Final report

On the postdoctoral proposal entitled

”Isolation and structure determination of biologically active metabolites of Hungarian macrofungi”

Proposal number: 124476
Project type: postdoctoral (PD)
Project start: 2017/09/01
Closing date: 2019/07/31
Principal investigator: Attila Ványolós

1. Introduction

The vernacular term mushroom is applied for macrofungi with distinctive fruiting bodies observable to the naked eye.[1] The number of different mushroom species is estimated to be 140,000, of which only 10% are known to science, and about 700 species are known to possess significant pharmacological properties.[2] Hungary thanks to its varied relief is one of the European countries with higher biodiversity in wild mushrooms, some of them with remarkable gastronomic importance and several others with significant therapeutic potential. More than 3000 mushrooms are indigenous to Hungary, but to date, however, there has been very few detailed scientific study of these mushrooms in terms of their potential pharmacological benefits. The most important objectives of the proposed project were to carry out pharmacological and chemical screenings of Hungarian mushroom species; perform a preparative chemical work with selected fungal species; determine the structures of the isolated compounds; to investigate the cytostatic, antioxidant, ion channel inhibitory and antimicrobial activity of the pure compounds, and finally to identify novel compounds as potential candidates for drug development.

2. Results

Collection, identification and screening for GIRK channel activity of mushrooms native to Hungary

As part of the screening study the GIRK channel inhibitory effects of some mushroom extracts obtained from species indigenous to Hungary were evaluated. Totally, 40 extracts of 10 higher basidiomycete mushrooms were assessed: *Gymnopus dryophilus, Gymnopus fusipes*, *Hebeloma sacchariolens*, *Hypholoma fasciculare*, *Hypholoma lateritium*, *Laetiporus sulphureus*, *Megacollybia platyphylla, Rhodocybe popinalis Tricholoma populinum* and *Tricholomopsis rutilans*. To the best of our knowledge no other study has previously investigated the potential GIRK channel activity of mushrooms. According to the results obtained some fungal species display significant inhibitory activity on GIRK channel. Among the fractions with different polarities, fraction A (*n*-hexane fractions with the more lipophilic constituents) and fraction B ($CHCl_3$-soluble compounds) proved to be active (i. e., at least 50% decrease in the current at 0.1 mg/mL concentration). The aqueous (fraction D) and aqueous MeOH (fraction C) extracts did not exert considerable activity on GIRK channel. However, there are some exceptions: neither fraction B of *R. popinalis* nor fraction A of *T. populinum* exerted remarkable blocking effect on the GIRK channel, whereas fractions C of *L. sulphureus* and *H. lateritium* proved to have notable inhibitory activities. Fraction B of *H. lateritium* proved to be the most effective (53% decrease on GIRK current) at the lower (0.01 mg/mL) concentration among the

tested fungal extracts. Therefore, this species was chosen for in-depth chemical analysis in order to identify the secondary metabolites responsible for the observed ion channel activity.[3]

Isolation and structure determination of bioactive metabolites from *Hypholoma lateritium*

Twelve compounds (**1**–**12**) were isolated from the methanol extract of brick cap mushroom (*Hypholoma lateritium)* (Figure 1.). The structures of the compounds were elucidated using extensive spectroscopic analyses, including NMR and MS measurements. Lanosta-7,9(11)-diene-12β,21α-epoxy-2α,3β,24β,25-tetraol (**1**) and 8-hydroxy-13-oxo-9*E*,11*E*-octa-decadienoic acid (**2**) were identified as new natural products, together with ten known compounds, from which 3β-hydroxyergosta-7,22-diene (**4**), demethylincisterol A2 (**5**), cerevisterol (**6**), 3β-*O*-glucopyranosyl-5,8-epidioxyergosta-6,22-diene (**7**), fasciculol E (**9**), and uridine (**12**) were identified in this species for the first time.

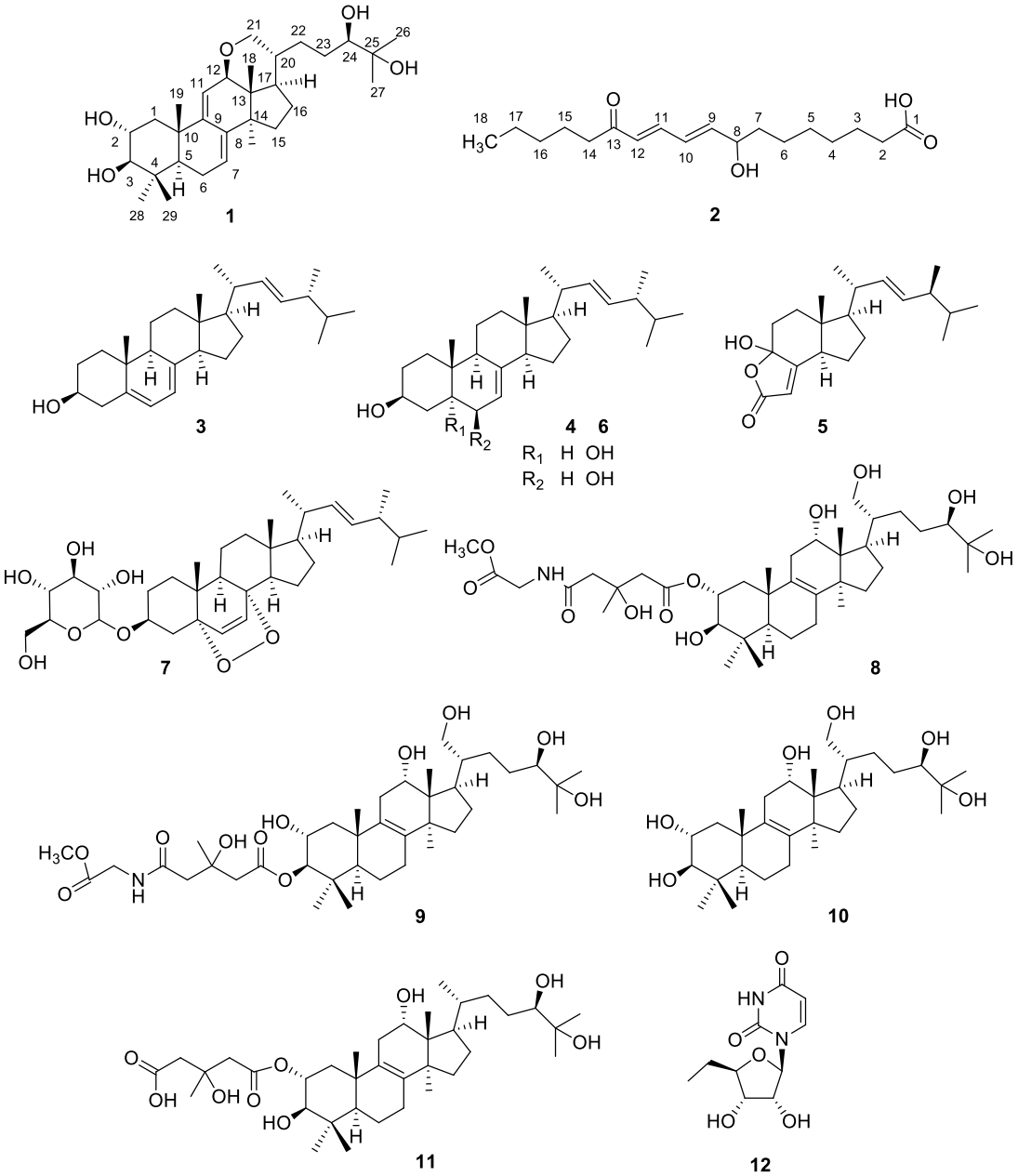


Figure 1. Structure of compounds (**1**-**12**) isolated from *Hypholoma lateritium*

The isolated triterpenes (**1**, **3**–**11**) were investigated for their toxicity in vivo using bdelloid rotifer assays. Most of the examined steroids in general showed low toxicity, although the effects of the compounds varied in a wider range from the non-toxic lanosta-7,9(11)-diene-12β,21α-epoxy-2α,3β,24β,25-tetraol (**1**) to the significantly toxic cerevisterol (**6**), with substantial dependence in some cases on the presence of nutrient in the experimental environment. This study provides the most exhaustive chemical analysis of the mushroom *Hypholoma lateritium*, affording not only novel information on the characteristic secondary metabolites of this species, but also valuable results of in vivo toxicity assays of isolated compounds.[4]

Evaluation of the ion channel activity of mushroom metabolites (**1**, **3-11)** revealed that lanosta-7,9(11)-diene-12β,21α-epoxy-2α,3β,24β,25-tetraol (**1**) demonstrates remarkable blocking activity on GIRK current (IC_{50} 395.1 ± 31.8 nM). Investigation of the selectivity of the GIRK inhibitory effect proved that lanosta-7,9(11)-diene-12β,21α-epoxy-2α,3β,24β,25-tetraol (**1**) has only weak inhibitory activity on hERG channel (7.91 ± 2.83% at 100 μM), exerting more than three orders of magnitude lower blocking activity on hERG channel than on GIRK channel. Considering its intense blocking effect and high selectivity, **1** could be a potential promising agent in treatment of atrial fibrillation.[3]

Isolation of antiproliferative secondary metabolites from *Scleroderma bovista*

In preliminary experiments we revealed the antiproliferative properties of different extracts prepared from *Scleroderma bovista* (results not published), therefore we conducted a research for the identification of compounds responsible for the observed biological activity. The detailed chemical analysis of the methanol extract obtained from the lyophilized sporocarps of *Scleroderma bovista* resulted in the identification of seven compounds (Figure 2.).

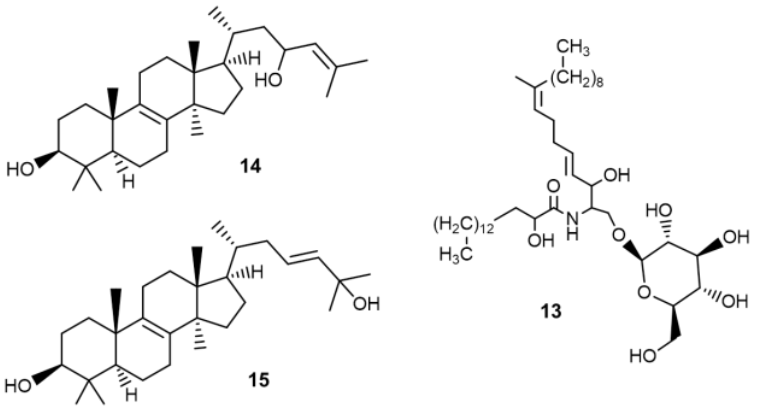


Figure 2. Structures of compounds **13**-**15** isolated from *Scleroderma bovista*

All compounds have been identified for the first time from this species. **3**-**4** and **6**-**7** have ergostane structures: 3β-hydroxyergosta-7,22-diene (**3**), ergosterol (**4**), cerevisterol (**6**) and 3β-O-glucopyranosyl-5,8-epidioxyergosta-6,22-diene (**7**). Compound **13**, known as cerebroside B possesses a ceramide unit linked to a glucose moiety. Compound **14** is 23-hydroxylanosterol, while (**15**) is lanosta-8,23-dien-3β,25-diol. The isolated compounds **7** and **13-15** were tested for their in vitro antiproliferative activity by MTT method on four different human cancer cell lines (HeLa, A2780, MDA-MB-231 and MCF-7). Compounds **14** and **15** having lanostane structures, proved to be the most active against three cell lines (HeLa, A2780 and MCF-7).[5]

Bioactivity guided isolation of antioxidant and antibacterial compounds from *Tapinella atrotomentosa*

In a previous study we highlighted the antimicrobial potential of extracts of *Tapinella atrotomentosa*, among other fungal species native to Hungary.[6] Bioassay-guided fractionation of the chloroform extract of *Tapinella atrotomentosa* led to the isolation of four secondary metabolites (**16**-**19**). Two of the compounds are lactones, osmundalactone (**16**) and 5-hydroxy-2-hexen-4-olide (**17**), while **18** and **19** were identified as terphenyl quinones, spiromentins C and B, respectively (Figure 3.).

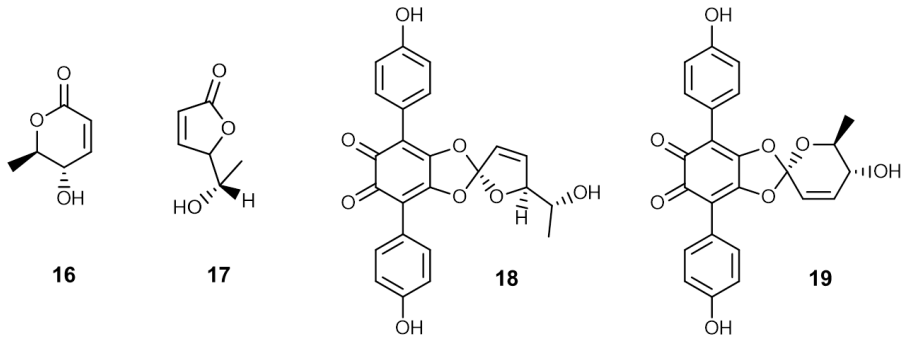


Figure 3. Structure of compounds **16**-**19** isolated from *Tapinella atrotomentosa*

The isolated fungal metabolites were evaluated for their antibacterial activities against several Gram-positive and –negative bacteria. In addition, their synergistic effect with cefuroxime against *methicillin-resistant Staphylococcus aureus (MRSA)* was also evaluated. Compounds **16**-**18** proved to possess significant antibacterial activity against multiresistant *Acinetobacter baumannii* and *extended-spectrum beta-lactamase (ESBL)*-producing *Escherichia coli* (Table 1.). The investigation of the antioxidant effect of the isolated compounds in DPPH and ORAC assays revealed that spiromentins C (**18**) and B (**19**) have remarkable antioxidant activity (Table 2.).[7]

Table 1. Antibacterial activity of compounds **16**–**19** expressed in MIC values

	Calculated MIC values (μg mL^{-1})			
Compound	MACI	ESBL *E. coli*	*Mor. catarrhalis*	MRSA
16	10	10	–	250
17	6	10	50	250
18	20	10	50	250
19	–	100	–	–

MACI: multiresistant *Acinetobacter baumannii*, ESBL *E. coli*: extended-spectrum beta-lactamase producing *Escherichia coli, Mor. catarrhalis*: *Moraxella catarrhalis*, MRSA: methicillin-resistant *Staphylococcus aureus*

Table 2. Antioxidant activity of compounds **16**-**19** in ORAC assay

Compound	**ORAC antioxidant activity (mmol TEg^{-1})**
16	0.74 ± 0.30
17	3.85 ± 0.34
18	16.21 ± 0.38
19	11.23 ± 0.58
Ascorbic acid	6.97 ± 0.01

Investigation of the chemical profile of *Porodaedalea chrysoloma*

The chemical analysis of the methanol extract of *Porodaedalea chrysoloma* afforded the isolation of five compounds (**3**-**4**, **20**-**22**). The first two are phenolic derivatives: methyl (*E*)-3-(4-methoxycarbonylphenoxy)-acrylate (**20**) is a new natural product, while methyl 3-(4-methoxycarbonylphenoxy)-propionate (**21**) was isolated from a natural source for the first time (Figure 4.). The triterpene steroids ergone (**22**), 3β-hydroxyergosta-7,22-diene (**3**), and ergosterol (**4**) have not been previously identified in this species. The isolated fungal metabolites **1**-**5** were evaluated for their antioxidant activity. Compounds **20** and **3** proved to possess considerable antioxidant effect in the ORAC assay with 2.21 ± 0.34 and 5.02 ± 0.47 mmol TE/g, respectively.[8]

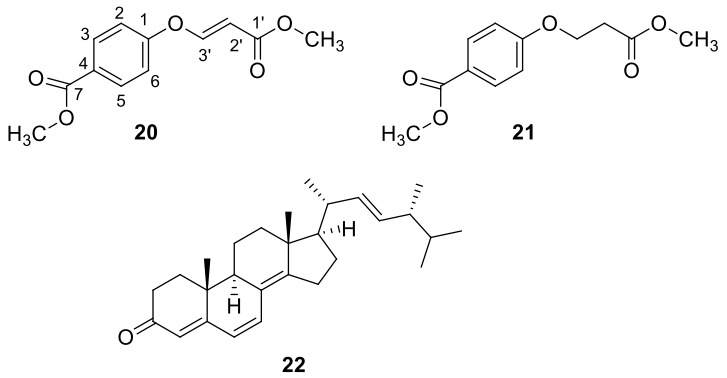


Figure 4. Structure of compounds isolated from *Porodaedalea chrysoloma*

Other results and achievements

Related to the topic of this project – based on the results obtained – the following dissertation of the PhD student Bernadett Kovács was defended:

”From cyclic peptides to terphenyl quinones: biologically active metabolites from Hungarian mushrooms” (2019).

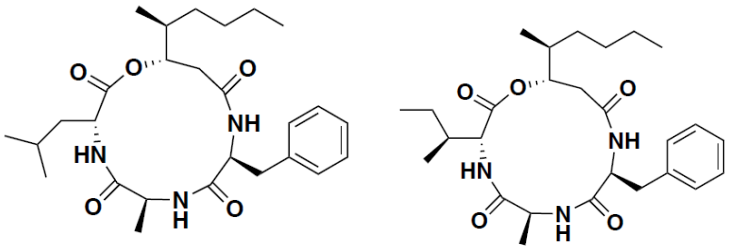
Poster presentation "Hungarian mushrooms as untapped source of natural products: from screening studies to biologically active metabolites" was selected to receive the Best Poster Award at the Conference of the Society for Medicinal Plant and Natural Product Research in Basel, 2017.

3. Future perspective

The closing date of this project has been changed from August 2020 to July 2019, so the originally 3 years decreased to 2 years. Therefore some of the experiments planned for the original term of 3 years are already performed, but their publication is not yet finished, it will be completed in the following period. Based on these results several articles are expected to be submitted and appear in the next 1-1.5 years. These planned publications and some of those already appeared will finally serve for the dissertations of PhD students András Sárközy and Bayar Chuluunbaatar.

The above mentioned experiments include but not limited to the isolation of cyclic peptides beauveriolide I and III with immunomodulatory property from *Cordyceps sp.* (Figure 5.), identification of triterpene steroids with unusual structures from *Fuscoporia torulosa*, examination of the anti-

inflammatory property of metabolites from *Hypholoma lateritium,* and isolation of antioxidant compounds from *Meripilus giganteus.*



23 **24**

Figure 5. Structures of beuaveriolides I (**23**) and III (**24**) isolated from *Cordyceps sp*.

4. Summary

In the frame of this research project the detailed chemical and pharmacological investigation of several mushroom species have been performed. The results obtained highlight the remarkable structural diversity of fungal metabolites (steroids, terphenyl quinones, cyclic peptides, depsipeptides, lactones, ceramides etc.) with promising pharmacological properties (antiproliferative, ion channel modulating, antibacterial, antioxidant etc.). Among the main outcomes of this project the identification of triterpenes with GIRK channel blocking and anti-infalmmatory properties from the edible mushroom *Hypholoma lateritium* as well as the immunomodulatory cyclic peptides from the medicinal mushroom *Cordyceps sp*. could deserve special attention because of their potential application as leading molecules in drug development.

References

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