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Chemo-sensitizing activity of natural cadinanes from *Heterotheca inuloides* in human uterine sarcoma cells and their *in silico* interaction with ABC transporters

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ABSTRACT

Sensitizing activities exerted by 3,4-dihydro-7-hydroxycadalene (**1**), *rac*-3,7-dihydroxy-3(4*H*)-isocadalen-4-one (**4**) and (1*R*,4*R*)-4*H*-1,2,3,4-tetrahydro-1-hydroxycadalen-15-oic acid (**9**), the major cadinanes isolated from *Heterotheca inuloides,* towards multidrug-resistant MES-SA/MX2 and parental MES-SA epithelial human uterine sarcoma cell lines were evaluated. We also evaluated the *in silico* interactions (expressed as $\Delta G_{binding}$ in kcal/mol) of cadinanes **1**, **4** and **9** in an *in vitro* assay, and also tested several structurally related natural compounds with the multidrug resistance protein (MDR1, P-glycoprotein), human multidrug resistance protein 1 (MRP1), and breast cancer resistance protein (BCRP) structures as pharmacological targets using AutoDock and AutoDock Vina. Compound **1** potentiated the cytotoxicity of doxorubicin and mitoxantrone drugs in resistant MES-SA/MX2 cells, compared to cells treated with each drug alone. Compound **1** could reverse the resistance to doxorubicin 12.44 fold at a concentration of 5 μM. It also re-sensitized cells to mitoxantrone 3.94 fold. Hence, compound **1** may be considered as a potential chemosensitizing agent to overcome multidrug resistance in cancer. The docking analysis suggested that there are interactions between cadinanes from *H. inuloides* and MDR1, MRP1, and BCRP proteins mainly through π-π interactions and hydrogen bonds.

1. Introduction

Cancer is a group of related diseases characterized by the continuous proliferation of cells as the result of cumulative mutations in genes that regulate the cell cycle and genomic instability [1,2]. In many cases, the initial response to conventional cytotoxic chemotherapies is successful, but some patients relapse due to the proliferation of cancer cells tolerant or resistant to chemotherapeutic agents, which severely limits the effectiveness of chemotherapy, leading in many cases to patient death [3,4]. The phenomenon of drug resistance in cancer cells is multicausal, but the overexpression of certain ATP-binding cassette (ABC) membrane transport proteins such as MDR1 (known as permeability glycoprotein or P-glycoprotein), MRP1 (multidrug resistance-associated protein-1), and BCRP (breast cancer related protein), located in the luminal membrane of endothelial cells of organs involved in elimination of exogenous and endogenous substances under normal physiological conditions, has been considered the main contributor to resistance and chemotherapy failure, because these proteins expel a wide variety of clinically relevant molecules from their target cells, maintaining concentrations below toxic levels [5,6]. Reducing the multi-drug resistance of cancer cells should significantly improve the response of cancer cells to cytotoxic agents. A strategy against cancer multi-drug resistance associated with the overexpression of ABC membrane transporter proteins involves the simultaneous administration of sensitizing substances that increase intracellular concentration of the drugs by inhibiting their expulsion [7]. Uterine sarcomas are aggressive mesenchymal tumors that cause approximately 3–7% of all uterine malignancies and represent a significant therapeutic problem

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because of their poor prognosis [8,9]. The cancer resistance to treatment in some patients emphasizes the need for new treatment strategies [10]. The use of natural products that sensitize the cells and increase the efficacy of drugs represents an alternative in the management of cancer chemotherapy-resistance [11] and thus, broad research has been carried out on MDR1 inhibitors isolated from plants [12]. Various natural products with cadinane skeleton display a broad variety of biological activities of [13–17] including the potential to modulate drug resistance [18]; besides, there are other type of sesquiterpenes that also display this activity [19,20].

Heterotheca inuloides Cass. (Asteraceae) is a plant endemic to Mexico that is widely used in traditional medicine by indigenous, rural, and urban communities. This medicinal plant, commonly known as "Mexican arnica", is used to treat different conditions associated with inflammatory processes, skin conditions, gastrointestinal problems, and urinary infections [21], as well as for the empirical treatment of chronic diseases such as diabetes and cancer. Phytochemical studies of *H. inuloides* have led to the isolation and identification of more than 140 chemical compounds present in the flowers, stems, and roots, comprising sesquiterpenoids, phenolic compounds, and phytosterols [22]. As other species of the genus *Heterotheca*, Mexican arnica is characterized by cadinane sesquiterpenes, which have a broad range of bioactivity [23,24].

Considering previous studies on the MDR modulation mediated by sesquiterpenes [19,20], specially by dimeric cadinanes [18], the potential of the major cadinanes *H. inuloides* to sensitize cancer cells were examined in multidrug-resistant variant MES-SA/MX2 of the epithelial human uterine sarcoma cell line MES-SA. The *in silico* interaction of these natural cadinane sesquiterpenes with the MDR1, MRP1 and BCRP transporters was also explored, leading to an improved understanding of the molecular mode of action of these compounds.

2. Materials and methods

2.1. Natural cadinane compounds

Natural products 3,4-dihydro-7-hydroxycadalene (**1**), 7-hydroxycadalene (**2**), 4-methoxyisocadalene (**3**), *rac*-3,7-dihydroxy-3(4*H*)-isocadalen-4-one (**4**), 1-hydroxy-1(4*H*)-isocadalen-4-one (**5**), (4*R*,10*S*)-δ-cadinen-15-oic acid (**6**), (4*R*,10*S*)-3,4,7,10-tetrahydrocadalen-15-oic acid (**7**), (4*R*)-3,4-dihydrocadalen-15-oic acid (**8**), (1*R*,4*R*)-4*H*-1,2,3,4-tetrahydro-1-hydroxycadalen-15-oic acid (**9**), and 7-hydroxycadalen-14-al (**10**) (Fig. 1) were isolated by conventional chromatographic techniques from the acetone extract of *H. inuloides* as in previous studies in our group [25]. Due to quantity shortages, only major components (**1**, **4**, **9**) were evaluated in the biological assay.

Table 1
Modulation of doxorubicin and mitoxantrone cytotoxicity in drug-sensitive MES-SA and drug-resistant MES-SA/MX2 cell lines by natural cadinanes.

	Doxorubicin		Mitoxantrone	
	IC_{50} μM (RF index)[b]		IC_{50} μM (RF index)[b]	
	MES-SA/MX2	MES-SA	MES-SA/MX2	MES-SA
Drug alone	2.414 [a]	0.050 [a]	0.513 [a]	0.029 [a]
1	0.194 (12.442)	0.010 (5.233)	0.132 (3.946)	0.012 (2.547)
4	1.416 (1.705)	0.015 (3.370)	1.181 (0.441)	0.014 (2.079)
9	0.823 (2.934)	0.046 (1.084)	0.525 (0.992)	0.023 (1.307)
Verapamil	0.110 (21.890)	0.045 (1.114)	0.269 (1.936)	0.0014 (2.074)
Resveratrol	0.822 (2.936)	0.016 (3.077)	1.518 (0.337)	0.012 (2.538)

[a] Required amount of drug alone to reduce the growth of the cell lines by 50%.
[b] Reversal fold index (RF) = (IC_{50} of drug alone)/(IC_{50} of drug in combination with modulator).

2.2. Chemicals

Waymouth's MB 752/1 medium, McCoy's 5A medium, sulforhodamine B sodium salt, trypan blue, doxorubicin (doxorubicin hydrochloride), mitoxantrone (mitoxantrone dihydrochloride), verapamil [2-(3,4-dimethoxyphenyl)-5-[2-(3,4-dimethoxyphenyl)ethyl-methylamino]-2-propan-2-ylpentanenitrile], trichloroacetic acid (TCA), DMSO, resveratrol and tris (tris(hydroxymethyl)aminomethane) were obtained from Sigma Aldrich (St. Louis, MO, USA). Heat inactivated fetal bovine serum (Gibco®) and Antibiotic-Antimycotic (100X) (Gibco™) were purchased from Thermo Fisher Scientific (MA, USA).

2.3. Chemo-sensitizing potential to doxorubicin and mitoxantrone

Epithelial human uterine sarcoma cell lines MES-SA (ATCC® CRL-1976™) and the resistant phenotype MES-SA/MX2 (ATCC® CRL-2274™) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). MES-SA cells were cultured in McCoy's 5 medium, while MES-SA/MX2 cells were cultured in a medium containing a 1:1 mixture of Waymouth's MB 752/1 medium and McCoy's 5. In both cases, the culture medium was supplemented with 10% heat inactivated bovine serum and 1% antibiotic-antimycotic (100 X) (Gibco™). Cells were cultured at 37 °C in a humidified incubator in an atmosphere containing 5% CO_2 media in the presence or absence of inhibitors. The viability of the cells used in the experiments exceeded 95%, as determined by a trypan blue assay.

The chemo-sensitizing potential of natural cadinanes on MES-SA and MES-SA/MX2 cells was determined through cell proliferation inhibition using the sulforhodamine B (SRB) assay [26]. *In vitro*, proliferation assays of MES-SA and MES-SA/MX2 cells were performed for

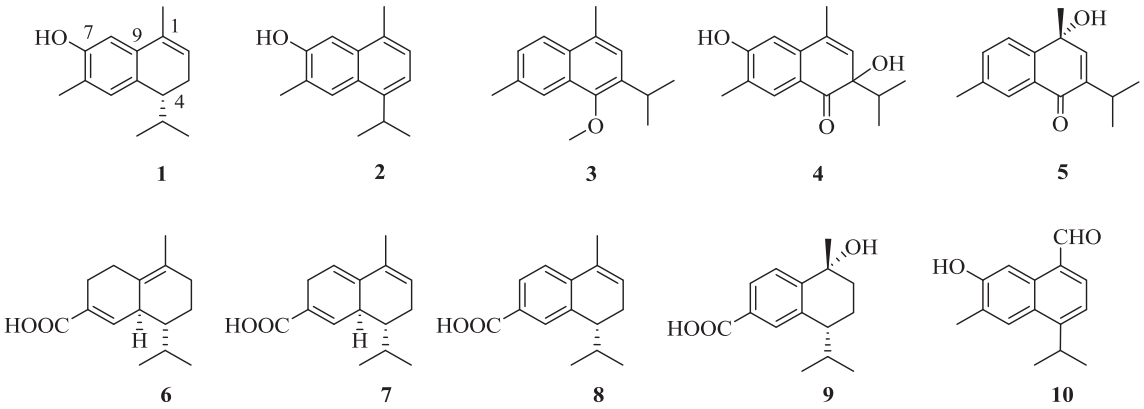

Fig. 1. Structures of the |evaluated compounds.

Table 2
Comparison of the binding affinities for cadinanes and proteins MDR1, MRP1 and BCRP calculated with AutoDock and AutoDock Vina.

Compound	kcal/mol					
	MDR1 (P-gp)		MRP1		BCRP	
	AutoDock	AutoDock Vina	AutoDock	AutoDock Vina	AutoDock	AutoDock Vina
1	− 7.02	− 7.0	− 6.24	− 5.9	− 6.27	− 7.8
2	− 6.47	− 7.0	− 5.95	− 6.3	− 6.18	− 8.0
3	− 6.49	− 7.2	− 5.99	− 6.3	− 5.85	− 8.5
4	− 7.74	− 7.4	− 6.90	− 6.7	− 6.83	− 7.9
5	− 7.79	− 7.7	− 6.60	− 6.1	− 6.31	− 8.6
6	− 7.22	− 6.9	− 8.42	− 6.5	− 7.25	− 7.7
7	− 6.89	− 7.4	− 8.07	− 6.7	− 7.25	− 8.8
8	− 6.77	− 6.7	− 8.40	− 6.6	− 7.17	− 7.8
9	− 7.06	− 6.7	− 8.14	− 6.4	− 7.54	− 7.5
10	− 6.38	− 7.5	− 6.51	− 6.2	− 5.88	− 7.8

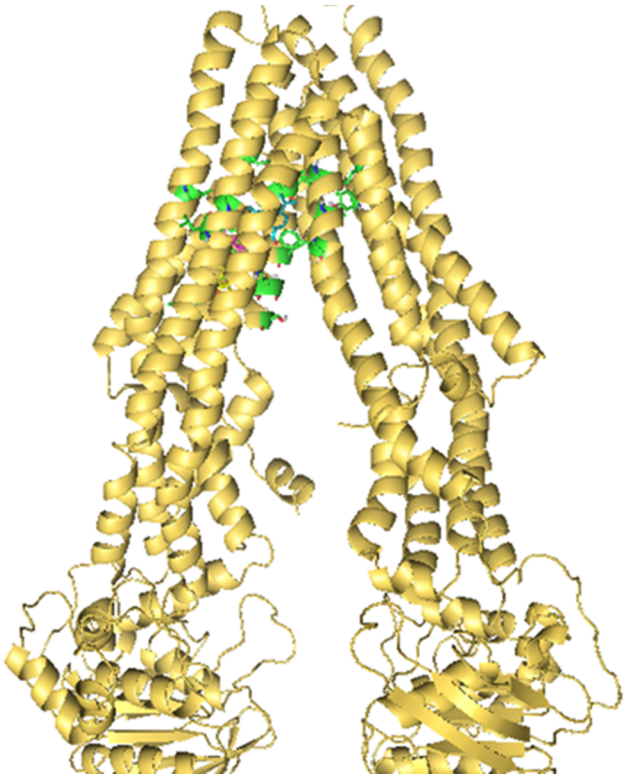

Fig. 2. Interaction of cadinanes **1–10** with the structure of the MDR1 protein. The compounds mostly bind in the same locations, in middle or upper regions of the structure.

doxorubicin and mitoxantrone alone, as well as these drugs combined with the tested compounds (at 5 μM), with the applied concentrations of doxorubicin and mitoxantrone ranging between 0.01 and 10.00 μM. Verapamil and resveratrol (5 μM) were used as positive control in combination with each drug. All assays were performed in triplicate. Stock solutions of all compounds were prepared in DMSO at a maximum concentration of 0.5%. The detailed methodology of cytotoxic assay was described in a previous work [27].

2.4. Ligand molecular docking

Three-dimensional structures of the multidrug resistance protein (ABCB1/P-gp/MDR1) homolog MsbA (PDB: 4M1M) [28], human multidrug resistance protein 1 (ABCC1/MRP1) (PDB ID: 2CBZ) [29], and breast cancer resistance protein (ABCG2/MXR/BCRP) (PDB ID: 5NJ3) [30] were downloaded from the RCSB PDB repository. The corrected mouse P-gp structure (MDR1a; PDB ID: 4M1M; UNIPROT ID: P21447) was selected as the template protein, because it is the one most closely related for construction of the homology models for human P-gp [31]. The corrected PDB ID: 4M1M structure shares nearly 87% protein sequence identity, and a drug translocation pathway 96% identical to human MDR1 [28]. All the geometries were optimized first using the semi-empirical method Austin Model 1 (AM1) [32]. MGL tools 1.5.4 (The Scripps Research Institute) with AutoDock [33] were used to dock inhibitors, to identify the active entities for ligand interaction sites in MDR1, MRP1 and BCRP protein structures. The structures of compounds **1–10** were built using previously reported X-ray crystallography data [25]. Molecules were docked over the entire protein to identify their interaction sites. Lamarckian genetic algorithm (LGA) [34] was implemented in AutoDock, while an optimized algorithm that combined various local optimization procedures, including genetic algorithms, particle swarm optimization, simulated annealing and others was used for AutoDock Vina [35]. The final positions of the compounds were ranked by lowest interaction energy values (expressed in kcal/mol and binding residues). H-bond interactions between compounds and the protein were explored. The output obtained from AutoDock and AutoDock Vina was further analyzed with PyMOL software package (Schrödinger, LLC, NY, USA) and Discovery Studio Visualizer (Dassault Systems, BIOVIA Corp., CA, USA). Validation of the employed computational protocol was established through comparison of docking results carried out using data of co-crystallized ligand, with the docking pose of experimental crystal data [29,30,36]. A docked pose with a Root Mean Square Deviation (RMSD) value < 2 Å was considered successfully performed [37].

2.5. Data analysis and statistics

Proliferation inhibition assays were performed in triplicate. The drug concentration log values and the percentage of cell proliferation inhibition were fitted to generate dose-response curves using the Origin *7* software (OriginLab Corp., MA, USA). The 50% cell proliferation inhibition concentration (IC_{50}) values were calculated by linear regression analysis and interpolation on the fitted regression curves. The reversal fold index resistance values were calculated by determination of the ratio of the IC_{50} of the drug alone and the IC_{50} of the drug with the evaluated compound performed in parallel.

3. Results and discussion

3.1. Sensitizing activity in human sarcoma cell lines

We evaluated the activity of natural cadinane sesquiterpenes **1**, **4** and **9** as drug-resistance sensitizers using the multidrug sensitive MES-SA uterine sarcoma cell line (MES-SA/MX2). In the assay, the

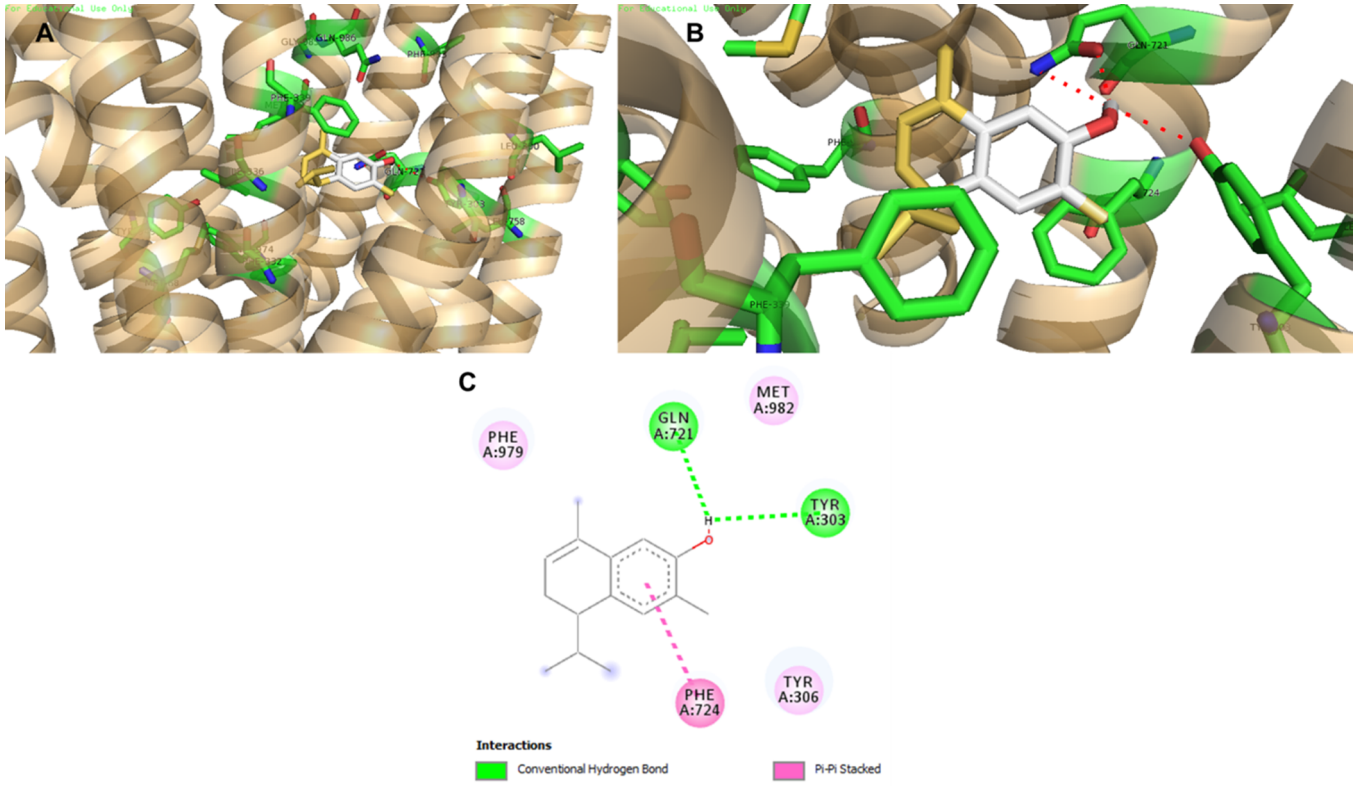

Fig. 3. **A** and **B**: Interactions of compound **1** with MDR1. **C**: 2D image showing interactions with the Gln-721 and Tyr-303 residues, as well as π- π interaction with the Phe-724 residue. Interactions are represented as: H-bond with amino acid backbone (green circle and dashed line); π- π interaction (magenta circle).

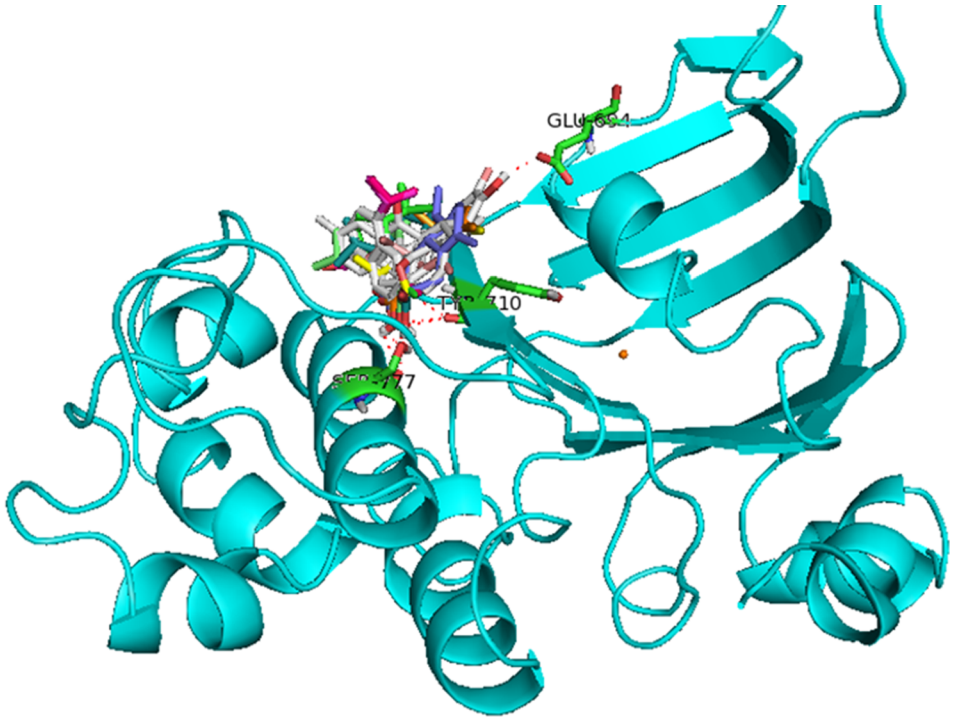

Fig. 4. Interaction of the compounds **1–10** with MRP1 protein. Hydrogen bonds with residues Glu-694, Ser-777 and Tyr-710 can be observed.

concentrations of the drug required for 50% inhibition of cell proliferation for the resistant and wild type cells line (MES-SA/MX2) and MES-SA were 2.414 and 0.050 μM, respectively, in the case of doxorubicin; and 0.513 and 0.029 μM, respectively, in the case of mitoxantrone (Table 1). These results imply that 48-fold and 17-fold higher concentrations of doxorubicin and mitoxantrone, respectively, are required to cause the same effect in the MES-SA/MX2 (resistant) cell line as in the MES-SA (wild-type) cell line. Compound **1** increased the sensitivity to doxorubicin and mitoxantrone in the MES-SA cells as well as MES-SA/MX2 cells. Although compound **1** has a broad spectrum of

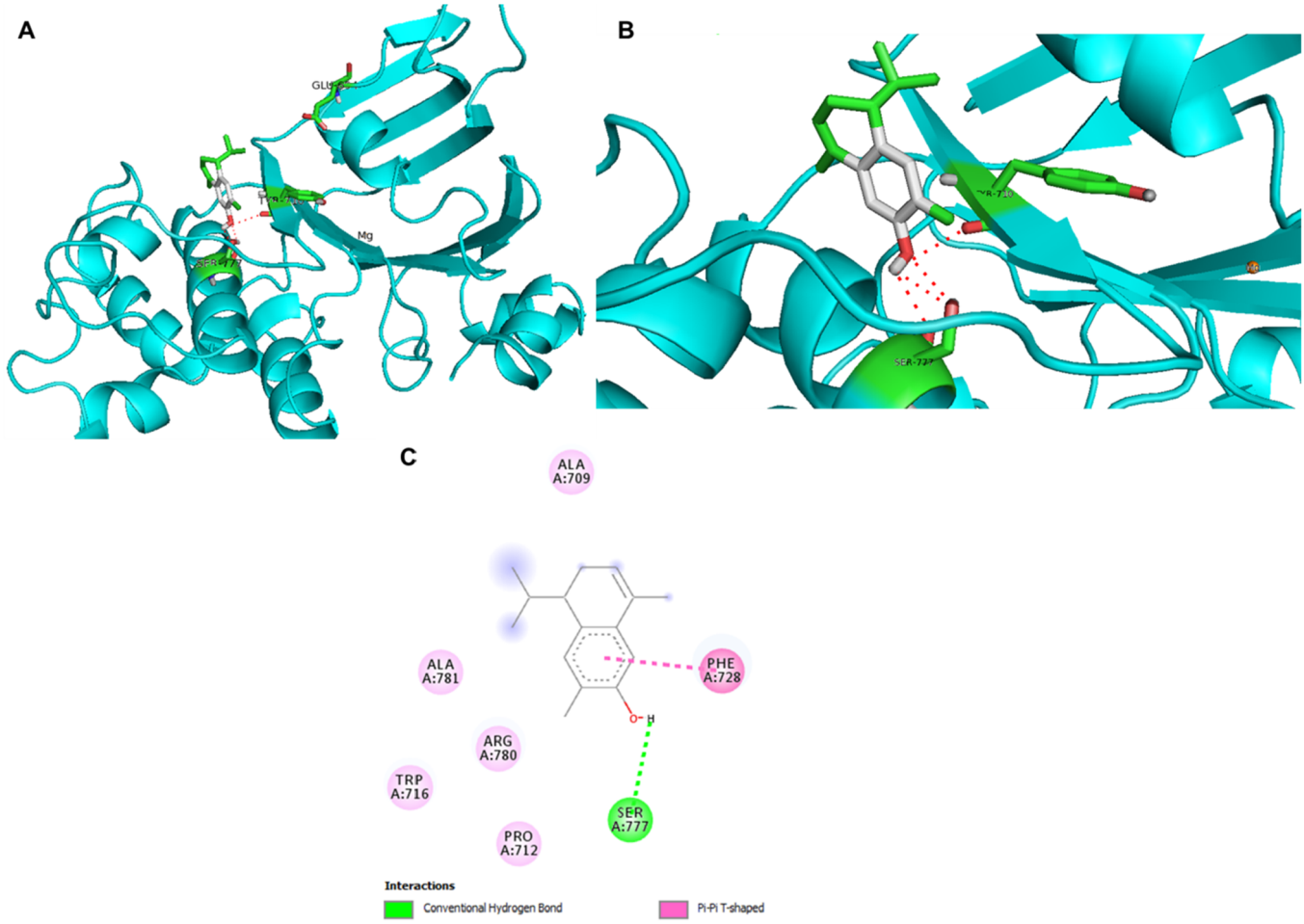

Fig. 5. (**A**): Interaction between compound **1** and MRP1 in an allosteric region near the catalytic site of MRP1. (**B**): Hydrogen bonds between compound **1** and residues Ser-777 and Tyr-710. (**C**): 2D image showing the interaction with the residue Ser-777 and the interaction of the π- π type with the residue Phe-728.

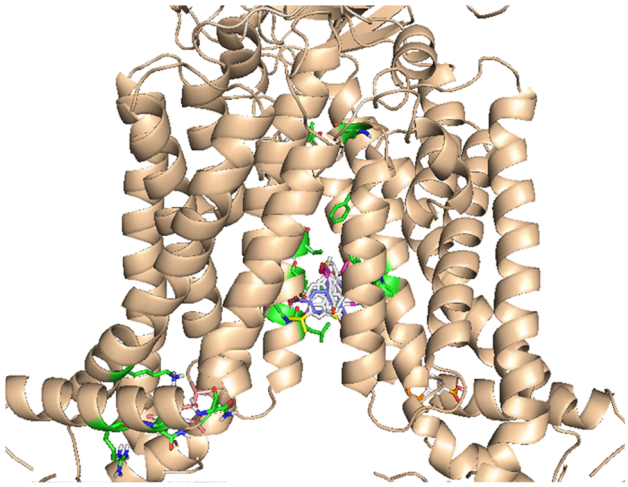

Fig. 6. Interaction of compounds **1**–**10** with BCRP protein.

biological activities, including the modulation of NFκB and Nrf2 transcription factors [22,24], this is the first time that the drug-resistance modulation activity of **1** has been reported. Compounds **4** and **9** were also able to sensitize the MES-SA/MX2 cell line to doxorubicin; despite, they were not better than the control verapamil, a drug used in therapeutic practice.

The natural product resveratrol was also used as a positive control, since its coadministration with antineoplastic drugs improves the treatment of drug-resistant tumors [38,39], an effect that can be attributed to its ability to modulate the expression of ABC transporters [40]. Of note, compound **1** had reversal fold (RF) indexes greater than those of resveratrol (RF), indicating the potential of this compound to sensitize the resistant strain of MES-SA cell line.

Therefore, the data obtained from the reversal activity of the cadinanes of *H. inuloides* provide useful information for the development of new sensitizing agents.

3.2. Results of in silico study

The overexpression of ABC membrane transporter proteins in cancer cells confers resistance to antineoplastic agents, such as mitoxantrone, thus these proteins are considered an important target for reversing chemotherapy resistance [41,42]. In an effort to find new inhibitors of multidrug resistance, compounds **1**, **4** and **9**, previously evaluated *in vitro* against resistant cell lines, and a series of structurally analogous compounds isolated from *H. inuloides* were studied *in silico* to predict the possible interactions and affinity to MDR1, MRP1 and BCRP proteins. The output of Autodock Vina in most cases was very close to that of AutoDock. The results are consistent with published data indicating that the energies given by AutoDock Vina and AutoDock are very similar [43] (See Table 2). Some authors recommend the use of AutoDock Vina because it improves the accuracy of the binding mode predictions [35,44] and because it executes more quickly and more accurately ranks larger molecules than does AutoDock [45]. Results obtained when carrying out the docking with each of the three proteins are shown in more detail below.

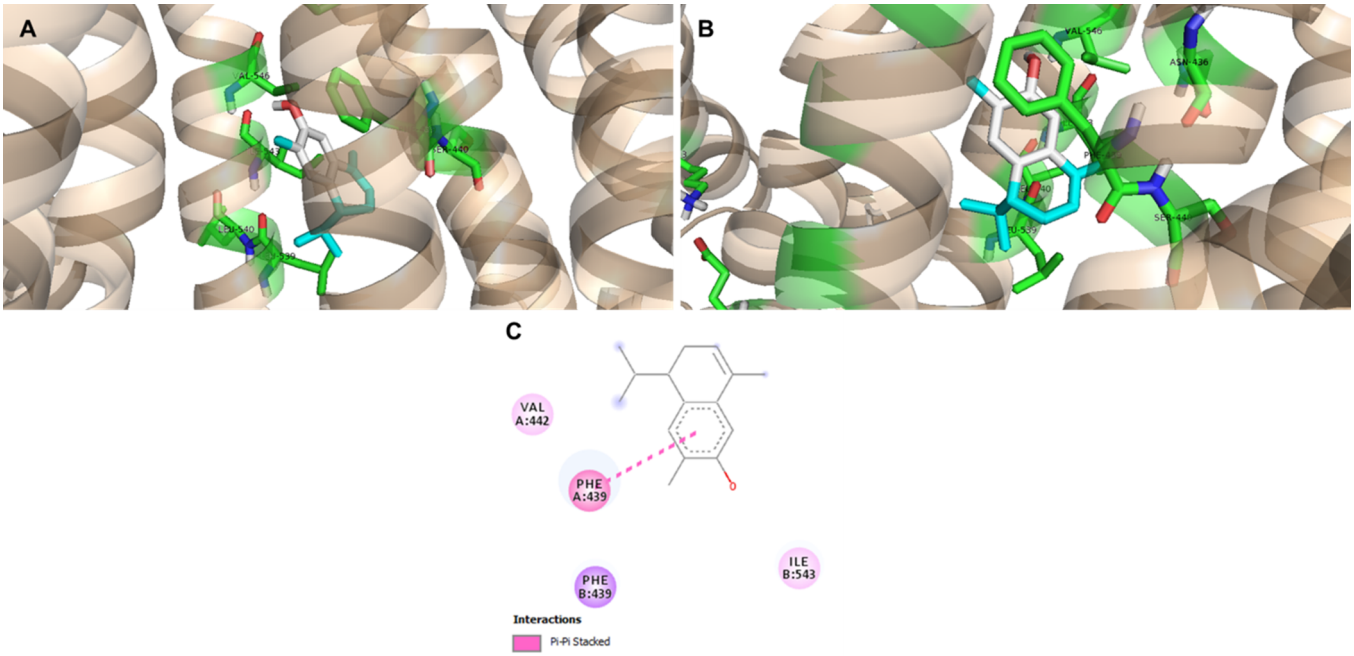

Fig. 7. Interaction of compound **1** with BCRP protein. **A** and **B**: Compound 1 is positioned in the center of the BCRP channel and also the interaction between **1** and the BCRP protein implies the formation of hydrogen bonds with the residues Val 546, Ser-440 and Asn-436**C**: 2D image. A π- π interaction with the residue Phe-439 can be observed.

3.2.1. Docking with MDR1 (P-glycoprotein/ABCB1)

The results obtained with AutoDock and AutoDock Vina showed that binding energies as well as the region where the natural cadinanes from *H. inuloides* bind to the protein are very similar. Most of them bind to the P-glycoprotein in the middle or upper region through interactions such as hydrogen bonding and π-π interactions (Fig. 2). In particular, cadinanes **1**, **4** and **9**, evaluated in the *in vitro* model against cell lines, were located in the upper region of the MDR1 channel, which could affect the function-activity of the translocation pathway. Compounds **1** (Fig. 3), **4** and **9** formed hydrogen bonds with the Gln-721, while compounds **4** and **9** showed interaction with the Tyr-306 residue (see Figs. S1 and S2 in supplementary material). These amino acids have been defined as part of the hydrophobic pocket of the protein; the formation of hydrogen bonds with the hydroxyl group contribute to the stronger binding by forming efficient hydrophobic interactions [36,46,47]. Tyr-306 along with other residues (mainly aromatic) may play important roles in the access of drugs such as paclitaxel to the binding site of MDR1 [48]. Other compounds, such as **8**, formed hydrogen bonds with the Gln-986 residue, while compound **10** formed hydrogen bonds with the Gln-721 and Tyr-303 residues. 1-Hydroxy-1(4*H*)-isocadalen-4-one (**5**) was found to be the strongest-docking compound (Table 2). Interaction energies towards the P-glycoprotein ranged from −7.79 to −6.38 kcal/mol with AutoDock and from −7.7 to −6.7 with AutoDock Vina, respectively. Previous reports indicated that the type and size of substituents in structurally related compounds confer important pharmacophoric characteristics, strongly affect the reversal activity and are critical in the efficacy as MDR1 inhibitors [49–51].

3.2.2. Docking with MRP1 (ABCC1)

Compounds **1–10** bind to the same region of the protein, near to the catalytic site, interacting mainly through hydrogen bonds with residues Ser-777, Tyr-710 and Glu-694 (Fig. 4). Additionally, compounds **1** and **4** showed π-π type interaction with the residues Phe-728 (Fig. 5) and Tyr-710 (Fig. S3), respectively, while **9** had hydrogen bonds with the Arg-780 residue (Fig. S4). Compounds **6–8** had similar interactions to those of **9**. Compounds **6–9** showed the highest affinities for MRP1 protein, with values ranging from −8.14 to −5.95 kcal/mol with AutoDock and −6.7 to −5.95 kcal/mol for Autodock Vina (see Table 2). These results indicate that the cadinanes could inhibit MRP1 in an allosteric manner.

3.2.3. Docking with BCRP (ABCG2)

Compounds were bound in the region where cholesterol binds, which could provoke inhibition of this receptor (Fig. 6). Compounds **1** and **9** were positioned at the center of the protein channel through π-π type interactions with the residue Phe-439 and hydrogen bonds with the residues Ser-440 and Asn-436 (Figs. 7 and S5). This observation is consistent with previous studies, which show that these residues interact with a BCRP inhibitor [52]. Additionally compound **1** showed hydrogen bonds with the Val-546 residue. Compound **4** was positioned at the base of the receptor of the intracellular zone of the protein forming hydrogen bonds with residues Asn-387, Arg-383, Asn-391 and Lys-473 (Fig. S6). In addition, compounds **4** and **8** bind at the base of the receptor in the intracellular zone, so they could inhibit by another mechanism of action. The binding energies of compounds **6–9** obtained using both methods were similar, whereas those obtained for compounds **1–5** and **10** differed slightly between the two methods.

4. Conclusions

We studied the activity of the major cadinanes present in *H. inuloides* as modulators of multidrug resistance in MES-SA/MX2 cells and the affinity for these cadinanes toward MDR1, MRP1, and BCRP1 proteins to define the location of the ligand-binding sites. The results showed that compound 3,4-dihydro-7-hydroxycadalene (**1**) sensitizes MES-SA/MX2 cell lines and potentiates the effects of doxorubicin and mitoxantrone. The negative binding energies obtained for *in silico* analysis using AutoDock and AutoDock Vina docking programs suggested that there are interactions between cadinanes from *H. inuloides* and ABC transporters. The results obtained using AutoDock and AutoDock Vina were in some cases very similar to each other; thus, this consistency shows the likelihood of cadinanes to inhibit ABC transporters. Due to structural similarities among the studied cadinanes, *in*

silico studies showed that these compounds share interactions with common residues and that the phenyl ring plays an important role in π-π interactions and hydrophobic interactions. This is the first time that modulation of MDR by these cadinanes has been studied *in vitro* and *in silico*, providing evidence of promising activity, better than previously reported for these types of substances.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103091.

References

[1] M.S. Lawrence, P. Stojanov, P. Polak, G.V. Kryukov, K. Cibulskis, A. Sivachenko, S.L. Carter, C. Stewart, C.H. Mermel, S.A. Roberts, A. Kiezun, P.S. Hammerman, A. McKenna, Y. Drier, L. Zou, A.H. Ramos, T.J. Pugh, N. Stransky, E. Helman, J. Kim, C. Sougnez, L. Ambrogio, E. Nickerson, E. Shefler, M.L. Cortés, D. Auclair, G. Saksena, D. Voet, M. Noble, D. Dicara, P. Lin, L. Lichtenstein, D.I. Heiman, T. Fennell, M. Imielinski, B. Hernandez, E. Hodis, S. Baca, A.M. Dulak, J. Lohr, D.A. Landau, C.J. Wu, J. Melendez-Zajgla, A. Hidalgo-Miranda, A. Koren, S.A. McCarroll, J. Mora, R.S. Lee, B. Crompton, R. Onofrio, M. Parkin, W. Winckler, K. Ardlie, S.B. Gabriel, C.W.M. Roberts, J.A. Biegel, K. Stegmaier, A.J. Bass, L.A. Garraway, M. Meyerson, T.R. Golub, D.A. Gordenin, S. Sunyaev, E.S. Lander, G. Getz, Mutational heterogeneity in cancer and the search for new cancer-associated genes, Nature 499 (2013) 214–218, https://doi.org/10.1038/nature12213.

[2] Y. Yao, W. Dai, Genomic instability and cancer, J. Carcinog. Mutagen. 05 (2014) 1–8, https://doi.org/10.4172/2157-2518.1000165.

[3] K.O. Alfarouk, C.M. Stock, S. Taylor, M. Walsh, A.K. Muddathir, D. Verduzco, A.H.H. Bashir, O.Y. Mohammed, G.O. Elhassan, S. Harguindey, S.J. Reshkin, M.E. Ibrahim, C. Rauch, Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp, Cancer Cell Int. 15 (2015) 1–13, https://doi.org/10.1186/s12935-015-0221-1.

[4] G. Housman, S. Byler, S. Heerboth, K. Lapinska, M. Longacre, N. Snyder, S. Sarkar, Drug resistance in cancer: an overview, Cancers (Basel) 6 (2014) 1769–1792, https://doi.org/10.3390/cancers6031769.

[5] R. Masereeuw, F.G.M. Russel, Regulatory pathways for ATP-binding cassette transport proteins in kidney proximal tubules, AAPS J. 14 (2012) 883–894, https://doi.org/10.1208/s12248-012-9404-z.

[6] T. Ozben, Mechanisms and strategies to overcome multiple drug resistance in cancer, FEBS Lett. 580 (2006) 2903–2909, https://doi.org/10.1016/j.febslet.2006.02.020.

[7] S. Guo, L. Lv, Y. Shen, Z. Hu, Q. He, X. Chen, A nanoparticulate pre-chemosensitizer for efficacious chemotherapy of multidrug resistant breast cancer, Sci. Rep. 6 (2016) 1–11, https://doi.org/10.1038/srep21459.

[8] C. Benson, A.B. Miah, Uterine sarcoma – current perspectives, Int. J. Womens. Health. 9 (2017) 597–606, https://doi.org/10.2147/IJWH.S117754.

[9] T. Cuppens, S. Tuyaerts, F. Amant, Potential therapeutic targets in uterine sarcomas, Sarcoma 2015 (2015) 1–14, https://doi.org/10.1155/2015/243298.

[10] S. Ricci, R.L. Stone, A.N. Fader, Uterine leiomyosarcoma: Epidemiology, contemporary treatment strategies and the impact of uterine morcellation, Gynecol. Oncol. 145 (2017) 208–216, https://doi.org/10.1016/j.ygyno.2017.02.019.

[11] M. Stompor, M. Świtalska, J. Wietrzyk, Synthesis and biological evaluation of acyl derivatives of hydroxyflavones as potent antiproliferative agents against drug resistance cell lines, Z. Naturforsch. C. 73 (2017) 87–93, https://doi.org/10.1515/znc-2017-0093.

[12] J. Yu, P. Zhou, J. Asenso, X.-D. Yang, C. Wang, W. Wei, Advances in plant-based inhibitors of P-glycoprotein, J. Enzyme Inhib. Med. Chem. 31 (2016) 867–881, https://doi.org/10.3109/14756366.2016.1149476.

[13] Y. Dai, L. Harinantenaina, P.J. Brodie, M.W. Callmander, S. Randrianasolo, E. Rakotobe, V.E. Rasamison, D.G.I. Kingston, Isolation and synthesis of two antiproliferative calamenene-type sesquiterpenoids from *Sterculia tavia* from the Madagascar rain forest, Bioorg. Med. Chem. 20 (2012) 6940–6944, https://doi.org/10.1016/j.bmc.2012.10.012.

[14] H.Y. Lee, J.T. Kwon, M. Koh, M.H. Cho, S.B. Park, Enhanced efficacy of 7-hydroxy-3-methoxycadalene via glycosylation in in vivo xenograft study, Bioorg. Med. Chem. Lett. 17 (2007) 6335–6339, https://doi.org/10.1016/j.bmcl.2007.08.071.

[15] C.-B. Ouyang, X.-M. Liu, Q. Liu, J. Bai, H.-Y. Li, Y. Li, Q.-X. Wang, D.-D. Yan, L.-G. Mao, A. Cao, M.-X. Guo, Toxicity assessment of Cadinene sesquiterpenes from Eupatorium adenophorum in mice, Nat. Prod. Bioprospect. 5 (2015) 29–36, https://doi.org/10.1007/s13659-014-0050-2.

[16] J.L. Rodriguez-Chavez, Y. Rufino-Gonzalez, M. Ponce-Macotela, G. Delgado, In vitro activity of "Mexican Arnica" Heterotheca inuloides Cass natural products and some derivatives against Giardia intestinalis, Parasitology 142 (2015) 576–584, https://doi.org/10.1017/S0031182014001619.

[17] J.L. Rodríguez-Chávez, E. Coballase-Urrutia, A. Nieto-Camacho, G. Delgado-Lamas, Antioxidant capacity of "Mexican Arnica" Heterotheca inuloides Cass natural products and some derivatives: their anti-inflammatory evaluation and effect on *C. elegans* life span, Oxid. Med. Cell. Longev. 2015 (2015) 1–11, https://doi.org/10.1155/2015/843237.

[18] Q.M. Li, J.G. Luo, R.Z. Wang, X.B. Wang, M.H. Yang, J. Luo, L.Y. Kong, Involucratusins A-H: unusual cadinane dimers from *Stahlianthus involucratus* with multidrug resistance reversal activity, Sci. Rep. 6 (2016) 1–11, https://doi.org/10.1038/srep29744.

[19] F. Muñoz-Martínez, P. Lu, F. Cortés-Selva, J.M. Pérez-Victoria, I.A. Jiménez, Á.G. Ravelo, F.J. Sharom, F. Gamarro, S. Castanys, Celastraceae Sesquiterpenes as a new class of modulators that bind specifically to human P-glycoprotein and reverse cellular multidrug resistance, Cancer Res. 64 (2004) 7130–7138, https://doi.org/10.1158/0008-5472.can-04-1005.

[20] D. Torres-Romero, F. Muñoz-Martínez, I.A. Jiménez, S. Castanys, F. Gamarro, I.L. Bazzocchi, Novel dihydro-β-agarofuran sesquiterpenes as potent modulators of human P-glycoprotein dependent multidrug resistance, Org. Biomol. Chem. 7 (2009) 5166, https://doi.org/10.1039/b915678j.

[21] A. Aguilar Contreras, Plantas medicinales del herbario IMSS: cuadros básicos por aparatos y sistemas del cuerpo humano, Instituto Mexicano del Seguro Social, Ciudad de México (1994).

[22] J.L. Rodríguez-Chávez, V. Egas, E. Linares, R. Bye, T. Hernández, F.J. Espinosa-García, G. Delgado, Mexican Arnica (Heterotheca inuloides Cass. Asteraceae: Astereae): ethnomedical uses, chemical constituents and biological properties, J. Ethnopharmacol. 195 (2017) 39–63, https://doi.org/10.1016/j.jep.2016.11.021.

[23] G. Delgado, M.del.S. Olivares, M.I. Chávez, T. Ramírez-Apan, E. Linares, R. Bye, F.J. Espinosa-García, Antiinflammatory constituents from *Heterotheca inuloides*, J. Nat. Prod. 64 (2001) 861–864, https://doi.org/10.1021/np0005107.

[24] V. Egas, E. Millán, J.A. Collado, T. Ramírez-Apan, C.A. Méndez-Cuesta, E. Muñoz, G. Delgado, Effect of natural and semi-synthetic cadinanes from *Heterotheca inuloides* on NF-κB, Nrf2 and STAT3 signaling pathways and evaluation of their in vitro cytotoxicity in human cancer cell lines, Bioorg. Med. Chem. 25 (2017) 3135–3147, https://doi.org/10.1016/j.bmc.2017.03.069.

[25] V. Egas, R.A. Toscano, E. Linares, R. Bye, F.J. Espinosa-García, G. Delgado, Cadinane-type sesquiterpenoids from *Heterotheca inuloides*: absolute configuration and anti-inflammatory activity, J. Nat. Prod. 78 (2015) 2634–2641, https://doi.org/10.1021/acs.jnatprod.5b00571.

[26] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer drug screening, J. Natl. Cancer Inst. 82 (1990) 1107–1112, https://doi.org/10.1093/jnci/82.13.1107.

[27] J. Rodríguez-Chávez, E. Coballase-Urrutia, G. Sicilia-Argumedo, T. Ramírez-Apan, G. Delgado, Toxicological evaluation of the natural products and some semisynthetic derivatives of Heterotheca inuloides Cass (Asteraceae), J. Ethnopharmacol. 175 (2015) 256–265, https://doi.org/10.1016/j.jep.2015.08.055.

[28] J. Li, K.F. Jaimes, S.G. Aller, Refined structures of mouse P-glycoprotein, Protein Sci. 23 (2014) 34–46, https://doi.org/10.1002/pro.2387.

[29] O. Ramaen, N. Leulliot, C. Sizun, N. Ulryck, O. Pamlard, J.Y. Lallemand, H. van Tilbeurgh, E. Jacquet, Structure of the human multidrug resistance protein 1 nucleotide binding domain 1 bound to Mg^{2+}/ATP reveals a non-productive catalytic site, J. Mol. Biol. 359 (2006) 940–949, https://doi.org/10.1016/j.jmb.2006.04.005.

[30] N.M.I. Taylor, I. Manolaridis, S.M. Jackson, J. Kowal, H. Stahlberg, K.P. Locher, Structure of the human multidrug transporter ABCG2, Nature 546 (2017) 504–509, https://doi.org/10.1038/nature22345.

[31] S. Jain, M. Grandits, G.F. Ecker, Interspecies comparison of putative ligand binding sites of human, rat and mouse P-glycoprotein, Eur. J. Pharm. Sci. 122 (2018) 134–143, https://doi.org/10.1016/J.EJPS.2018.06.022.

[32] M.J.S. Dewar, E.G. Zoebisch, E.F. Healy, J.J.P. Stewart, AM1: a new general purpose quantum mechanical molecular model, J. Am. Chem. Soc. 107 (1985) 3902–3909, https://doi.org/10.1021/ja00299a024.

[33] G.M. Morris, R. Huey, W. Lindstrom, M.F-. Sanner, R.K. Belew, D.S. Goodsell, A.H. Olson, AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility, J. Comput. Chem. 30 (2009) 2785–2791, https://doi.org/10.1002/jcc.

[34] G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew, A.J. Olson, Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, J. Comput. Chem. 19 (1998) 1639–1662, https://doi.org/10.1002/jcc.20634.

[35] O. Trott, A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J. Comput. Chem. 31 (2009) 455–461, https://doi.org/10.1002/jcc.21334.
[36] S.G. Aller, J. Yu, A. Ward, Y. Weng, S. Chittaboina, R. Zhuo, P.M. Harrell, Y.T. Trinh, Q. Zhang, I.L. Urbatsch, G. Chang, Structures of P-glycoproteins reveals a molecular basis for poly-specific drug binding, Science 323 (2009) 1718–1722, https://doi.org/10.1126/science.1168750.
[37] O. Carugo, S. Pongor, A normalized root-mean-spuare distance for comparing protein three-dimensional structures, Protein Sci. 10 (2008) 1470–1473, https://doi.org/10.1110/ps.690101.
[38] S.C. Gupta, R. Kannappan, S. Reuter, J.H. Kim, B.B. Aggarwal, Chemosensitization of tumors by resveratrol, Ann. N. Y. Acad. Sci. 1215 (2011) 150–160, https://doi.org/10.1111/j.1749-6632.2010.05852.x.
[39] J. Meng, F. Guo, H. Xu, W. Liang, C. Wang, X.-D. Yang, Combination therapy using co-encapsulated resveratrol and paclitaxel in liposomes for drug resistance reversal in breast cancer cells in vivo, Sci. Rep. 6 (2016) 1–11, https://doi.org/10.1038/srep22390.
[40] S. Karthikeyan, L.H. Sugeerappa, N. Rajendra Prasad, Resveratrol modulates expression of ABC transporters in non-small lung cancer cells: molecular docking and gene expression studies, J. Cancer Sci. Ther. 6 (2014) 497–504, https://doi.org/10.4172/1948-5956.1000314.
[41] M. Munoz, M. Henderson, M. Haber, M. Norris, Role of the MRP1/ABCC1 multidrug transporter protein in cancer, IUBMB Life 59 (2007) 752–757, https://doi.org/10.1080/15216540701736285.
[42] A. Rajagopal, A.C. Pant, S.M. Simon, Y. Chen, In vivo analysis of human multidrug resistance protein 1 (MRP1) activity using transient expression of fluorescently tagged MRP1, Cancer Res. 62 (2002) 391–396.
[43] A. Castro-Alvarez, A. Costa, J. Vilarrasa, A. Castro-Alvarez, A.M. Costa, J. Vilarrasa, The performance of several docking programs at reproducing protein–macrolide-like crystal structures, Molecules 22 (2017) 136, https://doi.org/10.3390/molecules22010136.
[44] Z. Wang, H. Sun, X. Yao, D. Li, L. Xu, Y. Li, S. Tian, T. Hou, Comprehensive evaluation of ten docking programs on a diverse set of protein–ligand complexes: the prediction accuracy of sampling power and scoring power, Phys. Chem. Chem. Phys. 18 (2016) 12964–12975, https://doi.org/10.1039/C6CP01555G.
[45] M.W. Chang, C. Ayeni, S. Breuer, B.E. Torbett, Virtual screening for HIV protease inhibitors: a comparison of AutoDock 4 and Vina, PLoS One 5 (2010) e11955, https://doi.org/10.1371/journal.pone.0011955.
[46] I. Abraham, S. Jain, C.-P. Wu, M.A. Khanfar, Y. Kuang, C.-L. Dai, Z. Shi, X. Chen, L. Fu, S.V. Ambudkar, K. El Sayed, Z.-S. Chen, Marine sponge-derived sipholane triterpenoids reverse P-glycoprotein (ABCB1)-mediated multidrug resistance in cancer cells, Biochem. Pharmacol. 80 (2010) 1497–1506, https://doi.org/10.1016/J.BCP.2010.08.001.
[47] M. Lv, J.-G. Qiu, W.-J. Zhang, Q.-W. Jiang, W.-M. Qin, Y. Yang, D.-W. Zheng, Y. Chen, J.-R. Huang, K. Wang, M.-N. Wei, K.-J. Cheng, Z. Shi, Wallichinine reverses ABCB1-mediated cancer multidrug resistance, Am. J. Transl. Res. 8 (2016) 2969–2980 (accessed January 30, 2019), http://www.ncbi.nlm.nih.gov/pubmed/27508017.
[48] J. Zhang, D. Li, T. Sun, L. Liang, Q. Wang, Interaction of P-glycoprotein with anti-tumor drugs: the site, gate and pathway, Soft Matt. 11 (2015) 6633–6641, https://doi.org/10.1039/C5SM01028D.
[49] M.L. Kennedy, F. Cortés-Selva, J.M. Pérez-Victoria, I.A. Jiménez, A.G. González, O.M. Muñoz, F. Gamarro, S. Castanys, A.G. Ravelo, Chemosensitization of a multidrug-resistant Leishmania tropica line by new Sesquiterpenes from Maytenus magellanica and Maytenus chubutensis, J. Med. Chem. 44 (2001) 4668–4676, https://doi.org/10.1021/JM010970C.
[50] J.M. Pérez-Victoria, B.M. Tincusi, I.A. Jiménez, I.L. Bazzocchi, M.P. Gupta, S. Castanys, F. Gamarro, A.G. Ravelo, New natural Sesquiterpenes as modulators of daunomycin resistance in a multidrug-resistant leishmania tropica line‖,⊥, J. Med. Chem. 42 (1999) 4388–4393, https://doi.org/10.1021/JM991066B.
[51] C.P. Reyes, F. Muñoz-Martínez, I.R. Torrecillas, C.R. Mendoza, F. Gamarro, I.L. Bazzocchi, M.J. Núñez, L. Pardo, S. Castanys, M. Campillo, I.A. Jiménez, Biological evaluation, structure−activity relationships, and three-dimensional quantitative structure−activity relationship studies of dihydro-β-agarofuran Sesquiterpenes as modulators of P-glycoprotein-dependent multidrug resistance, J. Med. Chem. 50 (2007) 4808–4817, https://doi.org/10.1021/JM070290V.
[52] N. Ji, Y. Yang, C.-Y. Cai, Z.-N. Lei, J.-Q. Wang, P. Gupta, Q.-X. Teng, Z.-S. Chen, D. Kong, D.-H. Yang, VS-4718 antagonizes multidrug resistance in ABCB1- and ABCG2-overexpressing cancer cells by inhibiting the efflux function of ABC transporters, Front. Pharmacol. 9 (2018) 1236, https://doi.org/10.3389/fphar.2018.01236.