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Accessing biological actions of *Ganoderma* secondary metabolites by *in silico* profiling

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ABSTRACT

The species complex around the medicinal fungus *Ganoderma lucidum* Karst. (Ganodermataceae) is widely known in traditional medicines, as well as in modern applications such as functional food or nutraceuticals. A considerable number of publications reflects its abundance and variety in biological actions either provoked by primary metabolites, such as polysaccharides, or secondary metabolites, such as lanostane-type triterpenes. However, due to this remarkable amount of information, a rationalization of the individual *Ganoderma* constituents to biological actions on a molecular level is quite challenging. To overcome this issue, a database was generated containing meta-information, *i.e.*, chemical structures and biological actions of hitherto identified *Ganoderma* constituents (279). This was followed by a computational approach subjecting this 3D multi-conformational molecular dataset to *in silico* parallel screening against an in-house collection of validated structure- and ligand-based 3D pharmacophore models. The predictive power of the evaluated *in silico* tools and hints from traditional application fields served as criteria for the model selection. Thus, the focus was laid on representative druggable targets in the field of viral infections (5) and diseases related to the metabolic syndrome (22). The results obtained from this *in silico* approach were compared to bioactivity data available from the literature. 89 and 197 *Ganoderma* compounds were predicted as ligands of at least one of the selected pharmacological targets in the antiviral and the metabolic syndrome screening, respectively. Among them only a minority of individual compounds (around 10%) has ever been investigated on these targets or for the associated biological activity. Accordingly, this study discloses putative ligand target interactions for a plethora of *Ganoderma* constituents in the empirically manifested field of viral diseases and metabolic syndrome which serve as a basis for future applications to access yet undiscovered biological actions of *Ganoderma* secondary metabolites on a molecular level.

1. Introduction

The species complex of *Ganoderma* is one of the most important sources for medicinal mushrooms (Lindequist et al., 2010). Especially Asian countries, like the People's Republic of China, Japan, and Korea have developed a long-standing and strong tradition in using *Ganoderma* preparations for the prevention and treatment of various diseases (Paterson, 2006). Major medicinal properties related to this macro-fungal source include, *e.g.*, anticancer, antibiotic, and antiviral activities, as well as immune response-stimulating, anti-hypertensive, and blood lipid lowering effects (Barros et al., 2008). In many studies, bioactivities have been determined on a phenotypic level (primary bioassays) performed with complex multi-component mixtures, without chemical characterisation or without specifying the molecular target (secondary bioassays). This might be due to time-consuming isolation and identification processes, as well as cost-intensive target-specific in-depth pharmacological tests. However, in regard to analytical quality control measures of commercially relevant *Ganoderma* preparations or in the search for novel drug leads, there is an urgent need to especially assess the biological effects of secondary metabolites in a fast and straightforward way.

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In the case of *Ganoderma* constituents the amount of available structural information is substantial; in the category of secondary metabolites, more than 200 different lanostane-type triterpenes have been described (Paterson, 2006). Moreover, in past years many additional bioactive secondary metabolites were described for this fungal material due to considerable progress in techniques for dereplication, isolation, analysis, and pharmacological evaluation. Major *Ganoderma* constituents are triterpenes of the fungal cell membrane, *e.g.*, ergosterol and derivatives such as ergosterol peroxide, γ-ergostenol, α-dihydroergosterol, ergosta-4,6,8(14),22-tetraen-3-one, or (3β,5α,8α,22E)-5,8-epidioxy-ergosta-6,9(11),22-trien-3-ol. Furthermore, according to quantification studies, ganoderic acids A, AM1, B, C1, C2, D, DM, F, G, H, K, Me, Mk, S, T, TR, Y, and ganoderenic acids A, B, D, as well as ganoderols A, B, ganoderiol F, ganodermatriol, ganoderal A, Me ganoderate D, ganoderate G, and lucideric acid A, can be considered as major lanostane-type triterpenes (Liu et al., 2012; Yan et al., 2013; Zhao et al., 2006). *Ganoderma* constituents which are not often reported in literature and thus are considered as minor components include, *e.g.*, lucialdehyde E, ganoderiol C, lucidenic acid J, or ganodermaside A.

In general, these secondary metabolites are isolated only in low amounts and therefore are not commercially available, which represents a bottleneck for in-depth biological characterisation.

To overcome these issues and moreover as a way to close the knowledge gap of this large pool of pharmacologically almost untapped pure constituents, the application of *in silico* based methods might be a promising strategy for prioritising bio-assays.

The generation of pharmacophore models, which can be used for virtual screening to predict and rationalize a compounds' biological activity, requires structural data of either the protein or of known ligands; at best both is available. In an attempt to increase the propensity of identifying bioactive molecules it is further crucial to properly validate the models, to understand and to critically review the obtained *in silico* results (Scior et al., 2012). This study is focused on a selection of pharmacologically relevant targets for which already sufficient data is available, *i.e.*, druggable targets. Here, two distinctly different disease areas, *i.e.*, viral infections and pathological conditions related to the metabolic syndrome, have been chosen on the basis of the traditional application of *Ganoderma* products in these fields (Lindequist et al., 2010; Sanodiya et al., 2009).

Metabolic syndrome is an umbrella term that comprises multiple individual pathological conditions, *e.g.*, obesity, elevated blood pressure, glucose intolerance, and altered lipid metabolism, with the primary outcome of increasing the risk for cardiovascular disease (Grundy et al., 2004). Accordingly, the pathogenesis of the metabolic syndrome is very complex and involves many pharmacological targets of which we selected 22 well-characterised ones for this study.

For the investigation of *Ganoderma* antiviral activities, the focus was laid on druggable targets of the human rhinovirus (HRV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and the influenza virus.

Especially in natural product research, with an abundance of possible structural scaffolds, which can interact with a huge number of pharmacological targets, a rationalised strategy for the identification of bioactivity and the discovery of leads is essential. *In silico* prediction tools such as ChemGPS and PASS or the concept of protein fold topology have proven to be especially effective in this respect (Goel et al., 2011; Larsson et al., 2005; McArdle and Quinn, 2007). Moreover, as reported before, the technique of pharmacophore-based parallel screening has been successfully applied to natural compound databases in our research group (Rollinger, 2009; Rollinger et al., 2009). Here, each pharmacological target of interest is represented by one or more validated pharmacophore models (structure- or ligand-based). Each model is composed of steric and electronic features which are necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger or block its biological response (Wermuth et al., 1998). Hence, when screening a compound structural database against a well-defined set of pharmacophore models, pharmacological profiles of these molecules can be predicted.

In sum, the concept of *in silico* parallel screening allows for combining two spaces, both the biological space (*i.e.* the pharmacological target) and the chemical space (*i.e.* the compound structure), and thus might give valuable hints for the straightforward access of biological activities of *Ganoderma* compounds. Moreover, with this strategy, the aim is to systematically access the longstanding tradition of applying this fungal remedy for viral infections and disorders related to metabolic syndrome on a molecular level.

2. Results and discussion

2.1. Ganoderma database

As a starting point for this study, an extensive literature search was performed to collect structural, as well as available bioactivity information of *Ganoderma* secondary metabolites.

The genus *Ganoderma* contains several species which are not easily distinguishable; thus, it is often referred to as a species complex (Szedlay, 2002). In most cases, reliable species delimitation is only possible by interpretation of morphological–ecological characteristics in combination with molecular phylogenetic data. In literature, a clear identification of species is often neglected. However, traditionally used *Ganoderma* species (*e.g. G. lucidum*, *Ganoderma applanatum*, *Ganoderma tsugae*) contain a very similar pattern of constituents (*e.g.* lanostane-type triterpenes) and therefore the whole genus *Ganoderma* was considered for evaluation.

This survey led to the generation of a 3D multi-conformational *Ganoderma* molecular structure database (*i.e. Ganoderma* DB) containing a total number of 279 constituents (see Table S1 in Supporting information; the sd-file of the database is available as Supplementary material). For about 45% of *Ganoderma* compounds only structural information was collected since no data on biological activity was reported exclusively for these constituents. Concerning structure classes, the *Ganoderma* DB is mainly composed of lanostane-type triterpenes (97%). This chemical class can be subdivided in 188 triterpene acids and esters, 66 triterpene alcohols and ketones, 3 lanostane peroxides, 2 glycosidic lanostanes, and 7 miscellaneous terpenoids (see Fig. 1 for examples). Among other scaffolds, 9 long-chain saturated and unsaturated fatty acids and derivatives thereof, one benzofuran and two hydroquinone derivatives are reported as constituents from *Ganoderma* sp. (Table S1).

2.2. Pharmacophore model collection and in silico profiling

In silico profiling of the *Ganoderma* DB was performed using a set of previously generated 3D chemical feature-based pharmacophore models. In general, a pharmacophore model represents the binding mode of a certain compound to a specific drug target. A model represents chemical features such as H-bond donors or acceptors, hydrophobic groups, and positive or negative ionizable moieties which encode chemical functionalities of a ligand. However, ligands for the same target may adopt different binding modes; thus, multiple models might be required to cover all of these binding modes (Schuster, 2010). A major selection criterion for choosing a pharmacological target for the generation of a robust pharmacophore model is the availability of sufficient data.

Accordingly, focus was laid on targets in the field of viral infections and the area of diseases related to the metabolic syndrome. This led to the selection of an array of well-defined in-house struc-

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