Chemico-Biological Interactions 250 (2016) 12–26

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Discovery of new selective cytotoxic agents against Bcl-2 expressing cancer cells using ligand-based modeling

Nour H. Aboalhaija [a], Malek A. Zihlif [c], Mutasem O. Taha [b, *]

[a] Department of Pharmaceutical Sciences, Faculty of Pharmacy, Zarqa University, Az-zarqa, Jordan
[b] Drug Discovery Unit, Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Amman, Jordan
[c] Department of Pharmacology, Faculty of Medicine, University of Jordan, Amman, Jordan

ARTICLE INFO

Article history:
Received 8 October 2015
Received in revised form
28 February 2016
Accepted 2 March 2016
Available online 5 March 2016

Keywords:
Bcl-2
Pharmacophore
QSAR
MLR
kNN
Virtual screening
Anticancer screening

ABSTRACT

Bcl-2 is an anti-apoptotic protein involved in cancer resistance to cytotoxic therapies making it an interesting target for inhibitors design. Towards this end, we implemented an elaborated ligand-based computational workflow that combines exhaustive pharmacophore modeling and quantitative structure-activity relationship (QSAR) analysis to explore the structural features required for potent Bcl-2 inhibitors employing 98 known Bcl-2 inhibitors. Genetic function algorithm (GFA) coupled with k nearest neighbor (kNN) or multiple linear regression (MLR) analyses were employed to generate predictive QSAR models based on optimal combinations of pharmacophores and physicochemical descriptors. The optimal QSAR-selected pharmacophore models were validated by receiver operating characteristic (ROC) curve analysis and by comparison with crystallographic structures of known inhibitors co-crystallized within Bcl-2 binding pocket. Optimal QSAR models and their associated pharmacophore hypotheses were validated by identification and experimental evaluation of new selective cytotoxic compounds against Bcl-2 expressing cancer cells. The hits were retrieved from the National Cancer Institute (NCI) structural database. Several potent hits were captured. The most potent hits illustrated IC_{50} values of 4.2 and 2.60 μM against MDA-MB-231 cancer cell-line.

1. Introduction

Cancer is a global health problem and a leading cause of death worldwide [23,55]. Chemotherapy has significantly improved during the last decade largely due to the introduction of effective drug combinations and treatment schemes [40,44].

B-cell lymphoma 2 (Bcl-2) family of proteins plays critical role in regulating cell death through intrinsic (mitochondrial) route of apoptosis [17,48]. Structurally, Bcl-2-family of proteins can be divided into two groups: The first is anti-apoptotic and includes Bcl-2, Bcl-X_L and Mcl-1. These are comprised of four Bcl homology (BH) domains. The second group is proapoptotic and can be further classified into two subgroups; (i) effector proteins, which includes Bax and Bak, and these are composed of three BH domains, and (ii) the proapoptotic activator proteins (e.g., Bim, Bid), which contain only one domain (BH3) [25,47,52].

The anti-apoptotic Bcl-2-family members have binding grooves capable of accommodating BH3 domain of pro-apoptotic family members, preventing oligomerization of those members that leads to initiation of apoptosis cascade [47,67].

Bcl-2, an anti-apoptotic Bcl-2-family member overexpressed in a wide range of human cancers, supports survival of respective cancer cells via evading apoptosis. Potent Bcl-2 inhibitors were reported to exhibit potent anticancer activities against leukemia cells (HL-60) and breast cancer cells (MDA-231) over-expressing Bcl-2 [19,68]. Moreover, in clinical context, Bcl-2 over-expression is manifested in the form of resistance to traditional cytotoxics and apoptotic delays in response to radiation therapy [31,70]. Therefore, Bcl-2 is considered as promising molecular target for the design of new anticancer drugs that focus on overcoming cancer resistance to apoptosis [12,19,24,68].

Pioneer Bcl-2 inhibitor drug candidates, including the structural analogues ABT-737 and Navitoclax from Abbott Laboratories (Fig. 1), have progressed to Phase I/II clinical evaluation in cancer [41]. However, their relatively large size (Mwt = 975 in the case of Navitoclax) makes it important to develop selective Bcl-2 inhibitors with lower molecular weights. This led to the development of small molecular inhibitors of Bcl-2 such as Obatoclax (Fig. 1). Obatoclax

* Corresponding author.
E-mail address: mutasem@ju.edu.jo (M.O. Taha).

http://dx.doi.org/10.1016/j.cbi.2016.03.006
0009-2797/

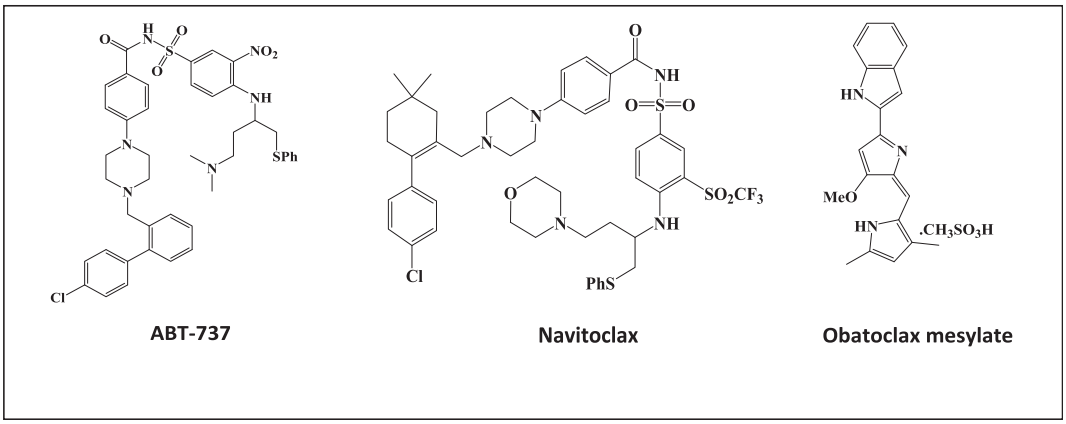

Fig. 1. Examples of Bcl-2 inhibitors in development.

(GX15-070) is a pan-Bcl-2 inhibitor with lower binding affinity to Bcl-2 family members than ABT-737. It is currently in Phase I/II clinical trials [29]. Unfortunately, a recent study on mice has shown that treatment with bolus injection of obatoclax has failed to reach pharmacologically effective levels in blood; and dose escalation was limited due to significant neurologic toxicity [13]. These issues prompted continuous efforts towards new Bcl-2 potent inhibitors [73].

Despite the great current interest in Bcl-2-family members, the number of recent modeling studies aimed at design and discovery of new inhibitors against Bcl-2-family members, including Bcl-2, is still inadequate [10,11,36,39,45,54,71]. Most modeling efforts aimed for new anti-Bcl2 inhibitors focused on structure-based methodologies including docking studies [11], virtual screening using docking-based pharmacophores [39,71] or virtual screening implementing molecular dynamics-based binding models derived from Bcl-2 crystallographic structures [45,54]. On the other hand, related ligand-based modeling efforts (against Bcl-2-family) are basically limited to two studies only (up to our best knowledge). Both published anti-Bcl-x_L pharmacophore models [10,36].

Although crystallographic structures are considered rather reliable for drug design purposes, they can be limited by inadequate resolution, crystallization-related artifacts of protein-ligand complex, lack of information about protons and ionization states of ionizable amino acid residues [5,28,56,61].

The limited number of anti-Bcl2 ligand-based modeling efforts combined with drawbacks inherent in structure-based design modeling methodologies prompted us to attempt our well-established strategy of combining pharmacophore exploration with classical quantitative structure activity relationship (QSAR) to explore the binding requirements for potent Bcl-2 inhibitors within a rather large and potent list of inhibitors. The resulting pharmacophores were used as *in silico* search queries for the discovery of new potential Bcl-2 inhibitors. Similar computational workflows were used for the discovery of novel inhibitors against several targets involved in pathological conditions [1–4,6,7,9,28,50,57,62].

Fig. 2 illustrates the overall performed computational workflow. The process commenced by exploring the pharmacophoric space of 98 Bcl-2 inhibitors using HYPOGEN/CATALYST module of DiscoveryStudio suite (Biovia Inc., USA). Subsequently, genetic function algorithm (GFA) coupled with multiple linear regression (MLR) analysis or k-nearest neighbor (kNN) analysis were employed to search for best possible QSAR models (self-consistent and predictive) that combine optimal 3D pharmacophores with other physicochemical descriptors.

QSAR-selected pharmacophores were validated by evaluating their abilities to successfully classify a list of compounds as actives or inactives (i.e., by assessing their receiver-operating characteristic (ROC) curves). Moreover, as an additional validation, we compared their pharmacophoric features with crystallographic ligand/Bcl-2 complexes to identify similarities in critical interactions within ligand-Bcl-2 complexes. Eventually, optimal QSAR-selected pharmacophores were used as 3D search queries to screen the national cancer institute (NCI) virtual molecular database for new potential Bcl-2 inhibitors. Several hits gave low micromolar selective cytotoxicities against MDA-MB-231 cancer cells (breast cancer cells over-expressing Bcl-2). The chemical identities and purities of active hits were validated by nuclear magnetic resonance and mass spectroscopy.

2. Experimental

2.1. Molecular modeling

2.1.1. Software and hardware

The software packages that have been utilized during this study are the following.

- DiscoveryStudio, version 2.5.5, Biovia Inc. (www.biovia.com), USA.
- CS ChemDraw Ultra 7.0.3, Cambridge Soft Corp. (http://www.cambridgesoft.Com), USA.
- MATLAB (Version 7.4.0.287- R2007a), MathWorks Inc. (www.mathworks.com).

2.1.2. Data set

Literature review was performed to identify as many structurally diverse Bcl-2 inhibitors as possible. Accordingly, the structures of 98 Bcl-2 inhibitors (**1**–**98**, Table S1 under Supplementary Materials) were collected from three recently published articles [15,43,69]. Their bioactivities were expressed as binding inhibition constants (Ki, nM). The logarithms of measured Ki values were used in the pharmacophore modeling, as well as, quantitative structure activity relationship modeling thus correlating the data linearly to free energy change [5,6,27,63].

In the cases where Ki was reported as being less than 1 nM (compound no. **21**, **22**, **32**, **34**, **36**, **39**, **40**, **41**, **43**, **44**, **45**, **47**, **48**, **49**, **50** and **51**) we assumed their Ki = 0.7 nM (which corresponds to the Ki of the most potent compound in the collection). In few cases Ki was reported as being higher than 1000 nM (compound no. **87** and **92**) we assumed their Ki = 1000 nM, similarly, Ki was reported as