Journal of Taibah University Medical Sciences (2023) 18(3), 461–469

Taibah University

Journal of Taibah University Medical Sciences

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Original Article

Reinstating apoptosis using putative Bcl-xL natural product inhibitors: Molecular docking and ADMETox profiling investigations

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Received 11 July 2022; revised 17 September 2022; accepted 25 October 2022; Available online 14 November 2022

الملخص

أهداف البحث: في حين أن التوازن الدقيق في أفراد الأسرة المؤيدة للاستماتة والمضاد للاستماتة من عائلة بروتين الخلايا البائية 2 (بي سي إل - 2) يمثل ملف إشارة طبيعي. أدى الميل في التوازن نحو أفراد الأسرة المضادة للاستماتة إلى تقوية أشكال مختلفة من السرطانات مع ميزة البقاء على قيد الحياة ومقاومة العلاج. تحريض موت الخلايا المبرمج هو نهج علاجي رئيسي في اكتشاف أدوية السرطان وتثبيط ورم الغدد الليمفاوية للخلايا البائية الكبيرة جدا (بي سي إل – إكس إل) هو هدف سريري طويل الأمد لعلاج السرطان.

طريقة البحث: في هذه الدراسة ، نجمع بين الأساليب بمساعدة الكمبيوتر للإبلاغ عن المجلدات المفترضة. قبل حملة الفحص الافتراضية الخاصة بنا ، أجرينا استراتيجية تجربة إعادة تخزين للمثبط المرتبط بالأشعة السينية لبروتين بي سي إل – إكس إل مع بعض برامج الإرساء المتاحة تحت تصرفنا للحصول على البرنامج بأفضل كفاءة لهذا الفحص. ظهرت آي جيم دوك لإعادة إنتاج المعلومات البلورية بالأشعة السينية واستخدمت لإرساء مكتبة الترابط التي تم تطويرها من مركبات أبحاث طبية متنوعة مع ملفات تعريف مضادة للاستماتة من خلال عائلة بي سي إل–2.

النتائج: من بين المركبات الموجودة في المكتبة ، سجل Alpha-manogstin -زانثونويد طبيعي يتم الحصول عليه من اللحاء والنسغ المجفف من زَهرَةُ الحَجَر. ، وهي شجرة استوائية تنتمي إلى عائلة الكلوزية أو حاملات النقط الشجرية-. (إضافة توضيحية من المترجم)- و Oubain -مادة سامة مشتقة من النبات-. (إضافة توضيحية من المترجم)- نتائج ذات قيم طاقة ملزمة تبلغ kcal / mol123.025- و kcal / mol122.271- على التوالي والتي تزيد عن kcal / mol120.8- التي تمت ملاحظتها بالمعيار.

الاستنتاجات: كشفت هذه المركبات عن إمكانية تقارب ملزمة أكثر من ABT-737 وهو مثبط قياسي للبروتين. بالإضافة إلى ذلك ، لا تتفاعل هذه السقالات فقط مع بقايا النقاط الساخنة ذات الصلة لتثبيط Bcl-xL ولكنها تمتلك أيضًا نقطة نهاية جيدة للحركية الدوائية والسمية الممتازة التي يجب أخذها في الاعتبار لمزيد من الاختبارات وتطوير الأدوية.

الكلمات المفتاحية: عائلة بي سي إل – 2؛ بي سي إل – إكس إل؛ الالتحام الجزيئي؛ الفحص الافتراضي؛ موت الخلايا المبرمج.

Abstract

Objectives: While a fine balance in the pro-apoptotic and anti-apoptotic family members of the B-cell lymphoma-2 (Bcl-2) protein family represents a normal signaling profile, a tilt in balance towards anti-apoptotic family members has fortified different forms of cancers with survival advantage and resistance against treatment. Induction of apoptosis is a key therapeutic approach in cancer drug discovery, and the inhibition of the anti-apoptotic B cell lymphoma extra-large (Bcl-xL) is a long-

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Peer review under responsibility of Taibah University.

Production and hosting by Elsevier

 https://doi.org/10.1016/j.jtumed.2022.10.014

standing clinical target for cancer therapy. In this study, we combined computer-aided approaches to report putative binders for this target.

Methods: Before our virtual screening campaign, we conducted a redocking experiment strategy of the x-ray bound inhibitor of the Bcl-xL protein with some of the available docking software at our disposal to determine the software with the best efficiency for this screening. iGEMDOCK emerged to reproduce the x-ray crystallographic information and was used to dock the library of ligand, which was developed from diverse literature reporting compounds with anti-apoptotic profiles through the Bcl-2 family.

Results: Of the compounds in the library, alpha-mangostin and oubain scored as hits with binding energy values of −123.025 kcal/mol and −122.271 kcal/mol, respectively, which is more than −120.8 kcal/mol observed by the standard.

Conclusions: These compounds revealed a more binding affinity potential than ABT-737, which is a standard inhibitor of the protein. In addition, these scaffolds not only interact with relevant and hotspot residues for the inhibition of Bcl-xL but also possess good pharmacokinetic and excellent toxicity, an endpoint that should be considered for further testing and drug development.

Keywords: Apoptosis; Bcl-2 family; Bcl-xL; Molecular docking; Virtual screening

Introduction

Cancer is a disease with multifactorial and multifaced etiology.[1] Factors such as environmental factors, genetics, and sedentary lifestyle have been identified solely and in concert to influence the causation of the disease.[2–4] Cancer is characterized by abnormal growth, which stems from an inappropriate response to growth signals.[5,6] Generally, cells do not grow until told to do so, suggesting that the growth of cells under normal parlance is under strict regulation by an array of genes and a complex network of proteins. However, once their cellular machinery is hijacked, possibly as a result of mutation, uncontrolled growth ensues and cancer can result. If not caught early enough, cells grow into a tumor mass and invade nearby and/or distant tissue.[7]

One in four people die of cancer, making it the second leading cause of death globally.[8] Apart from the high proliferation rate, one way in which cancer cells become dominant is by inducing the growth of capillaries from pre-existing blood vessels towards their site—an event called angiogenesis.[9] This neovasculature system provides tumors with nutrients and oxygen just like normal cells and is important for their growth and metastasis.[10] Since the cellular phenotype of tumor cells is different from normal cells, one can assume that the immune system (being the first-line defense mechanism) should recognize them as foreign and thus induce a cascade of immunogenic responses whose downstream effects will culminate in the demise of the cells.[11] However, during tumorigenesis, the immune system is hijacked. Normal cells around the primary tumor site are also taken over to better suit their purpose by forming an immunosuppressive tumor microenvironment (TME) with the aim of evading the immune system.[12,13] One important thing about these cancer cells is that they are resistant to cell death, especially programmed cell death (a homeostatic cell death mechanism that is initiated to remove foreign cells).[14] Thus, induction of apoptosis represents a therapeutic standpoint for combating several cancer types such as head and neck cancer,[15] breast cancer,[16] and colorectal cancer.[17]

Programmed cell death or apoptosis mainly occurs via two mechanisms: extrinsic and intrinsic pathways. While the former is dependent on the expression of death cell receptors, the latter is mitochondrial-dependent.[18] The decision of which pathway the cell follows is a factor of the stimulus in question. In the intrinsic mode of apoptosis, the B-cell lymphoma 2 (Bcl-2) family of proteins that are native to the outer mitochondrial membrane mediate this type of cell death.[19] Their activity, after inducing mitochondrial outer membrane permeabilization, results in the release of cytochrome *c*, which leads to cell death via a series of caspase activation processes.[20] This event is different from the mitochondrial permeability transition event that occurs in the inner membrane of the mitochondria.[21]

The Bcl-2 family is a diverse group of proteins that contains three different subgroups: pro-apoptotic, anti-apoptotic, and pro-apoptotic BH3-only subgroup. While pro-apoptotic members such as Bcl-2 antagonist/killer and Bcl-2-associated X protein act in favor of apoptosis, the anti-apoptotic Bcl-2 and Bcl-extra-large (Bcl-xL) prevent apoptosis induction.[22] A dynamic balance between the pro-apoptotic and anti-apoptotic subgroup determines whether a cell dies or lives. Following cellular insult, normal cells upregulate the activity of the pro-apoptotic family member in such a way that their activity will be sufficient to cause cell death. However, in cancer, there is increased activity of the anti-apoptotic family members such as Bcl-xL, thus preventing the induction of apoptosis.[23] This suggests that inhibition of anti-apoptotic family members such as Bcl-xL could help cause cancer cell death. With the impact of natural product scaffolds in the discovery of various approved drugs for human disease and their increasing interest in drug discovery today, in this study, we screened natural products (obtained from diverse literature)[24–27] whose cytotoxic mechanism of action is dependent on inhibition of the Bcl-2 family to identify potent binders for Bcl-xL using a well-validated docking approach. The drug-likeness and toxicity characteristics of the candidates were also evaluated.

Materials and Methods

Ligand search and preparation

We searched PubMed (https://pubmed.ncbi.nlm.nih.gov/) to obtain plant-based bioactive compounds with anti-

apoptotic potential. A total of 35 compounds had the mechanism of action of downregulating the activity of one or more of the Bcl-2 anti-apoptotic family of proteins. The three-dimensional structural information of these ligands was recruited from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in Simulation Description Format and the converted to Protein Data Bank (PDB) format. Chemdraw 3D 16.0 was used to minimize the ligands and were saved in PDB format for further use.

Protein preparation

The coordinate of the anti-apoptotic Bcl-xL protein was obtained from the PDB (https://www.rcsb.org/) as 3ZK6 and treated accordingly using BIOVIA Discovery Studio 2019 to remove extra crystallized compounds and water molecules. Missing residues were fixed using the "Misc" functionality in Autodock software, and polar hydrogens were also added. The amino acid residues that make up the active site of the protein were detected using CASTp.[28] These residues were noted and used for site-specific docking.

Docking validation

To proceed with our virtual screening campaign, iGEMDOCK,[29] SAMSON,[30] and MOE,[31] which are the software we have at our disposal, were used to redock the co-crystallized ligand (WEHI-539) back into the protein—an event called redocking. The ligand and protein of the PDB code 3ZK6 were separated using Discovery Studio 2019 (http://www.accelrys.com) and were redocked using the three software programs accordingly. This method is similar to what we have used in our previous studies.[32,33]

Molecular docking and post-docking analysis

Molecular docking was carried out using iGEMDOCK 2.1, a software with a hybrid scoring function (from simple empirical and a pharmacophore-based function) to reduce false positives. For this study, the population size was set to 300; generations were pegged at 80, and the number of solutions was put to 3. Other parameters were left in their default setting.

Drug-likeness and ADMETox evaluation

The drug-likeness properties of the prospective drug candidates was predicted using molinspiration (https://www.molinspiration.com/cgi-bin/properties). On the other hand, the pharmacokinetic and toxicity endpoint were predicted using the absorption, distribution, metabolism, excretion, and toxicity lab (ADMETlab) webserver.[34]

Results

Molecular docking validation

Validation in docking takes several approaches. Here, a redocking experiment strategy was used to determine which software would be best in docking the ligand library by measuring the ability of the software at our disposal to reproduce the geometry of the ligand in the crystallographic information of 3ZK6 as a yardstick. In the redocking experiment, the ligand of a PDB crystal structure was redocked into its active site and an root-mean-square deviation (RMSD) value was computed between the crystal conformer and the relative docked pose. RMSD value less than 2.0 Å is considered good but values closer to 0.0 Å are excellent, meaning that the software is a good fit for structure-based drug discovery of the protein target. Figure 1 shows the reproducibility index of the crystal information of 3ZK6 by iGEMDOCK, MOE, and SAMSON. From the figure, the geometry of the pose generated from MOE was relatively different from that of the crystal information. The one produced by SAMSON still had a resemblance in some portion of its ring, whereas that yielded by iGEMDOCK was consistent with the crystal conformer. While MOE and SAMSON software reproduced pose with RMSD values of 3.6 Å and 2.3 Å, respectively, the iGEMDOCK generated pose had a value of 0.8 Å, suggesting its further use for this virtual screening study.

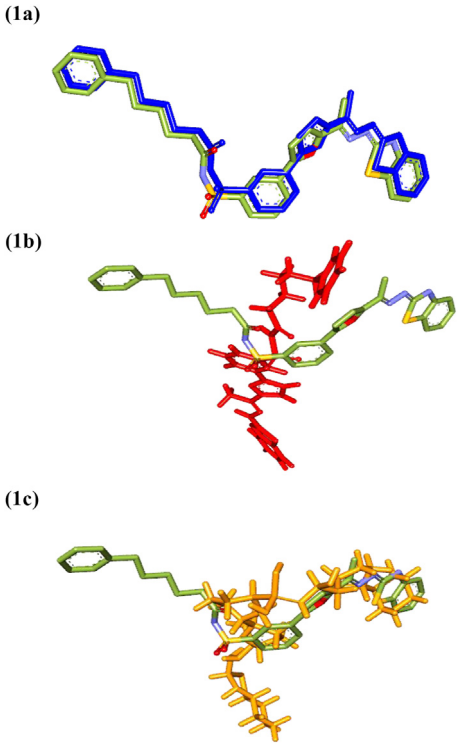

Figure 1: Superimposition of the co-crystallized ligand and the redocked pose of 3ZK6 using three different software programs (a) iGEMDOCK (b) MOE (c) SAMSON. Green = color of crystal conformer, other colors are with respect to the software

Molecular docking

The result of the docking simulation of all 35 compounds is shown in Figure 2. Apparently, the major energy type that constitutes the interaction between these ligands and the Bcl-xL protein are the van der Waals (VDW) energy and

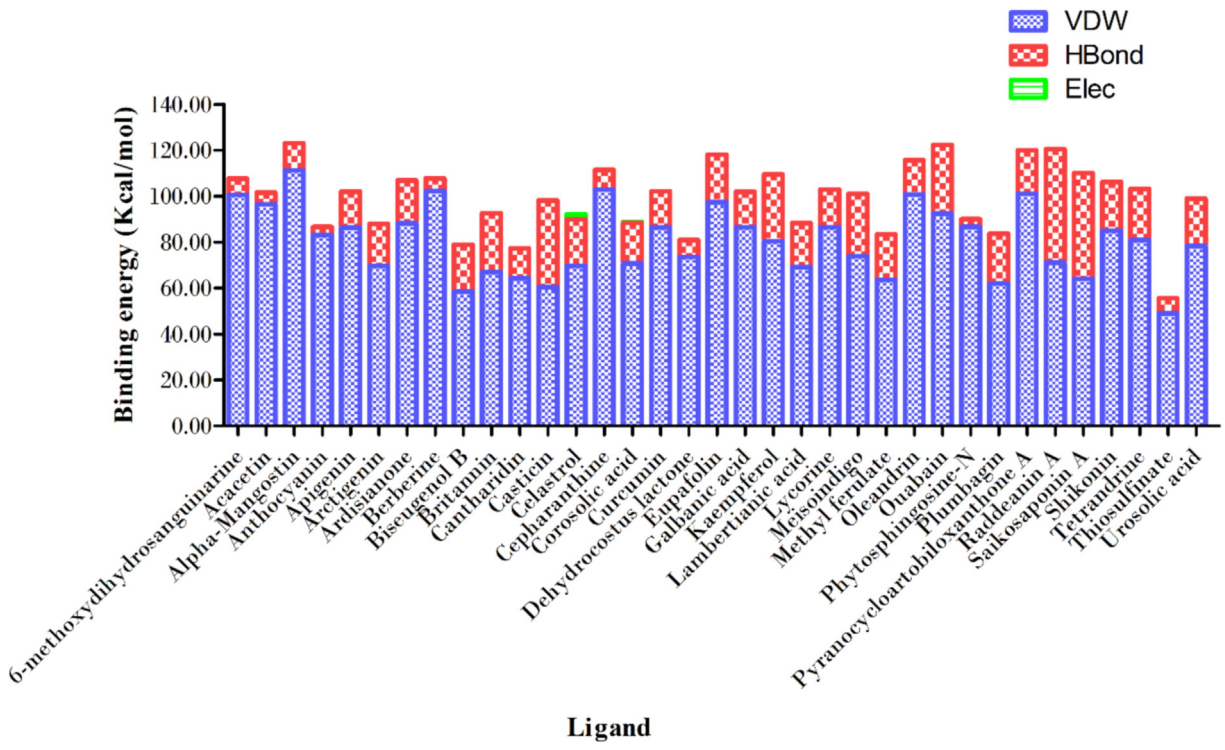

Figure 2: Binding energy values of the ligand.

Table 1: Binding energy information of the standard drug and potential hits.

Standard	VDW	HBond	Elec	Energy
ABT-737	−91.1	−30.46	0.76	−120.800
Alpha-Mangostin	−111.246	−11.7791	0.00	−123.025
Oubain	−92.4354	−29.8353	0.00	−122.271

Table 2: Lipinski's rule of five of the selected hits.

Ligands	Molecular weight	Hydrogen bond donor	Hydrogen bond acceptor	LogP	Number of violations
Alpha-mangostin	410.466	3	6	5.089	1
Oubain	584.659	8	12	−1.515	2

hydrogen bond, to a lesser extent—the electrostatic force. From the result, only celastrol and corosolic acid were bonded by the three energy types, and others were mediated by VDW and hydrogen bond alone. Alpha-mangostin (with an energy value 123.025 kcal/mol) was the ligand with the highest binding affinity, whereas thiosulfinate was the least binder with −55.5368 kcal/mol as its energy score.

The binding energy of the standard ABT-737 (Table 1) was used as the cutoff value for this study, ligands with binding energy less than −120.8 kcal/mol were discarded, and those with binding energy above this set bar were regarded as hits for this study; thus prospective drug candidates for the Bcl-xL target. It is important to state that there is no standard drug in the market with a molecular mechanism specific for the inhibition of Bcl-xL. The consideration for ABT-737 drug binding energy as the standard for this screening was based on the fact that its mechanism is dependent in part on the binding and inhibition of Bcl-xL.[35] Alpha-manogstin and oubain with binding energy values of −123.025 kcal/mol and −122.271 kcal/mol, respectively (Table 1), were the only candidates with binding energy greater than −120.8 kcal/mol. This suggests that they show a better binding affinity for the target than the standard drug and thus were considered for further *in silico* screening.

ADMETox and drug-likeness properties

The goal of bringing a drug to the clinic is beyond the high-binding property of the drug to its target alone but also its drug-like attitude. The pharmacokinetic and pharmacodynamic properties of the drug also goes a long way to a

Table 3: Pharmacokinetic and pharmacodynamic prediction of selected hits.

Parameters	Oubain	Alpha-mangostin
Absorption		
Caco-2 (log cm/s)	−6.413	−4.847
HIA	+++	−
Distribution		
BBB Penetration (cm/s)	−−	−−−
Metabolism		
CYP1A2 inhibitor	−−−	−
CYP1A2 substrate	−−	−
CYP2C19 inhibitor	−−−	++
CYP2C19 substrate	−−	−−−
CYP2C9 inhibitor	−−−	++
CYP2C9 substrate	−−	+++
CYP2D6 inhibitor	−−−	+
CYP2D6 substrate	−−	−−
CYP3A4 inhibitor	−−−	−−
CYP3A4 substrate	−−−	−−−
Excretion		
Clearance (ml/min/kg)	1.538	6.820
Half-time	0.138	0.345
Toxicity		
hERG Blockers	−−	−−−
DILI	−−−	+
AMES Toxicity	−−−	−
Carcinogenicity	−	−−−

Note: HIA = human intestinal absorption; BBB = blood brain barrier; CYP = cytochrome p450 enzyme family; hERG = human ether-a-go-go; DILI = drug-induced liver injury. For endpoint classification, the prediction probability values are metamorphosed into six symbols: 0−0.1(−−−), 0.1−0.3(−−), 0.3−0.5(−), 0.5−0.7(+), 0.7−0.9(++), and 0.9−1.0(+++).

successful clinical trial of the drug. Poor ADMETox property is the major cause of attrition of most drugs in the clinic, making profiling for the drug-like properties and ADME endpoint an important pursuit in early drug discovery endeavors. Table 2 shows the drug-like properties of alpha-mangostin and oubain using the Lipinski's rule of five—he proposed that a small-molecule drug for drug development should not violate more than one of the following: molecular weight <500 Da, the number of hydrogen bond donor ≤ 5, the number of hydrogen bond acceptor ≤ 10, and octanol−water partition coefficient not more than 5.[36] It could be deduced from the table that while alpha-mangostin only violated one of the rules of five, oubain violated two. This suggests that oubain will raise an alert for being poor for drug development under Lipinski's yardstick, although this inference is too early without considering its ADMET features.

The ADME and toxicity properties were computed using ADMETlab webserver, and relevant parameters are reported in Table 3. From the absorption end, Caco-2 is a model membrane for evaluation of the ability of a drug to permeate the intestinal wall in the *in vitro* and *in silico* parlance. A compound with Caco-2 value >-5.15 log cm/s is considered likely to permeate the Caco-2 membrane.[37] The prediction reveals oubain to have a permeability value of −6.413 log cm/s and −4.847 for alpha-mangostin. An implication for this is that while the former possesses a poor permeability feature, the latter has a good likelihood of getting into the systemic circulation. Human intestinal absorption was also predicted as an indicator of permeability, it could be deduced that both prospective candidates have a good prospect for oral bioavailability and intestinal absorption. This is easily noticeable from the probability value of both candidates—alpha-mangostin has a probability value of 0.317, which translates to 31.7% and oubain carries a probability value of 0.99, which translates to 99% (not shown in the table). Interestingly, a compound with an absorbance value less than 30% is considered poorly absorbed, suggesting alpha-mangostin and oubain as excellent candidates for intestinal permeation. Considering the distribution endpoint of the two prospective drug candidates, both show no propensity to traverse the blood−brain barrier and thus will not interfere with normal central nervous system signaling functions (Figure 3).

Cytochrome p450 is an important family of drug-metabolizing enzymes that is responsible for the metabolism of about two-thirds of known drugs with oral administration in humans. This metabolizing property has been mainly attributed to the following five isoenzymes: 1A2, 3A4, 2C9, 2C19, and 2D6. The interference (whether induction or interference) of a drug with these proteins may result in a drug−drug interaction response.[38] The metabolism portion of table … shows the endpoint result of these prospective drug candidates; a (−−−/−−) means the candidate has no affinity for the protein, whereas a (+++/++) result shows a strong affinity for any of the proteins. Interestingly, oubain proved not to be a substrate or an inhibitor to any of the isoenzymes and hence an excellent drug for metabolism. Meanwhile, alpha-mangostin has a fair property with only interference with 2C9 mainly—it is both a substrate and inhibitor for the protein. Overall, this still suggests a good metabolic property for these prospective drug candidates.

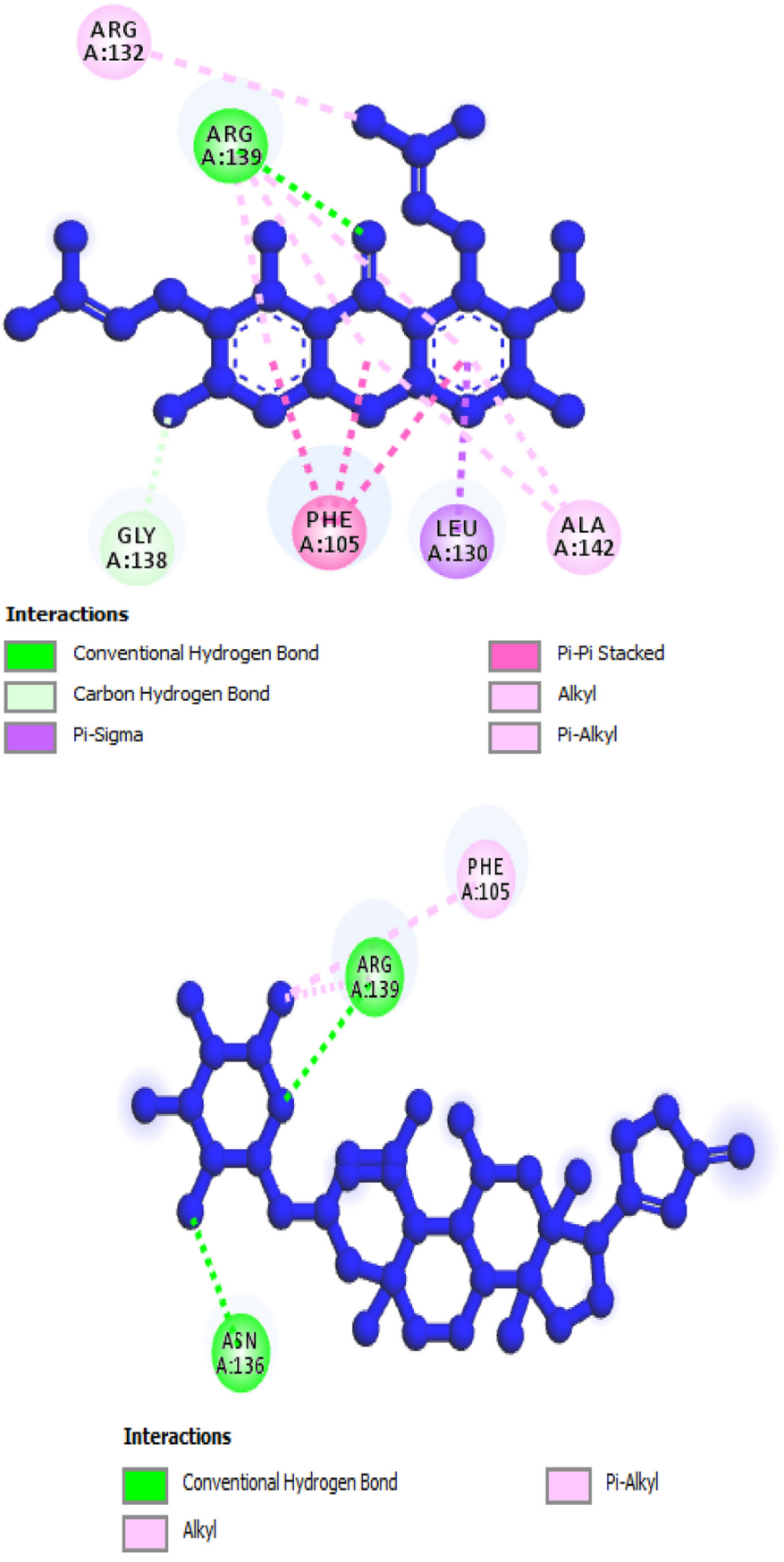

Figure 3: Binding interaction of the hit compounds, alpha-mangostin, and oubain at the active site of Bcl-xL.

The clearance rate of a drug is an important pharmacokinetic property in pharmacology and it mainly depends on the half-life and clearance (CL) of the drug. A drug with <5 value is considered poor for excretion, whereas that which is ≥ 5 is regarded as excellent. Oubain has an endpoint value of 1.538 while alpha-mangostin has 6.820. One interpretation for this is that alpha-mangostin has a high clearance property, whereas oubain has a low one. However, the half-life descriptor was in favor of both candidates as excellent candidates with good excretion profiles. This is because the empirical decision of the parameter has it that compounds with values within the range of 0–0.3 are excellent. Looking at the toxicity parameters, both prospective drug candidates—alpha-mangostin and oubain—have no impact in any of the toxicological descriptors, though alpha-mangostin is likely to cause drug-induced liver injury.

Discussion

Sustained proliferative signaling, evading immune destruction, invading vasculature, resisting cell death, shunning growth suppressors, and reprogramming cellular metabolism all represent, in part, the functional capabilities or hallmarks of cancer cells.[39] Generally of these features, induction of cell death is regarded as the utmost aim in cancer therapy.[40] One important approach to that is the inhibition of the anti-apoptotic family members (especially Bcl-xL) of the Bcl-2 protein family. Bcl-2 family members have this name because of their common BH domain, and are divided into anti-apoptotic, BH3-only, and pro-apoptotic members. While the pro-apoptotic family members are the pore former and induce apoptosis, the anti-apoptotic group inhibits cell death—both in a manner that involves coordinated interactions with the BH3-only peptides.[41] In cancer, the anti-apoptotic family member Bcl-xL is overexpressed and its inhibition portends a critical way for the induction of cell death.

Several clinically approved drugs got their core scaffold from the natural bioactive compound structure of the product.[42,43] Natural product scaffolds have a good history of pharmacological relevance and have been a good source of inspiration for developing lead candidates by medicinal chemists. With this motivation, we sought to identify putative natural product binders of the Bcl-xL protein. To this end, we developed a library of natural products for inhibition of this protein by conducting a search of the literature for compounds that have potential to induce apoptosis. Emphasis was paid to those whose mechanism of action was dependent on the inhibition of the Bcl-2 family of proteins, and a total of 35 compounds were obtained. Molecular docking studies of these compounds with the Bcl-xL protein revealed alpha-magostin and oubain as excellent binders due to binding energy value, which was far better than ABT-737—a standard drug.

Alpha-mangostin made a network of interactions including hydrogen bond, pi and alkyl bond with different amino acid residues at the active site of the protein. More specifically, it made hydrogen bonds with Arg139 and Gly138; while the former was conventional, the latter was a carbon hydrogen type. This ligand also interacted with hydrophobic amino acids such as Phe105, Lue130, and Ala142 in a reaction coordinated by $\pi-\pi$ stacked, pi-alkyl, pi-sigma, and alkyl bonds. The interaction of these hydrophobic residues with the ligand was as a result of its xanthene ring, which is similar to the hydrophobic core binding scaffold of popular standard inhibitors like ABT-737 and other synthetic inhibitors. On the other hand, oubain only interacted with three amino acid residues (Arg136, Arg139, and Phe 105) on the target. The interaction was facilitated with hydrogen bond, pi-akyl, and alyl bond. Interestingly, it interacted using only the cyclohexane ring of its structure. This should not raise any flags because emphasis has been on hotspot residues for the inhibition of protein targets that mediate protein–protein interactions.[44] Thus, the molecular interaction by oubain is still sufficient to inhibit the protein. The Bcl-xL protein active site is a protein domain containing four different pockets, namely P1, P2, P3, and P4. Residues that reportedly facilitate the inhibition of this protein includes Arg139, Phe105, Tyr195, Arg132, and Glu96.[46] This suggests that interactions with some of these residues leads to effective inhibition of the protein. Interestingly, alpha-manogstin and oubain interacted with about three of these residues. Their interactions were stacked using other supporting residues on the active site like Leu1130, Asn136, Ala142, and others. Similarly, previous docking simulation studies have also reported the effective inhibition of Arg139, Phe105, and Arg132 with flavonoids and synthesized compounds.[47,48]

This mechanistic interaction system would not make any sense in the drug discovery parlance if these potential drug candidates did not do well pharmacokinetically and pharmacodynamically. Interestingly, both candidates fit well with the benchmark of Lipinski's postulation for orally bioavailable drugs, with alpha-mangostin being excellent. Also, these two compounds seem to have a good absorption and toxicity character. In addition, their half-life and metabolic features gives a hope in sight for good performance in the clinic.

Conclusions

In this study, we identified alpha-mangostin and oubain as potent binders and inhibitors of Bcl-xL based on high binding energy values through a well-validated molecular docking protocol. The property possessed by these compounds coupled with their excellent drug-like and profound pharmacokinetic and pharmacodynamic properties put them forward as prospective drug candidates for Bcl-xL that should be considered for further testing on their ability to induce apoptosis via the inhibition of the Bcl-xL protein.

Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not for-profit sectors.

Conflict of interest

The authors have no conflict of interest to declare.

Ethics approval

Not applicable.

Consent for publication

Not applicable.

Authors' contributions

IDB - Conceptualization, Methodology, Software, Validation, Formal analysis, investigation, resources, data curation, visualization, project administration, and writing; **ATO** - Validation, Data curation, visualization project administration, writing, reviewing, and editing; **AKO** - Formal analysis, investigation, data curation and project administration; **OKB** - Validation and visualization; **NO** - data curation and visualization; **MS** - Formal analysis, investigation and project administration; **FTA** - Formal analysis, investigation, visualization, reviewing; **TOO** - Data curation, visualization and project administration; **TIA** - Formal analysis, Investigation, visualization and project administration. All authors have read and approved the final version of the manuscript.

Availability of data and materials

Not applicable.

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How to cite this article: Boyenle ID, Ogunlana AT, Kehinde Oyedele A-Q, Olokodana BK, Owolabi N, Salahudeen A, Aderenle OT, Oloyede TO, Adelusi TI. Reinstating apoptosis using putative Bcl-xL natural product inhibitors: Molecular docking and ADMETox profiling investigations. J Taibah Univ Med Sc 2023;18(3):461−469.