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Synthesis, activity, metabolic stability, and pharmacokinetics of glucocorticoid receptor modulator–statin hybrids

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Abstract—The synthesis, activity, metabolic stability, and pharmacokinetics of steroidal and nonsteroidal glucocorticoid receptor modulator–statin hybrids is reported. Potent steroidal antagonist–statin hybrids like **22** (h-GR binding $IC_{50} = 7$ nM) and nonsteroidal modulator hybrids like **16** (h-GR binding $IC_{50} = 2$ nM) were discovered. Appending a 'statin'-like diol-acid group to the modulators dramatically improved metabolic stability (and in some cases hepatocyte activity), but did not impart hepatoselectivity.

Metformin has become a cornerstone of antidiabetic treatment both as a mono-therapy and in combination with other agents.[1] Although its precise mechanism of action is still under investigation, its predominant activity is inhibition of hepatic glucose production (HGP). Its therapeutic and commercial success has stimulated research on multiple targets whose regulation may moderate HGP.[2] Antagonists of the glucocorticoid receptor (GR) have been explored as potential antidiabetic agents that inhibit HGP.[3] Because of the possibility of undesired effects due to extrahepatic GR antagonist exposure, attempts have been made to target them to the liver.[4] Steroidal GR antagonists, like mifepristone (RU-486) (**1**), are well known members of this class (Fig. 1). However, they are unselective and show significant progesterone receptor (PR) activity leading to their abortive properties. Recently, several classes of nonsteroidal GR ligands, including a series of GR selective modulators from our laboratory have been identified.[5,6] Both the steroidal and nonsteroidal GR antagonists are typically widely distributed after oral dosing potentially leading to both hepatic and systemic effects. We have sought to alter the pharmacokinetics of

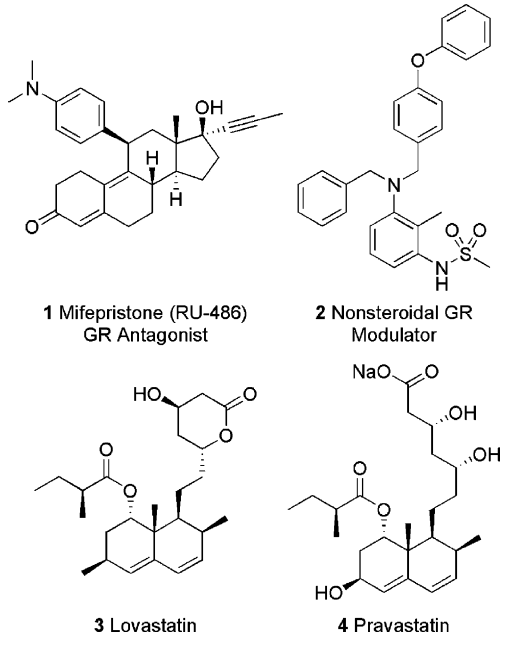

Figure 1. GR ligands and HMG CoA reductase inhibitors.

Keywords: Glucocorticoid receptor modulator; Statin.
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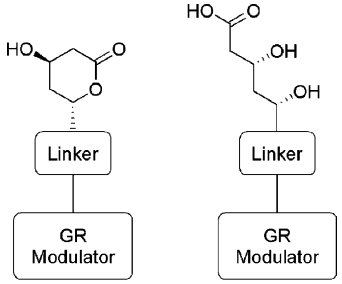
GR antagonist mifepristone (**1**) and the GR selective nonsteroidal modulator **2** to increase their hepatic selectivity.

One example of liver selective small molecule therapeutics is the statin class of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors.[7] These compounds, which prevent coronary heart disease by lowering low-density lipoprotein cholesterol levels, show preferential distribution and action in the liver. However, some potentially beneficial extrahepatic effects have also been studied.[8] Lovastatin (**3**) and pravastatin (**4**) are two statins that have polar sidechains that structurally represent the class. Inactive hydroxylactones, like **3**, upon ingestion and absorption, undergo lactone hydrolysis to the active diol-acids. Frequently the lipophilic lactones are able to passively cross membranes more efficiently than the polar diol-acids. Several diol-acids, like pravastatin (**4**), are passively and actively taken up by transporters (frequently the organic ion transporter) into hepatocytes.[9] This increases their hepatoselectivity. Pravastatin (**4**) is orally absorbed without the necessity of administering the corresponding lactone.

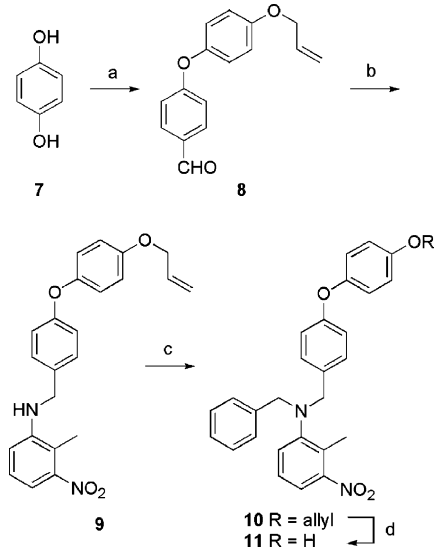
We prepared hydroxylactone GR antagonist/modulator-hybrids **5** and diol-acid GR antagonists/modulator-hybrids **6** in order to liver target GR antagonists or modulators (Fig. 2). In the case of hybrids with the aniline bearing site of mifepristone (**1**), the GR binding activity was expected to be similar to **1** because the appended groups would project outside of the ligand-binding domain of the GR.[10] Reported structure activity studies also indicated large groups could be linked to this site without large reductions in potency.[11] The activity of the hybrids of the nonsteroidal GR modulator **2** was less predictable. The site of attachment and linker would need to be experimentally determined. If these hybrids target the GR modulators to the liver they could be optimized for dual lipid-lowering activity. Alternatively, HMG CoA activity could be potentially removed by altering the diol stereochemistry.

Two sites in the nonsteroidal modulators tolerated large polar substituents. At these positions, GR modulator-statin hybrids were prepared using an aryl ether linker. The core of one modulator was prepared from hydroquinone **7** (Scheme 1). Allylation followed by a nucleophilic aromatic substitution reaction with 4-



5 GR Modulator Lactone Hybrid
6 GR Modulator Diol-Acid Hybrid

Figure 2. GR ligand–statin hybrids.



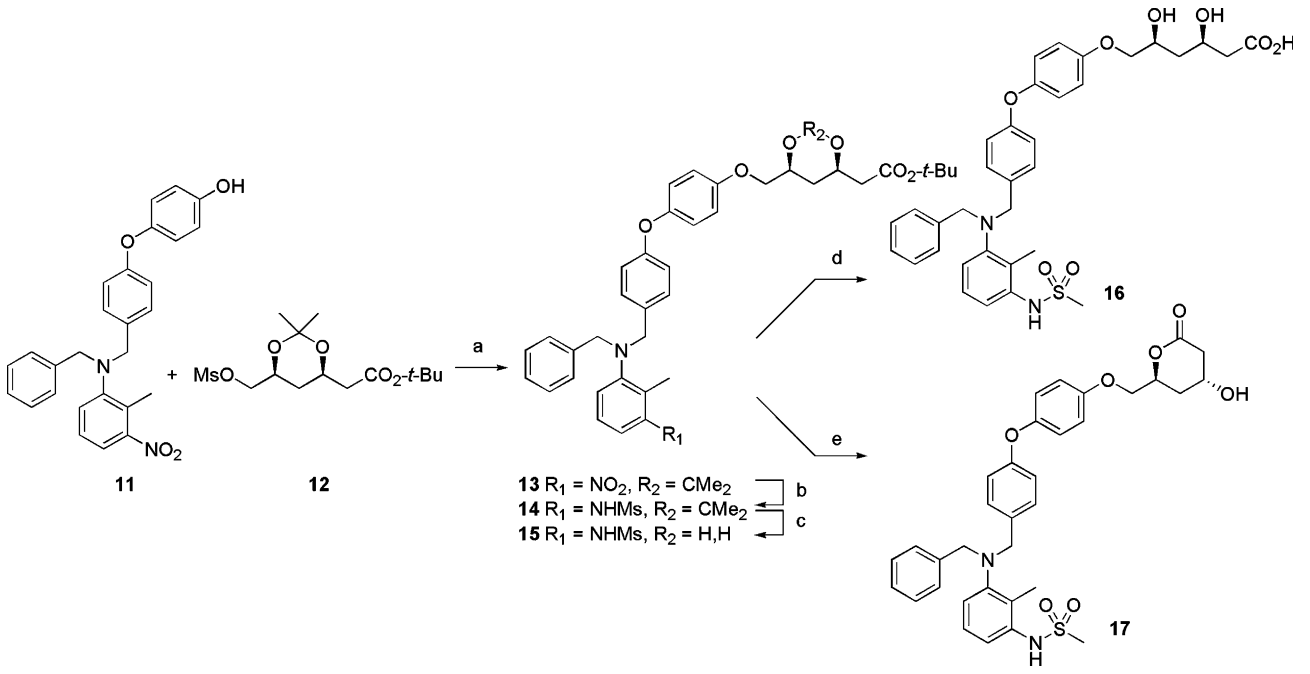
Scheme 1. Reagents and conditions: (a) i. allyl iodide, K_2CO_3, acetone, 40 °C, 12 h, 41%, ii. 4-fluorobenzaldehyde, K_2CO_3, DMF, 100 °C, 12 h, 81%; (b) 2-methyl-3-nitroaniline, AcOH, DCE, rt, 4 h, $Na(OAc)_3BH$, 12 h, 85%; (c) benzylbromide, *i*-Pr_2NEt, DMF, 90 °C, 12 h, 94%; (d) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, rt, 1 h, 95%.

fluorobenzaldehyde provided aldehyde **8**. Reductive amination with 2-methyl-3-nitroaniline followed by alkylation with benzyl bromide gave allyl ether **10**. Deprotection using tetrakis(triphenylphosphine)palladium[0] in the presence of phenylsilane yielded phenol **11**.[12]

The key portion of the statins was appended from a known precursor via precedented alkylation chemistry (Scheme 2).[13] Phenol **11** and mesylate **12** reacted to give ether **13** in 62% yield. Conversion of nitroarene **13** into methylsulfonamide **14**, followed by deprotection, gave the diol-acid **16** in good yield. Lactone **17** was prepared from *t*-butyl ester **15** by treatment with trifluoroacetic acid. Steroidal GR antagonist–statin hybrids **21**–**24** were prepared from a known intermediate using a similar strategy.[14]

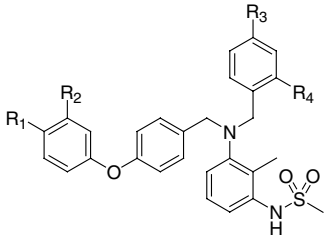
The antagonists and modulators were assayed in a h-GR binding assay, monitoring for the displacement of radiolabeled dexamethasone. Functional activity was assessed in a GR alkaline phosphatase reporter whole cell assay (GRAF).[6a] A second assay measuring the blockade of dexamethasone-induced activity of tyrosine amino transferase (TAT) in freshly isolated rat hepatocytes was also employed. Since rat hepatocytes have transporters (the organic ion transporter among others), it was hoped diol-acid hybrids would show improved activity in this assay. Weaker activity might also be observed in the GRAF assay if the statin substituents lowered cell permeability.

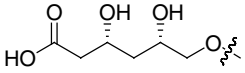
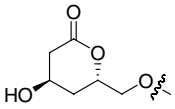
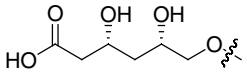
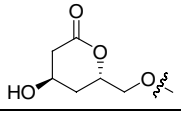
The nonsteroidal GR modulator **2** binds potently to h-GR ($IC_{50} = 2.7$ nM) (Table 1). It also has excellent selectivity over other nuclear hormone receptors (>100×), including human progesterone receptor (h-PR), mineralocorticoid (h-MR), androgen receptor (h-



Scheme 2. Reagents and conditions: (a) K_2CO_3, 18-crown-6, DMSO, 80 °C, 18 h, 62%; (b) Fe, NH_4Cl, EtOH, H_2O, 80 °C, 1 h; MsCl, pyr, rt, 1 h, 84%; (c) 3 N HCl, THF, EtOH, rt, 12 h, 97%; (d) NaOH, EtOH, rt, 2.5 h, 72%; (e) TFA, CH_2Cl_2, 0 °C → rt, 2 h, 82%.

Table 1. GR binding and functional assay results for nonsteroidal modulators **2** and **16**–**20**



Compds	R_1	R_2	R_3	R_4	h-GR Binding IC_{50}, nM[a]	GRAF IC_{50}, nM[a]	Rat TAT IC_{50}, μM[a]
2	H	H	H	H	2.7	240	>30
16		H	H	H	2.1	300	>30
17		H	H	H	2.1	440	>30
18	H	H	F	F	21	385	>30
19	H		F	F	15	760	>30
20	H		F	F	25	670	>30

[a] Values are the geometric means of two experiments (na = not active, nd = not determined).

AR), and estrogen receptor (ER_α and $_\beta$) (data not shown). However, sulfonamide **2** has modest functional activity in the GRAF assay and is inactive in the rat hepatocyte TAT assay. *para*-Substituted hybrids

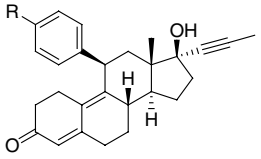
diol-acid **16** and lactone **17** have similar activity to the parent structure and are equally selective for h-GR. Diol-acid **16** shows little species selectivity (r-GR binding $IC_{50} = 2.6$ nM). *meta*-Substituted hybrids of the h-GR selective difluoride **18** were also prepared. Although less potent, these hybrids (**19** and **20**) also maintained the activity of the parent **18**. Interestingly, the diol-acid **16** and lactone **17** show similar activity, as do the other pair **19** and **20**. Although potent binding statin hybrids were identified their hepatocyte functional activity did not improve.

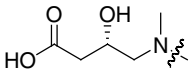
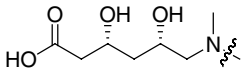
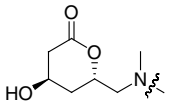
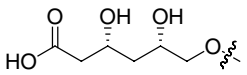
Steroidal GR antagonists–statin hybrids were also evaluated (Table 2). The steroidal core is less selective due to its potent antagonism of h-PR. A shorter monohydroxyacid **21** showed diminished activity relative to mifepristone (**1**). However, the longer chain diol-acid **22** and corresponding lactone **23** retain similar h-GR binding potency to the parent **1**. Aniline hybrids **22** and **23** also have potent functional antagonist activity (GRAF $IC_{50} = 18$ and 23 nM, respectively). Ether hybrid diol-acid **24** has potent binding activity but has significantly weaker GRAF activity, relative to mifepristone (**1**) and closely related aniline **21**, indicating it is probably not cell permeable. Interestingly, all the compounds show good potency in the rat hepatocyte TAT assay. This suggests diol-acid **24** and the other steroid hybrids are actively transported into hepatocytes.

The metabolic stability of representative GR modulator and antagonist–statin hybrids were evaluated in rat and human microsomes and hepatocytes. For the lactone **17**, the appearance of the corresponding diol-acid **16** was also monitored. Diol-acid **16** was metabolically stable when exposed to rat, or human, liver microsomes (incubation period = 60 min). The parent dibenzylaniline **2** is rapidly metabolized in microsomal incubations indicating the diol-acid group stabilizes the core structure. This is a desirable property for a liver targeting group. Lactone **17** under identical conditions converted to the stable diol-acid **16** during the incubation period. Diol-acid **16** underwent metabolism by both rat and human hepatocytes. In rat hepatocytes, metabolism occurred more extensively consuming all but 5%, whereas 60% of the parent remained at the end of the in human hepatocytes (6 h studies). The identity of the metabolites was not determined but they are less polar than the diol-acid **16**. This led us to speculate that they are dehydration products. During these experiments it was also noted that lactone **17** converts to acid **16** in buffer (half life not determined). This may also be the cause of their similar activity in the in vitro assays.

Pharmacokinetic studies at 5 mpk in Sprague–Dawley rats of open chain acid **16** showed the compound was cleared rapidly ($Cl_p = 5.2$ L/h kg), had a high volume of distribution ($V_\beta = 6.2$ L/kg), modest AUC (1.0 μg h/mL)

Table 2. GR binding and functional assay results for steroidal antagonists **1** and **21**–**25**



Compds	R	h-GR binding IC_{50}, nM[a]	h-PR Binding IC_{50}, nM	GRAF IC_{50}, nM[a]	Rat TAT IC_{50}, μM[a]
1	NMe_2	1.1	2.9	4.8	0.27
21		37	200	76	0.22
22		6.8	32	18	0.13
23		2.3	3.8	23	0.14
24		9.2	47	2900	0.56

[a] Values are the geometric means of two experiments (na = not active, nd = not determined).

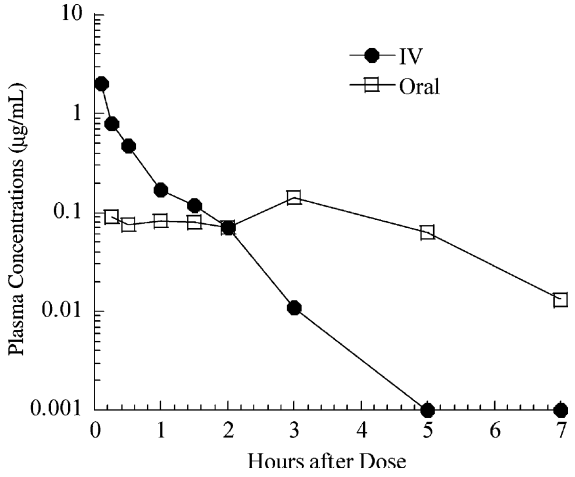


Figure 3. Rat pharmacokinetics of GR modulator–statin hybrid **16**.

and a short half life ($t_{1/2} = 48$ min) after iv dosing. Bioavailability was moderate ($F = 54\%$) (Fig. 3). Although the high clearance may be consistent with liver uptake, liver levels at $t = 7$ h were low (0.024 μg/g) in the iv animals and the orally treated animals were not appreciably higher (0.1 μg/mg). Diol-acid **19** gave a similar profile. Oral administration of the corresponding lactone **20** provided nearly identical blood and liver levels of diol-acid **19** when compared with oral dosing of **19** itself. The data is consistent with lactone hydrolysis prior to absorption. No lactone **20** was detected in either plasma or liver.

Steroidal hybrid **24** had a similar profile to the nonsteroid **16**. Steroidal diol-acid **24** was stable in rat and human liver microsomes (>90% remaining after 60 min). Good stability was also observed in rat (75% remaining) and human (>95% remaining) hepatocytes after a 6 h incubation. As with the nonsteroidal diol-acids, this profile represents a substantial improvement over the rapid metabolism of mifepristone (**1**). Oral/iv pharmacokinetic studies at 5 mg/kg in Sprague–Dawley rats indicated steroidal antagonist **24** had a high iv clearance consistent with liver uptake. However, low bioavailability (<5%) and liver levels (<5 μg/g) after iv or oral dosing were observed.

Nonsteroidal GR selective modulator–statin hybrids and steroidal GR antagonist–statin hybrids were prepared that have similar nuclear hormone activity as their potent parent compounds. The dihydroxyhexanoic acid side chains improved the metabolic stability of both the steroidal antagonist and nonsteroidal modulator. It also improved the cellular activity of the steroid hybrids in hepatocytes. These are valuable properties for a polar low molecular weight group to confer. However, neither class of hybrids achieved the necessary liver levels (or liver selectivity) to provide sufficient GR blockade to lower blood glucose levels. The successful realization of liver targeting for these series with other structural motifs will be reported in future publications.[11]

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