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Synthesis and activity of novel bile-acid conjugated glucocorticoid receptor antagonists

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Abstract—A series of potent steroidal glucocorticoid receptor antagonists has been discovered. After conjugation to cholic acid, the compounds retained an affinity for GR in vitro and had modest in vivo efficacy.

The glucocorticoid receptor (GR) is ubiquitously expressed in the body and regulates a myriad of functions. In the liver, the endogenous GR ligand cortisol leads to increased hepatic glucose production via the upregulation of key gluconeogenic enzymes.[1] The antiglucocorticoid RU-486 (**1**, Fig. 1), or mifepristone, has been shown to acutely reduce hepatic glucose output suggesting that GR blockade might be a useful approach for treating the hyperglycemia associated with type 2 diabetes.[2] However, RU-486 also has deleterious extrahepatic effects that limit its utility as a chronic diabetes therapy.[3] Recent data in rodents further imply that selective hepatic GR blockade would be beneficial to diabetic patients.[4] In this study, the authors note that hepatic GR knockout mice with diabetes had lower hepatic glucose output when fasted. This is correlated with dramatically reduced phosphoenolpyruvate carboxykinase (PEPCK) mRNA induction. The therapeutic utility of steroidal GR antagonists could potentially be expanded if they could be targeted to the liver.

Our strategy for accomplishing this involves the coupling of a potent GR antagonist with cholic acid in hopes of engaging the bile-acid transporter machinery to concentrate the drug in the liver.[5] The linchpin of this strategy is the 11β aryl substituent, shown by the GR crystal structure with RU-486 (**1**) bound to prevent helix 12 of the GR from adopting its active conformation.[6] GR forms complexes with agonists that allow helix 12 to close over the ligand binding site.[6] Because the 11β aryl substituent is solvent exposed, attachment of cholic acid or other bulky substituents is tolerated. A-348441 (**2**), a cholic acid conjugate of RU-486, was discovered by successfully applying this strategy.[7,8]

As a follow-on strategy, we sought to redesign the steroidal GR antagonist (**3**) to improve potency and then prepare the bile-acid conjugates **4** in hopes that improved in vitro potency of the parent GR antagonist would result in improved potency in vivo.

An 11β aryloxy group might provide an alternate means of attachment for the bile-acid. The corresponding nonconjugated ethers were prepared by an S_n2' cuprate addition to known epoxide **5**, followed by acidic hydrolysis and ketal deprotection (Scheme 1).[9]

Compounds were first evaluated in a GR binding assay (Table 1). A radiolabeled form of GR agonist dexamethasone was used as the reference ligand. The functional assay, abbreviated GRAF ('**GR** linked to Alkaline Fosfatase'), was a cell-based format using CHO cells engineered to express GR.[7] They have been further modified such that GR activation stimulates the

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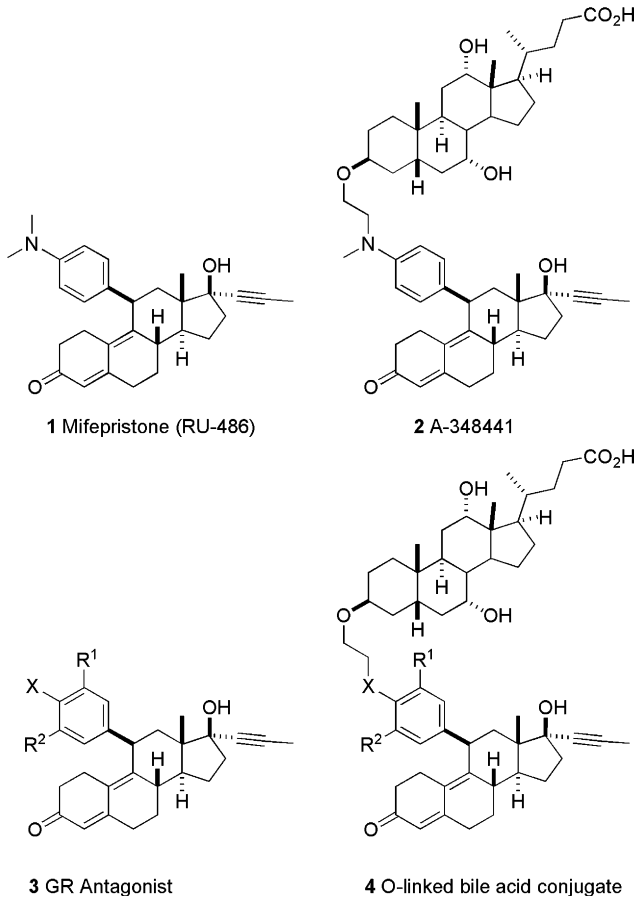
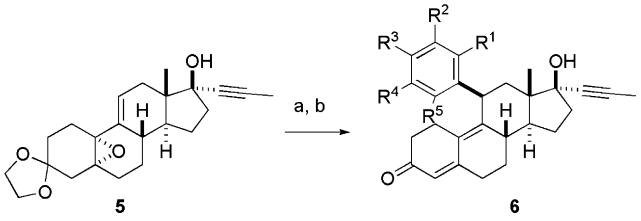


Figure 1. Representative steroidal GR antagonists.



Scheme 1. Reagents and conditions: (a) ArBr, Mg, I_2, THF, reflux 15 min, then 0 °C, CuI, **5**; (b) 2 M HCl, THF, rt, 5 min (20–50% 2 steps).

secretion of alkaline phosphatase. Effective antagonists in this assay decreased dexamethasone stimulated production of alkaline phosphatase.

The SAR for this series of analogs showed that a variety of 11β substituents were tolerated by the receptor. *para*-OMe substitution led to known compound **6a**, which was roughly equipotent to RU-486 (**1**) in both binding and functional assays.[10] Similarly, the literature compound **6b**, with a *para*-SMe substituent, also had comparable potency.[10] *para*-*O*-Alkyl derivatives of various sizes were tolerated (**6c**–**e** and known compound **6f**).[10] *para*-Phenols tended to be less active than their O-alkylated homologs (compare the significant drop in potency of **6g** relative to **6h**). *ortho*-Substitution, as evidenced by **6i**–**l**, was tolerated only if the substituents were small. A simple *meta*-substituent in compounds **6h** and **6c** led to a boost in potency, while *meta*-di-substituted analogs were less potent than their mono-substituted comparators (**6m** and **6d**). A significant drop in potency was observed if both *meta*-substituents were larger than methyl (**6n**). Compounds **6k** and **6o** indicated that the position of difluoro substituents on the aromatic ring could modulate potency.

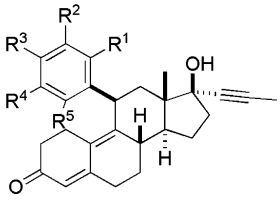
Compound **6h** stood out as an exceptionally potent antagonist, likely the most potent steroidal GR antagonist reported to date.[11] It was 10 times more potent than RU-486 (**1**) in the GR binding assay and 5 times more potent in the GRAF functional assay. The *meta*-dimethyl analog **6m** was also shown to be about twice as potent in vitro as RU-486 (**1**). The biphenyl ether **6e** was found to be equipotent. These three compounds were selected for conjugation to cholic acid and evaluation in vivo.

The cholic acid coupling partner was prepared as described in Scheme 2. The synthesis of alcohol **7** was completed as described in the literature.[12] The ethylene glycol linker was then activated as the mesylate to provide **8**. The GR antagonist portion of the molecule was again prepared from epoxide **5**. Phenols **9a**–**c** were alkylated with allyl bromide. The protected aryl bromides (**10a**–**c**) were then subjected to the cuprate coupling to epoxide **5**. After the addition, acidic hydrolysis and ketal deprotection proceeded smoothly. The allyl protecting group was removed using phenyl silane under Pd^0 catalysis. The two halves of the conjugate, **8** and **11a**–**c**, were coupled over the course of 24 h with slight warming in the presence of base. Attempts to increase the reaction rate by raising the temperature led to lower product recovery, presumably due to decomposition of the starting materials. Saponification of the methyl ester furnished conjugates **4a**–**c**.

Bile-acid conjugates **4a**–**c** were found to be potent GR antagonists (Table 2). The bile-acid did cause a decrease in binding affinity and functional potency relative to the parent compounds (**6h**, **6e**, and **6m**). Nevertheless, compounds **4a**–**c** were taken into a prednisolone challenge experiment using male CD rats.[7] In this experiment, efficacy of the GR antagonist was related to how much it blunted the effects of GR agonist prednisolone on two hepatic parameters, tyrosine aminotransferase (TAT) upregulation and glycogen deposition. To assay for liver-targeting, the attenuation of prednisolone induced lymphopenia, a systemic parameter, was also monitored. The ideal liver-selective GR antagonist would have effects on TAT and glycogen and no effect on lymphopenia.

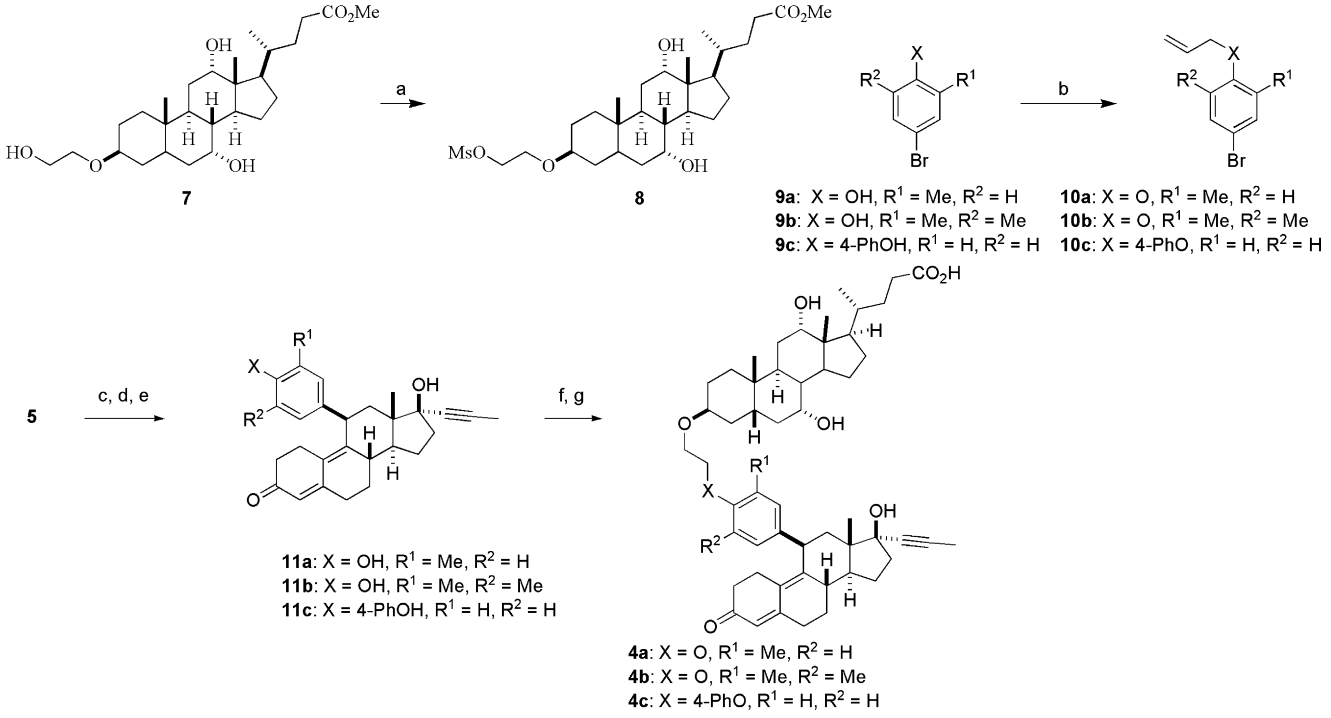
When administered as a single 100 mg/kg oral dose to rats followed by the prednisolone challenge, RU-486 (**1**) effectively nullified the effect of prednisolone on TAT and liver glycogen. It was also systemically available as was demonstrated by reversal of prednisolone induced lymphopenia (Table 2). A-348441 (**2**) was also efficacious against the hepatic parameters but had no effect on lymphopenia. GR antagonist **6h** effectively

Table 1. SAR for 11β-aryl substituents



Compound	R^1	R^2	R^3	R^4	R^5	GR IC_{50}[a] (nM)	GRAF IC_{50}[b] (nM)
1	H	H	NMe_2	H	H	1.1	5.0
6a	H	H	OMe	H	H	0.9	7.6
6b	H	H	SMe	H	H	0.6	1.8
6c	H	Cl	*O*-allyl	H	H	0.4	4.7
6d	H	Cl	*O*-allyl	Me	H	1.5	20.9
6e	H	H	4-OMePh	H	H	0.7	4.2
6f	H	H	OPh	H	H	1.5	18.8
6g	H	Me	OH	H	H	4.5	28.6
6h	H	Me	OMe	H	H	0.1	1.1
6i	OMe	H	OMe	H	OMe	481.5	nd
6j	OMe	H	OMe	H	F	6.1	43.0
6k	F	H	OMe	H	F	0.7	10.1
6l	Me	H	OMe	H	H	9.0	32.0
6m	H	Me	OMe	Me	H	0.4	2.8
6n	H	OMe	OMe	OMe	H	60.7	nd
6o	H	F	OMe	H	F	3.2	15.0

Human GR binding assay. GRAF cellular functional assay.
[a] Values are means of two experiments.
[b] nd, Not determined.



Scheme 2. Reagents and conditions: (a) MsCl, Et_3N, THF, rt, o.n., (37%); (b) allyl bromide, Cs_2CO_3, rt, 20 h (45%); (c) **10a**–**c**, Mg, I_2, THF, reflux 15 min, then 0 °C, CuI; (d) 2 M HCl, THF, rt, 5 min (21% 2 steps); (e) $Pd(Ph_3)_4$, $PhSiH_3$, CH_2Cl_2, rt, 1 h (55%); (f) **8**, *n*-Bu_4NI, Cs_2CO_3, THF, 55 °C, 24 h (93%); (g) 1 M LiOH, THF.

blocked hepatic TAT and lymphopenia in the periphery, similar to RU-486 (**1**). Conjugate **4a** was only marginally active in vivo. It had no effect on glycogen and minimal, equal effects on TAT and prednisolone induced lymphopenia. Compound **4b** showed minimal effects on glycogen deposition. Conjugate **4c** was active in this

Table 2. In vitro and in vivo data for bile-acid conjugates **4a–c** and comparison to antagonists A-348441 (**2**), **6h**, and RU-486 (**1**)

Compound	GR IC_{50}[a] (nM)	GRAF IC_{50} (nM)	TAT (%)	Gly.[c] (%)	Lymph. (%)	Plasma Conc.[b,c] (nmol/mL)	Liver Conc.[b,c] (nmol/g)
1	1.1	5	101	77	104	3.9	26.5
2	1.2	10	72	86	9	4.5	26.5
6h	0.1	1.1	97	nd	67	4.8	18.1
4a	3.1	30.7	31	0	20	4.5	22.2
4b	4.9	200	0	12	0	nd	nd
4c	2.5	23.5	44	51	40	nd	nd

[a] Values are means of two experiments.
[b] Data collected 7 h after 100 mg/kg oral dose. There were 5 animals per group.
[c] nd, Not determined.

Table 3. Rat ADME properties for selected compounds

Compound	Microsomal stability[a] (% remaining)	Plasma binding[b] (% bound)
1	70	95.6
2	98	95.9
6h	87	nd
4a	95	98.3
6e	96	nd
4c	100	nd

[a] Twenty minutes incubation period.
[b] nd, Not determined.

model. However, **4c** was significantly less active than RU-486 (**1**) and, like **1**, had peripheral as well as hepatic effects.

In order to better understand the disparity between potency in vitro and lack of in vivo efficacy of the O-linked conjugates, we first measured the concentration of **4a** in rat liver 7 h after administration of the GR antagonist (Table 2). The concentration of **4a** in the liver compared favorably to effective liver concentrations of RU-486 (**2**) and A-348441 (**1**).

Conjugates **4a** and **4c** were also examined for stability in rat liver microsomes (Table 3). Both compounds were inert to oxidative metabolism. This was also true for A-348441 (**1**). Apparently conjugation to cholic acid had the effect of protecting these steroidal GR antagonists from oxidative clearance as the parent compounds **6e** and **6h** as well as RU-486 (**1**) were rapidly degraded.

After discounting low liver concentration and metabolic instability as reasons for the modest efficacy of the conjugates, we measured protein binding of conjugate **4a** in rat plasma and found it to be >98% bound.

In summary, a series of 11β aryloxy modified steroid GR antagonists was discovered to have comparable or superior potency, in vitro, to RU-486 (**1**). However, despite achieving a comparable liver concentration of **4a**, the conjugate was not as effective as RU-486 (**1**) or its conjugate A-348441 (**2**) in vivo. Differences in protein binding do not fully explain the observed differences in in vivo activity. Clearly other mechanisms worked in concert to limit the efficacy of **4a**. Additional metabolism experiments could shed light on the fate of conjugates **4a–c** in vivo. Further studies in this area would also focus on a re-examination of the conjugation of potent GR antagonists such as **6h**.

Acknowledgments

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