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Triterpenoids from *Inonotus obliquus* and their antitumor activities

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Inotodiol (PubChem CID: 44422314)
Inonotsuoxide A (PubChem CID: 44422304)
Lanosteol (PubChem CID: 246983)
Betulin (PubChem CID: 72326)
Betulinic acid (PubChem CID: 64971)
Oleanolic acid (PubChem CID: 10494)
3β-Hydroxy-lanosta-8,24-diene-21-al (PubChem CID: 44581610)
Trametenolic acid (PubChem CID: 12309443)

ABSTRACT

Three new lanostane-type triterpenes, inonotusanes A–C (**1**–**3**), and a new naturally occurring one, 3β-hydroxy-25,26,27-trinorlanosta-8,22*E*-dien-24-oic acid (**4**), together with sixteen known triterpenoids (**5**–**20**), including 13 lanostane derivatives, 2 lupanes and 1 oleanane-type triterpene were isolated from the sclerotia of *Inonotus obliquus*. Their structures were elucidated by 1D and 2D NMR spectroscopy and HRMS. Compounds **6**, **8**, **18** and **20** exhibited strong cytotoxicity against A549 tumor cell lines, with IC_{50} values of 2.34, 1.63, 8.39 and 5.39 μM, respectively. Seven compounds (**3**, **9**, **10**, **12**, **18**–**20**) exhibited moderate cytotoxicity against A549, HT29, Hela or L1210 tumor cell lines.

1. Introduction

Inonotus obliquus (Pers.: Fr.) Pil., belonging to the family Hymenochaetaceae, is widely distributed in Europe, Asia, and North America. This mushroom has been used as folk medicine for cancer for more than four centuries in Russia and western Siberia [1–3]. Previous phytochemical investigations of *I. obliquus* mostly described lanostane triterpenes, most of which were exclusive to *I. obliquus* in nature, which showed antitumor activities *in vitro* and *in vivo*, including cytotoxicity against L1210, P388 and KB tumor cells (inotodiol, 14-al, inonotsuoxide A and B) and prolonging the survival days of P388-bearing mice (inotodiol) [4–9]. Recently, other antitumor triterpenes have been reported (inonotusol F, 3β,22-dihydroxylanosta-8,24-dien-11-one and trametenolic acid) [10]. In our research for additional

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bioactive compounds from *I. obliquus*, two new lanostane stereoisomers (inonotusanes A and B, **1** and **2**), two 25,26,27-trinorlanostanes, inonotusane C (**3**, new compound) and 3β-hydroxy-25,26,27-trinorlanosta-8,22*E*-dien-24-oic acid (**4**, new natural product), as well as 16 known triterpenoids (**5**–**20**) were found in the EtOAc fraction of *I. obliquus* (Fig. 1). Compound **4** was mentioned one time in 1974, during the side-chain degradation of lanosterol, but without any spectroscopic data [11]. To our knowledge, 25,26,27-trinorlanostanes are rich in the fungus *Ganoderma lucidum* Karst (Polyporaceae), which may be considered as a distinctive feature [12–16]. But their presence is unusual in the family Hymenochaetaceae. And our results could contribute to chemotaxonomic studies of the *Inonotus* genus. MTT tests showed **6**, **8**, **18** and **20** exhibited strong cytotoxicity against A549 tumor cell line, with IC_{50} 2.34, 1.63, 8.39 and 5.39 μM, respectively. Herein, we describe the structure elucidation of the new compounds **1**–**3** and the new natural product **4**. The structure–activity relationships on cytotoxicity against the tested tumor cell lines are also discussed.

2. Experimental

2.1. General

^{1}H and ^{13}C NMR spectra were obtained on Bruker AV-400 instruments (Bruker, Zurich, Switzerland) using TMS as internal standard for chemical shifts. Chemical shifts (δ) were expressed in ppm with reference to the TMS resonance. HRESIMS were recorded on an Agilent 1100 series LC/MSD ion trap mass spectrometer. Optical rotations were measured with a SGW-1 polarimeter (Shanghai Jingke, China). IR spectra

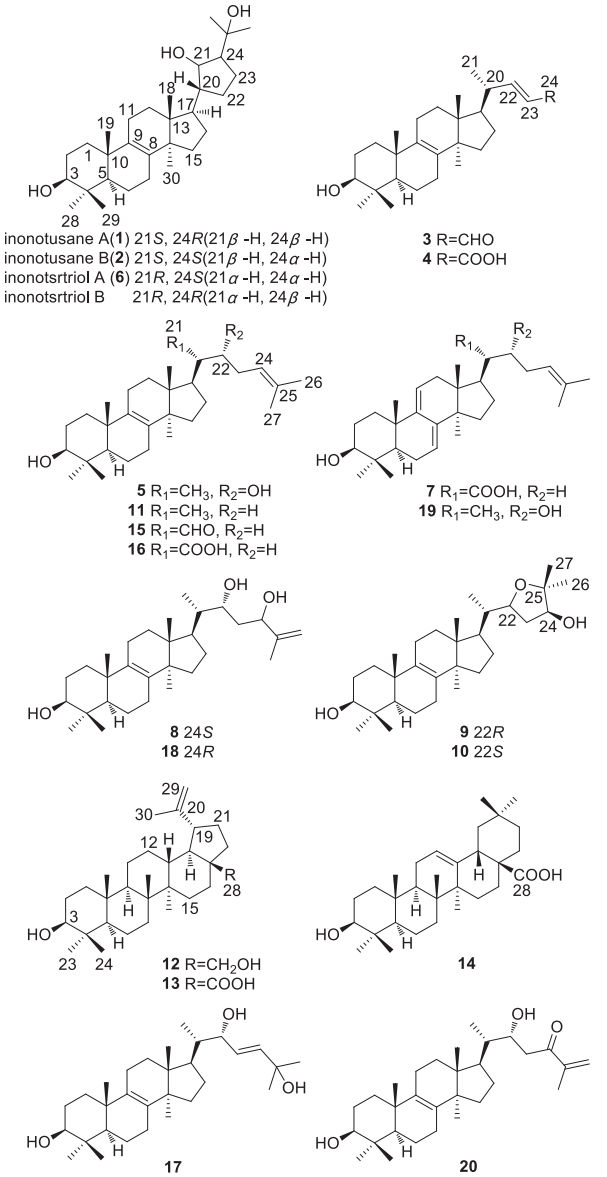

Fig. 1. Structures of compounds **1**–**20** and inonotstriol B.