Bioorganic & Medicinal Chemistry Letters 25 (2015) 4020–4023

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification of dialkyl diacetylene diols with potent cancer chemopreventive activity

Chang-Yong Lee [a], Ji Ho Yun [b], Kyungsu Kang [b], Chu-Won Nho [b], Dongyun Shin [a,*]

[a] College of Pharmacy, Gachon University, 191 Hambakmoe-ro, Yeonsu-gu, Incheon 406-799, Republic of Korea
[b] Functional Food Center, Korea Institute of Science and Technology, 290 Daejeon-dong, Gangneung 210-340, Republic of Korea

ARTICLE INFO

Article history:
Received 15 February 2015
Revised 17 April 2015
Accepted 28 May 2015
Available online 6 June 2015

Keywords:
Cancer
Chemoprevention
Diyne
Quinone reductase
Stereoisomeric

ABSTRACT

An increasing importance of chemoprevention for controlling cancer risks prompted the discovery of new active cancer chemopreventive agents. In this study, we designed and synthesized substituted hexa-2,4-diyne-1,6-diols, more structurally simplified, tunable, and easily preparable than natural gymnasterkoreaynes, and evaluated their cancer chemopreventive activities by measuring concentration of doubling quinone reductase activity (CD), cell viability, and chemopreventive index (CI). Most of the diols exhibited good CD activity and low cytotoxicity. In particular, tetradeca-5,7-diyne-4,9-diol and 2-methyltetradeca-5,7-diyne-4,9-diol showed the best cancer chemopreventive activity, approximately equipotent to that of sulforaphane. And, by synthesizing optically active stereoisomers of selected active compounds, the effect of stereochemistry was also studied. Eventually, we produced a chemopreventive compound for in vivo study.

Cancer chemoprevention is defined as the inhibition, retardation, and/or reversal of the carcinogenesis steps that include initiation, promotion, and progression by chemicals with otherwise low cytotoxicity.[1–3] Chemoprevention was categorized in three main areas by Russo group: (1) prevention of carcinogenesis in healthy individuals; (2) inhibition or retardation of cancer in individuals with pre-malignant lesions or; (3) secondary prevention or inhibition of cancer recurrence in patients already having treatment for a primary cancer.[4] Many studies have been reported on mechanisms for chemoprevention, especially prevention of carcinogenesis.[5,6] Among them, detoxification of toxic quinones by quinone reductase (QR, also called NQO1) is one of recognized mechanisms for chemoprevention. Quinone reductase, a representative phase II detoxification enzyme, is revealed to have a close relationship with prevention of cancer by blocking cancer initiation. Thus, QR induction is used as a biomarker of chemopreventive activities and their potency.[7,8]

Up to date, various natural compounds with chemopreventive effects were isolated mostly from dietary plants, and most of them were reported to show their activities by preventing carcinogenesis. Some representative agents include sulforaphane from cruciferous vegetables, lycopene from tomato products, and resveratrol from grapes.[9,10] In particular, oltipraz, a dithiolethione class chemopreventive agent, has evaluated its anti-cancer activity in phase II clinical trial, though it failed due to the low efficacy and side effects.[11]

In previous publications, we reported the isolation of gymnasterkoreaynes B, E, and G from *Gymnaster koraiensis* by activity-guided fractionations and their potent cancer chemopreventive effects.[12] These compounds induced phase II detoxification enzymes known to have cytoprotective functions, such as glutathione-*S*-transferase, UDP-glucuronosyltransferase, NAD(P)H: quinone oxidoreductase (NQO1), and glutathione reductase (GSR), in normal and HepG2 human hepatocarcinoma cells. The gymnasterkoreaynes are naturally occurring polyacetylenic compounds. Our group recently completed the total synthesis of gymnasterkoreaynes E and G, which contain octa-4,6-diyne-2,3,8-triol as a major functional backbone (Fig. 1).[13] We also described the structure–activity relationship (SAR) of these polyacetylenes on cancer chemopreventive activity.[14] In that study, important structural information regarding the use of diyne triols in cancer chemoprevention was revealed: the reduction of the diyne moiety to a saturated alkyl group fully eliminated both chemopreventive activity and cytotoxicity, and the variations of the terminal alkyl groups had significant effects on the induction potency of quinone reductase and cellular toxicity.

Structurally, gymnasterkoreaynes G, E and B have *cis*-olefin and three stereocenters. Such molecular complexities limited the synthesis of optically pure form of active stereoisomers and also the production of a large amount of active compounds for in vivo study. Hence, on the basis of the established SAR, we hypothesized

* Corresponding author. Tel.: +82 32 899 6111; fax: +82 32 820 4829.
E-mail address: dyshin@gachon.ac.kr (D. Shin).

http://dx.doi.org/10.1016/j.bmcl.2015.05.098
0960-894X/

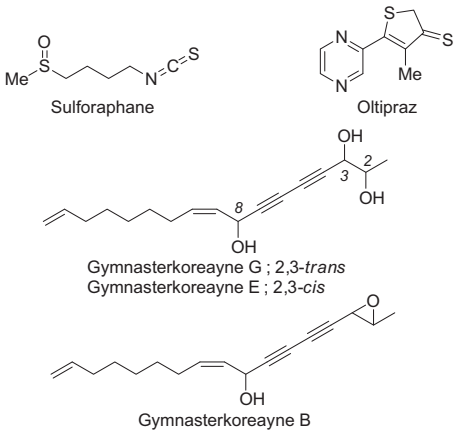


Figure 1. Structures of gymnasterkoreayne E, G, and B.

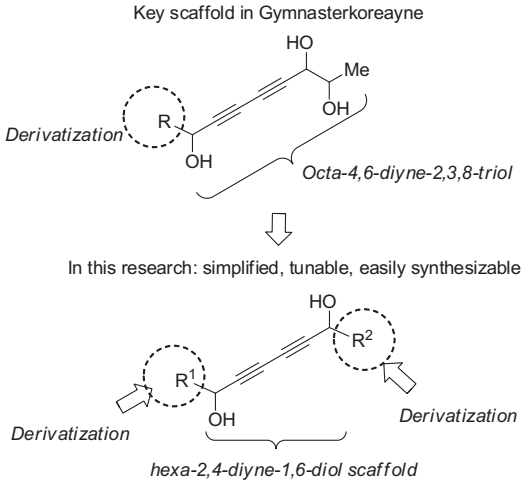
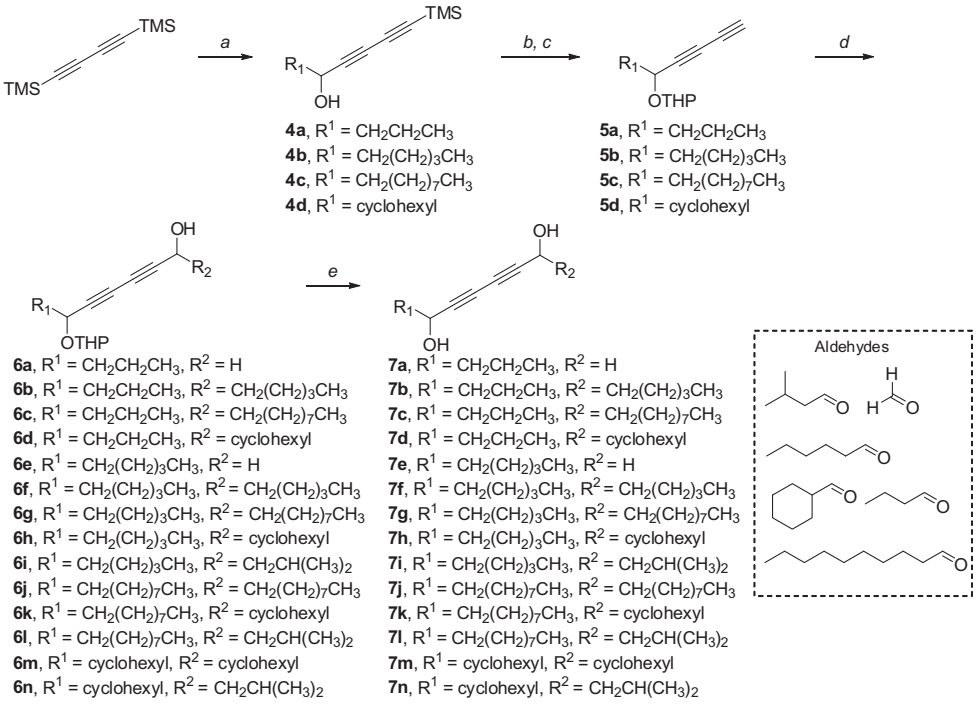


Figure 2. Design of diyne diols based on diyne triols.

that a structurally simplified diyne-based scaffold which retains diacetylenic diol moiety might have similar biological activities and is much easier to synthesize as stereoisomeric mixtures or single stereoisomers than diacetylenic triols. Herein, we report the synthesis and cancer chemopreventive activities of new diyne diols and eventually, creation of compounds for in vivo study (Fig. 2).

Syntheses of the diacetylene diol analogues were conducted using the previously established synthetic procedure by our group, as illustrated in Scheme 1. Starting from the commercially available 1,4-bis(trimethylsilyl)buta-1,3-diyne, various alkyl aldehydes were installed on both ends of 1,3-butadiyne. Generation of acetylenic anions from 1,4-bis(trimethylsilyl)buta-1,3-diyne through a metal-silicon exchange reaction by treating methyl lithium–lithium bromide in THF, followed by the addition of alkyl aldehyde produced acetylenic alcohols **4a**–**4d** in excellent yields.[15] Sequential protection of the secondary alcohol with THP and desilylation of terminal trimethylsilyl by TBAF gave compounds **5a**–**5d**. In the THP protection step, two diastereomeric mixtures were detected in thin-layer chromatography and were barely separable, and so both diastereomers were used for the next step without separation. Terminal alkynes were deprotonated by ethylmagnesium bromide and treated with alkyl aldehydes to give THP-protected dialkyl hexa-2,4-diyne-1,6-diols **6a**–**6n**. Finally, deprotection of THP under the condition of a 9:1 mixture of 70% AcOH in H_2O and THF gave the desired diols **7a**–**7n**. After synthesis of the derivatives, we recognized that **7b**, **7e**, **7f**, **7j**, and **7m** had been previously reported.[16–20] Overall, 14 diyne diols were synthesized by modification of both terminals with different alkyl groups. By fixing the left terminal with *n*-propyl, *n*-pentyl, *n*-nonyl, or cyclohexyl groups, the right terminal was derivatized with other alkyl groups.

We exploited the potency of cancer chemopreventive activity induced by dialkyl diacetylene diols by measuring the quinone reductase (QR) assay in Hepa1c1c7 murine hepatoma cells. The QR assay, a useful tool for the evaluation of cancer chemopreventive activity, was performed according to the Prochaska modified method.[8,21] The potency of the cancer chemopreventive activity



4a, $R^1 = CH_2CH_2CH_3$
4b, $R^1 = CH_2(CH_2)_3CH_3$
4c, $R^1 = CH_2(CH_2)_7CH_3$
4d, R^1 = cyclohexyl

5a, $R^1 = CH_2CH_2CH_3$
5b, $R^1 = CH_2(CH_2)_3CH_3$
5c, $R^1 = CH_2(CH_2)_7CH_3$
5d, R^1 = cyclohexyl

6a, $R^1 = CH_2CH_2CH_3$, R^2 = H
6b, $R^1 = CH_2CH_2CH_3$, $R^2 = CH_2(CH_2)_3CH_3$
6c, $R^1 = CH_2CH_2CH_3$, $R^2 = CH_2(CH_2)_7CH_3$
6d, $R^1 = CH_2CH_2CH_3$, R^2 = cyclohexyl
6e, $R^1 = CH_2(CH_2)_3CH_3$, R^2 = H
6f, $R^1 = CH_2(CH_2)_3CH_3$, $R^2 = CH_2(CH_2)_3CH_3$
6g, $R^1 = CH_2(CH_2)_3CH_3$, $R^2 = CH_2(CH_2)_7CH_3$
6h, $R^1 = CH_2(CH_2)_3CH_3$, R^2 = cyclohexyl
6i, $R^1 = CH_2(CH_2)_3CH_3$, $R^2 = CH_2CH(CH_3)_2$
6j, $R^1 = CH_2(CH_2)_7CH_3$, $R^2 = CH_2(CH_2)_7CH_3$
6k, $R^1 = CH_2(CH_2)_7CH_3$, R^2 = cyclohexyl
6l, $R^1 = CH_2(CH_2)_7CH_3$, $R^2 = CH_2CH(CH_3)_2$
6m, R^1 = cyclohexyl, R^2 = cyclohexyl
6n, R^1 = cyclohexyl, $R^2 = CH_2CH(CH_3)_2$

7a, $R^1 = CH_2CH_2CH_3$, R^2 = H
7b, $R^1 = CH_2CH_2CH_3$, $R^2 = CH_2(CH_2)_3CH_3$
7c, $R^1 = CH_2CH_2CH_3$, $R^2 = CH_2(CH_2)_7CH_3$
7d, $R^1 = CH_2CH_2CH_3$, R^2 = cyclohexyl
7e, $R^1 = CH_2(CH_2)_3CH_3$, R^2 = H
7f, $R^1 = CH_2(CH_2)_3CH_3$, $R^2 = CH_2(CH_2)_3CH_3$
7g, $R^1 = CH_2(CH_2)_3CH_3$, $R^2 = CH_2(CH_2)_7CH_3$
7h, $R^1 = CH_2(CH_2)_3CH_3$, R^2 = cyclohexyl
7i, $R^1 = CH_2(CH_2)_3CH_3$, $R^2 = CH_2CH(CH_3)_2$
7j, $R^1 = CH_2(CH_2)_7CH_3$, $R^2 = CH_2(CH_2)_7CH_3$
7k, $R^1 = CH_2(CH_2)_7CH_3$, R^2 = cyclohexyl
7l, $R^1 = CH_2(CH_2)_7CH_3$, $R^2 = CH_2CH(CH_3)_2$
7m, R^1 = cyclohexyl, R^2 = cyclohexyl
7n, R^1 = cyclohexyl, $R^2 = CH_2CH(CH_3)_2$

Scheme 1. Synthesis of diyne diols. Reagents and conditions: (a) MeLi–LiBr, THF, 0 °C to rt; then aldehyde, 78–83%; (b) DHP, *p*-TsOH, CH_2Cl_2, rt, 77–88%; (c) TBAF, THF, rt, 82–87%; (d) EtMgBr, 0 °C to rt; then aldehyde, 67–78%; (e) 70% AcOH in H_2O/THF (9:1), 40 °C, 71–83%.