Bioorganic & Medicinal Chemistry Letters 21 (2011) 168–171

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Tetrahydroquinoline glucocorticoid receptor agonists: Discovery of a 3-hydroxyl for improving receptor selectivity

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ARTICLE INFO

Article history:
Received 21 September 2010
Revised 4 November 2010
Accepted 8 November 2010
Available online 11 November 2010

Keywords:
Glucocorticoid
Transactivation
Transrepression
Non-steroidal
Tetrahydroquinoline

ABSTRACT

We have previously disclosed a series of glucocorticoid receptor (GR) ligands derived from 6-indole-1,2,3,4-tetrahydroquinolines through structure–activity relationship (SAR) of the pendent C6-indole ring. In parallel with this effort, we now report SAR of the tetrahydroquinoline A-ring that identified the importance of a C3 hydroxyl in improving GR selectivity within a series of non-steroidal GR agonists.

The glucocorticoid receptor (GR) belongs to the steroid receptor subfamily of nuclear hormone receptors[1] (NHRs) including the estrogen receptor (ER), mineralocorticoid receptor (MR), androgen receptor (AR), and progesterone receptor (PR). Glucocorticoids (GCs) such as prednisolone **1** and dexamethasone **2** (Fig. 1) are widely prescribed in the clinic to treat inflammatory and autoimmune disorders. Although effective therapeutic agents, the use of GCs must be managed against their propensity to induce a variety of side effects.[2] The transrepression (TR) of pro-inflammatory genes that encode for cytokines and other inflammatory mediators is thought to form the basis for the beneficial anti-inflammatory effects of GCs while direct transactivation (TA) of specific genes, such as those involved in gluconeogenesis, leads to their unwanted side effects.[3] Discovery of GR ligands that selectively favor TR over TA has been the focus of considerable research effort and remains a difficult task.[4–11]

In addition to TR/TA selectivity, the development of new non-steroidal GR-selective ligands possessing drug-like properties can be challenging.[12] We recently identified GR ligands based on 6-indole-1,2,3,4-tetrahydroquinolines, exemplified by **3**.[13] Initial SAR in the series focused on the pendent C6-indole ring to improve GR selectivity while maintaining TR activity. Concurrent with this effort, we explored SAR of the tetrahydroquinoline A-ring of **3** that subsequently identified new GR-selective agonists possessing steroid-like TR activity.

GR binding was determined using a radiolabeled dexamethasone competitive binding assay with baculovirus-expressed GR.[4] To assess the functional activity of the new ligands, GR-mediated TA was measured in a co-transfection (CTF) assay using a luciferase reporter containing a glucocorticoid response element (GRE).[14] Potency (EC_{50}) was determined from half-log concentration–response curves and maximal efficacy was determined relative to dexamethasone. To evaluate TR activity of the new ligands, a CTF E-selectin repression assay in HepG2 cells was used in order to determine repression of transcriptional activation mediated by

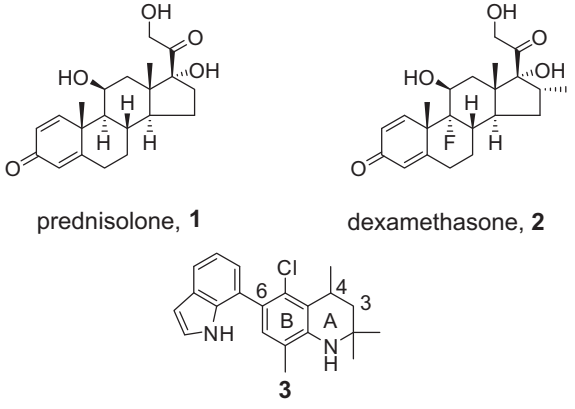

Figure 1. Steroidal glucocorticoids and 6-indole tetrahydroquinoline.

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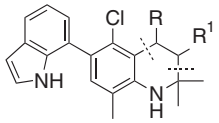
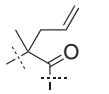
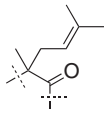
doi:10.1016/j.bmcl.2010.11.047

Table 1
In vitro assay results[a]



Compd[b]	R + R[1]	GR binding K_i (nM)	PR binding K_i (nM)	GRE activation		E-selectin repression assay		IL-6 repression assay	
				Eff. (%)	EC_{50} (nM)	Eff. (%)	IC_{50} (nM)	Eff. (%)	IC_{50} (nM)
1	Prednisolone	5.3 ± 0.3	—	130 ± 7	5.3 ± 3.6	100 ± 2	4.1 ± 0.8	97 ± 1	23 ± 2
3		0.6 ± 0.1	13 ± 1	110 ± 6	20 ± 3	92 ± 3	9.4 ± 2.9	67 ± 8	59 ± 23
7		5.8 ± 0.7	70	77 ± 9	235 ± 25	76 ± 11	13 ± 11	57	—
8		4.5 ± 2.2	14 ± 8	106 ± 10	95 ± 13	82 ± 7	4.7 ± 1.6	nt	nt
9		3.5 ± 0.7	25	223 ± 15	7.4 ± 0.5	123 ± 1	2.1 ± 1.9	92 ± 12	36 ± 14
10		6.0 ± 1.4	420	174 ± 36	215 ± 40	71 ± 5	18 ± 10	47 ± 7	64 ± 18
11		340	270	—	—	57 ± 8	—	nt	nt
12		1.7 ± 0.4	1200 ± 200	144 ± 6	7.1 ± 4.5	101 ± 3	1.1 ± 0.2	96 ± 5	16 ± 4
(−)**12**		0.9 ± 0.1	2400 ± 700	133 ± 34	0.8 ± 0.3	79 ± 6	0.2 ± 0.03	103 ± 10	1.6 ± 0.5
(+)**12**		85 ± 12	1400 ± 500	48 ± 18	483 ± 44	40 ± 7	—	56	100
13		16 ± 4	1300 ± 180	157 ± 15	105 ± 14	84 ± 4	14 ± 4	95 ± 18	37 ± 34
17		nt	nt	—	—	64 ± 6	—	—	—
19		436	3000	—	—	27 ± 5	—	49	—
20		416	1900	—	—	—	—	nt	nt
22		10 ± 3	1100 ± 150	71 ± 5	370 ± 35	69 ± 10	24 ± 8	40	—

[a] EC_{50} and IC_{50} values determined from half-log concentration response curves. Agonist efficacies represent the percentage maximal response in comparison to dexamethasone (100%). E-selectin repression efficacies represent the percent of maximal inhibition of the response induced by TNFα and IL-1β. IL-6 repression efficacies represent the percent of maximal inhibition of the response induced by IL-1β. Standard errors (SEM) represent the mean value of at least two separate experiments with triplicate determinations. If no SEM is noted, value is from a single determinant. M-dash (—), not active and denotes <20% efficacy or potency >1 μM. nt, denotes not tested.
[b] Except where noted, compounds were tested as racemates.

NFκB or AP-1[15] while an IL-6 ELISA assay[4] determined inflammatory cytokine repression in primary neonatal human dermal fibroblast (NHDF) cells.

Tetrahydroquinoline **3** (Table 1) is a GR agonist possessing high E-selectin and partial IL-6 repression efficacy. Although an effective ligand for GR, **3** is highly lipophilic ($c\log P = 6.9$) and showed significant PR binding affinity ($K_i = 13$ nM). We sought to investigate the A-ring of **3** to minimize receptor cross-reactivity while in turn improving TR activity. In an effort to reduce the high lipophilicity of **3** the strategy of incorporating polar functionality into the A-ring was undertaken. Dihydroquinoline **4**[13] (Scheme 1) served as a convenient starting point from which both the C3 and C4 positions of **3** could be examined. Toward this end, **4** was oxidized (borane–THF, hydrogen peroxide) followed by subsequent C6 bromination (*N*-bromosuccinimide) to yield intermediate bromide **5**. Swern oxidation of **5** led to the formation of ketone **6** which could be alkylated with an appropriate electrophile (potassium *tert* butoxide, THF) followed by palladium-catalyzed Suzuki reaction using 1*H*-indol-7-ylboronic acid to provide C3 racemic ketone analogs **8–11**. Alternatively, Suzuki reaction of **5** or **6** generated C3 hydroxyl or ketone analog **12** and **7**, respectively.

The results of analogs **7–11** are summarized in Table 1. Introduction of a ketone at C3 (**7**) resulted in a less potent (~10-fold) and efficacious GR agonist with reduced GR binding affinity relative to **3**. Although, **7** offered an improved $c\log P$ (5.5) over **3**, it was inactive in the IL-6 repression assay. Incorporating substitutents at the C4 position of **7** such as methyl (**8**), allyl (**9**), or prenyl (**10**) provided full agonists with single nanomolar binding affinity. GR agonist potency of **7** was enhanced by either a C4 methyl or allyl group, with **9** exhibiting an in vitro profile similar to predniso-