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Discovery and SAR study of novel dihydroquinoline containing glucocorticoid receptor ligands

Hidenori Takahashi,* Younes Bekkali, Alison J. Capolino, Thomas Gilmore, Susan E. Goldrick, Richard M. Nelson, Donna Terenzio, Ji Wang, Ljiljana Zuvela-Jelaska, John Proudfoot, Gerald Nabozny and David Thomson

Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, Ridgefield, CT 06877, USA

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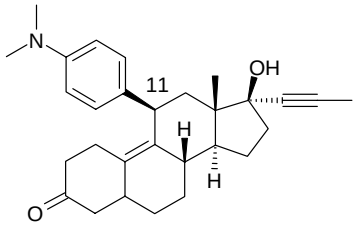
Abstract—We report the discovery of a novel class of glucocorticoid receptor (GR) ligands based on 1,2-dihydroquinoline molecular scaffold. The compounds exhibit good GR binding affinity and selectivity profile against other nuclear hormone receptors.

Synthetic glucocorticoids are widely used to treat many serious inflammatory and autoimmune disorders.[1] However, a major drawback in the clinical use of synthetic glucocorticoids is their association with a number of severe and life-threatening adverse events, such as enhanced bone resorption and muscle weakening. Discovery of glucocorticoid receptor (GR) agonists that are dissociated, that exhibit a reduced incidence or a reduced severity of side effects while maintaining potent anti-inflammatory activity, is currently an intensely sought goal.[2] There are also additional efforts underway to identify selective GR antagonists with the expectation that these may be useful in treating diabetes.[3]

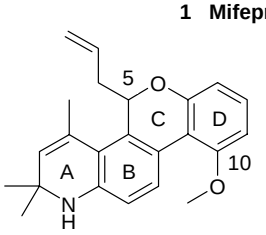
Mifepristone (RU-486) **1** has been reported as a GR antagonist and shown to be effective for blocking gene transcription mediated by endogenous glucocorticoids. The utility of Mifepristone has been demonstrated for treatment of Cushing's syndrome, diabetes, glaucoma, and depression.[4] Although structural similarity between Mifepristone and endogenous glucocorticoids is observed, it also shares structural features with a series from Abbott/Ligand, exemplified by **2**,[5] that is reported to be dissociated in both cellular and in vivo systems.

Based upon literature reports it is likely that this series evolved from a series of progesterone receptor (PR) selective ligands exemplified by **3**.[6] Literature data have indicated that both series of nuclear receptor ligands, **2** and **3**, may bind to the receptors in a similar manner, and that the choice of substituents at the C5-position determines the degree of dissociation, while an optimally sized functional group on C10-position in AL-438 is essential for the desired GR/PR selectivity profile (Fig. 1).

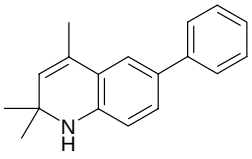
This article discloses our initial attempts to identify a novel series of dissociated GR ligands that is also



1 Mifepristone (RU-486)



2 AL-438



3 LG001447

Figure 1. Steroidal and non-steroidal nuclear receptor ligands.

Keywords: Glucocorticoid receptor; Ligand; SAR.
* Corresponding author. Tel.: +1 203 798 5049; fax: +1 203 798 5297; e-mail: htakahas@rdg.boehringer-ingelheim.com

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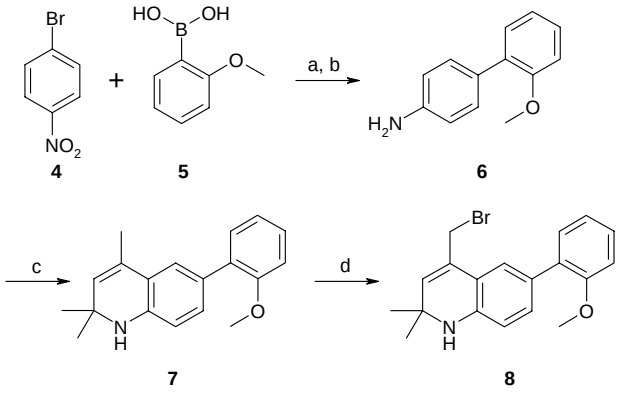
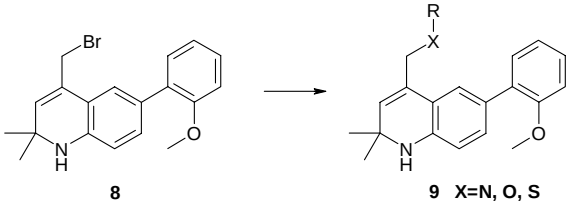
structurally related to RU-486 wherein we append the C11-position substituents of RU-486, believed to be necessary for dissociation and GR/PR selectivity, to the A-ring rather than the C-ring, while also opening up the C-ring.

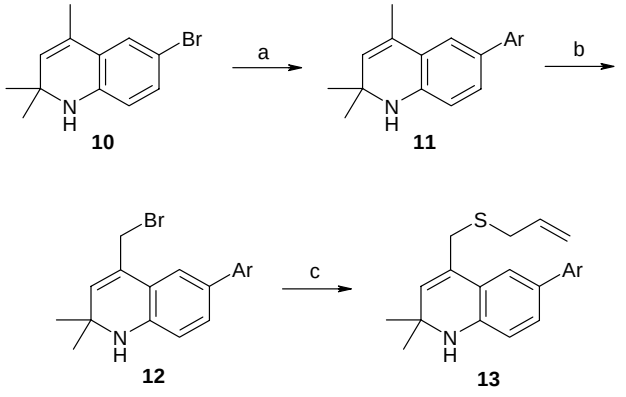
We had initially focused on identifying short and versatile synthetic routes that would allow us to explore a variety of substituents and substitution patterns on the dihydroquinoline core efficiently (Schemes 1–3). We



Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$ (5 mol%), DMF, 160 °C, 15 min, microwave, 70%; (b) Pd/C, NH_4COOH, MeOH, rt, 3 h, 99%; (c) acetone, iodine (30 mol%), $MgSO_4$, 160 °C, 25 min, microwave, 43%; (d) *N*-bromosuccinimide, CH_3CN, −20 °C, 30 min, 65%.



Scheme 2. Reagents: X = N; allylamine, K_2CO_3, DMSO, rt, 50%; X = O; allylalcohol, NaHMDS, THF, rt, 80%; X = S; RSH, K_2CO_3, DMSO, rt 30–75%.



Scheme 3. Reagents and conditions: (a) $Pd(OAc)_2$ (0.1 mol%), (2-biphenyl)dicyclohexylphosphine, KF, $ArB(OH)_2$, toluene, THF, rt, 16 h, 41–77%; (b) *N*-bromosuccinimide, CH_3CN, −20 °C, 30 min, 50–75%; (c) K_2CO_3, DMSO, allyl mercaptan, rt, 16 h, 20–65%.

chose the bromide **8** as an advanced intermediate to explore the effect of C4-position substitution. The synthesis of **8** is depicted in Scheme 1. A palladium catalyzed cross-coupling[7] of 1-bromo-4-nitrobenzene and 2-methoxy-phenylboronic acid, followed by palladium catalyzed hydrogen transfer reaction, afforded a precursor for the Skraup reaction. Treatment of aniline **6** with catalytic amount of iodine and acetone afforded dihydroquinoline scaffold in modest yield. Bromide intermediate **8** was reacted with a variety of nucleophiles to furnish the desired analogs in acceptable overall yield (Scheme 2).

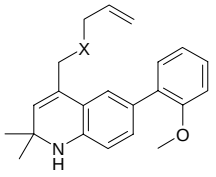
For SAR studies of substituents on the phenyl ring at the C6-position of the 1,2-dihydroquinoline, which we envisioned as mimicking the D-ring of AL-438, we chose known compound **10**[6] as the key branching point intermediate. For the cross-coupling reaction, we applied modified Suzuki coupling conditions reported by Buchwald et al.[8] due to a low yield of the products obtained by applying standard Suzuki cross-coupling conditions. Subsequently, compound **11** was brominated under the same conditions as described in Scheme 1, followed by displacement of the bromide atom by allyl mercaptan in the presence of potassium carbonate, as base, to give the desired analogs **13** in acceptable yields (Scheme 3).

All the compounds were tested in binding assays against a panel of human nuclear hormone receptors (GR, PR, estrogen receptor (ER), and mineralocorticoid receptor (MR)) using a fluorescence polarization competitive binding assay.[9]

Introduction of an allyl group containing a sulfur linker, in the C4-position of 1,2-dihydroquinoline scaffold, gave the novel, non-selective GR ligand **16** (IC_{50} = 360 nM). Those compounds with nitrogen **14** or oxygen **15** replacements for the sulfur linker did not exhibit either GR or PR binding activity at concentrations up to 2000 nM (Table 1).

Replacing the allyl substituent on compound **16** had a remarkable effect on GR binding affinity, and we learned that the SAR at this position was not straightforward. For example, (i) reduction of the allyl group to a *n*-propyl group resulted in a loss of binding affinity, (ii) phenethyl and phenyl groups were tolerated but a benzyl group was not, (iii) branched butyl and cyclic

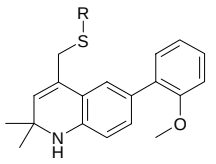
Table 1. GR and PR binding affinity with different C4-position linkers

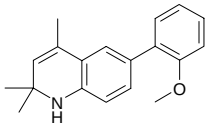


Compound	X	GR IC_{50}[a] (nM)	PR IC_{50} (nM)
14	NH	>2000	>2000
15	O	>2000	>2000
16	S	360	460

[a] Values are means of two experiments.

Table 2. GR and PR binding affinity with different C4-position linkers





11A

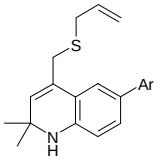
Compound	R	GR IC_{50}[a] (nM)	PR IC_{50} (nM)
16	Allyl	360	460
17	Propyl	1015	1600
18	Cyclopentyl	548	>2000
19	Cyclohexyl	371	>2000
20	2-Methylbutyl	303	>2000
21	3-Methylbutyl	703	>2000
22	Phenyl	804	>2000
23	Benzyl	>2000	660
24	Phenethyl	194	>2000
25	4-Chlorophenyl	800	>2000
26	$-C(O)CH_3$	>2000	620
27	$-CH_2C(O)OCH_3$	>2000	>2000
28	$-CH_2C(O)NHCH_3$	>2000	>2000
11A		>2000	>2000

[a] Values are means of two experiments.

alkyl groups were generally tolerated, and (iv) polar substituents such as ketone, ester, amide were not tolerated (Table 2). Surprisingly, phenethyl derivative **24** demonstrated improved GR binding affinity (IC_{50} = 190 nM) and exhibited more than 10-fold selectivity over PR binding (IC_{50} > 2000 nM). Overall, replacement of the allyl group with a phenethyl group resulted in a 2-fold improvement in GR binding affinity, and a significant improvement in PR binding selectivity. Additionally, both benzyl **23** and acetyl **26** derivatives demonstrated higher binding affinity to PR than GR. The SAR analysis at this position suggested that minor modifications modulated both GR binding affinity and PR selectivity. Lastly, simple methyl substituted derivative on C4-position **11A** showed no binding affinity to either GR or PR. These results suggest that it is required to employ optimally sized substituent and precise positioning for improved GR binding affinity.

Subsequently, we explored the effects of varying the substitution pattern on the phenyl ring at the C6-position of the dihydroquinoline core (Table 3). We focused on testing the steric and electronic effects of substituents on the phenyl ring. Shifting the methoxy group on the phenyl ring from the *ortho*-position **16** (GR IC_{50} = 360 nM) to the *meta*-position **36** (GR IC_{50} = 1000 nM) resulted in an approximately 3-fold loss in GR binding potency, while maintaining PR binding affinity resulting in **36** being a moderately PR selective ligand. The 4-methoxyl phenyl analog **38** displayed a significant loss in both GR and PR binding affinity. When the 2-methoxy group was replaced with hydrogen **29**, fluorine **30**, methyl **31**, ethoxy **32**, trifluoromethyl **33**, nitro **34**, and phenyl **35**, those compounds all demonstrated a loss of GR binding affinity. These results suggested that there is a size limitation for substituents on these positions. Neither did the di-methoxy phenyl analogs **39**, **40**, and **41**. On the other hand, both 2-methoxy-5-fluoro **42** and 2-chloro-3-hydroxy **43** disubstituted analogs displayed good GR binding affinity and significant binding selectivity over PR. Adding a fluorine group at the 5-position of the 2-methoxyphenyl ring resulted in a 2-fold increase in GR binding affinity and moderate improvement of selectivity profile. For substituents on the phenyl ring at the C2-position, although a hydrogen-bonding motif or a halogen atom was favored, precise positioning of hydrogen-bonding functional group was required in order to achieve improved GR binding affinity. Overall the SAR of this position displayed that minor modifications resulted in a substantial loss of both GR and PR binding affinity.

Table 3. GR and PR binding affinity with different C6-position substituents

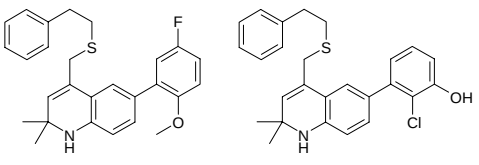


Compound	Ar	GR IC_{50}[a] (nM)	PR IC_{50} (nM)
16	2-MeO-phenyl	360	460
29	Phenyl	>2000	>2000
30	2-F-phenyl	>2000	360
31	2-Me-phenyl	>2000	>2000
32	2-EtO-phenyl	>2000	>2000
33	$2\text{-}CF_3$-phenyl	>2000	>2000
34	$2\text{-}NO_2$-phenyl	>2000	>2000
35	2-Ph-phenyl	>2000	>2000
36	3-MeO-phenyl	1000	460
37	3-EtO-phenyl	>2000	>2000
38	4-MeO-phenyl	>2000	>2000
39	2,3-Di-MeO-phenyl	>2000	>2000
40	2,5-Di-MeO-phenyl	>2000	>2000
41	2,6-Di-MeO-phenyl	>2000	>2000
42	2-MeO-5-F-phenyl	190	1400
43	2-Cl-3-OH-phenyl	129	960

[a] Values are means of two experiments.

Additionally, we prepared compounds that combined the optimal C4- and C6-position substituents (Table 4). These combination molecules contained the C4-position phenethyl and the C6 2-methoxy-5-fluorophenyl, to give derivative **44**, or the C6 2-chloro-3-hydroxyphenyl to give derivative **45**. Compound **44**

Table 4. GR, PR, ER, and MR binding affinity of compounds with the best substituent combinations at C4- and C6-positions



44 **45**

Compound	GR IC_{50}[a] (nM)	PR IC_{50} (nM)	ER IC_{50} (nM)	MR IC_{50} (nM)
44	84	>2000	>2000	>2000
45	220	1400	>2000	>2000

[a] Values are means of two experiments.

displayed a 4-fold improvement in GR binding affinity (IC_{50} = 84 nM) and a good selectivity profile across a panel of nuclear hormone receptors. Overall, the SAR study at the C4-position led to a 2-fold improvement in GR binding affinity, specifically by replacing the allyl group with a phenethyl group. The SAR study at the C6-position also led to a 2-fold improvement of GR binding affinity via the addition of a fluorine atom at the 5-position. The combination of these two independent findings resulted in a 4-fold improvement in GR binding affinity and a significant improvement in the selectivity profile (>20-fold) against PR. This additive result suggests that the C4- and C6-positions are contributing independently to the overall binding potency.

We tested the functional activity of these compounds using an IL-1-induced IL-6 assay in human foreskin fibroblasts.[10] Although both compounds **44** and **45** did not show inhibition of IL-6 production (agonist activity) in this assay, these compounds demonstrated their ability to prevent a response to dexamethasone induced-GR transactivation of an MMTV reporter gene in HeLa cells.[11] In the latter assay, **44** and **45** demonstrated an IC_{50} = 45 and 260 nM, respectively. These data suggest that these ligands are selective GR antagonists.

In summary, a novel and potent series of selective GR ligands was identified. We found that variation of both the substituents at the C4- and the C6-positions can impact both GR binding affinity and GR/PR binding selectivity. The most potent ligand identified, compound **44**, is a novel, potent, and highly selective GR antagonist.

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9. Fluorescence polarization competitive binding assays were performed to quantitate the ability of test compounds to displace ligands from GR, MR, ER, and PR in solution. Binding reactions were assembled in 96-well microplates. Baculovirus lysate containing either GR or MR was incubated with 5 nM tetramethyl-rhodamine conjugate of dexamethasone, and test compound dilutions in an assay buffer containing 10 mM TES, 50 mM KC1, 20 mM sodium molybdate, 1.5 mM EDTA, 0.04% w/v CHAPS, 10% v/v glycerol, and 1 mM DTT, pH 7.4. For the PR assay, baculovirus lysate containing PR was incubated with 5 nM tetramethyl-rhodamine conjugate of RU486 and the test compound dilutions. The ER binding assay was performed using the ER Competitor Assay kit from Panvera (Invitrogen part number P2614). This assay uses purified baculovirus-expressed human ER and fluorescein conjugate of a proprietary ER ligand (Fluormone^{TM} ES2). IC_{50} values shown are means of a single experiment done in duplicate 11-point concentration–effect curves. Bekkkali, Y.; Gilmore, T.; Spero, D. M.; Takahashi, H.; Thomson, D. S.; Wang, J. *PCT Int. Appl.*, WO2004018429.
10. Human foreskin fibroblasts were stimulated with 1 ng/mL recombinant human IL-1 in the presence of test compound. After 24 h, the degree of GR agonist activity (transrepression) was determined by measuring IL-6 in the tissue culture media.
11. HeLa cells stably transfected with MMTV luciferase construct were preincubated with 20 nM dexamethasone for 15 min. After preincubation, cells were treated with the compounds or their vehicle (0.2% DMSO) and incubated for 6 h. After the incubation, the induction of luciferase was measured. IC_{50} values are means of two experiments.