Bioorganic & Medicinal Chemistry 22 (2014) 256–268

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

New highly toxic bile acids derived from deoxycholic acid, chenodeoxycholic acid and lithocholic acid

Ferenc Májer [a], Ruchika Sharma [a], Claire Mullins [a], Luke Keogh [a], Sinead Phipps [b], Shane Duggan [b], Dermot Kelleher [b], Stephen Keely [c], Aideen Long [b], Gábor Radics [a], Jun Wang [a], John F. Gilmer [a,*]

[a] School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland
[b] Cell and Molecular Biology, Institute of Molecular Medicine, Trinity Centre for Health Science, St James's Hospital, Dublin 8, Ireland
[c] Molecular Medicine Lab, RCSI, Smurfit Building, Beaumont Hospital, Beaumont, Dublin 9, Ireland

ARTICLE INFO

Article history:
Received 12 July 2013
Revised 27 September 2013
Accepted 16 November 2013
Available online 23 November 2013

Keywords:
Bile acid
Cytotoxicity
Lipophilicity
Deoxycholic acid
Ursodeoxycholic acid

ABSTRACT

We have prepared a new panel of 23 BA derivatives of DCA, chenodeoxycholic acid (CDCA) and lithocholic acid (LCA) in order to study the effect of dual substitution with 3-azido and 24-amidation, features zindividually associated with cytotoxicity in our previous work. The effect of the compounds on cell viability of HT-1080 and Caco-2 was studied using the 3-[4,5-dimethylthizol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Compounds with high potency towards reduction of cell viability were further studied using flow cytometry in order to understand the mechanism of cell death. Several compounds were identified with low micromolar IC_{50} values for reducing cell viability in the Caco-2 and HT1080 cell lines, making them among the most potent BA apoptotic agents reported to date. There was no evidence of relationship between overall hydrophobicity and cytotoxicity supporting the idea that cell death induction by BAs may be structure–specific. Compounds derived from DCA caused cell death through apoptosis. There was some evidence of selectivity between the two cell lines studied which may be due to differing expression of CD95/FAS. The more toxic compounds increased ROS production in Caco-2 cells, and co-incubation with the antioxidant *N*-acetyl cysteine blunted pro-apoptotic effects. The properties these compounds suggest that there may be specific mechanism(s) mediating BA induced cell death. Compound **8** could be useful for investigating this phenomenon.

© 2013 Elsevier Ltd. All rights reserved.

1. Introductionz

Bile acids (BAs) are oxidative metabolites of cholesterol whose principal biological function is the solubilisation of enteral nutrients, facilitating their absorption.[1] BAs modulate their own biosynthesis, transport and distribution through interactions with cell surface and nuclear receptors.[2] These interactions have long range influence on metabolism but their evolution was probably a response to BA cytotoxicity. Particularly when in their unconjugated from, BAs can induce cell death in a wide range of mammalian cell types.[3] This phenomenon is relevant to the pathophysiology of hepatobiliary diseases and to BA promoter effects in colorectal and esophageal cancer.[1,4]

BAs cause cell lysis and necrosis–an effect attributed to a non-specific capacity to disrupt cell membranes. However BAs can also induce cell death at sublytic concentrations (a property they share with detergents such Triton-X).[5] Sublytic effects are due in part to ligand independent activation of the extrinsic or death receptor pathway (CD95/Fas).[6] These effects may be mediated inside-out by membrane activation of PLA_2, NAD(P)H oxidase, which causes intracellular ROS increases.[6] DCA causes ligand independent activation of EGFR and MAP kinases in some cell types, opposing the apoptotic effects due to Fas activation.[7] DCA effects on cell viability are therefore complex and sometimes self-attenuating. Increases in ROS can follow mitochondrial damage which can be direct effect (intrinsic pathway) or an amplification of death receptor activation. ER stress is also increasingly being investigated as a mechanism of BA induced cell death.[6]

A primary chemical trigger for these events continues to evade elucidation but they are widely believed to be non-specific in origin and are they are usually attributed to membrane perturbations, consistent with observations about the importance of lipophilicity to BA toxicity. However it is likely that there are specific effects at work too. Katona et al. reported the synthesis of enantiomeric BA pairs of LCA, DCA and CDCA.[8] LCA, DCA and CDCA had the same experimental critical micellar concentration values as their enantiomeric partners (*ent* LCA, *ent* DCA, *ent* CDCA) but the naturally occurring stereoisomers were more cytotoxic.[9] This indicates that at least some of the pro-apoptotic features of BAs are due to stereospecific interactions, most likely with protein receptors. We recently reported on the properties of extended library of BAs

* Corresponding author. Tel.: +353 1 896 2795; fax: +353 1 896 2793.
E-mail address: gilmerjf@tcd.ie (J.F. Gilmer).

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.11.029

deliberately enriched with new members of widely differing physicochemical properties.[10] A correlation was observed between cytotoxicity and lipophilicity (r^2 >0.6) in this group, which was particularly strong in homologous subsets (r^2 >0.95). However, the toxicity of LCA, CDCA and DCA was not well predicted by these correlations. The study concluded that lipophilicity is a necessary but not sufficient property for BAs to exhibit cytotoxicity.

Much of the focus on the cellular effects of BAs has been concerned with explaining and preventing their contribution to hepatobiliary diseases. What if the pro-apoptotic properties of BAs could be amplified, producing compounds with useful cytocidal activity? Some of the azide analogs in our previous work exhibited unexpectedly high cytotoxic activity towards esophageal cells. We also identified simple amides of UDCA and DCA that were more toxic than the parent BAs. In the present study we have prepared and studied 23 new BA analogs integrating features that were individually associated with inhibition of cell viability in our previous work (Fig. 1). The effects of the compounds on cell viability was characterised using two cell lines with different intrinsic/extrinsic pathway susceptibility Caco-2 derived from colorectal adenocarcinoma and HT1080 from a lung fibrosarcoma.

2. Synthetic chemistry

A new panel of 23 BA (Fig. 2) derived from DCA, CDCA and LCA was prepared using synthetic approaches that we described previously for incorporating 3-azido and 24-amido functionality.[10,11] Some elaboration of the BA 7- and 12- positions was also explored. In order to obtain the 3β-azido, 7α-acetyloxy derivatives of CDCA (**1**–**3**), first the CDCA was converted to7α-hydroxyl acetate in two steps (Scheme 1). The 3-OH group on **24** was mesylated using methanesulfonyl chloride in DCM in the presence of Et_3N. The SN2 substitution on the mesylate using sodium azide afforded the 3β-azido intermediate, which gave **1** following treatment with aqueous base. The carboxylic acid was activated using N-OH-Su or HOBt monohydrate, treated with either ammonia or cyclopropyl amine to obtain 3β-azido, 7α-acetyloxy-24-amido CDCA derivatives **2**-**3**, respectively. We found the OAc group in **1**–**3** to be highly resistant to hydrolysis under basic conditions. In order to produce **4**–**6**, CDCA methyl ester was selectively acetylated on position 3, followed by mesylation of the 7-OH in the presence of tertiary base. This spontaneously eliminated when the reaction mixture in pyridine was allowed to stand at rt producing the 7,8-ene. Alkene **4** was activated at C24 and amidated to give **5** and the 24-cyclopropylamido compound **6**.

A panel of five amido analogs of DCA was synthesized with 3-β-azido group on the A-ring (**7**–**11**) (Scheme 2). The key intermediate **26** was obtained in three steps form DCA, mesylated on position 3 and azide introduced.[10,11] Using standard coupling procedures, this was reacted with each of five amines to afford 3β-azido, 24-amido (**7**), 24-cyclopropylamido (**8**), 24-benzylamido (**9**), 24-cyclohexylamido (**10**) and 24-propylamido (**11**) analogs.

Preparation of LCA derivatives (**12**–**17**) was carried out using similar approaches to those used to obtain the DCA derivatives (Scheme 3). The alpha azido DCA derivatives **18**, **19**, which are epimers of **7** and **8**, required introduction of the 3-azide with retention of configuration (Scheme 4). This was achieved using a modified Mitsunobu on the selectively protected DCA to produce the β-bromide **28**. Azide substitution and deprotection gave **29** which was amidated using HOBt/EDC and the appropriate amine.

We produced some CDCA analogs with 3α- or 3β-azido orientation without 7-acetoxy protection which had proven resistant to removal in **1**–**3** (Scheme 5). To synthesise the 3-β-azido CDCA, the 3-hydroxyl group of the methyl ester protected CDCA was regioselectively mesylated in cold DCM using stoichiometric amount of methanesulfonyl chloride in the presence of two equiv of triethylamine. The mixture was worked up immediately after completing the addition of the reagent then the mesylate was converted to azide in the next step. The methyl ester was hydrolysed, and compounds **20** and **21** obtained through mixed anhydride (ehtylchloroformate) which was reacted with the appropriate amine.

Synthesis of **23**, required introduction of the azide group at position-3 with retention of configuration (Scheme 6). Mitsunobu conditions on **30** using methanesulfonic acid were used to afford the 3-β-mesylate (**31**). This was reacted with sodium azide yielding. The methyl ester group was hydrolysed to obtain the free carboxylic acid **23**.

3. Effects on viability of Caco-2 and HT1080 cell lines

Caco-2, a cell line derived from a human colorectal epithelial tumour or HT1080 lung fibrosarcoma cells at 70% confluence were treated with the test compounds in serum free media for 1 h and

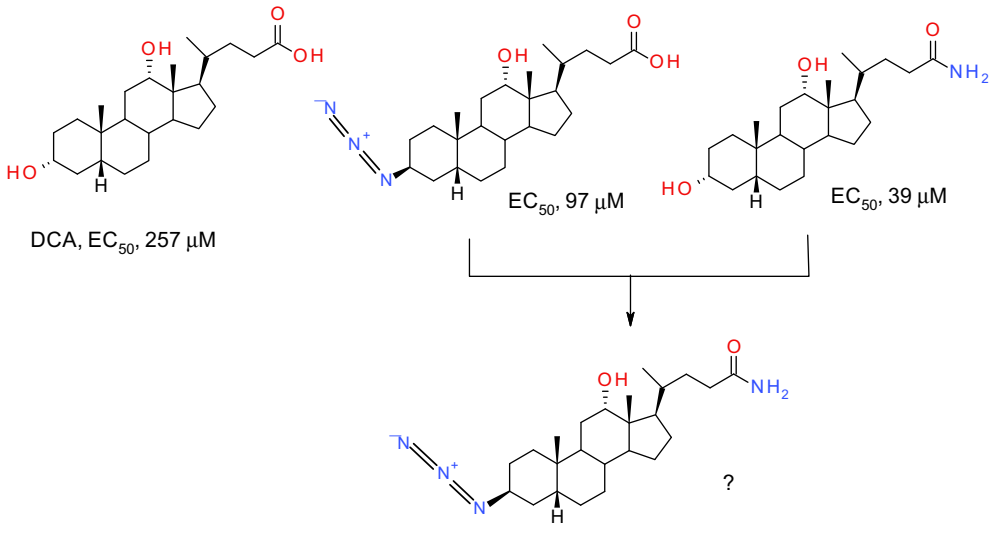

Figure 1. EC_{50} values for the inhibition of HET-1A (normal esophageal) cell viability by DCA and its amido and 3-azido analogues. The present study sought to characterise the effects of incorporating both types of modifications into a single novel BA analogue.

Download English Version:

https://daneshyari.com/en/article/10584243

Download Persian Version:

https://daneshyari.com/article/10584243

Daneshyari.com