Bioorganic & Medicinal Chemistry Letters 30 (2020) 127402

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Topical 'dual-soft' glucocorticoid receptor agonist for dermatology

Kevin N. Dack[a,*], Patrick S. Johnson[a], Krister Henriksson[a], Stefan Eirefelt[a], Martin A. Carnerup[a], Martin Stahlhut[a], Anna K. Ollerstam[b]

[a] LEO Pharma A/S, Industriparken 55, 2750 Ballerup, Denmark
[b] AstraZeneca, Laboratory Animal Sciences, Drug Safety and Metabolism, IMED Biotech Unit, Pepparedsleden 1, Mölndal, Sweden

ARTICLE INFO

Keywords:
Dermatology
Non-steroidal
SEGRA
Soft-drug
Glucocorticoid receptor agonist

ABSTRACT

Steroidal glucocorticoids (GR agonists) have been widely used for the topical treatment of skin disorders, including atopic dermatitis. They are a very effective therapy, but they are associated with both unwanted local effects in the skin (skin thinning/atrophy) and systemic side effects. These effects can limit the long-term utility of potent steroids.

Here we report on a topically delivered non-steroidal GR agonist, that has the potential to deliver high efficacy in the skin, but due to rapid metabolism in the blood & liver ("dual-soft") it should have greater systemic safety than existing treatments. In addition, compared to less selective steroidal GR agonists, the new non-steroidal Selective Glucocorticoid Agonists (SEGRAs) have the potential to avoid the skin atrophy observed with existing topical steroids.

Due to its potential for reduced skin atrophy and low systemic exposure, LEO 134310 (**17**) may be suitable for long term topical treatment of skin diseases such as atopic dermatitis and psoriasis.

For more than 70 years steroidal glucocorticoids (GR agonists) have been widely used for the topical treatment of skin disorders, including atopic dermatitis.[1] They are a very effective therapy but are associated with both unwanted local effects in the skin (skin thinning/atrophy) and systemic side effects. These effects can limit the long-term utility of potent steroids.[2]

The glucocorticoid receptor (GR) is a transcription factor belonging to the nuclear hormone receptor family. Ligand activation triggers both positive and negative regulation of gene transcription and subsequently pleiotropic effects on metabolism and the immune system. Glucocorticoids are widely prescribed for the treatment of inflammatory and autoimmune conditions and are highly effective, but their therapeutic use can be limited due to adverse effects following chronic administration.[2]

Numerous attempts to dissociate anti-inflammatory effects from systemic metabolic side effects, using new non-steroidal Selective Glucocorticoid Agonists (SEGRAs), have been described3 wherein the rigid steroidal core is replaced by alternative non-steroidal scaffolds. An alternative scaffold of note is the suitably substituted indazole system,[4] wherein the *N2* position of the indazole ring system has been shown, by X-ray crystallography,[5,7] to be a mimic of the A-ring ketone of steroidal agonists, which can form hydrogen bonds to Gln570 within the GR binding site.

Substitution on the *N1*-aryl can access a region in the GR that is often referred to as the 'meta-channel',[6] that has proven beneficial for obtaining greater GR selectivity over related steroid receptors. A good example of this is **AZD7594**, (Fig. 1) an inhaled development candidate.[7]

In contrast to the small inhaled doses, a topically applied product must avoid photosensitivity and/or phototoxicity, especially when applied to sun exposed skin on face, hands and limbs. The ICH guidelines[8] require further testing if the compound absorbs in the range > 290 nm and most *N1*-aryl-indazoles have strong UV absorbance in that range and may require further de-risking of parent and metabolites *in vitro* and in *in vivo*.

Therefore, we initially designed and investigated isosteric novel templates based around pyridyl and benzoyl amides, which could use the carbonyl oxygen as an isostere for the indazole N-2 position. These isosteres have a smaller chromophore, than an indazole, and consequently a lowered risk of phototoxicity. We theorized that maintaining coplanarity between the carbonyl and the aryl would give the closest mimetic of the indazole. In particular, the pyridyl amide is expected to be almost planar due to the attraction between the amide NH and pyridyl N, whereas, the benzamide, due to steric clash between the amide N—H and ortho Ar-H of the benzoic acid core, is significantly twisted out of plane. (Fig. 2).

* Corresponding author.
E-mail address: kevin.dack@leo-pharma.com (K.N. Dack).

https://doi.org/10.1016/j.bmcl.2020.127402
Received 12 May 2020; Received in revised form 6 July 2020; Accepted 7 July 2020
Available online 13 July 2020
0960-894X/

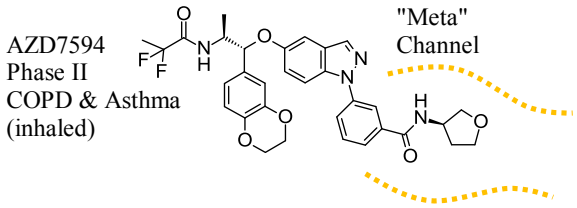


Fig. 1. AZD7594.

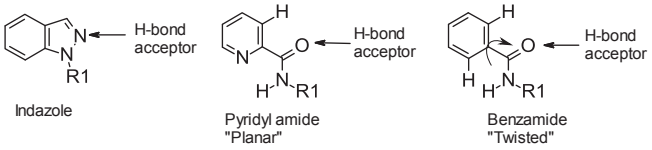
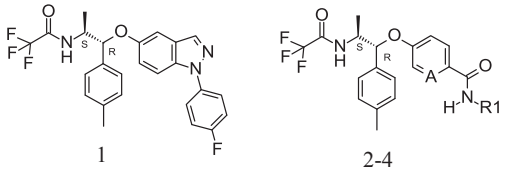


Fig. 2. Indazole isosteres.

Table 1
Indole Isosteres.



Entry	A	R^1	hPBMC[a] IC_{50} (nM)	Comment
1			2	Indazole
2	CH	Phenyl	~5000	Partial agonist
3	N	Phenyl	26	Full agonist
4	N	Cyclohexyl	9	Full agonist

[a] Inhibition of LPS-induced TNF-α release in peripheral blood mononuclear cells (PBMCs) – geometric mean of minimum of 2 independent experiments.

A small range of initial analogues (**2–4**, Table 1) were made to explore the potential for achieving agonist activity in a hPBMC assay[9] stimulated with LPS to secrete TNFα. The benzamide **2** was a weak, partial agonist, but the more planar pyridyl amide **3** gave full agonism and was only 10-fold less potent than an indazole comparator (**1**). Saturating the pendant phenyl to a cyclohexyl **4** improves the potency further and removes a potential aniline toxicophoric structural alert.

The pyridyl amides were further explored by making a larger range of amides (data not shown) to look for vectors to access the 'meta-channel'. Amongst the numerous analogues prepared, one of the most promising was the 3-(*N1*-Boc) piperidine amide **5** (Fig. 3), with a full-agonist IC_{50} of 8 nM

We next turned our attention to introducing a metabolically soft spot. The concept of 'soft drugs' design[10] is to rapidly convert an active parent molecule by metabolism to a less active derivative to reduce systemic effects. In a recent publication,[11] LEO scientists described 'dual-soft' PDE4 inhibitors that can act locally when applied topically and are then metabolized extra-hepatically in the blood as the

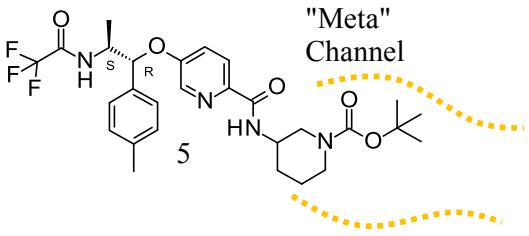
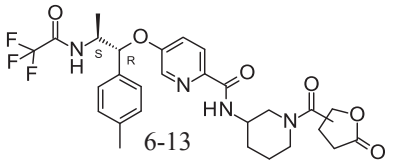


Fig. 3. Compound **5.**

Table 2
Optimisation of piperidine and lactone moieties for potency and blood instability.



Entry	X[a]	Lactone	hPBMC[b] IC_{50} (nM)	Blood[c] T½ (mins.)	KC[d] T½ (mins)
6	*S*	*5-R*	7	> 120	> 360
7	*R*	*5-R*	90	51	nd
8	*S*	*5-S*	73	83	nd
9	*R*	*5-S*	107	44	nd
10	***S***	***4-R***	**7**	< **10**	> 360
11	*R*	*4-R*	21	< 10	> 360
12	*S*	*4-S*	8	102	> 360
13	*R*	*4-S*	34	17	nd

[a] X = Piperidine enantiomer.
[b] Inhibition of LPS-induced TNF-α release in peripheral blood mononuclear cells (PBMCs) – geometric mean of minimum of 2 independent experiments.
[c] Stability half-life in human whole blood.
[d] Stability half-life in cultured human keratinocytes.

molecules enter the systemic circulation, thus limiting the systemic adverse effects. Crucial to this concept is having assays to determine both the stability in the skin (good keratinocyte stability) and rapid metabolism in whole blood to less active metabolites.

A series of compounds were prepared in which the Boc-protecting group of **5** was replaced by a set of esters and lactones to explore the balance of GR agonist potency and 'soft drug' potential. It was challenging to find esters that could achieve a suitable balance of potency with both rapid hydrolysis by esterases in blood and stability in keratinocytes, however, the lactones **6–13** (Table 2) provided some examples that satisfied these criteria. The *(S)*-piperidine enantiomers (**6**, **10** and **12**) had full agonist PBMC IC_{50} < 10 nM and were more potent GR agonists than their *R*- enantiomers. In addition, the *(4R)*-lactones (**10** & **11**) were shown to be very unstable in human whole blood and rapidly hydrolyzed to the open hydroxy acids in < 10 min but were also sufficiently stable in human keratinocytes (half-life > 360 min). The lactone hydrolysis was mainly paraoxonase (PON) mediated, as the addition of EDTA stabilized[12] the lactones in assays. Compound **10** (**LEO 131928A**) had an encouraging, balanced profile and was investigated further.

LEO 131928 (**10**) was rapidly metabolized (Fig. 4) in blood and hepatocytes to give the hydroxy acid **14** as the major metabolite. This was consistent across all major species (human, dog, pig, rat and mouse). The acid **14** was > 100-fold less active as a GR agonist in human PBMCs and therefore **LEO 131928** fits the profile of a 'dual-soft' molecule.

In addition, **10** was sufficiently stable towards chemical hydrolysis at pH 5.5 (> 95% remaining after 16 h at 37 °C) but slowly hydrolyzed at pH 7 (82% remaining after 16 h at 37 °C). The photostability was excellent and **10** was (as predicted) also non-phototoxic in 3 T3 cells (NRU Assay)[13] with viability IC_{50} > 125 μg/mL in both the presence and absence of UV light.

With an optimized meta-channel moiety with favorable dual-soft

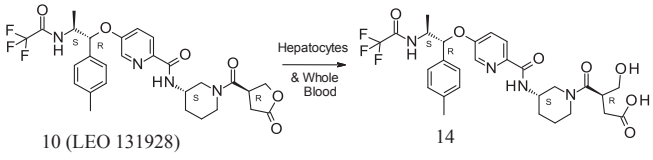
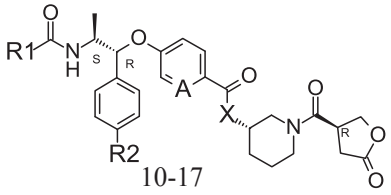


Fig. 4. Whole blood and hepatic metabolism of cmpd. **10.**

Table 3
Selected SAR.



Entry	R^1	R^2	A/X	hPBMC[a] IC_{50} (nM)
10	CF_3	Me	N /NH	7
15	CF_3	Me	CH / O	2
16	CF_3	Me	CH / NH	79
17	***2R*-THF**	**cPr**	**CH / O**	**2**
18	*2S*-THF	cPr	CH / O	22
19	CF_3	cPr	CH / O	1

[a] Inhibition of LPS-induced TNF-α release in peripheral blood mononuclear cells (PBMCs) – geometric mean of minimum of 2 independent experiments.

properties in hand, we then reconsidered alternatives to the pyridyl amide as an indazole isostere and investigated benzoate esters as they should also maintain coplanarity between the phenyl and carbonyl (Table 3). The pyridyl esters analogues had insufficient chemical resistance to aqueous hydrolysis and were not pursued further, however, the benzoate analogue of **10** (i.e. **15**) had increase GR agonist potency and all other properties were similar to **10**.

As previously noted with comparison of **2** and **3**, the benzamide analogue (**16**) was 10-fold weaker than **10** and supported the hypothesis of attaining coplanarity between the aryl and the amide carbonyl.

To select a development candidate, we investigated a larger range of analogues in both the pyridyl amides and benzoate esters, in which the amide R^1 and the aryl substituent R^2 were varied to get the optimal balance of properties such as potency, solubility and stability.[14] From these analogues, compound **17** was selected as a development candidate for further *in vitro* and *in vivo* profiling.

LEO 134310 (**17**) has low nM potency in the TNFα stimulated PBMC assay, comparable to dexamethasone, and like **10** and most analogues containing the *(4R)*-lactone moiety, it is rapidly hydrolyzed to the less active (hPBMC IC_{50} = 260 nM) hydroxy acid **20** in whole blood and hepatocytes of human and all main laboratory mammals (Fig. 5). Importantly, the stability in skin keratinocytes was very good (half-life > 6 h). Due to the extrahepatic metabolism via blood and other tissues, the *in vivo* PK in rat and dog after intravenous dosing gave systemic clearance greatly in excess of liver blood flow (500 mL/min/kg in rat).

The *in vitro* and *in vivo* safety and efficacy was investigated in greater detail and is covered in greater depth in an *article in press*.[15] Briefly, **LEO 134310** was > 100-fold selective for GR over the related AR, MR and PR (data not shown) and clean when assessed in all off-target assays, hERG, Cyp and DDI assays and genetic toxicology testing. It also demonstrated efficacy after topical application in a mouse TPA induced ear inflammation model,[16] with a greatly increased therapeutic index over betamethasone valerate with regards to efficacy relative to systemic adverse effects. In addition, **LEO134310** demonstrated target engagement and biomarker changes equivalent to betamethasone valerate when applied to human skin *in vitro* and had significantly less

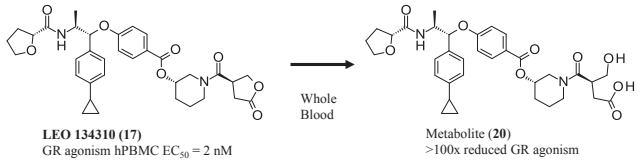


Fig. 5. Whole blood metabolism of cmpd. **17**.

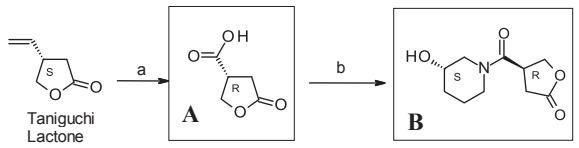


Fig. 6. Route to key chiral building blocks **A** and **B**: **Steps: a.** $RuCl_3$, $NaIO_4$, CCl_4, MeCN, H_2O, RT (70%) **b.** HATU, DIPEA, CH_2Cl_2, RT, 3-(S)-hydroxypiperidine 3 h (90%).

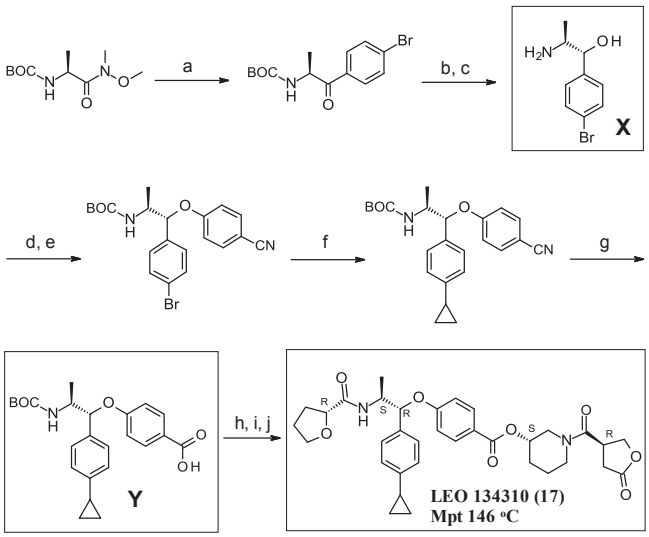


Fig. 7. Discovery route to **LEO 134,310** (**17**): **Steps: a.** 4-Bromo-phenylmagnesium bromide, THF, 0 °C-RT, 16 h (43%) **b.** $Al(O^iPr)_3$, IPA, 60 °C, 16 h (97%) **c.** TFA, CH_2Cl_2, 0 °C-RT, 4 h (72%) **d.** 4-fluoro-benzonitrile, NaH, NMP, 60 °C, 1 h (81%) **e.** BOC_2O, DMAP, THF, RT, 2 h (72%) **f.** ^{c}Propyl boronic acid, $(^c\text{Hexyl})_3P$, $Pd(OAc)_2$, Toluene/water (10:1), 120 °C (96%) **g.** 32% aq. NaOH, EtOH, 65 °C, 72 h (92%) **h.** EDC, DMAP, CH_2Cl_2, **B**, RT, 18 h (94%) **i.** 4 M HCl in dioxane, RT, 1 h (100%) **j.** HATU, DIPEA, DMF, (R)-Furoic acid (71%).

skin atrophy compared to betamethasone valerate when applied for 28 days to minipig skin *in vivo*.[15]

The Medicinal Chemistry route[14] to **LEO 134310** (**17**) is illustrative of the synthesis towards most of the analogues discussed herein.

The key 'dual-soft' lactone acid **A** is prepared by oxidative cleavage of the Taniguchi lactone[17] which is then coupled to 3-(S)-hydroxypiperidine to give the lactone alcohol fragment **B** (Fig. 6).

The central beta amino alcohol **X** (Fig. 7) is prepared by disclosed methods[14] starting from an aryl-Grignard addition to the Weinreb amide of Boc *(S)*-alanine and subsequent chelation controlled Meerwein-Ponndorf-Verley stereoselective reduction[18] of the ketone followed by deprotection. Arylation of the amino alcohol **X** is conducted using sodium hydride in *N*-methyl pyrrolidinone to control O-arylation over *N*-arylation, reprotection and palladium catalyzed cross-coupling, with cyclopropyl-boronic acid, followed by nitrile hydrolysis gives the benzoic acid building block **Y**.

The benzoate ester linkage is formed between benzoic acid **Y** and alcohol **B** using EDC and DMAP and finally, the Boc-protection is replaced with the *(R)*-Furoic amide to give **LEO 134310** (**17**). Further Process Chemistry improvements to the synthesis will be disclosed in a future publication.

In conclusion, we have applied the concept of 'dual-soft' drug design towards identifying **LEO 134310** (**17**) as a potent and selective GR agonist that shows target engagement and relevant GR mediated biomarker changes in mouse and pig after topical application. The potential for the unwanted systemic side-effects of topical glucocorticoids appears to be reduced preclinically by the rapid 'deactivation' in blood to the hydroxy acid metabolite, with > 100-fold less GR potency. **LEO**

134310 was taken to Phase I studies[19] to ascertain the safety, toleration and efficacy – results will be disclosed in due course.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank the numerous LEO Pharma colleagues involved in the discovery and early development of **LEO 134310**.

Appendix A. Supplementary data

The synthesis of **17** and all intermediates used in its preparation are described along with the protocols for the PBMC functional assay, and the whole blood and keratinocyte stability assays. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127402.

References

1. Ahluwalia A. *Mediators Inflamm.* 1998;7(3):183–193. https://doi.org/10.1080/09629359891126.
2. Hengge UR, et al. *J Am Acad Dermatol.* 2006;54(1):1–15. https://doi.org/10.1016/j.jaad.2005.01.010.
3. Sundahl N. *Pharmacol Therapeutics.* 2015;152:28–41. https://doi.org/10.1016/j.pharmthera.2015.05.001.
4. Hemmerling M. *Bio & Med Chem Letts.* 2016;26:5741–5748. https://doi.org/10.1016/j.bmcl.2016.10.052.
5. Biggadike K, et al. *J Med Chem.* 2008;51:3349–3352. https://doi.org/10.1021/jm800279t.
6. Biggadike K, et al. *Bioorg Med Chem Lett.* 2011;21:1126–1133. https://doi.org/10.1016/j.bmcl.2010.12.121.
7. Hemmerling M, et al. *J Med Chem.* 2017;60:8591–8605. https://doi.org/10.1021/acs.jmedchem.7b01215.
8. Photosafety evaluation of pharmaceuticals S10, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/s10-photosafety-evaluation-pharmaceuticals.
9. It was possible to distinguish partial and full agonists in the TNFα release assay indicating that both transactivation and transrepression mechanisms contributed to the cellular response. Unless noted, compounds mostly behaved as full agonists. Reference SEGRA compounds (and hydrocortisone) known to mediate reduced GR transactivation behaved as partial agonists in this assay. However, the mechanistic details leading to their partial effects were not addressed. Experimental protocol is included in the Supplementary Notes.
10. (a) Bodor N. *Med Res Rev.* 1984;4(4):449–469. https://doi.org/10.1002/med.2610040402;

    (b) Aprile S, et al. *Drug Discovery Today.* 2019;24:2234–2246. https://doi.org/10.1016/j.drudis.2019.08.007.
11. Larsen J, Lambert M, Pettersson H, Vifian T, Larsen M, Ollerstam A, Hegardt P, Eskilsson C, Laursen S, Skak-Nielsen T, Hansen LM, Knudsen NØ, Stilou TG, Nielsen SF. Discovery of LEO 39652 a Novel "Dual-soft" PDE4 Inhibitor for Topical Treatment of Atopic Dermatitis, article in press.
12. (a) Mackness MI. *Biochem Biophys Res Commun.* 1998;242. https://doi.org/10.1006/bbrc.1997.7872;

    (b) Draganov DI, et al. *J Lipid Res.* 2005;46:1239–1247. https://doi.org/10.1194/jlr.M400511-JLR200.
13. Kim K. *Toxicol. Res.* 2015;31:97–104. https://doi.org/10.5487/TR.2015.31.2.097.
14. Johnson PS, Dack KN, Henriksson K. Preparation of aminoalkoxypyridinecarbonyl-amino- piperidine carboxyate derivatives and analogs for use as non-steroidal glucocorticoid receptor modulators. WO 2017046096 A1.
15. Eirefelt S, Stahlhut M, Svitacheva N, Ewald DA, Marstrand TT, Ollerstam A, Norsgaard H. Non-clinical evaluation of a novel non-steroidal glucocorticoid agonist with low systemic exposure and reduced potential for skin atrophy, article in press.
16. Stanley PL, Steiner S, Havens M, Tramposch KM. *Skin Pharmacol.* 1991;4(4):262–271. https://doi.org/10.1159/000210960.
17. Kieseritzky V, et al. *OPRD.* 2014;18(5):643–645. https://doi.org/10.1021/op500096j.
18. Yin J, et al. *J Org Chem.* 2006;71(2):840–843. https://doi.org/10.1021/jo052121t.
19. Clinical Trial to Assess Safety, Tolerability and the Pharmacodynamic Effect of Different Concentrations of a New Anti-inflammatory Substance in Subjects with Chronic Plaque Psoriasis (NCT03669757).