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Designing of Protein Kinase C β-II Inhibitors against Diabetic complications: Structure Based Drug Design, Induced Fit docking and analysis of active site conformational changes

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Abstract:
Protein Kinase C β-II (PKC β-II) is an important enzyme in the development of diabetic complications like cardiomyopathy, retinopathy, neuropathy, nephropathy and angiopathy. PKC β-II is activated in vascular tissues during diabetic vascular abnormalities. Thus, PKC β-II is considered as a potent drug target and the crystal structure of the kinase domain of PKC β-II (PDB id: 2I0E) was used to design inhibitors using Structure-Based Drug Design (SBDD) approach. Sixty inhibitors structurally similar to Staurosporine were retrieved from PubChem Compound database and High Throughput Virtual screening (HTVs) was carried out with PKC β-II. Based on the HTVs results and the nature of active site residues of PKC β-II, Staurosporine inhibitors were designed using SBDD. Induced Fit Docking (IFD) studies were carried out between kinase domain of PKC β-II and the designed inhibitors. These IFD complexes showed favorable docking score, glide energy, glide emodel and hydrogen bond and hydrophobic interactions with the active site of PKC β-II. Binding free energy was calculated for IFD complexes using Prime MM-GBSA method. The conformational changes induced by the inhibitor at the active site of PKC β-II were observed for the back bone Cα atoms and side-chain chi angles. PASS prediction tool was used to analyze the biological activities for the designed inhibitors. The various physicochemical properties were calculated for the compounds. One of the designed inhibitors successively satisfied all the *in silico* parameters among the others and seems to be a potent inhibitor against PKC β-II.

Keywords: PKC β-II, HTVs, SBDD, IFD, Prime MM-GBSA, Chi angles, ADME/Tox

Background:
The PKC family belongs to the AGC superfamily of serine/threonine kinase. The PKC enzymes play a key role in intracellular signal transduction for specific hormonal, neuronal, and growth factor stimuli **[1-2].** This family comprises more than ten isoforms categorized into three classes (classical, novel, and typical) based on their structure and cofactor regulation **[3-7].** PKC β-II is a predominant isozyme activated in vascular tissues during hyperglycemia, the underlyingfactor for diabetic vascular abnormalities. These classes have conserved N-terminal regulatory region (C1 and C2) and C-terminal catalytic region or kinase domain. The N and C terminal domains are connected by a proteolytically labile hinge sequence **[8].** The catalytic or kinase domain requires three sites

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of phosphorylation for full activation of the PKC **[9, 10].** Phosphorylation of threonine 500 (Thr 500) occurs in the activation loop, which subsequently leads to autophosphorylation at threonine 641 (Thr 641) in the turn motif and serine 660 (Ser 660) in the hydrophobic motif **[11, 12].**

The classical PKC (α, β-I, β-II, and γ) is activated by DAG, phosphatidylserine (PS), and calcium. These enzymes contain two cysteine-rich zinc finger-like motifs in C1 region, which are essential for phorbol ester binding and for interacting with DAG, and a putative calcium binding domain in C2 region. The novel PKC (δ, ε, η, and θ) requires DAG and PS, but is insensitive to calcium. Since, the C2 region (calcium binding domain) is absent in the novel PKC, they are generally activated by DAG and PS. The typical PKC (ζ and 1/ λ) isozymes require only PS but neither DAG nor calcium for full activation. In the structural features, one of these cysteine-rich zinc finger-like motifs in the C1 region and calcium binding domain in the C2 region are missing in the typical PKC **[13-14].**

PKC β-II is an essential isozyme among the PKC isoforms, which is activated in vascular tissues during hyperglycemia–induced situation, leading to diabetic complications **[15-17].** This enzyme regulates in the pathogenesis of diabetic complications such as retinopathy, nephropathy, neuropathy, angiopathy, and cardiac dysfunction **[18].** The PKC β-II activation may increase retinal endothelial permeability, basement membrane protein synthesis, and stimulate angiogenesis **[19-21]**. When the diabetic animals were treated with PKC β-II inhibitors, pathological changes such as reduced retinal blood flow, increased retinal mean circulation time, and diabetes-induced retinal vascular permeability and leakage were observed **[22, 23].**

The first structural information of catalytic or kinase domain of PKC β-II with bound bisindolylmaleimide (BIS), a potent and competitive inhibitor of ATP is available in the Protein Data Bank (2I0E). Our study aims to evaluate the structural features of PKC β-II that provides essential information for developing potent inhibitors using SBDD approach. Five stranded β sheets (β1-β5) in the N-terminal lobe (residues 339-421) and two α helices (αB and αC) in the C-terminal lobe (residues 426-620) constitutes the kinase domain of PKC β-II. These two terminal lobes are connected by a linker region defined by residues 422-425 **[24].** The designed SBDD inhibitors (SBDDIs) were subjected for Induced Fit Docking followed by Prime MM-GBSA evaluations. These results were compared with IFD of BIS-PKC β-II complex. Finally, the conformational changes (Cα and χ (chi) angles) and interactions studies (hydrogen bond and hydrophobic) were analyzed in the PKC β-II-SBDDIs complexes. These designed inhibitors may be potent in the *in vitro* and *in vivo* studies of diabetes complications and may help to design new potent inhibitors for PKC β-II in future.

Methodology:

Protein preparation

The crystal structure of human PKC β-II with BIS inhibitor complex was retrieved from the Protein data bank (PDB: 2I0E) for HTVs and IFD studies. The raw crystal structure consisted of a dimer (A and B chains) molecule with waters where bond orders, topologies, formal atomic charges, ionization, and tautomeric states were not available. The A chain of PKC β-II alone was taken for further molecular modeling studies. The A chain was employed for protein preparation where removing waters, assigning H-bond, formal charges, ionization, and tautomeric states were carried out. Moreover, the amino acid flips were assigned and non Hydrogen atoms were literately minimized until RMSD reaches 0.3Å.

Ligand energy minimization

Staurosporine is a natural compound **[25]**, which is uniquely potent as an inhibitor against PKC in *in vitro* studies **[26, 27]**. Based on the ADME/TOX properties, the Staurosporine (PubChem Compound: CID 44259) related sixty molecules (data not shown) were obtained from PubChem Compound database. Using Ligprep module, all the compounds were energy minimized by addition of hydrogens, 2D to 3D conversion, realistic bond lengths and bond angles, low energy structure with correct chiralities, ionization states, tautomers, stereochemistries and ring conformation (Schrodinger LLC 2009, USA).

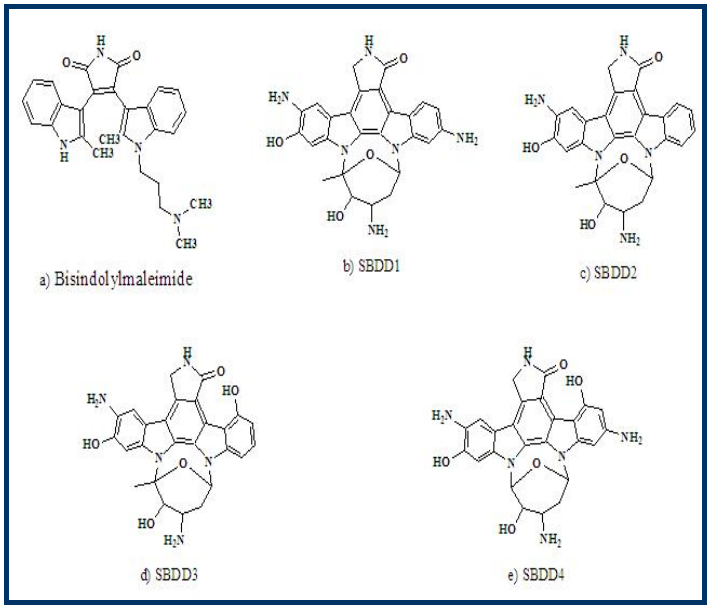

Figure 1: Chemical diagrams of Bisindolylmaleimide and inhibitors used in the SBDD study.

High Throughput Virtual screening

HTVs was carried out against the active site key residues Glu 421, Val 423, Thr 404, and Asp 470 of PKC β-II **[24]** for filtering the sixty compounds. HTVs was performed by Glide module that requires a previously calculated grid receptor with a set of ligands. During the receptor grid generation process, the energy minimized complex of PKC β-II with BIS was loaded in the workspace; the active site of the receptor was calculated automatically by picking the BIS. The grid box represents the shape and properties of the receptor active site that provides progressively more accurate scoring function and energy of complex, when the ligand is docked. Glide searches for favorable interactions between one or more ligands and the active site receptor. In HTVs, the grid allows the ligand binding where more than one possible and meaningful conformations were generated with the rigid receptor. However, van der Waals radii of non-polar atoms were calculated using Glide to decrease close contact penalties between the ligands and the active site residues. The docking score glide energy, glide

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emodel and non-bonded interactions were calculated in the HTVs results. Based on these results, the active site residues of PKC β-II and inhibitors synthetic viability, four new inhibitors were designed using SBDD approach **(Figure1)**. The designed SBDD inhibitors were energy minimized using Ligprep.

Induced Fit Docking studies

Upon any ligand binding, many proteins usually undergo side-chain or back bone (Cα atom) movements or both in reality. But in the conventional docking methods, as the receptor is held rigid and the ligand is free to move, these methods may provide misleading results. Hence to overcome these problems, the IFD (flexible docking) methodology was employed using Glide software (Schrödinger LLC 2009, USA). In SBDD studies, IFD is one of the essential factors to predict accurate ligand binding and concomitant structural movements in the active site of the receptor **[28]**. The human PKC β-II- BIS complex was energy minimized and subjected for IFD with the SBDDIs. The grid was generated to specify the active site by choosing the BIS in the receptor. In IFD study, the active site of PKC β-II conforms more closely to the shape and binding mode of the ligand. The energetically favorable docked complexes were obtained and the best poses were chosen by docking score, glide energy, and glide emodel. The hydrogen bond and hydrophobic interactions were analyzed using Ligplot **[29]** and PyMol **[30].**

Binding free energy calculation

The ligand binding energies and ligand strain energies were calculated for the SBDDIs and BIS inhibitor using Prime MM-GBSA panel (Schrödinger LLC 2009, USA). In this study, the best pose from the IFD complexes was chosen to obtain the binding free energy calculation. The combination of parameters viz., OPLS-AA, molecular mechanics energies (E_{MM}), an SGB solvation model for polar solvation (G_{SGB}), and a nonpolar solvation term (G_{NP}) composed of the nonpolar solvent accessible surface area and van der Waals interactions were subjected for carrying out Prime MM-GBSA. The total binding free energy: ΔGbind = Gcomplex – (Gprotein + Gligand)

Prediction of biological activity and ADME

PASS (Prediction of Activity Spectra for Substances) is a prediction tool for predicting the biological activity of the chemical structures on the basis of structural orientation **[31, 32].** The SBDDIs were tested for their biological activity using PASS. Moreover, Using Qikprop3.2 module, the energy minimized ligands were evaluated for their Absorption, Distribution, Metabolism, and Excretion (ADME) properties. Qikprop is quick, accurate and predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules. It provides the ranges of molecular predicting properties for comparing the properties of a particular molecule with those of 95% of known drugs (Schrödinger LLC 2009, USA).

Conformational changes of Cα and χ angles

The crystal structure of PKC β-II (2I0E_A) and the IFD complexes have been taken for analyzing conformational changes of the active site Cα atoms. The four IFD complexes were superimposed with 2I0E_A reference structure for matching the structure based alignment using Chimera **[33].** Hence, Needleman-Wunsch algorithm and BLOSUM-62 matrix were used to align amino acid residues, followed by the alignment of the helix and strand Cα atoms of the crystal structure with the respective helix and strand Cα atoms of the IFD complexes. Then, the active site residues of side-chain chi angles (χ_1 (N-Cα-Cβ-Cγ), χ_2 (Cα-Cβ-Cγ-Cδ), χ_3 (Cβ-Cγ-Cδ-Cε), and χ_4 (Cγ-Cδ-Cε-Cζ)) were analyzed with all the SBDD complexes for understanding conformational changes at the active site of PKC β-II. The side-chain chi angles were calculated and analyzed using Chimera and MolProbity **[34]** software.

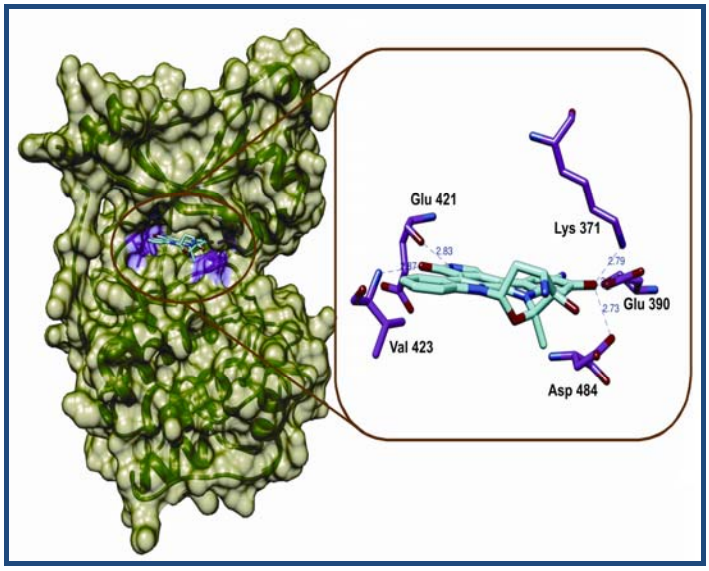

Figure 2: Hydrogen bond interactions (blue dot line) of SBDD2 with active site residues

Results and Discussion:

BIS inhibitor was redocked at the active site of PKC β-II. The binding of BIS - PKC β-II complex has closely mimicked the crystal structure of PKC β-II (2I0E_A). This inhibitor has maintained three hydrogen bond interactions with Glu 421 (O), Val 423 (N) and Thr 404 (OG1) residues as found in the crystal structure. Hydrophobic interactions were observed viz Leu 348, Gly 349, Phe 353, Val 356, Ala 369, Met 420, Tyr 422, Asn 471, Met 473, Ala 483, and Asp 484 with a docking score -8.67, glide energy -57.66, and glide emodel -84.85. The binding free energy was -55.27 Kcal/mol **Table 1 (see supplementary material)**. The superimposition of crystal and docked complexes has yielded rms deviation of 0.19Å.

SBDD1 bound PKC β-II complex has docking score -10.71, glide energy -62.59, and glide emodel -103.57. Upon inhibitor binding, hydrogen bond interactions with Lys 371 (NZ), Glu 390 (OE2), Glu 421 (O) and Val 423 (N) residues and hydrophobic interactions with Phe 353, Ala 369, Thr 404, Met 420, Tyr 422, Met 473 and Asp 484 were observed at the active site. The inhibitor O4 atom formed two hydrogen bond interactions with Glu 390 (OE2), 2.76Å and Lys 371 (NZ), 3.05Å. The binding free energy was -47. 7 Kcal/mol **Table 1 (see supplementary material)**. The PASS has predicted PKC inhibitor activity for the SBDD1.

SBDD2 has five hydrogen bond and five hydrophobic interactions with Lys 371 (NZ), Glu 390 (OE2), Glu 421(O), Val 423 (N) and Asp 484 (OD1) and Phe 353, Ala 369, Met 420, Tyr 422 and Met 473, respectively at the active site of PKC β-II. The inhibitor O4 atom has three hydrogen bond interactions with

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the active site residues. The O4 atom act as a hydrogen bond acceptor with Lys 371 (NZ), (2.79Å) and a bifurcated donor with Asp 484 (OD1), (2.73Å) and Glu 390 (OE2), (3.31Å) in the H-bond interactions **(Figure 2)**. The inhibitor binding exhibits a docking score -10.93, glide energy -64.74 and glide emodel -108.30. The binding free energy was found to be -57.80 Kcal/mol for this complex **Table 1 (see supplementary material)**. SBDD2 has showed PKC inhibitor activity by PASS prediction.

Docking score -11.54, glide energy -63.92, and glide emodel -110.88 were obtained in the SBDD3 bound PKC β-II complex. This inhibitor has maintained six hydrogen bond interactions with Lys 371 (NZ), Glu 390 (OE2), Glu 421(O), Val 423 (N & O), and Asp 484 (OD2) and five hydrophobic interactions with Leu 348, Phe 353, Val 356, Ala 369 and Met 420 at the active site of PKC β-II. The inhibitor O4 atom has three hydrogen bond interactions that have shown a hydrogen bond acceptor with Lys 371 (NZ), (2.69Å) and a bifurcated donor with Asp 484 (OD1), (2.66Å) and Glu 390 (OE2), (3.16Å). The binding free energy -53.14 Kcal/mol was obtained in the SBDD3 bound conformation in the PKC β-II **Table 1 (see supplementary material)**. PASS confirmed that this inhibitor has potential inhibitor activity against PKC.

SBDD4 docked orientation at the active site of PKC β-II has shown four hydrogen bond interactions with Lys 371 (NZ), Thr 404 (OG1), Glu 421 (O), and Tyr 422 (OH) and six hydrophobic interactions with Leu 348, Gly 349, Phe 353, Val 356, Met 473, and Asp 484. Docking score -11.26, glide energy -67.94, and glide emodel -105.06 were observed in this complex. The binding free energy between the SBDD4 and the active site of PKC β-II was calculated to be -48.67 kcal/mol **Table 1 (see supplementary material)**. The ligand has PKC inhibitor activity that was observed using PASS.

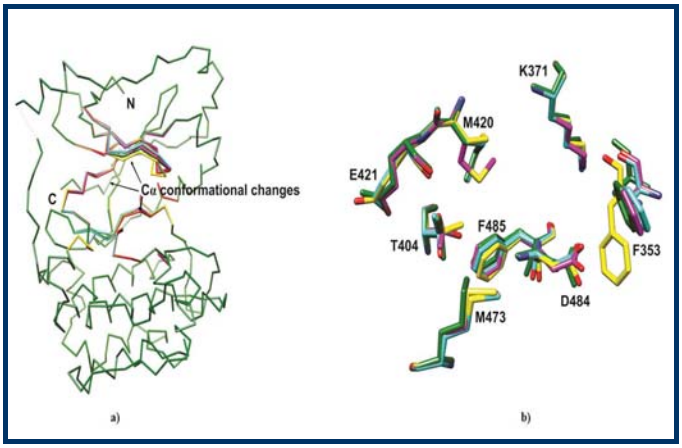

Figure 3: a) Superimposed docked structures of PKC β-II in ribbon representation. **b)** Side-chain conformational changes in the PKC β-II complexes. BIS, SBDD1, 2, 3, and 4 bound complexes are colored in red, green, magenta, cyan and yellow, respectively

Conformation changes of Cα and side-chain χ angles

The Cα conformational changes were analyzed for all the SBDDIs complexes with crystal structure of PKC β-II. SBDD 1, 2, 3, and 4 complexes showed RMSD values 0.255 Å, 0.226 Å, 0.244 Å, and 0.213Å, respectively **(Figure 3a).** The side-chain conformational changes were analyzed by measuring chi angles at the active site residues for all the complexes. Leu 348, Val 356, Glu 390, Leu 394, Tyr 422 and Val 423 residues showed slight side-chain conformational changes. However, Phe 353, Lys 371, Thr 404, Met 420, Glu 421, Met 473, Asp 484 and Phe 485 residues showed more side-chain conformational changes **(Figure 3b, Table 2 (see supplementary material).** These results have shown that SBDDIs induced conformational changes at the active site.

Analysis of physicochemical properties

The physicochemical properties were calculated for SBDDIs using Qikprop simulation. The molecular weight of the designed inhibitors are less than 500Da (expect SBDD4) and QPlogPo/w (octanol/water partition coefficient) for all the inhibitors is less than five. The partition coefficient of octanol/gas (QPlogPoct), water/gas (QPlogPw) and brain/blood (QPlogBB) values are satisfied by all the SBDDIs. The aqueous solubility (QPlogS) and the skin permeability (QPlogKp) were predicted for the inhibitors which are in the allowed solubility and permeability range **Table 3a (see supplementary material)**. Total solvent accessible surface area (SASA), hydrophobic component of the SASA (FOSA) and hydrophilic component of the SASA (FISA) were predicted for the inhibitors that were abiding the ranges specified in the Qikprop. Qualitative human oral absorption was predicted where SBDD2 and 3 have favorable oral absorption score of 3 and 2 respectively. However, SBDD1 and 4 have shown low oral absorption (score: 1). Polar nitrogen and oxygen van der Waals surface area (PSA) of SBDDIs fulfill the limit in physicochemical calculation **Table 3b (see supplementary material)**.

Conclusion:

In this study, sixty inhibitors similar to Staurosporine were screened and based on the screening and the PKC β-II active site residues four new inhibitors were designed using SBDD approach. These inhibitors were subjected for IFD studies to observe the interactions between the SBDDIs and the active site of PKC β-II. These inhibitors were compared with redocked BIS- PKC β-II complex using docking score, glide energy, glide emodel, hydrogen bond and hydrophobic interactions, and binding free energy. All the SBDDIs have better docking score, glide energy and glide emodel compared to BIS bound PKC β-II complex. However, BIS, SBDD2 and SBDD3 showed good binding free energy. Hydrogen bond and hydrophobic interactions were analyzed for SBDDIs that have exhibited favorable interactions with the active site residues.

In physicochemical properties, all the SBDDIs showed favorable results. All the inhibitors satisfied partition coefficient of QPlogPoct, QplogPw and QplogBB and surface area calculations of SASA, FOSA, FISA, and PSA. According to molecular weight calculation, SBDD1, 2 and 3 compounds are below 500 Da. The human oral absorption properties showed that SBDD1 and SBDD4 have low oral absorption. SBDD3 compound has favorable oral absorption. Surprisingly, SBDD2 has high oral absorption value in the physicochemical properties calculations. SBDD1, 2, and 3 compounds satisfied all the *in silico* parameters like docking score, glide energy, glide emodel, binding free energy, ADME, hydrogen bond and hydrophobic interactions. Hence, SBDD2 has more potent

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inhibition in the PKC β-II and favorable physicochemical properties than all the designed inhibitors. The potent inhibition of SBDD2 could be revealed through *in vivo* and *in vitro* studies and it may be used to design new potent inhibitors against diabetes complications.

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Supplementary material:

Table 1: Docking score, glide energy, glide emodel, hydrogen bond interactions and binding free energy of Induced fit docked complexes

Inhibitor	Inhibitor atom	H-Bond interaction	Residues	Distance of D...A (Å)	Docking score	Glide energy (Kcal/mol)	Glide emodel (Kcal/mol)	ΔG bind (Kcal/mol)
BIS	O20	O-H…N	VAL 423 (N)	2.93	-8.67	-57.66	-84.85	-55.27
	N13	N…H-O	GLU 421(O)	2.91				
	O14	O…H-O	THR 404 (OG1)	3.22				
SBDD1	O2	O…H-N	VAL 423 (N)	2.83	-10.71	-62.59	-103.57	-47.47
	N3	N-H…O	GLU 421 (O)	2.65				
	O4	O-H…O	GLU 390 (OE2)	2.76				
	O4	O…H-N	LYS 371 (NZ)	3.05				
SBDD2	O2	O…H-N	VAL 423 (N)	2.87	-10.93	-64.74	-108.30	-57.80
	N3	N-H…O	GLU 421 (O)	2.83				
	O4	O-H…O	ASP 484 (OD1)	2.73				
	O4	O…H-N	LYS 371(NZ)	2.79				
	O4	O-H…O	GLU 390 (OE2)	3.31				
SBDD3	O5	O-H…O	VAL 423(O)	2.60	-11.58	-63.92	-110.88	-53.14
	O2	O…H-N	VAL 423 (O)	2.94				
	N3	N-H…O	GLU 421 (O)	2.79				
	O4	O-H…O	ASP 484(OD2)	2.66				
	O4	O…H-N	LYS 371 (NZ)	2.69				
	O4	O-H…O	GLU 390 (OE2)	3.16				
SBDD4	N3	N-H…O	GLU 421(O)	2.84	-11.26	-67.94	-105.06	-48.67
	N5	N…H-N	LYS 371 (NZ)	3.04				
	N4	N…H-O	TYR 422 (OH)	2.92				
	O2	O…H-O	THR 404 (OG1)	3.04				

Table 2: Docked complexes side-chain Chi angles (χ1, χ2, χ3, and χ4) conformational changes

Residues	SBDD1				SBDD2				SBDD3				SBDD4			
	χ1	χ2	χ3	χ4	χ1	χ2	χ3	χ4	χ1	χ2	χ3	χ4	χ1	χ2	χ3	χ4
Leu 348	294.8	168.3	-	-	293.6	165.3	-	-	299.7	174.8	-	-	292.5	170.1	-	-
Phe 353	45.8	84.0	-	-	49.4	82.9	-	-	49.4	84.6	-	-	309.0	105.9	-	-
Val 356	187.0	-	-	-	184.9	-	-	-	188.4	-	-	-	185.6	-	-	-
Lys 371	153.2	166.7	161.9	157.7	183.2	186.0	182.7	183.9	159.1	169.6	169.0	167.5	158.8	170.4	173.4	170.5
Glu 390	176.5	165.1	12.7	-	176.6	158.9	23.0	-	175.7	160.8	22.0	-	176.3	166.1	4.5	-
Leu 394	278.4	166.9	-	-	275.8	161.5	-	-	279.6	168.8	-	-	284.4	172.9	-	-
Thr 404	310.0	-	-	-	43.8	-	-	-	309.0	-	-	-	195.6	-	-	-
Met 420	300.2	289.4	299.1	-	217.7	162.1	292.2	-	294.5	293.5	296.4	-	309.7	306.8	297.7	-
Glu 421	93.5	188.4	129.2		86.6	186.2	137.9	-	157.9	187.6	56.3	-	158.6	184.5	53.4	-
Tyr 422	182.7	55.5	-	-	181.4	53.8	-	-	184.4	56.4	-	-	189.2	58.1	-	-
Val 423	183.4	-	-	-	184.8	-	-	-	182.3	-	-	-	180.2	-	-	-
Met 473	288.1	174.2	283.4	-	293.0	157.2	194.5	-	311.4	189.9	183.0	-	292.5	166.9	189.3	-
Asp 484	156.6	52.2	-	-	208.9	167.2	-	-	195.5	119.8	-	-	171.6	54.5	-	-
Phe 485	296.1	89.6	-	-	302.0	104.2	-	-	295.7	94.6	-	-	293.0	92.0	-	-

Table 3a: Calculation of Lipinski's rule of five, partition coefficient and skin permeability

Inhibitor (Range)	MW (130.0-725.0)	Volume (500.0 – 2000.0)	donorHB(†) (0.0 – 6.0)	acceptHB(†) (2.0 – 20.0)	QPlogPo/w (–2.0 – 6.5)	Rule of Five ($)	QPlogPoct (8.0 – 35.0)	QPlogPw (4.0 -45.0)	QPlogS (–6.5 –0.5)	QPlogBB (–3.0 –1.2)	QPlogKp (–8.0 -1.0)
SBDD1	484.51	1246.35	8	8.7	-0.13	2	31.8	23.8	-2.5	-2.3	-8.5
SBDD2	469.49	1217.78	6.5	7.7	0.79	2	28.7	20.7	-2.8	-1.6	-7.3
SBDD3	485.49	1243.20	7.5	8.4	0.31	2	31.0	22.8	-2.6	-1.9	-7.7
SBDD4	500.51	1271.56	9	9.4	-0.60	2	33.5	25.9	-2.3	-2.6	-8.9

Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution. Values are averages taken over a number of configurations, so they can be non-integer. $ Number of Lipinski's rule of five violations.
QPlogPoct, QPlogPw, QPlogS and QPlogBB were predicted partition coefficient of octanol/gas, water/gas, aqueous solubility and brain/blood, respectively. QPlogKp was predicted skin permeability (log *Kp*).

Table 3b: Surface area calculation and Human oral absorption

Inhibitor(Range)	SASA (300.0 – 1000.0)	FOSA (0.0 – 750.0)	FISA (7.0 – 330.0)	Human oral absorption	PSA (7.0 – 200.0)
SBDD1	655.22	161.71	326.20	1	169.69
SBDD2	637.62	163.66	264.44	3	142.25
SBDD3	654.84	163.92	289.44	2	158.27
SBDD4	670.18	164.12	350.17	1	184.17

SASA: Total solvent accessible surface area, FOSA: Hydrophobic component of the SASA (saturated carbon and attached hydrogen), FISA: Hydrophilic component of the SASA (SASA on N, O, and H on heteroatoms), PSA: Van der Waals surface area of polar nitrogen and oxygen atoms.

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