

BRUSELAS: HPC Generic and Customizable Software Architecture for 3D Ligand-Based Virtual Screening of Large Molecular Databases

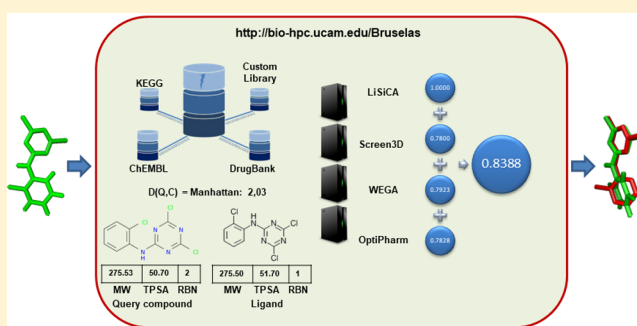
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Supporting Information

ABSTRACT: BRUSELAS (balanced rapid and unrestricted server for extensive ligand-aimed screening) is a novel, highly efficient web software architecture for 3D shape and pharmacophore searches in *off the cuff* libraries. A wide panel of shape and pharmacophore similarity algorithms are combined to avoid unbiased results while yielding consensus scoring functions. To evaluate its reliability, BRUSELAS was tested against other similar servers (e.g., USR-VS, SwissSimilarity, ChemMapper) to search for potential antidiabetic drugs. A web tool is developed for users to customize their tasks and is accessible free of any charge or login at <http://bio-hpc.eu/software/Bruselas>. Source code is available on request.



1. INTRODUCTION

Virtual screening (VS) is a set of *in silico* techniques that aims to increase the chances of finding novel hit and lead compounds. Despite its moderate computational cost, VS remains one of the preferred options for assessing large chemical spaces and for reducing the time spent on *in vivo* experimentation.¹ This approach has gained importance during recent decades for studying of a variety of diseases such as diabetes, malaria, and Parkinson's disease.^{2–12} The wide diversity of VS techniques may be classified into two major groups, depending on whether the 3D structure of the target is known (structure-based, SBVS) or not (ligand-based, LBVS). LBVS relies on a knowledge of small molecules that bind the target of interest and encompasses a collection of methods, including similarity searching, pharmacophore modeling, and QSAR (quantitative structure activity relationship).¹³ In the search for a cost-effective solution, similarity searching is the favorite approach, whenever possible, due to its simplicity.¹⁴ The underlying idea behind similarity searching is to build a fingerprint for molecules that can be easily compared, while simultaneously maintaining the information necessary to identify similar biological activity. An alternative to this approach is pharmacophore modeling, which aligns two or more molecules to identify the shared pharmacophore features between them. In this case, molecular similarity is represented in terms of compounds whose pharmacophoric features are quite similar to the pattern identified.¹⁵

To provide computational services accessible to any users, several VS web servers have emerged concomitant with the development of web technologies. Usually, online VS servers focus on the application of one or many techniques and allow users to set a collection of parameters, including the databases to be screened and the similarity algorithms to be applied.¹⁶ Although VS is widely used, some critical issues remain unsolved. For example, millions of compounds have been studied and their details published in the literature, but only those fulfilling drug-related requirements are of interest. In addition, computational resources may also represent a bottleneck when the chemical space is very large or the tasks to perform are computationally expensive.¹⁷ Finally, the large number of existing compounds, tools, and techniques complicates the development of research on VS.

To help to solve such critical issues, we have developed BRUSELAS (balanced rapid and unrestricted server for extensive ligand-aimed screening), which is a software architecture focused on 3D LBVS. BRUSELAS integrates several software tools (e.g., Obabel, WEGA, DRAGON) and hides their details from the final user (Table 1). As an additional feature, it maintains a large database of compounds imported from publicly accessible databases and curated for their use in VS. That database allows the server to suggest a suitable library containing the most promising compounds for

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Table 1. List of Software Tools Embedded in BRUSELAS

task	tool	references
calculation of descriptors	DRAGON	18
chemical databases	DrugBank, ChEMBL, KEGG, DIA-DB	19–22
remove salts from conformers	Standardizer	23
calculation of conformers	Omega2	24
shape similarity	WEGA, LiSiCA, Screen3D, OptiPharm	25–28
pharmacophore modeling	SHAFTS	29
molecular conversion	Obabel, Molconvert	23 and 30
3D visualization	Jmol	31

each given query molecule. The screening phase is complemented with a collection of statistical functions to make predictions more effective. Since efficiency is a crucial point in VS, BRUSELAS profits from a high performance computing (HPC) platform for delivering results with a delay of a few minutes only. To ease the use of the architecture and the interpretation of its predictions, a web tool has been developed and is available without fee or login at <http://bio-hpc.eu/software/Bruselas>.

The present work aims to help to make VS available to general scientists by merging advanced features in a transparent and friendly web-based environment. It provides a software architecture for the configuration and execution of 3D

virtual screening tasks, which implements a workflow specifically designed for handling a large number of compounds. Such an architecture can be potentially used by researchers in a number of areas related to drug discovery. On the other hand, it opens up a way to investigate these areas of research, using highly customizable tools in web environments, which can take advantage of the most recent computational advances, e.g., distributed computing, to predict those compounds with the greatest possibilities of becoming drugs in the future. This manuscript is structured as follows: Section 2 introduces an overview of BRUSELAS architecture, the underlying workflow implemented and the main tools it uses. Section 3 assesses the use of the web tool and highlights its main features and the options available. In an attempt to provide a fair but critical assessment of the developed tool, Section 4 compares the reliability and the accuracy of the architecture with other similar servers by using two well-known drugs as queries. The main conclusions reached concerning BRUSELAS in its current version as well as forthcoming research lines are outlined.

2. MATERIALS AND METHODS

2.1. Screening Workflow. BRUSELAS implements the workflow illustrated in Figure 1. The protocol starts when a user requests a new screening task from the web tool. Although several parameters are available for customizing the tasks, only three of them are mandatory: the screening algorithm(s), the database(s) to be screened and the e-mail address to which the

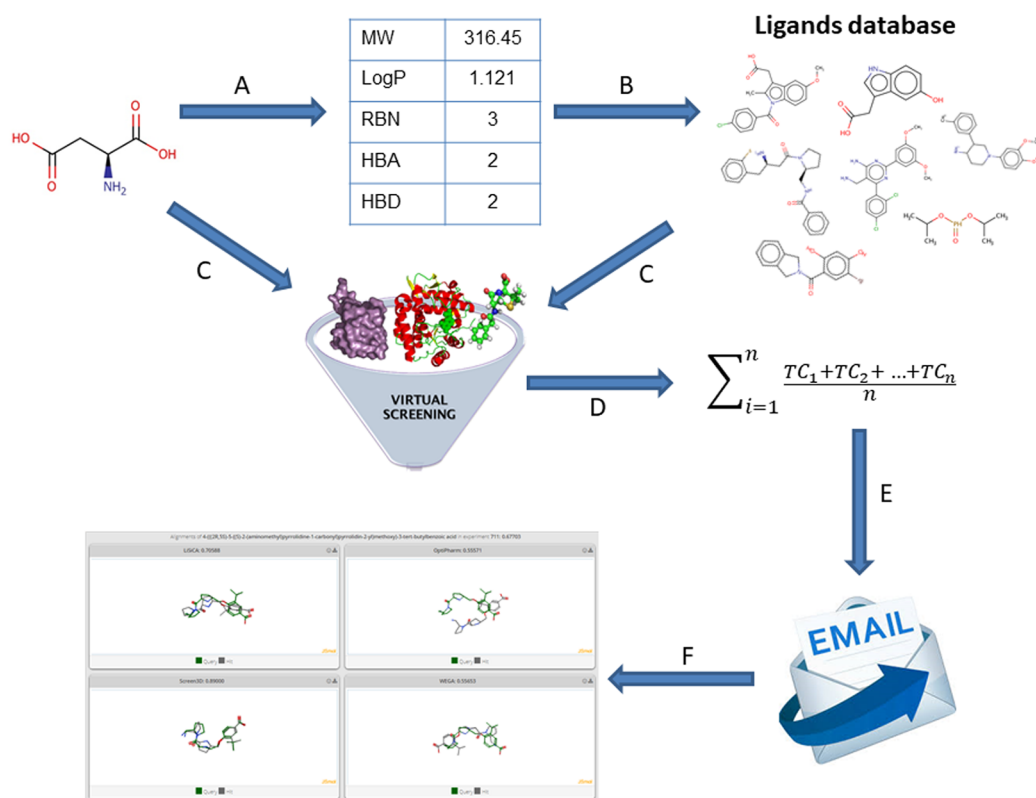


Figure 1. Graphic representation of BRUSELAS workflow for virtual screening tasks. (A) If the server needs to build a library, it calculates the selected descriptors from the query. (B) The calculated descriptors are used for selecting the most promising compounds from the database. (C) Once the library is calculated, both query and library are supplied for 3D screening. (D) If needed, the individual scores are combined in a global score. (E) Users are notified by e-mail when the results are available on the website. (F) Users can analyze the results on the web tool.

results will be sent. Users can choose to submit their own libraries or ask the server to build one *ad hoc* for the given query. If the users decide to screen the BRUSELAS libraries, they can customize the selection of the compounds that will be included in their library. Among the parameters of this step is the set of molecular descriptors that will be used, along with a distance function (e.g., Manhattan), to find the most promising compounds for the 3D screening stage. The descriptors of the ligands are already calculated with the DRAGON¹⁸ tool and stored in a relational database to save time, whereas the ones from the query are calculated every time, unless the users upload their own library. DRAGON is a powerful tool which is able to calculate almost 4900 descriptors. Since this is a very large number of descriptors, the server makes an initial proposal with the most common ones (molecular weight, number of rotatable bonds, number of hydrogen acceptor/donor atoms, topological polar surface area, and octanol–water partition coefficient), but the users have the freedom to modify the proposal by selecting those that best suit their needs (Figure 1A). Then, a fingerprint is built with the selected descriptors, which is used to reduce the number of ligands that will continue to the 3D screening stage (Figure 1B). Next, both the query and the library are sent to a supercomputing cluster for detailed 3D ligand-based screening (Figure 1C). In cases where many similarity algorithms are involved, the server has to deal with several scores arising from each of them. This situation is handled by applying a consensus scoring function, which combines the individual scores into a single one for each compound (Figure 1D). Once the screening is done, all the output generated by the algorithms is interpreted, adapted to BRUSELAS, and stored in the relational database. Finally, the users are notified by e-mail that their results are available on the website (Figure 1E).

2.2. Compound Database. As mentioned above, BRUSELAS may have to propose a suitable library for screening when the user does not supply one. For this reason, the server maintains a database of compounds which are imported from the Food and Drug Administration (FDA) data set deposited in DrugBank¹⁹ (1760 compounds), the set of active compounds in ChEMBL 21²⁰ (1 578 131), KEGG²¹ Compounds and Drugs subsets (23 667) and an *in-house* library of antidiabetics, DIA-DB²² (186). Although a 2D representation of such compounds can be freely obtained, they have been curated for VS in a preparation protocol because most of the similarity algorithms supported by BRUSELAS require many conformations to cover the chemical space. Accordingly, the first step of the protocol is to obtain the 3D representation of all the compounds to better handle flexibility. To find the 3D conformer that is most likely to occur in Nature, a Merck molecular force field (MMFF94) is applied since it has been demonstrated to be suitable for small organic molecules.³² Next, salts are removed with the standardizer²³ tool, and up to 10 conformers are calculated with the omega2²⁴ tool for those compounds having between 1 and 10 rotatable bonds. In this step, we set a root-mean-square distance (RMSD) of 0.7 to ensure a diversity of conformers and leave the rest of parameters at their default values. Conformer generation is repeated with less restrictive conditions for those compounds that do not generate any conformer previously. Finally, those compounds containing a heavy metal (e.g., chromium) or a boron atom bonded to a halogen that are not supported by the force field MMFF94 are not imported. The curation process results in a data set of 7 473 006 conformers

available for screening. This number represents an average of eight conformers for compounds with up to 10 rotors. Additionally, a collection of key terms, including bibliography, descriptions, and related targets, is extracted from the source databases and imported into BRUSELAS. Such information may be used for the creation of suitable libraries when users want to narrow the scope of the job to a specific disease or target.

2.3. Methods Available for 3D Virtual Screening. BRUSELAS supports both similarity and pharmacophore searching, both of which can be launched from the same interface. In order to obtain the most accurate predictions, BRUSELAS does not rely on one single similarity algorithm, but it proposes four 3D algorithms, each of a different nature, which can be combined in the same task to avoid biased results. The available algorithms are WEGA,²⁵ LiSiCA,²⁶ Screen3D,²⁷ and OptiPharm.²⁸ The server takes care of the format conversions to make each algorithm work with the required molecular format. Such algorithms represent four different complementary approaches for calculating similarity. Whereas WEGA evaluates similarity by means of weighted atomic Gaussian functions, LiSiCA is based on graph theory. On the other hand, Screen3D calculates a similarity score based on the atomic distances between query and ligands. Note that BRUSELAS configures Screen3D to handle both the query and the ligand, enabling the molecules to be rotated, in order to make more accurate estimations. The last approach, OptiPharm, is a parallelized evolutionary algorithm that needs large populations to analyze the search space and find accurate solutions.

Pharmacophore modeling is performed by SHAFTS,²⁹ which is a pharmacophore matching method based on a feature triplet hashing and searching algorithm. SHAFTS is suitable for large-scale VS with single or multiple bioactive compounds, and it has been successfully used in many studies, such as the search of p90 ribosomal S6 inhibitors and the development of B-Raf(V600E) inhibitors.^{33,34}

2.4. Consensus Scoring Functions. LBVS predictions may be influenced by the algorithm used to assess similarity, leading to biased results. This issue might be addressed by combining multiple similarity algorithms, but it requires the fusion of individual scores. Although consensus scoring functions are frequently used in SBVS,³⁵ they are rarely used in LBVS because ligand-based servers do not allow the combination of different algorithms. However, BRUSELAS does not restrict the selection of similarity algorithms to one single task; the output provides three consensus functions: the mean, the weighted mean, and the maximum score. Whereas the weighted mean is used to assign a customized rate to each algorithm and is especially useful to return unbiased results by a concrete algorithm, the mean function rates every algorithm with the same weight. Furthermore, the maximum score function returns the best score assigned by any of the algorithms applied. This set of functions covers all the possible approaches, from the most optimistic with the maximum score to the most conservative with the mean score.

2.5. Computation Time. The screening of large data sets is a time-consuming process whose duration usually grows with the size of the data set. HPC techniques are typically applied to speed up that task, and they have gained importance in the context of VS during recent years.³⁶ BRUSELAS also profits from HPC by running similarity calculations on a supercomputing cluster. With this approach, the screenable

A

Type of calculation Search Library building Score Submit

Algorithm: **ⓘ**

LISICA ☐ OptiPharm ☐ Screen3D ☐ WEGA ☐

Select query molecule: **ⓘ**

☐ Upload Ligand

☐ Ligand (Smiles)

☐ Draw Ligand

Select target library (optional): **ⓘ**

☒ BRUSELAS libraries

☒ DIA-DB ☒ DrugBank ☒ ChEMBL ☒ KEGG

☐ Custom library

Seleccionar archivo Ningún archivo seleccionado

B

Type of calculation Search Library building Score Submit

Descriptor extraction software: **ⓘ**

☒ DRAGON

Select descriptors (optional): A number between 1 and 100 descriptors must be selected. **ⓘ**

☒ Ghose-Crippen octanol-water partition coeff. (logP) ☒ molecular weight ☒ number of acceptor atoms for H-bonds (N,O,F)

☒ number of donor atoms for H-bonds (N and O) ☒ number of rotatable bonds

☒ topological polar surface area using N,O,S,P polar contributions

Distance function: **ⓘ**

☒ Manhattan distance ☐ Euclidean distance ☐ Range values

Compounds related with the following terms: **ⓘ**

Ex.: malaria, breast carcinoma, MAPK

C

Type of calculation Search Library building Score Submit

Consensus scoring function (optional): **ⓘ**

☒ Arithmetic mean ☐ Weighted arithmetic mean ☐ Maximum value

Similarity cutoff [0,1] (optional): **ⓘ**

Maximum number of hits (optional): **ⓘ**

D

Type of calculation Search Library building Score Submit

Job description (optional): **ⓘ**

Email:

SEND EXPERIMENT

Figure 2. Task configuration screen of BRUSELAS. (A) Selection of similarity algorithms, query and databases. (B) Users can configure the creation of an *off the cuff* library for screening. (C) Configuration of results and consensus scoring function. (D) A short text can be included in the notification e-mail.

Parameters of experiment 906

Submitted by	albanegas@alu.ucam.edu	Start/End date	30/03/2019 22:08:10 - 30/03/2019 22:25:45
Type of calculation	Similarity searching	Similarity software	WEGA, Screen3D, OptiPharm
Scoring cutoff	0	No. Results requested	100
Distance function	Manhattan distance	Consensus function	AVG
Description	acarbose + wega + screen3d + optipharm + drugbank		

[View more](#)

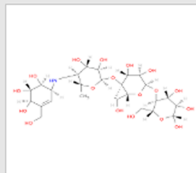
Results

Show entries

Search: Extended view ☐

Ranking ▲	Compound	Score[0,1]	Alignment
1	Acarbose	0.71856	
2	Ergotamine	0.62543	
3	Cefmetazole	0.59978	
4	Cefazolin	0.58376	
5	Deserpidine	0.55670	
6	Ertapenem	0.55237	
7	Dihydroergotamine	0.55136	
8	Piperacillin	0.54599	
9	Quinine	0.54555	

Query Molecule



  PyMOL mol2

References

[1] [3] [4] [5] [6] [7] [9] [10] [12] [13]

Figure 3. Analysis of results screen in BRUSELAS.

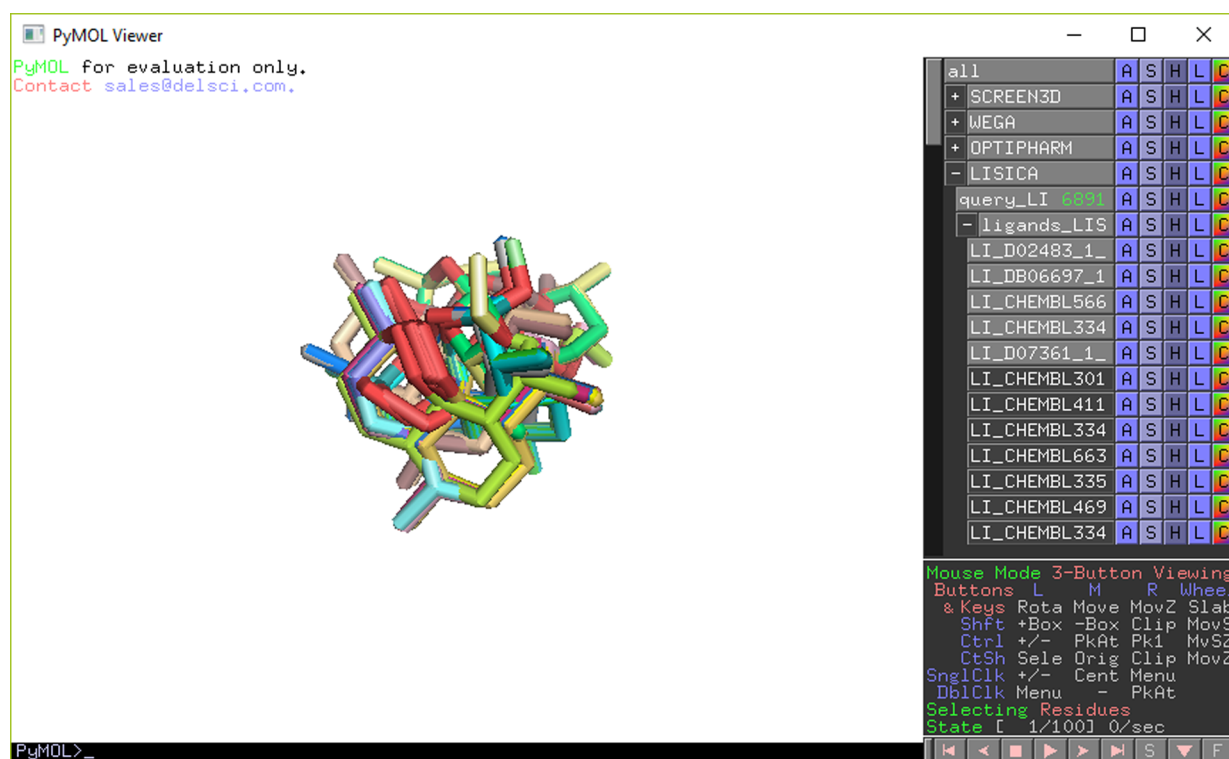


Figure 4. PyMOL session downloaded from BRUSELAS.

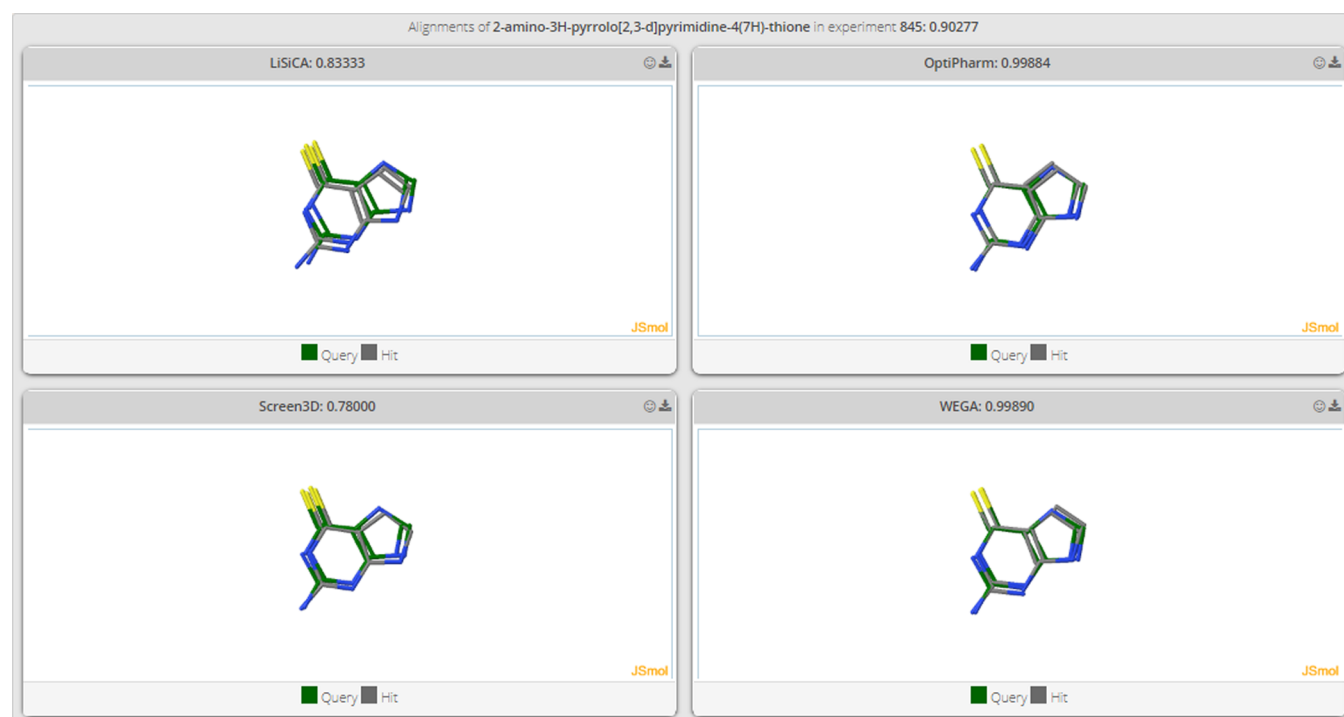


Figure 5. Alignment of query and ligand generated by all similarity algorithms in BRUSELAS.

compounds are grouped into small blocks, and then every group is compared against the query in parallel. As the server is currently configured, a standard task using the default parameters and involving the four algorithms available will deliver the results in less than 1 h. It should be noticed that, although the 100 best-ranked hits are returned by default, the library suggested by the server is larger. By using HPC and

limiting the number of conversions among formats, the results are rapidly delivered.

3. WEB TOOL USAGE

3.1. Input Data. Before starting the screening process, BRUSELAS needs to collect input parameters, which are gathered from the web tool. Although only a few fields are

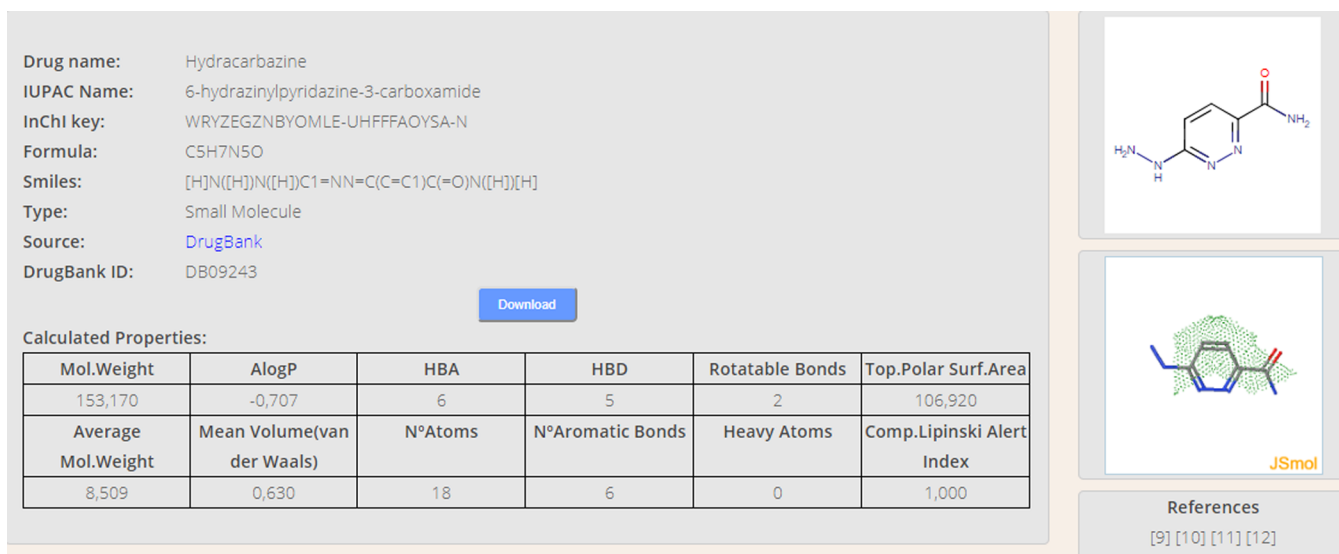


Figure 6. Detailed information on a compound selected from BRUSELAS' database.

required (query, library, similarity algorithm, and e-mail), the server accepts many other options to customize tasks, including the maximum number of hits to return and the scoring cutoff. Input parameters are organized in four tabs according to their purposes, as shown in Figure 2.

One of the most interesting abilities of BRUSELAS is the combination of many similarity algorithms in the same task and the screening of a custom database. Since the server relies on Obabel³⁰ and Molconvert²³ to carry out conversions, the most common formats are supported, including sdf, mol2, and pdb. Another distinguishing feature is the ability to create an *off the cuff* library on the basis of some key terms. When any keyword is introduced, the server searches for those compounds linked to the given terms and creates a library with them. This novel feature is useful for creating specific libraries for a given disease or focusing searches on a certain target. In addition, tasks can also be labeled with a short text, which is included in the notification e-mail, to differentiate them.³⁷

3.2. Analysis of Results. Users are informed by e-mail as soon as the calculation is completed.³⁷ The notification includes a link to the result explorer screen, which is organized in three blocks: the summary header, the query molecule and the list of hits (Figure 3). The header shows a summary of the input parameters used to set the task up. On the right, a 2D representation of the query is displayed. The user can manipulate the query and the results with the four buttons available under the query providing the following functionalities: (1) download the SMILES representation of the query; (2) download compound names and scores in an Excel file; (3) share the results with other users by e-mail; (4) download the hits in a PyMOL session where the five best-ranked compounds are selected by default (Figure 4); (5) download all the aligned ligands and the query in separate mol2 files for their offline manipulation. The third block lists candidate compounds sorted by score, which is expressed in terms of the Tanimoto coefficient, in descending order. Although this score is the final one calculated by BRUSELAS, individual scores predicted by each algorithm are also available in the same table by enabling the "Extended view" check. Next to the scores, the "Alignment" button opens a new window where both query

and ligand are conveniently aligned in the Jmol³¹ applet (Figure 5). This feature helps users to visualize, at a glance, how both molecules are aligned by each algorithm, with the possibility of downloading each aligned ligand individually.

3.3. Compound Explorer. The compound database maintained by BRUSELAS is available not only for screening but also for exploring its main properties. Compounds can be filtered by a variety of fields, including chemical name, International Union of Pure and Applied Chemistry (IUPAC) name, and SMILES representation. To obtain further information about a molecule, users just click on the name and they are directed to a detail screen (Figure 6), where additional information is displayed (e.g., typical molecular descriptors, link to the original source).

4. CASE STUDIES

4.1. Search for Malaria Drugs. Artemether is a drug frequently used in treatments against Malaria since its first approval in 2009, especially in Africa and Asia, either alone or in combination with other drugs such as Lumefantrine or Amodiaquine.^{38–41} With the aim of testing the reliability of BRUSELAS in the simplest case, we tested all the combinations of similarity algorithms with a copy of artemether as query. As expected, artemether was predicted as the best-ranked candidate with 100% similarity in all the cases. This result demonstrates that BRUSELAS behaves as expected in a simple case, independent of the combination of algorithms selected.

4.2. Antidiabetic Compounds. Type 2 diabetes mellitus (T2DM) is a frequent form of insulin resistance that maintains glucose homeostasis by increasing the release of insulin.⁴⁹ Acarbose is a glucosidase inhibitor which has been extensively applied in T2DM cases.^{50–53} In our case, acarbose was used as the query to conduct a second experiment with the aim of assessing the reliability of BRUSELAS predictions compared with some similar servers. Every server was supplied a copy of acarbose downloaded from ZINC⁵⁴ database, and the FDA approved drugs data set from DrugBank was screened when possible. In cases where acarbose is present in the selected database, it should be returned as the best-ranked hit. Moreover, the expected score should be as close as possible

Table 2. Assessment of the Efficacy of Some Relevant LBVS Servers in the Context of T2DM

VS server	similarity method	library to screen	rank acarbose	score	ref.
USR-VS	USR	ZINC	not found	—	42
	USCAT		not found	—	
SwissSimilarity	FP2	Drugs Approved	1	1.000	43
	ElectroShape 5D		1	0.907	
	Spectrophores		2	0.823	
	Shape-IT		not found	—	
	Align-IT		not found	—	
BRUSELAS ^a	All shape similarity	DrugBank	1	0.757	
	SHAFTS		not found	—	
Superimposé	Score1	Superdrug	not found	—	44
	Score1	Ligand Depot	not found	—	
	Sd_best_compare	Superdrug	not finished	—	
HybridSim-VS	FP2 + WEGA	DrugBank Approved	1	0.693	45
	MACCS + WEGA		1	0.685	
ChemMapper	FP2	DrugBank Custom	1	1	46
	USR		80	0.813	
	SHAFTS		not found	—	
wwLig-CSRre	LigCSR	DrugBank Approved	not found	—	47
iDrug	SHAFTS	MayBridge	not found	—	48
		ZINC Lead	not found	—	

^aThe full list of experiments performed on BRUSELAS is provided in the [Supporting Information](#).

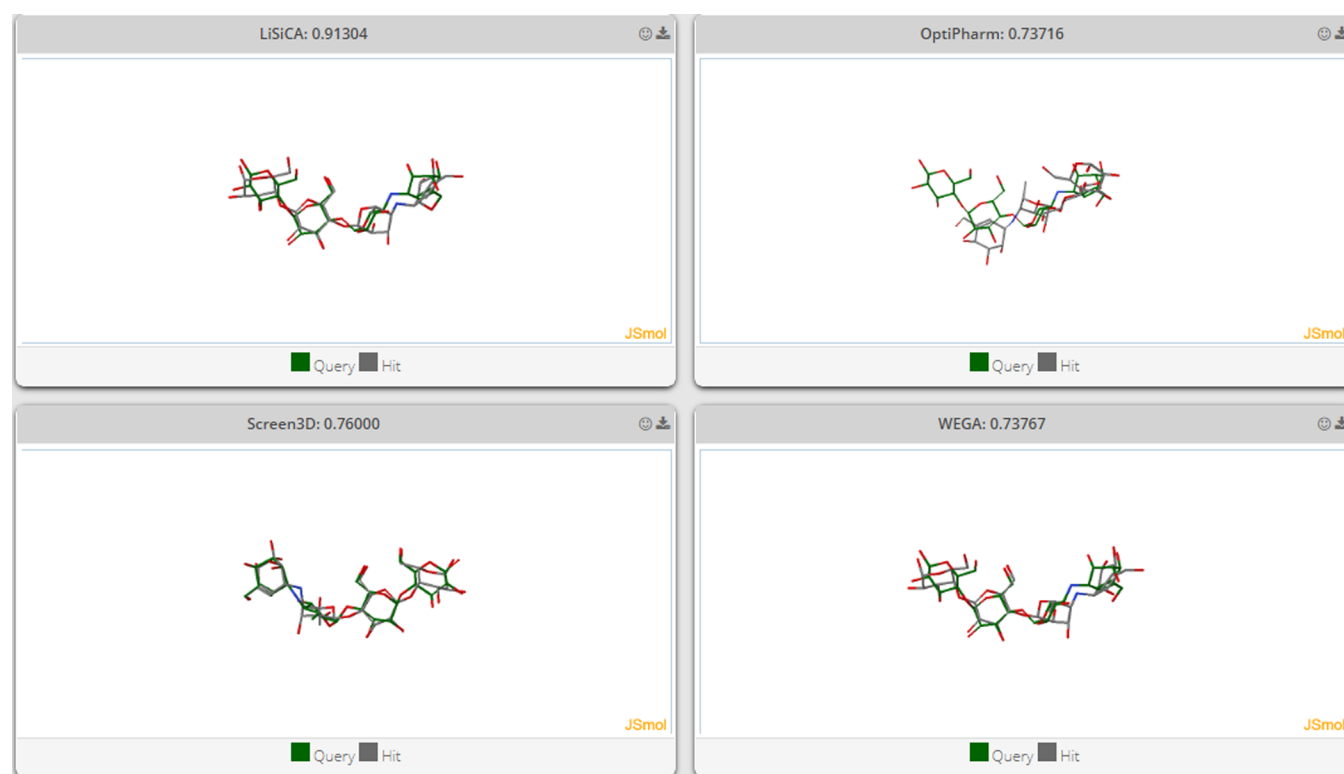


Figure 7. Alignments obtained for acarbose in the simulation combining the four algorithms.

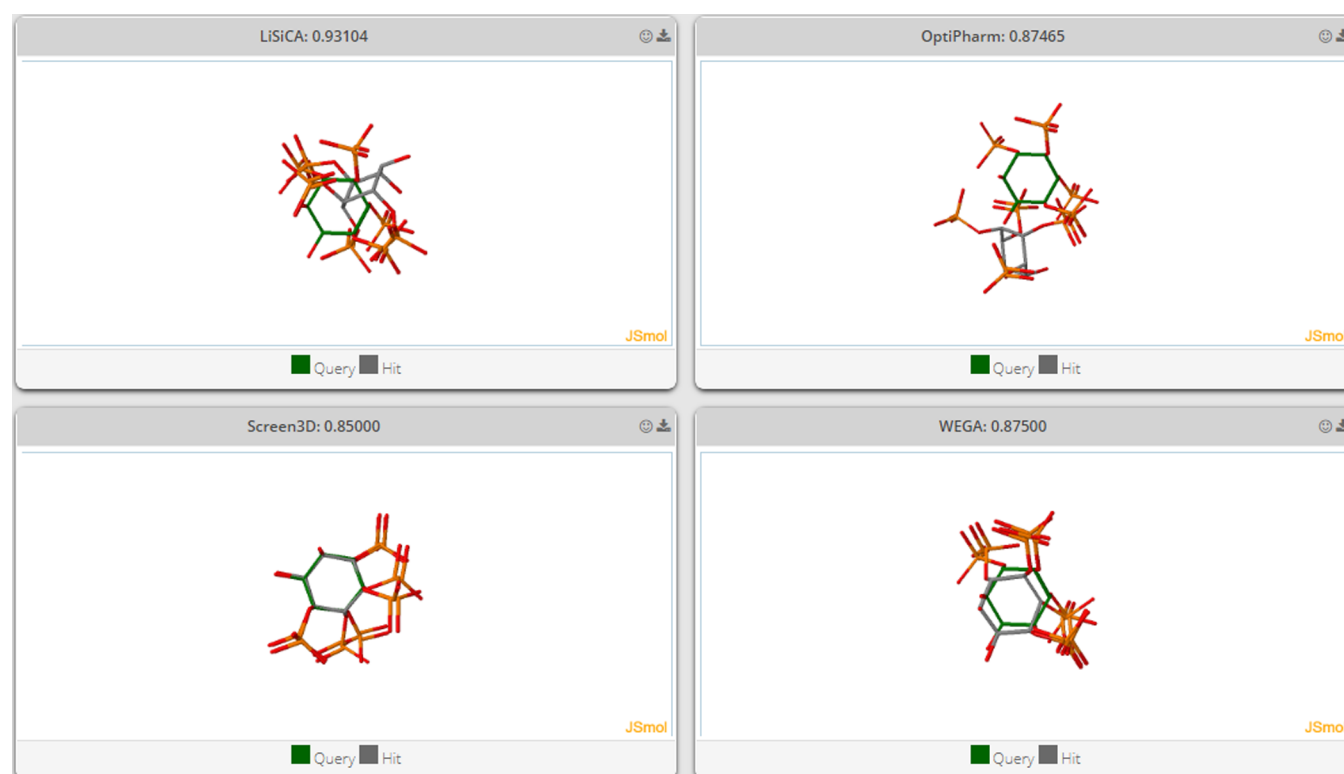
to 1, which represents total similarity in terms of the Tanimoto coefficient.

We split the tests into two groups: shape similarity and pharmacophore searching tasks. As regards shape similarity, Table 2 (see full details in [Supporting Information](#)) shows that BRUSELAS correctly predicted acarbose as the best-scored compound in all the searches, independent of the configuration chosen, resolving each task in less than half an hour. Figure 7 shows the alignments obtained for acarbose from all the

algorithms. As regards of the other servers, they can be classified into two groups, depending on whether they identified acarbose in the candidates list or not. The first group includes SwissSimilarity, HybridSim-VS, and ChemMapper. Although SwissSimilarity and ChemMapper exhibited the highest scores, the former did not find any result when screening with Shape-IT and Align-IT, and the latter only performs 2D screening and did not rank acarbose as the first option when using USR. On the other hand, HybridSim-VS

Table 3. Summary of the most relevant results obtained by BRUSELAS for TMI query using similarity and pharmacophore searches

similarity method	compound	score	ref
shape similarity	phosphoric acid mono((1S,2S,3R,4S,5R,6R)-2,3-dihydroxy-4,5,6-tris(phosphonooxy)cyclohexyl) ester	0.883	57
	phosphoric acid mono((1R,2R,3S,4R,5S,6S)-2,3-dihydroxy-4,5,6-tris(phosphonooxy)cyclohexyl) ester	0.811	
	inositol 1,3,4,6-tetraphosphate	0.771	
	phosphoric acid mono((1S,2S,3R,4S,5S,6S)-2,5-dihydroxy-3,4,6-tris(phosphonooxy)cyclohexyl) ester	0.751	
	L-adenosine triphosphate	0.567	58
Pharmacophore screening	D-myo-inositol 1,2,4,5-tetrakisphosphate	0.233	
	2-amino-9-(4-hydroxy-5-methoxytriphosphate tetrahydrofuran-2-yl)-1,9-dihydropurin-6-one (dGTP)	0.185	
	{[(S)-{[(S)-{[(2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy}(hydroxy)phosphoryl]oxy}(hydroxy)phosphoryl]amino}phosphonic acid	0.183	
	((2R,3S,4R,5R)-5-(7-amino-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyltriphosphoric acid	0.180	
	ATP analog	0.167	

**Figure 8.** Alignment of TMI and phosphoric acid mono((1S,2S,3R,4S,5R,6R)-2,3-dihydroxy-4,5,6-tris(phosphonooxy)cyclohexyl) ester obtained from the different algorithms.

always ranked acarbose as the best result, but the scores were far from those expected (0.693 was the highest score). We can see that BRUSELAS succeeded in all of the cases, and it gave acarbose higher scores than HybridSim-VS due to use of a consensus scoring function. The second group contains USR-VS, Superimposé and wwLig-CSRre, which either did not return any result or did not even finish the calculations with some of the configurations. The behavior of Superimposé may be explained by the fact that it only screens its own databases, which probably do not contain acarbose. USR-VS performed best in terms of computation time, but even though it does not screen the FDA approved drugs data set, it was expected to find acarbose in ZINC database because the query molecule was extracted from that database. Finally, wwLig-CSRre did not find the expected molecule when screening the same DrugBank subset as HybridSim-VS, suggesting a difference in the similarity algorithm used by both servers.

In terms of pharmacophore screening, all the servers providing this feature use the SHAFTS algorithm to assess similarity. It is observed that none of the servers (iDrug, ChemMapper and BRUSELAS) found acarbose in the list of hits, which suggests that the algorithm is unable to find the match.

4.3. Blood Anticoagulants. The medical challenge faced by an aging society with its need for anticoagulant drugs of increasing complexity encourages the search for new anticoagulant molecules. Heparin is widely used as an activator of antithrombin, although it has side effects. In a previous virtual screening campaign we discovered^{55,56} that the novel compound D-myo-inositol 3,4,5,6-tetrakisphosphate (TMI) is able to act as a heparin cofactor, binding with nanomolar affinity to antithrombin and causing its partial activation. Given its novel scaffold, it paves the way for the discovery of novel anticoagulants based on its molecular structure. Using

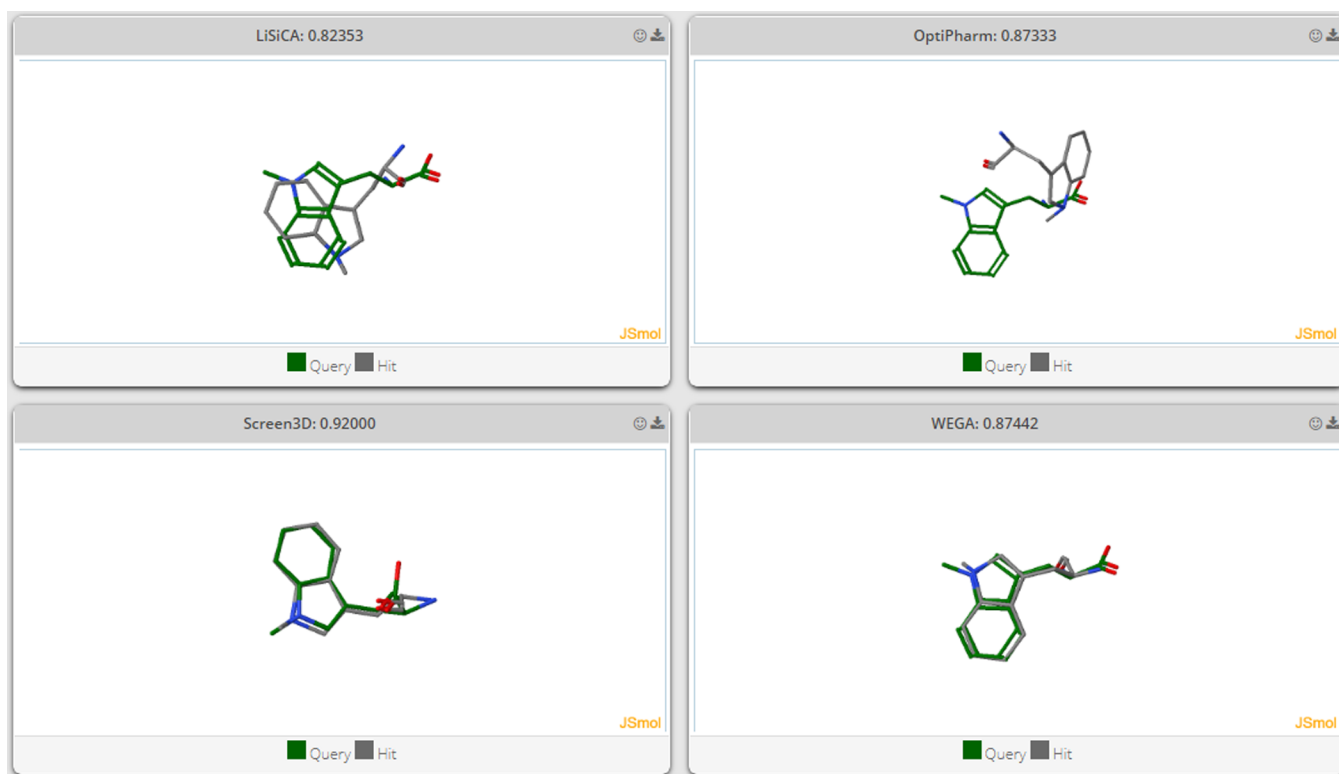


Figure 9. Alignment of Indoximod and (S)-2-amino-3-(1-methyl-1H-indol-3-yl)propanal obtained from the different algorithms.

BRUSELAS, we tested TMI using it as a query structure and performing both shape and pharmacophore searches against the ChEMBL database. Table 3 shows the most relevant hits obtained among the top scoring compounds. The full list of results is provided in Supporting Information.

Among the hits obtained by both methods were many different isomers of TMI, as was to be expected. For example, Figure 8 shows the alignment of TMI against one of the TMI isomers mentioned. It might be practical, then, to test these compounds experimentally as potential anticoagulants. Also of note was the fact that the method found ADP, which has previously been reported to act as a blood anticoagulant.⁵⁹ Other ADP related compounds, such as dGTP which has not previously been reported, might also be worth testing.

4.4. IDO1. The above examples looked at some representative uses of computational tools with a focus on drug discovery. Such approaches may help to rationalize the synthesis of new drugs for cancer therapy.⁶⁰ Indeed, there are several successful cases that target different steps in the cascade of reactions involved in tumor propagation, e.g., the tumor suppressor protein p53, proteins of the signal transducer and activator of transcription or molecular switches that control signaling pathways, to cite a few.^{61,62}

In spite of such efforts, patients with metastatic cancer represent one of the most urgent and challenging goals for medicine, where available therapies fail because malignant cells become drug-resistant during treatment. One of the most relevant achievements in this line has been the discovery of the cytosolic enzyme indoleamine 2,3-dioxygenase-1 (IDO1).⁶³ According to recent preclinical results, the inhibition of the IDO1 enzyme enhances the efficacy of classical chemotherapy, radiotherapy, and immune checkpoint therapy while bypassing their side effects.⁶⁴ This encouraging evidence, in turn, has led to the search for novel inhibitors by using both experimental

and theoretical methods.^{65,66} For the latter, Zheng, Yan and co-workers have recently used SBVS methods to propose novel IDO1 inhibitors.⁶⁵ However, to the best of our knowledge, there has been no systematic search using LBVS, so that this biological problem can be regarded as an optimal working example to test our software architecture. We selected three queries from the IDO1 inhibitors reported by the pharmaceutical industry, namely, indoximod, navoximod, and epacadostat.⁶⁷

It is of note that (S)-2-amino-3-(1-methyl-1H-indol-3-yl)propanal, whose alignments with indoximod are displayed in Figure 9, is located at the top of the list, as this molecule is known to be a potent inhibitory activity on IDO1, as demonstrated by Frédéric and co-workers, who combined docking predictions and *in vivo* experiments.⁶⁸ Our calculations, which were performed without imposing any bias or restriction, were led to similar results with a significantly lighter computational effort. Less expected were the rest of the compounds arising from both shape similarity and pharmacophore screening. For instance, (S)-methyl 2-amino-3-(1H-indol-3-yl)propanoate hydrochloride has been shown to act on Gap1, but to the best of our knowledge, it has not previously been tested in that context. We therefore conclude that the presented server correctly identified a known IDO1 inhibitor, which benchmarks the implemented protocol. Simultaneously, it is our hope that the new compound included in Table 4 may be used to expand the chemical space of novel and more efficient inhibitors beyond the more classical indoximod, navoximod, and epacadostat (see further details and results in the Supporting Information).

5. CONCLUSIONS AND FUTURE WORK

This contribution presents a novel software architecture, BRUSELAS, which performs 3D LBVS based on shape

Table 4. Best-Ranked IDO1 Inhibitors Proposed by BRUSELAS Using Indoximod, Navoximod, and Epacadostat as Queries on Shape and Pharmacophore Searches

similarity method	compound	score
shape similarity	(S)-2-amino-3-(1-methyl-1H-indol-3-yl)propanal	0.873
	(S)-methyl 2-amino-3-(1H-indol-3-yl)propanoate hydrochloride	0.819
	3-amino-2-(1H-indol-3-yl)-propionic acid methyl ester hydrochloride	0.809
	CHEMBL162163	0.809
	3-(2-(1H-tetrazol-5-yl)ethyl)-1H-indole	0.772
pharmacophore screening	2-amino-3-(1H-indol-3-yl)propionic acid methyl ester	0.308
	(S)-methyl 2-amino-3-(1H-indol-3-yl)propanoate hydrochloride	0.307
	SID103076391	0.302
	1-benzyl-2-propyl-1H-imidazo[4,5-c]quinolin-4-amine	0.266
	5,N ⁶ *-Dimethyl-N ⁶ *(2-methylpyridin-4-ylmethyl)benzo[cd]indole-2,6-diamine	0.263

similarity or pharmacophore searching. It also maintains a large collection of compounds to suggest a fitting library of ligands for the given query molecule. Aiming to satisfy researchers' needs, BRUSELAS intends to be a powerful platform for the application of LBVS techniques to drug discovery. To achieve this aim, the architecture exhibits a robust and flexible design complemented with a web tool to facilitate setting tasks and analyzing the results. BRUSELAS possesses distinguishing features that make it innovative in the field of LBVS. Those features are represented by the combined use of many similarity algorithms in the same task, the application of consensus scoring functions and the selection of keywords to focus searches on the desired disease or target. Additionally, tests proved that BRUSELAS outperforms many other similar servers in terms of reliability. It was one of the two servers succeeding in all the shape similarity searches, and it reached a good balance between speed and accuracy in comparison with others. Moreover, its applicability to different contexts has been demonstrated by searching blood anticoagulants and IDO1 enzyme inhibitors.

The comparison with similar servers suggests that BRUSELAS is of potential application in many other virtual screening studies. Nevertheless, some remaining challenges might be worth further study in the future. A more precise algorithm for choosing the most similar compounds based on the selected descriptors and the automatic assignment of weights to similarity algorithms are examples of such challenges. Moreover, a revision of the existing and newly published algorithms and an update of the libraries, which is a task that takes several months, have to be carried out regularly. Despite this, BRUSELAS has been demonstrated to be effective for the identification of new active molecules with innovative chemical scaffolds and to be reliable enough for experimental validation.

■ ASSOCIATED CONTENT

⑤ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jcim.9b00279.

Table S1, comparison of a collection of similarity web servers performing similarity searches with acarbose as the query molecule, which is an extension of Table 2,

containing all the experiments performed with BRUSELAS, considering the different similarity algorithms; Table S2, full list of candidates returned by the server when providing TMI as the query molecule, which is an extended version of Table 3; Table S3, complete list of candidates returned by BRUSELAS while looking for IDO1 inhibitors, contains similarities with indoximod, navoximod, and epacadostat and extends Table 4 (PDF)

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Notes

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■ ABBREVIATIONS

ADP, adenosine diphosphate; BRUSELAS, balanced rapid and unrestricted server for extensive ligand-aided screening; FDA, Food and Drug Administration; HPC, high-performance computing; IDO1, indoleamine 2,3-dioxygenase-1; IUPAC, International Union of Pure and Applied Chemistry; LBVS, ligand-based virtual screening; MMFF94, Merck molecular force field; QSAR, quantitative structure–activity relationship; RMSD, root-mean-squared distance; SBVS, structure-based virtual screening; SMILES, simplified molecular input line entry specification; T2DM, Type 2 Diabetes Mellitus; TMI, D-myo-inositol 3,4,5,6-tetrakisphosphate; VS, virtual screening.

■ REFERENCES

- (1) Jorgensen, W. L. The Many Roles of Computation in Drug Discovery. *Science* **2004**, 303 (5665), 1813–1818.
- (2) Dou, X.; Jiang, L.; Wang, Y.; Jin, H.; Liu, Z.; Zhang, L. Discovery of new GSK-3 β inhibitors through structure-based virtual screening. *Bioorg. Med. Chem. Lett.* **2018**, 28 (2), 160–166.
- (3) Nunes, R. R.; dos Santos Costa, M.; dos Reis Santos, B.; da Fonseca, A. L.; Ferreira, L. S.; Russo Chagas, R. C.; da Silva, A. M.; de Pilla Varotti, F.; Taranto, A. G. Successful application of virtual screening and molecular dynamics simulations against antimalarial molecular targets. *Mem. Inst. Oswaldo Cruz* **2016**, 111 (12), 721–730.
- (4) Tian, S. T.; Wang, X.; Li, L.; Zhang, X.; Li, Y.; Zhu, F.; Hou, T.; Zhen, X. Discovery of novel and selective adenosine A2A receptor antagonists for treating Parkinson's disease through comparative structure-based virtual screening. *J. Chem. Inf. Model.* **2017**, 57 (6), 1474–1487.
- (5) Tikhonova, I. G.; Sum, C. S.; Neumann, S.; Engel, S.; Raaka, B. M.; Costanzi, S.; Gershengorn, M. C. Discovery of novel agonists and antagonists of the free fatty acid receptor one (FFAR1) using virtual screening. *J. Med. Chem.* **2008**, 51 (3), 625–633.
- (6) Schuster, D.; Maurer, E. M.; Laggner, C.; Nashev, L. G.; Wilckens, T.; Langer, T.; Odermatt, A. The discovery of new 11 β -hydroxysteroid dehydrogenase type 1 inhibitors by common feature pharmacophore modeling and virtual screening. *J. Med. Chem.* **2006**, 49 (12), 3454–3466.
- (7) Salam, N. K.; Huang, T. H.; Kota, B. P.; Kim, M. S.; Li, Y.; Hibbs, D. E. Novel PPAR-gamma agonists identified from a natural product library: a virtual screening, induced-fit docking and biological assay study. *Chem. Biol. Drug Des.* **2008**, 71 (1), 57–70.
- (8) Mahmoudi, N.; de Julián-Ortiz, J. V.; Ciceron, L.; Gálvez, J.; Mazier, D.; Danis, M.; Derouin, F.; García-Domenech, R. Identification of new antimalarial drugs by linear discriminant analysis and topological virtual screening. *J. Antimicrob. Chemother.* **2006**, 57 (3), 489–497.
- (9) Desai, P. V.; Patny, A.; Sabnis, Y.; Tekwani, B.; Gut, J.; Rosenthal, P.; Srivastava, A.; Avery, M. Identification of novel parasitic cysteine protease inhibitors using virtual screening. 1. The ChemBridge database. *J. Med. Chem.* **2004**, 47 (26), 6609–6615.
- (10) Triballeau, N.; Acher, F.; Brabet, I.; Pin, J. P.; Bertrand, H. O. Virtual Screening Workflow Development Guided by the "Receiver Operating Characteristic" Curve Approach. Application to High-Throughput Docking on Metabotropic Glutamate Receptor Subtype 4. *J. Med. Chem.* **2005**, 48 (7), 2534–2547.
- (11) Evers, A.; Klabunde, T. Structure-based Drug Discovery Using GPCR Homology Modeling: Successful Virtual Screening for Antagonists of the Alpha1A Adrenergic Receptor. *J. Med. Chem.* **2005**, 48 (4), 1088–1097.
- (12) Rollinger, J. M.; Hornick, A.; Langer, T.; Stuppner, H.; Prast, H. Acetylcholinesterase Inhibitory Activity of Scopolin and Scopoletin Discovered by Virtual Screening of Natural Products. *J. Med. Chem.* **2004**, 47 (25), 6248–6254.
- (13) Vogt, M.; Bajorath, J. Predicting the performance of fingerprint similarity searching. *Methods Mol. Biol.* **2010**, 672, 159–173.
- (14) Kumar, A.; Zhang, K. Y. J. Hierarchical virtual screening approaches in small molecule drug discovery. *Methods* **2015**, 71, 26–37.
- (15) Kaserer, T.; Beck, K. R.; Akram, M.; Odermatt, A.; Schuster, D. Pharmacophore models and pharmacophore-based virtual screening: concepts and applications exemplified on hydroxysteroid dehydrogenases. *Molecules* **2015**, 20, 22799–22832.
- (16) Villoutreix, B. O.; Lagorce, D.; Labbé, C. M.; Sperandio, O.; Miteva, M. A. One hundred thousand mouse clicks down the road: Selected online resources supporting drug discovery collected over a decade. *Drug Discovery Today* **2013**, 18 (21–22), 1081–1089.
- (17) Scior, T.; Bender, A.; Tresadern, G.; Medina-Franco, J. L.; Martínez-Mayorga, K.; Langer, T.; Cuanalo-Contreras, K.; Agrafiotis, D. K. Recognizing pitfalls in virtual screening: a critical review. *J. Chem. Inf. Model.* **2012**, 52 (4), 867–881.
- (18) Todeschini, R.; Consonni, V. *Molecular Descriptors for Chemoinformatics*, 2nd ed.; Wiley-VCH: Weinheim, Germany, 2009.
- (19) Wishart, D. S.; Knox, C.; Guo, A. C.; Shrivastava, S.; Hassanali, M.; Stothard, P.; Chang, Z.; Woolsey, J. DrugBank: A Comprehensive Resource for in Silico Drug Discovery and Exploration. *Nucleic Acids Res.* **2006**, 34 (90001), D668–D672.
- (20) Gaulton, A.; Bellis, L. J.; Bento, A. P.; Chambers, J.; Davies, M.; Hersey, A.; Light, Y.; McGlinchey, S.; Michalovich, D.; Al-Lazikani, B.; Overington, J. P. ChEMBL: A Large-Scale Bioactivity Database for Drug Discovery. *Nucleic Acids Res.* **2012**, 40 (D1), D1100–D1107.
- (21) Kanehisa, M.; Sato, Y.; Kawashima, M.; Furumichi, M.; Tanabe, M. KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res.* **2016**, 44 (D1), D457–D462.
- (22) DIA-DB; Bioinformatics and High Performance Computing Research Group: 2015.
- (23) ChemAxon; ChemAxon Ltd., C..
- (24) Omega, OpenEye; OpenEye: 2016.
- (25) Yan, X.; Li, J.; Liu, Z.; Zheng, M.; Ge, H.; Xu, J. Enhancing Molecular Shape Comparison by Weighted Gaussian Functions. *J. Chem. Inf. Model.* **2013**, 53 (8), 1967–1978.
- (26) Lesnik, S.; Stular, T.; Brus, B.; Knez, D.; Gobec, S.; Janežic, D.; Konc, J. LiSiCA: A Software for Ligand-Based Virtual Screening and Its Application for the Discovery of Butyrylcholinesterase Inhibitors. *J. Chem. Inf. Model.* **2015**, 55 (8), 1521–1528.
- (27) Kalasz, A.; Sziš, D.; Imre, G.; Polgar, T. Screen3D: A Novel Fully Flexible High-Throughput Shape-Similarity Search Method. *J. Chem. Inf. Model.* **2014**, 54 (4), 1036–1049.
- (28) Puertas-Martín, S.; Redondo, J. L.; Pérez-Sánchez, H.; Ortigosa, P. M. OptiPharm: An evolutionary algorithm to compare shape similarity. *Sci. Rep.* **2019**, 9, 1398.
- (29) Liu, X.; Jiang, H.; Li, H. SHAFTS: a hybrid approach for 3D molecular similarity calculation. 1. Method and assessment of virtual screening. *J. Chem. Inf. Model.* **2011**, 51 (9), 2372–2385.
- (30) O'Boyle, N. M.; Banck, M.; James, C. A.; Morley, C.; Vandermeersch, T.; Hutchison, G. R. Open Babel: An open chemical toolbox. *J. Cheminf.* **2011**, 3 (1), 33.
- (31) Hanson, R. M. Jmol - a paradigm shift in crystallographic visualization. *J. Appl. Crystallogr.* **2010**, 43 (5), 1250–1260.
- (32) Halgren, T. A. Merck Molecular Force Field. I. Basis, Form, Scope, Parameterization, and Performance of MMFF94. *J. Comput. Chem.* **1996**, 17 (5–6), 490–519.
- (33) Lu, W.; Liu, X.; Cao, X.; Xue, M.; Liu, K.; Zhao, Z.; Shen, X.; Jiang, H.; Xu, Y.; Huang, J.; Li, H. SHAFTS: A hybrid approach for 3D molecular similarity calculation. 2. Prospective case study in the

discovery of diverse p90 ribosomal S6 protein kinase 2 inhibitors to suppress cell migration. *J. Med. Chem.* **2011**, *54* (10), 3564–3574.

(34) Kong, X.; Qin, J.; Li, Z.; Vultur, A.; Tong, L.; Feng, E.; Rajan, G.; Liu, S.; Lu, J.; Liang, Z.; Zheng, M.; Zhu, W.; Jiang, H.; Herlyn, M.; Liu, H.; Marmorstein, R.; Luo, C. Development of a novel class of B-Raf(V600E)-selective inhibitors through virtual screening and hierarchical hit optimization. *Org. Biomol. Chem.* **2012**, *10* (36), 7402–7417.

(35) Leelananda, S. P.; Lindert, S. Computational methods in drug discovery. *Beilstein J. Org. Chem.* **2016**, *12*, 2694–2718.

(36) Jaghoori, M. M.; Bleijlevens, B.; Olabarriaga, S. D. 1001 Ways to Run AutoDock Vina for Virtual Screening. *J. Comput.-Aided Mol. Des.* **2016**, *30* (3), 237–249.

(37) Please, ensure you add biohpc2015@gmail.com to your whitelist. If you do not receive a notification mail within 48 h after the creation of the job, check your spam folder.

(38) Esu, E.; Effa, E. E.; Opie, O. N.; Uwaoma, A.; Meremikwu, M. M. Artemether for Severe Malaria (Review). *Cochrane Libr* **2014**, No. 9, 1–85.

(39) Teklemariam, M.; Assefa, A.; Kassa, M.; Mohammed, H.; Mamo, H. Therapeutic Efficacy of Artemether-Lumefantrine against Uncomplicated Plasmodium Falciparum Malaria in a High-Transmission Area in Northwest Ethiopia. *PLoS One* **2017**, *12* (4), e0176004.

(40) Phyto, A. P.; von Seidlein, L. Challenges to Replace ACT as First-line Drug. *Malar. J.* **2017**, *16* (1), 296.

(41) Plucinski, M. M.; Ferreira, M.; Ferreira, C. M.; Burns, J.; Gaporayi, P.; João, L.; da Costa, O.; Gill, P.; Samutondo, C.; Quinija, J.; Mbounga, E.; de León, G. P.; Halsey, E. S.; Dimbu, P. R.; Fortes, F. Evaluating Malaria Case Management at Public Health Facilities in Two Provinces in Angola. *Malar. J.* **2017**, *16* (1), 186.

(42) Li, H.; Leung, K.-S.; Wong, M.-H.; Ballester, P. J. USR-VS: A Web Server for Large-Scale Prospective Virtual Screening Using Ultrafast Shape Recognition Techniques. *Nucleic Acids Res.* **2016**, *44* (W1), W436–441.

(43) Zoete, V.; Daina, A.; Bovigny, C.; Michielin, O. SwissSimilarity: A Web Tool for Low to Ultra High Throughput Ligand-Based Virtual Screening. *J. Chem. Inf. Model.* **2016**, *56* (8), 1399–1404.

(44) Bauer, R. A.; Bourne, P. E.; Formella, A.; Frömmel, C.; Gille, C.; Goede, A.; Guerler, A.; Hoppe, A.; Knapp, E. W.; Pöschel, T.; Wittig, B.; Ziegler, V.; Preissner, R. Superimposé: A 3D Structural Superposition Server. *Nucleic Acids Res.* **2008**, *36* (WebServer), W47–W54.

(45) Shang, J.; Dai, X.; Li, Y.; Pistolozzi, M.; Wang, L. HybridSim-VS: a web server for large-scale ligand-based virtual screening using hybrid similarity recognition techniques. *Bioinformatics* **2017**, *33* (21), 3480–3481.

(46) Gong, J.; Cai, C.; Liu, X.; Ku, X.; Jiang, H.; Gao, D.; Li, H. ChemMapper: a versatile web server for exploring pharmacology and chemical structure association based on molecular 3D similarity method. *Bioinformatics* **2013**, *29* (14), 1827–1829.

(47) Sperandio, O.; Petitjean, M.; Tuffery, P. wwLigCSRre: A 3D ligand-based server for hit identification and optimization. *Nucleic Acids Res.* **2009**, *37* (WebServer), W504–W509.

(48) Wang, X.; Chen, H.; Yang, F.; Gong, J.; Li, S.; Pei, J.; Liu, X.; Jiang, H.; Lai, L.; Li, H. J. iDrug: a web-accessible and interactive drug discovery and design platform. *J. Cheminf.* **2014**, *6* (1), 28.

(49) Ge, Q.; Chen, L.; Chen, K. Treatment of Diabetes Mellitus Using iPS Cells and Spice Polyphenols. *J. Diabetes Res.* **2017**, *2017*, 1–11.

(50) Liu, Z.; Zhao, X.; Sun, W.; Wang, Y.; Liu, S.; Kang, L. E. I. Metformin Combined with Acarbose vs. Single Medicine in the Treatment of Type 2 Diabetes: A Meta-Analysis. *Exp. Ther. Med.* **2017**, *13*, 3137–3145.

(51) Wettergreen, S. A.; Sheth, S.; Malveaux, J. Effects of the Addition of Acarbose to Insulin and Non-Insulin Regimens in Veterans with Type 2 Diabetes Mellitus. *Pharm. Pract. (Granada)*. **2016**, *14* (4), 832.

(52) Cadegiani, F. A.; Silva, O. S. Acarbose Promotes Remission of Both Early and Late Dumping Syndromes in Post-Bariatric Patients. *Diabetes, Metab. Syndr. Obes.: Targets Ther.* **2016**, *9*, 443–446.

(53) Li, J.; Niu, B.; Wang, X.; Hu, H.; Cao, B. A Case Report of Hereditary Neuropathy with Liability to Pressure Palsies Accompanied by Type 2 Diabetes Mellitus and Psoriasis. *Medicine* **2017**, *96* (19), e6922.

(54) Irwin, J. J.; Sterling, T.; Mysinger, M. M.; Bolstad, E. S.; Coleman, R. G. ZINC: A Free Tool to Discover Chemistry for Biology. *J. Chem. Inf. Model.* **2012**, *52* (7), 1757–1768.

(55) Adelt, S.; Plettenburg, O.; Stricker, R.; Reiser, G.; Altenbach, H. J.; Vogel, G. Enzyme-Assisted Total Synthesis of the Optical Antipodes d-myo-Inositol 3,4,5-Trisphosphate and d-myo-Inositol 1,5,6-Trisphosphate: Aspects of Their Structure–Activity Relationship to Biologically Active Inositol Phosphates. *J. Med. Chem.* **1999**, *42* (7), 1262–1273.

(56) Navarro-Fernández, J.; Pérez-Sánchez, H.; Martínez-Martínez, I.; Meliciani, I.; Guerrero, J. A.; Vicente, V.; Corral, J.; Wenzel, W. In Silico Discovery of a Compound with Nanomolar Affinity to Antithrombin Causing Partial Activation and Increased Heparin Affinity. *J. Med. Chem.* **2012**, *55* (14), 6403–6412.

(57) Hocek, M.; Silhár, P.; Shih, I. H.; Mabery, E.; Mackman, R. Cytostatic and antiviral 6-arylpurine ribonucleosides. Part 7: Synthesis and evaluation of 6-substituted purine l-ribonucleosides. *Bioorg. Med. Chem. Lett.* **2006**, *16* (20), 5290–5293.

(58) Quick, A. J. Anticoagulant Action of Adenosine Triphosphate. *Nature* **1963**, *200*, 469–470.

(59) Wassman, C. D.; Baronio, R.; Demir, Ö.; Wallentine, B. D.; Chen, C. K.; Hall, L. V.; Salehi, F.; Lin, D. W.; Chung, B. P.; Hatfield, G. W.; Richard Chamberlin, A.; Luecke, H.; Lathrop, R. H.; Kaiser, P.; Amaro, R. E. Computational identification of a transiently open L1/S3 pocket for reactivation of mutant p53. *Nat. Commun.* **2013**, *4* (1), 1407.

(60) Siddiquee, K.; Zhang, S.; Guida, W. C.; Blaskovich, M. A.; Greedy, B.; Lawrence, H. R.; Yip, M. L.; Jove, R.; McLaughlin, M. M.; Lawrence, N. J.; Sebt, S. M.; Turkson, J. Selective chemical probe inhibitor of Stat3, identified through structure-based virtual screening, induces antitumor activity. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104* (18), 7391–7396.

(61) Gao, Y.; Dickerson, J. B.; Guo, F.; Zheng, J.; Zheng, Y. Rational design and characterization of a Rac GTPase-specific small molecule inhibitor. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (20), 7618–7623.

(62) Muller, A. J.; DuHadaway, J. B.; Donover, P. S.; Sutanto-Ward, E.; Prendergast, G. C. Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. *Nat. Med.* **2005**, *11*, 312–319.

(63) Hou, D. Y.; Muller, A. J.; Sharma, M. D.; DuHadaway, J.; Banerjee, T.; Johnson, M.; Mellor, A. L.; Prendergast, G. C.; Munn, D. H. Inhibition of indoleamine 2,3-dioxygenase in dendritic cells by stereoisomers of 1-methyl-tryptophan correlates with antitumor responses. *Cancer Res.* **2007**, *67* (2), 792–801.

(64) Godin-Ethier, J.; Hanafi, L. A.; Piccirillo, C. A.; Lapointe, R. Indoleamine 2,3-dioxygenase expression in human cancers: clinical and immunologic perspectives. *Clin. Cancer Res.* **2011**, *17* (22), 6985–6991.

(65) Zhang, G.; Xing, J.; Wang, Y.; Wang, L.; Ye, Y.; Lu, D.; Zhao, J.; Luo, X.; Zheng, M.; Yan, S. Discovery of Novel Inhibitors of Indoleamine 2,3-Dioxygenase 1 Through Structure-Based Virtual Screening. *Front. Pharmacol.* **2018**, *9*, 00277.

(66) Prendergast, G. C.; Malachowski, W. P.; DuHadaway, J. B.; Muller, A. J. Discovery of IDO1 Inhibitors: From Bench to Bedside. *Cancer Res.* **2017**, *77* (24), 6795–6811.

(67) Navarro-Fernández, J.; Pérez-Sánchez, H.; Martínez-Martínez, I.; Meliciani, I.; Guerrero, J. A.; Vicente, V.; Corral, J.; Wenzel, W. In Silico Discovery of a Compound with Nanomolar Affinity to Antithrombin Causing Partial Activation and Increased Heparin Affinity. *J. Med. Chem.* **2012**, *55* (14), 6403–6412.

(68) Dolušić, E.; Larrieu, P.; Moineaux, L.; Stroobant, V.; Pilotte, L.; Colau, D.; Pochet, L.; Van den Eynde, B.; Masereel, B.; Wouters, J.

Frédérick, R. Tryptophan 2,3-Dioxygenase (TDO) Inhibitors. 3-(2-(Pyridyl)ethenyl)indoles as Potential Anticancer Immunomodulators. *J. Med. Chem.* **2011**, *54* (15), 5320–5334.