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Biologically Active Substances from Higher Fungi

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As a part of our search for naturally occurring bioactive metabolites of mushrooms, we have investigated the chemical constituents of more than 100 Basidiomycetes and Ascomycetes fungi, and isolated over 300 including 150 new terpenoids, phenolics and nitrogen-containing compounds. The isolation, structural elucidation and biologically activity of the natural products from the higher fungi are briefly discussed.

Key words higher fungi, natural products, bioactivities

China is extraordinary rich in higher fungi. To date about 10,000 species of fungi have been reported from the vast territory of China. Among them, nearly 6000 species, belonging to about 1200 genera, are higher fungi (excluding lichens). Higher fungi in bio-resources belong to the very productive biologically sources which produce a large and diverse variety of secondary metabolites. We have been interested in the biologically active substances present in untapped and diverse source of higher fungi from China. The isolation, structural elucidation and biologically activity of the new compounds have been reviewed previously.[1—3]

Recently several dozen new natural products and bioactive compounds were purified and characterized in selected mushrooms on the basis of using our knowledge on the collection of fruiting bodies, strain preservation, fermentation, biological screening and chemical investigation of higher fungi. The isolation, structural elucidation and biologically activity of these novel terpenoids, phenolics and nitrogen-containing compounds from basidiomycetes and ascomycetes fungi (Albatrellus confluens, Albatrellus dispansus, Boletus edulis, Boletopsis grisea, Bondarzewia berkeleyi, Cortinarius tenuipes, Cortinarius vibratilis, Daldinia concentrica, Engleromyces gotzii, Hebeloma versipelle, Hydnum repandum, Hygrophorus eburnesus, Lactarius deliciosus, Lactarius hatsudake, Lactarius hirtipes, Lactarius mitissimus, Lactarius rufus, Paxillus panuoides, Polyporus ellisii, Pulveroboletus ravenelii, Russula cyanoxantha, Russula foetens, Russula lepida, Russula nigricans, Sarcodon leavagatum, Sarcodon scabrosus, Shiraia bambusicola, Thelephora aurantiotincta, Thelephora ganbajun, Tremella aurantilba, Tricholomopsis rutilans, Tylopilus plumbeoviolaceus, Tylopilus virens, Tuber indicum, Xylaria euglossa, etc.) were reported.

A novel benzofuran lactone, named concentricolide, was isolated along with the four known compounds from the fruiting bodies of the xylariaceous ascomycete *Daldinia concentrica*. The structure of concentricolide was established by spectroscopic methods and X-ray crystallographic analysis. Its anti-HIV-1 activity was tested. Results showed that concentricolide inhibited HIV-1 induced cytopathic effects. The EC_{50} value was 0.31 μg/ml. The therapeutic index (TI) was 247. Concentricolide exhibited the blockage (EC_{50} 0.83 μg/ml) on syncytium formation between HIV-1 infected cells and normal cells.[4]

The identification of aromatic steroid hydrocarbons bearing a methyl group at positions 1, 2, 3, 4, or 6 in sediments and petroleum has been puzzling since possible steroidal precursors have not yet been reported in living organisms. Two new aromatic steroids were isolated from the fruiting bodies of *D. concentrica*, of which bears an unusual methyl group at position 1. We propose that the origin of these compounds is derived from the transformation undergone by their precursor due to microbial action. Compounds could be the long-sought, biological precursor steroids for organic matter in Earth's subsurface.[5]

Grifolin is a natural biologically active substance

isolated from the fruiting bodies of *Albatrellus confluens*. We, for the first time, have described a novel activity grifolin, namely its ability to inhibit the growth of tumor cells by the induction of apoptosis. Grifolin strongly inhibited of tumor cells lines: CNE1, HeLa, MCF7, SW480, K562, Raji and B95-8. Analysis of acridine orange (AO)/ethidium bromide (EB) staining and flow cytometry showed that grifolin possessed apoptosis induction activity to CNE1, HeLa, MCF7 and SW480. Furthermore, the cytochrome *c* release from mitochondria was detected by confocal microscopy in CNE1 cells after a 12 h treatment with grifolin. The increase of caspase-8, 9, 3 activities revealed that caspase was a key mediator of the apoptotic pathway induced by grifolin, and the under-expression of Bcl-2 and up-regulation of Bax resulted in the increase of Bax: Bcl-2 ratio, suggesting that Bcl-2 family involved in the control of apoptosis. Owing to the combination of the significant antitumor activity by inducing apoptosis and natural abundance of the compound, grifolin holds the promise of being an interesting antitumor agent that deserves further laboratory and *in vivo* exploration.[6] The antifungal activities of grifolin were also evaluated *in vitro* against 9 plant pathogenic fungi and *in vivo* against the plant disease of *Erysiphe graminis*.[7] Rufuslactone, a new lactarane sesquiterpene, was isolated from the fruiting bodies of the basidiomycete *Lactarius rufus*. It showed antifungal properties against plant pathogenic fungi.[8]

Lipid metabolism normally keeps a delicate balance between synthesis and degradation. When the balance is upset, hyperlipidemia may occur, which in turn can cause atherosclerosis, hypertension, diabetes etc. Modulators of lipid metabolism are expected to be useful in controlling these disorders. Obesity and hyperchole sterolemia are to relevant degree related to high nutritional fat intake. The key enzyme of dietary triglyceride absorption is pancreatic lipase. Inhibition of pancreatic lipase may therefore result in inhibition of fat absorption. Orlistat, a specific pancreatic lipase, is clinically used for preventing obesity and hyperlipidemia.

A compound called vibralactone was isolated from the culture broth of the polypore *Boreostereum vibrans* (Berk. & M. A. Curtis) Davydkina & Bondartseva (Aphyllophorales). It shows inhibition of pancreatic lipase with an IC_{50} of 0.4 μg/ml.[9]

A β-carboline compound, flazin isolated from *Suillus granulatus* has been shown weak anti-HIV-1 activity. Based on the structure of flazin, flazinamide [1-(5'-Hydromethyl-2'-furyl)-β-carboline-3-carboxamide] was synthesized and its anti-HIV activities were evaluated in the present study. The cytotoxicity of flazinamide was about 4.1-fold lower than that of flazin. Flazinamide potently reduced syncytium formation induced by HIV-1_{IIIB} with EC_{50} value of 0.38 μM, the EC_{50} of flazinamide was about 6.2-fold lower than that of flazin. Flazinamide also inhibited HIV-2_{ROD} and HIV-2_{CBL-20} infection with EC_{50} values of 0.57 μM and 0.89 μM, respectively. Flazinamide reduced p24 antigen expression in HIV-1_{IIIB} acute infected C8166 and in clinical isolated strain HIV-1_{KM018} infected PBMC, with EC_{50} values of 1.45 μM and 0.77 μM, respectively. Flazinamide did not suppressed HIV-1 replication in chronically infected H9 cells. Flazinamide blocked the fusion between normal cells and HIV-1 or HIV-2 chronically infected cells. It weakly inhibited activities of recombinant HIV-1 reverse transcriptase, protease or integrase at higher concentrations. In conclusion, the conversion of the carboxyl group in 3 position of flazin markedly enhanced the antiviral activity (TI value increased from 12.1 to 312.2) and flazinamide might interfere in the early stage of HIV life cycle.[10]

Higher fungi, among the many diverse organisms, are a major source of biologically active natural products. They have often been found to contain biologically active compounds, and they provide a rich variety of active secondary metabolites. There are potentially many compounds still to be discovered in higher fungi since until now only a relatively small number of higher fungi have been chemically investigated, and many of the remaining species are involved in interesting biological phenomena. These as yet unstudied species hold the promise of providing new natural products. That these fungi are often involved in interesting biological processes indicates not only that the new metabolites involved will be chemically interesting but also that the new metabolites may be biologically interesting and significant. The large biodiversity of higher fungi provides a huge resource for extending the chemodiversity of natural products and for finding new lead structures for medicinal chemistry.[11]

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