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Optimization and metabolic stabilization of a class of nonsteroidal glucocorticoid modulators

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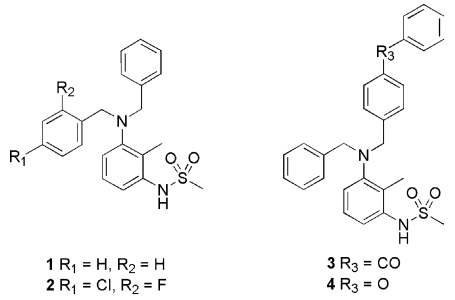
Abstract—The optimization of a series of nonsteroidal glucocorticoid modulators is reported. Potent selective GR ligands that have improved metabolic stability were discovered typified by the subnanomolar acid **12** (GR binding $IC_{50} = 0.6$ nM).

Nonsteroidal glucocorticoid receptor (GR) modulators have been explored as potential therapeutics for multiple diseases.[1] We recently reported a class of modulators that has potent GR affinity, moderate nuclear hormone selectivity, but poor metabolic stability.[2] A number of approaches were explored to improve the metabolic stability while optimizing their potency and selectivity. Two approaches for modification of the potential antidiabetic GR modulators are detailed herein.

Dibenzyl aniline sulfonamides, like **1** and dihalide **2**, served as leads for the series (Fig. 1). During optimization, substitution at the *para* position of the benzyl groups improved binding affinity and two of the most potent cores discovered were diphenyl ketone **3** and diaryl ether **4**. Rapid microsomal metabolism for these and related compounds was observed. Metabolite identification revealed that *N*-debenzylation was the primary metabolic pathway.

Human GR binding activity was determined in a radioligand competition binding assay using the labeled potent agonist [^{3}H]-dexamethasone. Functional activity was assessed in a cellular assay (GRAF). In the GRAF assay, genetically engineered cells expressing a glucocorticoid hormone response element and core promoter sequences linked to a secreted alkaline phosphatase reporter were employed to measure blockade of an agonist (dexamethasone) response. A second functional assay measuring the blockade of dexamethasone induced activity of tyrosine amino transferase (TAT) in freshly isolated rat hepatocytes was also employed. Nuclear hormone receptor selectivity was determined using binding assays for the human androgen receptor (h-AR), progesterone receptor (h-PR), mineralocorticoid receptor (h-MR), and thyroid-hormone receptor (h-TR$_\alpha$ and $_\beta$).



1 R_1 = H, R_2 = H
2 R_1 = Cl, R_2 = F
3 R_3 = CO
4 R_3 = O

Figure 1. GR modulator core structures.

Keywords: Glucocorticoid modulator; Antidiabetic.
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Table 1. GR binding, GR cellular functional inhibition, and rat microsomal stability for compounds **1**–**4**

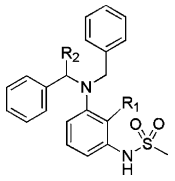
Compds	GR binding IC_{50}, nM[a]	GRAF IC_{50}, nM[a]	Rat microsomal $t_{1/2}$ (min)[b]
1	28	250	7.5
2	5.7	210	nd
3	13	340	20
4	2.7	240	4

[a] Values are geometric means of two experiments (nd = not determined).
[b] Microsomal incubations conducted at 10 μM with 0.25 mg/mL of microsomal protein.

The modulator cores **1**–**4** have potent GR binding activity, but modest cellular/functional activity and poor microsomal metabolic stability (Table 1). Diphenyl ether **4** is the most active binder with an IC_{50} of 2.7 nM. Compounds from this series were full antagonists in the GRAF assay. All four compounds are greater than eight times less potent in the GRAF assay relative to the binding assay. For comparison, the potent steroidal antagonist mifepristone (RU-486) has roughly equal IC_{50}'s in these assays. Rat microsomal metabolism for sulfonamides **1**, **3**, and **4** is rapid with the most stable compound being diphenyl ketone **3**. None have suitable stability for use as an orally dosed agent with the liver as a potential target organ.

A representative synthetic sequence is described in Scheme 1. Resorcinol monoacetate **5** was allylated, deprotected, and the resulting phenol reacted with 4-fluorobenzaldehyde. The product, aldehyde **6**, was then subjected to reductive amination reaction conditions in the presence of 2-methyl-3-nitroaniline and the product benzylated to provide nitroarene **7**. Deallylation with tetrakis(triphenylphosphine)palladium in the presence of phenylsilane provided the corresponding phenol **8**. The phenol could then be converted into a wide range of analogs by alkylation or Mitsunobu reaction. Alkylation with a halobutyric ester yielded nitroarene **9**. Nitro reduction and mesylation then gave the methylsulfonamide **10** that was hydrolyzed to acid **11**. Amide formation followed by hydrolysis yielded the modulator **12**. Although step intensive, the routes consist of simple individual protocols that work efficiently on small and multigram scale.

Table 2. GR binding, GR cellular functional inhibition and rat microsomal stability for compounds **13**–**15**

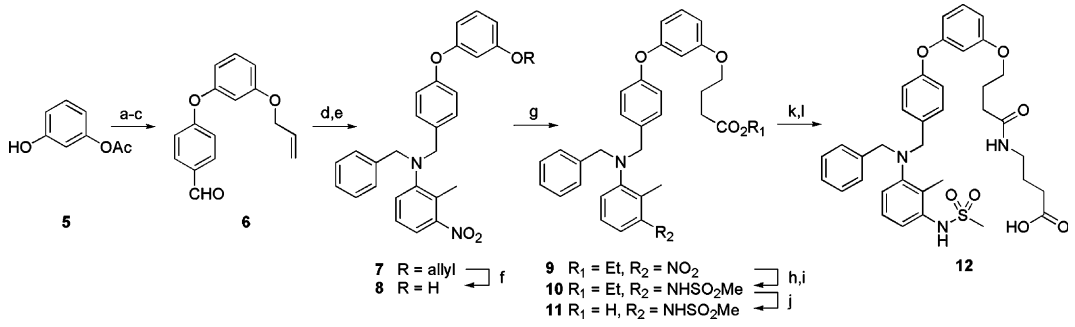


Compds	R_1	R_2	GR binding IC_{50}, nM[a]	GRAF IC_{50}, nM[a]	Rat microsomal $t_{1/2}$ (min)[b]
13	Me	Me	71	290	3.5
14	CH_2OH	H	56	840	4
15	Et	H	12	270	9

[a] Values are geometric means of two experiments (nd = not determined).
[b] Microsomal incubations conducted at 10 μM with 0.25 mg/mL of microsomal protein.

In order to improve metabolic stability multiple strategies have been explored. First, the steric hindrance around the dibenzylaniline nitrogen was increased. The polarity of the compounds was then increased and the metabolism tested. Other modifications designed to liver target the compounds were also evaluated for their effects on metabolic stability. Bile acid conjugation[3] and statin modulator hybrid formation[4] were explored and C-glucuronide formation[5] was considered.

The steric bulk around the aniline nitrogen could be modestly increased without a dramatic loss of potency (Table 2). For example, addition of a methyl group to the R_2 benzylic position as in **13** decreases the binding affinity by a factor of 2.5 without much impact on functional potency. Similarly, increasing the size of the R_1 substituent from Me **1** to CH_2OH **14** or ethyl **15** does not radically affect binding potency. Unfortunately, none of the substitutions improved metabolic stability. Larger substituents weakened GR affinity considerably (data not shown).



Scheme 1. Representative synthesis of a GR modulator: (a) allyl bromide, K_2CO_3, DMF, 80 °C, 12 h, 57%; (b) NaOH, THF, MeOH, H_2O, rt, 1 h; (c) 4-fluorobenzaldehyde, K_2CO_3, DMF, 100 °C, 12 h, 60% (two steps); (d) 2-methyl-3-nitroaniline, AcOH, DCE, rt, 4 h; $Na(OAc)_3BH$, 12 h, 82%; (e) benzylbromide, *i*-Pr_2NEt, DMF, 90 °C, 12 h, 84%; (f) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, rt, 1 h, 96%; (g) NaH, 4-bromo-butyric acid ethyl ester, DMF, 0 °C → rt, 12 h, 69%; (h) Fe, NH_4Cl, EtOH, H_2O, 80 °C, 1 h; (i) MsCl, py, rt, 1 h; (j) NaOH, THF, EtOH, rt, 2.5 h, 72%; (k) EDCI, HOBt, Et_3N, DMF, rt, 12 h, 93%; (l) NaOH, THF, EtOH, rt, 2.5 h, 74%.

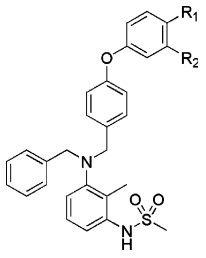
Adding polar groups around the periphery of the diphenyl ether core **4** proved to be a more effective strategy. Large substituents were tolerated on the terminal aryl ether ring (R_1 and R_2 positions—Table 3). A wide variety of functional groups provided potent compounds and most efforts focused on introducing groups that would be charged at physiological pH. Introducing amines that retained activity was challenging, but potent compounds like morpholine **16** were discovered. Unfortunately, these compounds did not show improved metabolic stability. Less polar amides like **17** nearly maintained the potency of the core **4** but were also metabolized rapidly. Ether **18** showed improved functional potency but also did not enhance stability. The same metabolism trend was observed in amide **19** and lactam **20**.

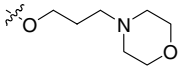
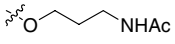
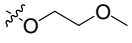
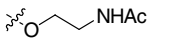
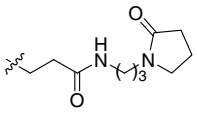
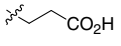
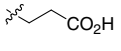
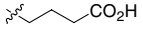
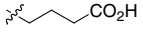
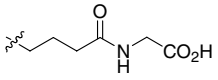
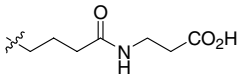
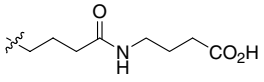
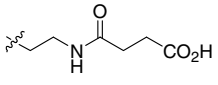
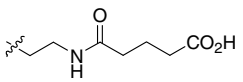
Introduction of carboxylic acids substantially improved the metabolic stability of this series. All of the acids tested had greater than 75% parent remaining after a 30 min incubation. For instance, the propionic or butyric acid sidechain in either the R_1 (**21** and **23**) or R_2 (**22** and **24**) position increased the microsomal half-life significantly. Moving the acid further from the core at the R_1 site (while including an amide in the linker) did not diminish the improved metabolic stability. For instance, amides **25**–**27** showed improved potency relative to the core **4**. The same was true at the R_2 site, where amides and ethers could also be included in the chain (**28**–**32**). Structurally related tetrazoles and acyl sulfonamides were also prepared (data not shown). They had moderate metabolic stability that fell between amides like **17** and the acids. Functional activity in the rat hepatocyte TAT assay for most of the metabolically stable compounds was moderate to weak. For instance, acid **21** has an $IC_{50} = 8.5\,\mu M$ (RU-486 $IC_{50} = 0.27\,\mu M$).

One of the most potent compounds found during this survey was modulator **12** that had subnanomolar binding IC_{50} for h-GR, good binding potency against rat GR ($IC_{50} = 1\,nM$, although rat hepatocyte TAT $IC_{50} < 30\,\mu M$), improved functional activity (GRAF $IC_{50} = 86\,nM$), and excellent microsomal stability. The compound also displayed excellent nuclear hormone receptor selectivity (>200-fold selective over AR, MR, PR, and TR_α and $_\beta$—data not shown).

In order to assess the in vivo antidiabetic potential of these compounds, we performed pharmacokinetic studies on several analogs. The systemically available antagonist RU-486 lowers blood glucose levels partially due to its suppression of hepatic glucose production.[6] Therefore, high liver drug levels were sought. Extrahepatic GR activity can be detrimental, including hypothalamic–pituitary–adrenal axis activation effects, so low peripheral exposure was desired. Representative profiles of the metabolically stable analogs were observed during oral and iv evaluations of amide **12** and acid **21** in Sprague–Dawley rats. Amide **12** had a modest half-life ($t_{1/2} = 2.2\,h$), low bioavailability ($F = 12\%$), and low liver levels 7 h post oral dose (0.56 μg/g) at 5 mpk. A high iv clearance ($CL_p = 12.7\,L/h\,kg$) greater than liver blood flow led to a low area

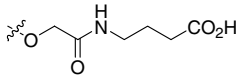
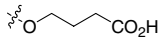
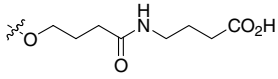
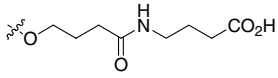
Table 3. GR binding, GR cellular functional inhibition, and rat microsomal stability for compounds **16**–**32** and **12**



Compds	Substituents	GR binding IC_{50}, nM[a]	GRAF IC_{50}, nM[a]	Rat microsomal $t_{1/2}$ (min)[b]
16 (R_2)		19	330	6
17 (R_2)		5.4	280	11
18 (R_2)		2.4	73	13
19 (R_2)		2.9	nd	30
20 (R_1)		2.2	180	12
21 (R_1)		34	500	>30
22 (R_2)		26	380	>30
23 (R_1)		8.8	670	>30
24 (R_2)		75	670	>30
25 (R_1)		1.6	120	>30
26 (R_1)		1.5	nd	>30
27 (R_1)		1.4	200	>30
28 (R_2)		11	460	>30
29 (R_2)		6.5	nd	>30

(*continued on next page*)

Table 3 (*continued*)

Compds	Substituents	GR binding IC_{50}, nM[a]	GRAF IC_{50}, nM[a]	Rat microsomal $t_{1/2}$ (min)[b]
30 (R_2)		11	660	nd
31 (R_2)		11	130	nd
32 (R_1)		3.9	640	nd
12 (R_2)		0.6	86	>30

Position of substituent listed in compds column. Unlisted substituent is hydrogen.

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

[b] Microsomal incubations conducted at 10 μM with 0.25 mg/mL of microsomal protein.

under the curve after a 5 mpk dose suggesting amide hydrolysis may be taking place. Acid **21** had a significantly lower iv clearance ($CL_p = 2.8$ L/h kg, 5 mpk) that resulted in improved area under the curve. Unfortunately, oral bioavailability (10 mpk) was still modest ($F = 10\%$) leading to low drug exposure.

A series of nonsteroidal GR modulators was optimized. Potent and selective compounds with improved metabolic stability were discovered with the most active analog being amide **12**. Early pharmacokinetic experiments revealed the basic cores have modest pharmacokinetic profiles. Related modulators with superior PK profiles and their in vivo activity in rodent models of diabetes will be reported shortly.

Acknowledgements

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References and notes

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