Synthesis and Structure–Activity Relationship Study of Biliatresone, a Plant Isoflavonoid That Causes Biliary Atresia

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Supporting Information

ABSTRACT: We report the first synthesis of the plant isoflavonoid biliatresone. The convergent synthesis has been applied to the synthesis of several analogs, which have facilitated the first structure–activity relationship study for this environmental toxin that, on ingestion, recapitulates the phenotype of biliary atresia.

KEYWORDS: Biliary atresia, biliatresone, isoflavonoid, structure–activity relationships

Biliary atresia (BA) is a disease affecting neonates that is characterized by rapidly progressive fibrotic damage to the extrahepatic biliary tree.¹ It is currently the most common indication for liver transplant in the pediatric population.² Outbreaks of BA in livestock in Australia have been correlated with ingestion of chenopods of the genus *Dysphania*, suggesting that ingestion of environmental toxins could be responsible for BA. We have recently disclosed that the plant isochalcone biliatresone, which is isolated from *Dysphania*, exerts a specific, localized destructive effect on the extrahepatic biliary system in a zebrafish model, recapitulating the BA phenotype.³ The inability to reproducibly access sufficient quantities of biliatresone from the natural source motivated us to develop an efficient synthetic pathway to biliatresone. We describe herein the first laboratory synthesis of biliatresone and the application of this route to the design, synthesis, and biological evaluation of analogs, which are critical tools for the study of the role of this toxin in causing BA.

Our retrosynthetic analysis for the synthesis of biliatresone 1 is shown in Scheme 1, in which the key intermediate 2, which would give 1 on methylation, is prepared by palladium-mediated reaction of substituted acetophenone 3 (A ring) with bromophenol 4 (B ring).⁴

Scheme 1. Retrosynthetic Analysis for the Preparation of Biliatresone 1

The requisite ketone 3 was prepared from 4,6-dimethoxy-1,3-benzodioxole 5 via (1) bromination (NBS, THF, 0 °C), (2) lithiation (*n*-BuLi, THF, −78 °C) and reaction of the derived aryl anion with acetaldehyde, and (3) oxidation (IBX, DMSO) (Scheme 2).

To establish the viability of the key aryl alkylation step, i.e., formation of 2 from 3 and 4 (Scheme 1), and the compatibility of this reaction with the highly oxygenated A ring of 3, we first

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Scheme 2. Synthesis of Acetophenone 3

examined the reaction of commercially available acetophenone 6 with the MOM ether of 2-bromophenol 7⁵ [Pd(dba)₂, DPE-Phos, NaOtBu, THF, 70 °C]. We were delighted to find that the desired product 8 was obtained in 75% isolated yield. However, extension of this reaction to more highly oxygenated acetophenone substrates to afford products 9–11 led to limited success, the results of which are outlined in Scheme 3.⁶

Scheme 3. Preliminary Data and Scope of the Palladium-Mediated Coupling Reaction

Introduction of an *ortho*-methoxy substituent into the acetophenone moiety afforded the alkylation product 9 in 40% yield, and the 2,6-dimethoxy product 10 was obtained in only 12% yield. When the reaction was extended to the preparation of the fully oxygenated acetophenone 11 required for the synthesis of bilitresone 1, no product was observed, establishing the sensitivity of the coupling reaction to A-ring functionalization.

We next screened a series of palladium sources, bases, and solvents in the reaction to form 11, using the UPenn High Throughput Experimental Center, the results of which are reported in Table 1 as ratios of product/internal standard.⁷ We observed consistently higher product formation with Xphos Pd G2 under all of the reaction conditions examined.

Table 1. Results of High Throughput Screening of the Reaction of 3 and 7 To Give Key Intermediate 11^a

entry	base (1.1 equiv)	solvent (0.3 M)	A	B	C	D	E	F
1	Cs ₂ CO ₃	dioxane	0.8	0.0	2.7	0.0	0.0	6.1
2	Cs ₂ CO ₃	toluene	1.2	0.2	2.0	0.0	0.0	6.3
3	NaOtBu	dioxane	4.4	3.1	2.4	1.0	4.8	4.6
4	NaOtBu	toluene	0.7	0.0	0.1	0.2	0.0	0.6

^aReported as ratios of product/internal standard: (A) Pd₂dba₃/DPEPhos; (B) PdCl₂ (DPPF); (C) Peppsi-IPr;⁸ (D) tBu₃P Pd G2;⁹ (E) tBuXPhos Pd G3;¹⁰ (F) Xphos Pd G2.¹¹

Because we observed more undesired side products with cesium carbonate as base, we opted to use Xphos G2 and sodium *t*-butoxide in dioxane on scale-up. We were delighted to find that under these conditions the desired product 11 was formed in 95% yield.

The conversion of 11 to bilitresone 1 is outlined in Scheme 4. Reaction of 11 with paraformaldehyde in the presence of

Scheme 4. Completion of the Synthesis of Bilitresone 1

potassium carbonate in DMF afforded the methylenated product 12 in 77% yield. Deprotection of the MOM ether (6 M aq. HCl, THF) then afforded bilitresone 1, the ¹H and ¹³C NMR spectra of which were identical to previously reported data.¹²

With an efficient synthesis of 1 in hand, we turned our attention to the preparation of analogs of 1 to establish the functionalities in bilitresone that are responsible for its toxicity. Using the same reaction sequence outlined in Schemes 3 and 4 (vide supra), we prepared the analogs shown in Figure 1 in which the substitution of both the A (red) and B (blue) rings of bilitresone was varied.¹³

Figure 1. Structural analogs 13–18 of bilitresone 1.

To test how these different structural changes affect toxicity, we separately exposed zebrafish larvae to equimolar concentrations (1 μg/mL) of bilitresone and to each of the structural analogs shown in Figure 1. Biliary toxicity was determined by qualitative assessment of intrahepatic and extra-hepatic bile ducts and gallbladder morphology 24–72 h after treatment, as previously reported.³ The role of the *ortho*-hydroxyl in the toxicity of 1 was studied by comparison of 1 with the B-ring methoxy analog 13, as well as the deoxy analog 16, and the *para*- and *meta*-hydroxylated isomers 17 and 18, respectively.

As illustrated in Figures 2 and 3, we found that while treatment of zebrafish larvae with 1 caused destruction of the gallbladder and extrahepatic ducts, with sparing of the intrahepatic ducts in all larvae, there was a significant reduction of toxicity with 17 (*para*) and a slight reduction in toxicity with 18 (*meta*). In contrast, removal of the B-ring hydroxyl, as shown in 16, afforded a compound that was lethal for all larvae even at low doses. It was not toxic to the biliary system, as all larvae treated with 16 had normal morphology of intra- and extra-hepatic biliary ducts and gallbladder, a likely reflection of toxic effects on nonbiliary cell types; however, nonspecific toxicity to the larvae prevented testing 16 at the same doses as used for 17 and 18.

Figure 2. Results of biological screening of bilatresone **1** and analogs **13–18** in zebrafish larvae. Each bar graph indicates the percentage of larvae ($n = 50$) with normal, minimally affected (small gallbladder) or severely affected (absent gallbladder) extrahepatic biliary morphology following 72 h incubation in 1% DMSO (solvent) with either bilatresone **1** or the analogs **13–18** (*0.5 $\mu\text{g/mL}$ 72 h; **0.25 $\mu\text{g/mL}$ 72 h).

Figure 3. Effect of bilatresone **1** and analogs **13–18** on extrahepatic biliary morphology in zebrafish larvae. Confocal projections through the liver and gallbladder of control larva (DMSO), and larvae treated with 0.5 $\mu\text{g/mL}$ bilatresone **1** or the analogs **13–18** for 72 h. All larvae were immunostained with an anti-Annexin-A4 antibody to reveal biliary morphology. Severe gallbladder injury is seen in larvae treated with **1**, **14**, and **18**. Mild gallbladder injury is seen in larvae treated with **13**, **15**, and **17**. Larvae were treated with a lower dose of **16** (0.25 $\mu\text{g/mL}$) because of severe toxicity at the standard dose. Intrahepatic duct morphology was within normal limits for all larvae.

In murine cholangiocytes (biliary epithelial cells) cultured as three-dimensional spheroids, in which bilatresone **1** caused loss of cholangiocyte polarity and spheroid collapse, **16** appeared to have nonspecific toxicity (as was observed in the fish larvae), although most lumens were closed. Analogs **17** and **18** were similar to **1** (see [Supporting Information](#)). While **17** was less toxic than **1** in fish, reductions in toxicity were not seen for **17** in this mammalian cell system ([Figure 4](#)).

We also tested the effect of A-ring modification of **1** using analogs **14** and **15** ([Figure 1](#)). The role of the dioxolane ring was studied by examination of the toxicity of **14**, in which the dioxolane is replaced by two methoxy groups, which led to a slight reduction in biliary toxicity in fish ([Figures 2 and 3](#)) but a marked increase in toxicity in mammalian cells ([Figure 4](#)).

In contrast, removal of the two methoxy groups from the A-ring of bilatresone to give **15** led to significantly lower biliary toxicity in both fish and cholangiocytes ([Figures 2–4](#)). Differential effects of **14**, **16**, and **17** in murine cholangiocytes ([Figure 4](#)) likely reflect differences between the *in vivo* fish assay and the cell-based cholangiocyte assay ([Figure 4](#)). This may involve differences in *in vivo* metabolism of these

Figure 4. Toxicity of bilatresone **1** and analogs **14–18** in cholangiocyte spheroids. Mouse cholangiocyte spheroids were treated with vehicle (DMSO), bilatresone **1**, and analogs **14–18** at varying doses as noted for 24 h, then fixed and stained. One hundred spheroids in each condition were assessed and lumens counted as either open, partially closed, or closed (see [Supporting Information](#)).

compounds or cell-type specific compensation for their toxicities.

Collectively these data underscore the importance of the electron-rich A ring for the biological activity of **1** (relative to **15**) and for the role of the orientation of the B-ring hydroxyl group, as illustrated in the dramatic decrease in toxicity going from bilatresone **1** (*ortho*-hydroxyl) to **13** (*ortho*-methoxyl) to **16** (no B-ring oxygen functionality), which demonstrates the importance of the B-ring oxygenation for toxicity. The importance of the position of the *ortho*-hydroxy group of **1** is further underscored by comparison of **1** with **17** (*para*-hydroxyl) and **18** (*meta*-hydroxyl), suggesting an important role of the *ortho*-hydroxyl in activation of the electrophilic enone in **1**.

This six-step synthesis of bilatresone **1** in 34% overall yield from **5** makes available for the first time gram quantities of this natural product for further biological and structure–activity relationship studies. Further studies to elucidate the mechanism of action of **1** is underway in our laboratories, and our results will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the [ACS Publications website](#) at DOI: [10.1021/acsmchemlett.7b00479](https://doi.org/10.1021/acsmchemlett.7b00479).

Experimental details and spectral characterization for all new compounds, as well as protocols for the bioassays ([PDF](#))

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Notes

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