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Synthesis and biological evaluation of novel steroidal pyrazoles as substrates for bile acid transporters

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Abstract—A series of novel steroidal pyrazoles was synthesized as substrates for bile acid transporters to explore their potential as drug carriers. The selected pyrazole fused bile acids were further conjugated with drugs and drug surrogates. Their in vitro transport activities were evaluated in human ileal bile acid transporter (hIBAT) and human liver bile acid transporter (hLBAT) expressing Chinese hamster ovary (CHO)-cells and *Xenopus laevis* oocytes. The results of synthetic efforts and transporter assays studies are described herein.

The Na^+/taurocholate co-transporting polypeptide (NTCP; SLC10A1), also termed the liver bile acid transporter (LBAT), and the apical sodium-dependent bile salt transporter (ASBT; SLC10A2), alternatively named the ileal (or intestinal) bile acid transporter (IBAT), are solute carrier transporters regulating the enterohepatic circulation of bile salts.[1] LBAT and IBAT are membrane co-transporters that mediate sodium dependent, electrogenic uptake of bile acids into hepatocytes and ileal enterocytes, respectively. In recent years interest in developing ligands for the human intestinal bile acid transporter (hIBAT) has been motivated by two distinct applications. First, inhibitors of hIBAT block the uptake of endogenous bile acids from the intestinal lumen resulting in increased fecal bile salt excretion and a compensatory up-regulation of de novo bile acid synthesis from cholesterol in the liver.[2] Enhanced bile acid biosynthesis via activation of cholesterol 7α-hydroxylase (CYP7A1) results in net reduction in plasma cholesterol, and hIBAT inhibitors are currently under clinical investigation as novel antihypercholesterolemic agents.[3] Second, the realization that the intestinal bile acid transport system represents a high capacity solute uptake pathway has prompted several groups to investigate chemical conjugation of bile acid moieties to drugs and diagnostic agents either via non-cleavable or enzymatically labile (i.e., prodrug) linkages. While the use of non-labile bile acid conjugates has been promoted as a method for achieving liver-selective targeting of drugs,[4] the latter approach has been proposed as an attractive strategy for improving systemic exposure to otherwise poorly orally bioavailable drugs.[5]

As part of our interest in developing novel ligands for the ileal and hepatic bile acid transporters, we have explored the synthesis of heterosteroids based on the cholanoic acid skeleton.[6,7] In this report we describe the synthesis of steroidal pyrazoles[8] and their interaction with bile acid transporters heterologously expressed in mammalian cells and *Xenopus* oocytes.

The bile acid building blocks, 3-ketocholanoic acid methyl esters **1a**–**c** were prepared in 70–92% overall yields following reported protocols.[9] The 3-keto bile acid esters **1a**–**c** were subsequently condensed with ethyl formate in the presence of sodium hydride in toluene to afford the 2-hydroxymethylene substituted intermediates **2a**–**c** in 81–94% yields. The bile acid derivatives **2a**–**c** were treated with hydrazines in ethanol at reflux temperature to yield the corresponding pyrazole fused bile acid methyl esters, which after saponification under standard conditions, afforded the steroidal pyrazoles **3a**–**g** in 63–95% overall yields. The glycine conjugated steroidal pyrazoles **4a**–**g** were prepared by coupling glycine *tert*-butyl

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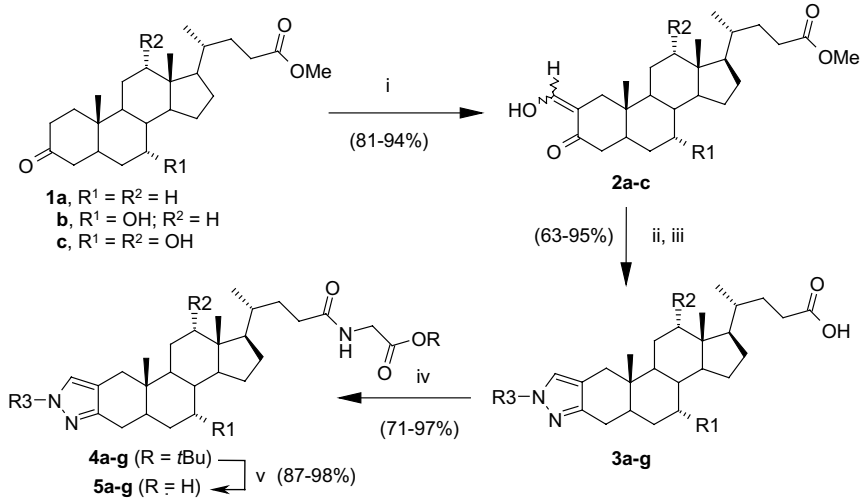
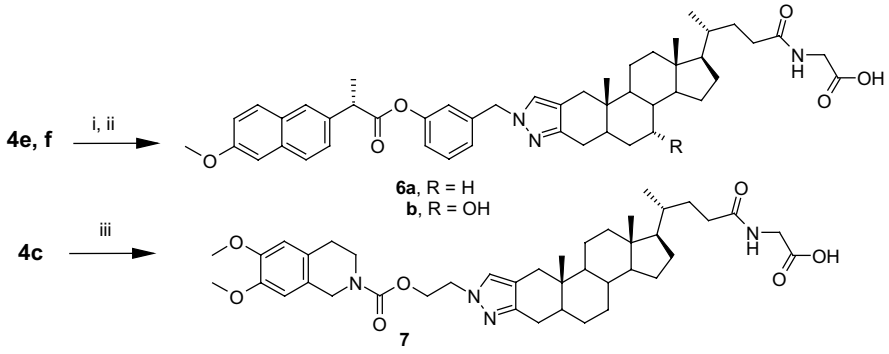


Scheme 1. Reagents and conditions: (i) NaH/HCO_2Et/cat. EtOH/toluene, rt, 12–24 h; (ii) H_2NNHR^3/EtOH, reflux, 8–12 h; (iii) 2 N NaOH/THF, rt, 5–8 h; (iv) EDAC/H–Gly–OBut·HCl/DIEA/DCM, rt, 12–18 h; (v) 4 M HCl in dioxane, rt, 5–8 h.

ester with **3a**–**g** under standard coupling conditions in 71–97% overall yields. Finally, the pyrazole annulated glycocholanoic acids **5a**–**g** were synthesized by treatment **4a**–**g** with 4 M hydrogen chloride (HCl) solution in dioxane in excellent yields (Scheme 1).

To investigate the possibility of using the steroidal pyrazole template as a drug carrier various drugs and drug surrogates were conjugated to functionalized substituents or linkers on the pyrazole moiety via an enzymatically cleavable covalent bond. Selected drugs and/or drug surrogate conjugates are described in Scheme 2. Naproxen conjugated steroidal pyrazoles **6a**–**b** were prepared by coupling naproxen with **4e**–**f** under standard conditions using trichlorobenzoyl chloride (TCBC) as coupling agent followed by treatment with 4 M HCl in dioxane in good yields. Similarly, the carbamate derivative **7** was synthesized in moderate yield by coupling pyrazole derivative **4c** with 3,4-dimethoxyquinoline using 1,1′-carbonyldiimidazole (CDI) as coupling agent.

The membrane transport activity of the steroidal pyrazoles and their drug conjugates[10] was evaluated in both human IBAT and LBAT in vitro transporter assays[11] (Table 1). Their affinity for the bile acid transporters was assessed from the inhibition of radio-labeled taurocholate uptake in recombinant CHO-cells. The functional transport activity was determined from an electrophysiology assay using transfected *Xenopus laevis* oocytes. The glycodeoxycholic acid (GDC) derivatives **5b**, **5d**, and **5f** showed good binding affinity in both hIBAT and hLBAT assays. In contrast, the glycolithocholic acid derivatives **5a** and **5c** and the glycocholic acid derivative **5g** showed high binding affinity for hLBAT but only weak affinity for hIBAT. The steroidal pyrazoles **5a**–**g** are weak to moderate substrates in hIBAT expressing oocytes (inducing 7–44% of the maximal current stimulated by the control substrate GDC). The Naproxen conjugated steroidal pyrazole derived from glycolithocholic acid **6a** showed lower binding affinity for hLBAT than that derived from glycodeoxycholic acid **6b** but both had very weak affinity for hIBAT. The drug conjugate **6b** showed weak substrate activity (12% I_{max}) whereas **6a** did not show any specific activity in the hIBAT expressing oocytes. The drug surrogate conjugated pyrazole **7** showed good binding affinity for hLBAT and reasonable affinity for hIBAT in radio-labeled competition uptake assays. Interest-



Scheme 2. Reagents and conditions: (i) (a) Naproxen/TCBC/pyridine/DCM, 0 °C to rt, 5 h; (b) **4e**–**f**/pyridine/DCM, 0 °C to rt, 8 h; (ii) 4 M HCl in dioxane, rt, 12 h; (iii) (a) **4c**/CDI/pyridine/DCM, 0 °C, 1 h; (b) 3,4-dimethoxyquinoline/pyridine/DCM, 0 °C to rt, 12 h.

Table 1. In vitro transport results of the steroidal pyrazoles **5a–g** and their drug conjugates **6a–b**, **7** in hIBAT and hLBAT expressing CHO-cells and *Xenopus laevis* oocytes

Entry	Steroidal pyrazoles and their drug conjugates				Competition assay[a] IC_{50} (μM)		Oocyte assay[b] % max GDC
	Number	R^1	R^2	R^3	hIBAT	LBAT	hIBAT
1	**5a**	H	H	H	60	1.6	26
2	**5b**	OH	H	H	1.6	0.3	21
3	**5c**	H	H	β-Hydroxyethyl	>300	0.7	17
4	**5d**	OH	H	β-Hydroxyethyl	22	0.2	7
5	**5e**	H	H	*m*-Hydroxybenzyl	0.9	0.3	44
6	**5f**	OH	H	*m*-Hydroxybenzyl	1.5	0.3	7
7	**5g**	OH	OH	*m*-Hydroxybenzyl	>300	8.6	22
8	**6a**	H	H	—	>300	63	0
9	**6b**	OH	H	—	>300	3.4	12
10	**7**	H	H	—	32	0.22	59

[a] IC_{50} data are generated from radio-labeled taurocholate competition assays in transporter expressing CHO cells at a highest test sample concentration of 300 μM.

[b] % Max values are relative to glycodeoxycholic acid (GDC) in transporter expressing *Xenopus laevis* oocytes at a test sample concentration of 200 μM.

ingly, conjugate **7** was well transported by hIBAT (59% of I_{max}), perhaps reflecting the influence of the β-hydroxyethyl linker moiety in permitting productive interactions with the transporter protein.

In summary, we have synthesized novel steroidal pyrazoles as substrates for bile acid transporters (hIBAT and hLBAT). The selected pyrazole fused bile acids were further conjugated with drugs and drug surrogates. The annulated pyrazoles and their drug conjugates showed good affinity for hLBAT as compared to hIBAT, and weak to moderate transport activity in hIBAT expressing oocytes. The results of this investigation demonstrated that the bile acid analogs derived from heteroannulation onto bile acid ring A at the C2–C3 positions can be substrates for bile acid transporters and be potentially used as shuttles for drug targeting.

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