Bioorganic & Medicinal Chemistry 22 (2014) 1412–1420

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

3-Ketone-4,6-diene ceramide analogs exclusively induce apoptosis in chemo-resistant cancer cells

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ARTICLE INFO

Article history:
Received 19 October 2013
Revised 16 December 2013
Accepted 26 December 2013
Available online 8 January 2014

Keywords:
Ceramide
Glucosylceramide synthase (GCS)
P-glycoprotein
Multidrug resistance
Anti-cancer drugs

ABSTRACT

Multidrug-resistance is a major cause of cancer chemotherapy failure in clinical treatment. Evidence shows that multidrug-resistant cancer cells are as sensitive as corresponding regular cancer cells under the exposure to anticancer ceramide analogs. In this work we designed five new ceramide analogs with different backbones, in order to test the hypothesis that extending the conjugated system in ceramide analogs would lead to an increase of their anticancer activity and selectivity towards resistant cancer cells. The analogs with the 3-ketone-4,6-diene backbone show the highest apoptosis-inducing efficacy. The most potent compound, analog **406**, possesses higher pro-apoptotic activity in chemo-resistant cell lines MCF-7TN-R and NCI/ADR-RES than the corresponding chemo-sensitive cell lines MCF-7 and OVCAR-8, respectively. However, this compound shows the same potency in inhibiting the growth of another pair of chemo-sensitive and chemo-resistant cancer cells, MCF-7 and MCF-7/Dox. Mechanism investigations indicate that analog **406** can induce apoptosis in chemo-resistant cancer cells through the mitochondrial pathway. Cellular glucosylceramide synthase assay shows that analog **406** does not interrupt glucosylceramide synthase in chemo-resistant cancer cell NCI/ADR-RES. These findings suggest that due to certain intrinsic properties, ceramide analogs' pro-apoptotic activity is not disrupted by the normal drug-resistance mechanisms, leading to their potential use for overcoming cancer multidrug-resistance.

1. Introduction

Ceramides, known as 'tumor suppressor lipids', regulate the anti-cancer signals implicated in apoptosis, cell cycle arrest, and autophagic responses.[1,2] Exposure to chemotherapeutic agents and radiation therapy lead to increased levels of endogenous ceramide, thereby inducing apoptosis through mitochondrial or non-mitochondrial pathways.[3] Similarly, synthetic ceramide analogs have been shown toimitate ceramide action on its downstream targets, leading to apoptosis and cell cycle arrest.[2] However, ceramide-metabolizing enzymes can decrease cellular ceramide levels, resulting in cell proliferation, migration, and survival.[4,5] One such enzyme, glucosylceramide synthase (GCS), converts ceramide into glucosylceramide on the cytosolic surface of the Golgi apparatus. Evidence shows a close relationship between GCS and P-glycoprotein (P-gp), an important multidrug-resistance (MDR) protein. GCS inhibitor treatment in multidrug-resistant cancer cells down-regulates the expression of *MDR1*, the P-gp-encoding gene.[6] Furthermore, drug-resistant cancer cells exposed to GCS inhibitors become sensitive to anticancer agents.[6–8] Ceramide analogs have the potential to act as inhibitors of these ceramide-metabolizing enzymes and thus maintain cellular ceramide levels necessary to induce cell death.[9] Therefore, ceramide analogs can exert their anti-cancer activities through two main approaches: (1) acting on ceramide downstream targets to promote cell death, and (2) inhibiting ceramide-metabolizing enzymes to increase cellular ceramide levels.

Due to the crucial role of ceramide in cell death regulation, hundreds of anticancer ceramide analogs have been synthesized and investigated in recent years.[10–16] Our previous work and that of many other research groups have shown that certain ceramide analogs preferentially inhibit the growth of chemo-resistant cancer cells in comparison to regular cancer cells.[17–22] Because of the relationship between GCS and multi-drug resistance, a hypothesis was formed that manipulation of glucosylceramide levels by inhibiting GCS is a useful way of inducing preferential killing of MDR cells.[18] In this work, we have tested this hypothesis by determining GCS activity in the chemo-sensitive and -resistant cancer cells.

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http://dx.doi.org/10.1016/j.bmc.2013.12.065

In this study, we designed 3-ketone-4,6-diene-containing ceramide analogs to determine whether extending the conjugated system in the backbone of the analogs would lead to an increase in the anticancer activity and selectivity towards chemo-resistant cancer cells. This design was based on the lead compounds 4,6-diene-ceramide and analog **3** as previously reported (Fig. 1).[22,23] The α-ketone–diene on the ceramide backbone and phenylacetyl functional group on the amide side chain are expected to increase the molecular rigidity of analog **406**, which in turn is expected to enhance its interaction with ceramide downstream targets and enhance cell death. We also seek to elucidate the mechanism of the cell death elicited by our compounds.

2. Results and discussion

2.1. Synthesis of ceramide analogs 401, 402, 403, 404, and 406

The syntheses of analogs **401**–**406** were achieved according to Chun's method as reported previously.[24] Condensation of serine methyl ester derivative **1** with methyl phenyl sulfoxide gave β-ketosulfoxide **3**. In Chun's paper, a diastereomeric mixture of **3** was mentioned. Since the sulfur atom of the sulfinyl functional group is a chiral center, theoretically, compound **3** consists of two diastereomers, **3R** and **3S** (Scheme 1). These two isomers (**3-up** and **3-down**, a higher spot and a lower spot observed by TLC) were successfully separated in this work through silica column chromatograph with petroleum ether/ethyl acetate 1.5:1 as the eluent. The ratio of **3-up** and **3-down** depends on the starting material (methyl phenyl sulfoxide) rather than the reaction. We could not designate the absolute configurations of **3-up** and **3-down**, because each compound has a very complex NMR spectrum, suggesting presence of conformational isomerism, which will be discussed in the followed section. Fortunately, as synthetic intermediates, **3R** and **3S** possess the same reactivity with the allylic bromide **2**, which was derived from the allylic alcohol ((*E*)-tridec-2-en-1-ol), to give 3-keto-4,6-diene **401**, thus isolation of **3R** and **3S** was not necessary in the actual synthetic procedure. Employing mild reaction conditions (K_2CO_3 in dimethylformamide (DMF) at room temperature), an all-trans diene **401** was produced executively. The all-trans configuration could not be directly confirmed by the ^{1}H NMR spectrum of **401** since the H-signals overlap in 6.2–6.1 ppm region. However, in its derivatives, ^{1}H NMR, COSY, and NOE spectra can determine the all-trans configuration. Selective reduction of 3-keto-4,6-diene (**401**) by diisobutylaluminum hydride (DIBAL-H) in tetrahydrofuran (THF) gave (*S*)-3-hydroxy-4,6-diene (**402**) as the predominant product in 50% yield. After treatment of **401** with trifluoroacetic acid in dry dichloromethane (DCM), 3-keto-4,6-diene-sphingosine **405** was obtained. D-Sphingosine (purchased from CNH Technologies, Inc., Woburn, MA), (*S*)-2-amino-3-hydroxy-N-tetradecylpropanamide (synthesis was described previously[23]), and compound **405** were amidated with phenyl acetyl chloride to give the ceramide analogs, **403**, **404**, and **406**, respectively.

2.2. Isomerism and NMR spectral properties of compound 3

As mentioned above, compound **3** was obtained as a mixture of **3-up** and **3-down** diastereomers. However, we could not assign R or S designations to the two. Each compound shows complex NMR spectral behaviors, which are fully displayed in the Supplementary Materials. A section of the ^{13}C NMR spectra for these diastereomersis is shown in Figure 2. Generally, two sets of peaks are seen for each single pure compound. The ratio of these two sets of peaks is 0.55:0.45 for both **3-up** and **3-down**. It is obvious that the two sets of peaks represent two relatively stable conformations of each compound. The hypothesized models of conformational isomerism are shown in Figure 3. The sp^2 hybridization of the nitrogen atom in the carbamate group results in a planar carbamate functional group (pink residue). Due to the presence of a bulky group (–$COCH_2SOPh$) on the carbon next to the N in the five-membered oxazoline ring, the steric effects lead to the –$COCH_2SOPh$ group locating in the space above or below the carbamate plane. Since these two poses cannot freely interchange, two relatively stable conformations are formed which are reflected by the two sets of signals in the NMR spectra. Figure 3 shows the two possible conformations of **3** in half-chair and envelope forms, respectively. In both conformations the α-H (in blue) is axial, which is consistent with the observations in ^{1}H NMR spectra. The starting material **1**, and ceramide analogs **401**, and **402** all show the same dualistic signals in NMR spectra with different ratios. Once the five-membered ring is opened, the compounds produced (**405** and **406)** lose this characteristic, suggesting that the ring is critical in restricting the conformational interchange. In our previous work, we observed similar dualistic signals in the six-membered ring compounds, *N*-Boc-carboline-3-carboxylic acid and its derivatives.[25] These observations indicate that the in-ring nitrogen amidation and the presence of a bulky group on the neighboring ring carbon play important roles in the formation of the two restricted conformations.

2.3. Ceramide analogs inhibiting cellular viability and clonogenic survival in MCF-7, MDA-MB-231, and MCF-7TN-R cells

In order to determine the cytotoxic effects of the ceramide analogs on MCF-7, MDA-MB-231, and MCF-7TN-R cells, MTT cell viability assays were performed using various concentrations of each analog ranging from 1 to 100 μM. The results are shown in Table 1. Compounds **401** and **406** were the most effective compounds across all cell lines with IC_{50} values of 4.05 ± 1.30 μM and 4.26 ± 1.48 μM, respectively, in the chemo-resistant MCF-7TN-R cell line. Interestingly, IC_{50} values for all analogs except analog **401** were lower in the chemo-resistant MCF-7TN-R cells compared to the sensitive MCF-7 cells, indicating that these compounds exhibit increased therapeutic potential in drug-resistant cancers (Table 1).

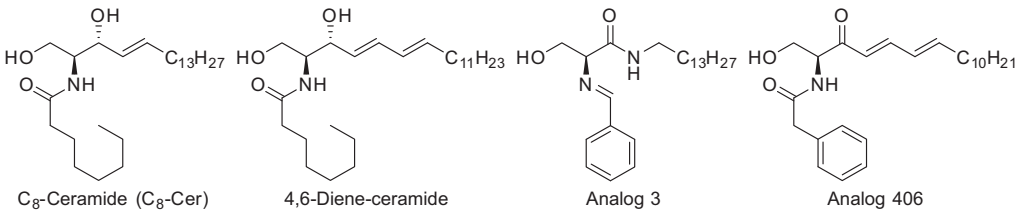

Figure 1. The structure of C_8-ceramide, 4,6-diene-ceramide, analogs **3** and **406**. C_8-ceramide is a short-chain ceramide widely used as a positive control in the studies of ceramide analogs.