

Model based design of inhibitors for c-jun

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Abstract:

Literature shows that various molecular cascades are activated by stress, UV rays and pollutants leading to wrinkle formation of the skin. These cascades start from five types of receptors (EGFR, PDGFR, PAFR, IL1R, TNFRB) and terminate with the production of matrix metalloproteinase's, which degrades collagen leading to wrinkle formation. Signaling pathway leading to wrinkle formation showed that c-jun is involved in these cascades. Therefore, c-jun is the preferential choice for inhibition to reduce the intensity of collagen degradation. Hence, the 3D structure of c-jun was modeled using segment based homology modeling by MODELLER 9v5. Evaluation of the constructed model was done by PROCHECK, WHAT CHECK and through RMSD/RMSF calculations. Ligands for the inhibitory sites were designed using LIGANDSCOUT. The interaction study of ligand and receptor was performed by AUTODOCK. A library of analogues was constructed for three known inhibitory sites. The receptor-analogue study was performed using the software MOLEGRO Virtual Docker. The analogues constructed from the designed novel reference ligands showed good binding with the receptor binding sites. It should be noted that these predicted data should be validated using suitable assays for further consideration.

Keywords: wrinkle formation; homology modeling; docking; inhibitors for c-jun**Background:**

Skin ageing is a hot research topic. Skin research has led to the development many products to keep skin younger and healthier for a long period. There are many preparations like Botox injections, laser treatment, fat graft implantation, etc., for skin ageing. However, these products are often expensive and usually have side effects. Hence, there is a need to analyze the molecular pathways for skin ageing and to inhibit potential targets. There are various symptoms for skin ageing but one of the most prominent sign of skin ageing is wrinkle formation. Wrinkle appears on the skin both due to intrinsic and chronological skin ageing (occurs with the passage of time) and premature skin ageing (influence of external factors like UV rays, stress and pollution). Data suggest that there are five receptors (EGFR, PDGFR, PAFR, IL1R and TNFRB) that are activated by stress. They stimulate their corresponding adapter proteins, mitogen activated protein kinases (MAPKs) and transfer the signal down to the nucleus to transcription factor activation protein 1 (AP-1). AP-1 further induces the transcription of matrix metalloproteinase's (MMPs).

MMPs(1,3,9) degrade collagen. The excessive degradation of collagen leads to loss of skin integrity towards wrinkle formation. Signaling cascade studies show that EGFR, PDGFR and PAFR through MAPK1 bring activation of AP-1. TNFRB through MAPK9 and IL1R through MAPK8 activation of AP-1 are known. However, different MAPKs were involved leading to activation of AP-1. Hence, if collagen degradation, activated by different stressors through different receptors is to be inhibited; AP-1 should be targeted [1]. AP-1 is made up of two subunits, namely c-fos and c-jun. It is known that c-jun expression is limiting AP-1 activation in human skin as c-fos concentration is constant, while c-jun concentration increases in photo-aged skin and old skin [2]. Moreover, c-fos-regulated genes exert a protective function and its deficiency makes the cells hypersensitive to a broad spectrum of DNA-damaging agents. Therefore, c-jun is a preferential choice for inhibition. c-jun is 331 residues long. It consists of a leucine zipper domain and a basic region. c-jun interacts with DNA by the leucine zipper domain.

In c-jun, the transcription activation domain residues are 91-186, the transcription repression domain residues are 31-57 and the promoter region for T antigen-dependent DNA unwinding (required for the initiation of polyoma virus DNA replication) residues are 91-186. The region 241- 252 residues of c-jun can increase DNA bending and binding at the AP-1 site [3]. Residues 252-281 in c-jun are the basic sub-domain, which is responsible for the sequence specific recognition site of DNA [4]. Studies performed so far have targeted MAPKs to inhibit the activation of c-jun. It has been shown that three types of

MAPK are involved in collagen degradation. Therefore, to completely inhibit the degradation of collagen (wrinkle formation) activated by various stressors, c-jun is targeted. However, the complete structure for c-jun is still not available for inhibitor design. Therefore, homology modeling is performed to construct the 3D structure for c-jun followed by inhibitor design through docking.

Methodology:**Homology modeling:**

3D structure modeling is the first step towards successful *in silico* drug designing. A suitable template with sufficient query sequence length coverage and sequence identity is not as yet available. Therefore, segment based homology modeling approach is used in this study. Fold recognition was employed for loop modeling in this procedure. BLAST and PSI BLAST were used for the selection of PDB templates and templates with > 40% sequence identity with the target sequence were selected (**Table 1 in supplementary material**). LOMETS (Local Meta Threading Server) was used to select templates for loop modeling. The server selects fold not only on the basis of secondary structure but also considers mutations, solvent accessibility and pairwise residue contacts [5]. Subsequently, the target sequence and templates were aligned using MODELLER 9v5 and thus, a 3D model was constructed. The constructed model was energy minimized in GROMACS force field using Steepest Descent Minimization Algorithms. The model was evaluated using PROCHECK, WHAT CHECK and RMSD, RMSF calculations. RMSD and RMSF calculations are good indicators of the uncertainty in the atomic coordinates and the favorable value is within 0.2 nm. PROCHECK validates the model for covalent bond distances, angles, atom nomenclature and stereo-chemistry [6]. What-check was used to evaluate the folding pattern of the model [7].

Ligand and receptor interaction:

Inhibitors are not yet available for c-jun. Therefore, it is important to design suitable inhibitors for c-jun. Ligands were designed using LIGAND SCOUT 2.02. The designed ligand and receptor interaction at desired position are performed using AUTODOCK version 1.5.2. Ligand analogues have been constructed using CHEMSKETCH and docking was performed by Molegro Virtual Docker (MVD). We used MVD because it showed higher docking accuracy than other state-of-the-art docking products (MVD: 87%, Glide: 82%, Surflex: 75%, FlexX: 58%) in the market [8]. During virtual screening, the following parameters were fixed: number of runs 10, population size 50, crossover rate 0.9, scaling factor 0.5, maximum iteration 2,000 and grid resolution 0.30. The docked results were evaluated on the basis of binding affinity, moledock score and re-ranking. Binding affinity

(kg/mol) is measured on the basis of known experimental data. The MolDock scoring function takes into consideration directional hydrogen bonding and charges. The docking scoring function, *E score*, is defined by the energy terms in equation 1 (see supplementary material). Re-rank-score in MVD provides an estimate for interaction.

Discussion:**Model evaluation:**

The constructed 3D model is shown in **Figure 1**. The results of PROCHECK parameters like Ramachandran plot (90.8 core, 7.1 allowed, 1.1 generous, 0.4 disallowed), main chain (6), side chain (5), bond length (97.8), bond angle (93.4) and list of all planar groups (100) were within favorable limits for the constructed model. The folding patterns evaluated by WHAT CHECK is similar to the comparative study of various tools for secondary structure prediction (GOR, JFRED, HNN, NN predict, PORTER, SOPMA and SSpro). Therefore, the constructed model holds good for folding patterns. The RMSD value calculated for model backbone after first fit to backbone at 9000 cycles (9ps time) gave a constant deviation of 0.125 nm. RMSF value calculated for the model was between 0.025-0.125 nm. The RMSD and RMSF values calculated were within the favorable limit of 0.2 nm.

Inhibition site:

Literature studies have shown that phosphorylation and consequently dephosphorylation brings about the activation of c-jun. The NH₂ terminal phosphorylation of c-jun in response to growth factors and UV rays is responsible for COOH terminal dephosphorylation. The NH₂ terminal amino acid that undergoes phosphorylation is serine 63, serine 73 and threonine 91. This initiates dephosphorylation of residues threonine 231, serine 243 and serine 249. Dephosphorylation decreases the electrostatic repulsions between the phosphate groups on c-jun and the DNA-binding site, hence enhancing c-jun binding on DNA [9-11]. Hence, the 3 phosphorylation sites should be inhibited

simultaneously in order to suppress the binding of c-jun on DNA and transcription of MMPs.

Ligand Design:

Novel ligands have been designed with ligand scout 2.02. The constructed ligand that showed interaction at desired phosphorylation inhibition sites by AUTODOCK were considered as reference ligand. We developed 3 reference ligands for the three inhibitory sites. Figure 2 shows the interactions of the reference ligand at the desired site on receptor. The reference ligand for site 1 shows interaction with ser 63 (desired) and asp 65. Similarly, reference ligand designed for site 2 shows interaction with ser 73 (desired) and glu77. The reference ligand for site 3 shows interaction with ser 83, gly 86, his 87, thr 90 and thr 91 (desired). The R₁ symbol on each reference ligand identifies the site, where desired interaction is seen between ligand and receptor. All the three reference ligands interacted well at the desired sites for corresponding phosphorylation.

Virtual screening:

The reference ligands however showed desired interaction on receptor but have low binding affinity with the receptor as demonstrated by MVD. Various analogues have been constructed by adding different functional groups (from + inductive effect to -inductive effect) at the sites R₁ and R₂ to obtain ligands having greater binding affinity with the receptor. Additional functional groups at R₁ increased the affinity of ligand at the desired inhibitory site. However, attachment of different functional groups at R₂ created an environment by interacting with neighboring residues as shown in **Figure 3**. The designed analogues also gave better calculations for binding affinity, moldock score and re-ranking score than their corresponding reference ligands, listed in **Table 2 (See supplementary material)**. Thus, the designed analogues showed interaction at desired inhibitory site with good docking score, for potential testing as a ligand.

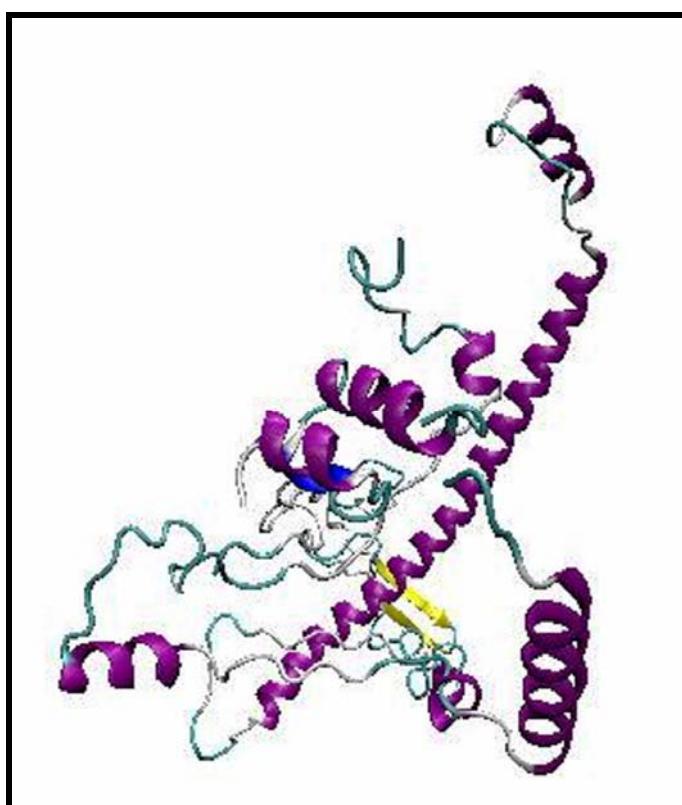


Figure 1: 3D model of c-jun. The model is generated using MODELLER.

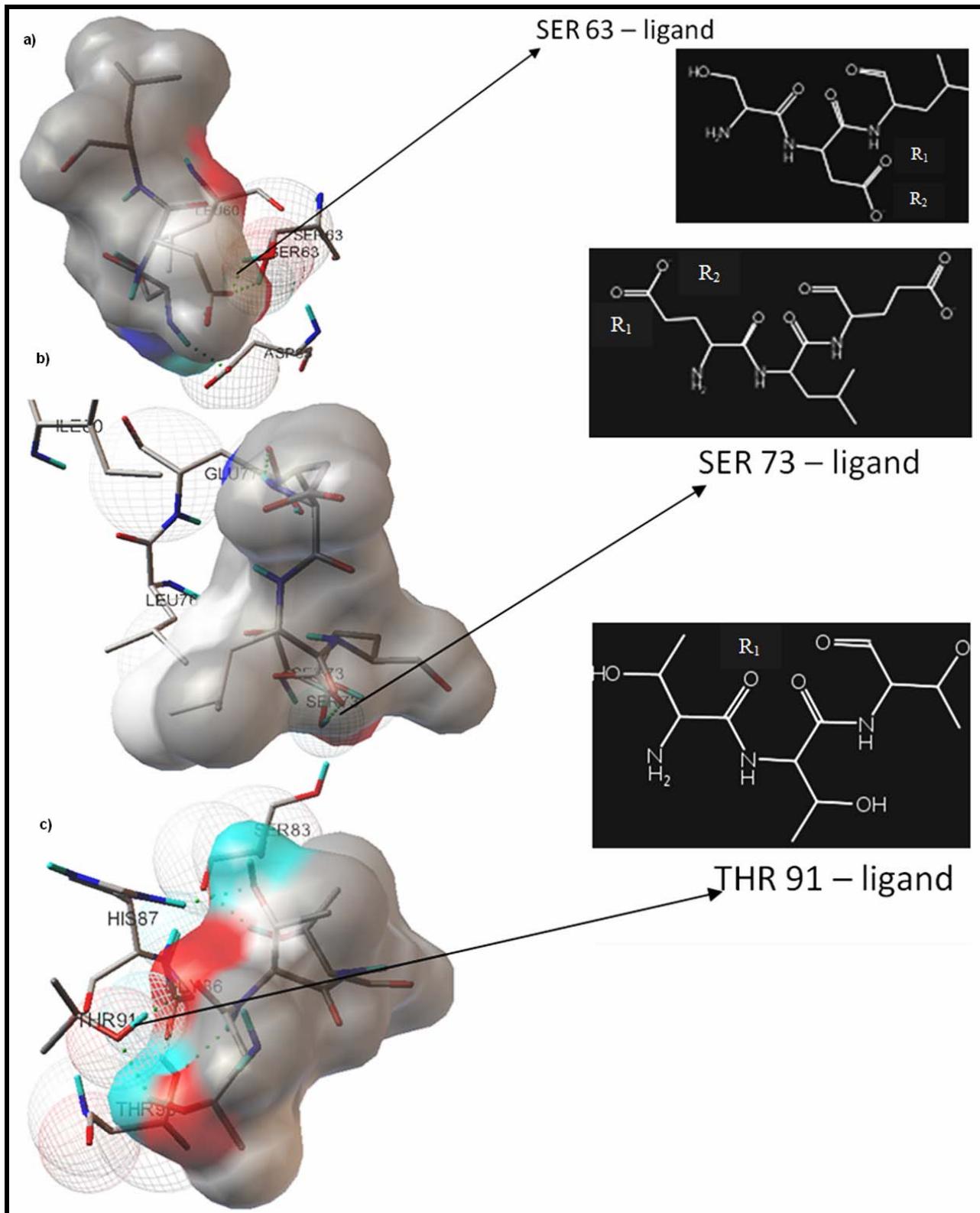
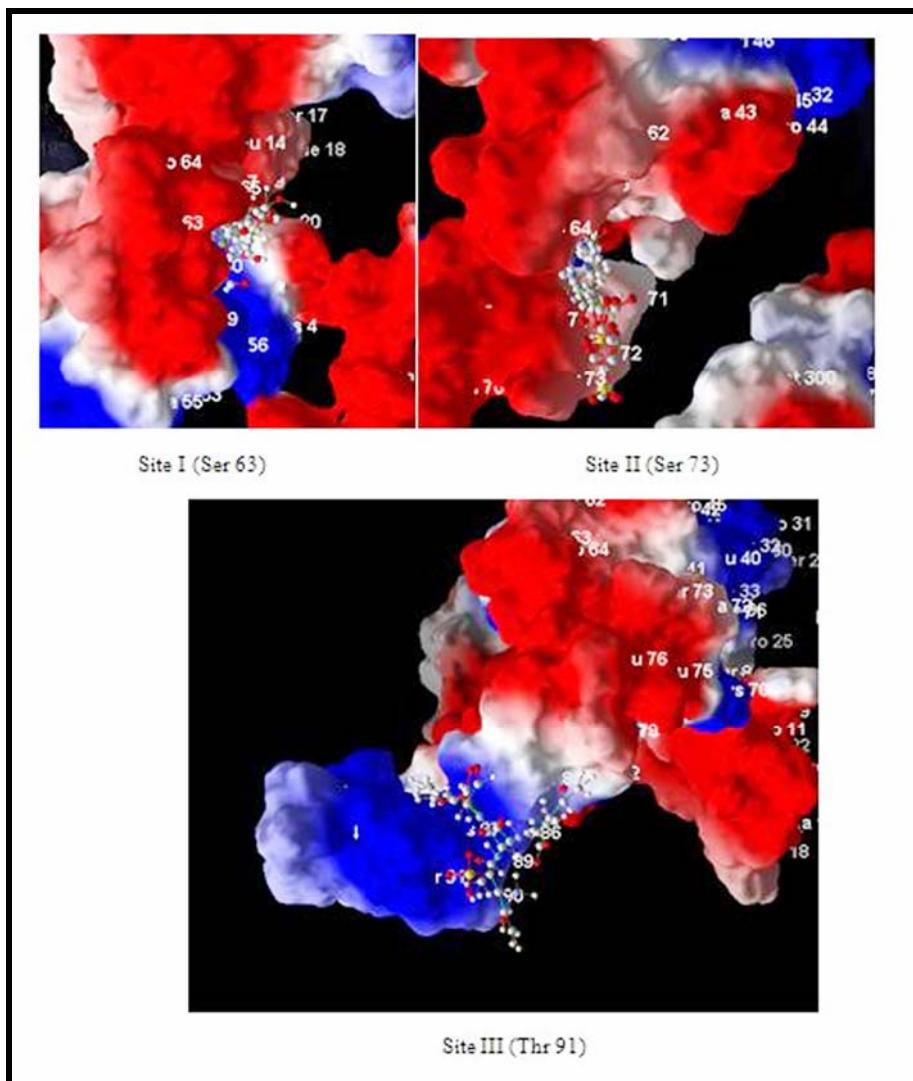


Figure 2: The docking view showing the binding of designed reference ligands with receptor at desired site.

**Figure 3:** The docking view of analogues and receptor for three different inhibition site.**Conclusion:**

Wrinkle formation is one of the most prominent signs of skin ageing. There is a tremendous demand for products that can completely inhibit collagen degradation. However, these products have side effects. Therefore, it is important to design inhibitors for targets like c-jun that are associated with wrinkle formation. Here, we show the use of modeling and virtual screening in the potential design of inhibitors for c-jun. It should be noted that these predicted data should be validated using suitable assays for further consideration in future studies.

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Supplementary material:

$$E_{\text{Score}} = E_{\text{inter}} + E_{\text{intra}}$$

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Equation 1

$$E_{\text{inter}} = \sum_{i \in \text{ligand}} \sum_{j \in \text{protein}} \left[E_{PLP}(r_{ij}) + 332.0 \frac{q_i q_j}{4r_{ij}^2} \right]$$

Where E_{PLP} is a piecewise linear potential and the second term describes the electrostatic interactions between charged atoms

$$E_{\text{intra}} = \sum_{i \in \text{ligand}} \sum_{j \in \text{ligand}} E_{PLP}(r_{ij}) + \sum_{\text{flexible bonds}} A[1 - \cos(m \cdot \theta - \theta_0)] + E_{\text{clash}}$$

Where double summation is between all atom pairs in the ligand, the second term is a torsional energy, θ is the torsional angle of the bond and E_{clash} , assigns a penalty of 1000 if the distance between two heavy atoms is less than 2.0 Å.

Table 1: List of PDB templates used for segment based homology modeling

Segment based Homology Modeling			
Tools used/purpose	Selected templates (PDB ID)	Length covered by PDB template on the protein sequence	Sequence/ fold identity between the PDB and reference protein sequence
BLAST & PSI BLAST (Selection of PDB templates for model construction)	2AMI 2PZJ 1FCY 1AF2 1JNM 1U7O	1-5 6-17 72-96 173-204 254-310 314-331	100% 66% 46% 40% 98% 50%
LOMETS (To fill the loops, regions not covered by BLAST PDB)	1ERD 2FFT 1DEV	30-69 97-170 206-246	> 50%

Table 2: Comparative study of binding affinity, moldock score and re-ranking score of reference ligand with analogues for three inhibitory sites

SITE I (ser 63)				
R1	R2	Binding affinity	Moldock score	Reranking score
O	O	-12.2805	-87.5834	-72.9544
COCl	O	-16.5375	-111.701	-84.5607
COCH3	O	-14.6547	-117.062	-83.3443
OSO3H	O	-13.0595	-120.418	-85.0854
COOEt	O	-16.4993	-120.514	-92.2664
C6H5O	O	-15.206	-122.575	-90.6628
H2OPO2	O	-13.9347	-130.74	-98.4258
C6H5OAc	O	-16.7329	-134.646	-99.2073
C6H5NO	O	-14.3909	-130.665	-95.9775
C6H5CHO	O	-11.7646	-134.708	-90.5285
C6H5OAc	CHO	-20.9032	-120.961	-93.2101
C6H5OAc	COCH3	-19.8917	-143.012	-103.415
C6H5OAc	ONO	-24.7791	-142.664	-103.735
C6H5OAc	OCHO	-19.8626	-146.693	-98.6499
C6H5OAc	SO2H	-19.0394	-144.512	-103.86
C6H5CHO	COOCH3	-19.4519	-124.963	-96.1255
C6H5CHO	CONH2	-17.2812	-144.175	-110.27
C6H5CHO	COCl	-19.116	-120.203	-89.3363
C6H5CHO	NO	-17.9934	-135.044	-103.948
C6H5CHO	OAc	-19.905	-138.993	-105.387
COPh	CONH2	-15.1967	-138.589	-101.398
COPh	COCl	-18.0481	-145.42	-101.962
COPh	NHAc	-14.2186	-147.456	-107.879
H2PO3	ONO	-15.8022	-121.942	-96.432
H2PO3	NHAc	-14.8584	-125.068	-97.9593
H2PO3	OAc	-20.6259	-128.349	-102.712
H2PO3	COOCH3	-20.9547	-129.354	-101.996

SITE II (ser 73)

R1	R2	Binding affinity	Moldock score	Reranking score
O	O	-10.0975	-97.968	-75.8075
H2PO3	O	-12.601	-100.64	-87.7476
NO	O	-12.4778	-110.199	-91.3151
COOPh	O	-19.5081	-114.199	-96.0297
OCH3	O	-15.5548	-115.03	-93.0747
OAc	O	-16.2034	-118.937	-94.9341
C6H5COOCH3	O	-20.6526	-119.826	-94.7233
C6H5OPO3H2	O	-20.0411	-105.614	-89.0779
C6H5SO2H	O	-17.75	-119.0791	-95.3803
C6H5OCHO	O	-20.1399	-124.793	-91.277
C6H5CHO	O	-18.8208	-129.938	-102.681
COOEt	CHO	-14.8759	-112.365	-93.4722
COOEt	COCl	-19.3856	-109.854	-94.6401
C6H5CHO	COOCH3	-24.4418	-116.134	-90.7422
C6H5CHO	COCH3	-25.0494	-110.399	-77.1874
C6H5CHO	COOEt	-24.4518	-109.6	-83.6871
C6H5CHO	CONH2	-22.3118	-107.413	-74.9499
C6H5OCHO	CHO	-21.9882	-121.115	-93.1812
C6H5OCHO	COOCH3	-27.3662	-116.949	-89.2061
C6H5OCHO	COCH3	-29.4138	-110.29	-75.225

SITE III (Thr 91)

R1	Binding affinity	Moldock score	Reranking score
O	-10.6525	-62.9956	-42.2497
COOCH3	-13.8865	-93.0221	-70.5025
COPh	-12.9626	-86.2756	-66.2633
ONO	-14.2588	-93.4583	-70.182
OAc	-13.6479	-93.0005	-70.1516
SO2H	-10.0113	-93.6338	-68.6467
NHAc	-10.2655	-94.0558	-74.6504
OSO3H	-11.8516	-96.7543	-71.6728
OPO3H2	-13.4183	-97.025	-68.2773
C6H5CHO	-11.9956	-98.2812	-74.4093
C6H5NO	-13.3098	-102.005	-73.7687
C6H5COCH3	-13.6466	-102.861	-75.4764
C6H5OAc	-14.6636	-112.187	-83.7989
C6H5COOCH3	-14.5597	-112.00	-78.0367
COOPh	-14.2241	-115.306	-83.0351