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Bile acid conjugates of a nonsteroidal glucocorticoid receptor modulator

Noah Tu,[a,†] J. T. Link,[a,*] Bryan K. Sorensen,[a] Maurice Emery,[a,‡] Marlena Grynfarb,[b] Annika Goos-Nilsson[b] and Bach Nguyen[a]

[a]*Metabolic Disease Research, Abbott Laboratories, Dept 4CB, Building AP-10, Rm. L-14, 100 Abbott Park Road, Abbott Park, IL 60064-6098, USA*
[b]*Karo Bio AB, Novum, SE-141 57 Huddinge, Sweden*

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Abstract—Bile acid conjugates of a selective nonsteroidal glucocorticoid receptor modulator were prepared and evaluated. Potent GR binding conjugates that showed improved metabolic stability were discovered. However, cellular potency and pharmacokinetics were not substantially improved.

The incidence of type 2 diabetes continues to rise increasing the need for safer and more efficacious medications for this debilitating disease.[1] One strategy for discovering anti-diabetics is to design compounds that inhibit hepatic glucose production (HGP).[2] One target of these efforts is to identify glucocorticoid receptor (GR) antagonists, or modulators, that inhibit the expression of key enzymes involved in HGP.[3] Due to the undesired effect of systemic GR antagonism, such as effects on the HPA axis or bone, researchers have sought to target these agents to the liver.[4] One strategy to accomplish this goal is to discover antagonists that are enterohepatically recirculated.[5] A tactic, which has been explored to increase the liver selectivity of small molecules is to conjugate them to enterohepatically recirculated bile acids.[6–8] Recently, we discovered a nonsteroidal series of potent and selective GR modulators that have modest functional activity and poor metabolic stability.[9] Typically, the most potent members of the series contain the core sulfonamide structure **1** and are widely distributed (systemically available) following oral dosing (Fig. 1). We conjugated these antagonists to bile acids to determine if their profile would improve.

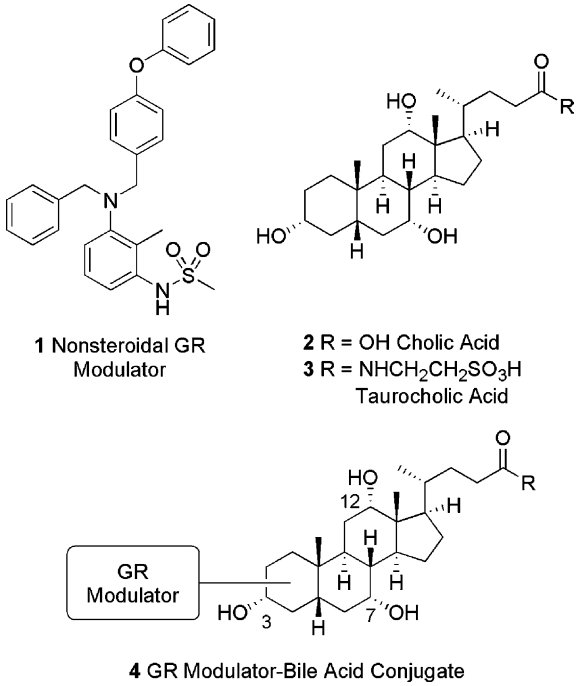

Figure 1. Representative nonsteroidal glucocorticoid receptor antagonist **1**, bile acids **2** and **3**, and generic bile acid conjugate **4**.

Keywords: Bile acid; Glucocorticoid receptor modulator; Conjugate.
* Corresponding author. Tel.: +1-847-935-6806; fax: +1-847-938-1674; e-mail: james.link@abbott.com
† Present address: S*BIO, 1 Science Park Road, Singapore 117528.
‡ Present address: Amgen, Thousand Oaks, CA 91320, USA.

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Our efforts centered on the bile acids cholic acid (**2**) and taurocholic acid (**3**). Cholic acid (**2**) is partially converted in vivo to taurocholic acid (**3**) and both compounds are enterohepatically recirculated in rodents and humans. Our initial plan was to discover GR modulators attached via a linker to a carboxylic acid that would reach outside of the GR ligand binding domain (LBD). These compounds could be coupled to appropriately functionalized bile acid pieces. This should lead to conjugates whose potencies are nearly equivalent to the unconjugated precursors. The GR modulator bile acid conjugates **4** prepared to evaluate this strategy were conjugated via the 3, 7, and 12 hydroxyl groups.

The GR modulators were prepared from 4-fluorobenzaldehyde **5** and a halophenol **6** (Scheme 1). Nucleophilic aromatic substitution yielded biphenyl ether aldehyde **7**. Reductive amination with 2-methyl-3-nitroaniline **8** provided aniline **9**. Alkylation with benzyl bromide gave nitroarene **10** that was reduced, and sulfonylated, to provide bromide **11**. Installation of different linkers, utilizing palladium mediated cross-coupling reactions, allowed for the preparation of a variety of conjugates. One example is the Negishi coupling[10] of bromide **11** with 3-ethoxy-3-oxopropylzinc bromide that, after hydrolysis, provided acid **12** suitable for coupling to an appropriately modified bile acid. Similar chemistry provided the GR modulators shown in Table 1.

Cholic acid **2** was converted to 3-β-hydroxyethyl ether **13** according to known procedures (Scheme 2).[11] The primary alcohol in triol **13** was then selectively converted to the corresponding bromide. Displacement with ammonia yielded amine **14**; ready for coupling and deprotection to provide the GR-modulator conjugates **4**.

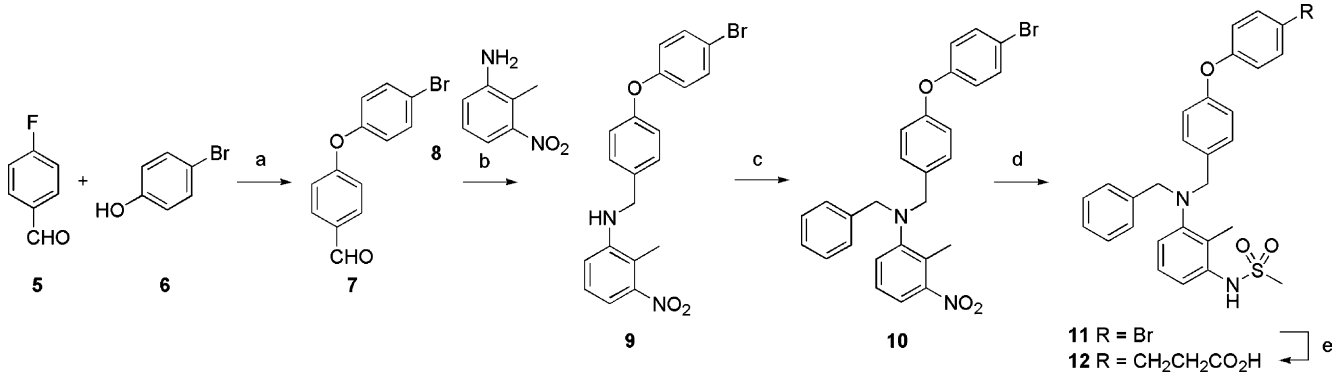
O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium tetrafluoroborate (TBTU)[12] mediated coupling of GR modulator acid **12** with cholic acid derived amine **14** yielded amide **15** (Scheme 3). Hydrolysis then gave the deprotected diol–acid conjugate **16**. Similar chemistry provided the conjugates described herein.

Compounds were assayed in a h-GR competitive binding assay measuring the displacement of the radiolabeled agonist dexamethasone. Binding selectivity against other nuclear hormone receptors, including the human progesterone receptor (h-PR), mineralocorticoid receptor (h-MR), androgen receptor (h-AR), estrogen receptor (h-ER), and thyroid-hormone receptor (h-TR α and β), was checked for important compounds. A reporter cell line (GRAF) expressing the glucocorticoid response element linked to the alkaline phosphatase reporter gene was utilized to assess the functional activity of the compounds (Table 1).[4]

A set of GR modulator acids was first prepared to determine the optimal site for conjugation. Due to the lipophilic nature of the GR LBD, it was hypothesized that if a potent modulator acid were discovered, the acid would extend outside of the pocket into solvent. The corresponding amide conjugates would then be expected to retain the potency of the parent acid. 3-β-(2-Aminoethoxy)-cholic acid was selected as a bile acid amine for conjugation. This was due to its equatorial linker chain that would minimize interaction between the GR modulator and the bile acid. Dibenzylaniline modulators **17** and **18** were first examined for modification. Linkers were incorporated at several positions. However, only the *para*-position of the benzyl group tolerated large substituents (data not shown). Addition of a three carbon acid chain produced acid **20** that has micromolar activity. Its corresponding conjugate **19** has improved (although modest) potency. This suggested the amide in **20** remained within the LBD, and that the *para*-position could function as a linking site.

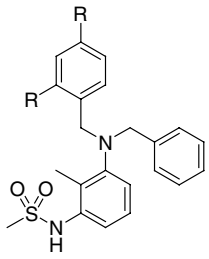
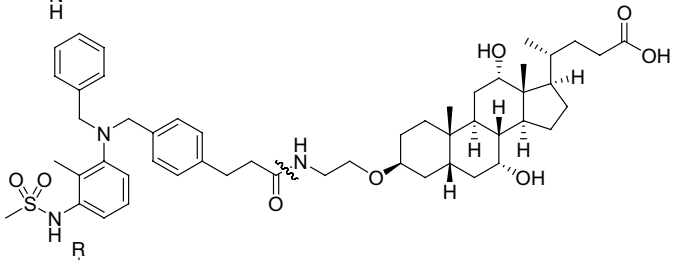
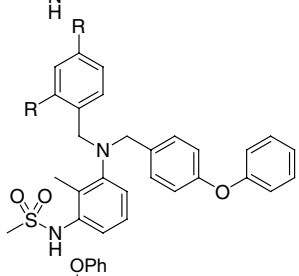
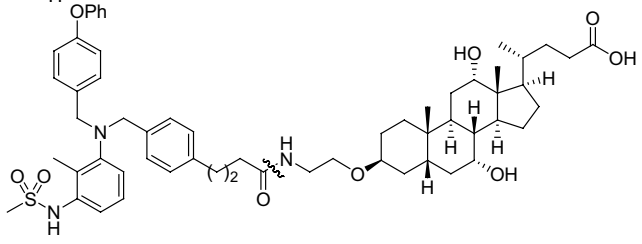
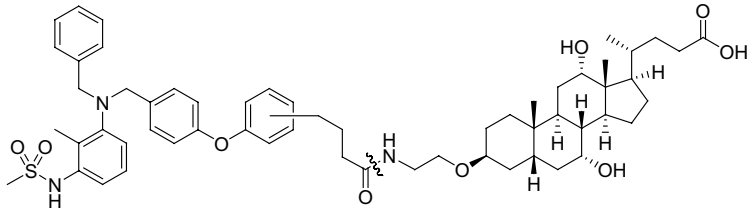
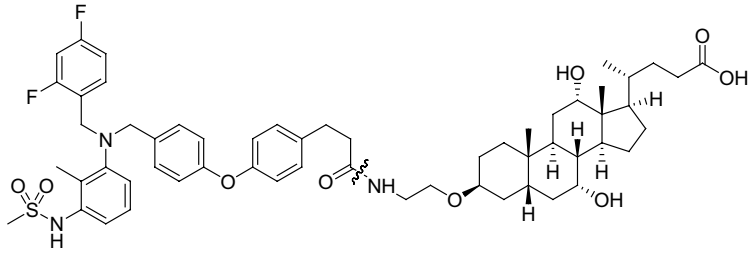
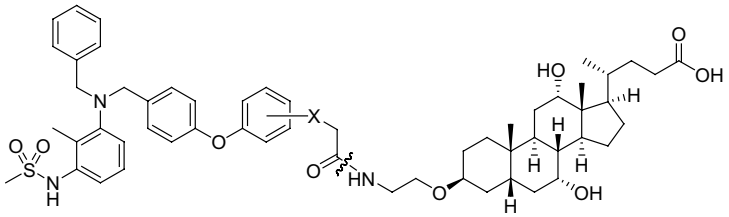
Due to the potency (h-GR binding $IC_{50} = 2.7$ nM, r-GR binding $IC_{50} = 1.3$ nM) of diaryl ether **21** the modulator core was enlarged.[9b] Acids **24** and **26** extended from the smaller aniline benzyl substituent showed moderate binding potency with the *meta*-isomer have better potency. Conjugation yielded **23** and **25** that have roughly twofold weaker binding potency than their parents. This is surprising when compared with the modulator conjugate pair **19** and **20** suggesting different binding modes.

Extending the length of the linker was accomplished by linking through the aryloxy group of **21** and **22**. Modi-

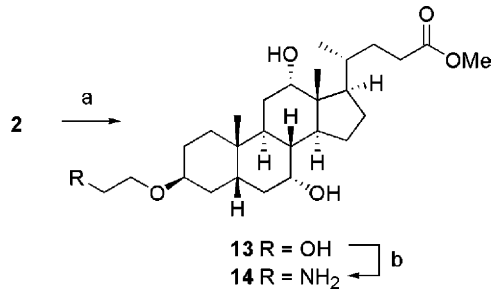


Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 100 °C, 12 h, 79%; (b) **8**, CH_3CO_2H, DCE, rt, 4 h; $Na(OAc)_3BH_3$, 12 h, 85%; (c) BnBr, *i*-Pr_2NEt, DMF, 95 °C, 12 h, 87%; (d) (i) Fe, NH_4Cl, 80 °C, 1 h; (ii) MsCl, pyr, rt, 1 h, 81%; (e) (i) $EtO_2CCH_2CH_2ZnBr$, $Pd(PPh_3)_4$, THF, 75 °C, 12 h, 77%; (ii) NaOH, THF, EtOH, H_2O, rt, 12 h, 98%.

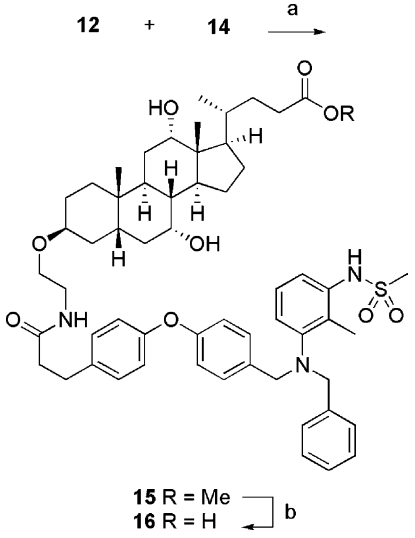
Table 1. GR binding and cellular GRAF activity for compounds **16**–**35**. In the case of the conjugates, the structure shown is for the *para*-substituted conjugate where the *para* refers to the aromatic ring to which the linker (between the bile acid and the GR modulator) is attached

Compds	Structure	GR Binding IC_{50} (nM)[a]	GRAF IC_{50} (nM)[a]
17 (R = H)		28	250
18 (R = F)		15	100
19 (**20**)		560 (>1000)	310 (>1000)
21 (R = H)		2.7	235
22 (R = F)		95	1800
para **23** (**24**)		260 (170)	ND (ND)
meta **25** (**26**)		93 (56)	ND (310)
para **27** (**28**)		50 (8.8)	ND (670)
meta **29** (**30**)		62 (270)	390 (ND)
31 (**32**)		270 (6.9)	ND (220)
para **16** (**12**) X = CH_2		17 (34)	450 (500)
meta **33** (**34**) X = O		57 (63)	590 (2300)

The activity of the unconjugated modulator acid is shown in parenthesis. The activity of the corresponding *meta*-isomer is also shown in some cases.
[a] Values are geometric means of two experiments (NA = not active, ND = not determined).



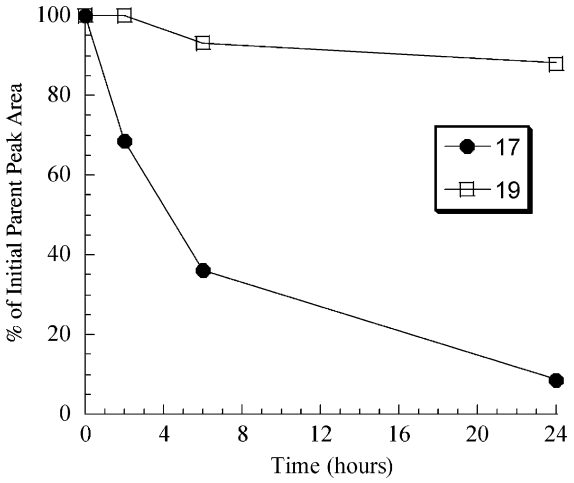
Scheme 2. Reagents and conditions: (a) Ref. 11; (b) (i) NBS, PPh_3, 0 °C → rt, 3 h, 79%; (ii) NH_3, MeOH, 100 °C, 16 h, 65%.



Scheme 3. Reagents and conditions: (a) TBTU, *i*-Pr_2NEt, DMF, 50 °C, 5 h, 83%; (b) LiOH, dioxane, H_2O, rt, 16 h, 95%.

fication of the phenoxy group gave the potent acid **28** and less potent *meta*-substituted isomer **30**. Conjugate **27** has 5–6-fold weaker binding to GR compared to its parent acid **28**. Interestingly, conjugation of acid **30** improved binding potency, as seen in **29**. These results suggest the linker region still remains within the GR pocket. Similar results were obtained with a one carbon shorter linker in conjugate **31** and parent acid **32**. Exchanging the two fluorines for protons decreases the potency of acid **12** relative to **32**. However, the binding potency of conjugate **16** is dramatically improved. The conjugate **16** is 20-fold selective over AR, MR, and PR (data not shown) retaining this important property of its parent **2**. The related *meta*-substituted ethers **33** and **34** show similar potency to one another. Comparison with **29** and **30** suggests this is coincidental rather than reflective of extending outside of the pocket. Functional activity, as assessed in the GRAF assay, was greater than tenfold worse than binding for all modulators and conjugates.

GR modulator-bile acid conjugate **19** was evaluated for metabolic stability in rat hepatocytes (Scheme 4). Conjugate **19** was remarkably stable relative to GR modulator **17** indicating that conjugation to cholic acid dramatically improved metabolic stability. This is an



Scheme 4. Metabolic stability of a nonsteroidal glucocorticoid receptor modulator **17** and the related bile acid conjugate **19** in rat hepatocytes.

important property for a liver targeting group to confer, allowing for a diversity of GR modulator structures.

In a pharmacokinetic study in Sprague–Dawley rats conjugate **16** (MW = 978.3) had lower bioavailiability (F<5%) than the nonconjugate acid **12** (F = 10%). This suggests that the conjugate is not passively absorbed, or actively transported, into the bloodstream by the rat ileal bile acid transporter (r-iBat). It was also hoped that conjugate formation would potentially improve cellular potency of the modulators. Initial GRAF results did not result in improvement (Table 2). However, this cell line does not contain bile acid transporters so poor activity may be expected (and perhaps even desired). To explore this possibility, compounds were analyzed in a second assay measuring the blockade of dexamethasone-induced activity of tyrosine amino transferase (TAT) in freshly isolated rat hepatocytes. Improvement of conjugate **16** relative to acid **12** was not observed and did not approach the potency of the steroidal antagonist RU-486. Biochemical studies of conjugate **16** to block the ability of rat hepatocytes to take up radiolabeled taurocholate (TCA) indicated the conjugate strongly interacted with the transporters. Interestingly, acid **12** also altered taurocholate uptake more potently than taurocholate itself. However, the compound was not enterohepatically recirculated as assessed by bile concentration measurements in bile cannulated rats.

Bile acid conjugates of a series of metabolically labile GR modulators were prepared. SAR analysis suggests

Table 2. Rat hepatocyte TAT and transport assay results for compounds **12** and **16**

Compds	GRAF IC_{50}, nM[a]	Rat hepatocyte TAT IC_{50} (μM)[a]	3H-Taurocholate uptake IC_{50} (μM)[a]
12	500	NA (>30)	1.3
16	450	NA (>30)	4
TCA	ND	ND	6
RU-486	4.8	0.27	ND

[a] Values are geometric means of two experiments (NA = not active, ND = not determined).

that the linkage point between the modulator and the bile acid was within the LBD. However, potent conjugates were discovered that have increased metabolic stability. Unfortunately, conjugation did not greatly improve cellular potency or the pharmacokinetic profile of the modulators. These results indicate that cholic acid does not function as a universal liver targeting group for all compounds. Rather, bile acid conjugates need to be evaluated on a case by case basis.

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