European Journal of Medicinal Chemistry 109 (2016) 36–46

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

# Silibinin derivatives as anti-prostate cancer agents: Synthesis and cell-based evaluations

Bao Vue [a], Sheng Zhang [a], Xiaojie Zhang [a], Konstantinos Parisis [a], Qiang Zhang [b], Shilong Zheng [b], Guangdi Wang [b, c], Qiao-Hong Chen [a, *]

[a] Department of Chemistry, California State University, Fresno, 2555 E. San Ramon Avenue, M/S SB70, Fresno, CA 93740, USA
[b] RCMI Cancer Research Center Xavier University of Louisiana, 1 Drexel Drive, New Orleans, LA 70125, USA
[c] Department of Chemistry, Xavier University of Louisiana, 1 Drexel Drive, New Orleans, LA 70125, USA

ARTICLE INFO

*Article history:*
Received 22 November 2015
Received in revised form
19 December 2015
Accepted 21 December 2015
Available online 24 December 2015

*Keywords:*
Silibinin derivatives
Anti-proliferative activity
Prostate cancer
Structure–activity relationship
Cell apoptosis

ABSTRACT

This study aims to systematically explore the alkylation effect of 7-OH in silibinin and 2,3-dehydrosilibinin on the antiproliferative potency toward three prostate cancer cell lines. Eight 7-*O*-alkylsilibinins, eight 7-*O*-alkyl-2,3-dehydrosilibinins, and eight 3,7-*O*-dialkyl-2,3-dehydrosilibinins have been synthesized from commercially available silibinin for the *in vitro* cell-based evaluation. The WST-1 cell proliferation assay indicates that nineteen out of twenty-four silibinin derivatives have significantly improved antiproliferative potency when compared with silibinin. 7-*O*-Methylsilibinin (**2**) and 7-*O*-ethylsilibinin (**3**) have been identified as the most potent compounds with 98- and 123-fold enhanced potency against LNCaP human androgen-dependent prostate cancer cell line. Among 2,3-dehydrosilibinin derivatives, 7-*O*-methyl-2,3-dehydrosilibinin (**10**) and 7-*O*-ethyl-2,3-dehydrosilibinin (**11**) have been identified as the optimal compounds with the highest potency towards both androgen-dependent LNCaP and androgen-independent PC-3 prostate cancer cell lines. 7-*O*-Ethyl-2,3-dehydrosilibinin (**11**) was demonstrated to arrest PC-3 cell cycle at the G0/G1 phase and to induce PC-3 cell apoptosis. The findings in this study suggest that antiproliferative potency of silibinin and 2,3-dehydrosilibinin can be appreciably enhanced through suitable chemical modifications on the phenolic hydroxyl group at C-7 and that introduction of a chemical moiety with the potential to improve bioavailability through a linker to 7-OH in silibinin and 2,3-dehydrosilibinin would be a feasible strategy for the development of silibinin derivatives as anti-prostate cancer agents.

Published by Elsevier Masson SAS.

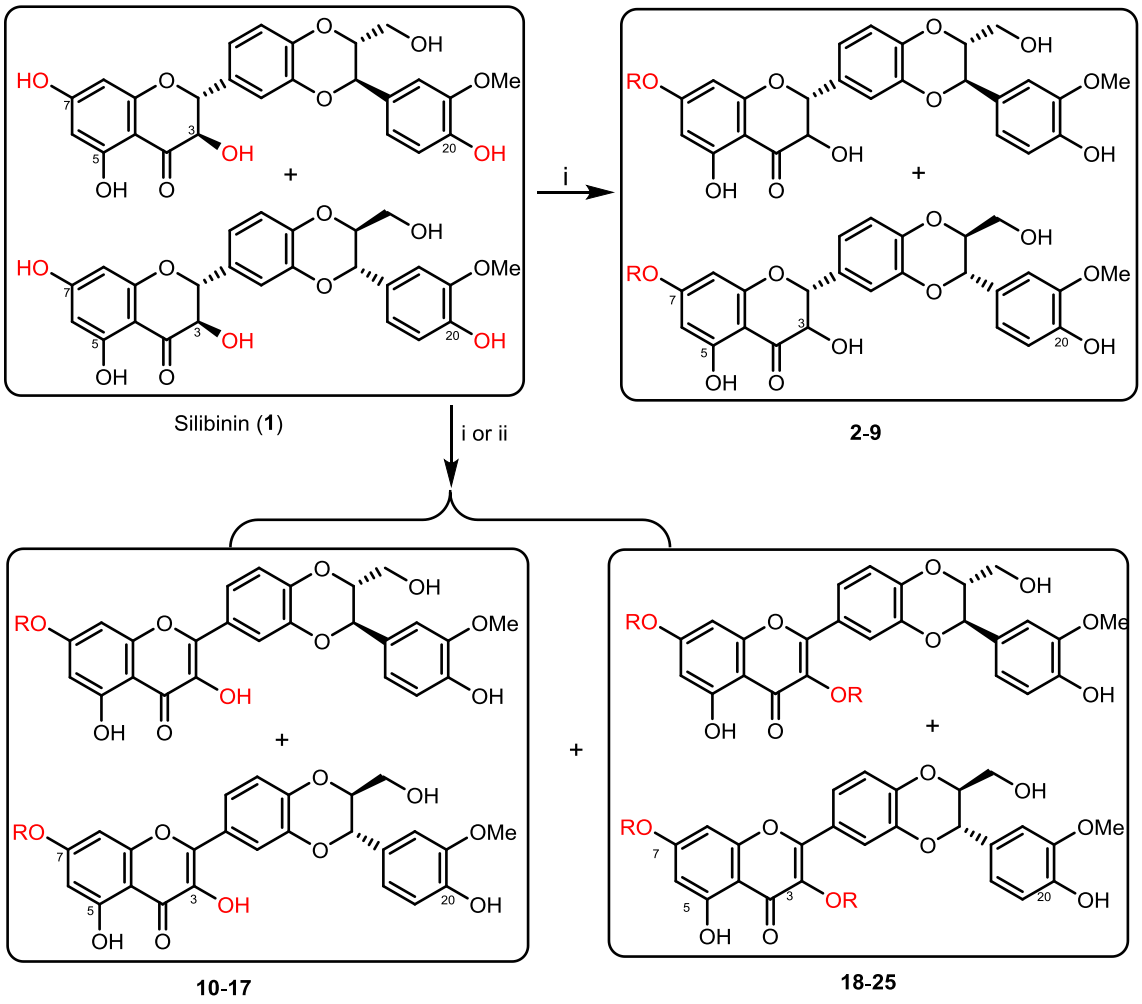
## 1. Introduction

Approximately 300,000 men worldwide, of whom 28,000 are U.S. men, die each year of castration-resistant prostate cancer due to inevitable refractory progression on the first-line treatment with docetaxel [1,2]. As a consequence, prostate cancer has been identified as the fifth and the second leading cause of cancer-related deaths in men worldwide and in American men, respectively. Several new treatments have recently been approved by the US Food and Drug Administration for patients with castration-resistant prostate cancer, but only with very little survival benefit [3,4]. It is thus an urgent need to continue searching for more effective therapeutics for this deadly disease.

Milk thistle (*Silybum marianum*) and its whole extract silymarin have long been used as chemotherapeutics for hepatotoxicity caused by poisoning mushroom and oxidative xenobiotics, and as dietary supplements for the prevention of hepatotoxicity in Europe and Asia [5]. Silibinin (**1**, Scheme 1), consisting of a 1:1 mixture of a pair of diastereoisomeric silybin A and silybin B, is the most abundant chemical components of silymarin [6]. Silybin A and silybin B are naturally occurring hybrid molecules of flavonoid and lignan. Several *in vitro* cell-based and *in vivo* animal studies have demonstrated that silymarin, silibinin, silybin A, and silybin B possess the potential in treating prostate cancer, but with moderate potency [7,8]. A phase I trial of silibinin-phytosome, a formula of silibinin, at a dose of 13 g/day suggested the safety profiles of silibinin in human but poor oral bioavailability due to first-pass metabolism of glucuronidation and sulfation. The phase II studies in patients with localized prostate cancer revealed that oral administration of high-dose silibinin-phytosome led to high

* Corresponding author.
*E-mail address:* qchen@csufresno.edu (Q.-H. Chen).

http://dx.doi.org/10.1016/j.ejmech.2015.12.041
0223-5234/Published by Elsevier Masson SAS.




| Compounds | R |
|---|---|
| **2**, **10**, **18** | Methyl |
| **3**, **11**, **19** | Ethyl |
| **4**, **12**, **20** | Propyl |
| **5**, **13**, **21** | Butyl |

| Compounds | R |
|---|---|
| **6**, **14**, **22** | Pentyl |
| **7**, **15**, **23** | Hexyl |
| **8**, **16**, **24** | Heptyl |
| **9**, **17**, **25** | Benzyl |

**Scheme 1.** Synthesis of silibinin derivatives (**2**–**25**). Reactants and conditions: (i) RX, $K_2CO_3$, DMF (or acetone); (ii) (a) Air, $CH_3COOK$, DMF, 60 °C, 6 h; (b) RX, $K_2CO_3$, DMF.

concentration of silibinin in blood but no detectable silibinin in prostate gland [9,10]. Hence, structure modifications of silibinin to engineer new chemical entities with enhanced potency and bioavailability are highly desirable and are the core focus of our research project.

We started this project from exploring the optimal structure moieties of silibinin suitable for modification by designing and synthesizing three groups of silibinin derivatives for *in vitro* cell-based evaluations against three prostate cancer cell lines. Silibinin was selected as our lead compound because it exhibited similar antiproliferative potency toward prostate cancer cells as each of its two optically pure antipodes (silybin A and silybin B) [11]. 7-*O*-Methylsilibinin (**2**) and 2,3-dehydrosilibinin have been demonstrated to possess enhanced antiproliferative potency toward human prostate cancer cells [12,13]. Additionally, 7-*O*-methylsilibinin (**2**), 7-*O*-benzylsilibinin (**9**), 7-*O*-methyl-2,3-dehydrosilibinin (**10**), 7-*O*-benzyl-2,3-dehydrosilibinin (**14**), 3,7-*O*-dimethylsilibinin (**18**), and 3,7-*O*-dibenzylsilibinin (**25**) have been reported by Kren and co-workers to show better inhibitory effects on P-glycoprotein modulatory activity than silibinin [14]. However, only methylated and benzylated derivatives of silibinin have been reported so far [15] while no synthesis and antiproliferative activities of other alkylated derivatives against prostate cancer cell lines have been systematically investigated. In this study, twenty-four alkylated derivatives of silibinin and 2,3-dehydrosilibinin have been synthesized and evaluated as anti-prostate cancer agents. Among them, eighteen new derivatives were prepared from silibinin for the first time. The above-mentioned six known compounds were also synthesized and evaluated for better understanding of the structure–activity relationship data. The findings from this study will pave the way for our further structural manipulations of silibinin to develop potential anti-prostate cancer agents. The design,