applied sciences

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Probing New Antileukemia Agents That Target FLT3 and BCL-2 from Traditional Concoctions through a Combination of Mass Spectrometry Analysis and Consensus Docking Methods

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Abstract: The search for new chemotherapeutics against leukemia is of great interest to researchers, owing to the limitation of the current drugs. In this research, new drug candidates against leukemia were probed through liquid chromatography-mass spectrometer (LC-MS) analysis of three traditional herbal concoctions, that provide the phytochemical profile of the samples. The identified compounds from the LC-MS were modeled for the analysis of their antileukemia activities, by using five different consensus methods, to combine the seven docking scores. The consensus methods are used to combine the docking scores to avoid losing promising drug candidates, due to a poor reproducibility of the docking scores across the different packages, due to differences in the scoring functions and training sets across the docking packages. The libraries of the potential drug candidates from the concoctions were constructed by searching the NIST database for molecules with a similar MS fragmentation. Venetoclax and gilteritinib, that target FLT3 and BCL-2 were ranked among the top hits, indicating the efficiency of this protocol without missing any potential drug. The results ranked rescinnamine and bisacodyl as new potential antileukemia agents that targets FLAT3, and BCL-2, including the mutated BCL-2 G101V receptor, that is known to be resistant to treatment with venetoclax.

Keywords: mass spectrometry; docking scores; consensus ranking; traditional concoctions; leukemia; drugs

Citation: Adeniyi, A.A.; Adeniyi, J.N.; Nlooto, M.; Singh, P. Probing New Antileukemia Agents That Target FLT3 and BCL-2 from Traditional Concoctions through a Combination of Mass Spectrometry Analysis and Consensus Docking Methods. *Appl. Sci.* **2022**, *12*, 11611. https://doi.org/10.3390/app122211611

Academic Editor: Tricia Naicker

Received: 27 September 2022
Accepted: 7 November 2022
Published: 15 November 2022

1. Introduction

The traditional experimental method for screening a large number of molecules towards biological targets is a high-throughput screening technique, but its demand for a large number of resources and time lead to the introduction of computer-aided virtual screening methods to decrease the time and economic costs [1]. The docking programs are used to filter potentially active compounds towards a protein target from a large library of compounds. These docking programs are computationally efficient, fast and of great impact in drug discovery, to search for the favorable position, orientation, and assumed conformation of molecules upon the binding and assigning docking scores to estimate the binding affinity [1–5]. The major challenge of relying on docking methods to screen a library of molecules against the protein target, is the possibility of providing false binding and ending up eliminating even the potent drug candidates from the list of top hits that will be considered. The reasons for the failure of the docking scores in some instances, are related to their training-set dependencies and scoring-function parameterization [1]. The approach

that has been found successful in limiting the possibility of throwing potential drugs into the trash bin of nonactive compounds and achieving a higher success rate, is to combine the results of multiple docking scores and then apply consensus ranking methods [1,6,7]. A higher success rate in the virtual screening processes has been obtained by combining results from different docking programs (i.e., consensus scoring) to obtain a final rank [1]. In addition to many traditional consensus methods that have been introduced, which make use of the average scores or the ranking of each molecule from different programs, a new exponential consensus ranking (ECR) was introduced recently that promises a good performance where the traditional consensus fails, due to the poor performance of one or more of the docking programs [1]. This study also employs the consensus docking approach in probing potential antileukemia agents, in combination with LC-MS. The three leukemia targets that are selected in this study are the Fms-like tyrosine kinase 3 (FLT3), B-cell lymphoma-2 (BCL-2), and the mutated BCL-2 G101V.

The abnormalities in FLT3, such as the over-expression of the gene or FLT3 mutations from internal tandem duplications (ITDs) within the FLT3 gene are linked to the poor prognosis factors in acute myeloblastic leukemia (AML) and acute lymphoblastic leukemia (ALL) [8–10]. FLT3 is considered a promising drug target in AML patients with FLT3 mutations [11]. The antileukemia drug that targets FLT3 is gilteritinib, which is known for the treatment of patients with relapsed/refractory FLT3-mutated AML [11,12]. It is classified as an ATP-competitive type I inhibitor and a selective FLT3 inhibitor that inhibits both FLT3-ITD and FLT3-TKD mutations [11,13].

BCL-2 is a founding member of the pro-survival class of proteins and functions by interacting with homology 3 (BH3) motifs of pro-apoptotic molecules to restrain its pro-apoptotic function [14]. The overexpression of BCL-2 proteins helps the cancer cells to evade treatment, therefore an effective step toward the treatment of cancers is the down-regulation of the anti-apoptotic BCL-2 proteins [15]. Venetoclax is the first BCL-2 antagonist approved, in 2016, for cancer treatment that is targeting the binding groove of BCL-2, that is usually used to interact with BH3 [14,16]. The binding of venetoclax to BCL-2 will prevent the BCL-2 BH3 protein-protein interaction and then promote the apoptosis in malignant cells, dependent on BCL-2 [14]. The first novel BCL-2 mutation G101V has been found in patients that initially responded to venetoclax treatment, but developed chronic lymphocytic leukemia (CLL) [14,17]. The BCL-2 G101V mutation provides resistance to the therapy by leading to some 180-fold reduction in the binding affinity of venetoclax and yet maintains an affinity for the BH3 motif of pro-apoptotic proteins [14].

This study considers the inhibitory activities of the proposed ligands from three samples of traditional concoctions that are named Emelia M, Mshikazi and Delosma H decoctions [18], against three leukemia targets BCL-2 (PDB 6o0k) and mutated BCL-2 G101V (PDB: 6o0l). To efficiently probe the inhibitory activities of the potential drug candidates, seven scoring functions and five consensus methods were used to combine the results. The library of potential drug candidates was generated from three traditional herbal samples that are locally recommended by traditional healers to possess anticancer agents. The potential bioactive compounds in the three traditional concoctions were predicted, based on their experimental MS fragmentations that match the MS fragmentation of molecules found in the NIST database. The combination of LC-MS and docking has been previously used by Mohammed et al. (2022), for the phytochemical profiling of Suaeda vermiculata extracts [19]. The authors made use of the molecular docking of the compounds identified in the LC-MS chemical profiling against three ATP-binding cassette proteins to identify the active anti-hepatocarcinoma compounds [19] In this work, we instead targeted the key fragments present in the three herbal samples using LC-MS and multiple docking instead of single molecular docking. This presents a fast and efficient method of analyzing traditional concoctions without necessarily tampering with the non-disclosure agreements made with the traditional healers as the source of their products is often kept secret to avoid knowledge theft. The study also included venetoclax and gilteritinib, which are known antileukemia drugs that target BCL-2 and FLAT3 (PDB: 6il3), respectively. The two drug candidates

serve as a benchmark for the docking scores and consensus methods on their ability to rank active compounds among the top hits. The interest is to use the co-crystalized ligands (venetoclax and gilteritinib) as the benchmark for the scoring functions and consensus methods to provide the correct binding site orientation and to rank the active compounds among the top 10 lead hits.

2. Methodology

The method employed in this work is best summarized as a LS-M analysis of the samples, followed by the identification of the ligands from the spectra, the docking of the identified ligands to the target, and the application of the consensus method to the docking score.

2.1. Generating Potential Drug Candidates from Traditional Herbs

Three samples of traditional herb medicine concoctions (labeled as A, B, and C) that are suggested to have antileukemia activities were collected from traditional healers. In this study, the samples were analyzed using LC-MS (ABSCIEX 4000 QTRAP hybrid triple quadrupole mass spectrometer with Shimadzu's front end). Twenty microliters of each sample were injected onto a Discovery C18 reverse-phase column (150 × 2.1 mm, Supelco) and separated using 0% to 95% linear water (Solvent A) and methanol (Solvent B) gradient over 30 min at 0.3 mL/min. The eluting analytes were first analyzed in the positive and then the negative ionization mode, each time, using an information-dependent acquisition (IDA) method where the ions between 200 and 1000 Da with intensities above 100,000 counts per second (cps) originating from an enhanced MS (EMS) survey scan, were selected and fragmented in the collision cell, and the fragments were recorded following an enhanced production (EPI) scan. The obtained LC-MS fragmentation of the samples was further analyzed to identify drug candidates that have a similar fragmentation from the NIST database (NIST 17 MS) that best matches the fragmentation, using the automatic mass spectral deconvolution and identification system (AMDIS 2.73) package that is designed for automating the complex process of extracting data from GC/MS data. It is worth pointing out that the identified molecules are not the exact molecules from the concoctions but the best representative target molecules from the NIST database. In all three samples, a total of 271 unique molecules (excluding all repetition) from the MS retention time, ranging from 0.0806 to 44.8792 min, were identified in the NIST database. The structures of the target molecules were downloaded from the NIST database, using their CAS numbering and were optimized, using the semi-empirical method PM7 for their virtual screening as potential inhibitors of FLAT3, BCL-2, and mutated BCL-2 G101V.

2.2. Docking and Scoring-Function

The downloaded 271 target structures from the NIST database were first optimized with the G16 package [20], using the semi-empirical method PM7 before their virtual screening, using different docking programs. Following the exclusion of the molecules with inorganic metals, such as silicon, from the list, a total of 262 molecules were screened against the three targets. A total of seven docking scores from five docking programs were used to analyze the inhibitory activity of the 262 identified molecules. The CAS number and the names of all of the 262 final molecules that were docked against the three target receptors are added to the Supplementary Table S1. In the four docking software packages that were used (Autodock, Molegro, Haddock, and Schrodinger), the binding site residues of the proteins were first identified and the ligands binding was restricted to the grid area of the selected binding site residues. The first two docking scores (Moledock and Rerank scores) are from the Molegro package [21], the third score (Vina score) is from Autodock vina [22], the fourth score (Glide score) is from Schrodinger package [23,24], and the last three are from the Haddock docking package [25,26], named according to the docking criteria (rigid Haddock(rigid)), flexible refinement (Haddock(flex)) and flexible refinement in water (Haddock(flex, water)). The default settings were used in all docking simulations

with little modifications. The search area distance of 25 Angstroms from the selected point on the binding site was used in Autodock-vina. The parameterization of the ligands for the Haddock docking was prepared using Acpype packages [27,28] and we chose Gasteiger charges for all of the atoms. The haddock was set to generate 50 rigid structures, 10 flexible refined poses, and finally 10 final flexible refined poses in water media. The Glide docking was carried out, using Schrodinger Python version 68013, using the default settings for the grid generation and docking.

2.3. Consensus Ranking of the Combined Docking Scores

A total of five different consensus methods were used to combine the results of the seven docking scores and to generate a better ranking of the top lead hits. The five consensus methods that were used are:

Rank by number (*RbN*) that combine the docking score of each molecule (s^j) using the average values over the number of the docking programs (n) [1,29].

$$RbN_i = \frac{1}{n}\sum_j s_i^j$$

Rank by rank (*RbR*) makes use of the rank of each molecule (r^j) and averages it over all of the docking programs (n) for each of the molecules [1,29].

$$RbR_i = \frac{1}{n}\sum_j r_i^j$$

Average of auto-scaled scores (*AASS*), that first normalizes each score between 0 and 1, using the minimum ($s^j{}_{min}$) and maximum ($s^j{}_{max}$) scores per each program and then averages over the number of the docking programs [1,30].

$$AASS_i = \frac{1}{n}\sum_j \frac{s_i^j - s_{min}^j}{s_{max}^j - s_{min}^j}$$

Z-score (*ZS*) that scales the score of each molecule (s^j), using the average (μ^j) and standard deviation (σ^j) of the scores per each program and averages over the number of the docking programs used [1,31].

$$ZS_i = \frac{1}{n}\sum_j \frac{s_i^j - \mu^j}{\sigma^j}$$

Exponential Consensus ranking (*ECR*) makes use of the exponential score of the rank of each molecule (r^j) and the expected value of the exponential distribution (σ) and them sums over all of the docking programs (n) for each of the molecules [1].

$$ECR_i = \frac{1}{\sigma}\sum_j \exp\frac{-r_i^j}{\sigma}$$

3. Results and Discussion

3.1. Identification of the Drug Candidates from the Concoctions Using LC-MS

Working with traditional concoctions is very challenging for the scientist because most of the traditional herbal practitioners withhold the names of the plants and other materials that were used in the preparation and the method of preparation is concealed for fear that someone else can steal their ideas. In this study, we demonstrate the fast means of identifying the biologically active compounds from the traditional concoctions without tampering with the non-disclosure agreements made with the traditional healers.

In this study, we analyzed three traditional concoctions collected from traditional healers in the Kwazulu-Natal province of South Africa, using LC-MS fragmentation. The identification of the closely similar drug candidates that best represent the LC-MS fragmentation was carried out using the automatic mass spectral deconvolution and identification system (AMDIS) package to analyze the fragmentation and the closely related molecules were identified from the NIST database. The three samples were analyzed to identify the potential antileukemia compounds, because of the unsubstantiated claims by the traditional healers on the anticancer activity of their products. A total of 271 molecules were generated from the MS fragmentation of the three traditional herbs samples that were analyzed with the AMDIS program and found to have related target structures in the NIST database that match best with the LC-MS fragmentation. A typical example of the LC-MS fragmentation that was used to identify 865043, which was found among the active compounds in this study, is shown in Figure 1.

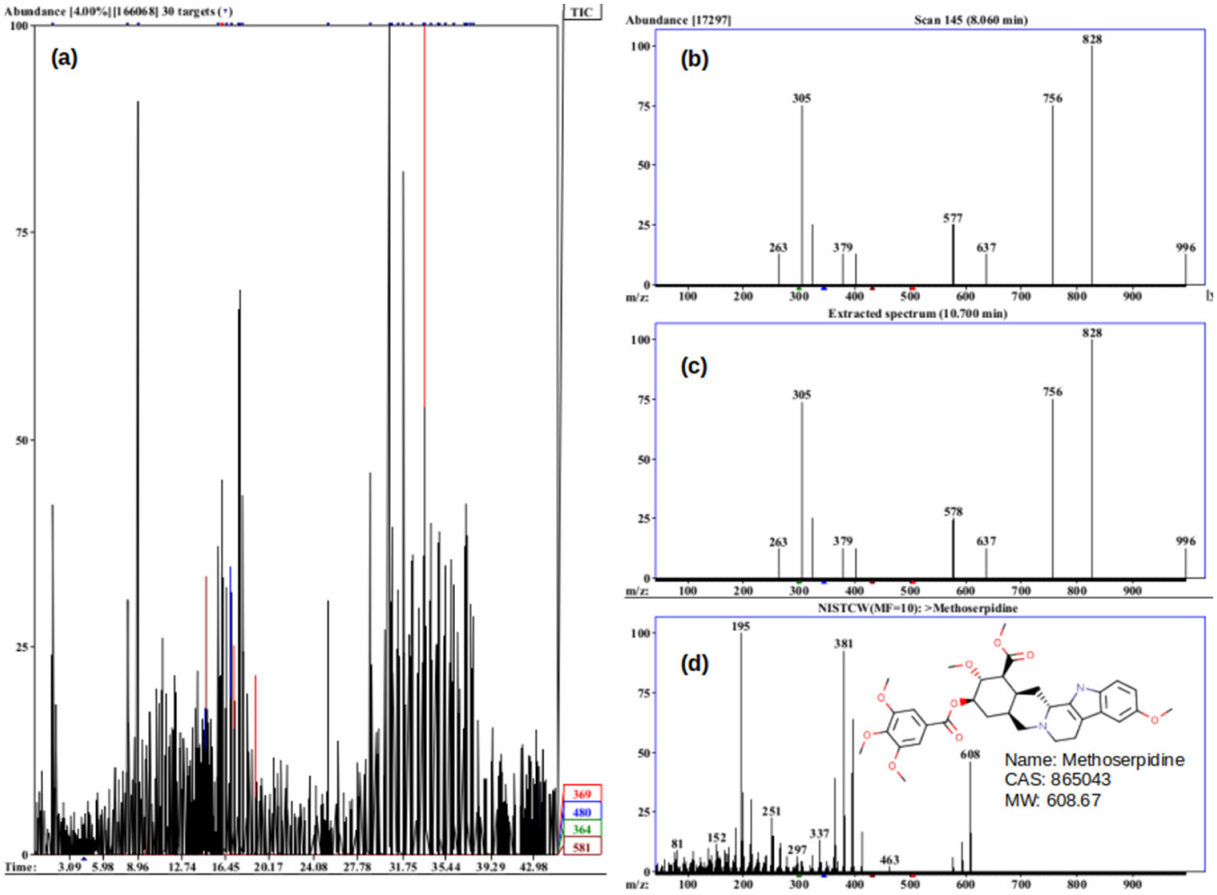

Figure 1. The (**a**) chromatogram, (**b**) scan, (**c**) extracted and (**d**) suggested target methoserpidine (CAS number: 865043) mass spectra derived from one of the three samples.

3.2. Docking Study

A total number of 262 molecules from the 270 identified molecules were used for the docking study after excluding those that contained inorganic metals, such as silicon. The docking study was conducted using the docking programs Molgro, Autodock Vina, Schrodinger Glide, and the Haddock programs. A total of seven docking scores were generated, two from Molegro (Moledock and Rerank scores), Vina, Glide, and the last three from Haddock, according to the scores obtained for the rigid, flexible, and flexible with water refinement, as described in the methodology.

The 262 potential drug candidates from the traditional herbs were screened for their antileukemia properties against two major leukemia targets BCL-2 and FLT3. The study also included the G101V mutant of BCL-2 (BCL-2-G101V) that has been recently reported to display a resistance to therapy [14]. The docking study included the co-crystallized ligands venetoclax [14,16] and gilteritinib [11,12] which are known antileukemia drugs against the target BCL-2 and FLT3, respectively, to serve as a benchmark for our study. The benchmark is used to determine the ability of the docking programs to effectively rank the experimentally proven inhibitor, among the top 10 lead hits and also the possibility of reproducing the experimental orientation. The results of the seven docking scores are analyzed using five consensus ranking methods RbN, RbR, AAS, ZS, and ECR, as described in the methodology.

3.3. Optimizing Ranking to Select the Top Hit Molecules

The list of the top 10 hit molecules from each of the seven docking scores used, is shown in Table 1, while those of the five consensus methods that were used to combine the seven scores are shown in Table 2. One of the most significant applications of the docking programs is to use it to screen the large library of molecules for the top hits that can bind strongly to the target. This has been of great use in the drug discovery research and has led to a significant decrease in the time and cost of discovering new drugs, compared to the traditional high-throughput screening. However, the challenge with the docking scores is that they are significantly different, based on their training set and parametrization method, which consequently leads to a different ranking of the screened molecules. This implies that no single docking score is completely reliable as it is possible to obtain a false hit and lose the promising molecules in the process.

Table 1. The top 10 hit molecules from the seven docking scores (Moledock, Rerank, Vina, Glide, Haddock(rigid), Haddock(flex) and Haddock(flex, water)) ranked according to their binding. The molecules from the traditional herbs are named, according to the CAS number that was used to extract them from the NIST database. The names of all of the molecules are added to Table S1 of the Supplementary Materials.

Molecule	Moledock	Molecule	Rerank	Molecule	Vina	Molecule	Glide	Molecule	Haddock (Rigid)	Molecule	Haddock (Flex)	Molecule	Haddock (Flex, Water)
6o0k													
1989522	−137.62	81232	−102.43	venetoclax	−12.10	venetoclax	−7.63	venetoclax	−21.34	venetoclax	−57.22	venetoclax	−75.17
113155	−137.02	57874	−102.08	215587	−10.20	3342981	−6.83	2642800	−19.92	gilteritinib	−52.12	865043	−54.66
24815245	−130.77	3648213	−98.16	71636	−10.20	67199660	−6.78	50293	−19.60	865043	−51.33	486475	−53.25
7235407	−130.72	865043	−96.37	113155	−10.10	6267023	−6.71	3848519	−18.81	18713514	−47.98	78308	−53.24
venetoclax	−129.01	58208	−93.33	1662017	−9.60	215587	−6.70	7173844	−18.34	603509	−46.70	85734	−53.07
67970	−128.31	1241947	−88.36	3342981	−9.10	28322023	−6.68	614266	−18.02	629970	−46.44	18713514	−51.34
486475	−127.40	101757	−87.69	77098	−9.10	72480	−6.64	22232719	−17.83	486475	−45.08	604320	−51.02
58208	−126.29	85734	−86.17	213467	−9.00	1771182	−6.61	603509	−17.71	78308	−44.36	32634687	−50.77
71636	−126.27	603509	−85.07	56495	−9.00	86884	−6.57	2227170	−17.69	85734	−43.66	603509	−50.63
3648213	−125.93	563042	−83.60	81232	−9.00	213467	−6.56	604320	−17.66	630024	−43.63	52645531	−50.25
6o0l													
venetoclax	−152.76	venetoclax	−110.00	venetoclax	−11.70	venetoclax	−8.45	544763	−19.38	28782425	−44.33	venetoclax	−55.58
24815245	−146.21	gilteritinib	−102.54	71636	−10.20	215587	−6.69	604320	−18.82	gilteritinib	−43.56	865043	−48.77
1989522	−142.78	3648213	−98.85	113155	−10.00	239010	−6.65	50146	−17.86	865043	−43.09	1164165	−47.11
604320	−137.89	57874	−96.80	81232	−9.70	86884	−6.58	531919	−17.77	67970	−42.30	2432908	−46.82
113155	−136.44	59052	−93.50	215587	−9.50	28322023	−6.54	39208009	−17.42	50146	−41.87	50146	−46.36
7235407	−134.60	865043	−90.17	213467	−9.40	1207698	−6.47	101757	−16.83	604320	−40.96	126147	−45.92
gilteritinib	−133.76	24815245	−89.95	1662017	−9.20	63077009	−6.43	117817	−16.75	venetoclax	−40.08	24815245	−45.08
865043	−132.21	563042	−89.81	56495	−9.10	1771182	−6.39	85698	−16.68	7173844	−39.51	629970	−44.27
85734	−130.39	1330865	−89.34	3342981	−9.00	72480	−6.39	1120281	−16.54	24815245	−39.27	28782425	−43.87
71636	−129.99	58208	−88.92	632520	−8.80	897789	−6.39	2259963	−16.32	117817	−39.04	32634687	−43.46
6il3													
24815245	−143.83	59052	−110.66	56495	−10.50	897789	−9.48	133493	−15.78	24815245	−49.40	1617909	−49.58
1989522	−141.97	111013	−105.01	213467	−10.40	520285	−8.91	111013	−15.07	gilteritinib	−45.96	59052	−47.70
venetoclax	−141.78	865043	−101.04	63077009	−10.40	239010	−8.00	608935	−15.03	85723	−44.74	1166525	−46.69
111013	−139.62	55334515	−100.24	113155	−9.70	3457485	−7.97	1207698	−14.88	642659	−44.60	101757	−45.61
113155	−139.41	2432908	−98.73	1662017	−9.70	2440224	−7.82	116165	−14.64	117840	−44.09	1031932	−45.36
604320	−136.22	24815245	−94.75	129000	−9.60	101757	−7.56	56495	−14.58	215587	−43.39	563042	−44.39
865043	−132.98	83885	−93.28	3342981	−9.60	531919	−7.44	58208	−13.93	213467	−43.16	126147	−44.23
59052	−131.80	57114	−92.62	239010	−9.50	15597769	−7.37	111068	−13.78	81812	−42.76	28782425	−44.12
gilteritinib	−131.66	84786	−92.60	venetoclax	−9.50	40817081	−7.10	215587	−13.76	1617909	−42.63	93765	−43.45
2432908	−128.74	1843056	−91.88	40817081	−9.30	85734	−7.10	614266	−13.72	1843056	−42.61	85734	−43.03

Table 2. The top 10 hit molecules from the five consensus ranking methods that were used to combine the seven docking scores. The molecules from the traditional herbs are named according to the CAS number that was used to extract them from the NIST database. The names of all of the molecules are added to Table S1 of the Supplementary Materials.

Molecule	RbN	Molecule	RbR	Molecule	AASS	Molecule	Z-Score	Molecule	ECR
6o0k									
865043	−50.835	865043	18.429	venetoclax	0.156	venetoclax	−2.015	venetoclax	0.513
85734	−48.062	603509	20.857	865043	0.229	865043	−1.676	865043	0.246
58208	−48.048	3848519	34.857	603509	0.272	603509	−1.398	603509	0.200
633318	−47.648	1662017	37.286	gilteritinib	0.280	85734	−1.238	85734	0.174
57874	−46.830	77098	38.857	85734	0.291	gilteritinib	−1.225	486475	0.174
3648213	−46.619	85734	39.000	58208	0.292	58208	−1.205	58208	0.159
81232	−46.535	venetoclax	39.429	3848519	0.298	1662017	−1.193	113155	0.149
357573	−46.287	58208	40.571	1662017	0.301	3848519	−1.158	81232	0.146
486475	−45.935	gilteritinib	49.714	563042	0.316	486475	−1.043	215587	0.143
563042	−45.685	52645531	51.571	486475	0.319	563042	−0.995	1662017	0.138
865043	−50.835	865043	18.429	venetoclax	0.156	venetoclax	−2.015	venetoclax	0.513
6o0l									
24815245	−56.941	venetoclax	6.857	venetoclax	0.027	venetoclax	−2.550	venetoclax	0.505
venetoclax	−56.263	gilteritinib	18.857	gilteritinib	0.190	gilteritinib	−1.562	865043	0.256
gilteritinib	−49.946	28782425	26.714	24815245	0.208	24815245	−1.495	gilteritinib	0.241
865043	−48.613	24815245	30.167	865043	0.217	865043	−1.414	604320	0.224
357573	−48.304	2259963	38.714	28782425	0.228	28782425	−1.318	24815245	0.217
3648213	−46.160	52645531	40.000	50146	0.242	50146	−1.142	50146	0.212
28782425	−45.071	865043	40.429	67970	0.253	67970	−1.122	28782425	0.172
1989522	−44.955	101757	45.857	531919	0.273	52645531	−1.012	84640	0.160
39208009	−43.894	1164165	47.857	52645531	0.275	531919	−0.982	3648213	0.147
28322023	−43.338	67970	53.143	604320	0.284	1164165	−0.948	215587	0.144
24815245	−56.941	venetoclax	6.857	venetoclax	0.027	venetoclax	−2.550	venetoclax	0.505
6il3									
24815245	−57.671	101757	25.143	24815245	0.224	24815245	−1.458	24815245	0.230
604320	−51.421	52645531	30.857	101757	0.237	101757	−1.374	111013	0.218
59052	−49.836	56495	36.857	56495	0.248	56495	−1.336	59052	0.210
111013	−49.366	531919	37.429	63077009	0.271	63077009	−1.194	56495	0.168
67970	−47.673	642659	39.143	213467	0.274	213467	−1.162	101757	0.164
2432908	−47.184	63077009	39.429	52645531	0.278	52645531	−1.157	113155	0.141
113155	−46.806	24815245	39.500	531919	0.279	531919	−1.153	213467	0.138
865043	−46.134	1843056	40.857	642659	0.279	642659	−1.133	gilteritinib	0.133
633318	−45.547	67199660	42.429	897789	0.280	897789	−1.125	215587	0.129
83885	−45.322	51234287	45.714	59052	0.281	215587	−1.114	1617909	0.127

3.4. Performance of the Docking Scores with Known Drugs

The ranking of known antileukemia drugs venetoclax and gilteritinib that target BCL-2 and FLAT3 are examined. The geometries of BCL-2 (PDB: 6o0k) and the mutated BCL-2 G101V (PDB: 6o0l) have a co-crystallized structure of venetoclax binding to their binding site. The results of the top 10 hits of seven docking scores are shown in Table 1. It is obvious from the results that the ranking of the molecules is significantly different from each other across the docking scores. It is obvious that gilteritinib, that is a known inhibitor of FLT3 (6il3), was ranked among the top 10 hit molecules only by the Moledock and Haddock(flex), in the absence of these two docking scores, this potent drug candidate would have been thrown into the trash bin of the non-active, by the other five docking scores. The performances of the docking scores were better for a known drug against BCL-2 (6o0k), which is venetoclax. Six of the seven docking scores, Moledock, Glide, and the three Haddock, ranked venetoclax among the best 10 hits, only the Rerank score got it wrong. The interaction of venetoclax with the mutated BCL-2 G101V is expected to be lower than the activity toward BCL-2, based on the experimental information, among the six that

found venetoclax as a promising BCL-2 inhibitor, Vina, Haddock(rigid), Haddock(flex) and Haddock(flex, water) ranked its inhibitory activity towards the mutated BCL-2 G101V lower than that of BCL-2, while Moledock and Glide gave it a better inhibitory activity towards the mutated BCL-2 G101V than BCL-2.

Using the consensus method to combine the results, the ECR ranked gilteritinib among the top 10 hits while it is excluded from the list of top 10 hits obtained from other consensus methods. Four of the consensus methods RbR, AASS, ZS, and ECR ranked venetoclax among the top 10 hits and even predicted it has the best inhibitor of the mutated BCL-2 G101V, by these four consensus methods. Furthermore, the known drug gilteritinib against FLAT3, is ranked among the top best inhibitors of the mutated BCL-2 G101V, by the docking scores (Moledock, Rerank, and Haddock(flex)), and also by all of the five consensus methods.

3.5. The Distribution of the Docking Scores

To understand the distribution of the scores obtained from the seven docking scores used for the ranking of the 262 potential drug candidates, from the three samples of the traditional concoctions and the two known drugs with the level of overlap of the docking scores in the ranking of the molecules, we normalized all of the docking scores using the normalizing expression explained for the consensus AASS. The plots of the distribution of the normalized docking scores of all of the molecules across the seven docking scores showing their level of overlap, are shown in Figure 2. The distributions for each of the seven docking scores are added to the Supplementary Figure S1.

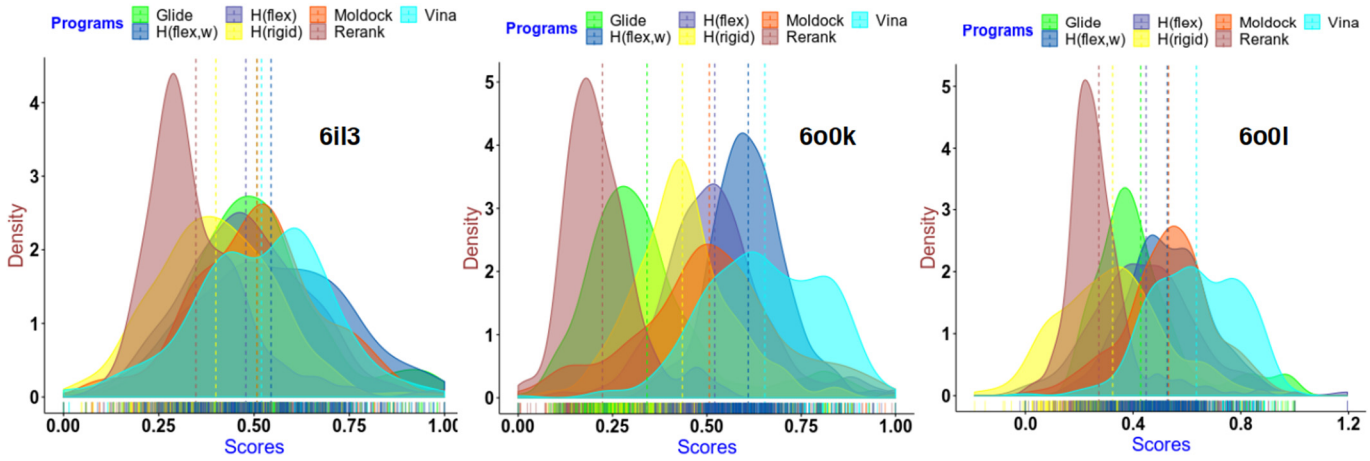

Figure 2. The distribution of the normalized docking scores of all of the molecules across the seven docking scores, showing the level of overlap and mean value (dash line).

The results of the distribution show that the scores of the ligands have a relatively normal distribution in the seven docking scores. The normal distribution is an indication that the molecules are grouped into three major groups, comprised of a small number of most active molecules on the extreme left, a large number of molecules as less active in the middle, and a small number of non-active molecules on the extreme right.

The pick of the distribution from the Rerank score is found towards the left of the others and is higher than others, indicating that it ranked many of the molecules as active, more than the other docking scores. The mean value of the docking scores from Moledock, Vina, Glide, and the three Haddock scores, are closer to each other than to the Rerank scores, indicating a much more similar distribution among the six excluding, the Rerank scores (Figure 2). Furthermore, the plots of the multi-variance relationship that show the level of correlation among the seven docking scores in the ranking of the molecules are shown in Figure 3. The plots of the multivariance analysis in Figure 3 clearly show that in the three targets, the ranking of the ligands by Moledock and Haddock(flex) have a significant level of correlation which also included Haddock(flex, water), in the target BCL-2 and its

mutated BCL-2 G101V. The scores from Haddock(flex) and Haddock(flex, water) are also significantly correlated and there is a relatively fair correlation between Glide and Vina.

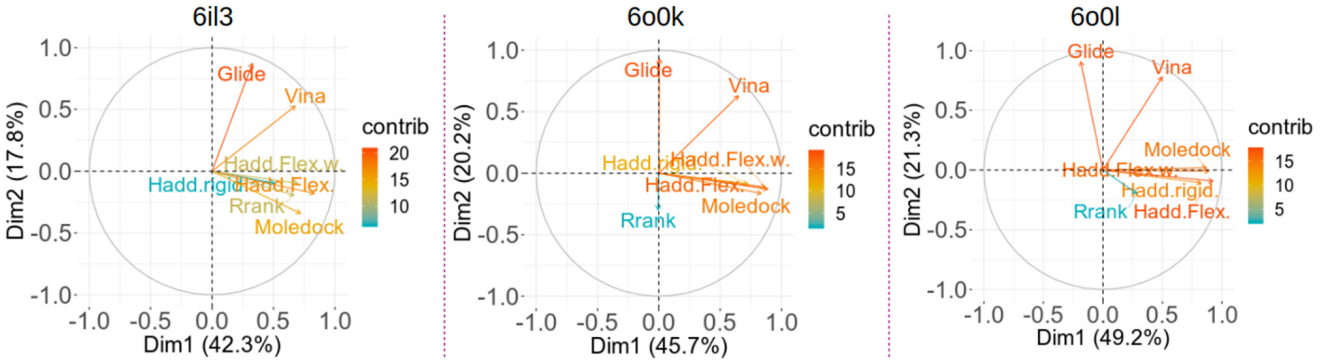

Figure 3. The distribution of the individual seven docking scores along with the two principal components with the quality of the contribution (cos2) of each to the PCA computed from the multivariance analysis to show their level of correlation. When the docking scores appear within close range to each other and are of a similar contribution to the PCA (similar color), then it shows a good level of correlation.

The distribution of the five consensus methods that were used to combine the docking scores, is shown in Figure S2. The methods RbN, RbR, AASS, and ZP also follow the normal distribution, indicating a few numbers of the molecules as active compounds on the extreme left side of the distribution. The distribution using the consensus ECR does not show a normal distribution; it has a higher number of molecules on the extreme left. Since the ranking of the ECR is different from the other consensus methods, in the sense that the active molecule will have the highest score values, it also clearly shows that the active compounds are a small proportion of the molecules on the extreme right of the distribution, in relation with the small number of active molecules found on the extreme left of the other consensus methods.

3.6. Potential Inhibitors BCL-2

In addition to the known drug venetoclax, the best-ranked inhibitors of BCL-2, by at least three of the seven docking scores among the 10 top hits, are 486475, 603509, 85734, and 865043. They are all captured among the top 10 hits of the five consensus methods except 486475, which is excluded in the RbR. Selecting the top 8 hits from the ECR and the top one from the other consensus methods, as as carried out for the target FLAT3, the promising potential inhibitors of BCL-2 from the three samples of the traditional concoctions are 865043, 603509, 85734, 486475, 58208, 113155, and 81232 of which, the known drug venetoclax is ranked best of all. The most promising, among the potential BCL-2 inhibitors from the traditional concoction, are 865043 and 603509, which are ranked best by many of the consensus methods and also by the docking scores.

Those ranked as potential inhibitors of the mutated BCL-2 G101V by at least three of the docking scores among the top 10 hits, are 24815245, 50146, 604320, and 865043 from the traditional concoctions and gilteritinib, which is a known inhibitor of FLAT3. The most promising potential inhibitors from the traditional concoctions that are included in the top 8 hits obtained from the ECR and the top 1 from the other consensus methods, are 865043, 604320, 24815245, 50146, 28782425, and 84640. The known inhibitor of BCL-2 (venetoclax,) is ranked best of all and also the known inhibitor of FLAT3 (gilteritinib) is ranked third. The best ranked across the consensus methods as the best inhibitors of the mutated BCL-2 G101V, are 865043 and 24815245, which were also ranked best for the native BCL-2 and FLAT3 targets, respectively.

3.7. The Difference in the Binding Site Orientation

The binding orientation obtained for the docking of known antileukemia drugs venetoclax and gilteritinib, that target FLAT3 and BCL-2 are shown in Figure 4 for the six docking scores (the seventh docking score Rank has the same binding site orientation as Moledock). Just as there is a significant difference in the ranking of the molecules, there is also a significant difference in their binding site orientation. In addition to the influence of the different score parametrization and training set, that leads to a difference in the docking scores [1], our result further shows that the difference in the docking poses plays a significant role in the observed disparity in the ranking of the molecules. Concerning the crystal structure binding orientation, none of the docking scores predicts the crystal structure binding orientation of gilteritinib to FLAT3. The binding orientation of venetoclax to BCL-2 was predicted by only Vina as the best pose, while the other scores predicted different orientations (Figure 4b).

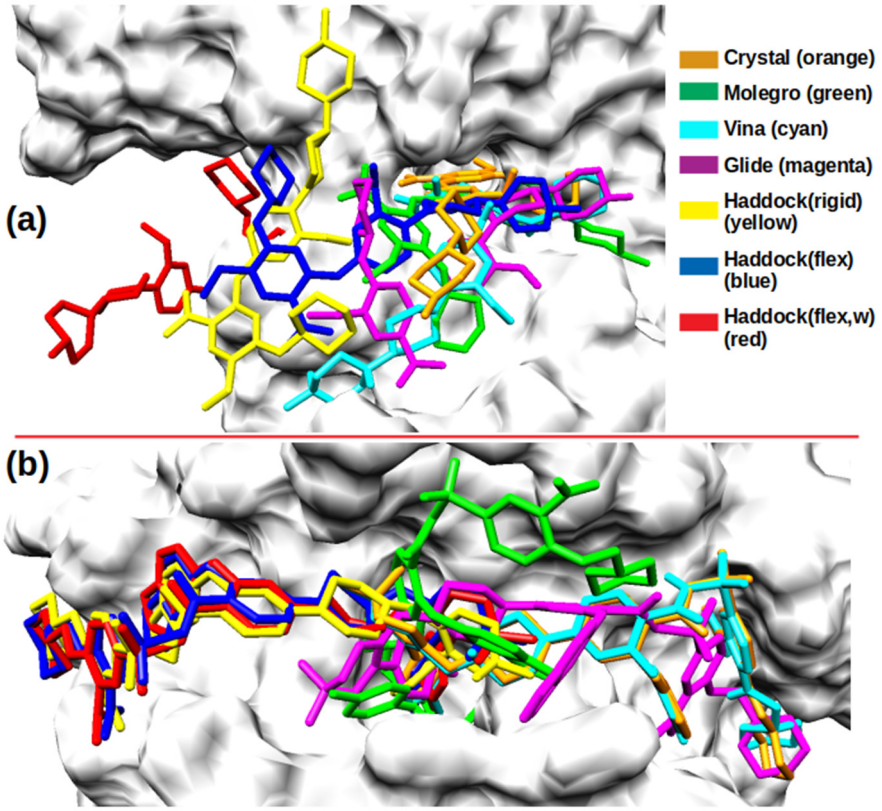

Figure 4. The binding site orientation of (**a**) gilteritinib to FLAT3 and (**b**) venetoclax to BCL-2 obtained from Molegro (green), Vina (cyan), Glide (magenta), Haddock(rigid) (yellow), Haddock(flex) (blue), and Haddock(flex, water) (red), in relation to their crystal structure crystal (orange).

3.8. The Top-Ranked Molecules from the Traditional Concoctions

The results of the analysis of the seven docking scores and five consensus methods show that the two potential inhibitors of FLAT3, from the traditional concoction are 24815245 (rescinnamine) and 101757 (benzenamine, N-phenyl-4-(phenylazo)). The two most prominent inhibitors of BCL-2 are 865043 (methoserpidine) and 603509 (bisacodyl), while that of the mutated BCL-2 G101V are 865043, and 24815245, which are already found to be the best inhibitors of BCL-2 and FLAT3, respectively. The two molecules methoserpidine (865043) and rescinnamine (24815245) that are found to be the most promising potential inhibitor of the mutated BCL-2 G101V, are very similar in structure with just a minor difference (Figure 5), but they are significantly different in function and toxicity, as explained below. The structures of these four molecules are shown in Figure 5. These molecules have been considered for biological applications, especially rescinnamine (that

targets FLAT3 and BCL-2 G101V in this study) and bisacodyl (that targets BCL-2 in this study) are known drugs in clinical applications. Rescinnamine is a vinca alkaloid that is derived from *Rauvolfia serpentina* and is used as an antihypertensive drug [32,33]. Bisacodyl is a stimulant laxative drug that is being used to treat constipation and found to be both safe and effective for long-term use [34,35]. Benzenamine, N-phenyl-4-(phenylazo), which can also be called benzeneazodiphenylamine, is part of the diphenylamine derivatives that are well known to act as an antioxidant, but not considered seriously for use in edible products for the fear of toxicity [36]. Methoserpidine is a synthetic isomer of reserpine that is being used as an antihypertensive drug but found to have a side effect that results in mental depression [37,38].

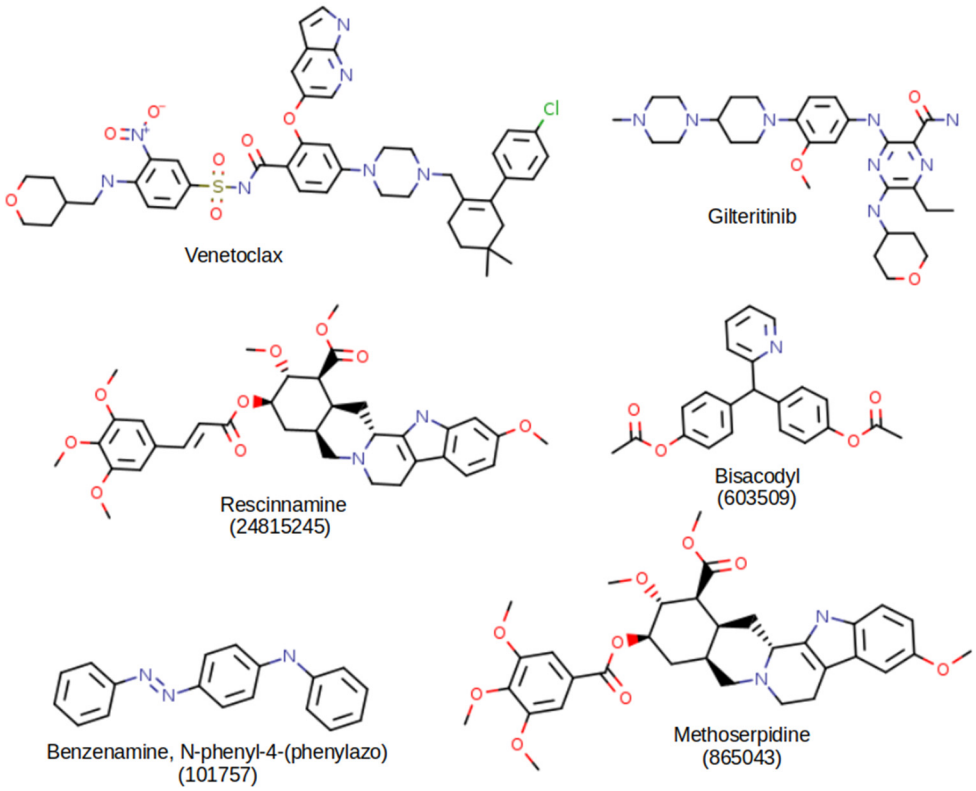

Figure 5. The known antileukemia drugs venetoclax and gliteritinib that target BCL-2 and FLAT3, respectively, and the four most promising antileukemia candidates predicted from the docking study.

The binding site residues interactions of rescinnamine with FLAT3 and the mutated BCL-2 G101V, and the interaction of bisacodyl with BCL-2 are shown in Figure 6 in an effort to compare their residue interactions with the residue interactions that defined the activity of the gilteritnib interaction with FLAT3 and the venetoclax interaction with BCL-2 and the mutated BCL-2 G101V, using their crystal interaction structures. The crystal interaction of venetoclax with BCL-2 and the mutated BCL-2 G101V are very similar in terms of protein residue interaction with only a minor difference that involves the Arg146 interaction in BCL-2, but Phe 153 interaction in the mutated BCL-2 G101V. The interaction of bisacodyl occupies some of the binding pockets that defined the activity of venetoclax, in its interaction with BCL-2, as they both share many residue interactions in common, such as Phe 104, Arg146, Tyr 108, Leu 137, Glue 136, Met 115, and Asp 111. The interaction of rescinnamine with the mutated BCL-2 G101V is also very similar to what was observed for the interaction of bisacodyl with BCL-2. The rescinnamine interaction with the mutated BCL-2 G101V was found to occupy a greater portion of the binding pockets that define the activity of venetoclax, as they shared many common residue interactions, such as Tyr 202, Val 148, Ala 100, Tyr 108, Phe 104, Asp 111, and Leu 137. The rescinnamine interaction with FLAT3 also shared some similar residue interactions that define the activity

of the gilteritinib interaction with FLAT3, such as residues Cys 695, Gly 697, Lys 614, and Asp 698.

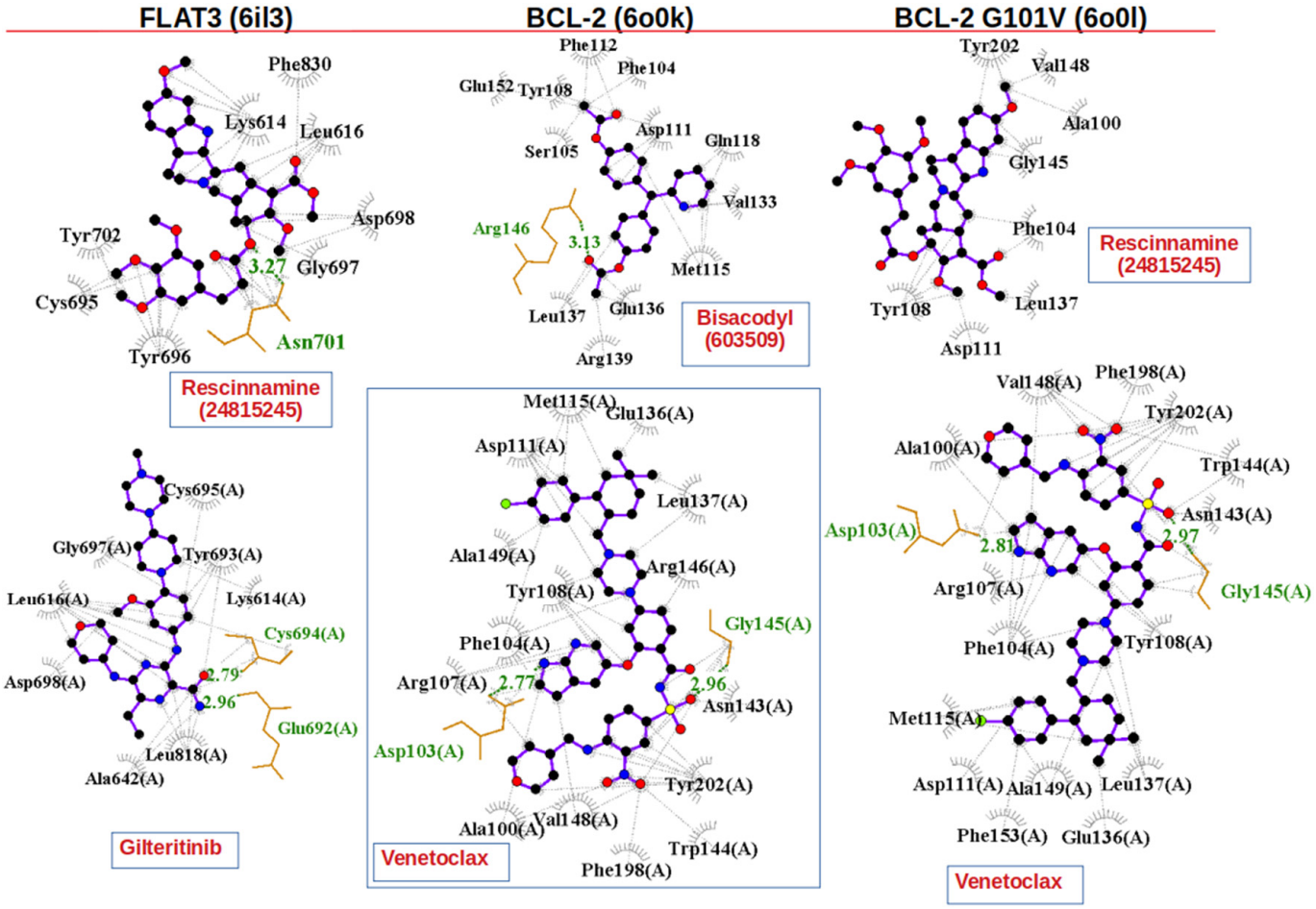

Figure 6. The docking pose binding site interaction of the rescinnamine (24815245) with FLAT 3 (6il3) and the mutated BCL-2 G101V (6o0l) and the interaction of bisacodyl (603509) with BCL-2 (6o0k), comparing the residue interaction with that of the crystal structure of the gilteritinib interaction with FLAT3 and the venetoclax interaction with BCL-2 and the mutated BCL-2 G101V.

4. Conclusions

A total of 262 potential antileukemia candidates were identified from the three samples, using LC-MS and were screened against leukemia targets FLAT3, BCL-2, and the mutated BCL-2 G101V, after excluding those that have inorganic metals, such as silicon.

The results from the ECR offered a better ranking of the potential drug candidates without excluding the benchmark molecules venetoclax and gilteritinib, which are known antileukemia drugs that target BCL-2 and FLAT3, respectively.

Among the top hit inhibitors from both the docking and consensus results, are 24815245 (rescinnamine) and 101757 (benzenamine, N-phenyl-4-(phenylazo)) that target FLAT3, as well as 865043 (methoserpidine) and 603509 (bisacodyl), that target BCL-2.

Methoserpidine and rescinnamine, which were found to be potential inhibitors of BCL-2 and FLAT3, respectively, were also predicted to be the top two promising inhibitors of the mutated BCL-2 G101V.

The binding interactions of the top hit potential drug candidates from the traditional concoctions are demonstrated to have similar residue interactions as the known drugs that target FLAT3 and BCL-2. This is an indication that the proposed potential drugs explored have similar binding pockets that define the activity of the known inhibitors of

these targets. A different study on the experimental anticancer activities of the concoctions using leukemia cancer cell lines is also under consideration.

Supplementary Materials: The following supporting information can be download at: https://www.mdpi.com/article/10.3390/app122211611/s1.

Author Contributions: Conceptualization, A.A.A.; Formal analysis, J.N.A.; Investigation, A.A.A. and M.N.; Project administration, M.N.; Supervision, P.S.; Writing—original draft, A.A.A.; Writing—review & editing, P.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Research Foundation (Grant No. 121276).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All of the necessary data that can help in the reproducibility of the results are included in the manuscript and also in the Supplementary Materials. The experimental LC-MS raw files were analyzed using the automatic mass spectral deconvolution and identification system (AMDIS) package version 2.73. The docking study was accomplished using Moledock version 2013.6.0, Autodock vina version 1.1.2, Glide in Schrodinger package version 2020, and Haddock docking package version 2.2. In all software used, the default parameters were used and all of the modifications are clearly stated in the methodology. Most of the data analysis and plotting are accomplished using Gnuplot version 5.2.

Acknowledgments: The authors would like to acknowledge the University of Kwazulu-Natal financial support and the Centre for High-Performance Computing (CHPC), Cape Town (South Africa), for the supercomputing facilities. P.S. thanks the National Research Foundation (NRF) South Africa for a Competitive Grant for unrated Researchers (Grant No. 121276).

Conflicts of Interest: The authors declare no conflict of interest.

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