Bioorganic & Medicinal Chemistry Letters 24 (2014) 1934–1940

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of a potent and dissociated non-steroidal glucocorticoid receptor agonist containing an alkyl carbinol pharmacophore

Hossein Razavi [a,*], Doris Riether [a], Christian Harcken [b], Jörg Bentzien [a], Roger M. Dinallo [a], Donald Souza [b], Richard M. Nelson [a], Alison Kukulka [a], Tazmeen N. Fadra-Khan [a], Edward J. Pack Jr. [a], Ljiljana Zuvela-Jelaska [b], Josephine Pelletier [b], Mark Panzenbeck [b], Carol A. Torcellini [b], John R. Proudfoot [a], Gerald H. Nabozny [b], David S. Thomson [a]

[a] Department of Medicinal Chemistry, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, PO Box 368, Ridgefield, CT 06877, USA
[b] Department of Immunology & Inflammation, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, PO Box 368, Ridgefield, CT 06877, USA

ARTICLE INFO

Article history:
Received 23 December 2013
Revised 27 February 2014
Accepted 3 March 2014
Available online 12 March 2014

Keywords:
Nuclear hormone receptors
Glucocorticoids
Non-steroidal
Dissociated
Agonist

ABSTRACT

Synthesis and structure–activity relationship (SAR) of a series of alkyl and cycloalkyl containing non-steroidal dissociated glucocorticoid receptor (GR) agonists is reported. This series of compounds was identified as part of an effort to replace the CF_3 group in a scaffold represented by **1a**. The study culminated in the identification of compound **14**, a *t*-butyl containing derivative, which has shown potent activity for GR, selectivity against the progesterone receptor (PR) and the mineralocorticoid receptor (MR), in vitro anti-inflammatory activity in an IL-6 transrepression assay, and dissociation in a MMTV transactivation counter-screen. In a collagen-induced arthritis mouse model, **14** displayed prednisolone-like efficacy, and lower impact on body fat and free fatty acids than prednisolone at an equivalent anti-inflammatory dose.

For well over a half of century, clinicians have relied on their staple arsenal of steroidal glucocorticoids (GCs) to combat inflammatory, immune and metabolic maladies.[1] However, the therapeutic uses of GCs in chronic diseases are associated with adverse effects such as glucose intolerance, muscle wasting, skin thinning, weight gain and osteoporosis that detract from their remarkable immuno-modulatory and anti-inflammatory activities.[2,3]

The glucocorticoid receptor (GR) is a member of the nuclear receptor superfamily and is comprised of multiple domains: an N-terminal transcription activation domain, a DNA binding domain that contains a dimerization interface, and a C-terminal ligand binding domain (LBD) that also contains a transcription activation domain. In its unbound state, the GR resides in the cytoplasm and is associated with, and stabilized by chaperones such as heat-shock proteins 70 and 90.[4] According to the proposed classical mechanism of GC signaling,[5] the binding of a GC agonist such as cortisol, the endogenous ligand in human, to the cytosolic receptor results in a cascade of events such as conformational changes, dissociation of the ligand bound receptor from chaperones, and translocation of the receptor–ligand complex into the nucleus. The ligand-activated GR functions as a monomer or a homo-dimer. Once inside the nucleus, the monomer binds to transcription factors such as NF-κB and AP-1, which prevents them from interacting with the DNA resulting in gene down-regulation. The glucocorticoid-mediated down-regulation of pro-inflammatory genes has been called transcriptional repression or 'trans-repression' (TR), and is thought to be the main mechanistic pathway for the anti-inflammatory activities of GCs.[6] Alternatively, the receptor–ligand homo-dimer binds to Glucocorticoid Response Elements (GREs) in the promoter region of genes that ultimately results in gene transcription. This glucocorticoid-mediated gene up-regulation, referred to as transcriptional activation or 'trans-activation' (TA), has been primarily associated with the aforementioned undesirable effects.[3]

The mechanism of GC action is intricate and still poorly understood. For instance, GC analogues with minor structural differences are reported to regulate differential gene expression in the same cell line.[7] The GR-mediated signaling is further complicated by factors such as post-transcriptional mechanisms and selective cofactor recruitment.[8] While the beneficial and the deleterious effects of GCs are generally considered to be due to on-target activities, their off-target cross-reactivity with other nuclear hormone receptors (NHR), such as the androgen receptor (AR), the mineralocorticoid receptor (MR), and the progesterone receptor (PR), also contributes to the observed side effects.[9]

* Corresponding author. Tel.: +1 (203) 778 7926; fax: +1 (203) 791 6072.
E-mail address: hossein.razavi@boehringer-ingelheim.com (H. Razavi).

http://dx.doi.org/10.1016/j.bmcl.2014.03.005
0960-894X/

Despite complexities, insights into the GC mechanism have rekindled hopes of finding an agent capable of eliciting the desired anti-inflammatory response while minimizing the unwanted side effects.[10] These discoveries have fueled the hypothesis that the two GC-mediated mechanistic pathways (i.e., TR and TA) could be segregated, thus separating the anti-inflammatory response from the unwanted side effects.[11] Since marketed GCs such as prednisolone and dexamethasone (Fig. 1) lack the desired NHR selectivity and are unable to differentiate between the two aforementioned mechanistic pathways, the search for a GR selective synthetic agent that is capable of dissociating the anti-inflammatory effects from the undesirable side effects has intensified.[12] Previous communications from our project group have described selective and dissociated GR agonists from a novel non-steroidal chemotype (e.g., **1a**), which is comprised of three main structural elements: an A-ring mimetic, a D-ring mimetic, and a four carbon linker bearing a hydroxyl and a trifluoromethyl group.[13] The trifluoromethyl carbinol group embedded in this linker is also featured in other GR agonists (e.g., **1b**, and **1c**).[14] The initial attempt to replace the CF_3 moiety in **1b** and **1c** with large non-fluorinated alkyl groups such as cyclohexylmethyl or benzyl provided moderate to good GR binders; however, these structural modifications resulted in the alteration of the functional activity from agonism to antagonism, thereby highlighting the critical role of this group as a function-regulating pharmacophore.[14] Herein, we report the continuation of our SAR efforts directed at the CF_3 group in the series depicted by **1a**.

As part of a strategy to prosecute the SAR in this scaffold, we examined the role of the CF_3 moiety on GR activity and strived to identify a suitable replacement for this group. We envisioned that an ideal replacement would retain the agonist activity that was displayed by its CF_3 counterpart. Moreover, it would serve as a function-regulating pharmacophore (vide supra) providing a handle to fine-tune the in vitro functional activity, which could translate into a desirable in vivo profile. Finally, the inclusion of such structural diversity might lead to an improvement in physicochemical properties, and mitigate any potential risks associated with unforeseen findings from this scaffold. Although the SAR based on **1b** and **1c** had indicated that the CF_3 group was requisite for agonist activity, we believed that these compounds contained sub-optimal A-ring mimetics and linkers. In fact, a recent report from Bayer Schering AG on this series has highlighted the deficiencies of the methylbenzoxazine group and the carboxamide linker (i.e., the A-ring mimetic and the linker depicted in compound **1c**), and has shown that their replacement resulted in improved GR selectivity, cellular activity and dissociation.[15] In this context, **1a** appeared to be a better starting point than **1b** and **1c**. Compound **1a** is a potent and selective GR ligand (Table 1). It is also a potent and efficacious inhibitor of IL-1-stimulated IL-6 production in human foreskin fibroblasts (HFF) cell lines, and has a dissociated profile in a TA reporter gene assay measuring induction at the MMTV promoter (10% maximal induction vs 100% for dexamethasone).[13b]

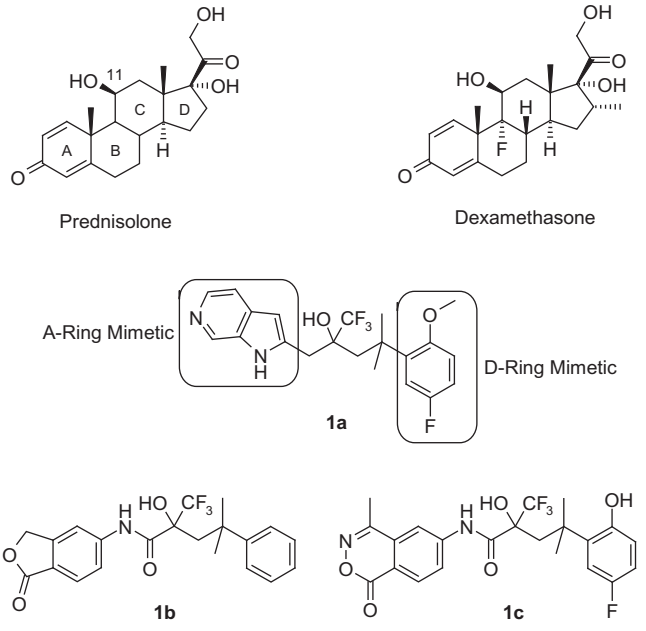

Figure 1. Examples of synthetic steroidal and non-steroidal glucocorticoids.

To rapidly access the alkyl derivatives in this series, we devised a synthesis whereby analogs could be generated from trifluoromethyl ketones. Syntheses of various CF_3 ketones have already been reported.[13b] According to the reaction sequence outlined in Scheme 1, CF_3 ketones were quantitatively converted to the corresponding carboxylic acids using the haloform reaction. The resultant acids were reacted with thionyl chloride in dichloromethane to afford the acid chlorides, which upon treatment with morpholine provided the corresponding amides in excellent yields. Next, morpholinyl amides in THF were reacted with various alkyl lithium reagents at −78 °C to provide the desired alkyl ketones in moderate to good yields. Finally, 2-methyl-azaindoles, prepared according to a previously reported method,[16] were sequentially treated with *n*-BuLi and *t*-BuOK at −78 °C. Reactions of the resultant carbanions with alkyl ketones furnished compounds **2**–**13** in low to moderate yields (Scheme 1).

Compounds **2**–**9** were initially evaluated for their affinity towards human GR, PR, and MR in a competitive binding fluorescence polarization assays using a tetramethyl rhodamine (TAMRA)-labeled dexamethasone probe for GR and MR, and a TAMRA-labeled mifepristone for PR.[17] The SAR indicated that analogs containing small alkyl groups (i.e., methyl and ethyl) were less potent GR binders than the CF_3 comparator (Table 1 cf. **2**, and **3** vs **1a**). These observed differences in potency were consistent with the reported effects of fluorine on increasing the affinity of a ligand towards its target and modulating its physicochemical properties.[18] Accordingly, we attributed the higher GR affinity of **1a** to the improved hydrophobic interactions between the lipophilic CF_3 group and the receptor, as well as the inductive effect of fluorine atoms that lowered the pK_a of the nearby hydroxyl group thereby enhancing its hydrogen bond donating ability (cf. calculated pK_a of OH group: **1a** = 12.1 vs **2** = 14.8).[19] The calculated pK_a values for the hydroxyl groups of the non-halogenated alkyl derivatives **2**–**9** were between 14.7 and 14.8. The minor pK_a differences among these analogs (e.g., calculated pK_a of OH group: **2** = 14.8; **4** = 14.7) suggested that the acidity of the OH group did not play a significant role in their observed binding potencies. In this series of compounds, the improvement in GR potency was correlated with the increase in the size of the alkyl moiety; however, the binding affinity plateaued beyond the isopropyl group indicating an upper limit for the relationship between size and potency (Table 1. cf. **2**, **3**, **4**, **5** and **6**). Compounds **4**–**6** also demonstrated good selectivity against PR and MR (i.e. >100 fold). In fact, the GR potency and NHR selectivity displayed by these compounds are comparable to those of the CF_3 analog (**1a**). Cycloalkyl derivatives **7** and **8** also showed potent GR activity, and >100 fold selectivity against PR and MR. However, increasing the ring size from cyclobutyl to cyclopentyl resulted in higher MR affinity and lower selectivity (Table 1 cf. **8** vs **9**).

Next, the panel was tested for their agonist activity in the IL-1-induced IL-6 production TR assay in HFF cell lines. Overall, all alkyl and cycloalkyl analogs were less potent and efficacious than **1a**. However, among the non-fluorinated alkyl groups, the