Isolation and structure determination of bioactive metabolites from *Clitocybe nebularis* and *Pholiota populnea*

Summary of PhD Thesis

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2023

INTRODUCTION

Mushrooms are visible fungi found worldwide, with over 5000 species identified. They are classified into Ascomycota or Basidiomycota. Some species are poisonous, while others are safe to eat. Mushrooms are known for their nutritional, medicinal, and bioremediation benefits due to their high nutrient and bioactive compound content. Triterpenoids, such as lanostane and ergostane derivatives, are common in mushrooms. These compounds possess various medicinal properties, including anti-inflammatory, antitumor, antioxidant, and immunomodulatory activities.

Cancer is a global health concern, with multidrug resistance (MDR) being a challenge in treatment. MDR is associated with altered cellular pharmacokinetics, including drug efflux mediated by ATP-binding cassette (ABC) transporters like P-glycoprotein (P-gp). Certain triterpenoids, like ginsenoside Rh2, have shown the ability to reverse drug resistance in cancer cells.

Anti-tumor activity refers to substances or treatments that inhibit tumor growth through mechanisms like cell division inhibition, apoptosis induction, inflammation reduction, or immune response enhancement. Fungal triterpenes, such as lepiotaprocerins and poricoic acid GM, have demonstrated anti-inflammatory effects.

The genus *Pholiota* is composed of saprotrophic mushrooms in the family Strophariaceae. *Pholiota* species are small to medium-sized, fleshy mushrooms, which are typically live on woods. The genus contains about 150 species, and has a widespread distribution, especially in temperate regions. According to the literature, the number of studies on the genus *Pholiota* has recently increased. This can be exemplified by *Pholiota nameko* and *Pholiota adiposa*, from which polysaccharides with antioxidant properties have been identified. From *Pholiota adiposa* ergosta-4,6,8(14),22-tetraen-3-one with antidiabetic effects, methyl gallate with antioxidant and anti-HIV activities were isolated besides of stigmasterol, and a novel spiroaxane sesquiterpene. Moreover, novel polyketides from *Pholiota sp*, and a highly potent antimicrobial styrene, bisnoryangonin were obtained from *Pholiota aurivella*. Despite previous investigations, several members of the genus *Pholiota* have not yet been investigated and their mycochemical, pharmacological potential remains unexplored.

The genus *Clitocybe* predominantly consists of saprotrophic species, decaying forest ground litter. There are estimated to be around 300 species in the widespread genus. Only a few representatives of the genus are thought to be edible; the majority are poisonous, many of which contain the toxin muscarine. Seldom are *Clitocybe* mushrooms harvested for food. *C. nebularis* was previously studied for macromolecules and polar compounds, therefore our study aimed at the characterization of the lipophilic metabolites.

AIMS OF THE STUDY

Recently, the research team of Department of Pharmacognosy at the University of Szeged started a screening program to investigate the pharmacological activities of Hungarian fungi and identify the bioactive compounds found in specific mushrooms. As part of the ongoing project, the primary goal of this study was to identify the biologically active components of *Pholiota populnea* and *Clitocybe nebularis* mushrooms, as well as to analyze their chemical and pharmacological properties. To accomplish the objectives, the following activities were undertaken:

- Review the literature and previous screening results related to the selected species, placing particular emphasis on its chemical composition and pharmacological properties.
- Grind the mushroom materials that have been collected and carrying out extraction.
- Perform solvent-solvent partition and various chromatographic methods to separate and isolate the pure components.
- Structure elucidation of the isolated compounds using NMR and MS methods (collaborating with the Institute of Pharmaceutical Analysis, University of Szeged, Szeged, Hungary, and Richter Gedeon Plc., Hungary). Provide characteristic NMR spectroscopic data for the undescribed constituents.
- Evaluate the isolated compounds for their pharmacological potential (at the Department of Pharmacognosy in collaboration with the Department of Medical Microbiology and Immunobiology, Szeged, Hungary).

MATERIALS AND METHODS

Samples of *Pholiota populnea* (Pers.) Kuyper & Tjall.-Beuk. (Strophariaceae) were collected in the autumn of 2017 near Szeged, Hungary, and identified by Attila Sándor (Mushroom Society of Szeged, Hungary). Fruiting bodies of *P. populnea* (4.2 kg) were stored at −20 °C until processing. A voucher specimen (No. H019) was deposited at the Department of Pharmacognosy, University of Szeged, Hungary. Samples of *Clitocybe nebularis* (Batsch) P. Kumm. were collected in 2017 from the environs of Sándorfalva and Csákányospuszta, Hungary and identified by A. Sándor (Hungarian Mycological Society) and V. Papp. Voucher specimens have been deposited in the mycological collection of the Hungarian Natural History Museum (VPapp-1110171).

The compounds were isolated by combination of various chromatographic techniques, such as flash chromatography (FC), gel filtration chromatography (GFC), preparative thin-layer chromatography (PTLC), and high-performance liquid chromatography (HPLC). Sephadex LH-20 and normal (NP) or reversed phase (RP) silica gel were applied as stationary phases. The structure

elucidation of the isolated compounds was performed by means of NMR (1D and 2D) and HRMS spectroscopy.

The pharmacological activities of the isolated compounds were assessed by various biological assays. Several compounds were tested to determine their antiproliferative properties against Colo205, Colo320, MCF-7, MRC-5, and A549 cell lines. Selected compounds underwent cytotoxicity screening, while others were subjected to a rhodamine 123 accumulation assay. Additionally, a checkerboard combination assay was performed specifically on the Colo320 colon adenocarcinoma cell line. The antimicrobial activity of several compounds was evaluated against both standard and clinical strains. Furthermore, the effects of certain compounds on COX-1, COX-2, 5-LOX, and 15-LOX enzymes were examined to evaluate their anti-inflammatory activity.

RESULTS AND DISCUSSION

Isolation of compounds from *Pholiota populnea*

The fresh mushroom material (4.2 kg) was ground in a blender and then percolated with MeOH (20 L) at room temperature. After concentration, the dry residue (151 g) was dissolved in 600 mL 50% aqueous MeOH and subjected to solvent–solvent partition with *n*-hexane (5 × 500 mL), $CHCl_3$ (5 × 500 mL), and EtOAc (5 × 500 mL) (**Figure 1**). The *n*-hexane-soluble phase (24 g) was subjected to NP-FC1 separation. Fractions with similar compositions were combined according to TLC monitoring (H1–H26). These fractions were further separated by FC and PTLC methods to obtain six compounds (**1**-**4**, **21**, and **22**). The chloroform-soluble phase part A and B (32.3 g) were further analyzed by using combined chromatographic techniques (FC, PTLC, GFC, and HPLC) to provide 10 compounds (**8**, **11**-**17**, **19**, and **20**). Investigation of the EtOAc-soluble phase (16.7 g) led to the isolation of 8 compounds (**5**-**7**, **9**, **10**,**18**, **24**, and **25**).

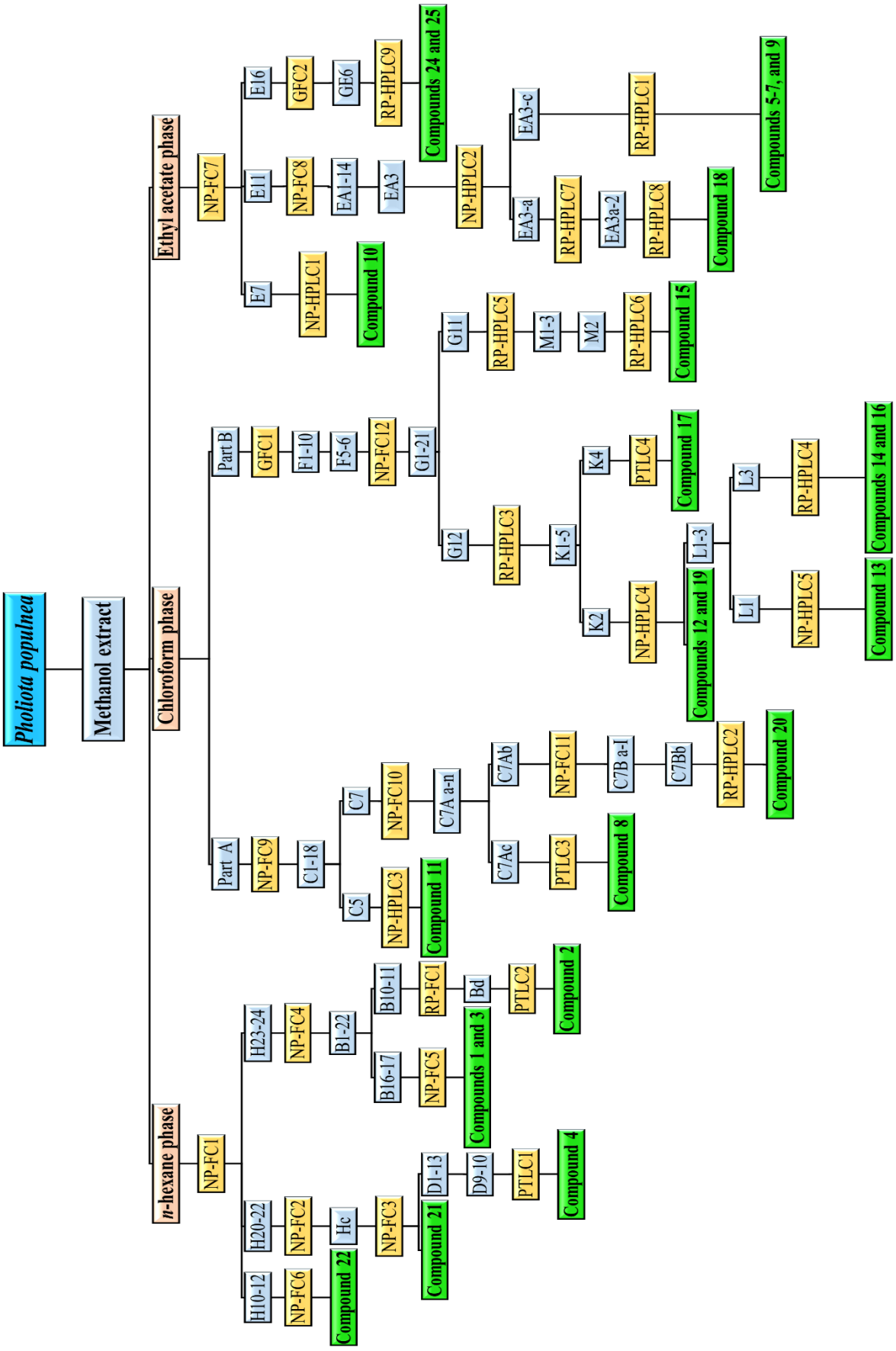


Figure 1. Isolation of compounds from *Pholiota populnea*

Isolation of compounds from *Clitocybe nebularis*

The fresh mushroom material (5.6 kg) was extracted with MeOH (20 L) at room temperature. After concentration, the MeOH extract (54 g) was dissolved in 50% aqueous MeOH and subjected to solvent-solvent partition using *n*-hexane (5 × 500 mL), $CHCl_3$ (5 × 500 mL), and then EtOAc (5 × 500 mL) (**Figure 2**). To study *Clitocybe nebularis* metabolites, the chloroform phase (2.12 g) was fractionated using NP-FC, and the combined fractions were analyzed using repeated RP-FC, which resulted in compounds **26** and **27**. Purification was carried out by multiple NP-FC followed by PTLC of the EtOAc (4.15 g) extract, leading to the isolation of compound **28**.

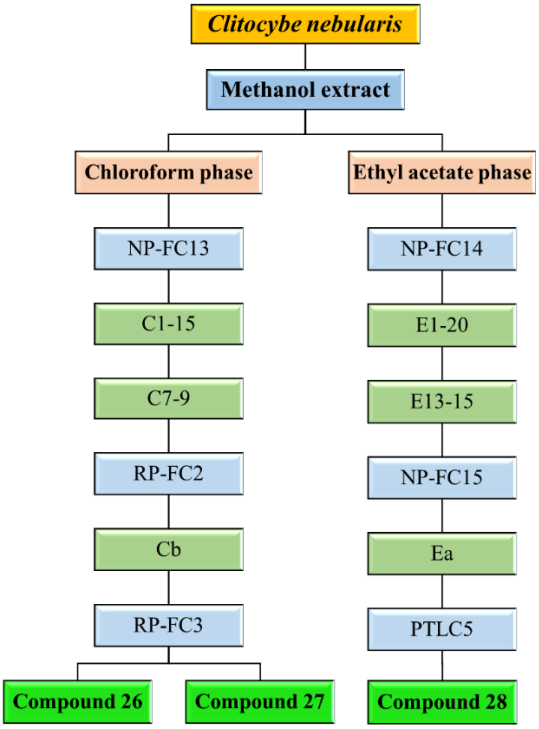


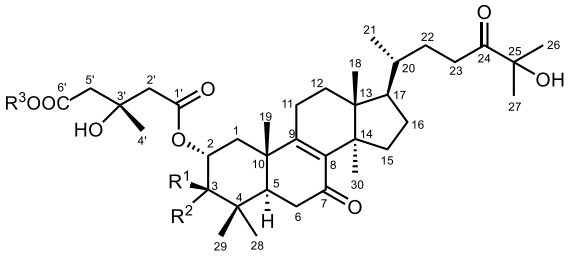
Figure 2. Isolation of the compounds from *Clitocybe nebularis*

Compounds from *Pholiota populnea* and *Clitocybe nebularis*

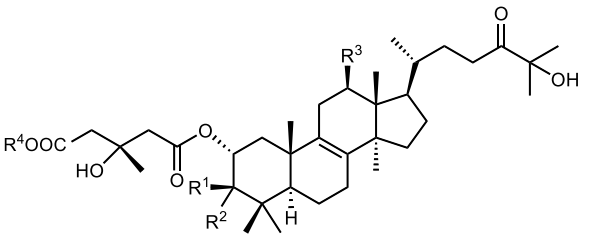
Investigation of the MeOH extract prepared from the edible mushroom *Pholiota populnea* led to the isolation of 25 compounds, among them 23 triterpenes and 2 nucleosides (**Figure 3**). Structure determination revealed 19 undescribed triterpenes, for which the trivial names pholiols A-S (**1**-**19**) were given. All compounds (**1**-**25**) were isolated and identified for the first time from this species.

Processing the extract of *Clitocybe nebularis* allowed to isolate 3 compounds (**26**-**28**) (**Figure 4**), which were found for the first time in this species.

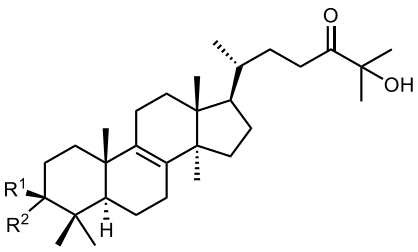
Molecular masses and molecular compositions were obtained from HRESIMS measurements. The structures were further determined using 1D and 2D NMR spectroscopy, which provided the most useful information. The constitutions and planar structures of the compounds were elucidated from the ^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, and HMBC experiments, and then the relative configurations of the chiral centers were determined with the aid of NOESY and ROESY spectra. As a result of our NMR studies, the complete ^{1}H- and ^{13}C assignments were accomplished for the new compounds and the previously published data for some known compounds where supplemented.



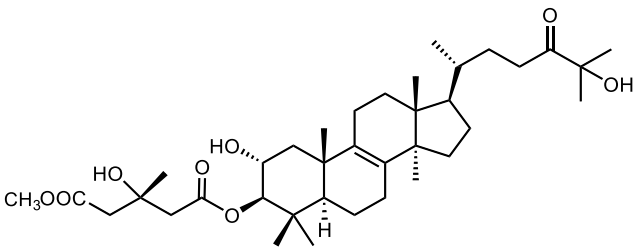
	R^1	R^2	R^3
1	OAc	H	H
2	OAc	H	CH_3
8	OH	H	CH_3
9	O		H



	R^1	R^2	R^3	R^4
3	OAc	H	OH	H
4	OAc	H	OH	CH_3
5	O		H	H
6	OAc	H	H	H
7	OH	H	H	H



	R^1	R^2
10	OH	H
11	O	



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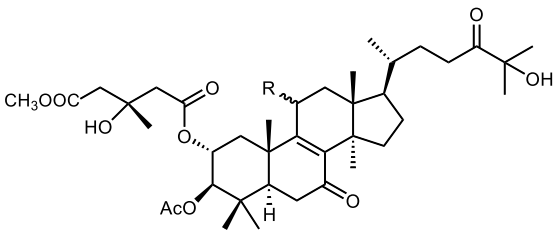
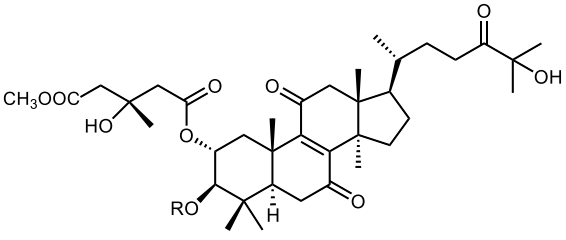
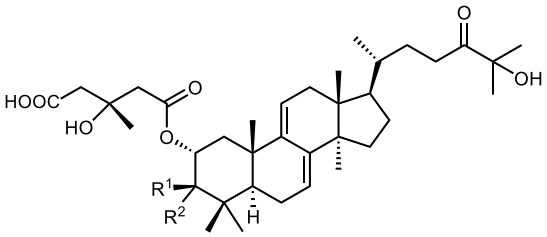


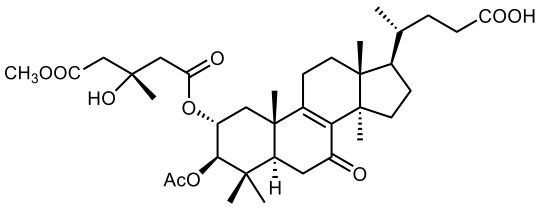
	R
13	βOH
14	αOH



	R
15	Ac
16	H



	R^1	R^2
17	OAc	H
18	O	



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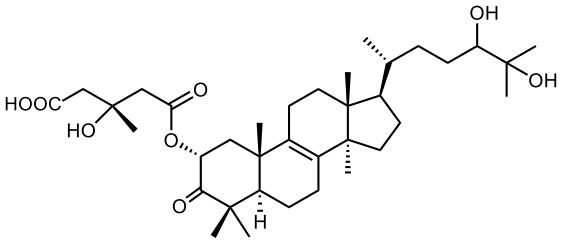

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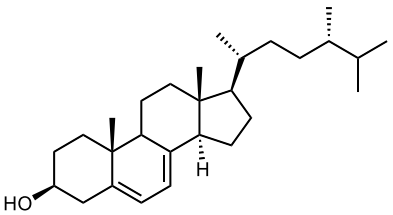

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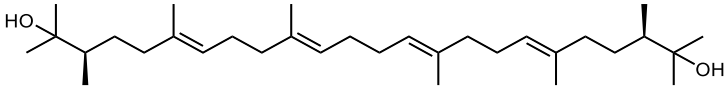

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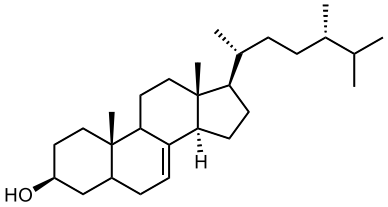

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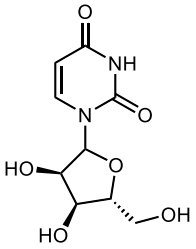

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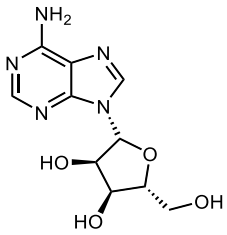

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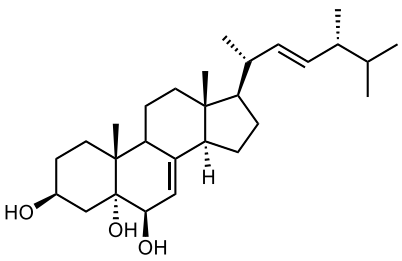
Figure 3. Structures of compounds **1**-**25** from *Pholiota populnea*



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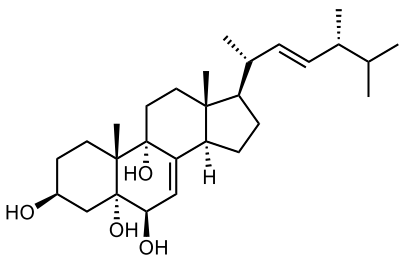

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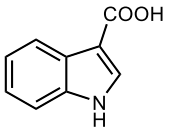

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Figure 4. Structures of compounds **26**-**28** from *Clitocybe nebularis*

Investigation of the chemical composition of *Pholiota populnea* resulted in the isolation of 19 undescribed triterpenes, which were named pholiol A to S, in addition to 6 known components. Among the 19 compounds, 18 had lanostane skeletons (pholiol A-R **1**-**18**), while 1 had a trinorlanostane skeleton (pholiol S **19**). Structure elucidation of pholiols revealed the presence of two unique moieties, 3-hydroxy-3-methylglutarate methyl ester (MeHMG) and 3-hydroxy-3-methylglutarate (HMG). MeHMG was found to be linked to either at C-2 (**2**, **4**, **8**, **13**-**16**, and **19**) or C-3 (**12**), while HMG was attached only at C-2 (**1**, **3**, **5**-**7**, **9**, **17**, and **18**). Our research afforded the first discovery of a lanostane triterpene containing either MeHMG or HMG moiety from the *Pholiota* genus. Further structural characteristic of pholiol series (**1**-**19**) is the presence of a keto group at C-24. (+)-Clavaric acid (**20**) is a known triterpenoid compound that is structurally related to fasciculic acid, which is a calmodulin inhibitor produced by *Naematoloma* (syn. *Hypholoma*) *fasciculare*. Compound **21** was identified as (3*S*,6*E*,10*E*,14*E*,18*E*,22*S*)-2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene, which is a linear triterpene polyol. Ergosterol (**22**) and 3*β*-hydroxyergosta-7,22-diene (**23**) are both sterols with broad distribution in fungal species. In the EtOAc extract of the *Pholiota populnea*, uridine (**24**) and adenosine (**25**) were detected. Both are nucleosides, which are building blocks of nucleic acids such as DNA and RNA.

Processing the extract of *C. nebularis* provided the isolation of three compounds, which had not previously been isolated from this species. With regard to their structures, the components can be divided into two groups: cerevisterol (**26**) and (22*E*,24*S*)-5*α*-ergosta-7,22-diene-3*β*,5,6*β*,9*α*-tetraol (**27**) belong to the group of triterpenes, while indole-3-carboxylic acid (**28**) is an organic acid.

Pharmacological activity of the isolated compounds

Antiproliferative assay of the compounds from Pholiota populnea

Pholiols L, M, O, Q, and S (**12**, **13**, **15**, **17**, and **19**), were investigated for their antiproliferative activity *in vitro* by MTT assay against Colo205, Colo320, MCF-7, A549, and MRC-5 cell lines. The anticancer drug doxorubicin was used as a reference agent. The highest antiproliferative effects were observed for pholiols M (**13**) and O (**15**) against the MCF-7 cell line, with IC_{50} values of 2.48 and 9.95 µM, respectively (**Table 1**). All other compounds exhibited weaker activity on the viability of the treated cancer cells, displaying IC_{50} values ranging from 28.07 to 89.84 µM.

Compounds **12**, **13**, **15**, **17**, and **19** were tested for their antiproliferative activity in the non-cancerous human embryonic lung fibroblast cell line (MRC) as well, and selectivity indexes (SI) were calculated. The relatively high SI values of pholiol M (**13**) indicated its strong and moderate tumor cell selectivity regarding the MCF-7 (SI > 40) and Colo205 (SI = 4.18) cell lines. SI values of 4.3 and 1.8 of pholiol O (**15**) indicated this compound to be moderately or slightly selective towards Colo205 cells,

while others did not display selectivity to cancerous cell lines over normal cells. Comparing their chemical structure of the most active compounds **13** and **15**, both belong to the class of lanostane triterpenes with 2,3-diester-7-on-8(9)-en functionalities. Additionally, compound **15** contains a 11-keto group while compound **13** has a 12-hydroxy group.

Table 1. Antiproliferative activity (IC_{50} μM) of compounds **12**, **13**, **15**, **17**, and **19** in human sensitive (Colo205) and resistant (Colo320) colon adenocarcinoma cells and normal embryonal fibroblast (MRC-5) cell line.

Comp	Colo205		Colo320		MCF-7		A549		MRC-5	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
12	32.41	0.89	28.07	4.62	**21.74**	0.88	53.73	1.27	70.86	1.22
13	**23.91**	0.026	32.51	2.97	**2.48**	0.16	>100	–	>100	–
15	**23.39**	0.060	42.14	0.15	**9.95**	0.37	51.53	1.61	42.89	1.34
17	49.97	0.52	69.19	2.67	46.28	1.86	51.21	1.04	>100	–
19	59.87	0.55	68.54	4.40	35.33	3.03	89.84	0.75	>100	–
Dox*	0.0617	0.0128	0.25	0.06	0.155	0.0027	1.04	0.097	0.52	0.018
DMSO	>2%	–	>2%	–	>2%	–	>2%	–	>2%	–

*Dox = doxorubicin.

Cytotoxic effect of the compounds from Pholiota populnea

The compounds **1**–**4**, **12**, **13**, **15**, **17**, **19**, **21**, and **22** isolated in good yield were tested for their cytotoxic activity on sensitive Colo205 and resistant Colo320 cell lines and on the normal MRC-5 embryonal fibroblast cell line using the MTT assay with doxorubicin as a positive control. Five compounds (**12**, **13**, **15**, **17**, **19**) were tested on MCF-7 and A549 cell lines, too. In the cytotoxic assay a higher cell population and a shorter exposure were used than in the antiproliferative test.

Among the studied compounds, ergosterol (**22**) showed substantial cytotoxic activity against the tumor cell lines with IC_{50} values of 4.9 μM (Colo205) and 6.5 μM (Colo320) (**Table 2**). Unfortunately, this compound was more potent against the MRC-5 cell line (IC_{50} 0.50 μM). Pholiols B (**2**), D (**4**), L (**12**), M (**13**), O (**15**), Q (**17**), S (**19**) and 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (**21**) possessed weak inhibitory activities (IC_{50} 26–93 μM) against the tested cell lines without any selectivity.

Comparing the antiproliferative and cytotoxic effects of the compounds remarkable difference displayed selectivity for the growing cell population without directly killing the exposed cells. Such a result was exhibited by pholiols M (**13**) and O (**15**), for which, no cytotoxic effect could be detected on MCF-7 cells (IC_{50} > 100 μM), proving their action exclusively on tumor cell proliferation.

Table 2. Cytotoxic activity in human sensitive (Colo205) and resistant (Colo320) colon adenocarcinoma cells and relative resistance ratio of compounds **1**–**4**, **12**, **13**, **15**, **17**, **19**, **21**, and ergosterol (**22**)

Compd	IC_{50} (µM)				RR[a]	IC_{50} (µM)					
	Colo205 (A)		**Colo320** (B)		B/A	**MCF-7**		**A549**		**MRC-5**	
	Mean	**SD**	**Mean**	**SD**		**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
1	>100	-	>100	-	**-**	-	-	-	-	>100	-
2	67.92	0.39	61.5	4.7	**0.90**	-	-	-	-	89.96	0.01
3	>100	-	>100	-	-	-	-	-	-	>100	-
4	51.36	0.1	48.94	0.65	**0.95**	-	-	-	-	54.18	3.00
12	40.33	1.64	33.92	1.84	**0.84**	43.69	0.03	93.61	1.94	66.08	1.36
13	35.93	0.44	67.22	3.86	1.87	>100	–	58.12	0.70	>100	–
15	31.52	0.91	91.52	4.96	2.90	>100	–	56.86	1.53	57.99	0.82
17	56.12	0.84	34.73	1.24	**0.62**	43.78	0.18	85.88	2.41	>100	–
19	57.50	0.96	57.52	2.36	1.0	42.99	0.61	83.65	6.06	55.27	0.41
21	26.7	0.33	27.48	1.56	1.03	-	-	-	-	29.06	2.2
22	**4.88**	0.57	**6.48**	0.22	1.33	-	-	-	-	**0.50**	0.09
Dox	3.32	0.08	11.96	0.88	3.60	5.73	1.02	10.22	0.07	3.60	0.35
DMSO	>2%	–	>2%	–		>2%	–	>2%	–	>2%	–

[a] RR (relative resistance ratio) = IC_{50} resistant/IC_{50} sensitive; Dox = doxorubicin

Comparison of the cytotoxic activities on Colo320 and Colo205 cells allowed for the detection of relative resistance (RR). The calculated RR values of the tested compounds showed selectivity against the resistant Colo320 cells of pholiols B (**2**) (RR 0.90), D (**4**) (RR 0.95), L (**12**) (RR 0.84) and Q (**17**) (RR 0.62) (**Table 2**).

Rhodamine 123 accumulation assay of the compounds from Pholiota populnea

The inhibitory activities of compounds **1**–**3**, **21**, and **22** on efflux function were evaluated by measuring the intracellular accumulation of rhodamine 123, within the Colo320 MDR cells. Tariquidar, a strong P-gp inhibitor, was used as positive control. The results revealed inhibition of P-gp MDR efflux pump activity manifested by pholiols A (**1**) and B (**2**) and aliphatic triterpene **21** (**Table 3**). Pholiol B (**2**) and the polyhydroxy-squalene derivative (**21**) exerted the highest anti-MDR effect in this bioassay with FAR values of 6.880 and 6.638, respectively.

*Checkerboard combination assay of the compounds from Pholiota populnea***:**

Compounds **2**, **4**, **21**, and **22** were tested for their capacity to reduce the resistance of the MDR Colo320 cell line to doxorubicin. **Table 4** reveals that pholiols B (**2**), D (**4**), and **21** interacted in a synergistic

Table 3. P-gp efflux pump inhibitory activity of compounds **1**–**3**, **21**, and **22** against MDR Colo320 cell line

Sample	conc. µM	FAR
tariquidar[a]	0.2	5.533
pholiol A (**1**)	20	3.418
pholiol B (**2**)	20	**6.880**
pholiol C (**3**)	20	0.967
compound **21**	20	**6.638**
ergosterol (**22**)	2	1.053
DMSO	2.00%	0.828

[a]Positive control.

manner, and combination indexes (CI) values at 50% of the ED_{50} were found to be 0.348, 0.660, and 0.082, respectively. The outstanding potency of **21**, designated as very strong synergism (CI = 0.082), is favorable. In this assay ergosterol (**22**) was found to have an additive effect in combination with doxorubicin.

Table 4. Chemosensitizing activity of compounds **2**, **4**, **21**, and **22** on Colo320 adenocarcinoma cells

compound	best ratio[a]	CI at ED_{50}[b]	SD	interaction
pholiol **B** (**2**)	23.2:1	0.348	0.051	synergism
pholiol **D** (**4**)	139.2:1	0.660	0.03	Synergism
compound **21**	2.9:1	0.082	0.057	very strong synergism
ergosterol (**22**)	3.6:1	1.03	0.12	nearly additive

[a]Best ratio: the best combination ratio between compound and doxorubicin. [b]CI at ED50: combination index value at the 50% growth inhibition dose.

It is noteworthy that the tetrahydroxysqualene derivative (**21**) has the capacity to potentiate the effect of doxorubicin in Colo320 cells by P-gp modulation and strong synergism with doxorubicin, and therefore it represents a promising new class of potential adjuvants of cancer chemotherapy.

Antimicrobial assay of the compounds from Pholiota populnea:

Compounds **1**–**4**, **21**, and **22** were tested and found to be inactive against *Escherichia coli* (ATCC 25922), *Salmonella enterica serovar Typhimurium* (14028s), *Staphylococcus aureus* (ATCC 25923), and *S. aureus* (27213) strains.

Antimicrobial assay of the compounds from Clitocybe nebularis:

The antimicrobial activity of compounds (**26**-**28**) was tested against bacterial strains of *Streptococcus agalactiae* (ATCC 13813), *Staphylococcus epidermidis* (ATCC 12228), *Moraxella catarrhalis* (ATCC

25238), *Haemophilus influenzae* (ATCC 49766), and *Proteus mirabilis* (HNCMB 60076) by disc diffusion method. Compounds **27** and **28** exhibited marginal inhibition on the growth of *M. catarrhalis*, while this strain showed resistance against compound **26**.

Anti-inflammatory assay of the compounds from Pholiota populnea:

The anti-inflammatory activity of compounds **3**, **5**-**11**, and **20**, was evaluated using COX-1, COX-2, 5-LOX, and 15-LOX assays. Dose-response experiments revealed that pholiols F (**6**) and (+)-clavaric acid (**20**) exhibited moderate anti-inflammatory activity against COX-2, with IC_{50} values of 439.8 and 766.7 µM, respectively. The other compounds had inhibition below 50% at the highest concentration tested (2.5 mM). Among the compounds, pholiols C (**3**), F (**6**), G (**7**), and I (**9**) exhibited the best inhibitory activity against 5-LOX, with IC_{50} values ranging from 194.5 to 519.7 µM. Pholiol F (**6**) was the most active against 5-LOX, with an IC_{50} value of 194.5 µM. However, all compounds were inactive against 15-LOX at the tested concentrations.

SUMMARY

Our study aimed to highlight the chemical and pharmacological potential of mushrooms *Pholiota populnea* and *Clitocybe nebularis*, focusing on previously undescribed compounds with cytotoxic, antiproliferative, and anti-inflammatory activity.

The isolation process involved grinding the samples, followed by percolation with methanol. The fractions with different polarities were then roughly separated using liquid-liquid extractions, resulting in *n*-hexane, chloroform, and EtOAc phases. The composition of these extracts was monitored by TLC investigations. Subsequently, various chromatographic methods, such as OCC, FC, PTLC, and HPLC, were employed for further separation, affording the isolation of 28 compounds in pure form. The structures of the isolated compounds were determined by means of spectroscopic methods, mainly MS and NMR spectroscopy.

In case of *P. populnea*, solvent-solvent extraction and different chromatographic separations of the extracts led to the isolation of nineteen novel triterpenes, named pholiol A-S (**1**-**19**), together with 6 know compounds, a lanostane type triterpene, (+)-clavaric acid (**20**), an acyclic triterpene, (3*S*,6*E*,10*E*,14*E*,18*E*,22*S*)-2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (**21**), two ergostane-type triterpenes, ergosterol (**22**), and 3*β*-hydroxyergosta-7,22-diene (**23**), and two nucleosides, uridine (**24**) and adenosine (**25**). The new compounds, pholiol A–S, are lanostane-type triterpenes (with exception of **10** and **11)** substituted with 3-hydroxy-3-methylglutaric acid and its 6-methyl ester. Characteristic structural features are the 24-keto group, 8/9

double bond, and in some compounds, 7- or/and 11-keto groups. Pholiol S (**19**) is an unusual compound with a trinor-triterpene structure, and pholiols Q (**17**), and R (**18**) with 7(8),9(11)-diene structure. All compounds (**1**–**25**) were isolated for the first time from this species. Pholiols M (**13**) and O (**15**) displayed antiproliferative activity against the MCF-7 cell line with IC_{50} of 2.48 and 9.95 µM, respectively. Furthermore, these compounds demonstrated tumor cell selectivity on MCF-7 cells [SI values of >40 (**13**) and 4.3 (**15**)]. The isolated metabolites (**1**–**4**, **12**-**19**, **21**, and **22**) were investigated for cytotoxic activity against Colo205, Colo320, A549, MCF-7, and nontumoral MRC-5 cell lines. Among the tested compounds, ergosterol (**22**) showed substantial cytotoxic activity against all cell lines with IC_{50} values of 4.9 µM (Colo205), 6.5 µM (Colo320), and 0.50 µM (MRC) with no tumor cell selectivity. However, pholiols B (**2**) (RR 0.90), D (**4**) (RR 0.95), L (**12**) (RR 0.84), and Q (**17**) (RR 0.62) were found to have selective cytotoxicity against the resistant Colo320 cells compared to the sensitive Colo320 cells. A P-glycoprotein efflux pump modulatory test on resistant Colo320 cells revealed that pholiols A (**1**), B (**2**), and the acyclic triterpene **21** have the capacity to inhibit the efflux-pump overexpressed in the cells. Moreover, the drug interactions of triterpenes with doxorubicin were studied by the checkerboard method on Colo320 cells. Pholiols B (**2**) (CI 0.348) and D (**4**) (CI 0.660) interacted in synergistic and acyclic triterpene **21** (CI 0.082) in a very strong synergistic manner. The anti-inflammatory activity of the isolated compounds (**3**, **5**-**11**, and **20**) was screened using COX-1, COX-2, 5-LOX, and 15-LOX inhibitory assays. The results showed that the lanostane derivatives exhibited moderate inhibitory activity against 5-LOX and COX-2 enzymes. Among the tested compounds, pholiol F (**6**) demonstrated the highest potency, with IC_{50} values of 194.5 µM and 439.8 µM against 5-LOX and COX-2, respectively. The isolated compounds were ineffective as antimicrobial agents.

For the first time, two steroids (cerevisterol (**26**) and [22*E*,24*S*)-5*α*-ergosta7,22-diene-3*β*,5,6*β*,9*α*-tetraol (**27**)] and an organic acid [indole-3-carboxylic acid (**28**)] were obtained from the chloroform and EtOAc fractions of *C. nebularis*. Their antimicrobial activity was assessed against human pathogen strains using the agar disc diffusion method. The susceptibility assay exhibited that compounds **27** and **28** have weak antimicrobial activity against *M. catarrhalis*.

Our findings indicate that mushrooms native to Hungary are a rich source of biologically active metabolites with great structural diversity. Especially, *P. populnea* is a promising source for discovery of new triterpenes with significant antiproliferative, and chemosensitizing activities on cancer cells. Therefore, understanding the mechanism of action of these compounds can provide a strong foundation for developing new, effective agents for the treatment of different types of cancer.

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LIST OF PUBLICATIONS RELATED TO THE THESIS

I. **Yazdani, M**., Béni, Z., Dékány, M., Szemerédi, N., Spengler, G., Hohmann, J., Ványolós, A. Triterpenes from *Pholiota populnea* as cytotoxic agents and chemosensitizers to overcome multidrug resistance of cancer cells. *Journal of Natural Products* **2022**; 85, 910-916. DOI: 10.1021/acs.jnatprod.1c01024 **IF: 4.803 (D1)**

II. **Yazdani, M**., Barta, A., Berkecz, R., Agbadua, O.G., Ványolós, A., Hohmann, J. Pholiols E–K, lanostane-type triterpenes from *Pholiota populnea* with anti-inflammatory properties. *Phytochemistry* **2023**, *205*, 113480. DOI: 10.1016/j.phytochem.2022.113480 **IF: 4.004 (Q1)**

III. **Yazdani, M**., Barta, A., Hetényi, A., Berkecz, R., Spengler, G., Ványolós, A., Hohmann, J. Isolation of the lanostane triterpenes pholiols L–S from *Pholiota populnea* and evaluation of their antiproliferative and cytotoxic activities. *Pharmaceuticals* **2023**, *16*, 104. DOI: 10.3390/ph16010104 **IF: 5.215 (Q1)**

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