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ORIGINAL RESEARCH

Design of Druglike Small Molecules for Possible Inhibition of Antiapoptotic BCL-2, BCL-W, and BFL-1 Proteins

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Abstract: New druglike small molecules with possible anticancer applications were computationally designed. The molecules formed stable complexes with antiapoptotic BCL-2, BCL-W, and BFL-1 proteins. These findings are novel because, to the best of the author's knowledge, molecules that bind all three of these proteins are not known. A drug based on them should be more economical and better tolerated by patients than a combination of drugs, each targeting a single protein. The calculated drug-related properties of the molecules were similar to those found in most commercial drugs. The molecules were designed and evaluated following a simple, yet effective procedure. The need for substantial computational resources often precludes researchers in many countries and small institutions from participating in the field. The procedure presented here offsets the problem by reducing the cost of involvement. The procedure can be used efficiently in the early phases of drug discovery to evaluate promising lead compounds in time- and cost-effective ways.

Keywords: small molecule mimetics, antiapoptotic proteins, computational drug design

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Introduction

The motivation for this work was twofold. First, to design new druglike small molecules that could form stable complexes with antiapoptotic BCL-2, BCL-W, and BFL-1 proteins. The designed molecules could potentially antagonize the proteins, thus facilitating apoptosis in cancer cells. The second objective was to present a procedure that could yield promising drug-like molecules using minimal programming skills and modest computational resources.

High-throughput experiments have yielded a large amount of information on various biomolecules. The design of new molecules with possible medical applications requires analyses of the accumulated information. The enormity of the task calls for the utilization of as many resources as possible.

Computer-based molecular design employs the methods of bioinformatics, medicine, biochemistry, biophysics, and other fields. Computational design has sped up research by identifying new molecules with possible medical applications prior to laborious experiments and expensive preclinical studies. However, substantial computational resources and programming proficiencies are usually needed to design computationally molecules with desired biological properties. This precludes researchers who lack computer resources in many countries and small institutions from carrying out studies in the field.

A simple, yet effective procedure presented here offsets the problem by lessening the need for high performance computing and requiring minimal computing skills. Using a single PC and open-source software, the procedure should help expand the number of those who are able to contribute to the field.

The availability of the procedure within a single document should also help those wishing to enter the field. Although highly valuable and much needed, comprehensive review papers on computational drug design may leave some researches wandering through a maze of various choices, unsure of the investments in time and money they would need to achieve their goals. The straightforward procedure described here can be used effectively in the early phases of discovery to evaluate promising lead compounds in time- and cost-effective ways.

Programmed cell death (apoptosis) is necessary for normal cellular functions and for the removal of damaged cells.[1] Malfunctioning apoptotic pathways can cause unrestrained cell growth and cancer development[2] Design of new molecules that can either restore or circumvent defective apoptotic pathways is vital for anticancer drug research.

Proteins play important roles in apoptosis. They can be either proapoptotic (prodeath) or antiapoptotic (prosurvival). Cell damage initiates stress signals. The stress signals stimulate proapoptotic proteins to bind and disable antiapoptotic proteins, thus preparing the cell for apoptosis.[3,4] Normally, this leads to the breakdown of the mitochondrial membrane,[5,6] causing the cell death.

Antiapoptotic and proapoptotic proteins from the BCL-2 family play important roles in apoptosis.[7,8] Many cancers overexpress BCL-2 antiapoptotic proteins.[9,10] The overexpression can prevent transmission of death signals and allow accumulation of abnormal cells.[11] This can lead to cancer advancement and poor chemotherapy response.[12] Blocking of antiapoptotic BCL-2 proteins is often necessary for apoptosis to occur.[13]

A new class of designed molecules, BH3 mimetics, may lead to death of cancer cells by hindering expressions of antiapoptotic BCL-2 family proteins.[14] A mimetic that targets a single protein can lead to drug resistance.[15] On the other hand, a drug that operates too broadly may harm healthy tissue. An alternative is to develop a drug that can simultaneously act on two or three proteins. Such a drug would have broad enough action to avoid drug resistance, yet be sufficiently specific to reduce injury of healthy cells. A single drug would be more economical and lead to fewer adverse effects than a combination with each drug targeting a single protein.

Small molecule ABT-737 (Protein Data Bank (PDB)[16] entry: 2YXJ) promotes apoptosis by inhibiting antiapoptotic BCL-2 and BCL-W proteins.[8,17] ABT-737 is effective even when a large number of the antiapoptotic proteins is present. The high-affinity ABT-737 molecule was designed using two molecules of modest affinities.[18] ABT-702 [ChemDB database[19] Compound ID (CID) 3965212] is a designed small molecule with anti-inflammatory effects in cells.[20] To the best of the author's knowledge, ABT-702 had not been studied previously for cancer treatment. Complexes of ABT-702 with BCL-2, BCL-W, or BFL-1

are not known. ABT-737 and ABT-702 showed no toxic effects in previous preclinical studies in mice.[17,21] In this work, ABT-702 and ABT-737 were used as templates to design computationally putative small molecule mimetics able to bind all three BCL-2, BCL-W, and BFL-1 proteins.

Computational design and study of novel molecules can be done using several existing programs. These include commercial programs like GOLD,[22] ZDOCK,[23] and LigandFit.[24] Some programs, like AutoDock[25] and DOCK[26] are not intuitive and require sizable computational skills and resources. Open-access Deep View,[27] ArgusLab,[28] Molinspiration,[29] and Osiris Property Explorer[30] programs used in this study are both intuitive and user-friendly. Furthermore, they do not require advanced programming skills.

A viable drug candidate should have bioavailability, metabolic stability, toxicity, and transport properties comparable to known drugs. These "druglike" properties depend on the compound's size, molecular flexibility, hydrophobicity, and electronic bond distributions.

The logarithm of the octanol-water partition coefficient of a compound, the logP value, is accepted in drug design as a reliable measure of the compound's hydrophilicity.[31] Low hydrophilicity corresponds to a high logP value. Previous studies showed that a compound is likely to be well absorbed by an organism if its logP value is less than 5.[31]

The logS value is another gauge of druglikeness. The logS value is the logarithm of the solubility measured in mol/liter, with units removed. The absorption of a compound is considerably influenced by its solubility. Generally, high logS values correspond to good absorption. Over 80% of commercial drugs have logS > -4, which corresponds to the solubility of 0.1 mmol/liter.[30] For the vast majority of commercial drugs the values of logS are between -5 and 1.[31]

The total polar surface area (PSA) of a molecule can reliably predict its transport through membranes, including its intestinal absorption and crossing of the blood-brain barrier.[32] The PSA values for most known drugs are less than 120 $Å^2$.[32]

Over 80% of commercially available drugs have molar masses below 500 g/mol.[30] Compounds with molar masses greater than 500 g/mol are difficult to absorb.

The number of rotatable bonds in a molecule determines its flexibility and can predict its oral bioavailability[33] Previous research in rats on over 1100 drug candidates[33] found that 10 or fewer rotatable bonds in a molecule indicated good oral bioavailability.

Lipinski's Rule of Five[34] provides a simple way to estimate molecular druglikeness. According to the rule, a druglike molecule has logP ≤ 5, molar mass <500 g/mol, 10 or fewer hydrogen bond acceptors, and 5 or fewer hydrogen bond donors. Molecules violating more than one of these rules likely are not viable drug candidates.

A simple procedure was employed in this work to design and evaluate new small molecules that could potentially promote death of cancer cells by binding antiapoptotic BCL-2, BCL-W, and BFL-1 proteins. The procedure described here could be employed to search for new small molecules with possible therapeutic applications.

Methodology

Computational tools

The following open-source software and servers were used: Protein Data Bank,[16] the ChemDB database,[35] Deep View,[27] ArgusLab,[28] Osiris Property Explorer,[30] and Molinspiration.[29]

Protein Data Bank (PDB) provides information about experimentally known structures and functions of various molecules. It also allows their visualizations and downloads. PDB is accessible to users with a wide range of computational skills. The ChemDB database allows online searches for chemicals by structural similarities.[19] Molecules can be easily downloaded from ChemDB in various file formats.

Deep View and ArgusLab can be downloaded from their respective websites. The websites offer excellent comprehensive tutoring, so that even a person with minimal or no programming experience should be able to successfully use the programs. User-friendly graphic and menu tools in Deep View allow easy visualizations and comparisons of molecules and their interactions.

ArgusLab program is intuitive and easy to learn. The program allows molecular design and docking calculations. To perform a docking calculation one first needs to specify a ligand (in this case a putative small molecule mimetic) and a molecule the ligand

should bind (in this case, the BCL-2, BCL-W, and BFL-1 proteins). The program then tries to dock the ligand to the binding site and reports on stable configurations it finds. ArgusLab can also carry out semiempirical geometry optimizations. Substitutions and deletions of atoms are easily done in ArgusLab. In addition, its molecular Builder Tool utility can be used for *de novo* molecular design.

Open-source programs Molinspiration and Osiris Property Explorer allow calculations of drug-related properties for a valid chemical structure. Osiris Property Explorer also predicts the molecule's potential mutagenic, tumorigenic, reproductive, or other risks. Molinspiration is a recognized internet server that averages about fifty thousand molecular property calculations per month. Molinspiration allows calculations of the logP, PSA, and molar mass values, as well as the numbers of rotatable bonds and H-bond acceptors and donors. The techniques used for calculations and validation studies are described elsewhere.[29] The Molinspiration and Osiris programs were employed to compute drug-related properties of the newly designed small molecules and to predict their potential toxicity.

The calculations of the logP and logS values are described on Osiris Property Explorer website.[30] Osiris calculations were originally optimized on training sets of more than 5000 compounds with measured logP values and more than 2000 compounds with measured logS values. Application of Osiris to an independent set of more than 5000 chemicals showed a very good correlation between calculated and experimental logP values.[30] Although sufficient, the accuracy of the logS assessment is not as good as the one for logP. The reason is that molecular logS values depend on topological factors that cannot be completely accounted for by atom types.

Osiris calculations for 15000 non-druglike chemicals and 3300 comercial drugs found that about 80% of the known drugs had positive values of the Drug Likeness parameter,[30] while almost all of the non-druglike chemicals had negative values.[30] A positive value of Drug Likeness indicates that the molecule consists mostly of building blocks (fragments) that are commonly found in commercial drugs.

Osiris can issue warnings to indicate that a structure may be toxic. However, the lack of the warnings does not absolutely guarantee that a given molecule is non-toxic. Nevertheless, the program has good predictive powers. When tested on substances known to be mutagenic, Osiris correctly predicted toxicity for 86% of them. Conversely, Osiris indicated that only 12% of tested commercial drugs were potentially harmful.[30]

None of the individual drug-related parameters guaranties that a molecule will be a suitable drug candidate. A promising drug candidate should have optimized values of all drug-related parameters and no indicated risk factors. Osiris combines the values of logP, logS, molecular mass, Drug Likeness, and toxicity risks in a single number, the Drug Score, which can be used to evaluate the compound's overall drug potential.

Dataset

Experimentally known three-dimensional (3D) structures of antiapoptotic BCL-2, BCL-W, and BFL-1 proteins were downloaded from Protein Data Bank (PDB),[16] entries 2O22, 1MK3, and 2VM6, respectively. PDB was also used to obtain the structure of ABT-737 (entry 2YXJ). The structure of the ABT-702 molecule was downloaded from the ChemDB databases[19] (CID 3965212).

The ChemDB database was then searched for molecules with structures similar to ABT-737 or ABT-702. Forty-seven such molecules were identified and docked to BCL-2, BCL-W, and BFL-1 in ArgusLab. The docking showed that six of these molecules formed stable complexes with all three proteins. The structures of the six molecules are shown in Figure 1.

The remaining 41 molecules formed either no stable poses or marginally stable poses with the proteins. New putative mimetics were designed using the above six experimentally known molecules as templates. New molecules that formed stable complexes with all three BCL-2, BCL-W, and BFL-1 proteins and had druglike properties were identified.

Procedure Employed

Figure 1 shows common features of the six experimental molecules. The pyrrole ring is present in each of the molecules and benzene in four. Linear C-H chains are found in the top three molecules in Figure 1. The figure shows that 5-atom rings connected via their C atoms were also present. Two of the molecules in

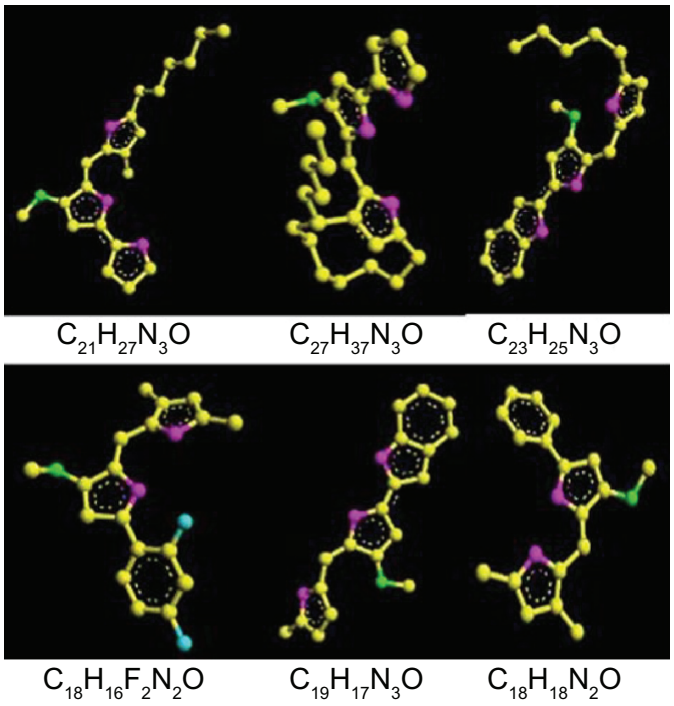

Figure 1. Structures of the six experimental small molecules. Color code: C-yellow, N-pink, O-green, F-blue. For simplicity, hydrogen atoms are not shown.

Figure 1 also had double-rings consisting of fused pyrrole and benzene (the third and fifth molecule, counting from left to right and top to bottom). None of the molecules contained more than three single rings or one double and two single rings.

The six molecules from Figure 1 were drawn in Osiris. The values of their logP, logS, Drug Likeness, Drug Score, and toxicity risks were calculated. Druglikeness calculated for the experimental molecules ranged between −18.6 for $C_{27}H_{37}N_3O$ to 2.58 for $C_{19}H_{17}N_3O$. Drug Scores were between 17% and 74%, and were obtained, respectively, for the same two molecules.

The next step was to identify which features of the experimental molecules increased and which decreased their druglike propensities. Osiris showed that the double-ring structure produced by pyrrole and benzene had positive druglikeness value and the Drug Score of 86%. After each experimental molecule was drawn in Osiris, its existing linear C-H chain was either extended or shortened, or new chains were added. Regardless of the chain's position within a molecule, it was observed that the longer the chain the lower the druglike propensities of the modified molecule. Therefore, the chains with more than three C atoms were excluded from considerations as possible building blocks for druglike molecules.

Figure 2 shows eight basic fragments whose combinations form all six experimental molecules. They will be referred to as fragments 1, 2, 3, etc., counting from left to right and top to bottom in Figure 2. It was found that fragment 8 had the lowest Drug Likeness of −5.02, Drug Score of 38%, and toxicity risks. Atomic substitutions of F in fragment 8 with Cl, Br, or I led to Drug Likeness scores in the range of −1.0 (for I) to −5.6 (for Br). All halogen substitutions gave Drug Score values below 50% and showed toxicity risks. Therefore, fragment 8 was excluded from further considerations.

Substitutions of atoms within fragments 1–7 were done in Osiris to see which substitutions enhanced druglike properties. Each carbon atom in the fragments was substituted, in turn, by nitrogen, oxygen, phosphorus, silicon, or sulfur. Similarly, each nitrogen atom was substituted, in turn, by carbon, oxygen, phosphorus, silicon, or sulfur.

Substitutions with phosphorus, silicon, or sulfur anywhere in the fragments led to negative Drug Likeness scores, with most values concentrated around −10. The Drug Likeness value was as low as −23.5 when N in pyrrole was substituted by Si. Substitutions with P, Si, or S also led to Drug Scores less than 50%. Substitutions of side-chain atoms in fragments 3–6 by P, Si, or S produced negative Drug Likeness and low Drug Scores. Given these findings, P, Si, and S were not considered further for atomic substitutions.

Substitutions of C atoms in fragment 7 with either N or O led to Drug Likeness scores between about −1 and −12 and Drug Scores below 50%. Similarly,

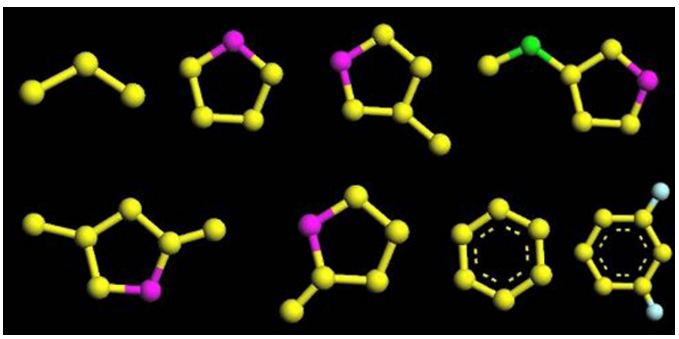

Figure 2. Basic building blocks (fragments) of the six experimental molecules. Color code: C-yellow, N-pink, O-green, F-blue. For simplicity, hydrogen atoms are not shown.

substitutions of C by either N or O in the side chains of fragments 4–6, or by N in the side chain of fragment 3, led to negative Drug Likeness and low Drug Scores. However, substitution of C by O in the side chain of fragment 3 led to Drug Likeness of 1.12, 86% Drug Score, and no indicated toxicity risks.

To summarize, the single-atom substitutions performed thus far left only fragments 1–7 and substitutions with C or N within the rings (except in fragment 7), and substitutions with O within the side chain of fragment 3, as having a potential to enhance druglike properties.

In the next step, two atoms were simultaneously substituted in the ring structures of fragments 2–6, with the goal of determining how these substitutions influenced druglike properties. Substitutions of C or N by two O within the rings produced negative Drug Likeness scores, Drug Scores less than 50%, and indicated toxicity risks. However, substitutions of an additional C atom in pyrrole (fragments 2–6) with N produced molecules with no toxicity risks, positive Drug Likeness, and Drug Scores between 79% and 86%, regardless of the relative positions of the two N atoms within the ring. Having more than two N atoms in a 5-atom ring had a negative influence on the molecule's druglike properties. Thus, four 5-atom rings with zero, one, or two N atoms were identified for further studies.

Next, the influence of double-ring structures was studied. Pyrrole ring was coupled with the four 5-atom rings to form double-rings having between one and three N atoms. The long C-H side chains were removed from the experimental molecules in Figure 1. Then, each of the rings in the structures that remained was replaced, in turn, by one of the double-rings. The overall goal was to see how the substitutions influenced the values of druglike properties. Figure 3 shows six double-rings found to improve druglike properties of the experimental molecules and to cause no toxicity risks. As will be shown in the Results and Discussion section, several new molecules with high druglikeness were obtained by linearly connecting the single- and double-rings via their C or N atoms.

Thirty molecules with the highest values of Drug Scores, optimal values of other druglike properties, and no indicated toxicities were chosen for further studies in Molinspiration. It should be noted that the main goal was not to find all possible molecular

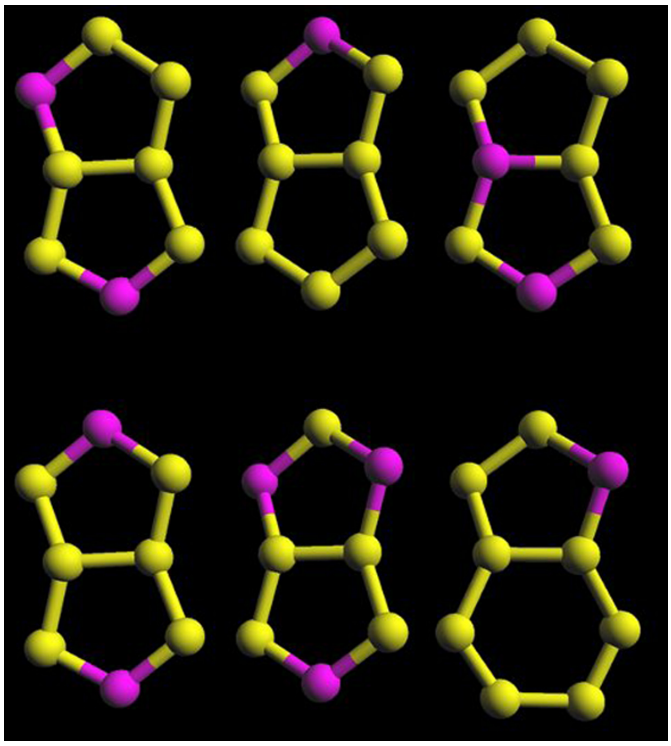

Figure 3. Six double-rings found to improve druglike properties of the experimental molecules and to cause no toxicity risks. Color code: C-yellow, N-pink, O-green, F-blue. For simplicity, hydrogen atoms are not shown.

configurations (~1000) that could be constructed from the single- and double-ring structures. The goal was to show that the described procedure could identify new putative druglike molecules that formed stable complexes with all three BCL-2, BCL-W, and BFL-1 proteins. On the other hand, given that a molecule could be drawn in Osiris in about 1–1.5 minutes, it is estimated that the search for all promising druglike structures with up to three single- or double-rings could be done in about 20–30 hours on a single PC.

Molinspiration was used to evaluate drug-related properties of the thirty molecules created in Osiris. The values of molar masses and PSA, as well as the numbers of rotatable bonds, H-bond acceptors, and H-bond donors were calculated. The designed molecules were ordered according to their overall Drug Scores. Molecules with no indicated toxic risks and optimal values of all other drug-related properties, as found by Osiris and Molinspiration, were used for docking studies in ArgusLab.

The Deep View program was used to identify the BH3 binding sites of BCL-2, BCL-W, and BFL-1

proteins. Amino acids within 8 Å of the binding sites were used for docking studies in ArgusLab. The Molecule Builder and Semiempirical Geometry Optimization functions in ArgusLab were used next to obtain 3D conformations of the molecules constructed in Osiris. The molecules were then used as ligands to perform docking to the BCL-2, BCL-W, and BFL-1 proteins. Docking was done using ArgusDock engine with flexible setting which allowed twisting of molecular bonds. Other docking parameters were as follows: AScore scoring function; ascore.prm parameter set; and 0.4 Å grid resolution. Binding energies of stable poses were calculated. Twelve small molecules that made the most stable complexes with all three proteins were identified.

Results and Discussion

Table 1 shows the binding energies obtained in ArgusLab for complexes of the BCL-2, BCL-W, and BFL-1 proteins and the 6 experimentally known small molecules downloaded from the ChemDB database. More stable complexes correspond to binding energies that are more negative. As can be seen in Table 1, the six experimental molecules bonded all three BCL-2, BCL-W, and BFL-1 proteins. The binding energies of the complexes ranged between –43.5 and –28.0 kJ/mol.

The structures of the twelve designed molecules are shown in Figure 4. All twelve molecules contain pyrrole, as do the six original experimental molecules. It should be noted that pharmaceutical industries use pyrrole to obtain compounds for drug preparations. Eleven of the twelve designed molecules contain three or fewer single- or double-ring structures. The first molecule in the second row of Figure 4 has

Table 1. The binding energies obtained via ArgusLab of the complexes formed by antiapoptotic BCL-2, BCL-W, and BFL-1 proteins and the six experimentally known small molecules.

Original molecule (CID)	BCL-2 (kJ/mol)	BCL-W (kJ/mol)	BFL-1 (kJ/mol)
22081373	–41.4	–41.0	–43.1
18177004	–43.5	–38.5	–33.9
18783077	–41.9	–41.0	–38.1
11544167	–43.5	–43.1	–41.4
18783063	–30.1	–31.4	–33.9
11673546	–28.0	–30.6	–30.1

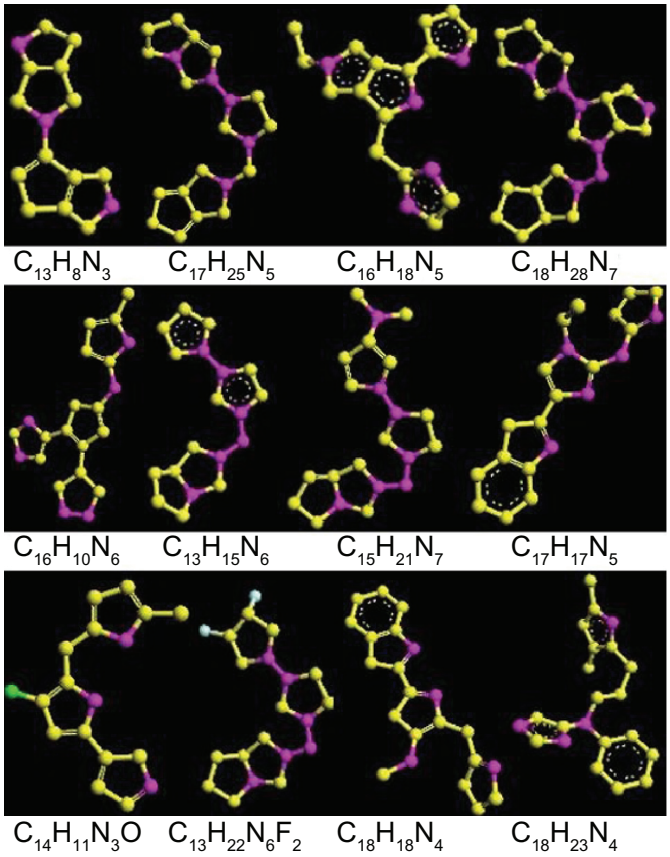

Figure 4. The structures of the twelve designed small molecules. Color code: C-yellow, N-pink, O-green, F-blue. For simplicity, hydrogen atoms are not shown.

four single-rings. All rings in the six experimental molecules are connected exclusively via C atoms. However, some rings in the designed molecules are also connected via C-N-C or N-N-N "bridges". Examples are molecules 4, 5, 6, 7, and 10 in Figure 4 (the numbering of the molecules is from left to right and from top to bottom of the figure).

While only one kind of double-ring (pyrrole fused to benzene) is found in the six experimental molecules, the double-rings shown in Figure 3 are present in the designed molecules in Figure 4. The third double-ring in Figure 3 is the most common one present in the designed molecules.

Table 2 shows drug-related properties of the designed molecules found by the Osiris and Molinspiration programs. Both programs predicted similar values for the molar masses, logP, and PSA. Molinspiration was used to find the number of rotatable bonds, H-bond acceptors, and H-bond donors. Toxicity risks and the values of Drug Likeness and Drug

Table 2. Drug-related properties of designed putative small molecule mimetics, calculated by Molinspiration and Osiris Property Explorer. The relevance and acceptable values of the listed properties are described in the text. In the table, MM stands for the molar mass; nrotb for the number of rotatable bonds; nON for the number of H-bond acceptors; and nOHNH for the number of H-bond donors.

Designed molecule	logP	logS	PSA (Å²)	MM (g/mol)	nrotb	nON	nOHNH	Drug likeness	Drug score
$C_{13}H_{10}N_3$	0.37	–1.17	31.1	213.3	1	3	2	3.63	96%
$C_{17}H_{25}N_5$	0.39	–1.70	13.0	298.4	3	4	0	5.03	93%
$C_{16}H_{11}N_5$	0.04	–1.67	60.1	283.4	4	5	2	3.48	93%
$C_{18}H_{25}N_7$	0.10	–1.56	44.0	337.4	3	7	2	4.68	92%
$C_{16}H_{15}N_6$	0.19	–2.69	80.6	296.4	4	6	5	3.30	90%
$C_{13}H_{26}N_6$	0.10	–3.18	29.9	260.3	3	6	1	4.21	90%
$C_{15}H_{29}N_7$	0.10	–3.22	29.9	302.4	4	6	1	4.94	88%
$C_{17}H_{13}N_5$	1.71	–2.90	54.6	291.4	4	5	1	2.58	86%
$C_{14}H_{15}N_3O$	2.31	–2.41	43.9	225.3	3	3	2	1.74	85%
$C_{13}H_{26}N_6F_2$	0.22	–3.81	29.9	296.3	3	6	1	2.87	82%
$C_{18}H_{13}N_4$	1.59	–3.80	49.1	290.4	4	4	1	2.96	82%
$C_{18}H_{23}N_4$	2.69	–3.62	39.3	302.5	6	4	3	3.28	81%

Score were calculated by Osiris. Twelve molecules were identified with no predicted toxicity, overall Drug Scores above 80%, and optimal values for all other drug-related properties.

The values of logP are less than 2.70 for all designed molecules, while the values of logS are between –1.17 and –3.81. Both of these sets of values are well within the accepted ranges for known drugs, as described in the Introduction section of this paper. The polar surface areas (PSAs) of all 12 molecules are less than 81 Å², well below the "druglike" value of 120 Å². All the molar masses are less than 338 g/mol. All designed molecules have between 1 and 6 rotatable bonds, between 1 and 7 H-bond acceptors, and 5 or fewer H-bond donors. All 12 molecules have positive Drug Likeness values, ranging from 1.74 to 5.03 (Table 2). As described previously, all these values are dependable predictors of the overall drug potential.[31–33]

Figure 5 shows the distributions of the logP, logS, molar mass, and Drug Likeness values for commercial drugs, non-druglike chemicals, and small molecules designed in this study. Graphs for commercial drugs and non-druglike chemicals were created using information from the Osiris Property Explorer website.[30] The green horizontal lines in the graphs indicate the approximate ranges of the values obtained for the designed molecules. As can be seen from Figure 5 the logP, logS, molar mass, and Drug Likeness values for all 12 designed molecules were comparable to those of the majority of commercial drugs.

Next, the 12 designed molecules were used as ligands in ArgusLab, as described in the Methodology section. Docking results showed that the designed molecules formed stable complexes with all three BCL-2, BCL-W, and BFL-1 proteins. The corresponding binding energies are given in Table 3.

Binding energies of the complexes formed by BCL-2 and the designed molecules were between –23.9 to –45.2 kJ/mol. These are compared to the binding energies between –28.0 and –43.5 kJ/mol of

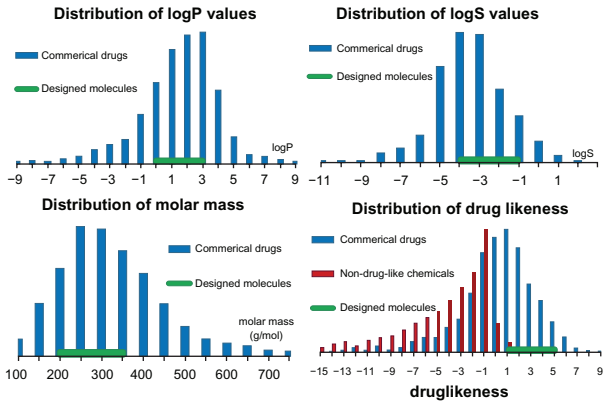

Figure 5. The distributions of the logP, logS, molar mass, and Drug Likeness values for commercial drugs, non-drug-like chemicals, and putative small molecule mimetics designed in this study. Data for commercial drugs (blue) and non-drug-like chemicals (red) were created using information from the Osiris Property Explorer website. The green lines indicate the approximate ranges of the values obtained for the designed molecules. The values on the vertical axis are arbitrary.

Table 3. The binding energies obtained via ArgusLab of the complexes formed by antiapoptotic BCL-2, BCL-W, and BFL-1 proteins and the twelve designed small molecules.

Designed molecule	BCL-2 (kJ/mol)	BCL-W (kJ/mol)	BFL-1 (kJ/mol)
$C_{13}H_{10}N_3$	–39.3	–35.2	–26.8
$C_{17}H_{25}N_5$	–33.1	–35.6	–37.3
$C_{16}H_{11}N_5$	–32.2	–33.1	–28.9
$C_{18}H_{25}N_7$	–31.0	–34.7	–33.5
$C_{16}H_{15}N_6$	–41.0	–38.5	–37.7
$C_{13}H_{26}N_6$	–28.5	–29.7	–26.8
$C_{15}H_{29}N_7$	–23.9	–25.5	–23.0
$C_{17}H_{13}N_5$	–34.3	–38.5	–33.9
$C_{14}H_{15}N_3O$	–40.6	–37.3	–34.7
$C_{13}H_{26}N_6F_2$	–26.0	–28.9	–26.8
$C_{18}H_{13}N_4$	–45.2	–45.2	–37.7
$C_{18}H_{23}N_4$	–43.5	–47.3	–38.5

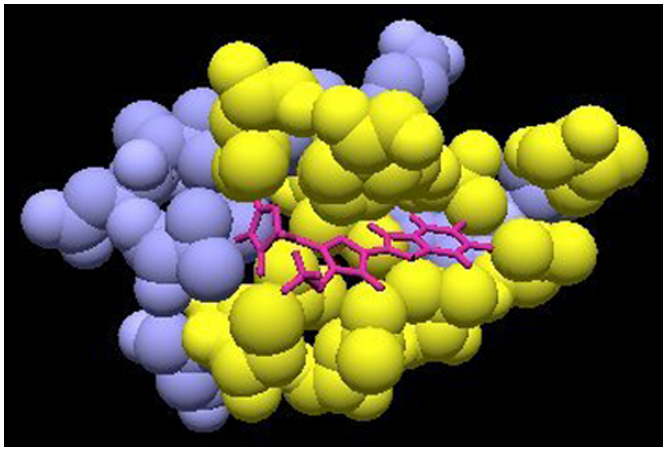

Figure 6. BFL-1 binding groove with designed $C_{18}H_{13}N_4$ molecule (purple ball-and-stick). The Noxa-binding residues of BFL-1 are indicated as yellow solid spheres. Other nearby BFL-1 residues are presented as purple solid spheres.

the BCL-2 complexes with the original 6 molecules from Table 1. The results are similar to measured binding energies of about –46.0 and –41.9 kJ/mol for BCL-2 complexes with $C_{33}H_{35}NO_6S$ and $C_{26}H_{21}NO_6S$ small molecules.[36]

Binding energies of BCL-W complexed with the designed molecules varied between –25.5 and –45.2 kJ/mol, and those with the original molecules between –30.6 and –43.1 kJ/mol. Complexes of BFL-1 with the designed molecules ranged between –23.0 and –38.5 kJ/mol and those with the original molecules between –30.1 and –43.1 kJ/mol.

The ranges of binding energies of complexes of the three proteins with both the designed and the original molecules did not significantly differ. However, the new molecules all had druglike properties, while the original ones lacked them.

Small molecules designed here bonded BCL-1 and BCL-W within the same binding sites as ABT-737[17] and cellular BH3-only proteins.[37] These findings suggest that the designed molecules might engage BCL-2 and BCL-W in a similar manner as ABT-737 to prevent them from sequestering BH3-only proteins.

Previous studies[38] showed that cellular proapoptotic Noxa protein bonded to a hydrophobic groove created by BFL-1 residues V44, V48, L52, C55, L56, L70, V74, V90, A94, F95, and I98. In the present work all designed molecules bonded within the BFL-1 site that included the above residues. Figure 6 shows one of the designed molecules, $C_{18}H_{18}N_4$, within the BFL-1 binding site. BFL-1 residues are shown as solid spheres and $C_{18}H_{18}N_4$ as magenta ball-and-stick.

Previous studies demonstrated that ABT-737[4,17] and ABT-263[39] (an orally bioavailable molecule related to ABT-737) promote cell death by inhibiting antiapoptotic proteins, instead of by damaging the DNA. This is advantageous, since cancer cells frequently have malfunctioning apoptotic pathways that allow them to evade apoptosis initiated by damaged DNA.[40] The designed molecules and ABT-737 have common structural features and interact within the same protein binding sites. These and previous findings[4,41] suggest that the molecules designed here might promote apoptosis in a similar way by inhibiting their target proteins, rather than by damaging the DNA. If future studies confirm this, the cancer-fighting potential of the designed molecules would be enhanced.

Given their structural similarities to the known ABT agents, their protein binding propensities, the calculated values of drug-related properties, and indicated non-toxicities, the molecules designed here may warrant further experimental studies.

Conclusions

This research identified twelve druglike small molecules that made stable complexes with antiapoptotic BCL-2, BCL-W, and BFL-1 proteins. The findings are novel because, to the best of the author's knowledge, small molecules that interact with all three of the proteins are not known. Drugs based on these molecules

could be useful against cancers with overexpressed BCL-2, BCL-W, or BFL-1. The agents might simultaneously inhibit two or three of the proteins, thus allowing apoptosis to proceed. A single drug should be more economical and lead to fewer adverse effects than a combination with each drug targeting a different protein.

The putative druglike molecules were designed and evaluated via a simple, yet effective procedure based on existing open-source programs and servers. A key advantage of the procedure is that good results can be achieved with modest computational resources and minimal programming skills. Furthermore, the availability of the straightforward procedure within a single paper should help those wishing to enter the field. The procedure described here can facilitate identification of putative biomolecules that merit further experimental studies. Reducing the cost of involvement in computational drug design should expand the number of researchers able to contribute to this exciting field.

All twelve designed molecules were found to have druglike properties, such as $\log P \leq 5$, $-5 \leq \log S \leq 1$, PSA < 120 Å^2, molar masses below 500 g/mol, fewer than 10 rotatable bonds and hydrogen bond acceptors, and fewer than 6 hydrogen bond donors. All of the molecules had positive Drug Likeness values with overall Drug Scores between 81% and 96%. They all formed stable complexes with BCL-2, BCL-W, and BFL-1 and bonded the same binding sites as cellular BH3-only proteins and the known ATB agents. The binding energies of the complexes ranged between about −23 and −47 kJ/mol. These findings, as well as previous laboratory and preclinical studies on ABT-737 and ABT-702, strongly suggest that the molecules designed here may act as BH3-like mimetics.

The study identified small molecules that formed stable complexes with BCL-2, BCL-W, and BFL-1 *in silico*. Although the laboratory syntheses of the designed molecules have not been done, it should be noted that experimentally available molecules were used as templates for all 12 designed molecules. Nevertheless, usefulness of the designed molecules for anticancer applications needs eventually to be evaluated *in vitro* and, if warranted, *in vivo*.

The progress in drug design can be achieved only by joined efforts of researchers from theoretical, computational, experimental, and clinical fields. It is the author's hope that this work would add to this worthwhile effort and enable international community to participate more fully in the search for useful druglike molecules.

Disclosure

This manuscript has been read and approved by the author. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The author and peer reviewers of this paper report no conflicts of interest. The author confirms that they have permission to reproduce any copyrighted material.

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