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R07. Identification of Antifungal Bisphosphocholines from Medicinal Gentiana Species

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IDENTIFICATION OF ANTIFUNGAL BISPHOSPHOCHOLINES FROM MEDICINAL GENTIANA SPECIES

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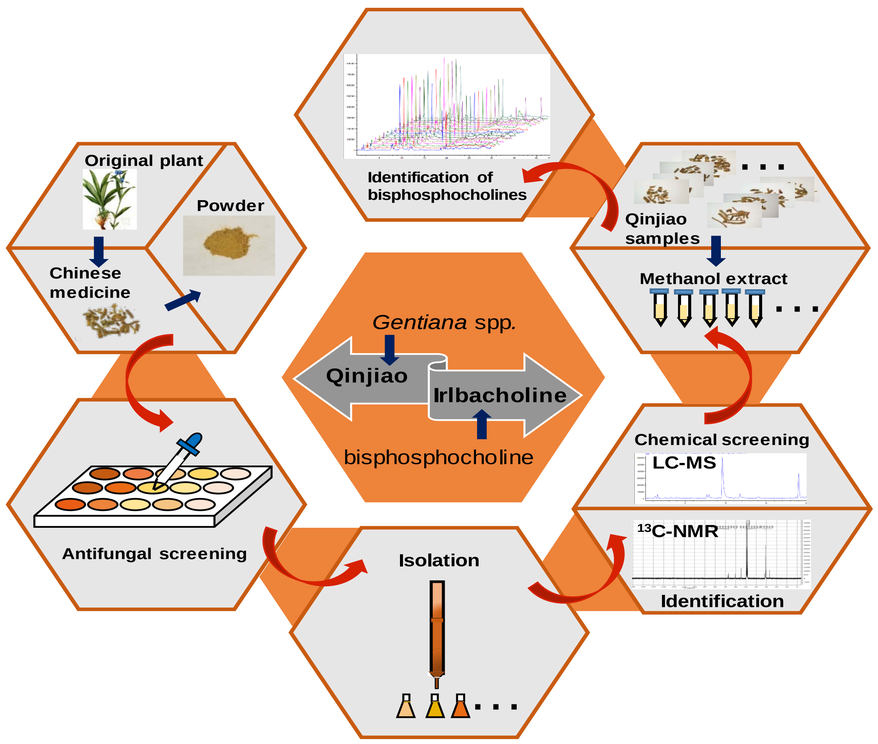
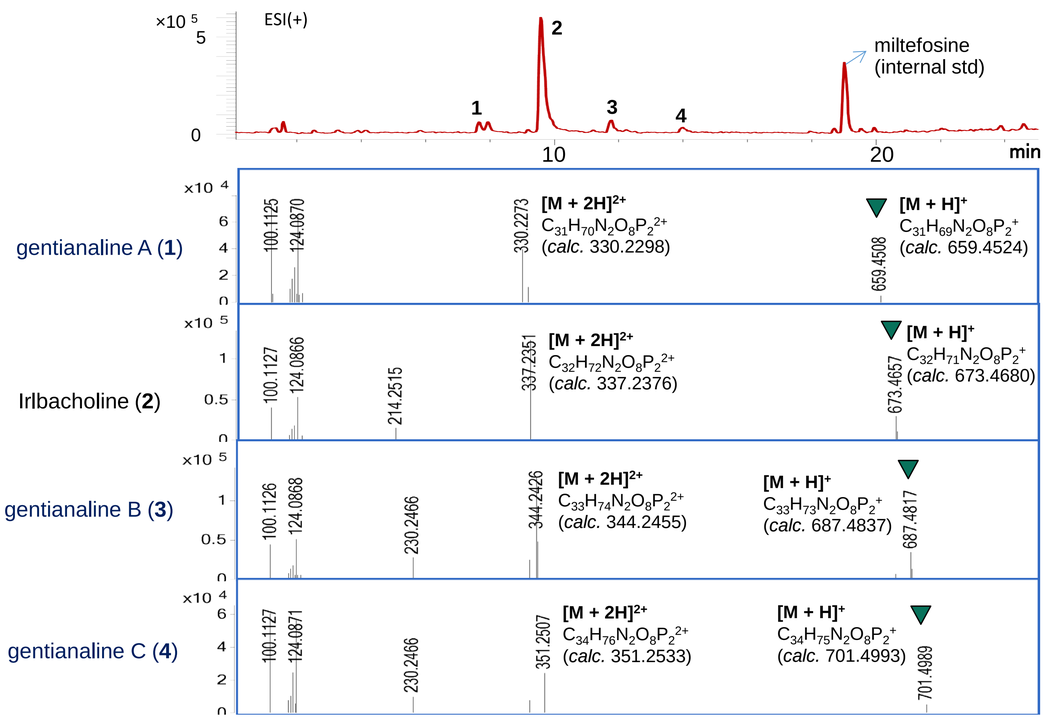
Abstract

Gentiana species including *G. crassicaulis*, *G. macrophylla*, *G. dahurica*, and *G. straminea* are used in traditional Chinese medicine (TCM) as "Qinjiao" for the treatment of rheumatism, hepatitis, and pain. Four antifungal bisphosphocholines [irlbacholine (**2**) and three new analogues, gentianalines A−C (**1**, **3**, and **4**)] were identified from *G. crassicaulis* by a bioassay-guided fractionation and structure elucidation approach. Subsequent chemical analysis of 56 "Qinjiao" samples (45 from *G. crassicaulis*, five from *G. macrophylla*, three from *G. dahurica*, and three from *G. straminea*) showed that bisphosphocholines were present in all four *Gentiana* species, with irlbacholine as the major compound ranging from 2.0−6.2 mg per gram dried material. Irlbacholine exhibited potent in vitro antifungal activity against *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Candida albicans*, and *Candida glabrata* with minimum inhibitory concentrations (MICs) values of 0.63, 1.25, 10.0, and 5.0 μg/mL, respectively. Identification of the bisphosphocholines, a rare class of antifungal natural products, in these medicinal plants provides scientific evidence to complement their medicinal use. The bisphosphocholines carrying a long aliphatic chain possess amphiphilic molecule-like properties with a tendency of retention in both normal and reversed-phase silica gel column chromatography, and thereby may be neglected in natural products discovery. This report may stimulate interest in this class of compounds that warrant the further study of other biological activities as well.

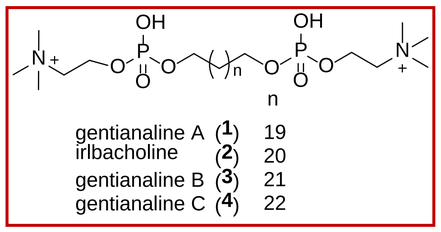
A Workflow for Identification of Antifungal Compounds



Identification of Bisphosphocholines by UHPLC-QToF-ESIMS



LC-MS Analysis of Bisphosphocholines in 56 *Gentiana* spp.



In Vitro Antifungal Activity of Irlbalcholine (2) and Extracts

Compound	IC50/MIC[a] (μg/mL)			
	C. neoformans ATCC 90113	*A. fumigatus* ATCC 204305	*C. albicans* ATCC 90028	*C. glabrata* ATCC 90030
irlbacholine (**2**)	0.42 / 0.63	0.87 / 1.25	3.7 / 10.0	2.08 / 5.0
fraction a[b]	1.15 / 2.5	2.06 / 2.5	15.0/ >20.0	7.3 / 20.0
EtOH extract	5.2 / 12.5	9.99 / 12.5	>200 / >200	34.4 / 100
$CHCl_3$ extract	14.9 / 25.0	44.3 / 100	>200 / >200	90.5/ 200
amphotericin B	0.19 / 0.63	1.1 / 2.5	0.22 / 0.63	0.21 / 0.63

[a]IC_{50}: concentration responsible for 50% growth inhibition of fungal cells; MIC: minimum inhibitory concentration (lowest concentration that allows no detectable growth). The highest test concentrations for compounds, fractions, and crude extracts are 20, 20, and 200 μg/ml, respectively. [b]A fraction contains irlbacholine as major compound and also gentianalines A−C as minor compounds determined by LC-MS.

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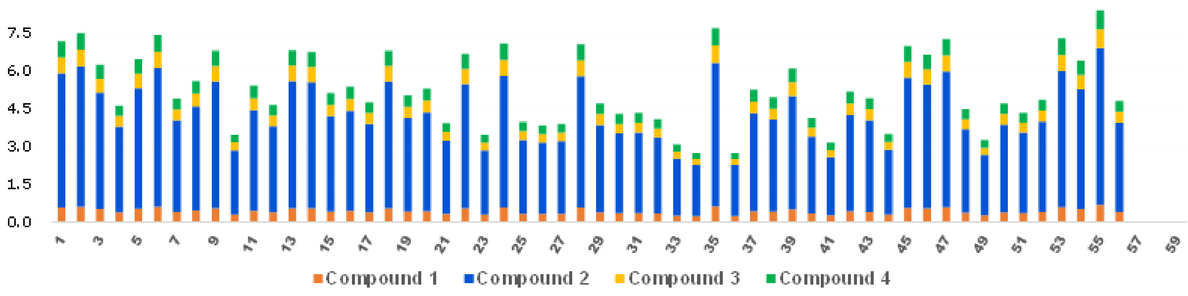
Contents of Antifungal Bisphosphocholines in 56 *Gentiana* spp.



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