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Denovo design and optimization of novel inhibitor molecules for HIV integrase

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Abstract: Infection of HIV virus; and AIDS is well understood in human and is characterized by a decrease in the number of helper T cells, which causes a severe immunodeficiency that leaves the body susceptible to a variety of potentially fatal infections. In the present study we chose integrase protein, the major target for antiviral drugs; as it facilitates the incorporation of viral DNA into chromosomes of target cells. Integrase of HIV type I (PDB ID: 1BIZ) was used as the receptor protein and new ligands molecules were generated using structure based *de novo* approach. Docking studies were performed to explore binding affinity and hydrogen bond interaction between the ligand and the 1BIZ protein molecule. Developed ligands were optimized according to the bio-safety and bioavailability parameters. By continuous parameters based sorting method; final molecules were screened and 10 different optimized molecules are proposed here as potential molecules that could be used as integrase inhibitors in treatment of AIDS.
Keywords: integrase, insilico, *denovo,* docking.

Introduction:

Human immunodeficiency virus type 1(HIV-1) is the etiological agent of acquired immunodeficiency syndrome (AIDS).[1] Following the infection, this retrovirus uses three key enzymes to propagate its life cycle; reverse transcriptase (RT), integrase (IN) and protease (PR).[2] Combinations of inhibitors of the RT and PR are currently the preferred clinical treatment for HIV infection and AIDS. However, targeting RT and PR still does not eliminate the virus from patients, making it necessary to explore other targets.[3] Attention has recently been focused on the HIV IN enzyme because it is one of the earliest steps in the viral life cycle and there is no native homologous process in the host cell.

Because of Integrase HIV virus is able to incorporate its genetic material into its host cell and this makes it a hot target among biologist and drug scientist to overcome with the AIDS problem. [4] Existing treatment approaches either lacks in targeted drug delivery or serious side effects. [5]

Integrase carry out integration process in following two steps: In the first step (termed 3'-end processing or 3'-P), the dinucleotide pair (pGT) is removed from each 3'-ends of viral DNA, to produce new 3'hydroxyl ends (CA-3'-OH). This reaction occurs in the cytoplasm, within a large viral nucleoprotein complex, the pre-integration complex (PIC). [6, 7] After entering the nucleus, the 3'-processed double strands DNA is joined to host target DNA (termed strand transfer). The joining reaction includes a coupled 5-bp staggered cleavage of the target host DNA and the ligation of processed CA-3'-OH viral DNA ends to the 5' phosphate end of the host DNA. Repair of the remaining gaps, although not understood at this time,

is probably accomplished by host cell DNA repair enzymes. [8, 9]

In-silico, *de-novo* structure generation and virtual screening methods are quick and less expensive to define new drug candidate. [10]The main objective of the presented study was to define a new class of molecules for the inhibition of target molecule. Integrase of HIV virus type I was considered as target molecule. [11]

Figure 1: Hiv integrase protein with putative site predicted by Ligsite[csc.]

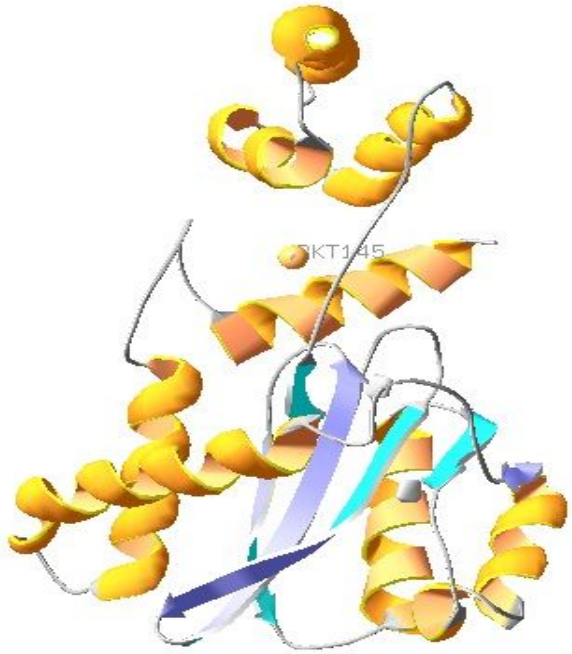
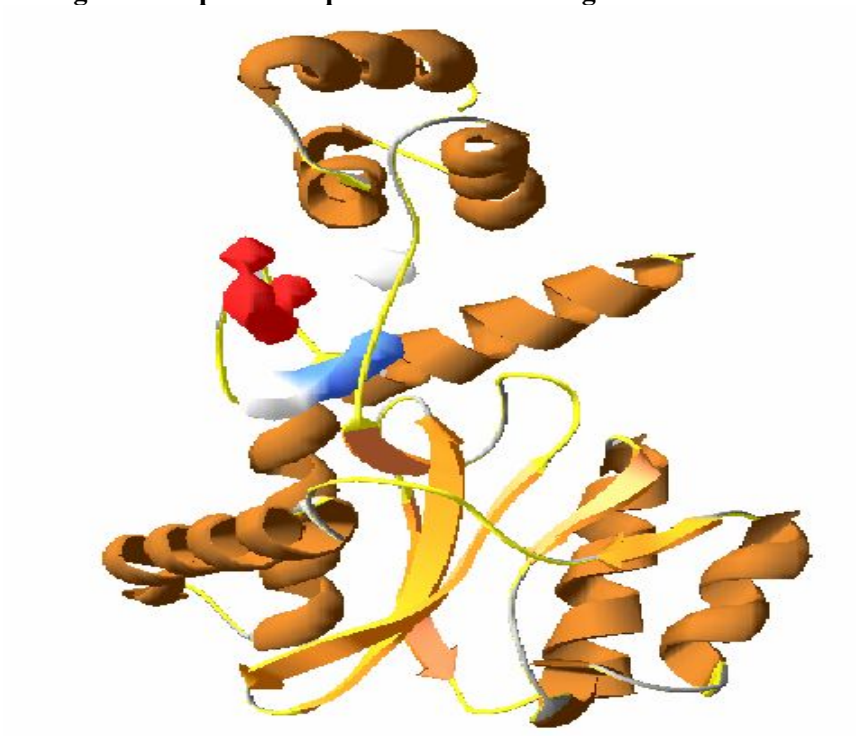


Figure 2: A pharmacophore model for integrase



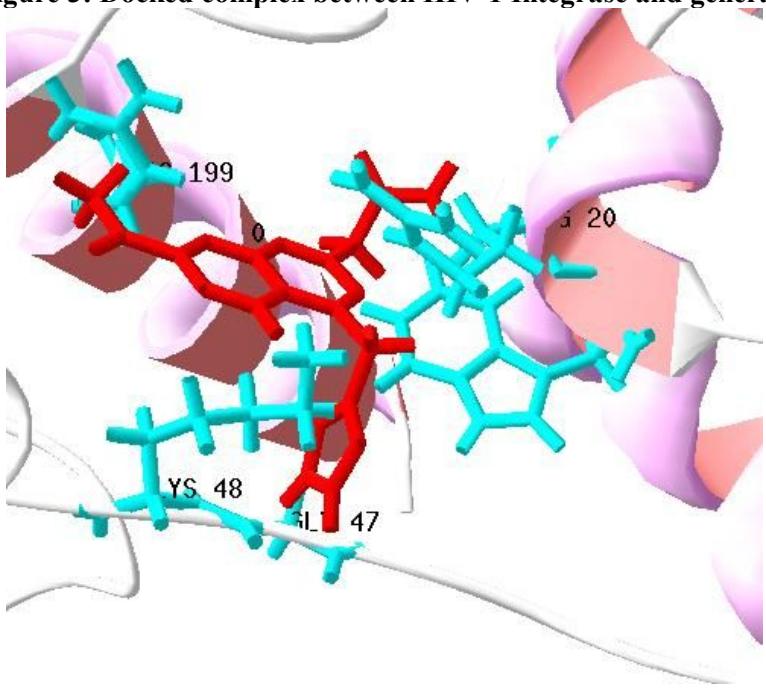
Experimental:

1. **Drug Target Identification:** Exhaustive search of available data over various internet resources regarding HIV molecular biology was carried out and Integrase was identified as a potential target molecule. Fine 3D structure with a resolution of 1.95Å of integrase was retrieved from the Protein Data Bank (PDB ID: 1BIZ). [12]Structure validation protocols were applied and finally a stable model was then used in further structure based protocols.
2. **Active Site Determination:** Using online tool "Ligsite[csc]" putative functional site present over the defined structure of integrase was identified and analyzed (Figure 1). Among the defined putative site Pkt 145 was identified comprising residue, Lys 46, Lys 48 and Asp 202 for the inhibition of integrase.[13]
3. **Lead Selection and Optimization:** Potent inhibitor molecules were searched and a series of lead molecules were defined considering their inhibition properties. Among the defined lead molecules di-keto (O=C(C)CC(=O)C), hydrazide (O=C(NNC(=O)C)C) and qinoline (n1cccc2ccccc12) were identified as the best lead molecules for the de *novo* generation of new molecules.[14] Ligands were generated by structure-based *de novo* approach. A total of around 78000 (*On an average 26000 using each lead molecule)* conformers were generated and a primary screening of ligands were done on the basis of their binding affinity with the receptor molecule. A pharmacophore model for receptor active site was derived using the POCKET module in LigBuilder.[15] Nitrogen atoms (blue) represent hydrogen-bond donor sites; oxygen atoms (red) represent hydrogen-bond acceptor sites; and carbon atoms (white) represent hydrophobic sites. Keeping the fact of structural and chemical complementation each of the selected lead molecules were then optimized using Ligbuilder's grow module (Figure 2). The generated molecules were analyzed using the PROCESS module in LigBuilder(Table 1). Each of the molecules was optimized over inhibition as well as bio-safety parameters.

Table 1: Chemical criteria used for PROCESS module in LigBuilder

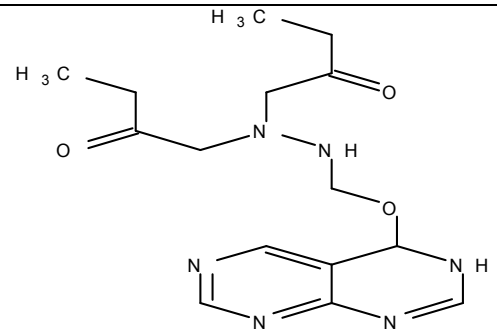
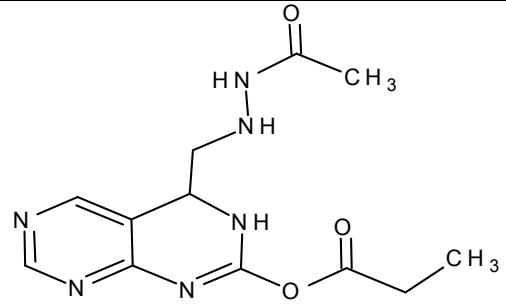
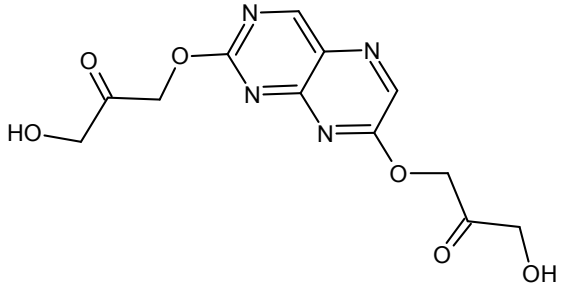
Maximal Molecular weight	500
Minimal Molecular weight	50
Maximal LogP	5.0
Minimal LogP	-5.0
Maximal PKD	5.0
Minimal PKD	1.0
Similarity cutoff	1.0
Number of molecules satisfied the criteria	500

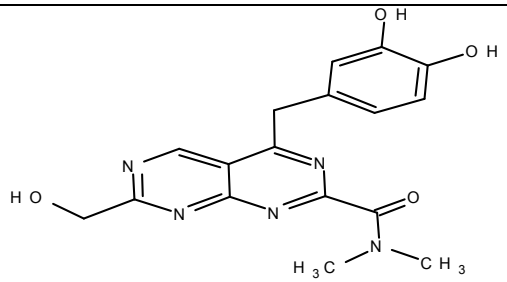
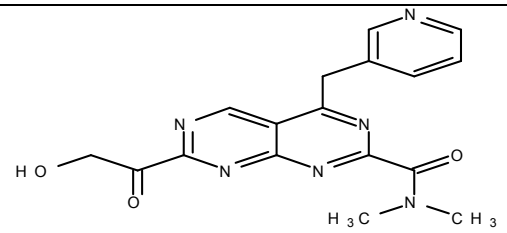
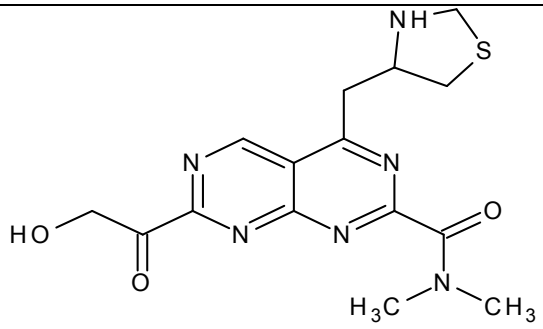
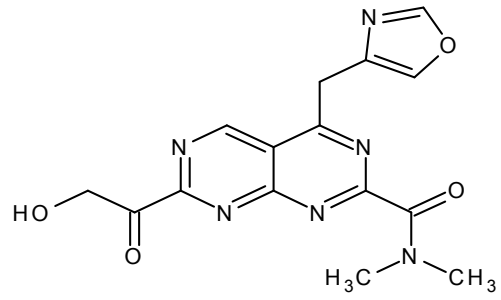
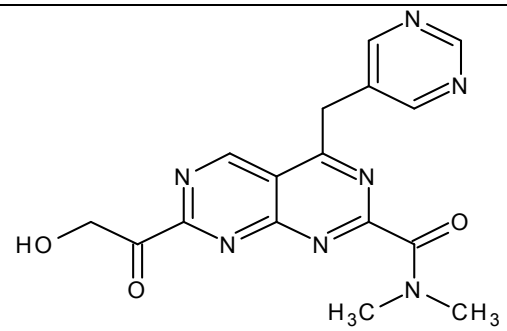
Figure 3: Docked complex between HIV-1 Integrase and generated molecule (LD009).



4. **Binding Affinity Exploration:** Degree of affection in between developed novel molecules and integrase in terms of structural and chemical complementation was explored by defining binding energy (ΔG) using the "Quantum" having force field "AMBER". [16] The average of results obtained in 3 runs of docking procedure for each of the developed molecules is considered. IC_{50} value was also explored for the efficacy check. The Swiss-Pdb Viewer was then used to generate images of protein structures docked with potential compounds (Figure 3).
5. ***In silico* ADME/Tox Prediction:** Bio-safety of the developed new molecules was also explored. Pharmacodynamic and pharmacokinetic behaviour of the developed molecules was studied over insilico tools like "Osiris property explorer" [17] and Molinspiration [18] to understand the behaviour of newly developed molecules inside the human body.
6. **Estimation of Synthetic Accessibility:** Using continuous parameter based sorting method finally screened molecules were then tested for their synthetic accessibility using "Sylvia". [19] SYLVIA ranks chemical compounds on a scale that reflects whether a structure can be synthesized by a straightforward synthesis route or whether it is a complex, challenging synthesis target. [20]
7. **Final Screening of Novel Ligand Molecules:** Averaging of the explored properties was used to define final ligand molecules. Overall 10 final molecules were selected as the best molecules for the target specific inhibition of integrase.

Table 2: Finally selected ligand molecules

SN	LIG.	Structure	IUPAC Name / SMILES
1.	LH007		1,1'-(2-{[(4*S*)-3,4-dihydropyrimido[4,5-*d*]pyrimidin-4-yloxy]methyl}hydrazine-1,1-diyl)dibutan-2-one O=C(CC)CN(NCOC1c2cncnc2/N=C\N1) CC(=O)CC
2.	LH011		(4*S*)-4-[(2-acetylhydrazinyl)methyl]-3,4-dihydropyrimido[4,5-*d*]pyrimidin-2-yl propanoate O=C(NNCC2c1c(ncnc1)\N=C(\OC(=O)CC)N2)C
3.	LD 004		1,1'-[pteridine-2,7-diylbis(oxy)]bis(3-hydroxypropan-2-one) O=C(CO)COc1nc2nc(OCC(=O)CO)ncc2nc1

4.	LD005		4-(3,4-dihydroxybenzyl)-7-(hydroxymethyl)-*N*,*N*-dimethylpyrimido[4,5-*d*]pyrimidine-2-carboxamide O=C(c1nc(c2c(n1)nc(nc2)CO)Cc3ccc(O) c(O)c3)N(C)C
5.	LD008		7-(hydroxyacetyl)-*N*,*N*-dimethyl-4-(pyridin-3-ylmethyl)pyrimido[4,5-*d*]pyrimidine-2-carboxamide CN(C)C(=O)c2nc(Cc1cccnc1)c3cnc(nc3n2) C(=O)CO
6.	LD009		7-(hydroxyacetyl)-*N*,*N*-dimethyl-4-(1,3-thiazolidin-4-ylmethyl)pyrimido[4,5-*d*]pyrimidine-2-carboxamide CN(C)C(=O)c2nc(CC1CSCN1)c3cnc(nc3n2) C(=O)CO
7.	LD011		7-(hydroxyacetyl)-*N*,*N*-dimethyl-4-(1,3-oxazol-4-ylmethyl)pyrimido[4,5-*d*]pyrimidine-2-carboxamide CN(C)C(=O)c2nc(Cc1cocn1)c3cnc(nc3n2) C(=O)CO
8.	LD012		7-(hydroxyacetyl)-*N*,*N*-dimethyl-4-(pyrimidin-5-ylmethyl)pyrimido[4,5-*d*]pyrimidine-2-carboxamide CN(C)C(=O)c2nc(Cc1cncnc1)c3cnc(nc3n2) C(=O)CO

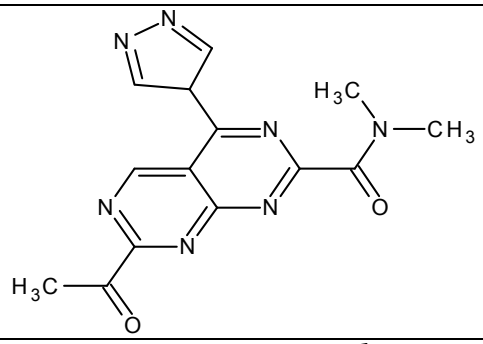
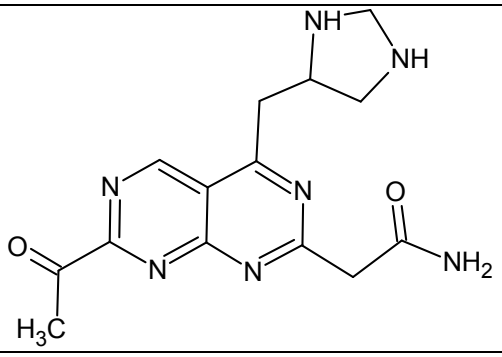
9.	LD014		7-acetyl-*N*,*N*-dimethyl-4-(4*H*-pyrazol-4-yl)pyrimido[4,5-*d*]pyrimidine-2-carboxamide O=C(c1ncc2c(nc(nc2n1)C(=O)N(C)C)C3C=NN=C3)C
10.	LD016		2-{7-acetyl-4-[(4*R*)-imidazolidin-4-ylmethyl]pyrimido[4,5-*d*]pyrimidin-2-yl}acetamide O=C(c1ncc2c(nc(nc2n1)CC(=O)N)CC3NCNC3)C

Table 3: Values calculated by Quantum

No.	IC50(Mol/L)	ΔG	RMSD	Flexible bond	Lipinski Rule
1.	8.39	-23.73	35.84	0	True
2.	7.19	-18.29	28.98	0	True
3.	1.21	-22.79	33.16	2	True
4.	2.31	-21.16	32.09	4	True
5.	2.25	-21.23	23.89	4	True
6.	4.60	-19.42	30.80	3	True
7.	1.49	-16.45	29.50	2	True
8.	5.12	-19.15	30.09	3	True
9.	5.18	-19.12	32.83	2	True
10.	4.11	-19.71	33.99	1	True

Table 4: Molecular Properties calculated by Osiris Property explorer

No.	cLogp	Solubility	Mol.wt	Drug-likeness	Drug score	Toxicity
1.	-1.59	-2.44	334.0	4.2	0.9	No
2.	-2.14	-0.91	360.0	5.05	0.91	No
3.	-5.84	-0.36	310.0	2.77	0.92	No
4.	0.07	-1.19	355.0	3.72	0.9	No
5.	-0.82	-1.28	352.0	3.77	0.9	No
6.	-1.52	-1.75	362..0	4.31	0.9	No
7.	-1.27	-1.55	342.0	3.08	0.9	No
8.	-1.45	-1.07	353.0	3.08	0.9	No
9.	1.65	-2.0	325.0	4.28	0.9	No
10.	-1.26	-1.4	329.0	4.07	0.92	No

Lig= Ligand Name, IC_{50}= Inhibitory Concentration 50, ΔG= Gibbs Energy, Tox.= Toxicity (Mutagenic, tumorigenic, irritant, reproductive effect)

Table 5: Toxic parameter calculated by Molinspiration

No	Lig	GPCR ligand	Ion channel	Kinase inhibitor	Nuclear-Receptor Ligand
1	LH007	-0.07	-0.53	-0.59	-0.78
2	LH011	-0.46	-0.53	-0.59	-1.07
3	LD 004	-0.79	-0.89	-0.87	-0.87
4	LD005	0.18	-0.21	0.02	-0.67
5	LD008	0.18	-0.02	0.13	-0.78
6	LD009	-0.05	-0.63	-0.43	-1.10
7	LD011	-0.10	-0.48	0.06	-0.82
8	LD012	0.26	-0.08	0.24	-0.95
9	LD014	0.14	-0.55	-0.23	-0.88
10	LD016	0.23	-0.47	-0.16	-1.02

Table 6: Synthetic Accessibility calculated by Sylvia

S.N.	1	2	3	4	5	6	7	8	9	10
Lig.	LH007	LH011	LD 004	LD005	LD008	LD009	LD011	LD012	LD014	LD016
SA	5.06	5.19	4.12	4.77	4.85	5.48	4.88	4.82	4.97	5.44

SA= Synthetic Accessibility

Results and Discussion:

Most of the finally selected molecules are the optimization products of diketo functional group while only few of hydrazine products are able to qualify rigorous selection procedure and none from qinoline. In silico finding suggests the diketo derivatives as the best inhibitory molecules for HIV integrase. Tables below shows the finally selected ligands(Table 2) and their molecules properties in which $IC_{50,}$ Binding energy(ΔG), RMSD, Flexible bond and Rule of '5' is calculated by using the "Quantum" (Table 3). Drug likeness (DL) and drug score (DS) is calculated by "Osiris Property explorer". A positive druglikeness value states that a molecule contains predominantly fragments which are frequently present in commercial drugs. The drug score combines druglikeness, cLogP, logS, molecular weight and toxicity risks in one handy value than may be used to judge the compound's overall potential to qualify for a drug. Bioactivity against different regular human body receptor is calculated by Molinspiration chemo-informatics (Table 5). Calculated distribution of activity scores for GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptor ligands and protease inhibitors compared with scores for about 100'000 "average organic molecules". The score allows efficient separation of active and inactive molecules. Synthetic Accessibility is predicted by using "Sylvia". The calculated synthetic accessibility scores agree with the values proposed by chemists to an extent that compares well with how individual chemists agree with each other.

Conclusion:

The purpose of this study is to generate novel compounds as HIV-1 integrase inhibitors. Ligands were generated by structure-based *de novo* approach for define lead molecules like diketo, hydrazide and quinoline and compute docking and ADME/Tox properties. Out of the selected lead molecules Listed Molecules shows the inhibitoriest effect at in-silico level. Here we proposed a new class of molecules as a potent inhibitor molecule for HIV integrase inhibition; which may opens up new ways for the effective treatment of AIDS.

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References:

1. Cotelle & Philippe, *Recent Patents on Anti-Infective Drug Discovery*,2006;1:15
2. Clercq & Erik De, *Pure Appl. Chem.*,2001.
3. D. V. Akimov et al., *Pharmaceutical Chemistry Journal*, 2002.
4. Vandekerckhove L. et al., *Review in Antiviral Therapy*, 2008.
5. Oyebisi Jegede et al., *AIDS Rev.*, 2008; 10:172.
6. Miller MD et al., *J Virol* , 1997; 71: 5382.
7. Yves Pommier et al., *Drug Discovery Today: Disease Mechanism,* 2006, Vol.3, No.2.

8. Esposito D and Craigie R., *Adv Virus Res,*1999; 52: 319.
9. Asante-Appiah E and Skalka AM, *Antiviral Res,* 1997, 36: 139.
10. Amy C. Anderson, *Chemistry & Biology*, 2003; 10: 787.
11. Jim A Turpin, *future- drug, Experimental Review, Anti-infective Theory,* 2003;
12. www.pdb.org/pdb/explore/explore.do?structureId=1BIZ
13. Laith Q. Al-Mawsawi, et al., *PNAS,* 2006; 103.
14. Vajragupta O and Boonchoong P, *Mahidol University Journal of Pharmaceutical Sciences*, 2002; 29 (3-4), 1-10.
15. Ying G et.al. Journal of Molecular Modeling, 2000; 6,498-516.
16. www.q-pharm.com/category/docking/
17. http://www.organic-chemistry.org/prog/peo/
18. http://www.molinspiration.com/cgi-bin/properties
19. www.molecular-networks.com.
20. Boda K., et al., *J. Comp.-Aided. Mol. Des.,* 2007, 21: 311.

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