Bioorganic & Medicinal Chemistry Letters 20 (2010) 7549–7552

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

SAR studies of gymnasterkoreayne derivatives with cancer chemopreventive activities

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ARTICLE INFO

Article history:
Received 1 June 2010
Revised 12 July 2010
Accepted 16 July 2010
Available online 21 July 2010

Keywords:
Structure–activity relation
Cancer
Derivatives
Chemopreventive
Gymnasterkoreayne

ABSTRACT

We synthesized diyne triols based on gymnasterkoreayne and evaluated their cancer chemopreventive activities in terms of the chemopreventive index (CI) to reveal the structure–activity relationship, and discovered more active compounds than natural diynes.

Cancer is an extremely concerning health issue worldwide in that it is one of the leading causes of death. Thus far, cancer has typically been treated by approaches such as surgery, chemotherapy, and radiation therapy. Chemoprevention is a novel concept that has recently attracted considerable attention; this strategy is mainly used to suppress, delay, or reverse carcinogenic processes using natural or synthetic substances having anti-cancer activities.[1] Therefore, this approach might be highly beneficial to patients in that it could reduce medical costs and improve the quality of life. Numerous studies and clinical trials have indicated that a series of certain chemicals exhibit chemopreventive activities that reduce the risk of certain cancers.[2]

Through epidemiological data and laboratory studies, it has already been demonstrated that numerous phytochemicals such as, sulforaphane (**1**), obtained from cruciferous vegetables; lycopene (**2**), obtained from tomatoes and tomato products; and resveratrol (**3**), a natural compound produced in grapes exhibit anti-cancer activity[2] (Fig. 1). Compounds exhibiting anti-cancer activities are typically classified as blocking agents or suppressing agents. Blocking agents such as allicin (**4**) and polyphenol antioxidants such as ellagic acid (**5**) prevent carcinogens from reaching the targets through the induction of detoxification enzymes. On the other hand, suppressing agents such as sulforaphane and oltipraz can inhibit carcinogenic transformation in normal cells at any stage of proliferation, while sulforaphane is also considered to act as a blocking agent at low doses.

The cancer chemopreventive activity of a compound is indicated by the chemopreventive index (CI), calculated as [concentration for 50% inhibition of cell viability (IC_{50})]/[concentration required to double quinone reductase (QR) induction (CD)].[3] QR induction is typically used as a biomarker for cancer chemoprevention.

Recently, our research team has focused on developing new cancer chemopreventive agents from Korean wild vegetables. The bioactivity-guided fractionation of the aerial parts of *Gymnaster koraiensis*, followed by the structural identification of its active components revealed that gymnasterkoreayne B (**6**) and G (**7**) exhibit chemopreventive activities having CI values of 9.57 and 10.51, respectively. Gymnasterkoreayne B and G, both of which are natural diynes,[4] were first isolated in 2002 and 2005, respectively.[5,6] Gymnasterkoreayne B has a 2,3-epoxy moiety and gymnas-

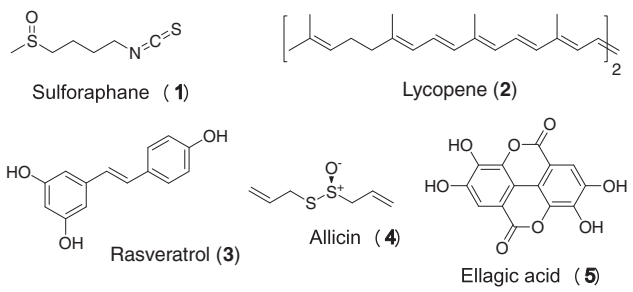

Figure 1. Examples of phytochemicals with anti-cancer activity.

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0960-894X/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.07.066

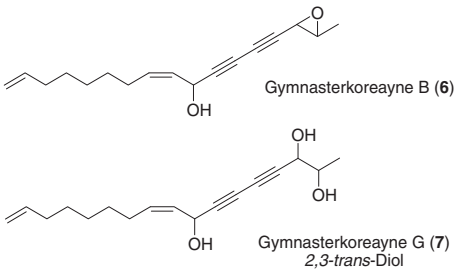


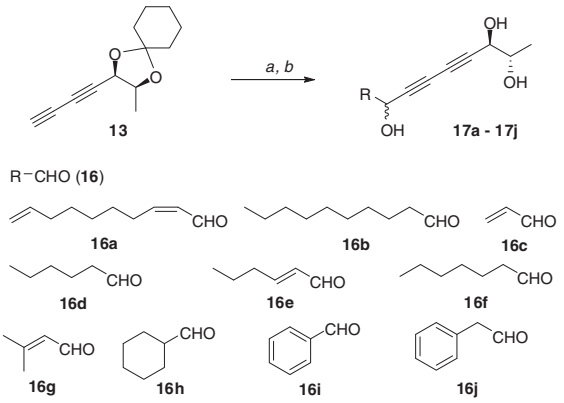
Figure 2. Structure of gymnasterkoreayne B and G.

terkoreayne G, a 2,3-*trans* diol moiety. The absolute stereochemistries of C2, C3, and C8 of these compounds have not yet been assigned (Fig. 2). To search for compounds having higher potency and less cytotoxicity, that is, a higher CI value, we carried out a structure–activity relationship (SAR) study. Herein, we disclose the synthesis of C2, C3-*cis/trans* 4,6-diyne-7-ol derivatives based on gymnasterkoreayne G and their SAR for cancer chemopreventive activities.

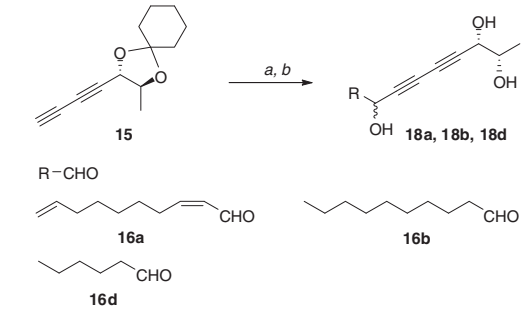
The preparation of the diyne intermediates **13** and **14** is shown in Scheme 1. First, a known (*S*)-2-(tert-butyldimethylsilyloxy)propanal (**8**)[7] is alkylized with 1,4-bis(tri-methylsilyl)buta-1,3-diyne (**9**) using MeLi–LiBr complex to afford *trans*- and *cis*-diastereomers **10** and **11** in 87% yield with a diastereomeric ratio of 78:22; these compounds were easily separated by flash column chromatography. Both of the TMS and TBS groups were removed simultaneously by the treatment of TBAF at room temperature. The diol moieties of **12** and **14** were ketalized by reacting them with 1,1-dimethoxycyclohexane and pyridinium *p*-toluenesulfonate (PPTS). As a result, two diyne intermediates **13** and **15** were obtained with 85% and 83% yields, respectively, in two steps. During the synthesis, we realized that these diynes should be immediately used for the next step of the synthesis because they were unstable, especially under solvent-free conditions.

2,3-*trans* Derivatives **17a**–**17j** for the SAR study were synthesized by introducing various aldehydes, as illustrated in Scheme 2. The generation of acetylenic anion from diyne **13** using ethylmagnesium bromide at room temperature, followed by the addition of aldehydes **16a**–**16j** provided the desired alcohols in 65–91% yield. Finally, the ketal was deprotected under 30% AcOH in H_2O/THF (1:3) to afford triols **17a**–**17j**.

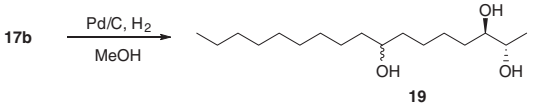
2,3-*cis*-Diol derivatives **18a**, **18b**, and **18d** were also obtained by the same procedure as that for the synthesis of 2,3-*trans* diols (Scheme 3). The aldehydes were selected based on the chemopreventive activities of 2,3-*trans* analogues. The fully hydrogenated



Scheme 2. Reagents and conditions: (a) EtMgBr, R-CHO, THF, 0 °C to rt, 55–78% for aldehydes; (b) 30% AcOH/THF (1:3), 50 °C, 73–87% for **17a**–**17j**.



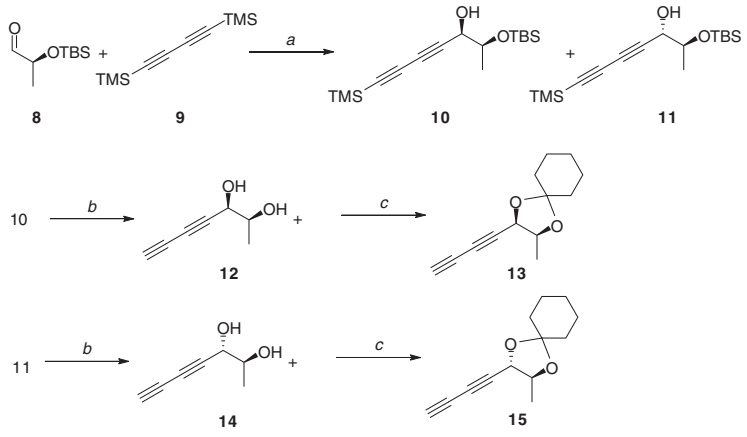
Scheme 3. Reagents and conditions: (a) EtMgBr, THF, 0 °C to rt, 58–77% for aldehydes; (b) 30% AcOH/THF (1:3), 50 °C, 82% for **18a**, 77% for **18b**, 85% for **18d**.



Scheme 4. Synthesis of fully hydrogenated triol.

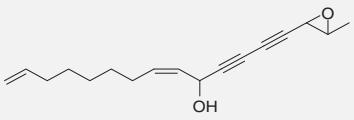
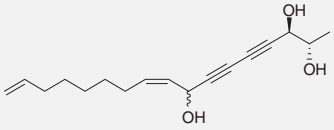
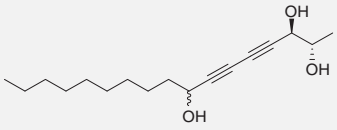
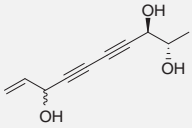
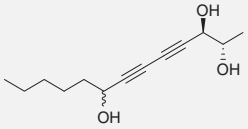
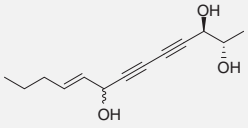
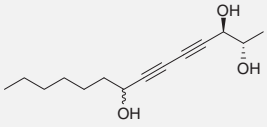
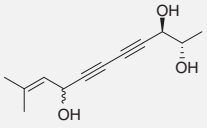
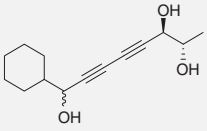
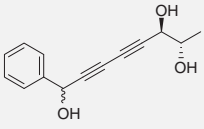
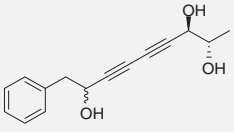
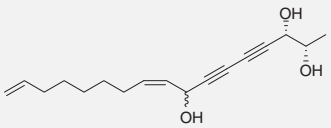
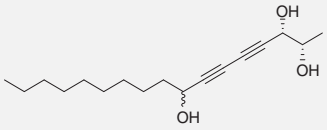
triol **19** was prepared by the hydrogenation of diyne **17b** using 10% Pd/C and H_2 in MeOH as a solvent. (Scheme 4)

The structures of the diyne derivatives prepared for this study and their anti-cancer activities are shown in Table 1. The cancer chemopreventive activity of these compounds was investigated



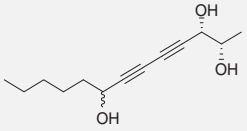
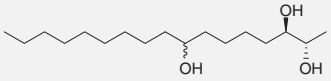
Scheme 1. Reagents and conditions: (a) MeLi–LiBr, THF, 0 °C to rt, 61% of **10**, 26% of **11**, respectively; (b) TBAF, THF, rt quantitative yields; (c) 1,1-dimethoxycyclohexane, PPTS, CH_2Cl_2, rt, 77% for **13** and 82% for **15**.

Table 1
Cancer preventive activities

No	Compound	Structure	CD[a] (μM)	IC_{50}[b] (μM)	CI
1			7.38	70.59	9.57
2	**17a**		8.44	88.63	10.51
3	**17b**		5.34	39.78	7.44
4	**17c**		158	176.95	1.12
5	**17d**		15.79	>500	>31.66
6	**17e**		28.59	422.18	14.77
7	**17f**		8.69	222.94	25.66
8	**17g**		23.86	405.99	17.02
9	**17h**		131.72	>500	>3.80
10	**17i**		36.84	>500	>13.57
11	**17j**		57.89	>500	>8.64
12	**18a**		13.72	252.22	18.39
13	**18b**		2.13	64.24	30.13

(*continued on next page*)

Table 1 (*continued*)

No	Compound	Structure	CD[a] (μM)	IC_{50}[b] (μM)	CI
14	**18d**		12.96	>500	>38.60
15	**19**		>500	>500	—

[a] QR induction activities were determined using the Prochaska modified bioassay with minor modifications.[8]
[b] Cytotoxicity.

in vitro by measuring the CI value, which is calculated as defined above.

Diyne triol **17a**, diastereomeric mixtures at C8, has a CI value of 10.51 that is comparable to that of gymnasterkoreayne B (CI value of 9.57). The two compounds have similar QR activities and cytotoxicity. Partially hydrogenated triol **17b**, which still retains diyne functionality, exhibited a slightly elevated CD value and higher cytotoxicity (CD = 5.34; IC_{50} = 39.78; CI = 7.44). When planning this study, we envisioned that the diyne moiety might be essential because of its biological activity considering the structural feature of gymnasterkoreaynes. To prove the same, fully hydrogenated triol **19**, which bears the same carbon skeleton as gymnasterkoreaynes B and G, was synthesized to evaluate the activities. As expected, triol **19** did not exhibit any QR induction activity and cell toxicity (CD >500; IC_{50} >500). These results provided basic structural information on the pharmacophore of gymnasterkoreayne products.

Based on the initial results of the SAR study, the chemopreventive activities of analogues **17c**–**17j**, which are derivatized at the left side chain of the diyne, were investigated, and the obtained results are summarized in Table 1. Vinyl compounds having a short alkyl group exhibited reduced QR activity and elevated cytotoxicity (CD = 158; IC_{50} = 422; CI = 1.12), whereas 2,2-dimethylvinyl analogue **17g** exhibited more potent activity and lower cytotoxicity (CD = 23.86; IC_{50} = 405.99; CI = 17.02). Diynes having medium-length chains (C5–C6), such as (*E*)-1-pentenyl (**17d**), *n*-pentyl (**17e**), and *n*-hexyl (**7f**) exhibited better activities in terms of the CI value. Among them, compound **17e** did not exhibit any cytotoxicity under 500 μM (CD = 15.79; IC_{50} >500; CI >31.66). Bulkier analogues such as cyclohexyl (**17h**), phenyl (**17i**), and phenylmethyl (**17j**) exhibited moderate CD activity, but little cytotoxicity. The above data on the structure–activity tendency suggested that a longer chain length of analogues induced better QR activity. However, in terms of cytotoxicity, compounds with medium-sized or bulkier side chains exhibited better profiles. In the interest of striking a balance, once can consider analogues having medium-sized chains to exhibit optimal cancer chemopreventive activity.

Triol **18a** and (*2S,3S*)-heptadeca-4,6-diyne-2,3,8-triol (**18b**) were more potent (CD = 13.72 and 2.13, respectively) and less cytotoxic (IC_{50} = 252.22 and 64.24, respectively) than the corresponding (*2S,3R*)-*trans*-diols. However, these two compounds were still sufficiently cytotoxic. *n*-Pentyl *cis*-diol **18d** did not exhibit any cytotoxicity under 500 μM, providing the best profile in terms of the CI value, which is similar to the result obtained for *n*-pentyl *trans*-diol **17d**.

In conclusion, we synthesized diyne triols based on gymnasterkoreayne G and evaluated their cancer chemopreventive activities in terms of the chemopreventive index (CI). The results of the structure–activity relationship revealed that the diyne moiety is an essential pharmacophore in these compounds. The chain length has a strong effect on the activity and cytotoxicity of these compounds. Among the analogues, two *n*-pentyl analogues **17d** and **18d** exhibited the best activity profiles in this study. (CD = 15.79 and 12.96; IC_{50} >500 and >500; CI >31.66 and >38.60, respectively). Further study is ongoing to see the effect of stereochemistry at C2, C3 and C8.

Acknowledgments

This study is supported by a KIST-Gangneung Institute Intramural Grant (2Z03411). The authors thank Dr. Hoh-Gyu Han (KIST) for helpful discussions and comments.

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