In silico Methods for Designing Antagonists to Anti-apoptotic Members of Bcl-2 Family Proteins

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Abstract: Designing antagonists to anti-apoptotic proteins of Bcl-2 family has become an important strategy in cancer chemotherapy. Using experimental techniques and computational methods, a few numbers of lead inhibitors to the anti-apoptotic proteins have been reported in the literature and a few of them are under clinical trials. In this review, the lead inhibitors designed using *in silico* methodologies are exclusively covered, systematically organized and critically evaluated. An orchestrated *in silico* strategy for screening and identifying efficient antagonists to the anti-apoptotic proteins has also been brought into fore.

Keywords: Apoptosis, Bcl-2 family proteins, Cancer, Drug designing, *In silico* tools, Neoplasm and Structural Bioinformatics.

INTRODUCTION

One of the leading causes of death in developed and developing countries (without any bias) is cancer disorders according to World Health Organization [1]. Cancer is a malignant neoplasm and the cancer cells are malignancy, invasion and metastasis in general. Although the count of all types of cancers is about 100, the following 13 types of cancers are commonly found in human: Bladder/Breast/ Colon/Endometrial/Gastric/Leukemia/Lung/Melanoma/ Non-Hodgkin Lymphoma/Pancreatic/Prostate/Renal/Thyroid cancer [2]. It is now well documented that cancer is caused by uncontrolled cell proliferation due to genetic disorders and/or abnormally enhanced life time of cells due to impairments of apoptosis. The cell proliferation processes are tightly regulated by oncogenes and tumor suppressor genes. The homeostasis of the functions of these genes are affected through hereditary genetic factors and environmental factors such as exposure to mutagens, carcinogens, radiations, infections and imbalanced diet habits [3-14]. Apoptosis plays crucial roles in many biological reactions from embryonic development to removal of damaged cells at the developed levels in all the eukaryotic organisms [15]. Defects in the apoptosis due to imbalance of anti-apoptotic and pro-apoptotic proteins, lead many diseases including cancer, ischemia, neurodegenerative disorders, AIDS etc. [16-18]. The apoptosis processes are characterized by two different mechanisms: intrinsic or mitochondrial pathway and extrinsic pathway [19, 20]. While the extrinsic pathway is activated by death receptors on the cell-surface, the intrinsic pathway is tightly regulated by Bcl-2 family of proteins (Fig. (**1**)). Proteins belonging to the Bcl-2 family are grouped into three distinct categories: pro-survival proteins, pro-apoptotic proteins and BH3–only proteins [21-25]. The proteins belonging to all these three groups are linked in one way or other to the mitochondrial apoptotic pathways. The pro-apoptotic proteins present in the outer membrane of mitochondria oligomerize to form a channel through which death signal cytochrome C is released and then it activates caspases leading to cell death [26, 27]. The anti-apoptotic proteins sequester the pro-apoptotic proteins and consequently terminate the cell-death signals [28-30]. Thus, designing antagonists to the anti-apoptotic/pro-survival proteins has become an important strategy in cancer chemotherapy.

In the past few years, computational tools have been extensively used in designing lead compounds for various diseases/disorders. Highly sophisticated free and commercial tools on molecular modeling, docking, dynamics and structure-activity predictions are now available to scrutinize the binding affinities, modes of interaction, specificities, bioavailability and toxicities of small and macromolecules [31]. From the year of 2000 to to-date, quite a large number of research papers have been published on designing lead inhibitors to the anti-apoptotic proteins of Bcl-2 family using various computational strategies that are schematically represented in (Fig. **2**). Interestingly, tens of lead anti-cancer compounds identified using the computational methods have been reported in the literature (Fig. (**3**)) and a few of them are right now under different stages of clinical trials. In these connections, the indispensable applications of the computational tools on designing *de novo* anti-cancer compounds are exclusively covered in the present review. Though many excellent review articles on apoptosis have been published from many eminent research groups in various scientific magazines, review articles providing a complete knowledge on the roles of bioinformatics tools for designing anti-cancer drugs have not yet been reported to our best knowledge. In this review article, we are providing a comprehensive essay on the roles of '*in silico*' tools used to

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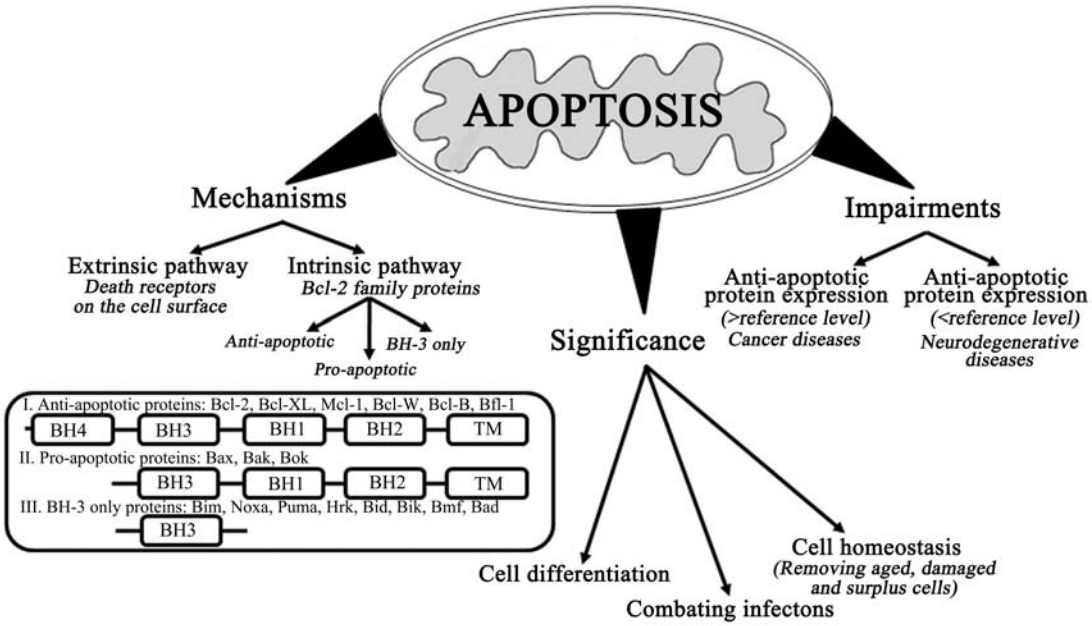

Fig. (1). The illustration outlines the significance, mechanisms and impairments of apoptosis. The proteins of Bcl-2 family are multi-domains molecules and their structural organizations are represented in the box-inset.

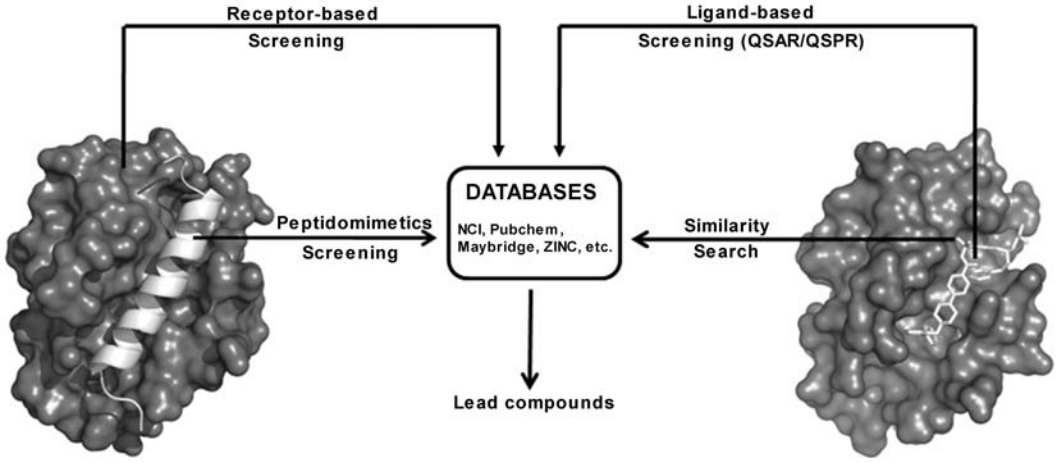

Fig. (2). Schematic representations of computational strategies being used to screen lead inhibitors from small molecular databases to the anti-apoptotic proteins of Bcl-2 family.

date on designing antagonists to the anti-apoptotic proteins of Bcl-2 family. We believe that this review fulfills the gap left unaddressed on the computer-aided anti-cancer drug discovery and it may also trigger exciting research on cancer biology in near future.

ANTAGONISTS OF ANTI-APOPTOTIC PROTEINS AS PROBED BY MOLECULAR DOCKINGS

To date, six structurally characterized anti-apoptotic proteins (Bcl-2, Bcl-W, Bcl-XL, Bcl-B, Mcl-1 and Bfl-1) from human beings have been reported in the literature. They are single chain polypeptide composed of 150-250 amino acids, structurally homologous and simple helical proteins. They have highly conserved five domains (BH1, BH2, BH3, BH4 and TM domains) and two well-defined binding grooves: BH-groove and BH3-binding groove. The BH1 & BH3 domains constitute the BH3-binding groove of the proteins which are capable of accommodating BH3-domain of the pro-apoptotic proteins and BH3-only peptides. Structurally, pro-apoptotic proteins are bereft of BH4 domain and have other domains such as BH1, BH2, BH3 and TM like anti-apoptotic proteins and functionally, they promote apoptosis, whereas the anti-apoptotic proteins resist apoptosis (Fig. (**1**)). Either directly (interacting with BH3-domain of the pro-apoptotic proteins) or indirectly (interacting with BH3-only peptides), the anti-apoptotic proteins prevent the formation of oligomeric structure of pro-apoptotic proteins in the outer membrane surface of

mitochondria through which death signal cytochrome C is released from the inner-membrane surface of the organelle. The cytochrome C activates caspases leading to cell death. It has been shown that the anti-apoptotic proteins, especially Bcl-2, Bcl-XL and Mcl-1 are over-expressed in many forms of cancers [32] and thereby, impairment of apoptosis is a 'hallmark of cancer', which has been well dealt in a few numbers of review articles [33-35]. In this background, small chemical molecules, which are capable of displacing the BH3-domains of the pro-apoptotic proteins from the BH3-binding groove of the anti-apoptotic proteins, are considered as lead compounds for designing efficient anti-cancer drugs.

In order to design lead compounds to any target proteins, understanding thermodynamic and structural parameters of complexes consisting of the proteins and the compounds is a prerequisite criterion. Ideally, this can be achieved using ITC (Isothermal Titration Calorimeter) and optical spectroscopic techniques (Circular dichroism, Fluorescence spectrometry etc.) at molecular level resolutions and using X-ray and NMR (Nuclear Magnetic Resonance) spectroscopic techniques at atomic level resolutions. However, these methods are expensive, time consuming and also applicable for the complexes depicting binding affinities in micromolar or below that concentration. Alternatively, computational methods in conjunction with the experimental approaches could be successfully used for identifying and designing lead compounds in a fast-track manner. HA14-1 is the first computer-based Bcl-2 inhibitor reported by Wang *et al.* in the year 2000 using virtual screening strategy [36]. In the study, 193,833 chemical compounds were retrieved from MDL/ACD database and docking complexes of each of the compounds with the Bcl-2 protein (modeled using experimental 3D structures of Bcl-XL) were generated by employing rigid protein – rigid ligand docking mode using Dock 3.5, a molecular docking program. The interaction between each of the compounds and the protein was analyzed on the basis of shape complementarities and binding energies and the virtual screening resulted in 53 compounds depicting 28 diverse scaffolds. The IC_{50} values of the 53 compounds were determined on the basis of their capabilities of displacing Flu-BakBH3 peptide bound on the BH3-binding groove of the Bcl-2 using fluorescence polarization assay (FPA) experiments. Based on the FPA data, they reported that HA14-1 may be a potential inhibitor to the Bcl-2 among the compounds considered by them and the HA14-1showed about 9 μM of IC_{50} to displace the peptide from the protein. Furthermore, they have successfully showed that the HA14-1 induces apoptosis of HL-60 cells using DNA fragmentation assay and mitochondrial membrane potential assay. However, the exact stereoisomer of the HA14-1 (the compound has 4 stereoisomer as it possesses 2 chiral centers) that can act as the potential antagonists to the Bcl-2 have been left unaddressed. The structures of the HA14-1 and other antagonists to the anti-apoptotic proteins reported in the literature have been depicted in (Fig. (**3**)). Details on the other antagonists that are identified using computational methods as preliminary strategies have been discussed in the subsequent sections given below.

In 2001, Enyedy *et al.* identified 35 inhibitors to the Bcl-2 protein from NCI (National Cancer Institute) small molecular 3D database consisting of 206,876 compounds using high throughput virtual screening strategies [37]. This work differed from the virtual screening work described in the above paragraph in two computational aspects: i) NCI database was used instead of ACD database; ii) protein-ligands complexes were generated using 'rigid protein - flexible ligand' docking mode instead of 'rigid protein - rigid ligands' dockings. Of the 35 compounds selected from the NCI using the method, compound **6** (as reported in the original paper) was shown as the most potent anti-cancer molecule as it showed better anti-proliferation activity against human myeloid leukemia cell lines (HL-60). The binding interaction between the compound **6** and the Bcl-XL (the template structure used to model the Bcl-2) have also been characterized at residue level using two-dimensional NMR methods, which authenticated that compound **6** interacted in the BH3-binding groove of the protein. However, the interaction between the compound and the Bcl-XL reported by them raises few concerns on the specificity of the compound for interacting with other anti-apoptotic proteins and its consequences on the cancer cells. Lugovskoy *et al.* collected 93 analogous of BH3I-1 and BH3I-2 (BH3 mimetics, small molecules that are capable of interacting on the BH3-binding groove of the anti-apoptotic proteins) from small molecular libraries of Chembridge and Chemnavigator using 'structural similarity search' strategy [38]. The compounds were then docked on the surface binding groove of the Bcl-XL using molecular docking routine TreeDock and search space for the docking points of atoms in the ligand on the protein could be defined on the basis of N^{15}-chemical shift perturbations observed from the N^{15}-HSQC spectra acquired for the Bcl-XL treated with the ligands. From the combined analyses of the NMR and the TreeDock data, they showed an excellent correlation between binding energies calculated by the TreeDock and binding affinities determined by the NMR methods for the complexes of the Bcl-XL with the BH3I-1, BH3I-2 and their analogous. From the screening method, they reported a novel compound BH3I-1SCH3, which showed binding affinity as strong as the BH3I-1 binds with the protein. Recently, Rega *et al.* carried-out high throughput virtual screening of 16,000 compounds of Maybridge database using molecular dockings and they short-listed 320 compounds on the basis of their docking energies with the Bcl-XL [39]. After mapping the binding modes of these compounds on the surface groove of Bcl-XL using C^{13}-filtered H^1 1D NMR experiments, the authors identified 4 compounds (BI-21C4, BI-21C5, BI-21C6 and BI-21C7) depicting new scaffolds, which were capable of inhibiting the binding of Bak-BH3 peptide to the Bcl-XL as demonstrated by cell viability assays (Fig. (**3**)).

ANTAGONISTS OF ANTI-APOPTOTIC PROTEINS AS SCREENED BY USING QSPR/QSAR METHODS

Quantitative correlations between chemical structures and biological activities could be deduced using quantitative structure property/activity relationship (QSPR/QSAR) studies on small chemical molecules. The strength of these methods is on the assumption that chemical molecules showing similar structures and properties must have similar

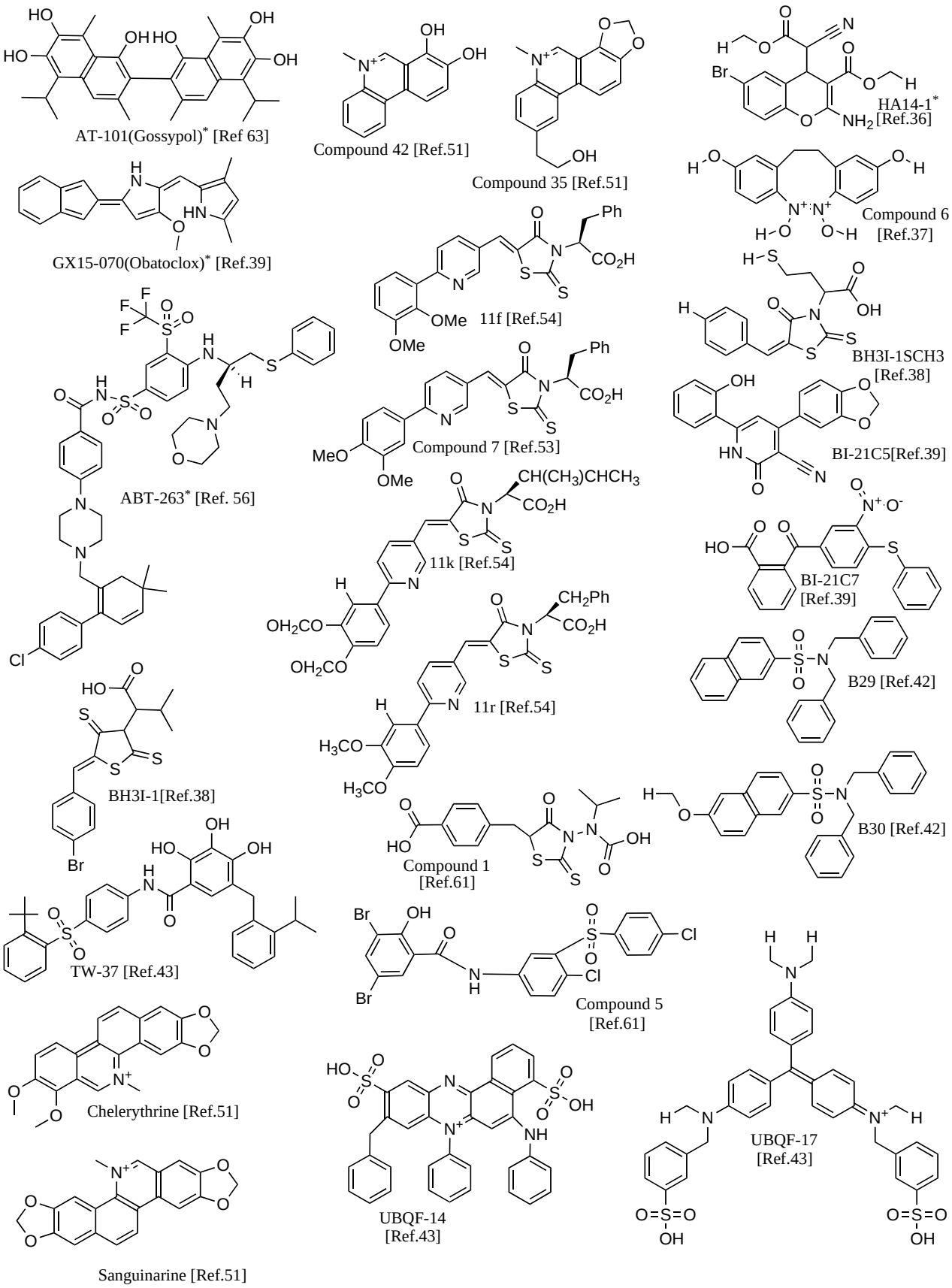

Fig. (3). Structures of lead antagonists to the anti-apoptotic proteins of Bcl-2 family, as reported in the literature. Molecules that are denoted by asterisk symbol are right now under clinical trials.

activities. There are six types of QSAR methods (1D-, 2D-, 3D-, 4D-, 5D- & 6D-QSAR) and each method is different from others particularly in descriptors considered for predicting activities of the molecules. A few numbers of research papers reporting potential inhibitors to the anti-apoptotic proteins using QSAR/QSPR methods have been published in the recent past years. Almerico *et al.* (2009) screened commercial compound database ZINC consisted of more than 2 million structures using a set of descriptors derived from the ABT-737, Gossypol and GX015070, which were in clinical trials at that time [40]. Using the strategy, they identified 2,229 drug-like compounds from the database and these compounds were then subjected to docking studies on open-cleft Bcl-XL structure (PDB ID: 1BXL). The studies brought into fore 17 compounds belonging to the sulfonamide class. Though the 17 compounds showed better docking energies than that of three reference compounds used in the study, the experimental validations of the predicted compounds have not yet been accounted to our best knowledge. The same research group of the work described above has recently generated a ligand-based 3D pharmacophore model using the three-dimensional structures and inhibitory activities of 42 biarylacylsulfonamides tested experimentally at the Abbott Laboratories [41]. The pharmacophore model consists of five essential structural features: two aromatic sites, two H-bond acceptor sites and one negative charged site. The model was then used to screen ZINC database and the resultant hits were further filtered by two consecutive docking runs (Glide-SP followed by Glide-XP) with the Bcl-XL, which resulted in six hits: ZINC00784464, ZINC00788197, ZINC03200686, ZINC03212331, ZINC03243504 and ZINC03356310. However, the experimental validations for the binding affinities of the six hits on the BH3-binding groove of the Bcl-XL and their biological actions on various cancer cell-lines have been left unaddressed, till now.

Mukherjee *et al.* reported three lead antagonists to the Bcl-XL after screening about 1.8 million compounds of Zinc database using receptor-based pharmacophore strategy at first time [42]. In order to generate receptor-based pharmacophores, they have used the Bcl-XL-Bak-BH3 complex and the virtual screening resulted in about 0.4 million promising inhibitors to the protein. The selected compounds were further subjected to various filters like molecular dockings (docking of small molecules with open-cleft Bcl-XL structure (PDB ID: 1BXL)), cross-dockings (docking of small molecules with two different open-cleft Bcl-XL structures extracted from the complexes of the protein with Bak-BH3 peptide (PDB ID: 1BXL) and N3B (PDB ID: 1YSI) small molecular ligand), docking pose-based descriptors and shape complementarities. On the basis of these computational filters, the authors identified a set of 45 small molecules belonging to various scaffolds and the capabilities of these compounds to displace fluorescein-labeled Bad-peptide from the His-tagged Bcl-XL protein were studied by using fluorescence polarization assay. Of the 45 computationally screened compounds, three compounds (J042, B29 and B30) depicting IC_{50} values in the micromolar concentrations were reported as reliable lead compounds for designing antagonists to the Bcl-XL. Recently, Pinto *et al.* has also used receptor-based pharmacophore strategy for identifying chemical inhibitors to the Bcl-XL [43]. However, the receptor-based structural analysis carried-out by Mukerjee *et al.* and Pinto *et al.* are differing from each other in the ligands used for mapping pharmacophores: in the former case, Bak-BH3 domain (hexadeca peptide) was the ligand, whereas in the latter case, 4 hexadeca peptides such as Bak, Bax, Bid and Hrk were considered as ligands. Moreover, in the latter case, common pharmacophores for surface groove of the Bcl-XL were identified by thoroughly analyzing the trajectories obtained from the molecular dynamic simulations of complexes (the Bcl-XL with each of the four hexadeca peptides). Using a consensus pharmacophore pattern, the small molecular databases NCI and ACD were screened and 429 hits were retrieved from the two databases. The 429 hits were docked on the surface groove of the Bcl-XL using DockDyn tool and the compounds were ranked on the basis of their binding energies. Top 15 compounds from the docking studies were then chosen and further assayed using a competitive fluorescence polarization method in order to verify their abilities to disrupt the Bcl-XL-Bak complex under *in vitro* conditions. From the combined analysis of data obtained from the pharmacophore screening, molecular docking and FPA assays, the authors reported two compounds, UBQF14 and UBQF17 (showing IC_{50} values in the micromolar range) as potent antagonists to the Bcl-XL of Bcl-2 family (Fig. (**3**)). We certainly learn from these types of studies that potential lead anticancer compounds are present in various small molecular databases (ACD, Chembridge, Chemnavigator, MayBridge, MDL, NCI, PubChem and ZINC) and those compounds would be effectively identified using appropriate search techniques such as similarity search, property-based search, ligand-based pharmacophore search, and receptor-based pharmacophore search. And these studies obviously suggest that search techniques, not the search space, are really matters for identifying lead compounds to the given target proteins, in general.

IN SILICO METHODS FOR PREDICTING SPECIFICITIES OF LEAD COMPOUNDS

Designing lead compounds with nanomolar affinities to given target molecules is essential but not sufficient because the lead compounds should exhibit specificities in addition to their higher affinities towards their target molecules. To date, quite a number of antagonists have been especially designed to the Bcl-2 and the Bcl-XL of the six anti-apoptotic proteins (Table **1**) and the binding interactions of the leads with the two proteins have also been extensively characterized using computational and experimental methods. Surprisingly, identification of lead compounds to Bcl-W, Bcl-B, Bfl-1 and Mcl-1 has been largely left unaddressed. It is important to mention that though all the six anti-apoptotic proteins have similar 3D structures, the percentage of sequence identities among the proteins are weak (< 30%) implying that they may target different types of tissues during apoptosis and may also differ in responses to different stress stimuli [44]. It has been shown that the Bcl-2 and the Bcl-XL are playing essential roles for regulating B-lymphocytes and platelet survival, respectively [45, 46]. Hence, administration of anti-cancer drugs interacting with the Bcl-2 and the Bcl-XL may

Table 1. Chemical Molecules Identified as Inhibitors to the Anti-apoptotic Proteins Upon Screening the Small Molecular Databases Using an Array of Computational Tools have been Listed in a Hierarchal Order. Primary Experimental Techniques Used to Validate the Computational Findings have also been Listed against to the Corresponding Studies

S. No	Target Protein	Lead Compounds	Small Molecule Databases Screened	HTVS Screening Tools	Experimental Methods	Refs.
1	Bcl-2	HA14-1	MDL/ ACD3D	Dock 3.5	FPA, Cell viability assay, DNA fragmentation assay	[36]
2	Bcl-2	Compound **6***	NCI 3D	Dock	FPA, Cell viability assay, NMR	[37]
3	Bcl-XL	BH3I-1SCH3	Chembridge, Chemnavigator	Treedock	FPA, NMR	[38]
4	Bcl-XL	BI-21C5 BI-21C7	Maybridge	FlexX	NMR	[39]
5	Bcl-XL	17 Compounds[#]	ZINC	Ligandscout, LigandFit	-	[40]
6	Bcl-XL	Compound **1*** Compound **5***	Inhouse database	GOLD	Cell culture, Flow cytometry	[61]
7	Bcl-XL	B29 B30	ZINC	GOLD	FPA	[42]
8	Bcl-XL	6 compounds[#]	ZINC	PHASE, Glide	-	[41]
9	Bcl-XL	UBQF-14 UBQF-17	ACD, NCI, Maybridge, DWD	DockDyn	FPA	[43]

*The IUPAC names of compounds **1**, **5** and **6** are 2-{5-[4-(dihydroxymethyl)benzyl]-4-oxo-2-thioxo-1,3-thiazolidin-3-yl}-3-methylbutanoic acid, 3,5-dibromo-N-{4-chlorophenyl)sulfonyl]phenyl}-2-hydroxybenzamide and 2,9-dimethoxy-5,6,11,12-tetrahydrodibenzo[c,g][1,2]diazocine 5,6-dioxide, respectively.

[#]ZINC IDs of 17 compounds reported in the ref. 40 and 6 compounds reported in ref.41 are herein shown.
ZINC IDs of the 17 compounds: 00828403, 01093553, 01112945, 01399215, 02336390, 02952637, 03017827, 03170711, 03225670, 03230589, 03300146, 03336378, 03660864, 04352892, 05433867, 06577840 and 06973945.
ZINC IDs of the 6 compounds: 00784464, 00788197, 03200686, 03212331, 03243504 and 03356310.

presumably cause either lymphopaenia or thrombocytopaenia or both of them to the patients suffering by cancers in which the proteins have no over expressions. Similarly, Mcl-1 and Bcl-W are essential for the sustainable production of haematopoetic stem cells and sperm cells, respectively [47, 48]. Moreover, BH3-only proteins regulating the functions of anti-apoptotic proteins are highly target-specific in their interactions [29]. For example, HRK inhibits the Bcl-XL but not the Mcl-1, whereas NOXA inhibits the Mcl-1 but not the Bcl-XL. The Bim-BH3-only peptide is capable of interacting with all the anti-apoptotic proteins. However, it shows different binding affinities to each of those proteins. Furthermore, it has been recently shown that the Bcl-B of human binds with Bax but not with Bak pro-apoptotic proteins implying that the Bcl-B may play essential roles in the Bax-mediated apoptosis exclusively [49]. Taken together, it could be easily realized that specific inhibitors to each anti-apoptotic protein are indispensable in order to avoid adverse side-effect from the chemotherapy treatments of a specific cancer overproducing the anti-apoptotic proteins in different concentrations.

There have been many attempts to improve specificities of lead compounds to the anti-apoptotic proteins and as well on designing anti-apoptotic protein-specific inhibitors [50-52]. Recently, Bernardo *et al*. have developed a small focused library of rhodanine derivatives as inhibitors to the anti-apoptotic proteins and have successfully shown that Bcl-XL-specific and Mcl-1-specific inhibitors depicting IC_{50} values in micromolar (< 10 μM) concentration [53]. They have shown from the library that compound **7** (as reported in the original paper) depicted 8 μM of K_i to displace Bak-BH3 peptide from the Mcl-1, whereas the compound was not active enough (> 100 μM) to displace the peptide from the Bcl-XL as determined by FPA methods. The K_d values of the compound were 10 μM and >750 μM for binding on the Mcl-1 and the Bcl-XL, respectively, as estimated using ITC technique. A possible rationale for the differential interactions of the ligand on the two proteins could be understood from the combined analyses of data from N^{15}-HSQC chemical shift perturbations and *in silico* docking studies on the complexes. The docking models showed that one of two methoxy groups in the phenyl ring of the compound **7** was not fitting into the BH3-binding groove of the Bcl-XL and exposed to charge-incompatible environment. It could be rationalized that the ligand should need to adapt a conformation in order to bring the exposed methoxy group into the groove and the resultant conformation of the compound may presumably not compatible to be accommodated in the groove. The authors also suggested that the Mcl-1 could accommodate the ligand to its BH3-binding groove as the groove is much wider than that of the Bcl-XL. In 2011, the same research group

(Bernardo *et al*. 2011) has shown a few more specific inhibitors to the Bcl-XL (compound **11i** & **11p**) and as well as to the Mcl-1 (compound **11b**,**11o**,**11r**,**11s** & **11t**) using FPA and *in silico* docking studies [54]. Thus, specificities of lead compounds to the anti-apoptotic proteins can be effectively analyzed and manipulated using docking strategies in conjunction with techniques that can be used for estimating dissociation constants of the proteins-ligands complexes.

It is also advisable to study the interactions of lead compounds with proteins that are non-homologous and having binding pockets similar to that of target proteins. At least, these types of studies can be carried-out using computational tools in the screening processes of lead compounds to avoid any failure in later stages of pre-clinical/clinical trials. In this context, it would be appropriate to discuss on the discovery of ABT-737 and ABT-263, which are structurally similar, highly potent inhibitors to the Bcl-XL. Oltersorf *et al.* demonstrated the structure-based methods to overcome non-specific interaction between a Bcl-XL inhibitor and human serum albumin (HSA) [55]. The inhibitor interacted with HSA through a part of its lipophilic surface area, which was exposed in the Bcl-XL complex. Thus, the authors synthesized a new compound with high hydrophilic moieties on the corresponding region and the resultant compound, ABT-737, showed greatly increased affinity (in nM) with the Bcl-XL and showed poor affinity with HSA. However, ABT-737 failed to clear the clinical trials due to its poor bioavailability. Interestingly, ABT-263, a structural analog of ABT-737, has been shown as potent inhibitor to the Bcl-XL with appreciable bioavailability [56]. We have recently proposed a rationalization for the differential bioavailability of the structurally similar compounds using an array of *in silico* strategies [57]. It was found that ABT-737 docked on CYP3A4, a metabolic enzyme present in intestine of human beings, with nearly 3 folds stronger binding affinity than the binding affinity of ABT-263 with the enzyme implying that pre-systemic metabolic rate of ABT-737 in the intestine must be several folds higher than that of ABT-263. Therefore, we attributed the differential bioavailability of the ABT-737 and the ABT-263 to their pre-systemic metabolic reactions that are presumably different from each other as we inferred from the docking complexes of the ligands on CYP3A4. There are many such 'specificity' studies in the literature and for instance, we could successfully design four *de novo* ribavirin analogous causing no adverse side-effect such as haemolytic anaemia in human beings upon thoroughly understanding the differential modes of interactions of the ribavirin (being used as anti-viral drug for the treatment of dengue infections) on the NS5-methyltransferase and JAK-2 (playing important roles on erythropoiesis) using molecular docking and dynamic strategies [58]. However, we are not intended to extend the discussions on those types of studies further herein, which are beyond this focused review on cancers. As evidenced by these studies, making use of the computational strategies at right direction on drug discovery processes may pave a way of designing leads possessing features to reduce the risk of late-stage attritions such as non-specificity, poor bioavailability and adverse side-effects.

COMPUTATIONAL TOOLS FOR PREDICTING BIOAVAILABILITY AND TOXICITY (ADMET) OF SMALL CHEMICAL MOLECULES

Predicting pharmacokinetics of compounds is an up-hill task owing to complex biological processes involving for absorption, metabolic reactions, distributions and clearance of drugs. In general, drugs taken orally are absorbed by intestine walls and then undergo metabolic reactions in the guts and liver followed by transportation to the target cells through blood circulation [59]. The extent of absorption of drugs through the intestinal walls of human beings could be reasonably predicted using Lipinski's rule-of-five [60]. Recently, Fullbeck *et al.* searched an in-house database with more than 4 million compounds using rule-of-five as a filter for identification of compounds showing 3D structural similarities to BH3I-1 and BH3I-2, which are well-studied antagonists to the Bcl-XL [61]. In their study, they identified 4 analogs of BH3Is and showed that 2 of them, **1** & **5** (as reported in the original paper), inhibited the protein much stronger than the inhibition of the BH3-Is on the protein, by using molecular docking and apoptotic cell death assays. Obviously, Lipinski's filter tremendously helped to identify 2 highly potent inhibitors to the Bcl-XL from more than 4 million compounds in the study, though one may have skeptical questions on the false-negative compounds in the database.

The bioavailability of drugs ingested orally is defined as the fraction of the drugs actively present in the blood circulation for their target functions. There were many attempts to predict the bioavailability of drugs on the basis of descriptors such as molecular volume, topological polar surface area (TPSA), lipophilicity, hydrogen bonding and so on. Many rules (Lipinski's rule-of-five, Veber's rule and Martin's rule) and many models (Yoshida's model, Andrew's model, Pintore's model, Turner and Maddalena's model, Ma's model, Moda's model and Wang's model) have also been described to predict the bioavailability of drugs. The merits and flaws of the rule-based methods and the models on the bioavailability have been extensively discussed in the two review articles published, recently [59, 62]. Studies on the validations of the models showed that none of the models proposed to-date is robust to predict accurate bioavailability of drugs/compounds. Comparative analysis on the performances of one model with others is also not possible as each model use different dataset and different descriptors for its bioavailability calculations. It has been suggested that there are two main reasons for unsuccessfulness of developing efficient *in silico* prediction tool on the bioavailability of drugs: i) multiple variables of complex processes governing the absorption and metabolic fate of molecules in human (ii) paucity of bioavailability data on approved drugs and compounds failed in clinical phases. The former barrier may be probably tackled by developing a meta-model integrating many individual models accounting every single process in bioavailability. The latter barrier may easily be addressed if pharmaceutical companies and drug research centers generously release their in-house bioavailability data to open sources. We may definitely expect a 'paradigm shift' on the prediction of bioavailability of small molecules, when the above said 'bottle necks' widely open in the near future.

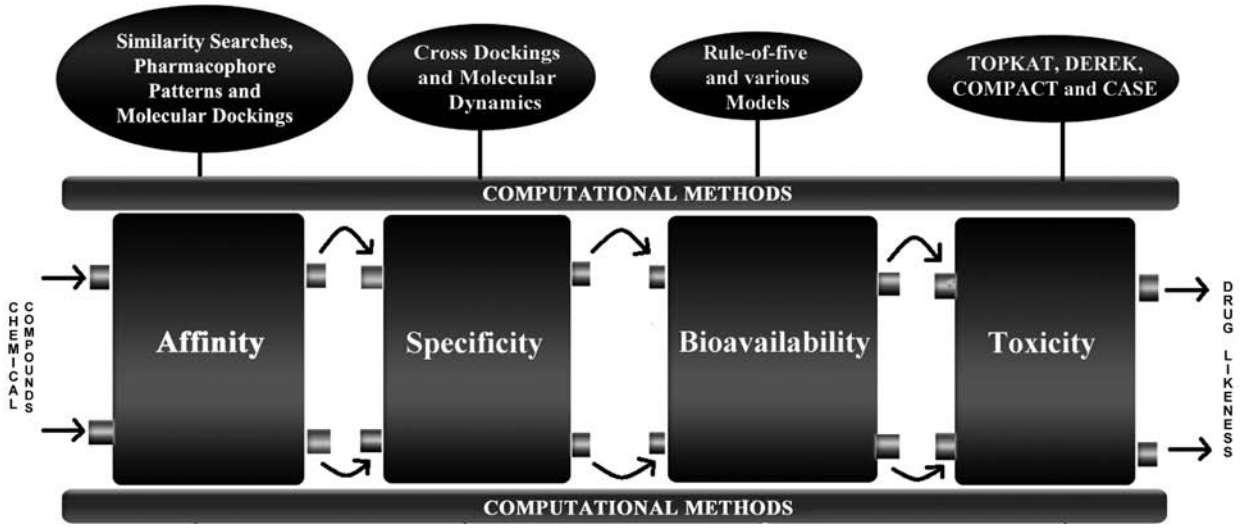

Fig. (4). A schematic representation of drug designing processes using four filters: Affinity filter, Specificity filter, Bioavailability filter and Toxicity filter. A few numbers of *in silico* tools that are being regularly employed for each filter are listed.

In addition to appreciable affinity, specificity and bioavailability, compounds must show no toxicity and adverse effects in human system to become desired drugs. About 20-40% of lead molecules fail either in pre-clinical or clinical trails (even at phase III) because of toxicity and adverse effects. It has been shown that Gossypol, shown at first as highly potent inhibitor to the Bcl-2, Bcl-XL and Mcl-1, failed in Phase I/II clinical trials due to its gastrointestinal toxicity [63]. Apogossypol, an analogue of Gossypol, has also showed some adverse effects in animal models of preclinical studies [64]. These types of failures are expensive and are also fuelling frustrations. Though there are a few numbers of computational tools (ACD/TOX suite, TOPKAT, DEREK, COMPACT and CASE) for predicting toxicity of compounds, they predict the toxicity of the compounds with about 50% concordance only [65]. In these backgrounds, it is high time of designing reliable *in silico* tools for predicting bioavailability and toxicity of lead compounds to avoid costly failures at pre-clinical/clinical trials.

CONCLUDING REMARKS

In this review article, all the break-through papers describing various screening strategies using *in silico* tools for identifying lead antagonists to the anti-apoptotic proteins of Bcl-2 family have been systematically organized, discussed and evaluated. In general, the *in silico* drug designing routes can be divided into four stages for sake of clarity as shown in the (Fig. (**4**)): (i) Affinity filters (ii) Specificity filters (iii) Bioavailability filters and (iv) Toxicity filters. The fitness of any compound to each filter can be systematically studied using an array of computational tools: 2D (two dimensional) & 3D (three dimensional) similarities screening and pharmacophores (QSPR, QSAR and ligand/receptor/peptide-based screening) searches are the main components of affinity filter; different types of molecular dockings (rigid/induced-fit/cross dockings) and molecular dynamic simulations play main roles in specificity filter; the bioavailability filter and toxicity filter employ many rule-based methods and models to identify drug-likeness compounds. The drug-likeness compounds predicted purely using an array of the *in silico* tools become drugs provided they successfully pass through the preclinical and clinical trails. Obviously, the *in silico* tools are indispensable to efficiently speed-up the drug designing process in cost effective modes within a stipulated time frame. It will be quite redeeming to have computational tools integrating all the four filters stated above in an organized manner and acting as beacons for the drug designing in an unequivocally manner in the near future.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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