Steroids 95 (2015) 88–95

Contents lists available at ScienceDirect

Steroids

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

Discovery of a novel isoxazoline derivative of prednisolone endowed with a robust anti-inflammatory profile and suitable for topical pulmonary administration

E. Ghidini [a,*], A.M. Capelli [a], C. Carnini [c], V. Cenacchi [b], G. Marchini [c], A. Virdis [d], A. Italia [e], F. Facchinetti [c]

[a] Chemistry Research and Drug Design Department, Chiesi Farmaceutici S.p.A., Parma, Italy
[b] Pharmacokinetic Department, Chiesi Farmaceutici S.p.A., Parma, Italy
[c] Pharmacology and Toxicology Department, Chiesi Farmaceutici S.p.A., Parma, Italy
[d] Nikem Research Srl, Baranzate di Bollate (Mi), Italy
[e] Chiman Srl, via Reggio Calabria, 12 Rottofreno (PC), Italy

ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form 27 November 2014
Accepted 15 December 2014
Available online 31 December 2014

Keywords:
Corticosteroid
Isoxazoline
Ovalbumin
Nuclear translocation
PK profile
GILZ

ABSTRACT

A novel glucocorticoids series of (GCs), 6α,9α-di-Fluoro 3-substituted C-16,17-isoxazolines was designed, synthesised and their structure–activity relationship was evaluated with glucocorticoid receptor (GR) binding studies together with GR nuclear translocation cell-based assays. This strategy, coupled with *in silico* modelling analysis, allowed for the identification of Cpd **#15,** an isoxazoline showing a sub-nanomolar inhibitory potency (IC_{50} = 0.84 nM) against TNFα-evoked IL-8 release in primary human airways smooth muscle cells. In Raw264.7 mouse macrophages, Cpd **#15** inhibited LPS-induced NO release with a potency (IC_{50} = 6 nM) > 10-fold higher with respect to Dexamethasone. Upon intratracheal (i.t.) administration, Cpd **#15**, at 0.1 μmol/kg significantly inhibited and at 1 μmol/kg fully counteracted eosinophilic infiltration in a model of allergen-induced pulmonary inflammation in rats. Moreover, Cpd **#15** proved to be suitable for pulmonary topical administration given its sustained lung retention ($t_{1/2}$ = 6.5 h) and high pulmonary levels (>100-fold higher than plasma levels) upon intratracheal administration in rats. In summary, Cpd **#15** displays a pharmacokinetic and pharmacodynamic profile suitable for topical treatment of conditions associated with pulmonary inflammation such as asthma and COPD.

1. Introduction

An important limitation of GCs therapy is that the desired anti-inflammatory effects are accompanied by side effects such as loss of muscle mass, redistribution of body fat, osteoporosis, diabetes, glaucoma and depression [1]. In patients with asthma and chronic obstructive pulmonary disease (COPD), the adverse effects of GCs chronic use can be limited by topical pulmonary delivery via inhalation [2]. Nevertheless, a degree of systemic exposure inevitably occurs which may raise safety concerns in elderly patients as well as in patients requiring high dose regimen [3]. Hence, there is the need to enhance local anti-inflammatory potency of topical GCs while limiting their systemic exposure in order to minimize unwanted side effects.

A considerable amount of research is aimed at discovering novel steroidal GR agonists with high anti-inflammatory potency upon topical application and limited systemic exposure. Despite these efforts, only few novel steroidal molecules showing significant structural changes with respect to existing drugs have been developed [4,5]. The present study attempts to fill this gap by describing the design, synthesis and pharmacological profile of a novel series of 6α,9α-di-Fluoro 3-substituted isoxazolines. **Cpd #15,** in particular, proved to be a suitable compound for pulmonary topical administration given its robust anti-inflammatory potency, prolonged lung retention and low systemic exposure upon intratracheal administration.

2. Experimental section

2.1. Chemicals and reagents

All commercially available chemicals and solvents were purchased from Aldrich-Sigma (St. Louis, MO). Steroidal derivatives

Abbreviations: GCs, glucocorticoids; NO, nitric oxide; GILZ, glucocorticoid-induced leucine zipper; ASMCs, airway smooth muscle cells.

* Corresponding author. Tel.: +39 0521 279913; fax: +39 05212762 545567.
E-mail address: e.ghidini@chiesi.com (E. Ghidini).

http://dx.doi.org/10.1016/j.steroids.2014.12.016
0039-128X/

(compounds **#1–18)** were synthesized in our laboratory following the route described in Scheme 1. Starting from commercially available derivative **#21** and for **#22**, the reaction proceeded in ethyl acetate and $NaHCO_3$, together with a few drops of water, by stirring at room temperature for six days (Scheme 1; A). When derivatives Cpd **#21** were prepared by *in situ* chlorination of the corresponding aldoximes with $BTMAICl_4$ (benzyltrimethylammonium tetrachloroiodate) [6] or bleach, the reaction proceeded in dry dichloromethane (DCM) and triethylamine (TEA) at room temperature for 3 h (Scheme 1; B). All reactions details are reported in the Supporting Information. The structures of these compounds are shown in Table 1 and the steroidal drugs are:

(16S,17R)-3′-(4-chlorophenyl)-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#1**; (16S,17R)-3′-(4-methoxyphenyl)-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#4**; (16S,17R)-3′-methylacetate-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#5**; (16S,17R)-3′-propyl-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#6**; (16S,17R)-3′-methyl-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#7**; (16S,17R)-3′-(hydroxymethyl)-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#8**; (16S,17R)-3′-hydroxy-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#10**; (16S,17R)-3′-(thiophen-3-yl)-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#11**; (16S,17R)-3′-(furan-3-yl)-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#12**; (16S,17R)-3′-(thiophen-3-yl)-6,9-difluoro-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#13**; (16S,17R)-3′-(furan-3-yl)-6,9-difluoro-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#14**; (16S,17R)-3′-bromo-6,9-difluoro-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#15**; (16S,17R)-3′-methyl-6,9-difluoro-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#16**; (16S,17R)-3′-(4-methoxyphenyl)-6,9-difluoro-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#17**; (16S,17R)-3′-phenyl-6,9-difluoro-11β,21-dihydroxy-4′H-pregna-1,4-dieno[16,17-d]isoxazole-3,20-dione, Cpd **#18**.

Preparation of Cpd **#2,** Cpd **#3** and Cpd **#9** was already described in literature. [7]

The purity of tested compounds determined by analytical UPLC was >98%. The standards Dexamethasone (Chart 1) is commercially available and was purchased from Acros Organics while Deflazacort active metabolite (Chart 1) was synthesized following a literature method [8].

Table 1
Compounds series.

Compound	R	X, Y
#1	p-Cl-Phenyl,	H, H
#2	COOEt	H, H
#3	COOH	H, H
#4	p-OMe-Phenyl	H, H
#5	CH_2OCOCH_3	H, H
#6	Propyl	H, H
#7	Methyl	H, H
#8	CH_2OH	H, H
#9	Br	H, H
#10	OH	H, H
#11	3-Thienyl	H, H
#12	3-Furyl	H, H
#13	3-Thienyl	F, F
#14	3-Furyl	F, F
#15	Br	F, F
#16	Methyl	F, F
#17	p-OMe-Phenyl	F, F
#18	Phenyl	F, F

2.2. Biological assay

2.2.1. Cell culture

Murine macrophagic cell line (RAW264.7) was purchased from ATTC (Manassas, USA) and cultured in RPMI 1640 medium (w/o Phenol Red) supplemented with 10% FBS, 2 mM glutamine, 100 U penicillin and 100 μg/ml streptomycin (Invitrogen), in an atmosphere of 5% CO_2 at 37 °C.

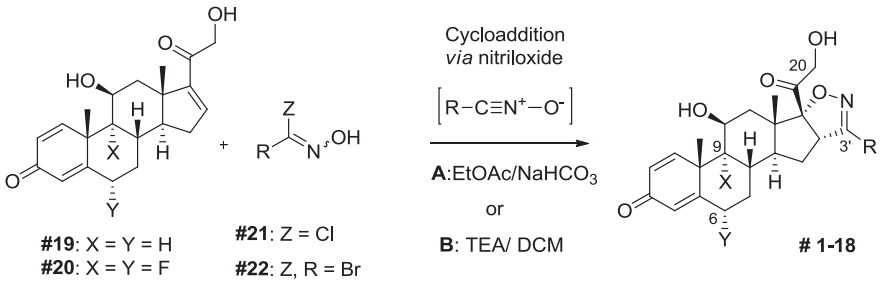
PathHunter™ CHO-K1 GR and MR Cell Line stably expressing EA-NLS-NRS and the ProLabel-tagged glucocorticoid and mineralcorticoid receptor respectively were purchased from DiscoverX (CA, United States). Cells were cultured in F-12 Nutrient Mixture (HAM) supplemented with 10% Fetal Bovine Serum (Invitrogen) plus 2 mM L-glutamine and antibiotics (100 U/ml Penicillin, 100 g/ml Streptomycin, 300 g/ml Hygromycin B, and 500 g/ml G418/Geneticin) in an atmosphere of 5% CO_2 at 37 °C.

Primary human airway smooth muscle cells (ASMCs) were purchased from LONZA (Basel, CH) and cultured in DMEM medium supplemented with 10% Fetal Bovine Serum, 2 mM glutamine, 100 U penicillin and 100 μg/ml streptomycin (Invitrogen), in an atmosphere of 5% CO_2 at 37 °C.

2.2.2. Nitric measurement assay protocol

RAW264.7 cells were seeded in 0.3 ml RPMI (w/o Phenol Red) containing 10% FBS in 48-well tissue culture plates at the density of 7.5×10^4 cells/well and grown for 24 h at 37 °C with 5% CO_2. Then cells were treated with different concentration of corticosteroids (10^{-11}M-10^{-6} M, final DMSO concentration 0.1%) for 15 min. before stimulation with lipopolysaccharide from *Escherichia coli* (100 ng/ml as final concentration) and incubated for 18 h in RPMI (w/o Phenol Red) supplemented with 10% FBS.

Accumulation of nitrite in the medium was measured by a colorimetric assay method based on the Griess reaction. Briefly,



Scheme 1. Compounds **1–18** were synthesized starting from enone, #19 or #20 and hydroximoyl chlorides derivatives **#21** or hydroxycarbonimidic dibromide **#22** via 1,3-dipolar cycloaddition of nitrile oxides.