Inverse Virtual Screening Studies of Selected Natural Compounds from Cerrado

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Several medicinal plants have been studied in recent years in Brazil. However, despite many efforts, the pharmacological mechanisms of many natural products are still unknown. Several biological assays *in vivo* and *in vitro* are needed to further address this issue, which increases the cost of these studies. The main goal of this study was to apply the methodology of inverse virtual screening (IVS), followed by docking studies, and refinement by molecular dynamics (MD) simulation and quantum mechanical/molecular mechanical to determine the pharmacological receptors for five selected natural products with the exception of the benzoxazinone isolated from the exudate of the radicle of the crop species which were mainly isolated from the Cerrado species, obtained from Cerrado, a typical Brazilian biome. Initially, the structures of the natural compounds were generated using the online software program sc-PDB, which searches for molecular targets deposited in the protein data bank. The ligands were docked against target proteins found in IVS step-forming complexes, which were refined again using MD simulations by ff03 force field for 1 ns. Finally, the binding energy for each complex was obtained by the ONIOM (PM6:UFF) method. As a result, these calculations suggested possible molecular targets for these natural compounds. Among the targets found were 1EH4, 2A4Z, 1H49, 1JT2, 2BNJ, and 3FW9, which are involved in cancer and rheumatoid arthritis pathologies, indicating that they are promising molecular targets. In this study, we proposed a biological assay for these natural compounds. The results indicate that structural changes may be proposed to generate compounds that are able to bind more strongly to the receptor and become new drug candidates, thus optimizing the search for lead natural compounds. © 2012 Wiley Periodicals, Inc.

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Introduction

The intake of herbs and leaves in the form of tea or *in natura* to treat diseases was one of the earliest uses of natural products. The history of the development of civilizations in the East and West, particularly the Egyptian, Greco-Roman, and Chinese civilizations, is rich with examples of natural resources being used in medicine, pest control, and defense mechanisms. Traditional Chinese medicine has developed efficiently, and even today, many plant species and medicinal preparations are studied to understand the mechanisms involved in the behavior and isolation of active compounds. Determining the potential supply of natural chemical by primitive ethnic and indigenous descriptions can be considered one of the most fundamental factors involved in the discovery of toxic substances and drugs over time. Living and learning with many different ethnic groups has provided valuable contributions to research on natural products and knowledge of the relationship between the chemical structure of a given compound and its biological activities.[1]

Many plant extracts and essential oils may be sources for the development of new drugs because they constitute an extensive source of bioactive substances, many of which are largely free of side effects. Every plant produces primary and secondary metabolites, which are essential for their survival and protection. Essentially, some secondary metabolites are produced as a result of adverse climate and soil conditions, and these secondary metabolites defend plants against herbivores, parasites, and pathogens.[2] Cerrado is the second largest Brazilian biome; it covers 2 million km^2, representing 23% of the area of the country, which characterized as an ecoregion of Brazil, particularly in Goiás and Minas Gerais States.[2] It has nutrient-poor soil rich in aluminum and experiences a prolonged drought throughout the year. These conditions stimulate the production of secondary metabolites such as alkaloids, terpenes, and phenolic compounds, which increase the chemical diversity of the flora of the biome.[2] In addition, this ancient biome is rich in biodiversity, hosting 160,000 species of plants, fungi, and animals. There are about 800 species of trees and large shrubs in this vegetation. If the flora of gallery forests, mesophytic forests and other habitats are included, the total number of vascular plant species is estimated to reach about 10,000.[3]

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Despite the investment of the pharmaceutical industry and the advent of modern techniques such as combinatorial chemistry combined with high throughput screening (HTS), a wide variety of compounds is produced from the reactions of random techniques; thus, there is an expected growth in the development of innovative molecules. However, few of them have reached the market. Conversely, natural products have attracted great attention because of their variety and the history of drug design using this source. Many natural molecules possess biological activity; these natural molecules are considered lead compounds. These natural molecules provide the initial structure for structural modifications to be performed to optimize pharmacological properties. Thus, these modified compounds can give rise to new drugs.[4,5]

It is estimated that there are 250,000 species of plants, 85% of which have not yet been investigated. In addition, there is a large collection of natural products of marine origin and microorganisms that have not been studied. Therefore, there are a large number of novel substances with strong biological potential to be studied.[5] The process of discovering new compounds begins with ethnobotanical and phytochemical studies following the complete isolation and characterization of chemical constituents. These compounds are screened through several pharmacological models to identify a possible therapeutic class. In Brazil, this process is conducted manually and usually in animal models or isolated organisms. These tests take time to produce results and are expensive, limiting the process of discovering new drugs.[6] Several genome projects have been performed in recent years. As a result, the structures of proteins complexed with lead compounds have been deposited in databases such as protein data bank (PDB). The PDB is the largest public database of three-dimensional structures of proteins obtained experimentally, mostly by X-ray crystallography or nuclear magnetic resonance. This database allows for the study of interactions between ligands and molecular targets.[7,8]

In parallel, cheminformatics approaches have evolved and are now indispensable tools in the process of discovering bioactive compounds. Cheminformatics combines multiple information sources and transforms them into knowledge for a particular purpose: quick decision making in the area of rational drug design. Thus, because new chemical entities need to be tested in a biological assay, cheminformatics methods can help to predict the pharmacological properties of these compounds.[9] Cheminformatics methods can be used to obtain information from the structural elucidation of ligands complexed with proteins previously deposited in the PDB and apply this knowledge to select molecular targets by structural similarity. This methodology is called inverse virtual screening (IVS).[10,11]

IVS is an important tool for the rapid identification of new molecular targets. The methodology starts with the structure of any compound and then performs a comparative analysis of this structure against ligands that are complexed with receptors previously described in the PDB database. Then, the interaction energy between the structure and receptors is predicted by a docking approach. Molecular docking is a key tool that has been used to predict the best-fitting position of the ligand into the receptor and to determine the binding energy.[12]

Molecular docking can be used to quantify the binding energy between ligands and their receptors. As a result, a complex with the atomic coordinates of the receptor and ligand can be viewed and analyzed by the researcher. Docking results can show how ligands are bound to a molecular target by intermolecular interactions. This information is useful in comparing the relative binding energy of a set of ligands with that of the same molecular target. Therefore, it is possible to predict the highest activity compound of this set.[11,13]

Although the docking methodology is very important for drug discovery, it has several limitations, such as a simplified scoring function and the simplification of conformational changes of macromolecules. However, this approximation allows a large number of compounds to be evaluated in a short time.[12] This strategy reduces the accuracy of virtual screening. Thus, the refinement of docking results by energy minimization is suggested to correct distortions, such as the unfavorable conformation of the lengths and angles of bonds and steric overlaps of atoms. Energy minimization is applied together with conformational analysis to optimize the geometry of the ligand–protein complexes that are formed. However, the methods of molecular dynamics (MD) do not explicitly include electrons.[14] Therefore, the calculated interaction energies may be the weakest part of this approach. Conversely, quantum mechanical(QM) methods incorporate electronic effects into their equations, but determining the potential energy surface as efficiently as MD methods requires a high computational cost. This limitation can be overcome using combined QM and molecular mechanical (MM) methods, which are also referred to as hybrid methods (QM/MM). These methods allow for the combination of two or more computational techniques into a single calculation, making it possible to investigate the chemistry of very large systems with high accuracy. The regions where chemical processes occur, for example, the binding site, are treated with adequate accuracy, whereas the rest of the system is treated at a lower level.[14] Among the several QM/MM approaches that are available, ONIOM-type methods have been used to study large systems as molecular targets (proteins).[16] In this method, the system is divided into two or three layers that can be treated with different QM and MM methods. This approach combines the advantages of both QM and MM methods, resulting in high accuracy and reduced computational cost.[17]

The aim of this study was to perform IVS methods followed by molecular docking, MD simulations, and QM/MM to suggest pharmacological receptors for the natural products shown in Figure 1.

Methodology

The compounds shown in Figure 1A were submitted to a search by the IVS program called sc-PDB,[18,19] as described previously,[20] against the molecular targets deposited in the PDB. Then, with the IVS results, the molecular docking method was performed to fit the ligand into the active site of the

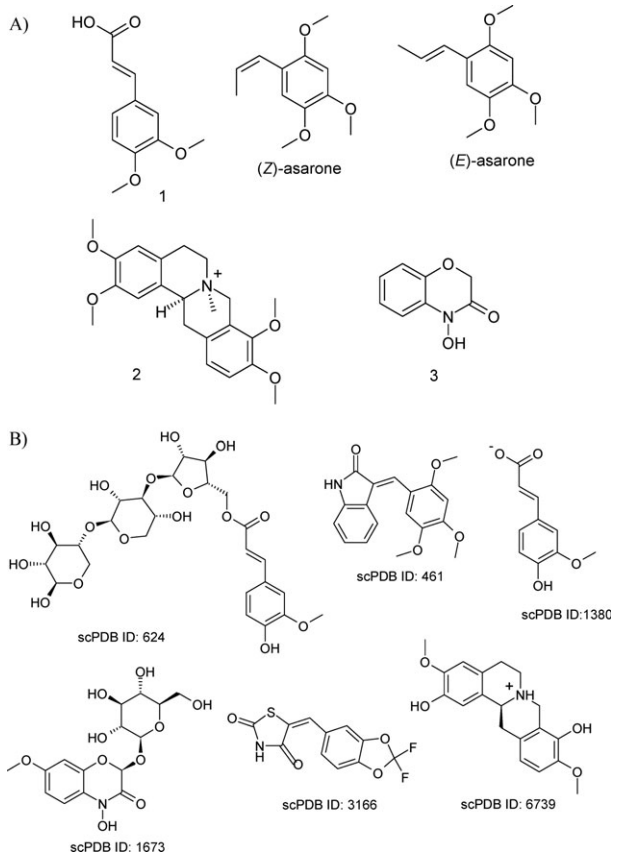

Figure 1. (A) Natural compounds from Cerrado; (B) resulted compounds obtained by IVS search with respective scPDB ID. scPDB ID is the annotation of ligands in sc-PDB database using mol2 file.[17,18]

target proteins. A redock step was performed to evaluate the method.[21] Natural compounds were generated using the software program GaussView 5.0[22] and refined by the semiempirical ***Parametric*** Method 6 (PM6),[23] implemented in the Gaussian 09W program.[24] Initially, the protein and ligands were prepared using Autodock tools, where only polar hydrogen was kept in the molecules. A grid box was generated for each molecular target found in the IVS step, covering the entire binding site. Then, all ligands were fitted rigidly to the receptors through Auto Dock Vina.[25]

The results of molecular docking were refined by energy minimization following MD simulations performed by Amber 11[26] using an ff03 force field. In the minimization step, the maximum number of cycles, 5000, were performed. The minimization switched from steepest descent to conjugate gradient after 1500 cycles. The generalized Born Model was used to simulate the implicit solvent.[27,28] The equilibrium framework was resubmitted to minimization calculations as described previously. The MD simulations were performed over a period of 100 ps for heating, following 1 ns for the production run. A value of 14 Å was used for nonbonded atoms.

Finally, the binding energies of all ligands were determined by single-point calculation using the ONIOM approach[17] implemented in the Gaussian 09W program.[24] Two hybrid layers were generated, where the amino acids of the active site and ligand were defined as higher layers and the rest of protein was characterized as the lower layer, through the PM6:UFF formalism.[29,30] Discovery Studio Visualizer 2.5[31] was used to analyze the results.

Results and Discussion

IVS was performed using the sc-PDB database. sc-PDB allows for structure-based drug design by identifying the binding sites suitable for the docking of drug-like compounds. Currently, sc-PDB comprises 8166 entries, including 1168 protein families and 1470 singletons.[17] This methodology was applied to the natural compounds mentioned previously (Fig. 1A). Six similar structures were obtained (Fig. 1B): these compounds share similar structures with the natural compounds. The results of the molecular targets are provided in Figure 2, which are described under the following PDB codes: 2A4Z, 1EH4, 1H49, 1JT2, 2BNJ, and 3FW9.[32–37]

IVS is able to find molecular targets and evaluate the respective binding energies. However, in this study, the Autodock Vina software program was used to predict the pharmacophoric conformation of the compounds. Autodock Vina improves the average accuracy of the binding mode predictions, and it is faster when compared with previous docking software. These characteristics make it amenable to the virtual screening approach. Then, the redock of crystallographic ligands was performed to validate the docking methodology. As shown in Figure 3, Autodock Vina can generally fit ligands with similar crystallographic structures. The only exception is scPDB ID 624, for which, the molecular target is 2BNJ (Fig. 3B). This result was expected because of the large number of rotating bonds.

Virtual screening and docking methodologies are widely used in academic and industrial research. However, docking results are limited by the rigidity of the molecular target. Therefore, it is not possible to provide a perfect and robust geometry of interaction between ligands and targets. Therefore, false-positive results can be determined, which will hardly yield experimentally validated results. Thus, bioinformatics tools have been proposed to solve this problem, which can be used to evaluate these methodologies.[37,38] However, all these strategies are still the subject debate.[37] In this study, MD simulations were used to identify false-positive results. MD simulations perform a conformational search of the complex obtained from docking on the potential energy surface. As a result, the rigidity of a molecular target during docking simulation is overcome using this approach. MD simulations were performed for all compounds studied using the Amber software program. After the MD simulations step, the binding energies of the molecules were obtained by the ONIOM methodology. It is important to stress that the main goal of this study was not to find a correlation between these binding energies and biological activity but to suggest molecular targets for which natural compounds can be used as lead compounds. Because the semiempirical PM6 method was incorporated into the higher layer of ONIOM, the accuracy of the results obtained using this method are considered to be better than those obtained using Autodock Vina.

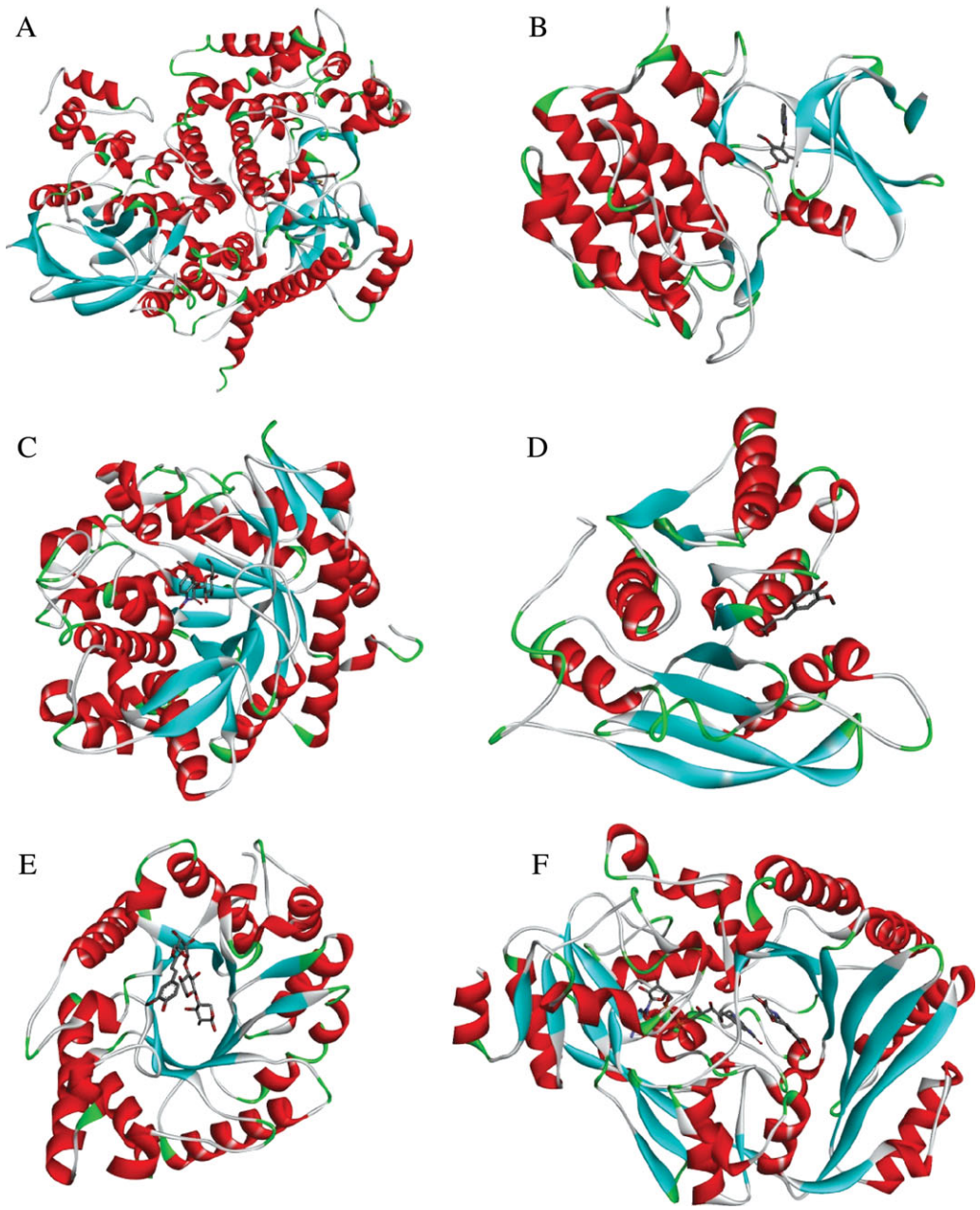

Figure 2. Molecular targets obtained from IVS search with respective PDB code. Alpha-helix, beta-sheets, and turns are in red, blue, and green, respectively. (A) 2A4Z; (B) 1EH4; (C) 1H49; (D) 1JT2; (E) 2BNJ; (F) 3FW9. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Among the natural compounds studied, the IVS results showed that 2A4Z is a molecular target for (*E*)-asarone (Table 1). The molecular target 2A4Z is an enzyme present in all living organisms. In higher plants, this enzyme has the function of defending against pests, activating plant hormones, performing lignification, and fostering cell wall catabolism. It has been observed that in legumes, such as corn and soybeans, this enzyme occurs as different isoenzymes and is involved in the development of pesticides or adjuvants. In addition, this target is a phosphoinositide 3-kinase (PI3K), which has been deemed a promising therapeutic target for the treatment of inflammatory and autoimmune diseases and cancer and cardiovascular disease. Recent studies have developed selective inhibitors, specific and orally active for this enzyme. These studies report that treatment with an oral PI3K affects the migration of neutrophils, protecting against rheumatoid arthritis. Furthermore, this treatment suppresses the progression of inflammation and joint damage in rat models of rheumatoid arthritis.[32,33] (*E*)-asarone forms hydrogen bonds with THR627 and van der Waals interactions with Met544, Trp552, Phe701, and Ile703, thus suitably fitting into the activity site (Fig. 4A). Thus, a favorable binding energy is evidenced by ONIOM (Table 1). This compound has carcinogenic and spasmolytic effects and induces structural chromosome aberrations in human lymphocytes.[39] Although biological assays have been well described, there are no experimental results regarding the respective receptor. The IVS results suggest that PI3K is the receptor for (*E*)-asarone. (*Z*)-asarone can bind to this receptor as well (Table 1 and Fig. 4B). However, this affinity is weaker than that observed for (*E*)-asarone.

In contrast with the (*E*)-asarone-2A4Z results, the IVS results revealed another molecular target for (*Z*)-asarone. Although *E* and *Z* asarones have *in vivo* carcinogenic effects, biological assays suggest that (*Z*)-asarone can bind to different molecular targets as a result of other biological effects, such as *in vitro* mutagenic activity and the induction of structural chromosome aberration in human lymphocytes.[39] The IVS results suggest that 1EH4 is the molecular target of (*Z*)-asarone (Fig. 4D). The 1EH4 comprises isoforms of casein kinase I. These proteins are involved in several biological functions, such as controlling heart rate, regulating DNA repair and cell morphology, and stabilizing cellular proteins. A specific isoform, Ckiδ, has been associated with Alzheimer's disease, suggesting that the presence of this protein is

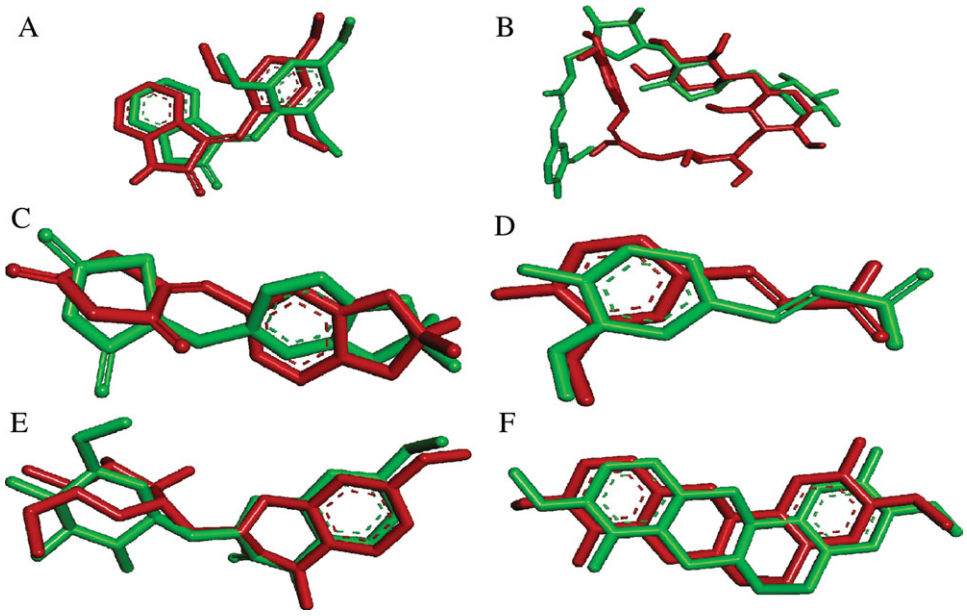

Figure 3. Redock of crystallographic ligands performed into respective molecular targets. The respective PDB code are in parenthesis. (A) scPDB ID 461 (1EH4); (B) scPDB ID 624 (2BNJ); (C) scPDB ID 3166 (2A4Z); (D) scPDB ID 1380 (1JT2); (E) scPDB ID 1673 (1H49); (F) scPDB ID 6739 (3FW9). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 1. IVS results and binding energy obtained by ONIOM (PM6:UFF) and AutoDock Vina.

Molecular target (PDB code)	ENERGY (kcal/mol)		
	LIGAND	ONIOM	DOCKING
2A4Z	scPDB ID: 461	−63.8	−5.2
	(*E*)-asarone	−488.0	−4.4
	(*Z*)-asarone	−16.8	−4.5
1EH4	scPDB ID: 3166	43.4	−7.0
	(*E*)-asarone	194.0	−6.1
	(*Z*)-asarone	−310.0	−6.1
1JT2	scPDB ID: 1380	−982.0	−3.8
	Compound **1**	ND	−4.0
2BNJ	scPDB ID: 624	−168.0	−5.5
	Compound **1**	ND	−6.3
3FW9	scPDB ID: 6739	−372.0	−11.5
	Compound **2**	−500.0	−10.7
1H49	scPDB ID: 1673	155.0	−7.2
	Compound **3**	−108.0	−5.9

associated with neurological degeneration.[34,35] These biological effects confirm and validate the method used. Figure 4D shows the binding mode of the complex Z-asarone with 1EH4. As shown, the methoxy moieties establish a H-bonding network with Val622 and reach a hydrophobic pocket composed of Trp552, Ile571, and Thr627, thus undergoing van der Walls interactions. Therefore, a favorable binding energy can be observed in Table 1. In addition, (*E*)-asarone can bind to this receptor, as suggested by Autodock Vina (Table 1 and Fig. 4C)

A similar analysis can be carried out for compound **1** (Fig. 1A)**.** Table 1 shows the IVS result for this compound. As shown, two molecular targets were found under PDB codes 1JT2 and 2BNJ.[36,37] The enzyme 1JT2 is present in *Clostridium thermocellum*, which is able to degrade the cell walls of plant cells. The cell wall is a polymeric backbone that provides integrity and protection to plant cells. With the knowledge of the active site of this enzyme as well as the amino acids that are essential for the interaction of this enzyme with its substrate, it is possible to develop potential inhibitors for this enzyme. In other words, 1JT2 is a feruloyl esterase, which cleaves ferulic acid's bonds into arabinoxylan and pectin.[36] In addition, the molecular target 2BNJ is a xylanase present in the yeast *Thermoascus aurantiacusthat*, which is able to degrade the polymer xylan, a major component of vegetable cell walls. The degradation of cell walls is important for the nutrition of herbivores, plant cell invasion by bacterial and fungal pests, the production of food and drink, and potentially the production of energy.[37] Following our IVS protocol, both receptors were submitted to MD simulations for 1 ns to validate the methodology. The MD simulations showed that compound **1** cannot bind to the active site of 1JT2 and 2BNJ. In other words, this approach could identify false-positive results. These results differ from those of the docking methodology, which showed favorable binding energy for both molecular targets, −4.0 kcal/mol and −6.3 kcal/mol, respectively (Table 1). Therefore, it was not possible to estimate the binding energy using ONIOM in this case.

The IVS results revealed that 3FW9[36] is a molecular target for compound **2** (Table 1). 3FW9 is a flavoprotein with oxyreductase activity. Figure 4E shows the most important intermolecular interaction between this natural compound from the Cerrado biome (**2**) and 3FW9. In this context, a H-bonding network was established between the mains chains of Gly156 and Phe155, and van der Waals interactions were formed among Tyr81, Trp140, and Leu257 (Fig. 4E). Furthermore, ONION and Autodock Vina were used to predict the binding energy. Both methods showed a favorable binding energy with the receptor, but they are different from each other. However, the ONIOM approach is more robust, producing more realistic results, as described above.

Finally, compound **3** was submitted to the same IVS protocol. The screening suggested the molecular target 1H49 for this compound (Fig. 4F). This enzyme is involved in macromolecular processes such as DNA repair and replication as well as protein synthesis and degradation. As shown, the ligand forms a hydrogen bond with Ser270. Table 1 summarizes the IVS, docking, and ONIOM results. As shown in table, much like the binding energy obtained for the **2**-3FW9 complex, the ONIOM, and Autodock Vina results differed as well.

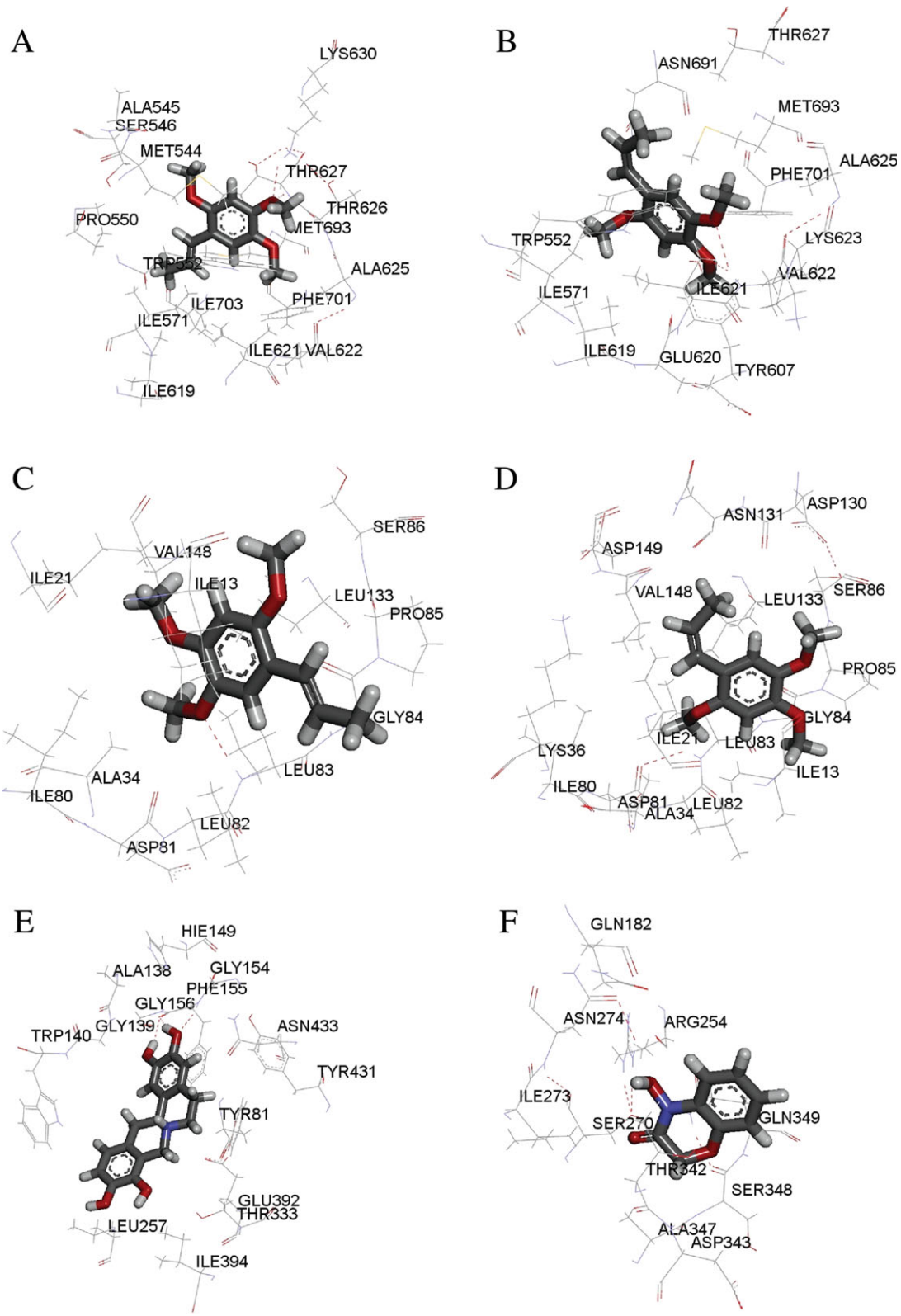

Figure 4. Pharmacophore map of natural compounds studied. The ligands and aminoacids are stick and wireframe, respectively. (A) 2A4Z-(*E*)-asarone complex; (B) 2A4Z-(*Z*)-asarone complex; (C) 1EH4-(*E*)-asarone complex; (D) 1EH4-(*Z*)-asarone complex; (E) 3FW9-**2** complex; (F) 1H49-**3** complex. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

All natural compounds studied were bound to their respective targets by intermolecular bonding interactions. The details of the pharmacophoric conformation and respective binding energy were described. In general, these ligands are recognized by hydrogen bonds and hydrophobic interactions. These data provide insight into the semisynthetic syntheses of new derivates, which can be designed by improving intermolecular interactions such as ionic, hydrogen, van der Waals, and dipole–dipole interactions. Furthermore, these results can motivate structure–activity relationships studies to improve the biological activity of this set of compounds. These results suggest that the pharmacological targets should be tested as a preliminary assay. The amino acids and their position in the binding sites of the selected molecular targets were described; thus, it was possible to identify the main interaction between the ligands and receptors and design new molecules that can fit more properly, suggesting new lead compounds. In addition, the IVS, docking, and MD simulation methodologies used in this study revealed molecular targets for a set of natural compounds and described how they can interact with active sites. Structural analysis, identification of active conformation and pharmacophores groups, and ligand binding studies were performed using IVS, docking, MD and QM/MM calculations. These methods enhance the accuracy of the results at a low computational cost. Our results, similar to those of previous studies, indicate that IVS requires additional methodologies to improve the prediction of the activity of secondary metabolites from natural sources.

Conclusions

All natural compounds were widely distributed in the Cerrado biome and are a part of a set of compounds with limited pharmacological data reported. Therefore, a HTS assay is needed to identify the biological activity of all of them. In addition, the experimental methods used for target identification and validation, including microarray, antisense, protein transcription factor, and haplotype analysis technologies, are, in general, expensive and time consuming. Therefore, it is difficult to perform large-scale target identification and evaluation using these methods. This situation is amplified by the progress of several genome and proteome projects that describe new molecular targets. In other words, this study suggests that *in silico* methods of searching for molecular target should be carried out before exhaustive experimental screening.

Finally, because several drugs exhibit promiscuous behavior, they can bind to multiple targets via pathological pathways, leading to an opportunity for drug development. However, this behavior can be responsible for several adverse effects as well. Thus, the identification and evaluation of multiple target compounds can be useful for the prediction of undesirable effects. Furthermore, the details of the respective pharmacological mechanism can be described in greater detail. Thus, IVS or the inverse docking approach (including refinement methods) can be considered a new tool for structure-based drug design.

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Keywords: IVS · inverse docking · natural products · sc-PDB · ONIOM · QM/MM

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[1] C. Viegas, Jr., V. Bolzani, E. J. Barreiro, *Quím. Nova* **2006**, *29,* 326.
[2] L. Gobbo-Neto, N. P. Lopes, *Quim. Nova* **2007**, *30,* 374.
[3] J. A. Ratter, J. F. Ribeiro, S. Bridgewater, *Ann. Botany* **1997**, *80,* 223.
[4] M. S. Butler, *J. Nat. Prod.* **2004**, *67,* 2141.
[5] R. Braz-Filho, *Quim. Nova* **2010**, *33,* 229.
[6] G. L. Patrik, An Introduction to Medicinal Chemistry, 4th ed.; Oxford University Press Inc., New York, **2009**.
[7] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov, P. E. Bourne, *Nucleic. Acids. Res.* **2000**, *28*, 235.
[8] H. M. Berman, K. Henrick, H. Nakamura, J. L. Markley, Nucleic. Acids. Res. **2007**, *35*, D301.
[9] C. G. Wermuth, The Practice of Medicinal Chemistry, 3rd ed.; Academic Press: New York, **2008**.
[10] D. Rognan, *J. Physiol.* **2006**, *99,* 232.
[11] F. H. Leite, A. G. Taranto, M. C. dos Santos Junior, A. Branco, M. T. de Araujo, J. W. de M. Carneiro, *Int. J. Quant. Chem.* **2010**, *110,* 2057.
[12] H. S. Lee, J. Choi, S. Yoon, *Genomics and Informatics* **2007**, *5,* 24.
[13] M. Vaqué, A. Ardévol, C. Bladé, M. J. Salvadó, M. Blay, J. F. Larrea, L. Arola, G. Pujadas, *Curr. Pharm. Anal.* **2008**, *4,* 1.
[14] I. Carvalho, M. T. Pupo, A. D. L. Borges, L. S. Bernardes, *C. Quim. Nova* **2003**, *3,* 428.
[15] A. Warshel, M. Levitt, *J. Mol. Biol.* **1976**, *103,* 227.
[16] A. G. Taranto, P. Carvalho, M. A. Avery, *J. Mol. Graph. Model.* **2008**, *27,* 275.
[17] F. Maseras, K. Morokuma, *J. Comput. Chem.* **1995**, *16,* 1170.
[18] E. Kellenberger, P. Muller, C. Schalon, G. Bret, N. Foata, D. Rognan, *J. Chem. Inf. Model.* **2006**, *46,* 717.
[19] J. Meslamani, D. Rognan, E. Kellenberger, *Bioinformatics* **2011**, *27,* 1324.
[20] A. P. Carregal, A. G. Taranto, M. Comar, L. A. Lima, J. M. Siqueira, *de Revista Eletrônica de Farmácia* **2011**, *8,* 71.
[21] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, A. J. Olson, *J. Comput. Chem.* **1998**, *19,* 1639.
[22] GaussView, Version 5, R. Dennington, T. Keith, J. Millam, GaussView, Version 5; Semichem Inc.: Shawnee Mission KS, **2009**.
[23] J. P. P. Stewart, *J. Mol. Model.* **2007**, *13,* 1173.
[24] Gaussian 09, Revision **A.1**, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N. J. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian 09, Revision **A.1**; Gaussian, Inc.: Wallingford CT, **2009**.
[25] O. Trott, A. J. Olson, *J. Comput. Chem.* **2010**, *31,* 455.
[26] D. A. Case, T. A. Darden, T. E. Cheatham, III, C. L. Simmerling, J. Wang, R. E. Duke, R. Luo, R. C. Walker, W. Zhang, K. M. Merz, B. P. Roberts, B.

Wang, S. Hayik, A. Roitberg, G. Seabra, I. Kolossvai, K. F. Wong, F. Paesani, J. Vanicek, J. Liu, X. Wu, S. R. Brozell, T. Steinbrecher, H. Gohlke, Q. Cai, X. Ye, J. Wang, M.-J. Hsieh, G. Cui, D. R. Roe, D. H. Mathews, M. G. Seetin, C. Sagui, V. Babin, T. Luchko, S. Gusarov, A. Kovalenko, P. A. Kollman, AMBER 11; University of California: San Francisco, **2010**.

[27] G. D. Hawkins, C. J. Cramer, D. G. Truhlar, *J. Phys. Chem.* **1996**, *100,* 19824.

[28] G. D. Hawkins, C. J. Cramer, D. G. Truhlar, *Chem. Phys. Lett.* **1995**, *246,* 122.

[29] A. K. Rappé, W. A. Goddard, III, *J. Phys. Chem.* **1991**, *95,* 3358.

[30] A. K. Rappé, C. J. Casewit, K. S. Colwell, W. M. Skiff, *J. Am. Chem. Soc.* **1992**, *114,* 10024.

[31] Accelrys Software Inc., Discovery Studio Modeling Environment, Release 2.5, San Diego: Accelyrs Software Inc., **2009**.

[32] M. Camps, T. Rückle, H. Ji, V. Ardissone, F. Rintelen, J. Shaw, C. Ferrandi, C. Chabert, C. Gillieron, B. Françon, T. Martin, D. Gretener, D. Perrin, D. Leroy, P. A. Vitte, E. Hirsch, M. P. Wymann, R. Cirillo, M. K. Schwarz, C. Rommel, *Nat. Med.* **2005**, *11,* 936.

[33] L. Verdoucq, M. Czjzek, J. Moriniere, D. R. Bevan, A. Esen, *J. Biol. Chem.* **2003**, *278,* 25055.

[34] N. Mashhoon, A. J. De Maggio, V. Tereshko, S. C. Bergmeier, M. Egli, M. F. Hoekstra, J. Kuret, *J. Biol. Chem.* **2000**, *275,* 20052.

[35] F. D. Schubot, I. A. Kataeva, D. L. Blum, A. K. Shah, L. G. Ljungdahl, J. P. Rose, B. C. Wang, *Biochemistry* **2001**, *40,* 12524.

[36] M. Vardakou, J. Flint, P. Christakopoulos, R. J. Lewis, H. J. Gilbert, J. W. Murray, *J. Mol. Biol.* **2005**, *352,* 1060.

[37] L. Hui-fang, S. Qing, F. Wei, *J. Mol. Graph. Model.* **2010**, *29,* 326.

[38] G. Lauro, A. Romano, R. Riccio, G. Bifulco, *J. Nat. Prod.* **2011**, *74,* 1401.

[39] C. Park, S. Kim, Y. Ahn, *J. Stored Prod. Res.* **2003**, *39,* 333.

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