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Synthesis and biological evaluation of novel, selective, nonsteroidal glucocorticoid receptor antagonists

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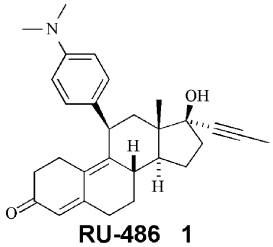
Abstract—We report the discovery of a novel class of glucocorticoid receptor (GR) antagonists based on the chromene molecular scaffold. The compounds exhibit good functional potency and an improved receptor selectivity profile for GR over other steroid receptors when compared to the classical steroidal GR-antagonist, RU-486.

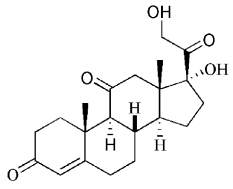
The glucocorticoid receptor (GR) is a member of the soluble intracellular steroid receptor superfamily that function as ligand-mediated transcription factors to control gene expression.[1] Glucocorticoids bind to the GR, and the resulting GR/ligand complex can either initiate gene transcription by binding to glucocorticoid receptor response sequences (GREs) on DNA or inhibit transcription by repressing the activity of other transcription factors such as NFκB or AP-1.[2] The initiation or repression of transcription events is determined by the topology of the GR/ligand complex, which is ultimately related to the structure of the GR-modulator.[3]

Glucocorticoid receptor antagonists such as RU-486 **1** have been shown to be effective at blocking gene-transcription mediated by endogenous glucocorticoids such as cortisone **2**. The utility of GR-antagonists in treating conditions that result from high levels of circulating glucocorticoids has been demonstrated by the use of RU-486 for the treatment of the Cushing's syndrome,[4] diabetes,[5] glaucoma,[6] and depression.[7] While RU-486 has demonstrated the potential therapeutic utility of GR-antagonists, it is limited by cross-reactivity with other steroid hormone receptors. Most notably, its activity as a progesterone receptor antagonist limits its pharmaceutical use as a GR-antagonist.

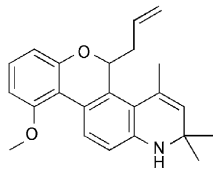
Nonsteroidal pharmacophores may offer a better opportunity for achieving the desired steroid receptor selectivity profile, however, there are only limited examples of nonsteroidal GR-antagonists reported in the literature.[8] Previous efforts at Abbott have focused on the optimization of chromene-based, receptor selective GR-modulators **3** that demonstrate an altered gene regulation profile compared to steroids.[9] In this work, we describe our efforts to optimize a novel,



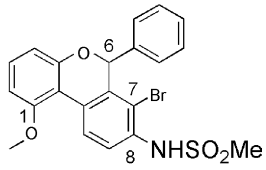
RU-486 1



Cortisone 2



AL-438 3



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Keywords: Glucocorticoid receptor (GR) antagonists; Chromenes; Nonsteroidal.
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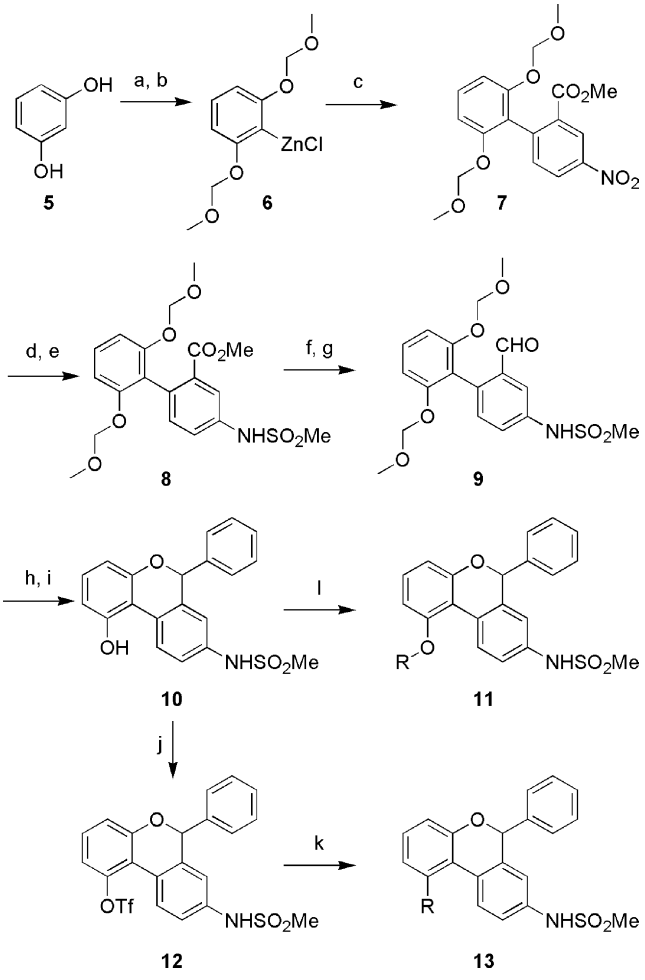
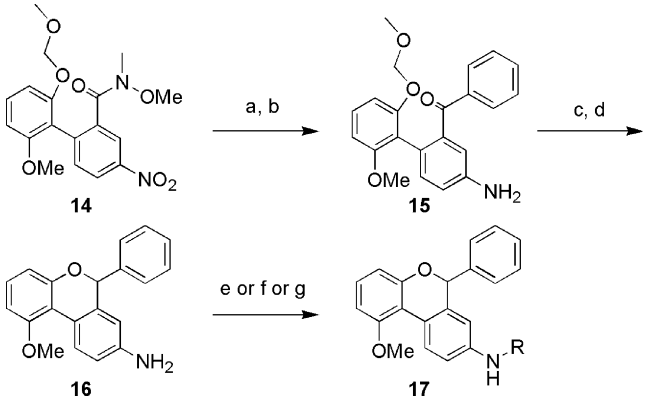
receptor-selective GR-antagonist based on the chromene scaffold. Screening of our compound repository identified chromene **4** as a novel, nonsteroidal GR-antagonist.

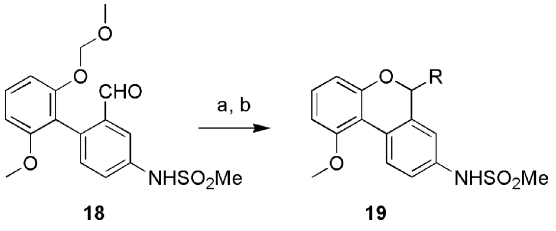
Herein, we describe our efforts to improve activity and increase selectivity of this lead compound. We have devised short and versatile synthetic routes that allow us to access a variety of substitution patterns on the chromene core (Schemes 1–3). Resorcinol **5** (Scheme 1) was dialkylated with MOMCl and the resulting ether was treated with *n*-BuLi followed by $ZnCl_2$ to generate the organozinc reagent **6** in situ. This compound was immediately reacted with methyl-2-bromo-5-nitrobenzoate under palladium-catalyzed conditions to form the biaryl intermediate **7** in good overall yield. The nitro arene was then reduced and the resulting amine was treated with MsCl. DIBAL reduction of methyl ester **8** to the corresponding alcohol and subsequent oxidation of the alcohol under TPAP/NMO conditions provided aldehyde **9** in high yields. Addition of PhLi at low temperature provided the corresponding benzhydrol, which was treated with acid to effect ring closure and simultaneous deprotection of the C1 hydroxyl.



Scheme 1. (a) NaH, DMF, MOMCl, 0 °C to rt, overnight, 99%; (b) *n*-BuLi, hexanes, THF, −30 °C, rt, 1 h, then $ZnCl_2$, 0 °C, rt, 2 h; (c) methyl-2-bromo-5-nitrobenzoate, $Pd(PPh_3)_2Cl_2$, 65 °C, overnight, 68% in two steps; (d) Fe, NH_4Cl, aq EtOH, 95 °C, 6 h, 52%; (e) $MeSO_2Cl$, pyridine, 0 °C to rt, overnight, 97%; (f) DIBAL, heptane, CH_2Cl_2, −78 to 0 °C, 2 h, 82%; (g) TPAP, NMO, powdered 4 Å MS, MeCN, rt, 2 h, 85%; (h) PhLi, THF, −78 °C, 2 h, then MeOH, 97%; (i) 2 M HCl in Et_2O, MeOH, rt, 20 h, 93%; (j) Tf_2O, Et_3N, CH_2Cl_2, −78 °C, 10 min, 0 °C, 20 min, 72%; (k) Bu_3SnR, $Pd(PPh_3)_4$, DMF, 120 °C, 14 h, 54–97%; (l) BSA, THF, 0 °C, 1 h, then KO*t*Bu, RX, 2 h, 29–78%.



Scheme 2. (a) H_2, 10% Pd/C, 4 atm, 1 h, quant.; (b) PhLi, Et_2O/cyclohexane, −5 °C, 1 h, 85%; (c) $NaBH_4$, EtOH, 65 °C, 1 h, 80%; (d) 4 N HCl in dioxane, MeOH, rt, 16 h, 87%; (e) RSO_2Cl, pyridine, $CHCl_3$, 80%; (f) RCOCl, PS–DIEA, CH_2Cl_2; (g) RCHO, DIEA, PS–BH_3, MeOH.

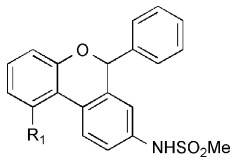


Scheme 3. (a) *p*-TsOH, MeOH, rt, 3 h, 99%; (b) $BF_3{\cdot}Et_2O$, CH_2Cl_2, −10 °C, RZnX, 9–58%.

Compound **10** can be used as a starting material for a variety of transformations such as alkylations to provide ethers **11** or via the triflate for Stille couplings to afford compounds **13**. Likewise, as shown in Scheme 2, biaryl intermediate **14** can be prepared in a similar fashion to **7** by starting with the Weinreb amide of 2-bromo-5-nitrobenzoic acid. Reduction of the nitro group and reaction with PhLi provided ketone **15**, which upon reduction to the alcohol and treatment with acid, yielded intermediate **16**. Aniline **16** was subjected to a variety of acylations, sulfonylations, and reductive aminations to furnish derivatives of general structure **17**. Following a similar route to the one delineated in Scheme 1, aldehyde **19** (Scheme 3) was prepared from 3-(methoxymethoxy)anisole.[10] Ring closure of **19** with *p*-TsOH and reaction of the resulting acetal with organozinc reagents under $BF_3{\cdot}Et_2O$ catalysis afforded compounds substituted at C6.

All compounds were tested for their binding affinity to the human glucocorticoid receptor and to the related progesterone, mineralocorticoid, androgen, and estrogen receptors in order to monitor selectivity. They were

Table 1. C1-SAR. Human GR-binding affinity[a] and whole cell GRAF functional assay[b]

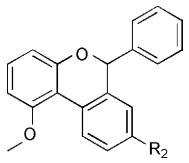


Compounds	R1	GR IC_{50} (nM)	GRAF IC_{50} (nM)
10	–OH	892 (559–1423)	1805
11a	–OMe	19 (17–21)	87
11b	–OEt	81 (70–92)	308
11c	$-OCF_2H$	2 (2–2)	57
13a	$-CH_2OH$	180 (150–220)	ND
13b	$-CO_2Me$	1204 (1382–1049)	ND
13c	–2-Thiophenyl	656 (639–673)	ND

[a] Binding affinities as measured by IC_{50}s (the concentration of compound required to inhibit 50% of the binding of [^{3}H]-dexamethasone). Values of IC_{50}s with ranges (in parentheses) are reported as geometric means of two experiments done in duplicate and values without ranges are from a single experiment done in duplicate.

[b] A genetically engineered, mammalian cell line expressing GR. IC_{50}s are determined from 12-concentration compound curves. (ND = not determined).

Table 2. C8-SAR. Human GR-binding affinity[a] and whole cell GRAF functional assay[b]



Compounds	R2	GR IC_{50} (nM)	GRAF IC_{50} (nM)
20	–H	10,000	1914
16	$-NH_2$	1937 (1273–2947)	2017
11a	$-NHSO_2Me$	19 (17–21)	87
17a	$-N(Me)SO_2Me$	1053 (949–1169)	210
17b	$-NHSO_2Et$	62 (61–63)	251
17c	$-NHSO_2N(Me)_2$	41 (26–67)	121
17d	$-NHSO_2-$ (2-thiophenyl)	18 (13–26)	93
17e	$-NHSO_2Ph$	146 (133–161)	137
17f	$-NHCOCH_3$	117 (106–131)	69
17g	–NHCOEt	160 (159–161)	124
17h	–NHCOPh	404 (294–554)	289
17i	–NHEt	564 (379–840)	382
17j	$-NHCH_2Ph$	910 (838–988)	402

[a,b] See Table 1.

also tested in a whole cell assay to measure functional cellular GR-antagonism (GRAF).[11]

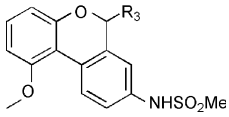
It was determined at an early stage that the bromine group at position 7 was not necessary for activity. In fact, removal of the bromine resulted in compound **11a** (Table 1), which was more potent and selective than the initial lead **4**. Subsequently all other analogs were synthesized with hydrogen at position 7.

Substitutions at C1 had a remarkable effect on activity. Replacement of the methoxy group with the difluoromethyl unit resulted in a more potent compound **11c**. Phenol **10**, larger substituents (e.g., **11b** and **13c**) or polar groups (**13a** and **13be**) had a detrimental effect on activity.

We then turned our attention to position 8 (Table 2), to examine the importance of the methanesulfonamide substituent. Removal of this unit altogether (compound **20**) led to loss of potency. Similarly, the free amine **16** and the *N*-methyl substituted sulfonamide **17a** were considerably less active than the initial sulfonamide **11a**. A parallel synthesis approach was undertaken to optimize the SAR at the amino terminus. A variety of sulfonamides, amides, and amines we synthesized from the free amino core **16**. We observed no significant improvement in activity from this exercise although, many compounds exhibited similar activities to the parent **11a**. Sulfonamides were in general better than amides and amines.

Thus far all the compounds had a phenyl group at C6. To explore a more diverse array of C6-substituents, we developed a robust and versatile synthesis that utilized Lewis-acid catalyzed addition of aryl, heteroaryl, and alkyl zinc reagents to a chromene–lactol intermediate

Table 3. C6-SAR. Human GR-binding affinity[a] and whole cell GRAF functional assay[b]



Compounds	R3	GR IC_{50} (nM)	GRAF IC_{50} (nM)
11a	Phe	19 (17–21)	87
19a	2-MeOPhe	812 (681–974)[c]	665
19b	3-MeOPhe	6 (5–6)	122
19c	3-MePhe	1	21
19d	4-MePhe	7 (6–7)[c]	ND
19e	3,5-MePhe	2	78
19f	3-FPhe	3 (3–4)[c]	76
19g	4-FPhe	29 (23–33)[c]	ND
19h	3-ClPhe	3 (3–3)	147
19i	4-ClPhe	10 (9–12)	129
19j	3,4-ClPhe	3 (3–4)	378
19k	4-EtOBn	548 (536–560)	ND
19l	2-Thiophenyl	7 (6–8)	66
19m	3-Thiophenyl	11 (10–12)	43
19n	3-PhOPhe	48 (44–53)	434
19o	4-PhOPhe	63	847
19p	3-EtO(CO)Phe	31 (28–34)	296
19q	4-EtO(CO)Phe	61 (39–94)	388
19r	3-HOPhe	216 (215–217)	ND
19s	2-Pyridyl	1430 (1428–1433)	ND
19t	3-Pyridyl	460 (366–578)	ND
19u	3-MeNHPhe	23	180
19v	Cyclohexyl	47 (43–50)	630
19w	*n*-Pentyl	21	ND

[a,b] See Table 1.

[c] Values are the geometric mean of three experiments done in duplicate.

Table 4. Human GR,[a] PR, MR, AR, and ER[a,d] binding affinities and whole cell GRAF functional assay[b]

Compounds	R1	R3	GR IC_{50} (nM)	PR-1 IC_{50} (nM)	MR-1 IC_{50} (nM)	AR-1 IC_{50} (nM)	ERa-1 IC_{50} (nM)	GRAF IC_{50} (nM)
RU-486			1	3	6000	89	ND	5
4			519 (378–814)[c]	10,000	454	ND	2281	430
11a	–OMe	–Phe	19 (17–21)	3355	22,897	6525	>10,000	87
11b	–OEt	–Phe	81 (70–92)	6896	893	>10,000	>10,000	308
11c	$-OCF_2H$	–Phe	2 (2–2)	367	162	8727	>10,000	57
19c	–OMe	3-MePhe	1	236	333	>10,000	ND	21
19d	–OMe	4-MePhe	7 (6–7)[c]	673	651	>10,000	>10,000	ND
19e	–OMe	3,5-MePhe	2	210	1220	>10,000	ND	78
19f	–OMe	3-FPhe	3 (3–4)[c]	476	683	>10,000	>10,000	76
19g	–OMe	4-FPhe	29 (23–33)[c]	1157	2484	>10,000	>1000	ND
19h	–OMe	3-ClPhe	3 (3–3)	498	2154	>10,000	>10,000	147
19l	–OMe	2-Thiophenyl	7 (6–8)	450	2252	>10,000	>10,000	66

[a,b] See Table 1.

[c] See Table 3.

[d] The following radiolabeled standards were used: [^{3}H]-progesterone (PR), [^{3}H]-aldosterone (MR), [^{3}H]-mibolerone (AR), [^{3}H]-estrodiol (ER).

derived from aldehyde **18** as shown in Scheme 3. Following this route, we prepared numerous analogs and were able to improve the potency by 10-fold to the single digit nanomolar range as exemplified by analogs **19c** and **19h** (Table 3). Substitution patterns on the phenyl ring clearly defined an SAR in this series. *meta*-Substituted phenyl analogs were more active than *para*, but both sites could accommodate polar groups (**19p**,**q**,**u**) and large substituents (**19n** and **19o**). Replacement of the phenyl ring, with a benzyl (**19k**) or pyridine rings, (**19s** and **19t**) led to a decrease in potency. However, thiophene (**19l** and **19m**) and cyclic (**19v**) or acyclic (**19w**) aliphatic groups were well tolerated.

Compounds that demonstrated potent GR-binding and functional antagonism were examined in a selectivity panel of steroid receptors (Table 4).

In our previous work with chromene-based GR-modulators we had discovered that the C1-substituent plays an important role in imparting GR-potency and selectivity.[12] Consistent with these findings, in the chromene series, the methoxy substituent at C1 (**11a**) was optimal for maximum selectivity. Small changes in the C1 substituent, for example, methoxy (**11a**) versus ethoxy (**11b**) led to a decrease in GR-potency and an erosion of the index of GR-selectivity. Even the more subtle change of C1-methoxy (**11a**) to C1-difluoromethoxy (**11c**) significantly altered the activity and selectivity profiles. Compound **11c** was more potent, however a disproportional increase in binding affinity for the MR receptor was also observed. Substitution patterns on the C-6 phenyl ring also played an important role in defining activity and selectivity. *meta*-Substituted compounds were in general more potent and selective than *para* (e.g., **19c** vs **19d** and **19f** vs **19g**) and larger substituents were more selective than smaller ones (e.g., **19h** vs **19f**). Refinements in the C6 substituent led to compound (**19c**) that demonstrated >200-fold selectivity for GR versus other steroid receptors and functional antagonism within 4-fold of RU-486.

In conclusion, we have discovered a novel class of chromene-based GR-antagonists. Optimized analogs in this series demonstrate potencies comparable to RU-486 and >200-fold selectivity versus the other steroid receptors.

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