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Structure-based virtual screening on a new open-source natural products database LOTUS to discover acetylcholinesterase ınhibitors

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ABSTRACT: Acetylcholinesterase (AChE) inhibitors have been used to delay the dementia progression in Alzheimer's Disease (AD). In 2017, a structure-based virtual screening (SBVS) protocol was made publicly available and successfully employed to discover chalcone derivatives and short peptides as AChE inhibitors. During the upgrading process of the SBVS protocol, an optimized version of the enhanced directory of useful decoys (DUDE) was released. This optimized DUDE was named DUDE-Z. In this article, the re-optimization of the upgraded SBVS protocol is presented. The optimization process made use of a machine learning package and library called recursive partitioning and regression tree (RPART) in R statistical computing software environment. The optimized SBVS protocol has the F-measure value of 0.322 against the DUDE-Z. The protocol was subsequently analyzed to efficiently screen on a newly released open-accessed natural products database LOTUS (https://lotus.naturalproducts.net/) to discover bioactive natural products as AChE inhibitors. The SBVS campaigns on 276,518 natural products identified 867 compounds as virtual hits, thirty-seven of which were identified as compounds found in the species from Kingdom Plantae.

KEYWORDS: Structure-based virtual screening; machine learning; acetylcholinesterase; natural products.

1. INTRODUCTION

Natural products have served as a useful source in the development of acetylcholinesterase (AChE) inhibitors [1]. Galantamine and rivastigmine are two successful examples of AChE inhibitors available in the market for the treatment of cognitive decline in Alzheimer's Disease (AD) [1, 2]. Galantamine is an alkaloid isolated from *Galanthus nivalis* [3], while rivastigmine is a semi-synthetic derivative of physostigmine, an alkaloid found in *Physostigma venenosum* [4]. Structures of galantamine, rivastigmine, and physostigmine are presented in Figure 1. On the other hand, an initiative for open knowledge management in natural products research was recently established [5]. The initiative was named LOTUS and provides us with an open-source natural products database, which has 276,518 compounds with their structures in SMILES and MOL formats [5]. The compounds are also linked to the organism. Therefore, the LOTUS database could serve as a convenient source for the discovery of bioactive natural products. Employing a retrospectively validated structure-based virtual screening (SBVS) protocol targeting AChE to screen on the LOTUS database is therefore of considerable interest.

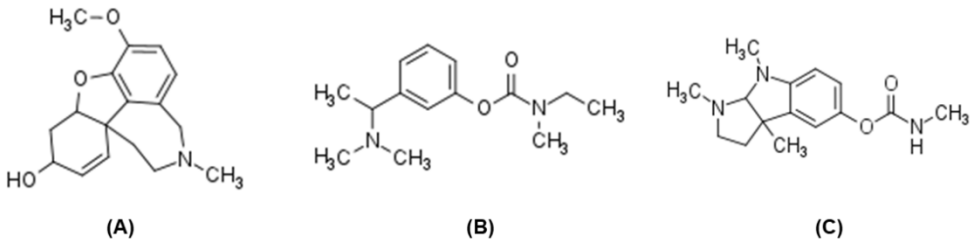

Figure 1. Structures of galantamine (A), rivastigmine (B), and physostigmine (C)

A retrospectively validated SBVS protocol to identify AChE inhibitors was publicly available in 2017 [6]. The protocol was validated against the enhanced directory of useful decoys (DUDE) [7]. The protocol

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was then employed to design chalcone derivatives and short peptides as potent AChE inhibitors [6]. Recently, the SBVS protocol was upgraded by using a different docking software and an upgraded version of the protein-ligand interaction fingerprinting (PLIF) program [6]. However, during the upgrading version, the optimized version of DUDE was released and named DUDE-Z [8]. Hence, the optimization of the SBVS protocol against DUDE-Z is of timely interest prior to its employment in virtual screening on the LOTUS database.

The research presented in this article aimed to optimize the upgraded version of the SBVS protocol targeting AChE and subsequently employ the optimized protocol to discover bioactive natural products as AChE inhibitors on the LOTUS database. The upgraded SBVS version targeting AChE was used and optimized against the DUDE-Z. The optimization process employed a machine learning technique called recursive partitioning and regressing tree (RPART) in the R statistical computing software environment [9]. The virtual screening on the LOTUS database of 276,518 compounds identified 867 virtual hits. Thirty-seven compounds of the hits were compounds in the Kingdom Plantae.

2. RESULTS

2.1. The SBVS protocol targeting AChE optimization

The conversion of the DUDE-Z dataset from SMILES format to PDB resulted in 84 active ligands and 4950 decoys in the PDB format. There were 15 active ligands missing during the conversion process, and it was found that those compounds were duplicates. Hence, the positive (P) and the negative (N) compounds in the DUDE-Z dataset used in this research were 84 and 4950, respectively. All PDB files were converted into pdbqt files successfully. The 3D structures of the DUDE-Z prepared in this research for further virtual screening both in PDB and pdbqt formats are available on request. The SBVS campaign against the dataset using the upgraded SBVS targeting AChE [6] resulted in the confusion matrix values as follows: True positive (TP), false negative (FN), false positive (FP), and true negative (TN) values were of 8, 76, 172, and 4778, respectively. The confusion matrix values corresponded to the F-measure value of 0.061, which was too low to be accepted for further use [7]. Fortunately, the retrospective SBVS against DUDE-Z provided us also with the PLIF bitstrings [10], which could be used further in the protocol optimization.

Table 1. Confusion matrices of the best-10 decision trees in the systematic modification of the prior distribution

No.	Prior Distribution	Data (unit)				F-measure
		TP	FN	FP	TN	
1.	0.80:0.20	55	29	124	4286	0.418
2.	0.82:0.18	52	32	114	4836	0.416
3.	0.83:0.17	52	32	114	4836	0.416
4.	0.77:0.23	56	28	132	4818	0.412
5.	0.81:0.19	53	31	121	4829	0.410
6.	0.85:0.15	35	49	53	4897	0.407
7.	0.86:0.14	35	49	53	4897	0.407
8.	0.79:0.21	55	29	133	4817	0.404
9.	0.88:0.12	34	50	51	4899	0.402
10.	0.89:0.11	34	50	51	4899	0.402

The first step of the optimization process was performed by systemically changing the docking scoring functions, i.e., the Gibbs free energy of binding (δG) [11], to be used as the cut-off value to select poses in the ensPLIF calculations [10]. This step resulted in the δG value of -7.6 kcal/mol as the best cut-off value, which provided us with the confusion matrix as follows: TP, FN, FP, and TN were 10, 74, 5, and 4945, respectively. The confusion matrix values corresponded to the F-measure value of 0.202. The dataset used for the retrospective validation is imbalanced [7, 8]. Therefore, the prior distribution of the P and N data could be further optimized in the RPART run [12] by systematic modification from 0.01:0.99 to 0.99:0.01 [6]. The confusion matrices of the decision trees with the top 10 F-measure values in the systematic modification of the prior distribution are presented in Table 1.

Employing machine learning techniques is vulnerable to overfitting [13]. The indication of overfitting [14] of the decision trees (Table 1) was then evaluated, and if there was an indication of overfitting, the complexity parameter was modified in the RPART re-run. After the analysis of the overfitting indication, it was discovered that the best decision tree was the one that resulted from the RPART run with the prior distribution of 0.89:0.11 and the complexity parameter value of 0.0126086. Further checking of the

possibilities of change correlations by the 1000 y-scrambling method [15] showed that 100% of the y-scrambled datasets have F-measure values less than 0.322. This indicated that the chance correlation was not observed [15]. The decision tree (Figure 2) has an F-measure value of 0.322. The corresponding interactions from ensPLIF descriptors presented in Figure 2 are presented in Table 2. Based on the corresponding residues in Table 2, Figure 3 is provided here to depict the interactions by using the AChE-native ligand huprine X complex from the PDB crystal structure (PDB ID: 1E66) [6] as the representative.

Table 2. The ensPLIF descriptors in the DUDE-Z optimized decision tree

No.	Descriptor	Corresponding Residue	Corresponding Interaction Type [16]
1	ensPLIF-22	Asp-72	hydrophobic
2	ensPLIF-28	Asp-72	ionic (residue as the anion)
3	ensPLIF-45	Phe-78	aromatic (edge-to-face)
4	ensPLIF-240	Trp-279	aromatic (face-to-face)
5	ensPLIF-253	Phe-290	hydrophobic
6	ensPLIF-296	Phe-330	aromatic (face-to-face)
7	ensPLIF-297	Phe-330	aromatic (edge-to-face)
8	ensPLIF-302	Phe-331	hydrophobic
9	ensPLIF-337	Trp-432	hydrophobic
10	ensPLIF-375	Tyr-442	H-bond (residue as the donor)

+

2.2. The prospective SBVS screening on the LOTUS database

The LOTUS database provided us with 276,518 natural products linked to information on their resources [5]. By filtering out compounds violating Lipinski's rules of 5 to get the drug-like compounds [17], 118,955 compounds remained. Since the optimized decision tree indicated aromatic moiety was essential (*vide supra*), the remaining compounds were filtered to have natural products compromising aromatic moiety. Hence, there were 59,310 natural products that were converted from their SMILES format into their PDB format and then subsequently into their pdbqt formats to be screened using the optimized SBVS protocol. The 3D structures converted from the SMILES format are available both in PDB and pdbqt formats on request. The SBVS campaigns identified 867 hits, which are provided as Supporting Information Table S2. By checking the hits to get information on corresponding natural resources in the LOTUS database (accessed on 28 August 2023) [5], 37 hits were identified as compounds from 34 species in the Kingdom Plantae. Those species are listed in Table 3.

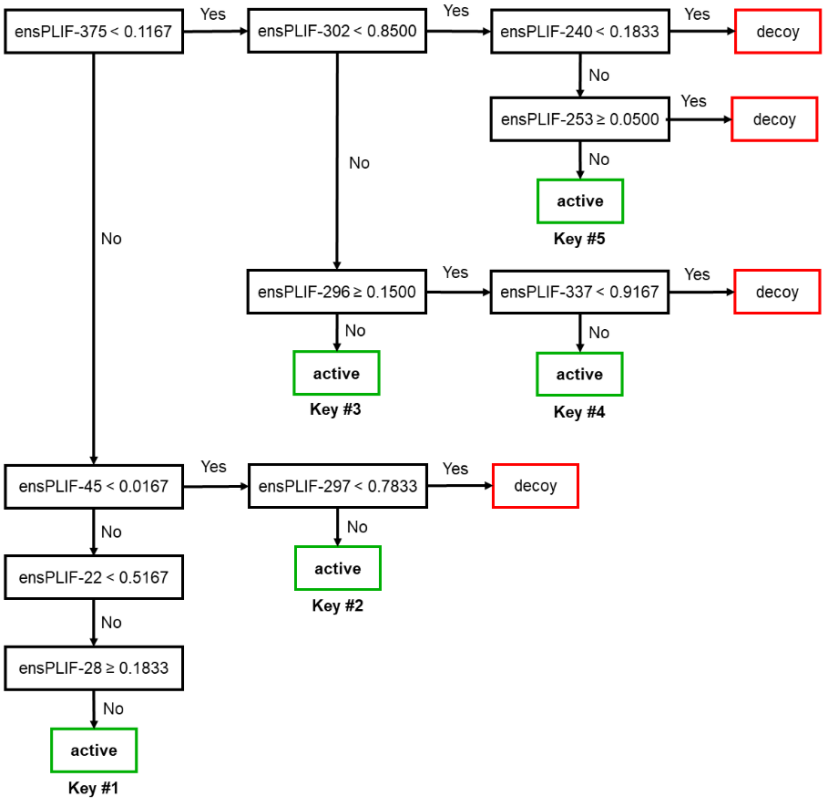

Figure 2. The DUDE-Z optimized decision tree for the SBVS protocol targeting AChE

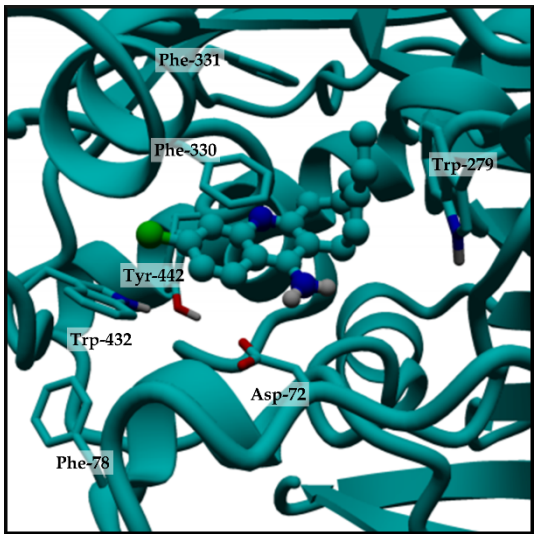

Figure 3. The complex of huprine X (in ball-and-stick mode) in the AChE binding pocket [6]. The secondary structure of the AChE is depicted in cartoon mode. Only the important residues in Table 2 are shown in the picture (in stick mode).

Table 3. Natural product resources from the Kingdom Plantae of the virtual hits

No	Species	LOTUS-ID of the Hits
1	*Anisotes longistrobus*	LTS0249698; LTS0249994
2	*Aplophyllum vulcanicum*	LTS0185900
3	*Biondia hemsleyana*	LTS0112585
4	*Caesalpinia crista*	LTS0207990
5	*Caesalpinia sappan*	LTS0081376
6	*Cephalotaxus harringtonii*	LTS0216275
7	*Cleome viscosa*	LTS0139264
8	*Clutia lanceolata*	LTS0274878; LTS0218683
9	*Cordia obliqua*	LTS0079882
10	*Croton eluteria*	LTS0022526
11	*Croton gratissimus*	LTS0182670
12	*Deguelia scandens*	LTS0124083
13	*Derris oblonga*	LTS0069449
14	*Diospyros loureiroana*	LTS0131712
15	*Diplostephium floribundum*	LTS0113861; LTS0245678
16	*Epimedium grandiflorum*	LTS0144034
17	*Eulophia nuda*	LTS0159378
18	*Gossweilerodendron balsamiferum*	LTS0190782
19	*Haemanthus multiflorus*	LTS0236502
20	*Hedysarum multijugum*	LTS0216479
21	*Justicia hayatai*	LTS0160742
22	*Justicia procumbens*	LTS0045639
23	*Justicia purpurea*	LTS0212665; LTS0180815
24	*Musa acuminata*	LTS0006491
25	*Packera bellidifolia*	LTS0240269
26	*Parartocarpus venenosa*	LTS0034682
27	*Rosmarinus officinalis*	LTS0246332; LTS0266849
28	*Sarcostemma viminale*	LTS0154149
29	*Scadoxus multiflorus*	LTS0236502
30	*Scutellaria rivularis*	LTS0086867
31	*Tephrosia apollinea*	LTS0071972
32	*Tylophora benthamii*	LTS0127740
33	*Tylophora ovata*	LTS0127740
34	*Vinca difformis*	LTS0164635

3. DISCUSSION

Aimed to provide a DUDE-Z optimized SBVS protocol targeting AChE, the upgraded version of the SBVS protocol [6] was run against the AChE ligands and decoys provided by DUDE-Z [8]. On the other hand, the natural products database LOTUS was recently made publicly available [5]. It was therefore irresistible to perform prospective SBVS campaigns on the LOTUS database by employing the optimized protocol.

As mentioned previously (vide supra), the SBVS campaign against the dataset using the upgraded SBVS targeting AChE [6] resulted in the confusion matrix values corresponding to the F-measure value of 0.061, which could not be employed for further use [7]. The PLIF bitstrings from the retrospective SBVS against DUDE-Z [10] were then used further in the protocol optimization. The resulting optimized SBVS protocol has an F-measure value of 0.322, which is higher than the original SBVS campaigns on DUDE (F-measure = 0.225) [7] and the most recent SBVS run (F-measure = 0.301) [18].

Based on Figure 2 and Table 2, there are 10 ensPLIF descriptors that play an important role in the optimized SBVS protocol, i.e., ensPLIF-22, -28, -45, -240, -253, -296, -297, -302, -337 and -375. In AChE, these ensPLIF descriptors related to the hydrophobic interaction with Asp-72, the ionic interaction with Asp-72 as the anion, the aromatic edge-to-face interaction with Phe-78, the aromatic face-to-face interaction with Trp279, the hydrophobic interaction with Phe-290, the aromatic face-to-face interaction with Phe-330, the aromatic edge-to-face interaction with Phe-330, the hydrophobic interaction with Phe-331, the hydrophobic interaction with Phe-342, and the H-bond with Tyr-442 as the donor, respectively (Table 2). Referring to the lock-and-key theory [19] and based on Figure 2 and Table 2, there are 5 types of keys for a ligand to be identified as an AChE inhibitor. Interestingly, except the key #3, all other keys involve aromatic interactions with Phe-78 (ensPLIF-45), Trp-279 (ensPLIF-240), or Phe-330 (ensPLIF-296 and -297) as one of the essential

interactions. Notably, both galantamine and rivastigmine have aromatic moieties (Figure 1). The SBVS protocol with the optimized decision tree script is provided as Supporting Information Table S1.

The optimized SBVS protocol was subsequently used to screen the LOTUS database [5] resulting in 34 potential species in the Kingdom Plantae to be developed further as AChE inhibitors (Table 3). Further experimental validation of the 2017 version of the SBVS protocol has provided us with novel chalcone derivatives and short peptides as AChE inhibitors [6]. Therefore, experimental validation of the optimized SBVS protocol presented in this article (Supporting Information Table S1) could be expected to assist us in the discovery of more novel AChE inhibitors [18]. Notably, some of the identified species presented in Table 3, e.g., Clutia lanceolate [20], Rosmarinus officinalis [21], and Caesalpinia crista [22], were reported to have activity as AChE inhibitors. The retrospective information [20–22] could serve as experimental validations of the in-silico approach to identify natural products as AChE inhibitors proposed in this article. Nevertheless, the compounds listed in Table 3 should be further verified in vitro to have information on the biomarker compounds in natural products with AChE inhibitory activities.

4. CONCLUSION

The upgraded SBVS protocol [6] was successfully optimized against the DUDE-Z dataset. The F-measure value of the optimized SBVS protocol was 0.322. Overfitting was avoided, and chance correlation was not observed in the optimized SBVS protocol. The virtual screening on the LOTUS database using the optimized SBVS identified 867 hits. Thirty-seven of the identified hits were compounds found in the 34 species in the Kingdom Plantae, some of which were reported as AChE inhibitors. These 37 identified hits could be explored further as biomarkers in the development of AChE inhibitors from natural products.

5. MATERIALS AND METHODS

5.1. Materials

The files to perform SBVS targeting AChE were provided by Istyastono et al. [6]. The optimized ligands and decoys in their SMILES format to perform the SBVS protocol optimization were obtained from the DUDE-Z database (https://dudez.docking.org/DUDE-Z-benchmark-grids/ACES/; accessed on 22 September 2022) [8]. The structures of the natural products in their SMILES format to perform the prospective SBVS were downloaded from the LOTUS database (https://lotus.naturalproducts.net/download/smiles; accessed on 1 October 2022) [5].

5.2. Instrumentation

The main machine used in the research presented in this article was a 64-bit Linux (Ubuntu 18.04.6 LTS) server with 8 Intel(R) Xeon(R) CPU E5-2680 v3 @ 2.50GHz as the processors and 16 GB of RAM. The software employed in the research presented in this article were YASARA-Structure [23] version 22.8.22, AutoDock Vina version 1.1.2 [11], PyPLIF HIPPOS [16] version 0.1.2 (installed in a miniconda environment with Python version 3.6.13 from https://anaconda.org/conda-forge/pyplif-hippos; accessed on 22 September 2022) [10], AutoDockTools-prepare (installed in a miniconda environment with Python version 2.7.13 from https://anaconda.org/insilichem/autodocktools-prepare; accessed on 22 September 2022) [24], and the RPART package in R statistical computing software version 3.4.4. [9].

5.3. Procedure

5.3.1. Optimization of the SBVS protocol targeting AChE

The optimized dataset, which consisted of 99 active ligands and 4950 decoys in their SMILES format, was downloaded from the DUDE-Z [8]. The same SMILES to PDB format conversion macro file from Istyastono et al. [6] was used to convert the structures from their SMILES format into their three-dimensional (3D) structure in protein data bank (PDB) format. The PDB files were then converted to the readily-to-dock pdbqt formats using the module prepare_ligand.py from the AutoDockTools-prepare [24]. The pdbqt files were then subjected to the SBVS protocol targeting AChE [6]. The results from the retrospective SBVS campaign were employed to optimize the quality of the SBVS protocol to reach the best protocol with the highest F-measure value. The screening campaigns provided us with the PLIF bitstring values which were valuable to be converted into ensemble PLIF (ensPLIF) values for further optimization as the descriptors [10]. The RPART package and library from R statistical computing software were used to generate the decision trees based on the ensPLIF values as the descriptor [10]. The F-measure value was calculated using the formula published by Cannon et al. [25].

5.3.2. Virtual screening on the LOTUS database

All 276,518 compounds in their SMILES format provided by the LOTUS database [5] were downloaded. The compounds were then filtered based on compliance with Lipinski's rules of 5 [17] and the availability of the essential moieties as suggested by the optimized SBVS protocol. The remaining compounds were then prepared similarly to those from the DUDE-Z (*vide supra*). The compounds in their pdbqt formats were then subjected to the optimized SBVS protocol. The virtual hits were then analyzed to identify the plants containing the hits resources by employing the information in the LOTUS database.

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Conflict of interest statement: The authors declare no conflict of interest.

Supporting information: The following supporting information is available online: Supporting Information Tables. The contents of the Supporting Information are: (i) Supporting Information Table S1 (The SBVS protocol with the optimized decision tree script), and (ii) Supporting Information Table S2 (The SBVS campaigns identified 867 hits).

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