

In Vitro and *In Silico* Approaches for the Antileishmanial Activity Evaluations of Actinomycins Isolated from Novel *Streptomyces smyrnaeus* Strain UKAQ_23

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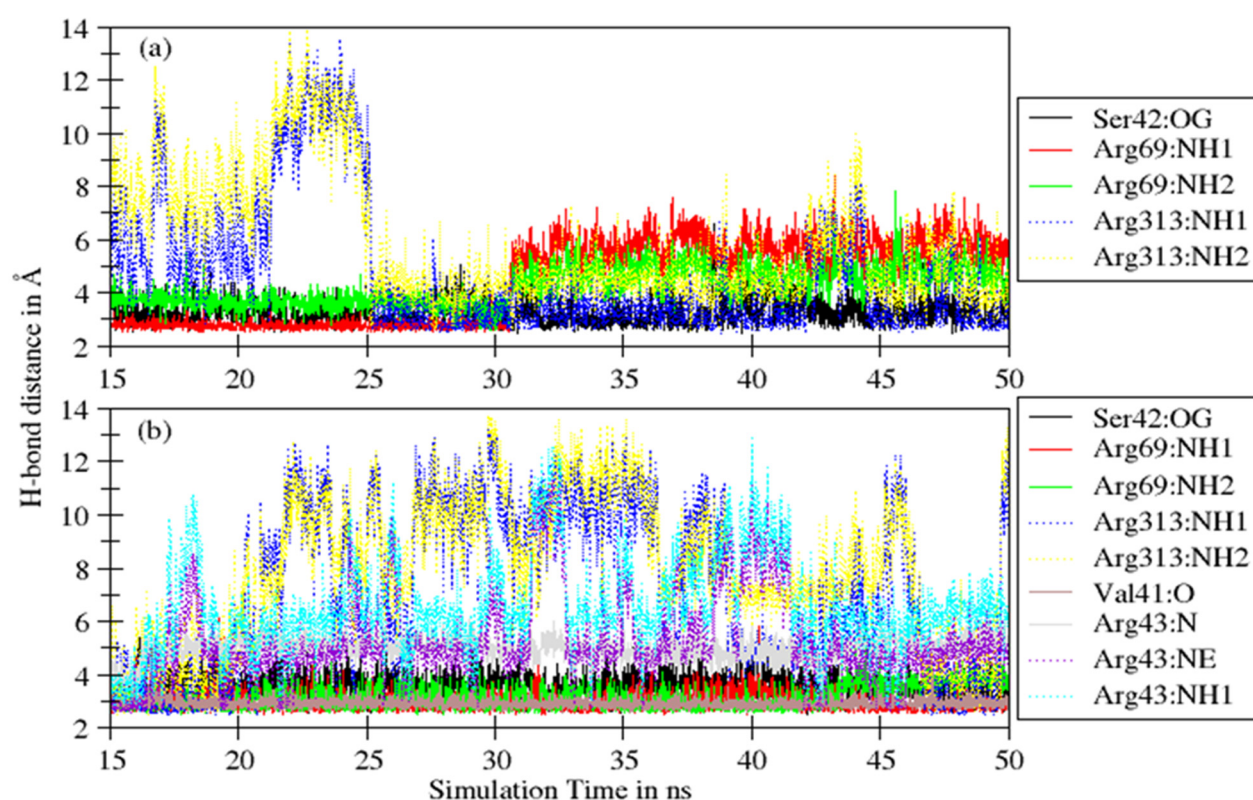


Figure S1. H-bond distances calculated between heavy atoms of protein (*i.e.*, O or N) and ligand (*i.e.*, O or N) for (a) actinomycin D and (b) actinomycin X₂ complexes. The hydrogen bonds were calculated using VMD, where the cutoff for Donor-Acceptor distance and angle was set to 3 Å and 20 degrees, respectively.

Table S1. The selected proteins for molecular docking studies with actinomycin D and actinomycin X₂ ligands targeting multiple pathways in *Leishmania major*. The table also lists the name of enzymes, their FASTA sequence, amino acid length, their PDB code (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
Trypanothione reductase	<i>Leishmania infantum</i>	MGSSHHHHHHSSGLVPRGSHMSRAYDLV VLGAGSGGLEAGWNAAVTHKKKAVVD VQATHGPPLFAALGGTCVNVGCVPKKLM VTGAQYMDLIRESGGFGWEMDRESLCPN WKTLLAAKNKVNSINESYKSMFADTEGL SFHMGFGALQDAHTVVVRKSEDPHSDVL ETLDTEYLIIATGSWPTRLGVPGDEFCTSN EAFYLEDAPKRMLCVGGGYIAVEFAGIFN GYKPCGGYVDLCYRGDLILRGFDTEVRKS LTKQLGANGIRVRTNLNPTKITKNEDGSN HVHFNDGTEEDYDQVMLAIGRVPRSQAL QLDKAGVRTGKNGAVQVDAYSKTSVDNI YAIGDVTNRVMLTPVAINEGAAPVETVFG GKPRATDHTKVACAVFSIPPITCGMTEEE AAKNYETVAVYASSFTPLMHNISGSKHKE FMIRIITNESNGEVLGVHMLGDSAPETIQSV GICMKMGAKISDFHSTIGVHPTSAEELCS MRTPAYFYESGKRVEKLSSNL	511	2JK6	Trypanothione pathway
Trypanothione synthetase-amidase	<i>Leishmania major</i>	MSSLQRASVSFNKPGHIPFAGVQGYAPGG VPAYSNKHDHYFSGERNIEDNIFGFKYQ CVEFARRWLLVRKGLLLPDVNWACHIFQ LKEVRDAATTESFAVLQVRNGTTTKPEAD ALLVYPSTDANPVGHVGTITEVGDDYVCV ADQNYRFHKWESSCAYKLLDHRDGIWT IIDDIDADEIEIPLGWLTFPGRANRPEGAPP VALHPSLHFKEPPKPYLLRRNFLPTESKAN WLDNMNPAERLFVEEFGMDVSRTRLEEK VVSYYESNHEFHLRCVAYGTQLHAIFMEA TAQVIESDEKLRLFAIPEEFWPRIRHSWKY QQTYISGRFDFAFNNETGEVKCFEYNADS ASTLLECGLIQQKWAESVGLDKQDTRGSG FAVERNLMKAWANSATGRVHFCVDEE REEQYTALYCMQAAEAVGLEGLCLIFDE FRFDDNGHVVDSDGVRVRNVWKTWMW ESAITDYAAAREERGENWKPSPKDKVRLC DLLLGDDWEILYFPMWKVIPSNAKILPMI YHNHPEHPAILKAEYELTDELKHHGYAK KPIVGRVGSNVIITSGDGVVHAESGGKYG KRNMIYQQFLFELKKQDDYYAIIIGGWMIG DAFSGTGIREDKSVITGVDSPFAAVRIKTD KLPHPVTLKDIDKMAEDE	652	2VOB	Trypanothione pathway
Tryparedoxin peroxidase	<i>Leishmania major</i>	MSCGNAKINSPAPSFEEVALMPNGSFKKIS LSSYKGKVVVLFYPLDFTFCPTFVIAFS DSVSRFNELNCEVLACSIDSEYAHLQWTL QDRKKGGLGTMAIPMLADKTKSIARSYGV	199	4K1F	Trypanothione pathway

		LEESQGVAYRGLFIIDPHGMLRQITVNDM PVGRSVEEVLRLLEAFQFVEKHGEVCPAN WKKGAPTMMKPEPNASVEGYFSKQ			
Squalene syn- thase	<i>Leishmania major</i>	MGFFSDSVAMMRVKWQMRSVKIQVPPEE TDLRFCYDIMNDVSRFAVVVAQLADQQ LRDAICIFYLVLRALDTLEDDMSVPVDVKL KELPKFHTHTSDMSWCMSGVGEGREREL LAKYPCVSREFKKLKKEYQDVIANICERM ANGMCEFLKRPVVTKDDYNQYCHYVAG LVGHGLTQLFARCGFEDPSLDDDLTSSNH MGLFLQKTNIIRDYYEDIREPPRMFWPKE IWGTYVTELKELKSESNNAAVQCLNAM VADALVHVPYIVDYLALRDPVFRFCAIP QVMAIATLKEVYNNPDFTQVKVKVSRPES CRIMLKATTLYSSLSMFRDYCVELQEKLD MQDASSVSIANSALAAIERIDLQLKKCQD VSYTRSLARYPGLGGQFLLTVMMDTVAGF FGGRKEIAGHA	414	Swiss Model (Template used: 3wca.pdb Query Coverage: 82% Sequence Identity: 61.83%)	Sterol biosynthetic pathway
Squalene monooxygenase	<i>Leishmania major</i>	MLYFFAAVLCAVSTLLLLNRTLRLRLSPA RTSYDYDVIIVGGSAGPVLAKALSDQGRK VLMVERTLFTKPDRIVGELLQPGGLNALK EVGMKECAETIGMPCHGYVVVDHKGKQ VDLPYRKGAAGVSFHFQDFVQSLRSHVFH NCKANVTMVEGTVNILTEGLSFSERAYG VEYTIAEKYEVPTKPFREDPPKTNPVAATV RKVATAPLVVMCDGGMSKWSRYQHYT PAYEYHSHFIGLVLKTIVRLPKEQRTVFFG KTGPILSYRLDDNELRLLDVYNKPTLPSLE KQSEWLIQDVAPCLPENMREQFVRVSKDT KSLRSMPMARYPPAFPSIKGYVGIGDHAN QRHPLTGGGMTCCFRDAIRLANSLNGIQS LRSVNQEEMAAIEDKIQAAILNYARYRYT HSCCINLLSWALYSVFSSPALRDACFDYFL CGGNCVGTGPMDLLAGLDPNVGSLFFHYC CVMLHGVANVMMRTGAYSESGKQLSNL EKLTNVASFFVDWERMKHAAYLLGKSTQI ALPLAKSEFYSMWRFVDPTSPLANISKRIK TMVYTKQFNGKQQRKPVGL	569	I-Tasser (Various templates were used including: 6c6n.pdb, 6fho.pdb, 6c6r.pdb)	Sterol biosynthetic pathway
Farnesyl pyrophosphate synthase	<i>Leishmania major</i>	MAHMERFQKVYEEVQEFLGDAEKRFEM DVHRKGYLKSMMDDTTCLGGKYNRGLCV VDVAEAMAKDTQMDAAAMERVLHDAC VCGWMIEMLAHFLVEDDIMDHSKTRRG KPCWYLHPGVTAQVAINDGLILLAWATQ MALHYFADRPFLAEVLRVFDVLDLTTIG QLYDVTSMVDSAKLDAKVAHANTTDYVE YTPFNHRRIVVYKTAYTYWLPLVMGLLV SGTLEKVDKKATHKVAMVMGEYFQVQD DVMDCTPPEKLGKIGTDIEDAKCSWLAV TFLTAPAEKVAEFKANYGSTDPAAVAVI KQLYTEQNLLARFEEYEKAVVAEVEQLIA ALEAQNAFAASVKVLWSKTYKRQK	362	4JZX	Sterol biosynthetic pathway

Glyceraldehyde-3-phosphate dehydrogenase	<i>Leishmania major</i>	MAPIKVGINGFGRIGRMVLQAICDQGLIG NEIDVVAVVDMSTNAEYFAYQMKYDTV HGRPKYTVEAVKSTPSVKTPDVLVNGHR IKCVKAQRNPADLPWGKLGVDYVIESTGL FTDKLQAEGLHKGGAKKVVISAPASGGAK TIVMGVNQHEYSPTSHHVSNASCTTNCL APIVHVLTKENFGIETGLMTTIHSYTATQK TVDGVSLKDWRGRAAAINIIPSTTGAAG AVGMVIPSTKGKLTGMSFRVPTPDVSVVD LTFRSTRETSIQEIDKAIKKAQTYMKDILG FTNDELVSDFINDNRSSVYDSKATLQNN LPGEKRFFKIVSWYDNEWGYSHRVVDLVR YMAATDAASAKM	361	Swiss Model (Template used: 1gyq.pdb Query Coverage: 99% Sequence Identity: 94.69%)	Glycolytic pathway
Triosephosphate isomerase	<i>Leishmania major</i>	MSAKPQPIAAANWKCNGTTASIEKLQVQ LNEHNISHDVQCVVAPTFFVHIPLVQAKLR NPKYVVSNAENAIKSGAFTGEVSMPIKLD LGINWVILGHSEERTYYGETDETVAQKVA DACKQGFMIACIGETLQQREANQTAKV VLSQTSIAITKLPKEAWDQIVLAYEPVWA IGTGKVATPEQAQEVHALLRKWVSEKIGT DVAAKLRILYGGSVSAGNAKTLYMKPDIN GFLVGGASLKPEFRDIIDATR	251	Swiss Model (Template used: 5cg7.pdb Query Coverage: 100% Sequence Identity: 91.63%)	Glycolytic pathway
Phosphoglycerate kinase	<i>Leishmania major</i>	MSLVLKKSIDDATVRDKKVLIRVDFNVVP KNGKITNDFRISALPTIQKVLKEGGSCIL MSHLGRPKGARMSPSPKGVGRGYEEAA TLRPVAARIAELLGQKVEFAPDCLDAAAY ASKLKNGDVLLLENVRFYAEESGSKKEEER DAMAKVLAASYADLYVSDAFGTAHRDSAT MTGIPKVLGAGYAGYLMEKEINYFSRVLN NPPRPLVAIVGGAKVSDKIELLDNMLSRIN YLVIGGAMAYTFQKAQGRKIGISMCEEDK LDLAKSLLKKAQERGVQVFLPVDHVCNK EFKAADSPLVTEVDVDPGYMALDIGPRTI HMYEEVIGRCKSAIWNGPMGVFEMPCYS KGTFAVAKAMGTGTQKNGLLSIIGGDS ASAAELSGEAKNMSHVSTGGGASLELLEG KTLPGVAILTDKDVKERGASCRFAFGVGS PSREACPLRCGHIFGGASIVREIVKLVVALL IGIFIGRRMSTKLIR	479	Swiss Model (Template used: 16pk.pdb Query Coverage: 86% Sequence Identity: 73.39%)	Glycolytic pathway
Pyruvate kinase	<i>Leishmania major</i>	MSQLAHNLTLSIFEPVANHRATRIVCTIGP STQSVEALKGLIQSGMSVARMNFSHGSHE YHQTINNVRQAAAELGVNIAIALDTKGP EIRTGQFVGGEAVMERGATCYVTTDPAFA DKGTKDKFYIDYQNLKSVVRPGSYIYIDDG ILILHVQSHEDQTLKCTVNAHTISDRRG VNLPGCDVDLPAVSAKDCADLQFGVEQG VDMIFASFIRSAEQVDVRKALGAKGHDI MIICKIENHQGVQNIDSIIIESDGIMVARG DLGVEIPAEEKVVAQKILISKCNVAGKPIV CATQMLESMTYNPRPTRAEVSDVANAVF NGADCVMLSGETAKGKYPNEVVQYMARI	499	Swiss Model (Template used: 3hqn.pdb Query Coverage: 100% Sequence Identity: 95.59%)	Glycolytic pathway

		CLEAQSAVNEYVFFNSIKKLQPIPMASAEAA VCSSAVNSVYETKAKVMVLSNTGRSARL VAKYRPNCPIVCVTTRLQTCRQLNITQGV ESVFFDAEKLGHDEGKEQRVAMGVGFAK SKGYVQTGDYSVVIHADHKVKGYANQTR ILLVE			
Phosphoglycerate mutase (2,3-diphosphoglycerate-independent)	<i>Leishmania major</i>	MSNLLLRPHKDLPRRKLIVVMDGLGIGP EDEYDAVHMASTPFMDAQRQNSRHFRSV RAHGTAUGLPTDADMGNSEVGHNALGA GRVALQGASLVDDALKSGEITYTGEGYRYL LGAFTEKESTLHLIGLLSDGGVHSRDNQI HSIIHAAKNGAKGIRVHVLYDGRDVPDG SSFRFTELEAVLARVRQDGCDAAIASGG GRMFVTMDRYDADWSIVERGWKAQVLG DARHFHSAKEAITTFREEDPKVTDQYPPF VVVDEQKPLGTIEDGDAVLCVNFGRDR VIEMTRAFEDDFDKFDRVRVPKVRYAG MMRYDGDGLGIPNNFLVPPPKLTRVSEYL CGTGLQIFACSETQKFGHVTYFWNGNRSG KVDEEHETFKEVPSDRVHFNEQPKMKSAE ITEAAIEALKSGMYDVVRINFPNGDMVGH TGDLRATIAGVEAVDESLAKLKDAVDSIN GVFIVTADHGNSDDMAQRDKKKGPMKD EKGNVPLTSLTSLSPVPVFIGGAGLDPRVA MRTDLPTAGLANVTATFINLLGFEAPEDY EPSLIFVEN	553	Swiss Model (Template used: 3igy.pdb Query Coverage: 100% Sequence Identity: 92.21%)	Glycolytic pathway
Fructose-bisphosphate aldolase	<i>Leishmania major</i>	MSRVTFQSQLPACNRIKTPYEELIATVKK LTPGKGLLAADESIGSCTKRFEPGLSNT EHRRQYRALMLEAEGLEQYISGVILHDET VSQKASNGKTFPEYLARGVVPGIKTDMG LCPLLEGAEGEQMTEGLDGYVKRASVYYK KGCRCCKWRNVYKIQNGTVSEPAVRFNA ETLARYAILSQMSGLVPIVEPEVMIDGKHD IDTCQRVSEHVWREVVAALQRHGVWEG CLLKPNMVVPGAESQTAAPQVAHYTV MTLARTMPAMLPGVMFLSGGLSEVQASE YLNAINNSPLPRPYFLSFSYARALQSSALK AWGGKDSGVAAGRRFLHRARMNSMA QLGKYKRADDDASSSSLYVKGNLY	371	Swiss Model (Template used: 2qap.pdb Query Coverage: 100% Sequence Identity: 95.96%)	Glycolytic pathway
Adenine phosphoribosyltransferase	<i>Leishmania major</i>	MSFKEISPNSFLLEDSPHLSQLLKKNYC SPVFSRPNVPRFADVSSITESPETLKAIRDFL VQRYRTMSPAPTHILGFDARGFLFGPMIA VELGIPFVLMRKADKNAGLLIRSEPYEKEY KEAAPEVMTIRYGSISKGSRVVLIDDLAT GGTALSGLQLVEASDAMVVEMVSILCIPFL KAAEKIHSTGHSRYKDIKFISLLSEEALTED NCGDSKNYTGPRVVSCGDVLSKHSQ	237	Swiss Model (Template used: 1qb7.pdb Query Coverage: 98% Sequence Identity: 92.27%)	Purine salvage pathway
Xanthine phosphoribosyltransferase	<i>Leishmania major</i>	MLPNHSCCKGFVDAQGRVFDGREYPMAS GIVATEDVIQANIKAMANTIAKDYKSLSH RDVRLSPGTAATAEAAEAPISYDNPLIISV	238	Swiss Model	Purine salvage pathway

		LKGSYIFTSDFIRYLGDCGLPHVDFVRLA SYNSDTTSTGQISMLAGLRFENLRGKHVLI VEDVCDSGRTLRLRDYIMEKFQPKSVKTL VMVNKEQAARKLDFDPEYFCLAGPNKYI VGYGFEVNDRYRDLRHIFILRDGEATRYP AKL		(Template used: 6mxc.pdb Query Coverage: 92% Sequence Identity: 56.36%)	
Deoxyhypusine Hydroxylase	<i>Leishmania major</i>	MSASNSCTVEEVKEYAKLLDPQEPLDSR MRELYRLKEDCLKTVAGVTVILEAIDTTDS VLLQHELAYNAGQSGREEAVPELERILRTT SYDVVTRHEAAEALGAIGSPLALQVLETH SAPTTEPEASIRETCELALARIAMKETKGD AAVAPPSGCEFVSVDPSAFSALYSSTDEP VPLTVEELEAVLLDTSGRTRLFRRYMAMF TLRNLATEAAVAALCRGLREDTISALFRH EVAFVLGQLERPSSQPALIAALKDEEEAP MVRHEAAEALGAIADPATLPVLESYATH HEPIVRDSCVVALEMHKYWAHFNSLAHQ QQEA	325	I-Tasser (Various templates were used including: 4d4z.pdb, 6fsq.pdb)	Hypusine biosynthetic pathway