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Computational designing of novel inhibitors to Bcl-B, an anti-apoptotic protein, using fragment-based drug designing approach

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Abstract

Apoptosis is a major cell death mechanism in multi-cellular organisms. Bcl-B (also known as Bcl2-L-10) is an anti-apoptotic protein and protects cells from apoptosis upon sequestering pro-apoptotic Bax protein. BIM, the BH3-only protein, binds on the BH3-binding groove of the protein and promotes Bax-mediated apoptosis. In this context, we retrieved 14 BH3-mimetics reported to the Bcl-B to date and the compounds were used for designing novel inhibitor to the protein using fragment-based drug designing (FBDD) method. The 14 BH3-mimetics showed 12 unique scaffolds and 51 non-redundant fragments. The 14 parent compounds, the 12 scaffolds and 51 fragments were docked on the BH3-binding groove of the protein and comprehensive analysis of the docking data resulted in a scaffold (1-phenyl-1H-pyrrole-2,5-dione) and a set of fragments, which were effectively used to design tens of novel small molecular compounds by means of CombiGlide. High-throughput virtual screening of the novel compounds on the binding groove of the protein brought into fore a de novo antagonist, (5-amino-2-(ethylamino)-N-(2-hydroxyphenyl)-3-(1-phenyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)benzenesulfonamide), which showed binding affinities on the Bcl-B about two folds greater than the binding affinities of the 14 parent compounds reported in the literature.

Keywords: Apoptosis, Bcl-B, CombiGlide, Fragment Based Drug Design, HTVS.

Introduction

Cancer, one of the leading causes of death in developing and developed countries, has no proper cure to date (http://www.who.org). In general, cancers are due to uncontrolled cell proliferations and impairment of apoptosis (L. PUSZTAI & al. 1996 [1]). Hence, resisting the apoptosis is one of the important hallmarks of cancers (D. HANAHAN & al. 2000 [2]). The apoptosis is an essential cellular process to maintain the cell and tissue homeostasis (N.N. DANIAL & al. 2004 [3] and the apoptotic pathways have been governed by intrinsic and extrinsic mechanisms (K.C. ZIMMERMANN & al. 2001 [4]). Bcl-2 proteins are the major players of the intrinsic pathways and the proteins are classified into three major classes: anti-apoptotic proteins, pro-apoptotic proteins and BH3-only proteins (J.M. ADAMS & al. 1998 [5]; J.C. REED, 1998 [6]). Delicate balance among these proteins keeps the apoptosis under the tight control, failing which leads to cause cancer or neurodegenerative diseases. Over expressions of various anti-apoptotic proteins have been reported in different types of cancers. Though the mechanism(s) by which anti-apoptotic proteins sequester the pro-apoptotic proteins have not yet been clearly addressed (J.E. CHIPUK & al. 2008 [7]; A. VILLUNGER & al. 2011 [8]), the compounds specifically binding on the BH3-groove of the anti-apoptotic proteins have been demonstrated to act as efficient anti-cancer compounds (P.H. BERNARDO & al. 2008 [9]; P.H. BERNARDO & al. 2010 [10]; P.H. BERNARDO & al. 2011 [11]; D. SIVAKUMAR & al. 2011 [12]; D. SIVAKUMAR & al. 2012 [13]).

The Bcl-B, one of the anti-apoptotic proteins, has been found to be overexpressed in breast, prostate, gastric, colorectal, lung and adenocarcinoma cancers (N. KE & al. 2001 [14]; M. KRAJEWSKA & al. 2008 [15]). The protein showed poor sequence similarities with other members of the Bcl-2 family (N. KE & al. 2001 [14]) and depicted specific interactions with Bax but not with Bak (D. ZHAI & al. 2008 [16]) implying that the Bcl-B plays an essential roles in the Bax-mediated apoptosis, exclusively. Moreover, the protein becomes pro-apoptotic protein upon interacting with an orphan nuclear receptor, Nur77 (F. LUCIANO & al. 2007 [17]). These findings clearly suggest that designing specific antagonists to the protein is essential to avoid side-effect in chemotherapeutic treatments of cancers that exclusively overproduce the Bcl-B. To date, 14 BH3-mimetics have been reported to the protein. In the present study, we have thoroughly studied the binding affinities and modes of structural interactions of the 14 small molecular chemical compounds and their unique scaffolds and fragments on the BH3-binding groove of the protein. Comprehensive analysis of the data uncovered a scaffold (1-phenyl-1H-pyrrole-2,5-dione) and a set of fragments (refer results and discussion), which were effectively used to design tens of novel small molecular compounds by means of CombiGlide (Schrodinger Inc, USA). High-throughput virtual screening of the novel compounds on the binding groove of the protein brought into fore a *de novo* antagonist (5-amino-2-(ethylamino)-N-(2-hydroxyphenyl)-3-(1-phenyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)benzenesulfonamide), which showed remarkably stronger binding affinities on the Bcl-B than the binding affinities of the 14 compounds reported in the literature to date. Structural features of the *de novo* compounds on interacting with the protein have been discussed in detail.

Materials and Methods

Retrieval of BH3-mimetics, scaffold identifications and fragment generations

The 14 small chemical molecular BH3-mimetics to Bcl-B reported in PubChem bioassay database (www.ncbi.nlm.nih.gov/pcassay) have been retrieved and the compounds were fragmented using RECAP (Retrosynthetic Combinatorial Analysis Procedure) algorithm (X.Q. LEWELL & al. 1998 [18]). The RECAP program uses a set of 11 cleavage rules for cleaving the active structures into the privileged fragments. The fragments generated were in 'SMILE' format by default, which were converted into pdb formats and then energy minimized by means of ArgusLab (http://www.arguslab.com) and Gromacs (B. HESS & al. 2008 [19]), respectively. The scaffolds of the compounds were identified by Scaffold Hunter 2.0 (S. WETZEL & al. 2009 [20]). Ligand efficiency of each BH3-mimetic retrieved from PubChem was determined by dividing binding affinity/GlideScore of a ligand by total number of non-hydrogen atoms present in the respective compound. Binding affinities (EC_{50}) and GlideScores of the compounds were obtained from the PubChem database and from molecular docking studies carried out in the present study, respectively.

Designing of novel compounds and HTVS studies

Glide, commercial molecular docking software (R.A. FRIESNER & al. 2004 [21]), was used to carry out the molecular interactions between the protein and small molecules. First step in the protein docking is protein preparation, in which hydrogen atoms were added to the protein structure and the resultant structure was energy minimized using OPLS2005 force field with the convergence cutoff of 0.3Å RMSD. Second, grid was set in such a way to cover the entire BH3-binding groove of the Bcl-B. Third, small chemical molecules were prepared by using LigPrep module, wherein original ionization state and structure specified chiralities were retained. The 14 BH3-mimetics, the 12 scaffolds and 51 fragments of the mimetics and tens of

novel compounds generated by means of CombiGlide were docked on the BH3-groove of the Bcl-B using Glide-XP and ranked based on their GlideScores, GlideEnergies and ligand efficiencies.

Results and Discussions

Comparative modeling of the hBcl-B and its interaction with the BH3-mimetics

The three-dimensional (3D) structures of the hBcl-B (Bcl-B from *Homo sapiens*) have not yet been elucidated by experimental techniques. In the present study, the 3D structure of the hBcl-B has been modeled using NMR structures of mBcl-B (Boo/Diva, 2KUA) reported recently, as a template by Modeller 9v8. The hBcl-B and mBcl-B (both proteins were considered with truncated transmembrane regions) are similar in length and share 48% sequence identities to each other. Both proteins have 'GWD' signature peptide in their BH2-domain. However, another characteristic signature peptide, 'NWGR', present in the BH1-domain of all anti-apoptotic proteins is found missing in the counterpart regions of hBcl-B and mBcl-B as well: instead of 'NWGR', the hBcl-B and the mBcl-B have 'SWSQ' and 'TWGR' in the corresponding positions, respectively (G.J.P. RAUTUREAU & al. 2010 [22]). The top ranked model structure was selected on the basis of 'Dope Score' and then the structure was subjected to 'Steepest descent' energy minimization algorithm with a tolerance of 1000 KJ/mol/nm, step size of 0.01 and maximum number of minimization steps as 50,000 by Gromacs 4.5.1. The energy minimized 3D structure of the protein was further validated using SAVS meta server (http://nihserver.mbi.ucla.edu/SAVS/): atom packing (-0.22), networks of non-covalent interactions (86.3%) and dihedral angle constraints (percentage of non-proline and non-glycine residues in the allowed, additionally allowed and generously allowed regions were 90.6%, 8.7% and 0.7%, respectively) were of high quality structures as represented by Z-score, Verify3D and Procheck, respectively (J. PONTIUS & al. 1996 [23]; J.U. BOWIE & al. 1991 [24]; R.A. LASKOWSKI & al. 1993 [25]). Structural alignments between the model structure and experimental structures of the Bcl-2 family anti-apoptotic proteins were analyzed by molecular visualization tool PyMol (http://www.pymol.org).

The theoretic 3D structure of hBcl-B was used to generate docking complexes with the 14 BH3-mimetics using Glide-XP and 10 of 14 compounds for which GlideScores are greater than tolerance limit (0 kcal/mol) have been listed in Table 1. Ligand efficiencies of the top-ten compounds have also been listed in the table. The ligand efficiencies (calculated using experimental EC_{50} values available for the compounds in the PubChem database to date) and GlideScores of the compounds were compared and the correlation co-efficient value was found to be 0.60. Nevertheless, the correlation value could be remarkably improved (R^2 = 0.91) if all the BH3-mimetics but a compound (PubChem ID: 109673, Table 1) that showing drastic deviation from others were considered for the analysis. Moreover, GlideScores and raw EC_{50} values of the 8 BH3-mimetics (EC_{50} values have not yet been reported for 2 of the 10 compounds, Table 1) were compared and correlation coefficient (R^2) was found to be 0.75 implying that theoretic scores and experimental affinities of the BH3-mimetics to hBcl-B are in good agreement to this extent. The 3D structures of all 14 compounds with their PubChem IDs have been depicted in the Figure 1.

Table 1: Various parameters derived from molecular dockings and experimental methods for the top-ten BH3-mimetics of the Bcl-B.

S. No.	PubChem IDs	Glide Score	Glide Energy	Ligand Efficiencies[1]	EC_{50} (μM)[2]	Ligand Efficiencies[3]
1	684642	-3.75	-30.32	0.18	8.51	0.41
2	4195720	-3.60	-34.29	0.11	8.66	0.26
3	650929	-3.36	-31.68	0.11	3.57	0.12
4	921149	-3.25	-30.72	0.15	1.46	0.07
5	1243212	-3.19	-37.06	0.10	NA[4]	ND[5]
6	2791433	-3.07	28.39	0.12	0.61	0.02
7	807871	-2.97	-26.95	0.16	0.32	0.02
8	44203217	-2.93	-27.67	0.10	0.62	0.02
9	1908777	-2.91	-40.23	0.09	NA	ND
10	109673	-2.75	-17.65	0.21	2.13	0.15

[1]Calculated by using GlideScores obtained from molecular docking studies.
[2]Taken from PubChem database.
[3]Calculated by using EC_{50} values.
[4]Not available; [5]Not determined.

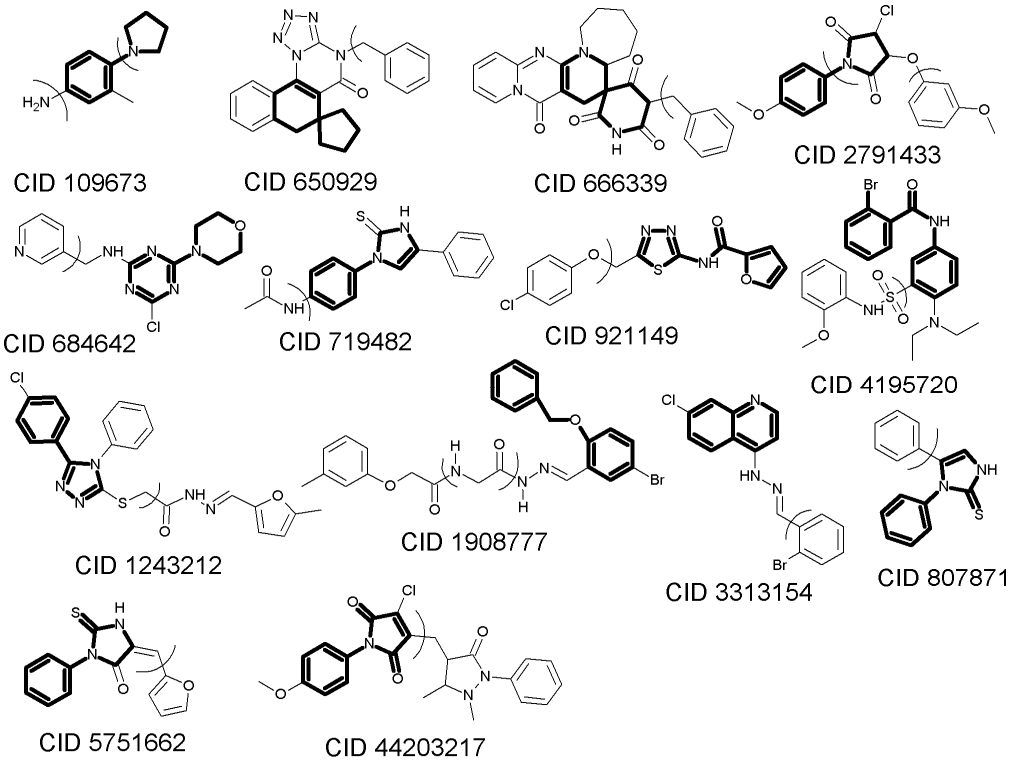


Figure 1: Two-dimensional structures of the 14 BH3-mimetics of the Bcl-B have been depicted along with their PubChem IDs. Scaffold portions and fragment parts of the compounds were denoted in dark color and curve braces, respectively.

Investigation on the scaffolds and fragments generated from the BH3-mimetics and FBDD

The 14 BH3-mimetics resulted in 12 unique scaffolds since the compounds CID 2791433 and CID 44203217 and the compounds CID 719482 and CID 807871 showed common skeleton structures of 1-phenylpyrrolidine-2,5-dione and 4-pheynlyimidazolidine-2-thione,

respectively (Figure 1). The 12 scaffolds were docked on the BH3-binding groove of the Bcl-B and ranked based on their GlideScores and GlideEnergies. The top two scaffolds, 2,4,8-triazaspiro[5.5]undec-9-ene-1,3,5-trione and 3-phenyl-2-thioxoimidazolidin-4-one, in the rank list were not promising to designing lead compounds by means of FBDD, since they were trapped in the deep dead-end pocket of the BH3-binding groove of the protein (Figure 2A). Hence, the compound 1-phenyl-1H-pyrrole-2,5-dione (Figure 2B), placed third position in the rank list, was used for generating 30 novel compounds upon coupling with 15 different fragments derived from the 14 BH3-mimetics (discussed below). The pyrrole ring of the scaffold (Compound 3) was substituted by the following chemical moieties: benzene ring was at position 1; carbonyl groups were present at second and fifth positions. The third and fourth positions of the pyrrole ring of the 1-phenyl-1H-pyrrole-2,5-dione had no substitutions and consequently, in the present study, both the positions of the scaffold were chosen for linking fragments to generate novel compounds.

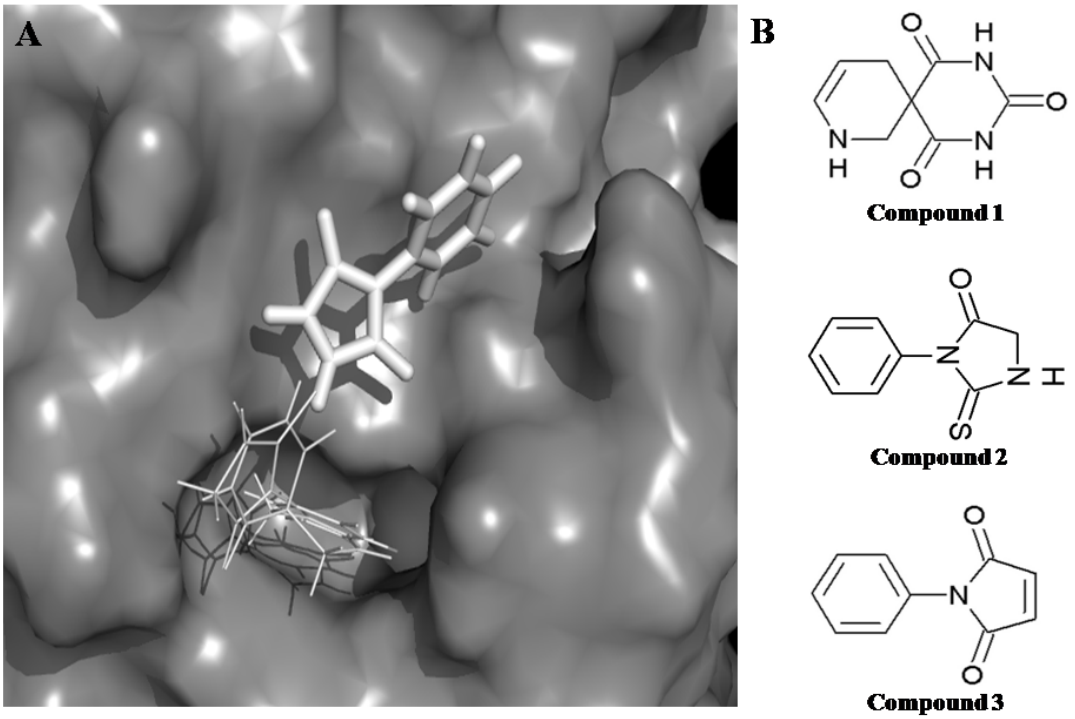


Figure 2: (A) Docking complexes of the scaffolds 2,4,8-triazaspiro[5.5]undec-9-ene-1,3,5-trione (Compound 1, shown in line model), 3-phenyl-2-thioxoimidazolidin-4-one (Compound 2, shown in line model) and 1-phenyl-1H-pyrrole-2,5-dione (Compound 3, shown in stick model) on the BH3-binding groove of the hBcl-B (shown in surface model). (B) Two-dimensional structures of the three scaffolds are depicted.

The 14 BH3-mimetics were broken into 76 fragments by means of RECAP algorithm and stringent examinations on the structural architectures of the fragments helped to identify 51 non-redundant fragments. Most of the fragments were in possession of either a six or a five member ring system. All the 51 fragments were docked on the BH3-binding groove of the Bcl-B using Glide-XP and sorted out based on their GlideScores and GlideEnergies. Moreover, the docked fragments were classified into various clusters based on their chemical properties and binding locations on the surface groove of the protein. The analyses brought-out 15 unique chemically diverse fragments shown in Figure 3 and these compounds only

were taken into further considerations for designing lead compounds to the Bcl-B by means of FBDD.

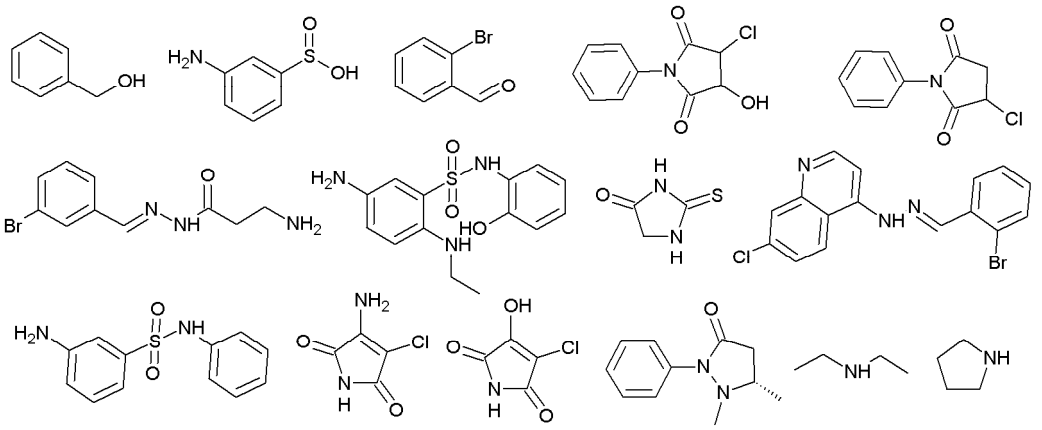
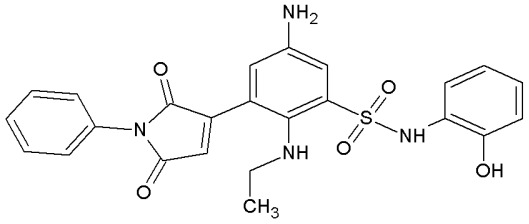
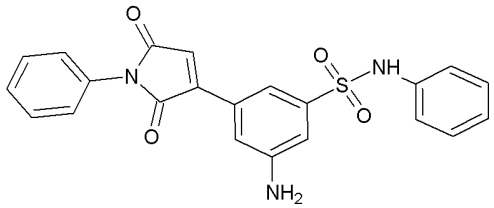


Figure 3: Two-dimensional structures of the 15 unique fragments identified from the 14 BH3-mimetics of the Bcl-B have been depicted.

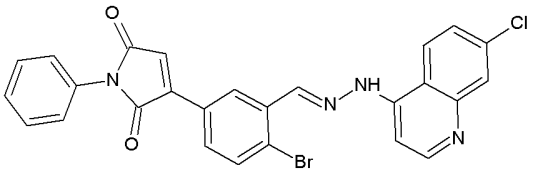
The 15 unique fragments (Figure 3) identified from the 14 BH3-mimetics (Figure 1) were linked systematically such that one fragment at a time either on the third or fourth positions of the 1-phenyl-1H-pyrrole-2,5-dione (Figure 2B) by using CombiGlide and the ligature exercise resulted in 30 novel small molecular chemical compounds. All the novel compounds constructed were energy minimized under solvated conditions and then docked on the BH3-binding groove of the hBcl-B by means of Gromacs and Glide-XP computational tools, respectively. Analyzes of the docking data revealed that all the compounds resided on the BH3-binding groove of the protein with the GlideScores ranging from -2.36 to -5.75. Of the 30 compounds, 8 compounds showed GlideScores less than -3.75 (a highest docking score for a BH3-mimetic (ID: 684642) taken from PubChem database) and structures of 3 compounds for which GlideScores are greater than -5.0 are depicted in Figure 4. From a quick inspection to the figure, it is obvious that compound A (5-amino-2-(ethylamino)-N-(2-hydroxyphenyl)-3-(1-phenyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl) benzenesulfonamide) could be a highly efficient inhibitor to the protein. The docking model of the compound with the protein is shown in Figure 5A and the residues of the protein such as His50, Phe53, Phe54, Tyr57, Leu67, Leu70, Met71, Ser74, Val75, Thr89, Leu90, and Phe93 were found to be within the 4 Å proximities of the compound. Moreover, the NH group present in the lead compound established a hydrogen bond interaction with NE2 atom of imidazole ring of His50 of the hBcl-B (Figure 5B). Strikingly, the compound A is a *de novo* inhibitor to the protein and also fully satisfies the Lipinski's rule-of-five (Molecular mass < 500 Da; Hydrogen bond donors ≤ 5; Hydrogen bond acceptors ≤ 10; log P in the range of -2.0 to 6.0). In these backgrounds, we strongly believe that the *de novo* compound reported herein may presumably act as potent prototype for designing efficient antagonist to the protein. Experimental validations of the *de novo* compound on inhibiting the activity of the protein in terms of its specificity and bioavailability are under progress in our laboratory.



Compound A (-5.75)



Compound B (-5.36)



Compound C (-5.15)

Figure 4: Two-dimensional structures of 3 highly efficient novel compounds generated from a scaffold and various fragments identified in the present study (refer to text). GlideScores obtained from molecular docking studies carried out for the novel compounds on the BH3-binding groove of hBcl-B are given in parenthesis.

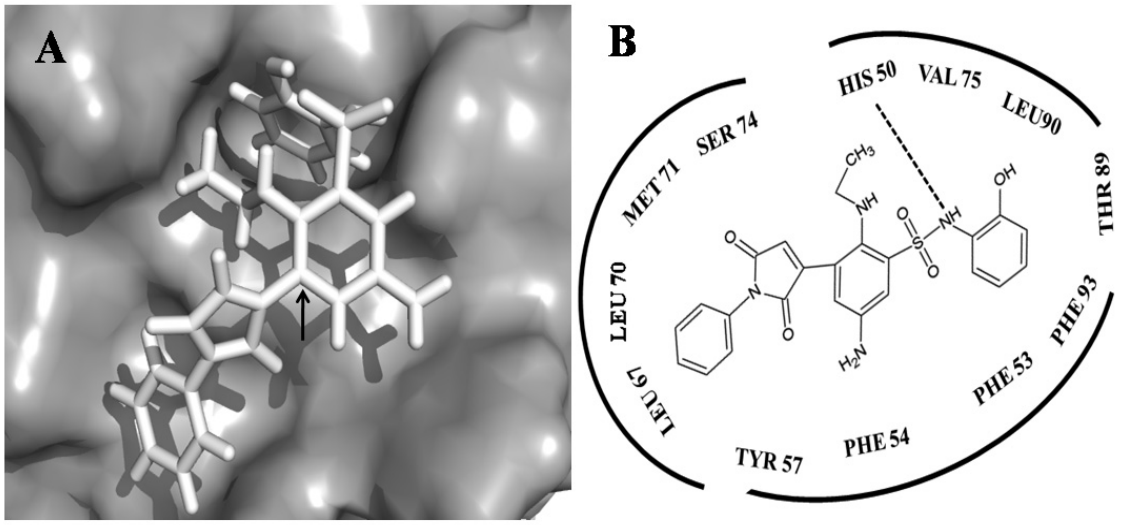


Figure 5: (A) Docking complex of a *de novo* compound, 5-amino-2-(ethylamino)-N-(2-hydroxyphenyl)-3-(1-phenyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)benzenesulfonamide (shown in stick model), on the BH3-binding groove of the hBcl-B (shown in surface model). The pointed arrow denotes the fragment-linking point on the scaffold. (B) Residues of hBcl-B that are within 4 Å to the ligand are schematically represented (Hydrogen bonding interaction is shown in a dotted line).

Concluding remarks

In the present study, the 3D structures of the hBcl-B from *Homo sapiens* have been homology modeled and validated using an array of structural validation tools. Using the theoretic structure, the binding modes and affinities of the 14 BH3-mimetics (reported to the protein to date), 12 scaffolds of the mimetics, 51 fragments of the mimetics and 30 novel compounds (designed by means of FBDD) were thoroughly investigated. The analyses disclosed that a *de novo* compound, (5-amino-2-(ethylamino)-N-(2-hydroxyphenyl)-3-(1-phenyl-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)benzenesulfonamide, showing about two folds times greater binding affinities than that of the 14 BH3-mimetics studied in the present work, is a promising lead for designing efficient antagonists to the hBcl-B. Structural characterizations of the *de novo* compound reported herein on interacting with the protein are under progress in our laboratory.

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