

## *Supplementary Material*

# **Antiprotozoal Activity of Thymoquinone (2-Isopropyl-5-methyl-1,4-benzoquinone) for the Treatment of *Leishmania major*-Induced Leishmaniasis: In Silico and In Vitro Studies**

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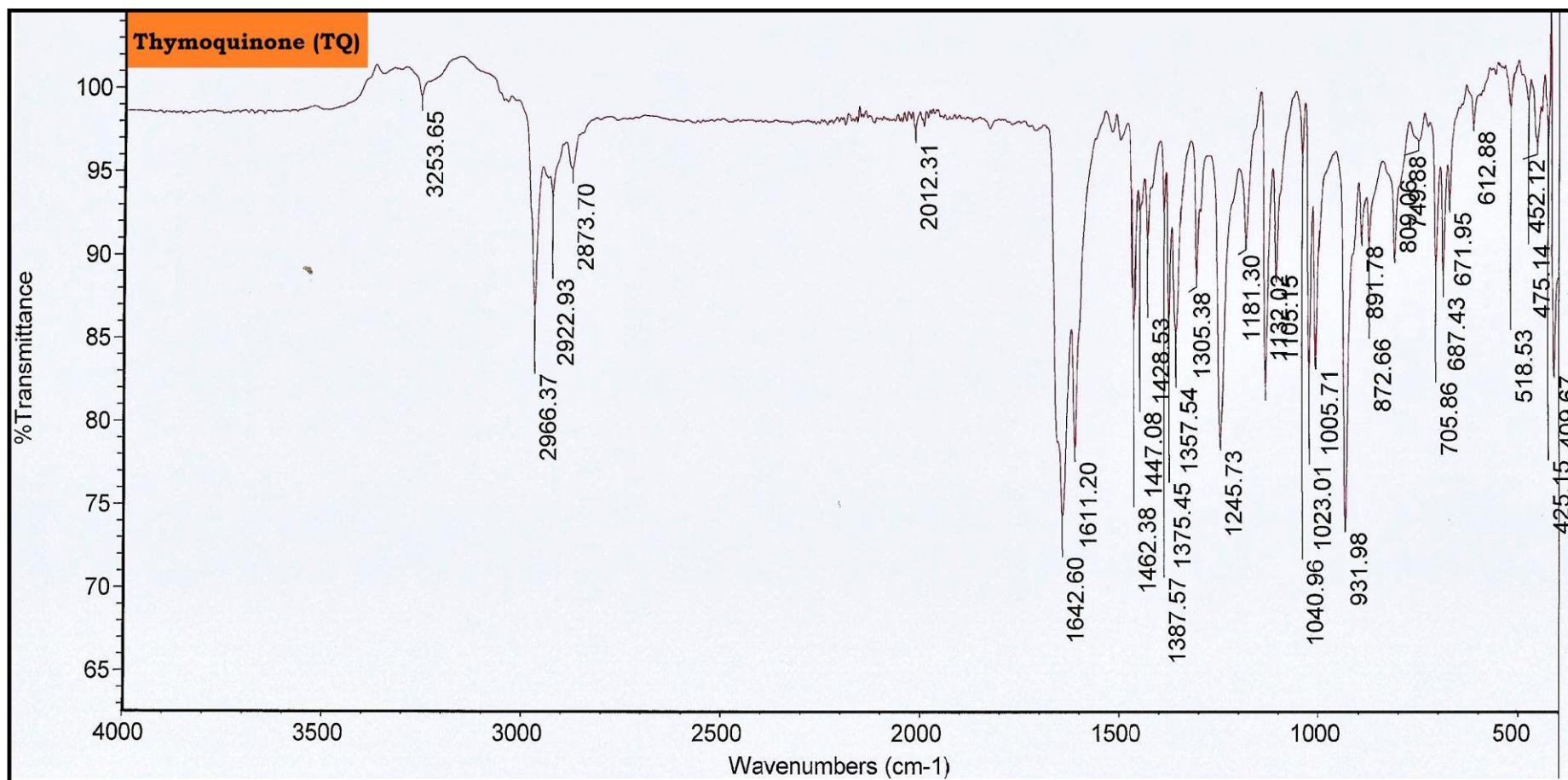
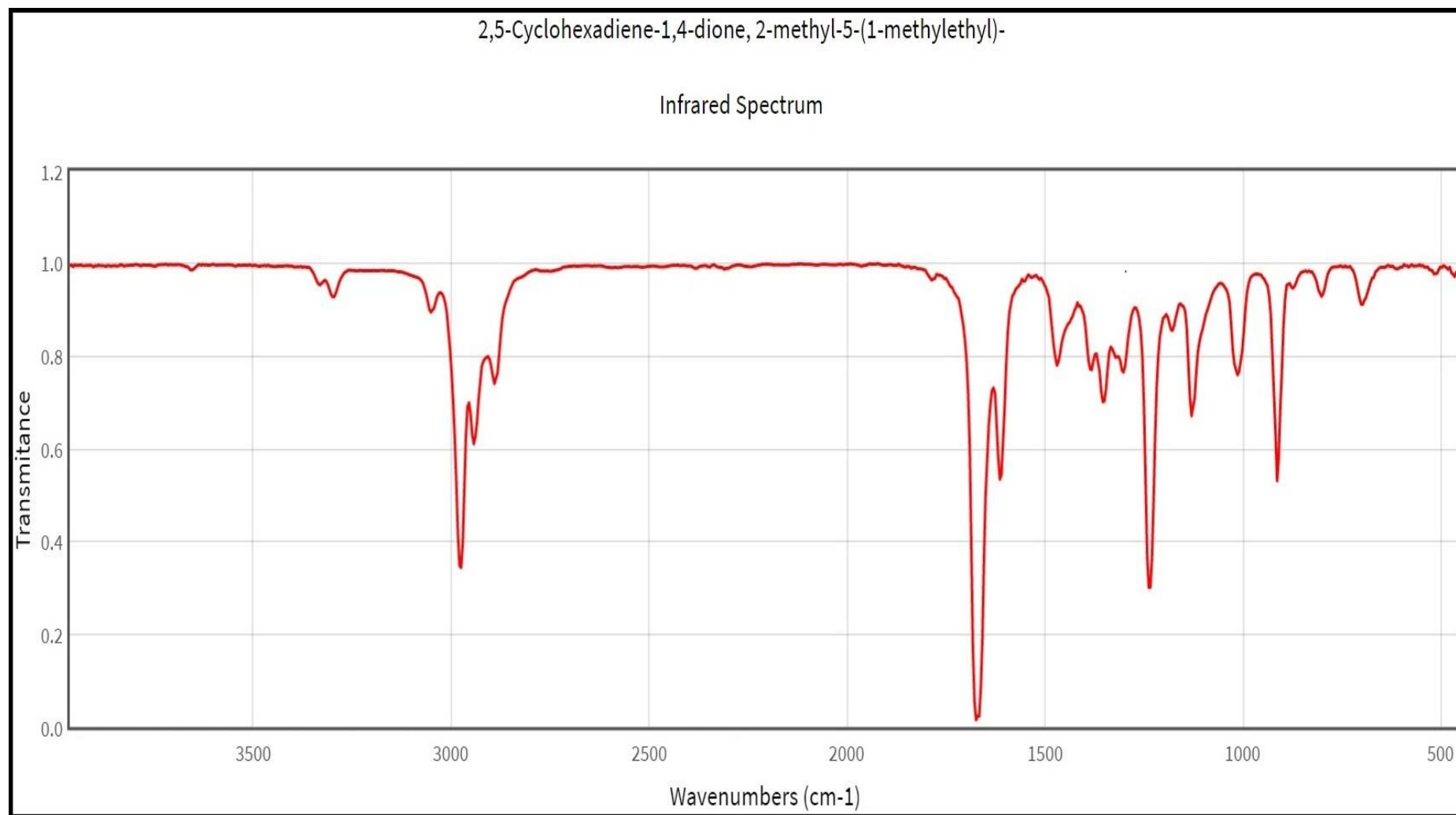
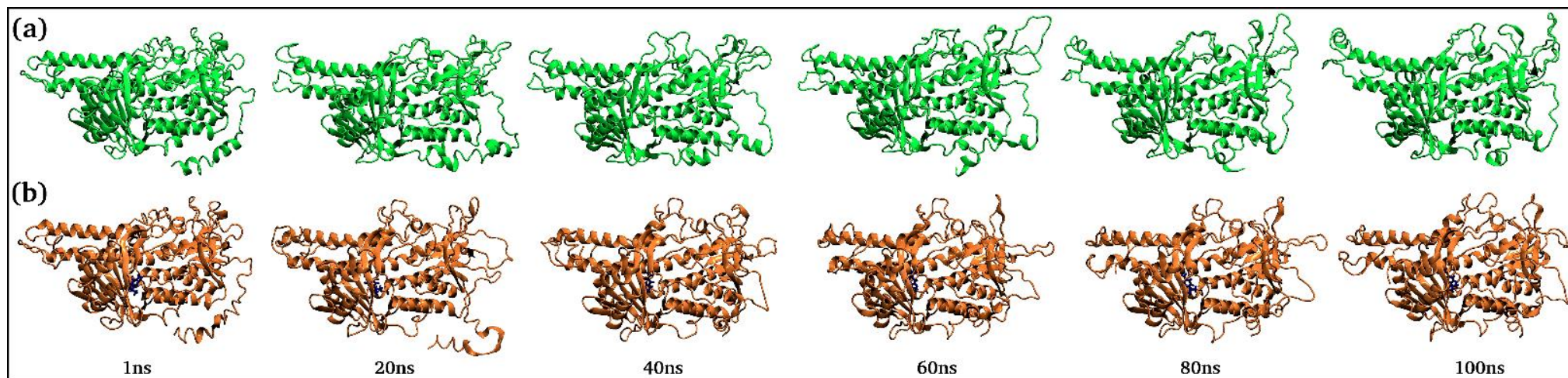


Figure S1. FT-IR spectra of thymoquinone (TQ).



**Figure S2.** FT-IR spectra of standard TQ. (Source : <https://webbook.nist.gov/cgi/cbook.cgi?ID=C490915&Mask=80> )



**Figure S3.** Snapshots of TQ-free (green) and bound states (orange) squalene monooxygenase protein at six different timescales, i.e., 1 ns, 20 ns, 40ns, 60 ns, 80 ns and 100 ns, during MD simulation study, indicating various conformational changes in the protein. As indicated, TQ remains in bound form throughout the simulation.

**Table S1.** The selected proteins for molecular docking studies with TQ ligand targeting multiple pathways in *Leishmania* spp. The table also lists the name of enzymes, their FASTA sequences, amino acid lengths, their PDB codes (if existed) or models (generated from homology modeling approach or threading approach), and the corresponding pathways where the protein is involved.

#	Enzyme	Organism	FASTA	Amino acid	PDB/Model	Pathway
1.	Trypanothione reductase	<i>L. infantum</i>	MGSSHHHHHHSSGLVPRGSHMS RAYDLVVLGAGSGGLEAGWNA AVTHKKKVAVVDVQATHGPPLF AALGGTCVNVGCVPKKLMVTGA QYMDLIRESGGFGWEMDRESLCP NWKTLIAAKNKVVNSINESYKSM FADTEGLSFHMGFGALQDAHTV VVRKSEDPHSDVLETLDTEYILIAT GSWPTRLGVPGDEFCSNEAFYL EDAPKRMLCVGGGYIAVEFAGIF NGYKPCGGYVDLCYRGDLILRGF DTEVRKSLTKQLGANGIRVRTNL NPTKITKNEDGSNHVHFNDGTEE DYDQVMLAIGRVPRSQUALQLDK AGVRTGKNQAVQVDAYSKTSVD NIYAIGDVTNRVMLTPVAINEGA AFVETVFGGKPRATDHTKVACA VFSIPPIGTCGMTEEEAAKNYETV AVYASSFTPLMHNISGSKHKEFMI RIITNESNGEVLGVHMLGDSAPEI IQSVGICMKMGAKISDFHSTIGVH PTSAEELCSMRTPAYFYESGKRVE KLSSNL	511	2JK6	Trypanothione pathway

2.	Mannosyltransferase (GPI-14)	<i>L. braziliensis</i>	MRNKTWLDRQSIGTLLLYGGLAR VLLIYAAFHDYYFRVKYTDIDY MIVVDGARELLHGGTPFDRTTYR YTPLLAVLVIPAVLIANPLGKVVF ALSDLGAAYYCFHMLLRFSTERS AKWMVVILILFNPVVLNVSTRGN SDMLISCMSMGVLAKFAEGRYFT AASILGFAVHFKIYPIIYALPLVLG VWERAKQERFFGRLAHTASVVV GCGLCFTVAFAVPTYVCYLVYGQ QYLDEGFIYHIIHREDHRHNFSPY WLLMYLNMGRRDLGVGVDYSA GLFAFLPQFAVLCYASWKLKNI AHACCVETILFVAFNKVCTVQYF VWFLPFLAFVFCEPAQPKWLTGV TNAPKSERPSMLSAVAAILMWSL MIPLWVWTAYGLEFEGRNHYGR LWIVSCVFYLATVSLAAWLGRLC YRSRITVSIKDHRPASKRA	432	I-Tasser (Various templates were used including: 5ezm.pdb, 5f15.pdb, 6snh.pdb)	Glycosylphosphatidylinosi tol-anchor biosynthesis
3.	Trypanothione synthetase- amidase	<i>L. major</i>	MSSLQRASVSFNKPGHIPFGAVQ GYAPGGVPAYSNNKHDHYFSGER NIEDNIFFGFKYQCVEFARRWLLV RKGLLLPDVNWACHIFQLKEVR DAATTESFAVLQVRNGTTTKPEA DALLVYPSTDANPVGHVGTITEV GDDYVCVADQNYRFHKWESSCA YKLKLDHRDGIWTIIDDIDADEIEI PLGWLTFFGRANRPEGAPPVALH	652	2VOB	Trypanothione pathway

			PSLHFKEPPKPYLLRRNFLPTESK ANWLDMNNPAERLFVEEFGMD VSRTRLEEKVVSYYESNHEFHLRC VAYGTQLHAIFMEATAQVIESDE KLRLFAIPEEFWPRIRHSWKYQQT YISGRFDFAFNNETGEVKCFEYN ADSASTLLECGLIQKWAESVGL DKQDTRGSGFAVERNLMAWA NSGATGRVHFCVDEEREEQYTAL YCMQAAEAVGLEGKLCILFDEFR FDDNGHVVDSDGVRVRNVWKT WMWESAITDYAAAREERGENWK PSPKDKVRLCDLLLGDDWEILYFE PMWKVIPSNKAILPMIYHNHPEH PAILKAEYELTDELRKHGYAKKPI VGRVGSNVIITSGDGVVHAESGG KYGKRNMIIYQQLFELKKQDDYY AIIGGWMIGDAFSGTGIREDKSVI TGVDSPFAAVRIKTDKLPHVTLK DIDKMAEDE			
4.	Tryparedoxin peroxidase	<i>L. major</i>	MSCGNAKINSPAPSFEVALMPN GSFKKISLSSYKGKWVVLFFYPLD FTFVCPTEVIAFSDSVSRFNELNCE VLACSIDSEY AHLQWTLQDRKKG GLGTMAIPMLADKTKSIARSYGV LEESQGVAYRGLFIIDPHGMLRQI TVNDMPVGRSVEEVLRLLEAFQF	199	<b>4K1F</b>	Trypanothione pathway

			VEKHGEVCPANWKKGAPTMKPE PNASVEGYFSKQ			
5.	<u>Squalene synthase</u>	<i>L. major</i>	MGFFSDSVAMMRVKWQMRSVKI QVPPEETDLRFCYDIMNDVSRFA VVVAQLADQQLRDAICIFYLVLR ALDTLEDDMSVPVDVKLKELPKF HTHTSDMSWCMMSGVGEGREREL LAKYPCVSREFKKLKKEYQDVIA NICERMANGMCEFLKRPVVTGD DYNQYCHYVAGLVGHGLTQLFA RCGFEDPSLDDDLTSSNHMGLFL QKTNIIRDYEDIREPPRMFWPK EIWGTYYTELKELKSESNNAAAV QCLNAMVADALVHVPYIVDYL ALRDPVFRFCAIPQVMAIATLKE VYNNPDTFQVKVKSREPESCRIM LKATTLYSSLSMFRDYCVLQEK DMQDASSVSIANSIAAIAERIDLQ LKKCQDVSYTRSLARYPGLGGQ FLLTVMDTVAGFFGGRKEIAGHA	414	Swiss Model ( <b>Template used:</b> 3wca.pdb <b>Query Coverage:</b> 82% <b>Sequence Identity:</b> 61.83%)	Sterol biosynthetic pathway
6.	Squalene monooxygenase	<i>L. major</i>	MLYFFAAVLCAVSTLLLLNRTLRS LRLSPARTSYDYDVIIVGGSIAGPV LAKALSDQGRKVLMMVERTLFTKP DRIVGELLQPGGLNALKEVGMKE CAETIGMPCHGYVVVDHKGKQV DLPYRKGASGVSFHFGDFVQSLRS HVFHNCKANVTMVEGTVNNILT EGLSFSERAYGVEYTIAEKYEVPT	569	I-Tasser (Various templates were used including: 6c6n.pdb, 6fho.pdb, 6c6r.pdb)	Sterol biosynthetic pathway



			KPFREDPPKTNPVAATVRKVATA PLVVMCDGGMSKWKSRYQHYTP AYEYHSHFIGLVLKTVRLPKEQRG TVFFGKTGPILSYRLDDNELRLLV DYNKPTLPSLEKQSEWLIQDVAP CLPENMREQFVRVSKDTKSLRSM PMARYPPAFPSIKGYVGIGDHAN QRHPLTGGGMTCCFRDAIRLANS LNGIQSLRSVNQEEMAAIEDKIQ AAILNYARYRYTHSCCINLLSWA LYSVFSSPALRDACFDYFLCGGNC VTGPMDLLAGLDPNVGSLFFHYC CVMLHGVANVMMRTGAYSESG KQLSNLEKLTNVASFFVDWERM KHAAYLLGKSTQIALPLAKSEFYS MWRFVDPTSPLANISKRIKTMVY TKQFNGKQRKPVGL			
7.	Farnesyl pyrophosphate synthase	<i>L. major</i>	MAHMERFQKVYEEVQEFLLGDA EKRFEMDVHRKGYLKSMMDTTC LGGKYNRGLCVVDVAEAMAKD TQMDAAAMERVLHDACVCGW MIEMPLQAHFLVEDDIMDHSKTRR GKPCWYLHPGVTAQVAINDGLIL LAWATQMALHYFADRPFLAEVL RVFHDVDLTTTIGQLYDVTSMD SAKLDKVAHANTTDYVEYTPF NHRRIVVYKTAYYTYWLPLVMG LLVSGTLEKVDKKATHKVAMVM	362	4JZX	Sterol biosynthetic pathway

			GEYFQVQDDVMDCTPPEKLGKI GTDIEDAKCSWLAVTFLTTAPAE KVAEFKANYGSTDPAAVAVIKQL YTEQNLLARFEEYEKAVVAEVEQ LIAALEAQNAFAASVKVLWSKT YKRQK			
8.	Glyceraldehyde-3-phosphate dehydrogenase	<i>L. major</i>	MAPIKVGINGFGRIGRMVLQAIC DQGLIGNEIDVVAVVDMSTNAEY FAYQMKYDTVHGRPKYTVEAVK STPSVKTPDVLVVNGHRIKCVKA QRNPADLPWGKLGVDYVIESTGL FTDKLQAEGLHGGAKKVVISAP ASGGAKTIVMGVNQHEYSPTSH HVVSNASCTTNCLAPIVHVLTK NFGIETGLMTTIHSYTATQKTVDG VSLKDWRGGRAAAINIIPSTTGA AKAVGMVIPSTKGKLTGMSFRVP TPDVSVDLTFRSTRETSIQEIDKA IKKAAQTYMKDILGFTNDELVSS DFINDNRSSVYDSKATLQNNLPG EKRFFKIVSWYDNEWGYSHRVVD LVRYMAATDAASAKM	361	Swiss Model ( <b>Template used:</b> 1gyq.pdb <b>Query Coverage:</b> 99% <b>Sequence Identity:</b> 94.69%)	Glycolytic pathway
9.	Triosephosphate isomerase	<i>L. major</i>	MSAKPQPIAAANWKCNGTTASIE KLVQVLNEHNISHDVQCVPVPTF VHIPLVQAKLRNPKYVVSANAI AKSGAFTGEVSMPILKDLGINWVI LGHSEERTYYGETDETVAQKVAD ACKQGFMVIACIGETLQQREANQ	251	Swiss Model ( <b>Template used:</b> 5cg7.pdb	Glycolytic pathway

			TAKVVLSTSAIATKLPKEAWDQ IVLAYEPVWAIGTGKVATPEQAQ EVHALLRKWVSEKIGTDVAAKLR ILYGGSVSAGNAKTLYMKPDING FLVGGASLKPEFRDIIDATR		<b>Query</b> <b>Coverage:</b> 100% <b>Sequence</b> <b>Identity:</b> 91.63%)	
10.	Phosphoglycerate kinase	<i>L. major</i>	MSLVLKKSIDDATVRDKKVLIRV DFNVPVKNGKITNDFRIRSALPTI QKVLKEGGSCILMSHLGRPKGAR MSDPSPEKGVRYEAAATLRPVA ARIAELLGQKVEFAPDCLDAAAY ASKLKNQDVLLLENVRFYAEEGS KKEEERDAMAKVLASYADLYVS DAFGTAHRDSATMTGIPKVLGA GYAGYLMEKEINYFSRVLNNPPR PLVAIVGGAKVSDKIELLDNMLS RINYLVIGGAMAYTFQKAQGRKI GISMCEEDKLDLAKSLLKKAQER GVQVFLPVDHVCNKEFKAADSP LVTESVDVPDGYMALDIGPRTIH MYEEVIGRCKSAIWNGPMGVFE MPCYSKGTFAVAKAMGTGTQKN GLLSIIGGDSASAAELSGEAKN MSHVSTGGGASLELLEGTLPGV AILTDKDVKERGASCRFAFGVGS PSREACPLRCGHIFGGASIVREIVK LVVALLIGIFIGRRMSTKLIR	479	Swiss Model ( <b>Template</b> <b>used:</b> 16pk.pdb <b>Query</b> <b>Coverage:</b> 86% <b>Sequence</b> <b>Identity:</b> 73.39%)	Glycolytic pathway

11.	Pyruvate kinase	<i>L. major</i>	MSQLAHNLTLSIFEPVANHRATRI VCTIGPSTQSVEALKGLIQSGMSV ARMNFSHGSHEYHQTTINNVRQ AAAELGVNIAIALDTKGPEIRTGQ FVGGEAVMERGATCYVTTDPAF ADKGTKDKFYIDYQNLSKVVRPG SYIYIDDGILILHVQSHEDQTLKC TVTNAHTISDRRGVNLPGCDVDL PAVSAKDCADLQFGVEQGVDMI FASFIRSAEQVG DVRKALGAKGH DIMIICKIENHQGVQNIDSIIIESD GIMVARGDLGVEIPA EKVVVAQK ILISKCNVAGKPVICATQMLESMT YNPRPTRA EVSDVANAVFNGAD CVMLSGETAKGKYPNEVVQYMA RICLEAQSAVNEYVFFNSIKKLQPI PMSAEEAVCSSAVNSVYETKAKV MVVLSNTGRSARLVAKYRPNCPI VCVTTRLQTCRQLNITQGVESVFF DAEKLGHDEGKEQRVAMGVGF AKSKGYVQTGDYSVVIHADHKV KGYANQTRILLVE	499	Swiss Model ( <b>Template used:</b> 3hqn.pdb <b>Query Coverage:</b> 100% <b>Sequence Identity:</b> 95.59%)	Glycolytic pathway
12.	Phosphoglycerate mutase (2,3- diphosphoglycerate- independent)	<i>L. major</i>	MSNLLLRPHKDLPRRKLIVVMD GLGIGPEDEYDAVHMASTPFMD AQRQNSRHFRSVRAHGTAVGLP TDADMGNSEVGHNALGAGRVA LQGASLVDDALKSGEIYTGEgyr YLLGAFTKEGSTLHLIGLLSDGGV	553	Swiss Model ( <b>Template used:</b> 3igy.pdb	Glycolytic pathway

			HSRDNQIHSIEHA AKNGAKGIR VHVLYDGRDVPDGSSFRFTEELE AVLARVRQDGCDAAIASGGGRM FVTMDRYDADWSIVERGWKAQV LGDARHFHSAKEAITTFREEDPK VTDQYYPPFVVVDEQGKPLGTIE DGDAVLCVNFRGDRVIEMTRAFE DEDFDKFDRVRVPKVRVYAGMMR YDGD LGIPNNFLVPPP KLTRVSEE YLCGTGLQIFACSETQKFGHVTYF WNGNRSGKVDEEHETFKEVPSD RVHFNEQPKMKSAEITEAAIEAL KSGMYDVVRINFPNGDMVGHTG DLRATIAGVEAVDESLAKLKDAV DSINGVFIVTADHGNSDDMAQR DKKGKPMKDEKGNVLPLTSHTLS PVPVFIGGAGLDPRVAMRTDLPT AGLANVTATFINLLGFEAPEDYEP SLIFVEN		<b>Query</b> <b>Coverage:</b> 100% <b>Sequence</b> <b>Identity:</b> 92.21%)	
13.	Fructose-bisphosphate aldolase	<i>L. major</i>	MSRV TIFQSQLPACNRIKTPYESEL IATVKKLTTPGKGLLADESIGSC TKRFEPIGLSNTTEEHRRQYRALML EAEGLEQYISGVILHDETVS QKAS NGKTFPEYL TARGVVPGIKTD MG LCPLLEGAEGEQMTEGLDGYVKR ASVYYKKGCRFCKWRNVYKIQN GTVSEPAVRFNAETLARYAILSQ MSGLVPIVEPEVMIDGKHDIDTC	371	Swiss Model <b>(Template</b> <b>used:</b> 2qap.pdb <b>Query</b> <b>Coverage:</b> 100%	Glycolytic pathway

			QRVSEHVVWREVVAALQRHGVIV EGCLLKPNMVVPGAESGQTAAP EQVAHYTVMTLARTMPAMPLPGV MFLSGGLSEVQASEYLNAINNSPL PRPYFLSFSYARALQSSALKAWG GKDSGVAAGRRAFLHRARMNS MAQLGKYKRADDDASSSSLYVK GNIY		<b>Sequence Identity:</b> 95.96%)	
14.	Adenine phosphoribosyltransferase	<i>L. major</i>	MSFKEISPNSFLLSDSHPLSQLLKK NYCWYSPVFSPRNVPRFADVSSIT ESPETLKAIRDFLVQRYRTMSPAP THILGFDARGFLFGPMIAVELGIP FVLMRKADKNAGLLIRSEPYEKE YKEAAPEVMTIRYGSISKGSRVVLI DDVLATGGTALSGLQLVEASDA MVMEMVSILCIPFLKAAEKIHSTG HSRYKDIKFISLLSEEALTEDNCG DSKNYTGPRVVSCGDVLSKHSQ	237	Swiss Model ( <b>Template used:</b> 1qb7.pdb <b>Query Coverage:</b> 98% <b>Sequence Identity:</b> 92.27%)	Purine salvage pathway
15.	Xanthine phosphoribosyltransferase	<i>L. major</i>	MLPNHSCCKGFVDAQGRVFDGR EYPMASGIVATEDVIQANIKAMA NTIAKDYKSLSHRDVRLSPGTAA TAEAAEAPISYDNPLIISVLKGSYI FTSDFIRYLGDCGLPHVVDVFLA SYNSDTTSTGQISMLAGLRFENLR GKHVLIVEDVCDSGRTLRLRDYI MEKFQPKSVKTLVMVNKEQAAR KLDFDPEYFCLAGPNKYIVGYGFE	238	Swiss Model ( <b>Template used:</b> 6mxc.pdb <b>Query Coverage:</b> 92% <b>Sequence Identity:</b> 56.36%)	Purine salvage pathway

			VNDRYRDLRHIFILRDGEATRYPA KL			
16.	Deoxyhypusine hydroxylase	<i>L. major</i>	MSASNSCTVEEVRKEYAKLLDPQ EPLDSRMRELYRLKEDCLKTVAG VTVILEAIDTTDSVLLQHELAYNA GQSGREEAVPELERILRTTSYDVV TRHEAAEALGAIGSPLALQVLET HSAPTTEPEASIRETCELALARIA MKETKGDAAVAPPSGCEFVSVDP SPAFSALYSSTDEPVPLTVEELEAV LLDTSGRTRLFRRYMAMFTLRNL ATEAAVAALCRGLREDTISALFR HEVAFVLGQLERPSSQPALIAALK DEEEAPMVRHEAAEALGAIADP ATLPVLESYATHHEPIVRDSCVVA LEMHKYWAHFNSLAHQQQEA	325	I-Tasser (Various templates were used including: 4d4z.pdb, 6fsq.pdb)	Hypusine biosynthetic pathway