Bioorganic & Medicinal Chemistry 26 (2018) 3909–3916

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and antitumor activity of bis(hydroxymethyl)propionate analogs of pterostilbene in cisplatin-resistant human oral cancer cells

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ARTICLE INFO

Keywords:
Pterostilbene
Resveratrol
Cisplatin
Oral cancer
Prodrug
Drug resistant

ABSTRACT

The aim of this study was to develop a new drug substance with low toxicity and effective inhibitory activity against cisplatin-resistant oral cancer. The naturally produced pterostilbene was selected as the lead compound for design and synthesis of a series of bis(hydroxymethyl)propionate-based prodrugs. All derivatives were screened for antiproliferative effects against the cisplatin-resistant oral squamous (CAR) cell line and the results indicated that several compounds demonstrated superior inhibitory activity compared with pterostilbene and resveratrol. Among them, the most promising compound, **12**, was evaluated for *in vivo* antitumor activity in a CAR xenograft nude mouse model. Obvious antitumor activity was observed at the lowest oral dose (25 mg/kg/day). Increasing the dose of **12** to 100 mg/kg/day reduced the tumor size to 22% of the control group. Based on these findings as well as the extremely low toxicity seen in the *in vivo* studies, we believe that compound **12** could serve as a new lead for further development.

1. Introduction

Although cancers of the head and neck comprise only approximately 6% of all cancer cases, they remain widespread and fatal.[1] Oral cancers are classified as head and neck cancers and account for about 85% of these cancers. Based on the histological tumor type, most oral cancers (about 90%) are squamous cell carcinomas (SCCs); other types are salivary gland tumors, lymphomas and sarcomas.[2] The identified risk factors related to the onset of oral cancer involve heavy tobacco and alcohol consumption, industrial inhalant exposure, and human papilloma virus (HPV) and Epstein-Barr virus (EBV) infections.[3] To date, the drugs most commonly used to treat oral cancer, often in combination with radiation therapy, are cisplatin (Platinol, Platinol-AQ),[4] 5-fluorouracil (Adrucil, Efudex, Fluoroplex)[5] and cetuximab (Erbitux).[6] Other chemotherapeutic drugs include docetaxel (Taxotere), paclitaxel (Taxol), gemcitabine, methotrexate (Abitrexate, Folex, Folex PFS, Mexate, Mexate-AQ) and bleomycin (Blenoxane).[7,8] Treatment with a combination of cetuximab and platinum-based chemotherapy involving cisplatin or carboplatin exhibits superior survival benefits and is the most powerful and commonly used first-line treatment for recurrent or metastatic oral cancer.[9] However, severe adverse effects and drug resistance often retard the use of platinum-based drugs.

Based on clinical observations, cisplatin is connected with renal failure, gastrointestinal adverse effects, hematological toxicity, neurotoxicity, nephrotoxicity and ototoxicity.[10] Compared with cisplatin, carboplatin causes less toxicity, but more myelosuppression.[11] Despite continuous improvement in the treatment of oral cancer, a low survival rate is found among oral cancer patients after receiving standard therapeutic regimens due to fast disease progression, drug resistance and a high rate of recurrence/metastasis. Therefore, efficacious drugs with fewer side effects and better safety profiles are still needed for the treatment of oral cancer.

Pterostilbene (4-[(*E*)-2-(3,5-dimethoxyphenyl)ethenyl]phenol) is a naturally occurring phytoalexin predominantly found in blueberries, grapes and wood of various trees.[12] Pterostilbene exhibits anticancer effects via various molecular mechanisms including modulation of signal transduction pathways,[13] cell cycle regulatory genes,[14] cell differentiation genes,[15] oncogenes and tumor suppressor genes.[16] Pterostilbene is a low- or non-toxic substance to humans. Experimental evidence showed that pterostilbene has potential for the prevention and treatment of various cancers, such as colon,[17] liver,[18] pancreatic,[19] lung[20] and breast.[21] However, the antitumor activity of pterostilbene

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https://doi.org/10.1016/j.bmc.2018.06.011
Received 27 April 2018; Received in revised form 29 May 2018; Accepted 7 June 2018
Available online 08 June 2018
0968-0896/

could be improved. The current study is aimed at the development of new pterostilbene derivatives for the treatment of cisplatin-resistant oral squamous (CAR) cell carcinoma. Thirteen new bis(hydroxymethyl) propionate analogs of pterostilbene (**12–18**, **20**, **22**, **23**, **24**, **25** and **27**) were designed and synthesized as target compounds.

2. Results and discussion

2.1. Design and synthesis

The rational design of these analogs is described below using compound **12** as a model substrate. The phenolic group (pKa 9.6)[22] on the 4′-position of pterostilbene is easily metabolized via transferase-catalyzed conjugation to yield water soluble glucuronide and sulfate metabolites, which are rapidly excreted.[23] Therefore, the half-life of pterostilbene is relatively short (ca. 105 min).[24] We have demonstrated previously that, compared with phenolic OH groups, aliphatic OH groups have higher pKa values[25] and therefore are more stable toward Phase II glucuronidation and sulfonation. Therefore, our target compound **12** was designed by incorporating a bulky bis(hydroxymethyl) alkanoate group on the 4′-position of pterostilbene. The pKa of **12** (13.6) is higher than the pKa of its parent compound pterostilbene, most likely making **12** less vulnerable than pterostilbene to metabolic conjugation, although the aliphatic OH group on **12** could still be capable of glucuronidation or sulfonation. In addition, compound **12** was designed to be a prodrug. The parent pterostilbene would be generated after *in vivo* hydrolysis of the 4′-position ester functionality of **12** by esterases. The pharmaceutical efficacy of target compound **12** is expected to be better than that of pterostilbene. To validate our postulates, various target compounds were synthesized and evaluated initially for antiproliferative activity against CAR cells.

As shown in Scheme 1, the commercially available starting materials, stilbenes **1–5**, variously substituted with OMe and OH groups at the 3,5,4′-position (**5** = resveratrol), were reacted with **6** in the presence of Et_3N to produce the corresponding esters **7–11**. HCl-promoted hydrolysis of these esters gave the corresponding targets **12–16**.

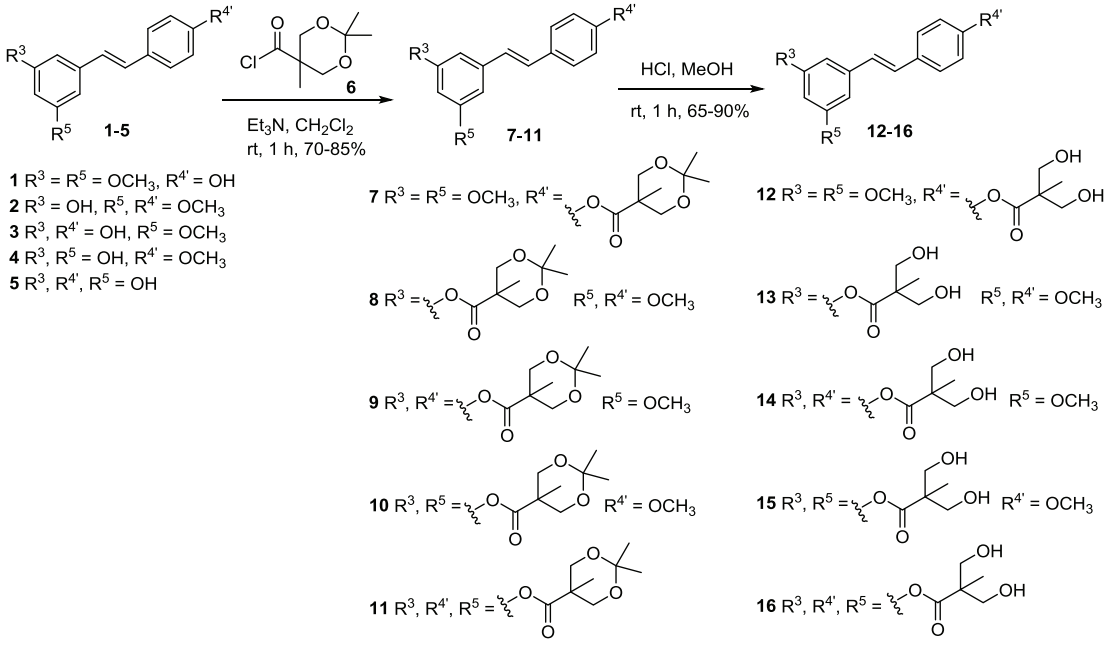
Next, as shown in Scheme 2, when treated with BCl_3·SMe in refluxing 1,2-dichloroethane for 5 h, compound **7** underwent partial demethylation to produce the desired monomethoxy **17** in 40% yield. The diol moiety of **17** was protected as a 1,3-dioxane under acidic conditions and subsequently the phenolic OH group was acetylated to produce intermediate **19**. Hydrolysis of **19** with HCl gave **20** in 63% yield. When compound **7** was treated with BCl_3·SMe_2 for an extended time (16 h), compound **18** with two phenolic OH groups was obtained as the major product in 35% yield. Compound **18** was readily converted to **22** via a three-step sequence of acetal formation, acetylation and acid-promoted hydrolysis. Treatment of compound **8** with excess BCl_3·SMe_2 gave **23** in 62% yield. A similar process was used for the synthesis of **24** from **10**. Finally, treatment of **14** with BCl_3·SMe_2 produced **25**, which underwent acetal formation and acetylation to give **26**. Acid-promoted hydrolysis of **26** produced **27** in 85% yield.

2.2. Biological evaluation

The synthesized target compounds and positive controls (pterostilbene, resveratrol and cisplatin) were screened against CAR cell lines. The results are summarized in Table 1. CAR cells were proven to be resistant to cisplatin (IC_{50} > 100 μM). When the phenolic OH groups on **1–4** were esterified with bis(hydroxymethyl)propanoic (BHMP) acid, the resulting derivatives **12–15** were all more potent than pterostilbene. Among them, compound **12** (IC_{50} = 32.58 μM) with a 4′-BHMP ester and 3,5-OCH_3 was superior to **13–15** (IC_{50} = 59.81–70.75 μM) and about three times more potent than pterostilbene (IC_{50} = 98.29 μM). However, compound **16** (IC_{50} = 124.1 μM) with BHMP esters at all three positions (4′,3,5) was less potent than the corresponding parent triol (resveratrol, IC_{50} = 88.26 μM).

Furthermore, the replacement of one OCH_3 of **12** with a hydrophilic OH led to reduced antiproliferative activity [**17** (3-OH, 5-OCH_3), IC_{50} = 63.96 μM] and replacement of both groups significantly reduced the potency [**18** (3,5-OH), IC_{50} = 132.7 μM]. Mono-acetylated **20** (IC_{50} = 50.30 μM) and di-acetylated **22** (IC_{50} = 115.8 μM) were more potent than their hydroxylated parent compounds **17** and **18**, respectively.

When the phenolic 3-OH of resveratrol was converted to a BHMP ester, the resulting compound **23** was less potent (IC_{50} = 113.5 μM) than resveratrol. Di-esterified compound **24** with BHMP at both position-3 and -5 exhibited greater potency (IC_{50} = 76.99 μM) than non-esterified resveratrol and mono-esterified **23** (based on data at 72 h). However, di-esterified compound **25** with BHMP at position-3 and -4′



Scheme 1.