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Systemic structure–activity relationship study of phenyl polyyne diols as potential chemopreventive agents

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ABSTRACT

The present study reports the cancer chemopreventive activities of phenyl polyyne diols derived from polyacetylene triol. Thirty-seven analogues based on a 1-phenylhexa-2,4-diyne-1,6-diol scaffold were prepared and their effects on QR activity were elucidated, as well as their cytotoxicities. We found that most of the derivatives based on phenylhexa-2,4-diyne-1,6-diol exhibited good QR induction activity and relatively low cytotoxicity and systemic structure–activity relationship was revealed. In particular, 4-fluorophenyl, 3-chlorophenyl, and 3,4-dioxolophenyl derivatives showed the best profiles in terms of QR induction, cytotoxicity, and CI.

Cancer is a major global healthcare issue. Many interventions have been developed to treat cancers, including chemotherapy, radiotherapy, and surgery. Carcinogenesis is a long-term process that involves multiple stages including initiation, promotion, progression, and metastasis. Cancer chemoprevention, defined as the use of chemicals to inhibit, reverse, or retard these stages, provides an attractive strategy to address human cancers and can be used by individuals who have a high risk for cancer development.[1] Cancer chemopreventive agents mostly target the initiation and promotion steps of carcinogenesis.[2,3] Various genotoxic stresses are known to cause cancer initiation, an irreversible step towards carcinogenesis. Phase II detoxification enzymes block tumor initiation by detoxifying or eliminating genotoxic carcinogens. Quinone reductase (QR, also known as NAD(P)H: quinone oxidoreductase), a representative phase II detoxification enzyme, is regarded as a surrogate biomarker for cancer chemoprevention.[4] To date, various naturally occurring phytochemicals and their synthetic derivatives have been discovered to induce QR and these therefore represent valuable sources of cancer chemopreventive agents.[5–8]

Our ongoing research into novel small molecules with cancer chemopreventive activity has recently focused on natural compound-based agents. In this connection, gymnasterkoreayne B (**1**), E (**2**), and G (**3**),[9,10] (Fig. 1) were isolated by activity-guided fractionation of *Gymnaster koraiensis* and shown to have high QR induction activity in vitro and in vivo, which resulted in anti-cancer and hepatoprotective effects.[11,12] Encouraged by this result, we carried out a preliminary structure–activity relationship study of this polyacetylene triol natural product, which has octa-2,4-diyne-1,6,7-triol as its basic scaffold. This study revealed important structural features that contributed to chemopreventive activity: (1) the diene moiety was essential for activity; (2) triol stereochemistry had mild effects on activity; (3) alkyl chain variation had significant effects on QR induction activity; overall, the n-pentyl derivative showed optimal effects in relation to its activity and toxicity.[13] More recently, we published a structure–activity relationship study of novel dialkyl diyne diols. This identified more potent chemopreventive compounds and explored the effect of stereochemistry by synthesizing all stereoisomers of the active compounds.[14]

The present study investigated the cancer chemopreventive activities of phenyl polyyne diols derived from polyacetylene triol, which are structurally simplified and easier to synthesize (Fig. 2). Thirty-seven analogues based on a 1-phenylhexa-2,4-diyne-1,6-diol scaffold were prepared and their effects on QR activity were elucidated, as well as their cytotoxicities.

The analogues were synthesized using the procedure reported previously by our group (Scheme 1).[13,15] Bis(trimethylsilyl)buta-1,3-diyne was mono-lithiated by incubation with methyl lithium–lithium bromide complex (MeLi–LiBr) in tetrahydrofuran (THF), followed by the addition of *n*-hexanal to afford 1-(trimethylsilyl)deca-1,3-diyn-5-ol (**6**).[16] In our previous study, the C5 substituent at one end of the diene triol core produced a good chemopreventive profile.[13] Subsequent protection of the resulting alcohol with dihydropyran (DHP) in the presence of an

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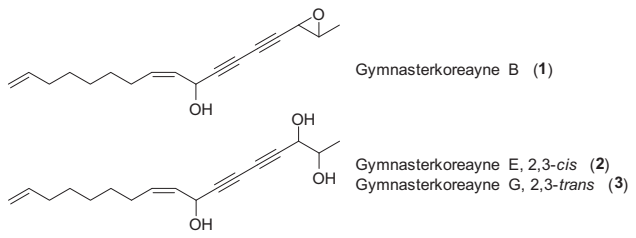


Figure 1. Structures of gymnasterkoreayne B, E, and G.

acid catalyst (PPTS), and removal of the terminal trimethylsilyl group by tetrabutylammonium fluoride (TBAF) provided tetrahydropyran (THP)-protected deca-1,3-diyn-5-ol (**7**). By treating ethylmagnesium bromide as a base, the terminal alkyne anion was completely generated, and then various substituted benzaldehydes and naphthaldehydes were added to produce benzyl alcohols (**8**). Finally, THP was deprotected in the presence of 70% AcOH/THF (9:1) to give a series of 1-phenylhexa-2,4-diyne-1,6-diol derivatives (**9**). Twenty-seven phenyl and two naphthyl compounds were synthesized, as listed in Table 1.

Induction of QR neutralizes potential carcinogens and this mechanism is believed to underlie cancer chemoprevention. The cancer chemopreventive activity of a compound is usually evaluated by measuring the concentration required to double QR activity (the CD value), cell viability (the 50% inhibitory concentration, IC_{50}), and the chemopreventive index (CI), which is calculated by dividing the IC_{50} by the CD. In this study, all diyne diols were evaluated for in vitro QR induction and cytotoxicity. Sulforaphane, which is a representative cancer chemopreventive organosulfur compound obtained from cruciferous vegetables, was used as a control agent and the maximum concentration used to test cell viability was 50 μM.

Our previous study of gymnasterkoreaynes indicated that the *n*-pentyl chain was relatively optimal in terms of its activity/toxicity ratio and ease of preparation. One end of each diyne diol compound therefore included an *n*-pentyl group. In the first round, 27 phenyl derivatives were synthesized and evaluated for QR induction activity and cell viability; these results are presented in Table 1. Various halides, alkyl, and alkoxy groups were introduced to the phenyl ring for this structure–activity study. The non-substituted phenyl compound, **9a**, showed a CD value of 6.5 μM and no toxicity at up to 50 μM. The mono-halogenated derivatives, **9b**–**9h**, exhibited good to moderate QR induction with CD values ranging from 1.3 to 22.3 μM, and low toxicity. The CD and cell viability results indicated that *meta* or *para* substitution with fluoride or chloride produced better CD values than *ortho* substitution. In particular, 3-chlorophenyl (**9d**), 4-fluorophenyl (**9f**), and 4-chlorophenyl (**9g**) compounds displayed CD values of 1.3, 3.2, and 3.3 μM, respectively, with no toxicity at up to 50 μM, with the exception of 3-chlorophenyl (IC_{50} = 41.8 μM). Displacement of hydrogen with bromide at the *meta* or *para* position did not enhance activity, as compared with the phenyl analogue, while cytotoxicity remained low. Substitution of a 4-*tert*-butyl group on phenyl (**9i**) preserved CD activity, but this compound was more toxic than the unsubstituted derivative (**9a**).

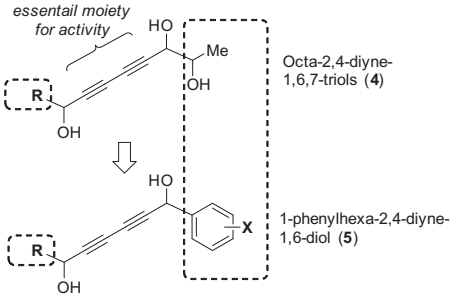
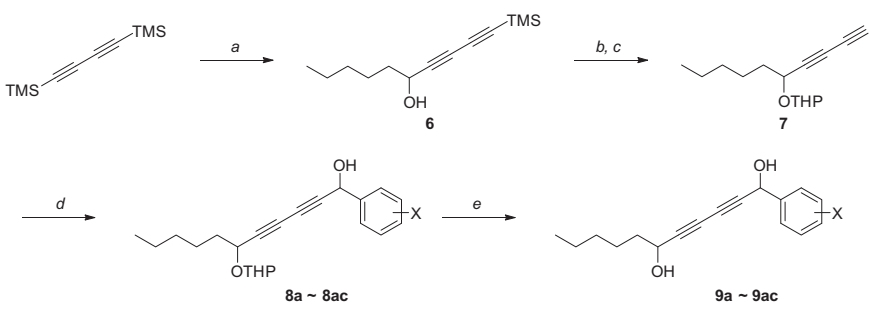


Figure 2. The 1-phenylhexa-2,4-diyne-1,6-diol scaffold.

To further investigate the effect of halide substituents on chemopreventive activity, nine di-halogen-substituted analogues (**9j**–**9r**) were synthesized and tested. Based on the biological results for the mono-substituted phenyl derivatives, which revealed that 3-chlorophenyl, 4-fluorophenyl, or 4-chlorophenyl derivatives were superior to the others, the 3- or 4-position in the phenyl ring was reserved for fluoride or chloride, with the exception of **9j**. Five derivatives, 2,5-di-Cl-Ph (**9j**) 3,4-di-F-Ph (**9k**), 3-Cl-4-F-Ph (**9l**), 3-F-4-Cl-Ph (**9m**), and 3,4-di-Cl-Ph (**9n**), exhibited CD values ranging from 4.4 to 7.0 μM, with low cytotoxicity. However, di-substitution with chloride at the 3,5- or 2,5 positions in the phenyl ring (**9o** and **9p**) resulted in a significant decrease in the CD value.

Next, to reveal further structure–activity information relating to cancer chemopreventive activity, nine alkyl or alkoxy analogues (**9s**–**9aa**) were prepared and their activities were evaluated. Among these, 3,4-ethylenedioxyphenyl (**9v**), 3,4-methylenedioxyphenyl (**9w**), and 2-fluoro-4,5-dimethoxylphenyl (**9aa**) exhibited potent induction of QR, with low cytotoxicity. Substitution at the 3 or 4 position of the phenyl group tended to enhance QR induction activity, which was consistent with the study of halide analogues. The CI value of the 3,4-methylenedioxyphenyl derivative (**9w**) was >28.2 (CD = 1.8 μM, IC_{50} > 50 μM), indicating that it was superior to sulforaphane. Compounds **9v** and **9aa** also showed good chemopreventive profiles. Other alkyl or alkoxy substitutions produced moderate activities. Replacement of phenyl with 1-naphthyl (**9ab**) or 2-naphthyl (**9ac**) resulted in analogues with slightly lower CD values (25.8 and 11.4 μM, respectively), while cytotoxicity remained low.



Scheme 1. *Reagents and conditions*: (a) MeLi–LiBr, THF, 0 °C; then *n*-hexanal, 77%; (b) DHP, PPTS, CH_2Cl_2, room temperature (rt), 86%; (c) TBAF, THF, rt, 98%; (d) EtMgBr, 0 °C, THF; then Ar-CHO, 45–87%; (e) 70% AcOH/THF (9:1), 40 °C.