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硕 士 学 位 论 文

紫草萘醌类似物细胞毒活性及  
构效关系研究

**Mechanisms of cytotoxicity and structure-activity  
relationship of naphthoquinone analogues in shikonin  
derivatives-induced tumor cell death**

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## 摘要

紫草素衍生物具有很强的细胞毒活性，其主要结构母核 1,4-萘醌与细胞毒活性的构效关系仍不清楚，本文选择不同取代类型的紫草萘醌化合物包括 1, 4-萘二酚、1, 4-萘二醌(NAP)、5, 8-二羟基-1, 4-萘醌 (DMNQ)、胡桃醌、紫草素及去氧紫草素等研究 1,4-萘醌的细胞毒活性规律及构效关系，为紫草素衍生物及其作用机制研究奠定基础。研究结果表明 1,4-萘醌类似物均具有细胞毒活性，与含巯基的亲核试剂如 N-乙酰半胱氨酸 (NAC) 和谷胱甘肽 (GSH) 形成 Michael 加成产物。NAC 或 GSH 可逆转紫草素萘醌类似物的细胞毒活性，其机制与 Michael 加成有关，推测紫草萘醌类化合物细胞毒活性产生机制可能与体内的半胱氨酸残基形成 Michael 加成所致。MTT 实验和 Western Blot 表明紫草萘醌类似物中紫草素和 DMNQ 活性较强，NAP 次之，推测 5,8-二羟基-1,4-萘醌结构为此类化合物抑制肿瘤细胞增殖的关键药效团，其诱导细胞凋亡的机制可能是通过线粒体和内质网两条途径发挥作用的。

此外，本文采用大黄素为内标，建立紫草素和异戊酰紫草素的 HPLC/ESI-MS/MS 定量分析方法，并应用于大鼠血浆中的两个化合物的含量测定。10% HCl 处理后的血样用乙酸乙酯和甲醇进行萃取回收，之后用 XB-C<sub>18</sub> 柱分离，甲醇-乙腈-水梯度洗脱 (10 mM 乙酸铵水: 0.05 % 甲酸乙腈: 甲醇, 10: 45: 45, v/v/v)，负离子模式多反应检测 (MRM)，紫草素和异戊酰紫草素的 LLOQs 分别为 0.5 ng/mL 和 9 ng/mL，其在线性区间的相关系数大于 0.99。日内和日间准确度及精密度好于 13.19%，两种分析物的相对及绝对回收率都超过了 74%，并无基质效应。由此提供了一种简单、快速和特效的大鼠中紫草素衍生物药物代谢动力学研究的方法。

**关键词：** 紫草萘醌 Micheal 加成 细胞毒活性

## Abstract

Shikonin derivatives, which have the basic structure of 1,4-naphthoquinone, can inhibited the growth of tumor cells included by HeLa or A549 cell lines. It is not clear 1,4-naphthoquinones the relationship between the unit induce tumor cell death and form Michael adduct with nucleophilic thiol groups in intracellular biomolecules. To evaluate the cytotoxicity of shikonin derivatives, 1,4-naphthoquinone analogues including shikonin, deoxyshikonin, juglone, 1,4-naphthalene-diol, 1,4-naphthoquinone(NAP), and 5,8-dihydroxy-1,4-naphthoquinone(DMNQ), were applied to investigate the features of cell growth inhibition, the structure-activity relationship and the mechanism of the cytotoxicities of 1,4-naphthoquinone in human lung cancer A549 cells. The results indicated that 1,4-naphthoquinone analogues inhibited the growth of A549 cells and formed Michael adduct with a thiol nucleophile such as N-acetylcystine (NAC) or glutathione (GSH), and preincubation of NAC/GSH with naphthoquinone derivatives eliminated the cytotoxicity of shikonin analogues in cultured cells. These results suggested that the cystein residues *in vivo* contribute to the cytotoxicity of naphthoquinone derivatives and Michael adduct formation was responsible for the severe the 1,4-naphthoquinone cytotoxicity. Western blot analysis showed that the toxicity of these compounds decreased in the series of shikonin ~ DMNQ > NAP, suggesting that side chain of shikonin, the hydroxyl of the side chain, and the double hydroxy substitute in naphthoquinone were responsible for the naphthoquinone's cytotoxicity. The mechanisms of 1,4-naphthoquinone analogues-induced tumor cell death involved in mitochondria pathway and the endoplasmic reticulum stress in A549 cells.

In addition, a high-performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC/ESI-MS/MS) method was

developed and validated for shikonin and isovalerylshikonin in rat plasma using emodin as internal standard (IS). The analytes were extracted from rat plasma with acetoacetate after 10% HCl treatment, methanol protein precipitation. The compounds were separated on a Ultimate<sup>TM</sup> XB-C<sub>18</sub> analytical column using a mobile phase of methanol / 10 mM ammonium acetate in water / acetonitrile containing 0.05% formic acid ( 45:10:45, v/v/v) with isogradient elution. All of the analytes were detected in negative ion mode using multiple reaction monitoring (MRM). LLOQs were 0.5 ng/mL for shikonin, and 9 ng/mL for isovalerylshikonin, respectively. Correlation coefficient (r) values for the linear range of the analytes were greater than 0.99. The intra-day and inter-day precision and accuracy were better than 13.19%. The relative and absolute recovery was above 74% and no matrix effects were observed for all the analytes. This validated method provides a simple, rapid and robust procedure for the pharmacokinetic studies of the shikonin analogues in rats (n = 4).

**Key Words:** 1,4-naphthoquinone; Micheal adduct; cytotoxicity.

## 缩略语说明

符号	英文含义	中文含义
PBS	Phosphate-buffered saline	磷酸盐缓冲液
MTT	thiazolyl blue tetrazolium bromide	四甲基偶氮唑盐
DMSO	Dimethyl Sulfoxide	二甲亚砜
OD	optical density	光密度
IC <sub>50</sub>	half-maximal inhibitory concentration	半数致死量
NAC	N-acetyl-L-cysteine	N-乙酰-L-半胱氨酸
GSH	Glutathione S-transferase	谷胱甘肽
PMSF	Phenylmethanesulfonyl fluoride	苯甲基磺酰氟
SDS	Sodium dodecyl sulfate	十二烷基磺酸钠
BcL-2	B cell lymphoma/ leukemia 2	
Bax	BcL-2 associated X protein	
P-JNK	phosphate c-Jun N-terminal Kinase	磷酸化 JNK
JNK	c-Jun N-terminal Kinase	氨基末端激酶
CYC	Cytochrome C	细胞色素 C
S/N	signal-to-noise	信噪比



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## 第一章 绪论

### 1.1 天然紫草素及其衍生物的研究进展

紫草为紫草科 (*Boraginaceae*) 多年生草本植物新疆紫草(*Arnebia euchroma* (Royle) Johnston)、紫草(*Lithospermum erythrorhizon* Sieb. Et Zucc.) 及黄花紫草(*Arnebia guttata* Bunge,又名内蒙紫草)的干燥根,有3个属的多种植物作为紫草而入药。这些植物主要分布于我国的东北、西北、西南地区以及其他一些国家和地区。其中拟紫草属植物新疆紫草(又名软紫草)[*Arnebia euchroma* (Royle) Johnston]和紫草属植物紫草(又名硬紫草)(*Lithospermum erythrorhizon* S. et Z.) 是药用紫草的主要来源<sup>[1]</sup>。

早在几个世纪之前,紫草就分别被东方和西方用来作为药物。作为传统中药的紫草始载于《神农本草经》,味甘,性寒,归肝经,功能凉血活血,解毒透疹。适用于麻疹或温热病发斑疹,用于血热毒盛斑疹紫黑、麻疹不透、疮疡、湿疹、水火烫伤。紫草具有抗菌、消炎止痛、抗病毒、抗癌、免疫调节、抗生育和止血等作用。此外,紫草对单纯疱疹病毒、乙型肝炎病毒、人类乳头瘤病毒、带状疱疹病毒及甲型肝炎病毒等均有抗病毒作用<sup>[2-4]</sup>。

对紫草的化学成分研究表明,紫草中含有多种化学成分,包括萘醌类、酚酸类、生物碱类、苯酚及苯醌类、三萜酸及甾醇类、黄酮类以及多糖类等。其中最引人注目的是紫草萘醌类化合物,表现出多种生理活性,作为天然色素应已用于医药、化妆品和印染工业中,具有很大的开发应用前景。

#### 1.1.1 天然紫草素衍生物的化学成分及结构

紫草的主要成分为多种萘醌(naphthoquinone)类色素,称为总色素,大



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