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VolSurf analysis of pharmacokinetic properties for several antifungal sesquiterpene lactones isolated from Greek *Centaurea* sp.

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Summary

Sesquiterpene lactones are terpenoid compounds characteristic of the Asteraceae (Compositae) possessing a variety of biological activities, such as cytotoxic, antitumor, antibacterial, and antifungal. The prediction of the pharmacokinetic profile of several antifungal sesquiterpene lactones, isolated from Greek taxa of *Centaurea* sp., was undertaken in this study using the VolSurf procedure. The molecules were projected on the following pre-calculated ADME models: Caco-2 cell permeability, plasma protein affinity, blood–brain barrier permeation and thermodynamic solubility. The *in silico* projection revealed a non optimal pharmacokinetic profile for the studied compounds. ADME *in silico* screening of a semi-synthetic derivatives virtual library has been performed in order to optimize the pharmacokinetic properties. A number of derivatives were proposed as it was predicted to have higher Caco-2 cell permeability, while the pharmacokinetic behaviour regarding BBB penetration, protein binding and solubility was mainly preserved.

Abbreviations: ADME – absorption, distribution, metabolism, excretion; BBB – blood brain barrier

Introduction

Natural products from plant, microbial, marine, or even mammalian sources have traditionally been a major drug source and continue to play a significant role in today's drug discovery environments, perhaps preferably as pure compound libraries or scaffolds for combinatorial chemistry [1, 2]. Based on this point of view, it is of interest to apply to natural products the virtual screening methods (target- and ligand-based virtual screening), which are well-established techniques for identifying new leads [3–6].

Since absorption, distribution, metabolism and excretion (ADME) are key properties in the process of drug discovery that need to be optimized early on, it is emerged the necessity of ADME property filtering in virtual screening [7]. Recently, computational efforts have been made to obtain models that describe and predict the pharmacokinetic behaviour of a compound [8, 9].

VolSurf [10] is a computational procedure that is specifically designed to produce descriptors related to pharmacokinetic properties, starting from 3D molecular field maps. In the standard

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Part of the results has been presented in: [36] 11th Panhellenic Pharmaceutical Congress [37] 15th European Symposium on Quantitative Structure-Activity Relationships & Molecular Modelling

procedure, GRID interaction fields [11] are calculated around the target molecules. The basic concept of VolSurf is to compress the information present in 3D grid maps into few 2D numerical descriptors, which are simple to understand and to interpret. The molecular descriptors obtained refer to molecular size and shape, to size and shape of both hydrophilic and hydrophobic regions and to the balance between them. The ADME models included in VolSurf predict Caco-2 cell (human intestinal epithelial cell line derived from a colorectal carcinoma) absorption [10], protein binding [12], blood–brain barrier (BBB) permeation [13], drug–water solubility [14], drug–DMSO solubility, metabolic stability [15], hERG (human Ether-a-go-go Related Gene) inhibition and volume of distribution.

Continuing our study on sesquiterpene lactones, in a first step we attempted to predict the pharmacokinetic properties of an interesting data set of potent antifungal sesquiterpene lactones, isolated from Greek taxa of *Centaurea* sp., by projection on the pre-calculated ADME models developed in VolSurf [16–18]. In a second step, we tried to optimize the pharmacokinetic properties of data set's selected molecules by ADME *in silico* screening of a semi-synthetic derivatives virtual library.

Materials and methods

Databases

The data set consists of 22 sesquiterpene lactones for which the isolation, structural elucidation and their antifungal activity in vitro was reported in our previously studies. Compounds **1–4**, **8–10**, **13**, **15–16** and **21** were isolated from *C. achaia* Boiss. & Heldr. [16], **5–7**, **11–12**, **17–19** and **22** from *C. thessala* Hausskn. ssp. *drakiensis* (Freyn & Sint.) Georg. and *C. attica* Nym. ssp. *attica* [17] and compounds **14** and **20** from *C. deusta* Ten [*C. alba* subsp. *deusta* (Ten) Nyman] [18]. The molecules were tested against nine fungi, using a modified microdilution technique, and their activity was expressed by the values of MIC (minimum inhibitory activity) and MFC (minimum fungicidal activity). As a control was used the commercial fungicide miconazole [16–18].

3D-Structures generation

The structures were generated using SYBYL molecular modeling package [19] running on a Linux workstation under the Fedora operating system, and their energies were minimized using the Powell method with a convergent criterion provided by the Tripos force field [20].

Computational methods

Caco-2 cell permeability, plasma protein affinity, BBB permeation and thermodynamic solubility of the studied compounds were predicted using VolSurf (version 4) (www.moldiscovery.com). We used the probes water (OH2), hydrophobic (DRY) and H-bonding carbonyl (O) to generate the 3D interaction energies and a Grid space of 0.5 Å.

Gview molecular graphics system (www.moldiscovery.com) was used in order to visualize the projection of our molecules on the models.

Results and discussion

Sesquiterpene lactones are natural products belonging in many families of plants, more distributed in the family Asteraceae (Compositae). They display a wide spectrum of biological activity [reviews: 21–25]; one of the most important is the antifungal activity [26–28].

The general mechanism of action is considered to be an alkylation of biological nucleophiles such as cysteine (cys) [29] and glutathione (GSH) or sulfhydryl-containing systems (phosphofructokinase [30] and glycogen synthetase [31]) by α,β-unsaturated carbonyl structures in a Michael-type addition.

The principal requirements for the exhibition of their biological activity are at least: (a) the presence of an exocyclic methylene conjugated to a γ-lactone and (b) the presence of a functional group, such as an epoxide, hydroxyl, chlorohydrin, unsaturated ketone or *O*-acyl adjacent to the α-CH_2 of γ-lactone, which can enhance the reactivity of the conjugated lactone toward biological nucleophiles [21].

However, these do not appear to be the only requisites in fungi or other living systems; other

requirements such as molecular accessibility and lipophilicity seem to play an important role for their antifungal activity [25]. Differences in the physiology of the individual fungal species must also be considered [32].

In this study we investigated the pharmacokinetic properties of 22 sesquiterpene lactones through projection on predefined models. The molecules belong to the classes of germacranolides, elemanolides and eudesmanolides (Figure 1), for which the antifungal activity was determined previously [16–18]. According to MICs and MFCs values it was demonstrated that germacranolides **1–8** possess greater antifungal potential than the other groups: elemanolides **9–12**, eudesmanolides **15–20** and the hydroxy ester derivatives **13–14**, **21–22**.

The interesting arisen finding that all compounds have shown to possess, a fungicidal potential higher than miconazole (the commercial fungicide used as a control) encouraged us to investigate their pharmacokinetic profile, using the VolSurf procedure.

The molecules were projected on the precalculated models: Caco-2 cell absorption, plasmatic proteins binding, BBB passage and thermodynamic solubility. From the plots (Figure 2a–d) it is predicted that the studied compounds can not be transported across the intestinal epithelium, they have a low affinity to the plasma-protein, they can not cross to the BBB and are medium-low aqueous soluble. However, it should be taken into consideration the prediction of protein binding, BBB and solubility, since several compounds cover an empty chemical space. The prediction for those molecules could be doubtful.

The results of the *in silico* study suggest a non optimal pharmacokinetic profile. The low permeability and the limited solubility suggest that the *per os* administration of these molecules could not be effective. A topical application may be preferable when applicable. The low penetration through the BBB suggests low toxicity at the central nervous system (CNS) level.

It is important to note that these natural compounds are predicted to have a pharmacokinetic behaviour quite different compared to miconazole. As it is observed in Figure 2a–d, miconazole is predicted to have higher Caco-2 cell permeability and higher affinity for the plasmatic proteins. However, the predicted permeability of miconazole across the BBB is higher than those predicted for our compounds. Conversely, our compounds are predicted to have a low BBB penetrating ability.

Therefore, in a further study our aim was to modify the chemical structure of the isolated sesquiterpene lactones in order to optimize the solubility and the permeability by the intestinal barrier. However, it should be noted that low polarity is one of the molecular requirements

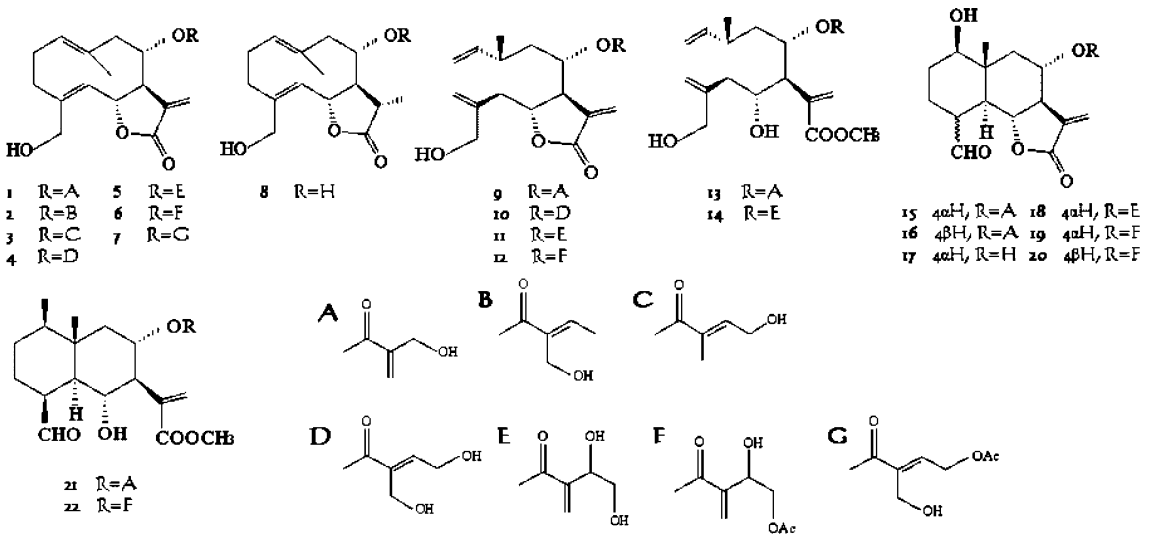

Figure 1. Chemical structure of the studied compounds.

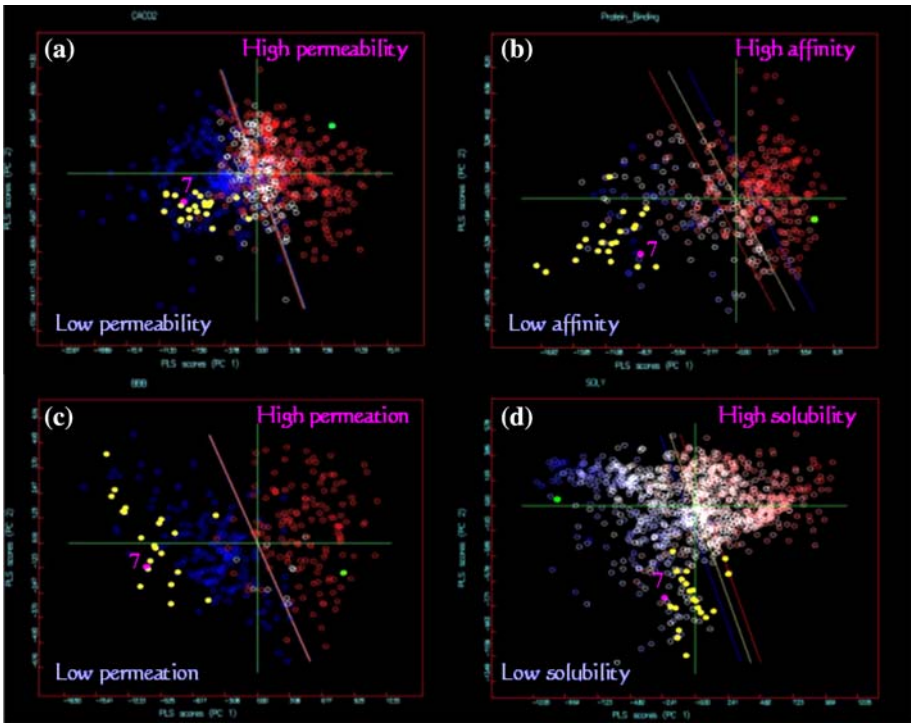

Figure 2. Projection of the studied compounds (compounds: **1**–**6**, **8**–**22** yellow; compound **7** pink; miconazole: green) on the precalculated VolSurf models (a) Caco-2 (b) protein binding (c) blood–brain barrier (d) solubility (Passive permeation is the basic assumption of Caco-2 and BBB models).

suggested for the antifungal activity of sesquiterpene lactones [25, 28, 33]. As it is observed from the reported tables of MICs and MFCs values [16–18], among the studied compounds, germacranolides which have retention times on a RP-18 column higher than the other groups are the most active. This supports the hypothesis of an inverse relationship between polarity and antifungal activity for sesquiterpene lactones.

Taking into account the aforementioned requirement, we focused our study only on the optimization of the Caco-2 permeability of our molecules. The idea was to modify the chemical structure of the compounds through substitution of a group, by 100 often used in synthetic chemistry fragments, maintaining at the same time the pharmacodynamic requirements.

Among the studied compounds the most interesting for *in silico* modifications considered to be compound **7**. Compound **7** (germacranolide), was demonstrated to be the most potent, possessing higher MICs values, against six of the nine examined fungi (*Aspergillus niger*, *A. ochraceus*, *A. versicolor*, *Penicillium ochrochloron*, *P. funiculosum* and *Trichoderma viride*) and higher MFCs values for two fungi (*Aspergillus niger*, *A. ochraceus*) with poor predicted Caco-2 permeability.

An interesting site to coordinate the fragments was the position of esterification C-8 (Figure 3).

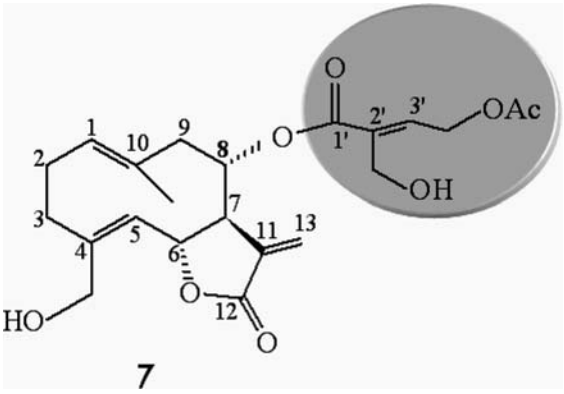

Figure 3. Chemical structure of compound **7**. Highlighted area indicates the site of substitution.

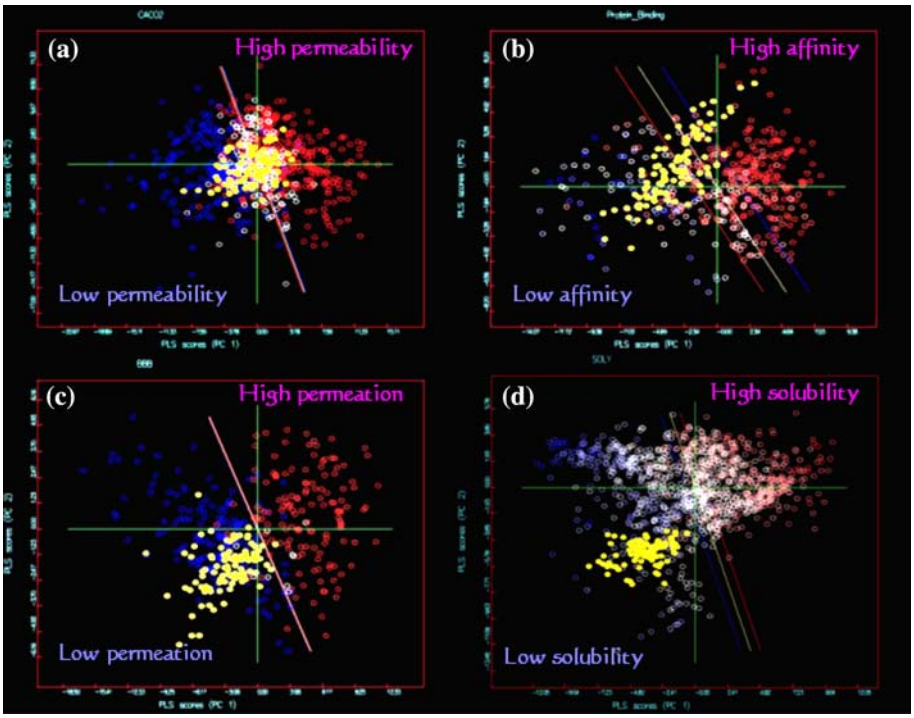

Figure 4. Projection of the 100 derivatives of molecules **7** (yellow colored circles) on the models (a) Caco-2, (b) protein binding, (c) BBB and (d) solubility.

As the biological activity of sesquiterpene lactones is generally attributed to the alkylating property of the α-methylene-γ-lactone moiety, we thought to maintain this moiety in compound **7** and modify the side chain that contains the second alkylating site, the conjugated carbonyl group.This introduction was anticipated to modify the molecule leading to a more permeable one. The 100 derivatives of compound **7** were generated and reprojected on Caco-2 permeability model. It was observed that several molecules are predicted to be permeable (yellow colored circles located in the red region of the model) (Figure 4a), while the pharmacokinetic behaviour regarding protein binding, BBB penetration and solubility was mainly preserved (Figure 4b–d). A part of the predicted Caco-2 permeable compounds (peroxides were excluded due to possible

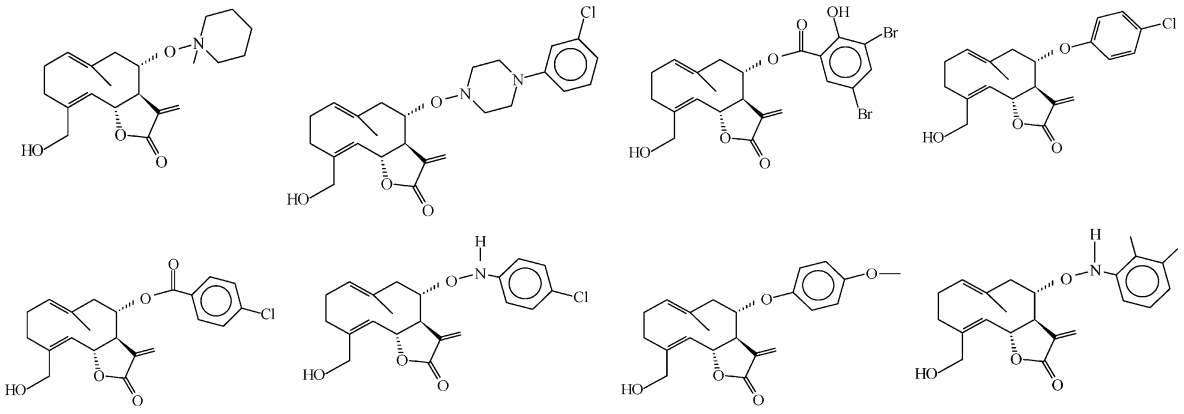

Figure 5. Structure of some permeable Caco-2 derivatives.

instability of these molecules in physiological conditions) could be proposed for a synthetic work in order to experimentally examine their pharmacokinetic profile (structures depicted in Figure 5).

Conclusion

In this study, it was investigated the pharmacokinetic profile of 22 sesquiterpene lactones, isolated from different Greek taxa of *Centaurea* sp., with the use of the computational method VolSurf. In general, it was predicted the studied compounds to exhibit poor pharmacokinetic properties. Our aim was to optimize the pharmacokinetic profile, by ADME *in silico* screening of a semi-synthetic derivatives virtual library. We identified several molecules which are predicted to permeate a Caco-2 cell-layer, but preserving at the same time the pharmacokinetic profile respect to protein binding, BBB penetration and solubility. A synthetic work concerning the proposed molecules could be interesting in examining their experimental pharmacokinetic properties.

Note

Color plates can be viewed in the online version of the paper.

Acknowledgements

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