Table 2A: Continued

Residue number	Residue	Chain	Residue number	Residue	Chain
433	SER	B	463	THR	A
433	SER	A	463	THR	B
433	SER	B	463	THR	A
433	SER	A	463	THR	B
433	SER	B	463	THR	A
433	SER	A	463	THR	B
463	THR	A	467	GLU	A
463	THR	B	467	GLU	B
464	SER	A	469	CYS	A
464	SER	B	469	CYS	B
464	SER	A	469	CYS	A
464	SER	B	469	CYS	B
464	SER	A	470	SER	B
464	SER	B	470	SER	A
464	SER	A	470	SER	B
464	SER	B	470	SER	A
466	GLU	A	470	SER	B
466	GLU	B	470	SER	A
466	GLU	A	470	SER	B
466	GLU	B	470	SER	A
466	GLU	A	470	SER	B
466	GLU	B	472	ARG	B
466	GLU	A	472	ARG	B
466	GLU	B	472	ARG	A
466	GLU	A	472	ARG	B
466	GLU	B	472	ARG	B
466	GLU	A	472	ARG	A
466	GLU	B	472	ARG	B
467	GLU	A	472	ARG	A
467	GLU	B	472	ARG	B
467	GLU	A	472	ARG	A
467	GLU	B	472	ARG	B
467	GLU	A	472	ARG	A
467	GLU	B	472	ARG	B
467	GLU	A	472	ARG	B
467	GLU	B	473	THR	A
467	GLU	A	473	THR	B
467	GLU	B	474	PRO	B

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