Bioorganic & Medicinal Chemistry Letters 20 (2010) 5835–5838

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of quinolines as selective glucocorticoid receptor agonists

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ARTICLE INFO

Article history:
Received 14 December 2009
Revised 27 July 2010
Accepted 28 July 2010
Available online 3 August 2010

Keywords:
Glucocorticoid
SEGRA
Transrepression
Transactivation
Quinoline

ABSTRACT

The dissociated glucocorticoid receptor (GR) agonist ZK 216348 is rendered GR-selective over other nuclear hormone receptors through replacing the methylbenzoxazine with a quinoline moiety. Compounds were shown to be efficacious in cell assays with respect to inflammation endpoints, along with reduced activity in a transactivation assay, hinting at an improved therapeutic window over corticosteroids.

Since their introduction into the market almost 60 years ago, glucocorticoids (GCs) maintain a dominant role in the treatment of inflammatory conditions,[1] for example, in the management of airway diseases,[2] rheumatoid arthritis,[3] eye and skin diseases (atopic dermatitis, contact eczema, and psoriasis).[4] Apart from their potent anti-inflammatory activity, long-term and high-dose treatment with orally applied GCs can lead to serious adverse effects, such as osteoporosis, diabetes, Cushing's syndrome, glaucoma and muscle atrophy. Recent efforts to elucidate the action of GCs on a molecular level have led to the hypothesis that the monomeric GC-bound glucocorticoid receptor abrogates transcription of pro-inflammatory gene products (transrepression),[5] while a dimeric GC–GR complex promotes inter alia expression of enzymes involved in catabolic processes (transactivation). Hence, interfering with the transrepression pathway should yield a beneficial anti-inflammatory effect, whereas triggering the transactivation pathway rather elicits typical GC side-effects. This rationale opened a path for improving on a venerable drug class in terms of therapeutic window by decoupling the transrepression from the transactivation activity.[6] Though refined and modified in the meantime,[7] it served as a valuable paradigm for the identification of novel, non-steroidal GR agonists, and a host of recent publications attests to this.[6–14] Our early efforts to identify such selective GR agonists (SEGRAs) led to the discovery of ZK 216348 ((+)-**1**, Scheme 1). The compound suppressed the induction of pro-inflammatory reactions while to a lesser degree affecting enzymes involved in glucose metabolism, which resulted in an improved systemic side-effect profile in animal models.[15] Since ZK 216348 suffered from poor selectivity, particularly towards the progesterone receptor (PR), we set out to identify SEGRAs with increased selectivity for the GR over other nuclear receptors to avoid any adverse effects mediated by the PR, androgen receptor (AR), or mineralocorticoid receptor (MR) (cf. Table 1).

Comparison of methylbenzoxazinones **1–4** (cf. Scheme 1 and Table 1) indicated that the undesired PR activity might be linked to the heterocyclic moiety. Furthermore, replacing the carboxamide with a methyleneamine functionality, as in **4**, appeared to reduce binding to AR and MR. In the absence of structural data, we decided to employ a parallel synthesis approach by opening epoxide **14** (cf. Scheme 2) with various amines to identify a suitable surrogate for the methylbenzoxazinone. In addition, we cleaved the methoxy ether to obtain the hydroxyl analogs, since GR affinity was observed both for anisole **2** and phenol **3**. From these efforts, naphthalene **6** emerged as a novel lead with a selective binding profile to GR comparable to that of dexamethasone (DEX), whereas its methylated congener **5** showed undetectable GR binding affinity. When replacing the naphthalene residue by a quinoline, a significant increase in GR binding affinity was recorded for phenol **8**, matching that of methylbenzoxazine **2**, but without PR, MR, or AR binding liabilities. The GR binding affinity of the analogous anisole **7** is reduced, yet comparable to that of **1**. In the 2-methylquinoline series, however, the most potent GR binder is methyl ether **9**. In summary, the quinoline series allows for the reconciliation of

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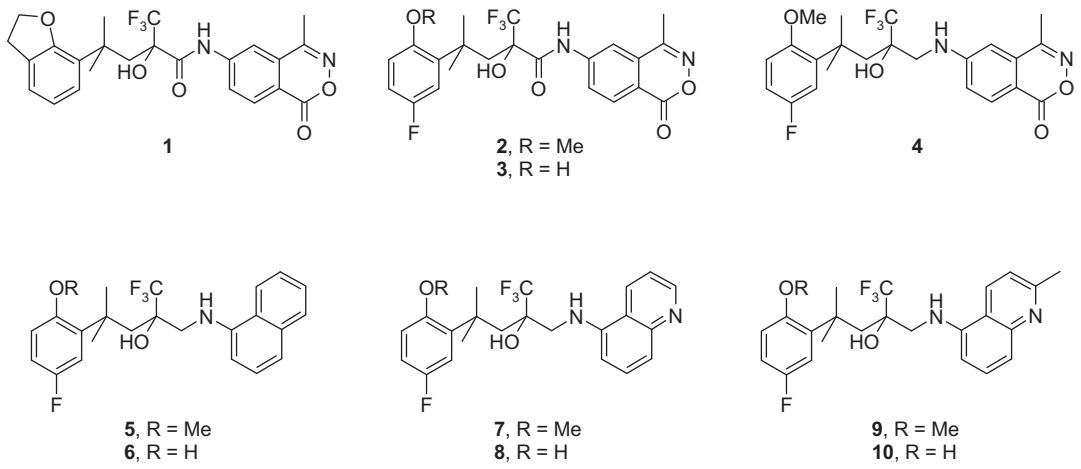
doi:10.1016/j.bmcl.2010.07.125



Scheme 1. Ref. 16.

Table 1
Binding profile against nuclear hormone receptors[a]

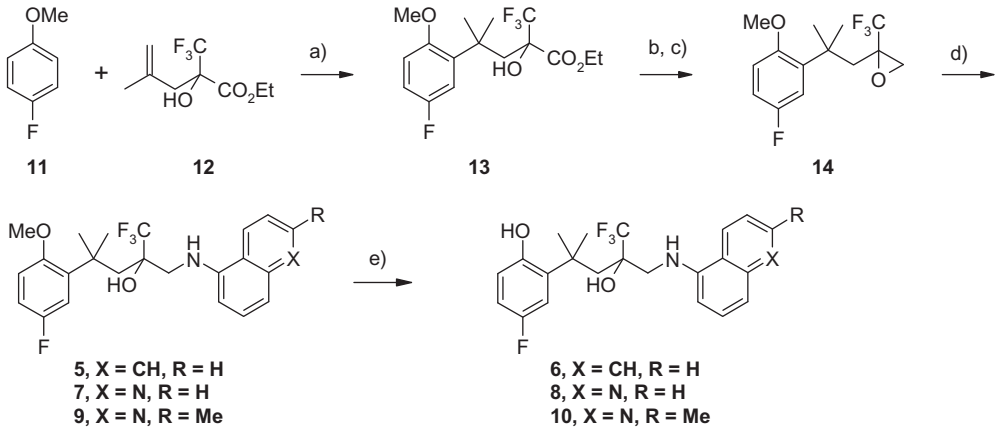
Compd	GR pIC_{50}	PR pIC_{50}	MR pIC_{50}	AR pIC_{50}
DEX[b]	7.9	<6.0	<6.0	<6.0
1	7.7	7.7	7.1	6.2
2	9.0	7.9	8.1	6.3
3	8.5	8.1	8.9	6.8
4	8.0	7.8	<6.0	<6.0
5	<6.0	<6.0	<6.0	<6.0
6	7.8	<6.0	<6.0	<6.0
7	7.7	6.0	<6.0	<6.0
8	9.0	<6.0	<6.0	<6.0
9	9.2	<6.0	<6.0	<6.0
10	8.2	<6.0	<6.0	<6.0

[a] Ref. 17.
[b] DEX, dexamethasone.

Table 2
Anti-inflammatory activity in THP-1 monocyte assays[a]

Compd	Transrepression/monocyte inhibition of IL-8 production	
	pIC_{50}	Max. efficacy[b] (%)
DEX[c]	8.6	100
1	7.5	52
3	7.3	78
6	<6.0	71
7	7.6	61
8	7.7	79
9	7.6	90
10	8.3	97

[a] Ref. 18.
[b] Maximal efficacy response is normalized to maximum efficacy of dexamethasone (= 100%).
[c] DEX, dexamethasone.



Scheme 2. Reagents and conditions: (a) $AlCl_3$, 10 °C–rt; (b) $LiAlH_4$, Et_2O; (c) PPh_3, EtO_2CNNCO_2Et, THF, 0 °C–rt; (d) $ArNH_2$, DMPU, 130 °C; (e) BBr_3, CH_2Cl_2.

strong GR affinity with selectivity over other nuclear hormone receptors.

In order to assess how GR binding translates into anti-inflammatory efficacy, compounds **6–10**, along with dexamethasone and methylbenzoxazines **1** and **3**, were evaluated in LPS-stimulated THP-1 monocytes for their capability to suppress IL-8 production (Table 2). Efficacy has been normalized to that of the dexamethasone reference.[18] Despite GR affinity similar to DEX, naphthalene **6** only poorly suppresses IL-8 formation. In contrast, the quinolines **7–10** are potent and efficacious agents: Compound **7** remains comparable to **1**, while a higher efficacy renders **8** comparable to **3**. 2-Methylquinolines **9** and **10** display an efficacy that almost matches that of DEX.

Next, we evaluated selected compounds in recombinant cell assays to assess their anti-inflammatory activity versus side-effect potential in a similar cellular context. Transrepression (TR) activity, the main driver for anti-inflammatory activity, was assessed in HeLa cell lines harboring a collagenase promoter coupled to a

Table 3
Anti-inflammatory and immunomodulatory (transrepression) and transactivation activity in recombinant cell assays[a]

Compd	Transrepression/transfected HeLa, LUC readout		Transactivation/transfected HeLa, LUC readout	
	pIC_{50}	Max. efficacy[b] (%)	pEC_{50}	Max. efficacy[b] (%)
DEX[c]	8.8	100	8.1	100
1	8.2	75	7.9	43
3	8.0	76	7.6	80
8	8.2	84	7.1	50
9	8.7	90	7.6	68
10	8.5	96	7.9	88

[a] Ref. 18.
[b] Maximal efficacy response is normalized maximum efficacy of dexamethasone (= 100%).
[c] DEX, dexamethasone.

luciferase gene. Transactivation (TA) activity, as surrogate marker for side effects, was determined with MMTV-luciferase transfected HeLa cells (Table 3).

The TR activity of quinoline **8** is similar to that of methylbenzoxazines **1** and **3**, whereas methylquinolines **9** and **10** appear to be slightly more potent and efficacious, almost reaching a DEX profile as in the THP-1 assay. However, this comes at the expense of increased efficacy and potency in the TA assay in the order **8** < **9** < **10** < DEX. Compound **8** showed the most favorable transactivation profile in the quinoline series, comparable to the benchmark ZK 216348 (**1**) and better than methylbenzoaxine **3**. Based on its GR affinity and selectivity profile, its activity in stimulated monocytes and its balanced transrepression/transactivation profile, compound **8** demonstrates that the quinoline class of SEGRAs overcomes drawbacks inherent in the benzoxazine series and has the potential to improve the dissociation profile.

Compounds **5–10** have been synthesized according to the sequence depicted in Scheme 2.[19] Friedel–Crafts alkylation of fluoroanisole (**11**) with alkene **12** furnished ester **13**.[20] Reduction to the diol and epoxide formation under Mitsunobu conditions gave **14**, which was opened with amines to provide compounds **5**, **7**, and **9**. Finally, methyl ether cleavage led to phenols **6**, **8**, and **10**. This straightforward process proved particularly versatile at the outset to identify a methylbenzoxazine surrogate through high-throughput syntheses with larger amine sets.

In summary, we have discovered quinolines that overcome the liabilities of our previously reported methylbenzoxazine lead ZK 216348. The prototype **8** displays an improved selectivity profile versus hormone receptors, an improved in vitro efficacy in monocytes, and a better balance between transrepression and transactivation activities as assessed in recombinant cells. In the course of our optimization efforts, we eventually identified a development candidate from this series, the topical SEGRA ZK 245186, which is currently being evaluated in clinical trials as a novel agent against atopic dermatitis[21] and ophthalmologic diseases.[22]

Acknowledgments

We thank Stefan Hünicke, Hans-Ulrich Klein, Angela Mücke, Roland Schreyer, Diana Schröder, Harry Vierhufe, and Ricardo Wieseke for their technical support.

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16. ZK 216348 is the (+) enantiomer of **1**; all other compounds are racemates.
17. Receptor binding assays. Extracts from Sf9 cells, infected with recombinant baculovirus coding for the human glucocorticoid receptor (GR), progesterone receptor (PR), androgen receptor (AR), or mineralocorticoid receptor (MR) were used for the receptor binding assays as described in Refs. 15,21. Tests were run in duplicate with a pIC_{50} value error of ±0.2.
18. Inhibition of IL-8 production: IL-8 synthesis was induced by stimulation of the human promyeloic cell line THP-1 with lipopolysaccharide (LPS). Cells were treated with LPS in the absence or presence of test compounds or reference (dexamethasone). For details see Ref. 15 Tests were run in duplicate with a pIC_{50} value error of ±0.2.
    Anti-inflammatory/immunomodulatory activity in vitro (transrepression): Inhibition of collagenase promoter activity. HeLa cells stably transfected with a luciferase reporter gene linked to the collagenase promoter were challenged with inflammatory stimulus (12-*O*-tetradecanoylphorbol-13-acetate (TPA)) in the presence of the reference (dexamethasone) or the test compounds. Tests were run in duplicate with a pIC_{50} value error of ±0.2.
    Transactivation activity in vitro: Induction of MMTV promoter activity has been used as surrogate marker for transactivation-mediated side effects. The MMTV promoter was linked to a luciferase reporter gene and HeLa cells were stably transfected with this construct. Cells were incubated with increasing concentrations of reference (dexamethasone) or test compounds. Tests were run in duplicate with a pEC_{50} value error of ±0.2.
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