Study on angiogenesis activity and mechanism of action of novel tanshinone derivatives produced by one-pot modification of tanshinone mixture from *Salvia miltiorrhiza*

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澳門大學碩士學位論文

通過一鍋法合成的丹參酮衍生物在斑馬魚模型上血管新生作用的系統性評價及機制研究

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Abstract

Study on angiogenesis activity and mechanism of action of novel tanshinone derivatives produced by one-pot modification of tanshinone mixture from *Salvia miltiorrhiza*

by Zhang Zherui

Thesis Supervisor: Prof. Simon Ming Yuen Lee

Chinese Medicinal Science

Chemicals present in low quantity in natural product constitute an important source of chemical diversity. However, isolation of sufficient amounts of these low abundant constituents for structural modification has been a challenge for several decades and subsequently halts research on the utilization of this important source of chemicals to identify novel scaffolds for drug discovery and development. In order to address the difficulty of chemical modification of low abundant constituents in natural products, a novel combinatorial modification approach called one-pot synthesis was used to diversify a partially purified tanshinone mixture isolated from Danshen (*Salvia miltiorrhiza*). This led to the isolation of ten new imidazole-tanshinones [DST-(1-10)] and one oxazole-tanshinone (DST-11), the structures of which were characterized by spectroscopic methods in combination with single-crystal X-ray crystallographic analysis.

Angiogenesis refers to the formation of new blood vessels from preexisting vasculature, and is essential in a series of physiologic processes such as embryonic development, wound healing and female reproductive cycle. Excessive or inefficient angiogenesis may lead to pathologic responses and diseases such as rheumatoid arthritis, diabetic retinopathy, tumor growth and metastasis as well as stroke, heart diseases and ulcers.

The angiogenic activities of the new tanshinone derivatives (DSTs) were determined in an experimental model of chemical-induced blood vessels damage in zebrafish. Of all the tested DSTs, DST-10 with a diketone functionality at C-13 and C-14 exhibited the most potent vascular protective and restorative activity with an EC_{50} value of 0.026 μM. Moreover, the mechanism underlying the pro-angiogenesis effect of DST-10 in injuried blood vessels zebrafish probably involved the VEGF/FGF-Src-MAPK and PI3K-P38 signaling pathways as shown by gene expression analysis and a blocking assay with pathways-specific kinase inhibitors. Meanwhile, DST-10 had been showed significant pro-migration and pro-invasion effetcs in HUVECs, but also exhibited obvious anti-proliferation effects against MCF-7, MDA-231 and A549 cancer lines *in vitro*.

Our results highlighted the potential of adopting a newly modified one-pot synthesis approach to enhance the chemical diversity and biological activities of constituents of natural products regardless of their abundance.

KEY WORDS： angiogensis, one-pot synthesis, tanshinone derivatives, zebrafish.

摘要

天然藥物中一些微量成分不僅豐富了化學多樣性，提供了寶貴的藥物資源，而且具備不容忽視的優良藥效。然而，這些微量成分的提取分離工藝難度較大，且較難獲得足夠的質量。其結構修飾更是受限已久，這些因素嚴重影響了微量化合物作為新型前體藥物的開發。為了克服此類瓶頸，一種倍受諸多化學家推崇的一鍋合成法被應用在大量及微量丹參酮的前期合成研究中，合成得到十個新型咪唑丹參酮衍生物 DST-（1-10）和一個惡唑丹參酮衍生物 DST-11。利用光譜技術結合 X-ray 單晶衍射法進行了結構鑒定。為後期的血管新生效果評價提供了物質基礎。

血管新生是一個重要的生理過程，它涉及胚胎發育，創傷癒合和女性生理週期等。血管新生的失調是諸多疾病的罪魁禍首，如血管新生過量將引發癌症、風濕性關節炎，而在另一方面中風、心肌缺血以及傷口難以癒合都與血管新生不足息息相關。

本課題利用 VRI 誘導的血管損傷斑馬魚模型篩選了十一種丹參酮衍生物的血管新生活性，並與常見的丹參酮（丹參酮 IIA，隱丹參酮 和丹參酮 I）進行了系統性比較。在所有十四種丹參酮類化合物中，在 C-13 和 C-14 位置上有二元酮的 DST-10 表現出較強的保護血管效果，其 EC_{50} 值僅 0.026 μM。而且 DST-10 潛在的血管新生作用機制通過即時定量 PCR 和特異性抑制劑實驗驗證出： DST-10 可能通過 VEGF/FGF-Src-MAPK 和 PI3K-P38 信號通路發揮作用。 與此同時 DST-10 在人臍靜脈內皮細胞和三個癌細胞上分別進行了評價，DST-10 不僅對人臍靜脈內皮細胞展示出明顯的促進遷移和侵入的效果，而且對 MCF-7，MDA-231，A549 三種癌細胞均展示了效果優越的抗增殖效果。我們的結果證實：利用一種新的一鍋法合成，不僅能夠增強化學多樣性，提升生物活性，而且能夠克服自然界產物中原材料稀缺的障礙，為以後合成新型化合物及利用其作為治療血管功能紊亂類疾病提供了依據。

關鍵字：血管新生; 一鍋合成法；丹參酮衍生物；斑馬魚.

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ABBREVIATIONS

%	percent
℃	degree celsius
Akt	protein kinase B
AP	alkaline phosphatase
CPT	cryptotanshinone
CV	caudal vein
DA	dorsal aorta
DLAV	dorsal longitudinal anastomontic vessel
DMSO	dimethyl sulfoxide
dpf	days post fertilization
dpt	days post treatment
DSTs	tanshinone derivatives
ECs	endothelial cells
EDTA	ethylenediaminetetraacetic acid
EGFP	enhanced green fluorescent protein

eNOS	endothelial nitric oxide synthase
ERK	extracellular signal-regulated kinases
F-12K	Kaighn’s modification of Han’s F12 medium
Flk	fetal liver kinase
Flk1	fetal liver kinase like
Flt-1	fms-like tyrosine kinase-1
Flt-4	fms-like tyrosine kinase-4
GFP	green fluorescent protein
h	hour
HIF	hypoxia-inducible factors
hpf	hours post fertilization
hpt	hours post treatment
HUVECs	human umbilical vein endothelial cells
ISV	intersomitic vessel
JNK	c-Jun amino-terminal kinases
KDR	kinase-domain region

MAPK	mitogen-activated protein kinases
MEK	mitogen-activated protein kinase kinase
min	minute
MMP	matrix metalloproteinases
MTT	3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltrtrazolium bromide
nM	nanomole
Nrp-1	neuropilin-1
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PCV	posterior cardinal vein
PH	pleckstrin homology
PI3K	phosphatidylinositol-3 kinase
PIGF	Placenta growth factor
PS	penicillin-streptomycin
Raf	rapidly accelerated fibrosarcoma
SA	segmental artery

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SIV	subintestinal vessel
SV	segmental vein
Tan I	tanshinone I
Tan IIA	tanshinone IIA
TCM	traditional Chinese medicine
UV	ultraviolet
VEGF	vascular endothelial growth factor
WT	wide-type
mg	microgram
XTT	2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide
μM	micromole

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