Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 5213–5224
Bioorganic & Medicinal Chemistry

Synthesis and biological properties of novel glucocorticoid androstene C-17 furoate esters

David A. Sandham,[a,*] Lucy Barker,[a] David Beattie,[a] David Beer,[a] Louise Bidlake,[a] David Bentley,[a] Keith D. Butler,[a] Sarah Craig,[a] David Farr,[a] Claire Ffoulkes-Jones,[a] John R. Fozard,[b] Sandra Haberthuer,[a] Colin Howes,[a] Deborah Hynx,[a] Sarah Jeffers,[a] Thomas H. Keller,[a] Paul A. Kirkham,[a] Janet C. Maas,[a] Lazzaro Mazzoni,[b] Andrew Nicholls,[a] Gaynor E. Pilgrim,[a] Elisabeth Schaebulin,[b] Gillian M. Spooner,[a] Rowan Stringer,[a] Pamela Tranter,[a] Katharine L. Turner,[a] Morris F. Tweed,[a] Christoph Walker,[a] Simon J. Watson[a] and Bernard M. Cuenoud[b,*]

[a]*Novartis Institutes for Biomedical Research, Horsham Research Centre, Wimblehurst Road, Horsham, West Sussex, RH12 5AB, United Kingdom*
[b]*Novartis Institutes for Biomedical Research, CH-4002 Basel, Switzerland*

Received 21 May 2004; accepted 22 June 2004
Available online 4 August 2004

Abstract—A series of novel corticosteroid derivatives featuring C-17 furoate ester functionality have been synthesised. Profiling in vitro and in vivo has resulted in the identification of a compound with a longer duration of action and a lower oral side effect profile in rodents compared to budesonide.

1. Introduction

Corticosteroids are clinically proven as the most effective treatment for all levels of severity of asthma. This utility resides in their gene transcriptional regulation effects on multiple biological mediators.[1] However, long-term systemic exposure to corticosteroids results in well-documented side effects such as childhood growth retardation, hypertension, osteoperosis, skin thinning, diabetes, weight gain, cataracts and adrenocortical insufficiency.[2] This side effect issue has been addressed by local delivery of corticosteroids with a high topical to systemic potency ratio, culminating in the development of low-dose inhaled corticosteroids (ICS). ICS, particularly those with potential for once daily dosing, constitute the gold standard in mild to moderate asthma treatment, either as monotherapy or more recently in combination with a long-acting β-2 agonist bronchodilator.[3]

Most of the ICS currently approved for asthma were originally developed as topical steroids for dermatological indications and subsequently adapted for inhalation.[4] Dependent on the nature of the delivery device and the patient's inhalation technique, up to 80% of the dose of ICS may be swallowed and hence be available for systemic absorption from the gastro-intestinal (GI) tract, as illustrated in Figure 1. Earlier generation ICS, such as budesonide **1** (Fig. 2) show significant oral bioavailability,[5] allowing potential for systemic side effects by this route in addition to those arising from direct pulmonary absorption. Subsequently, fluticasone **2** has been shown to undergo efficient inactivation via hepatic metabolism,[6] leading to negligible oral bioavailability. More recently, elegant pre-clinical studies by Biggadike and colleagues demonstrated the potential for plasma inactivation of compounds such as **3**, with a further reduction in side effect potential.[7] Conversely, the example of mometasone furoate **4**[8] indicates that it

Keywords: Glucocorticoids; Asthma; Duration of action; Side effects.
* Corresponding authors. Tel.: +44-1403-323827; fax: +44-1403-323837 (D.A.S.); fax: +41-61-3243991 (B.M.C.); e-mail addresses: david.sandham@pharma.novartis.com; bernard.cuenoud@pharma.novartis.com

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.06.027

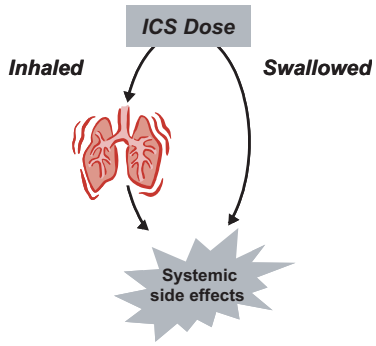


Figure 1. Origin of systemic effects in inhaled corticosteroids.

may be possible for an ICS to deliver very low systemic side effects without recourse to a priori soft drug design.[9] The pro-drug ciclesonide **5** is another ICS in development, which delivers low systemic side effects, possibly as a consequence of slow release due to high protein binding of the active metabolite in vivo.[10]

We sought to identify novel glucocorticoids with a long duration of action, suitable for once daily dosing and having low systemic side effects. A screening paradigm was devised to identify rapidly an appropriate pre-clinical in vivo profile for inhaled delivery, namely elimination of side effects upon oral administration, minimisation of side effects on inhaled administration and prolonged duration of action in a rodent asthma model. The starting point for this work was the 6α-fluoro 9α-chloro androstadiene C-17 propionate ester **6**, originally identified some years ago by Ciba-Geigy as a corticosteroid for potential dermatological indications with some separation of topical and systemic effects.[11] Following the example of mometasone, we sought to optimise **6** further by incorporating a range of substituted furoate esters at the C-17 position, as well as varying the C-21 methyl ester moiety.

Herein we disclose the synthesis and in vitro biological profiles of this novel class of molecules, together with the in vivo profiles of selected examples as potential novel inhaled corticosteroids for the treatment of asthma.

2. Compound synthesis

C-21 methyl ester derivatives were prepared according to Scheme 1. Starting from epoxyparamethasone **7**, oxidative cleavage according to literature conditions provided the acid **8** as a key building block.[12] Facile acylation of the hindered C-17 hydroxyl group was achieved via the intermediacy of a mixed anhydride,[12] either by treatment with an acid chloride in pyridine (Method A) or by coupling with the corresponding carboxylic acid in the presence of HATU (Method B) to give **9**. The synthesis was completed by esterification to furnish **10**, followed by acidic epoxide opening to deliver the target compounds **11a**–**n**, as indicated in Table 1. The first two steps of this process were amenable to parallel synthesis, with purification typically only required for the final products. Compound **11a** was also hydrogenated in the presence of Wilkinson's catalyst to provide 1,2-dihydro analogue **12**. The chlorohydrin acid **14** was prepared by treatment of intermediate **8** with HCl gas, to give chlorohydrin **13**, which was then selectively acylated at C-17, as shown in Scheme 2.

As an analogue of fluticasone propionate, C-21 fluorothioester **19** was prepared according to Scheme 3. Chlorohydrin **13** could be readily converted to thioacid **15** and the C-17 furoate ester was installed analogously to Scheme 1, to afford **16**. Finally, the fluoromethyl moiety in **19** was installed in a three-step sequence, via chloroester **17** and subsequent conversion to iodoester **18**.

3. Biological profiling

In vitro anti-inflammatory glucocorticoid agonist effects were assessed using an ELISA assay measuring the inhibition of the lipopolysaccaride (LPS) induced release of the inflammatory cytokine TNFα in a human U937

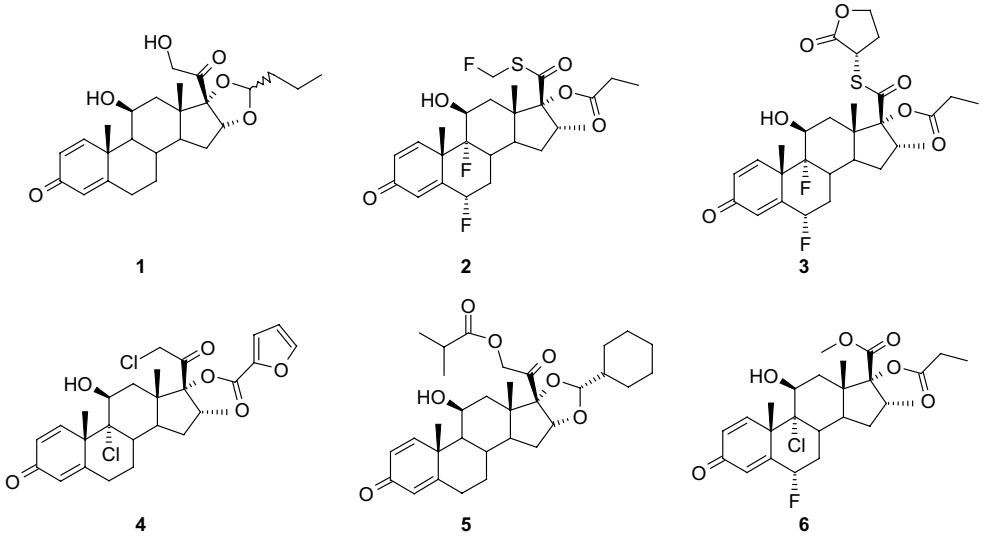
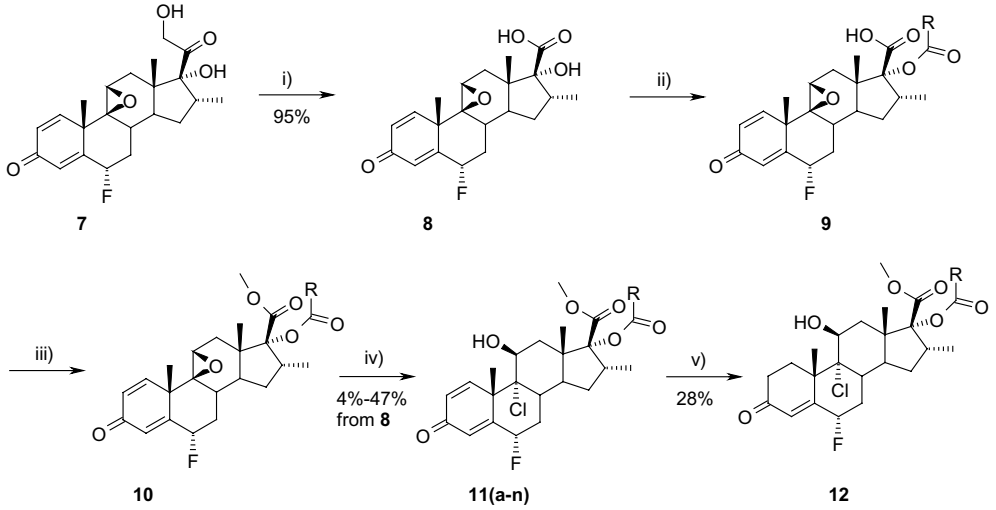


Figure 2. Selected corticosteroids.

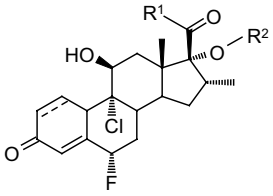


Scheme 1. Reagents and conditions: (i) H_5IO_6, THF–water, rt; (ii) Method A: RCOCl, pyridine 0 °C to rt or Method B: RCO_2H, HATU, DIPEA, DMF, rt; (iii) Me_2SO_4, DBU, EtOAc–DMF, rt; (iv) HCl gas, 1,4-dioxane, rt; (v) $(PPh_3)_3RhCl$, H_2 (3.5 bar), THF, rt.

macrophage-like cell-line.[13] This is considered as a functional measure of the transcriptional repression activity of the steroid, by which the majority of the beneficial therapeutic effects are believed to occur.[14] The relative potencies of compounds listed in Table 1 are expressed as the ratio of the IC_{50} value of the test compound relative to that of budesonide as an internal standard.

As an additional in vitro assay, glucocorticoid receptor binding affinity was determined for selected compounds,

Table 1. Cellular in vitro activity of novel steroids[a]



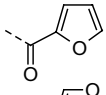
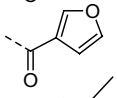
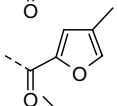
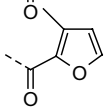
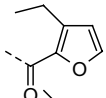
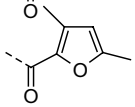
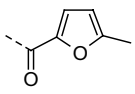
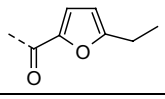
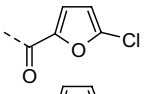
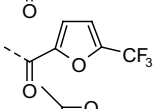
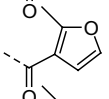
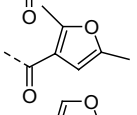
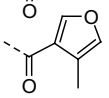
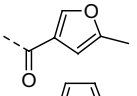
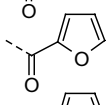
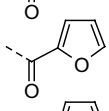
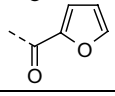
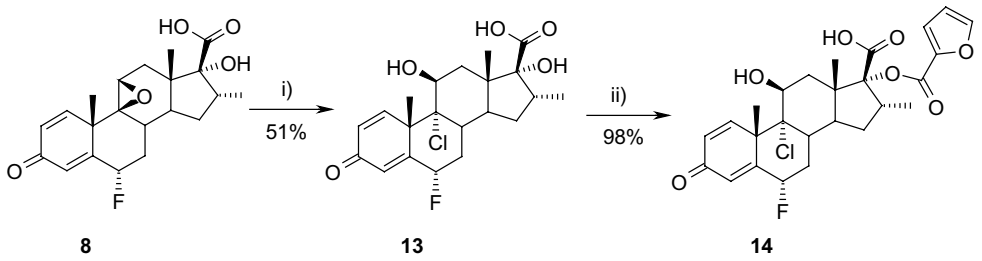
No.	R^1	R^2	TNF_α potency relative to **1**[b]
6	OCH_3		1.4
11a	OCH_3		4.6
11b	OCH_3		4.6
11c	OCH_3		5.1
11d	OCH_3		5.4
11e	OCH_3		4.1
11f	OCH_3		2.2
11g	OCH_3		0.5
11h	OCH_3		1.0

Table 1 (*continued*)

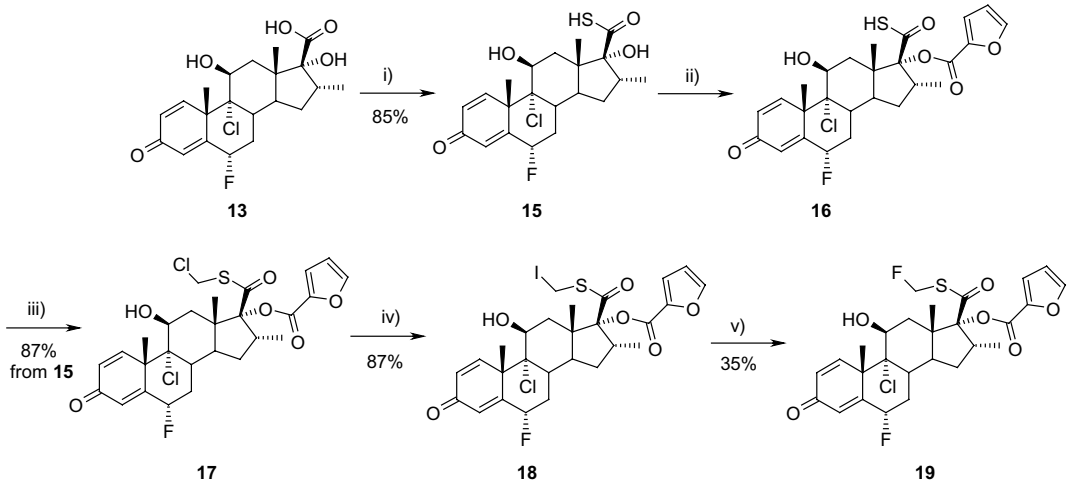
No.	R^1	R^2	TNF_α potency relative to **1**[b]
11i	OCH_3		1.5
11j	OCH_3		4.8
11k	OCH_3		2.4
11l	OCH_3		5.0
11m	OCH_3		1.1
11n	OCH_3		2.8
12	OCH_3		0.5
14	OH		<0.001
19	SCH_2F		3.6

[a] All compounds *except* **12** are $\Delta_{1,2}$.

[b] Ratio derived from the mean of at least two assays; mean IC_{50} of budesonide 248 ± 78 pM ($n = 18$).



Scheme 2. Reagents and conditions: (i) HCl gas, dioxane, rt; (ii) 2-furoyl chloride, pyridine, rt.



Scheme 3. Reagents and conditions: (i) NaSH, CDI, DMF, rt; (ii) 2-furoyl chloride, pyridine, rt; (iii) $BrCH_2Cl$, DBU, DMF, rt; (iv) NaI acetone, reflux; (v) AgF, MeCN, rt.

Table 2. In vitro human glucocorticoid receptor binding affinities

No.	Glucocorticoid K_i/nM[a]
1	0.3
11a	1.4
11b	5.3
11f	9.4
19	15.1

[a] Mean of at least two assays.

with a commercial fluorescence polarisation assay based on human glucocorticoid receptors expressed in insect SF-9 cells (Panvera Inc). K_i values are shown in Table 2.

Compounds were then further profiled in vivo as shown in Table 3, initially for effects on thymus weight reduction after chronic oral administration to rats over 4 days. Thymus weight is a very sensitive surrogate marker for systemic glucocorticoid activity in the rat and thus this model may be considered as a measure of likely side effects arising from GI tract absorption. Efficacy in the sensitised Brown Norway rat, employing ovalbumin (OA)-induced pulmonary eosinophilia was next assessed.[15] This provides a model for the greatly reduced levels of eosinophil influx seen in the lungs of asthmatic patients treated with glucocorticoids. To screen for the desired long duration of action, compounds were administered intratracheally (i.t.) as dry powder blends in lactose at a single dose 24 h prior to induction of eosinophilia by OA challenge. Finally, compounds

Table 3. In vivo efficacy and side effect profiling

No.	Thymus weight % control[a] p.o.	Eosinophilia reduction % control[b] i.t.	Thymus weight % control[a] i.t.
1	42±4*	29±11*	21±1*
6	57±3*	10±10	39±6*
11a	80±6	93±6**	18±1*
11c	63±4*	79±10**	30±3*
11e	93±7	−8±17	nd
11f	77±8	5±25	nd
11l	41±4*	83±16**	19±2*
11m	84±6	10±34	nd
19	66±6*	73±35*	24±2*

In each case compounds were dosed at 1 mg/kg.
* $p<0.05$ compared to vehicle.
** $p<0.005$ compared to vehicle.
nd = not determined.
[a] Relative to control animals treated with vehicle.
[b] Relative to control animals challenged with OA and treated with vehicle.

showing efficacy in this model were screened for systemic effects on thymus weight arising from chronic i.t. administration of lactose dry powder blends over 4 days, as a measure of likely glucocorticoid side effects due to direct pulmonary absorption.

4. Results and discussion

The unsubstituted 2 or 3-furoate esters **11a** and **11b** showed a clear increase in in vitro potency over **6**. Sub-

stituted analogues of the 2-furoate **11a** revealed 3- or 4-alkyl substituents in **11c–e** as being similar or marginally more potent than the parent compound. In contrast, 5-substituents in **11f–i** with the exception of trifluoromethyl derivative **11j** were clearly detrimental to potency. Compared with the 3-furoate parent compound **11b**, monosubstitution at the 2-, 4- or 5-positions appeared slightly detrimental in **11k**, **11m** and **11n**, whereas the 2,5-disubstituted furoate **11l** gave similar potency. In accord with literature precedent $\Delta_{1,2}$ dihydro analogue **12** gave reduced potency compared to **11a**,[16] while the corresponding C-21 acid **13** was essentially inactive, presumably as a consequence of the lack of cellular penetration.[9] Finally, compound **19** with the C-21 methyl ester replaced by an α-fluorothioester, showed a superior potency to both **1** and **6**.

As expected, all compounds tested showed a high affinity for the human glucocortiocoid receptor (Table 2), although there appeared to be no correlation between the rank order of potency in this binding assay and the functional activity described above.

Turning to in vivo profiling (Table 3), **1** and **6** both showed significant glucocorticoid side effects when administered orally or intratracheally, and **6** lacked any statistically significant duration of action for inhibition of eosinophilia in bronchioalveolar lavage (BAL) fluid at 1 mg/kg, while **1** showed only a small effect. In contrast, **11a** showed no significant oral side effects and complete inhibition of eosinophilia 24 h after 1 mg/kg dosing. For this compound, thymus weight reduction via the i.t. route was similar to **1** and slightly greater than **6**. 4-Methyl 2-furoate derivative **11c**, 3-furoate **11l** and fluorothioester **19**, all of which showed similar in vitro potency to **11a**, also gave a 24 h duration of action in the efficacy model, but with increased significant oral side effects compared to **11a**. Compounds **11e**, **11f** and **11m**, all of which showed lower in vitro potency than **11a** gave no significant oral thymus effects but exhibited little or no efficacy in the Brown Norway rat eosinophilia model.

The minimised thymus weight reduction via the oral route reflects either a high first pass metabolism and/or low absorption, and indeed **11a** could not be detected in the plasma of rats when dosed orally either as a suspension or solution at 4.6 mg/kg, with a lower limit of quantification of 0.05 μM. By contrast, **1** is reported to be 15% bioavailable in the rat after a 1.5 mg/kg oral dose.[17] The significant thymus weight reduction seen for all steroids via the i.t. route probably reflects the stability of the compounds in plasma. This was verified for compound **11a**, which showed no degradation in rat or human plasma over 30 min at 37 °C, indicating the C-21 methyl ester is apparently stable to plasma esterases under these conditions. One potential explanation for the duration of action observed in the efficacy model may be the physical consequence of slow dissolution of the dry powder formulation. In this regard, the much lower thermodynamic solubility of **11a** (0.0001 g/L) compared to budesonide (0.0045 g/L) at pH 6.8 in phosphate buffer could be a contributory factor.

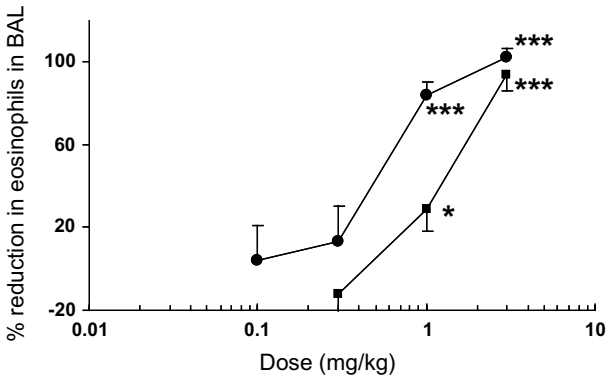


Figure 3. Comparison of the potencies of **1** and **11a** in the Brown Norway rat. Compounds dosed i.t. 24 h prior to OA challenge; (●) **11a**; (■) **1**; $*p<0.05$, $***p<0.001$ compared to OA challenged animals treated with vehicle.

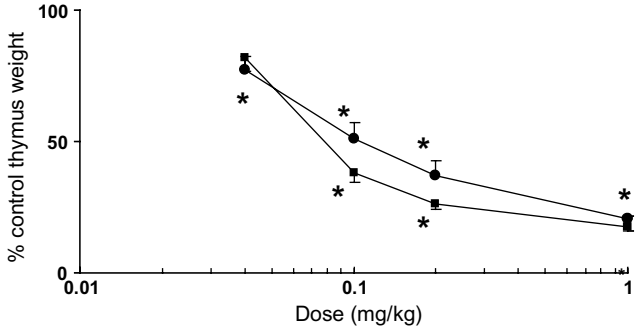


Figure 4. Comparison of the thymus weight reduction potencies of **1** and **11a** after i.t. administration. Compounds dosed i.t. as dry powder blends for 4 days; (●) **11a**; (■) **1**; $*p<0.05$ compared to animals treated with vehicle.

Given the favourable duration of action and oral side effect in vivo profile of **11a**, this compound was further profiled in the Brown Norway rat efficacy model with a full dose response (Fig. 3) compared with **1** head to head, clearly indicating its superior potency for reduction of eosinophilia in the BAL fluid after OA challenge.

Further dose–response comparison of the side effect profiles of the above compounds underscored the similar levels of systemic activity after i.t. administration (Fig. 4) and the difference in side effects after oral administration (Fig. 5). In the latter case, **11a** is at least 10-fold less potent than **1** in eliciting 50% thymus weight reduction.

5. Conclusions

In summary, novel androstadiene C-17 esters have been prepared and profiled for in vivo anti-inflammatory activity and side effects. Replacement of propionate by a furoate ester gave increased potency in vitro, as well as providing an increased duration of action and reduction of oral glucocorticoid side effects in vivo. The in vitro structure–activity relationship indicated a variety of substitution was allowed on the furan ring

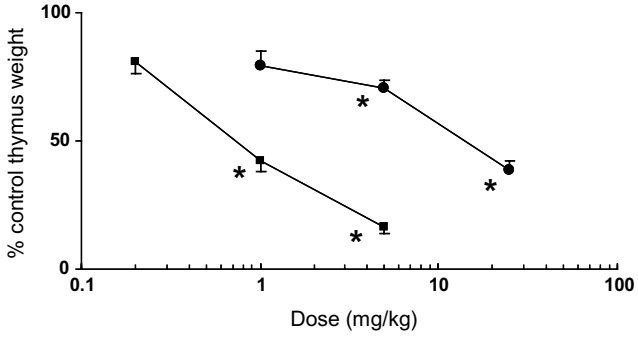


Figure 5. Comparison of the thymus weight reduction potencies of **1** and **11a** after oral administration. Compounds dosed orally as suspensions for 4 days; (●) **11a**; (■) **1**; $*p<0.05$ compared to animals treated with vehicle.

of the ester. In vivo screening enabled rapid identification of the unsubstituted 2-furoate ester **11a** as a compound with minimal oral absorption, together with superior efficacy and duration of action and similar i.t. side effect profile compared to budesonide in in vivo rodent models. Based on these results, compound **11a** may have potential to function as a long-acting once-daily inhaled corticosteroid. Further studies on **11a** and related compounds will be reported in due course.

6. Experimental section

Reagents and solvents were purchased from common commercial suppliers and used as received. Noncommercial acid chlorides were prepared by refluxing the corresponding furoic acids with thionyl chloride, followed by evaporation to dryness in vacuo. All reactions were carried out under an atmosphere of argon unless otherwise stated. TLC was performed on Merck Kieselgel 60 F_{254} plates and column chromatography was conducted on Merck Kieselgel 60 (230–400 mesh). Analytical HPLC was carried out on a reverse-phase Hypersil Elite C_{18} column (10 cm×0.3 cm) at 40 °C, using a gradient of 30–95% acetonitrile over 8 min, flow rate 0.50 mL/min, with detection at 254 nm. Mass spectrometry, including accurate mass determination, was performed using a Micromass Time-of-Flight LCT. (ES+ve) refers to mass spectra run in positive mode using electrospray techniques. ^{1}H NMR analyses were performed on Bruker ARX/AV 400 MHz instruments in $CDCl_3$ or DMSO-d_6. Chemical shifts (δ) are expressed in ppm relative to residual proton signals in solvent. Melting points were determined on a Gallenkamp capillary melting point apparatus and are uncorrected. Elemental analyses were performed on a LECO CHNS 932 instrument.

6.1. 9α-Chloro-6α-fluoro-11β-hydroxy-17α-(1-oxopropoxy)-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (6)

Prepared as previously described.[11]

Mp 197–198 °C; Anal. HPLC t_R 6.82 min, 99%; ^{1}H NMR ($CDCl_3$) 0.98 (d, 3H, $J=7$ Hz), 1.0 (s, 3H), 1.17 (t, 3H, $J=8$ Hz), 1.27–1.32 (m, 2H), 1.64 (s, 3H), 1.79–2.01 (m, 3H), 2.23–2.28 (m, 1H), 2.40 (q, 2H, $J=8$ Hz), 2.47–2.62 (m, 2H), 2.70 (dd, 1H, $J=4,14$ Hz), 3.33–3.36 (m, 1H), 3.73 (s, 3H), 4.57 (br s, 1H), 5.29–5.46 (2m, 1H), 6.37 (dd, 1H, $J=2,10$ Hz), 6.43 (s 1H), 7.12 (d, 1H, $J=10$ Hz); MS(ES+) *m/z* 483 $(M+H)^+$; Anal. Calcd for $C_{25}H_{32}ClFO_6 \cdot 0.2H_2O$: C, 61.73; H, 6.67. Found: C, 61.75; H, 6.92.

6.2. 9β,11β-Epoxy-6-fluoro-17α-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid (8)

To a suspension of epoxyparamethasone **7** (25.0 g, 64.10 mmol) in THF (300 mL), was added dropwise at 25 °C a solution of periodic acid (19.73 g, 85.54 mmol) in water (100 mL). The reaction mixture was stirred at 25 °C for 16 h, then concentrated to approximately half its original volume. Water (200 mL) was added slowly with vigorous stirring and the resulting precipitate was filtered off and dried under vacuum to give **8** (23.0 g, 95%) as an off-white solid: ^{1}H NMR ($CDCl_3$) 0.97 (d, 3H, $J=7$ Hz), 1.08 (s, 3H), 1.42 (s, 3H), 1.64 (q, 2H, $J=12$ Hz), 1.76–1.87 (m, 2H), 2.14 (d, 2H, $J=11$ Hz), 2.32 (q, 2H, $J=8$ Hz), 2.61–2.70 (m, 2H), 2.94–3.02 (m, 1H), 3.33 (br s, 1H), 5.36–5.52 (2m, 1H), 6.25 (dd, 1H, $J=2,8$ Hz), 6.45 (s, 1H), 6.53 (d, 1H, $J=10$ Hz).

6.3. 9α-Chloro-6α-fluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11a)

General procedure for acylation of C-17 α-hydroxyl group. Method A.

6.4. 9β,11β-Epoxy-6-fluoro-17α-[(2-furanylcarbonyl)oxy]-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid (9a)

To a stirred solution of **8** (4.0 g, 10.64 mmol) in pyridine (15 mL) at 25 °C was added dropwise 2-furoyl chloride (1.53 g, 11.70 mmol). The reaction mixture was stirred at room temperature for 2 h, then added dropwise to a vigorously stirred solution of 6 M aq HCl (45 mL). After 30 min of stirring at 25 °C, the resulting precipitate was filtered, dissolved in CH_2Cl_2, dried ($MgSO_4$), filtered and concentrated to give **9a** (4.9 g, 98%) as an off-white solid: ^{1}H NMR ($CDCl_3$) 1.02 (d, 3H, $J=8$ Hz), 1.08 (s, 3H), 1.44 (s, 3H), 1.61–1.77 (m, 2H), 1.84–1.95 (m, 2H), 2.07 (dd, 1H, $J=3,15$ Hz), 2.31–2.41 (m, 2H), 2.66–2.72 (m, 1H), 3.28–3.36 (m, 2H), 4.60 (br s, 1H), 5.39–5.54 (2m, 1H), 6.30 (dd, 1H, $J=2,10$ Hz), 6.49–6.55 (m, 2H), 6.56 (d, 1H, $J=10$ Hz), 7.18 (d, 1H, $J=3$ Hz).

6.5. 9β,11β-Epoxy-6α-fluoro-17α-[(2-furanylcarbonyl)-oxy]-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (10a)

To a stirred solution of **9a** (1.10 g, 2.34 mmol) in EtOAc (25 mL) at 0 °C, was added DBU (0.422 mL, 2.82 mmol), followed by Me_2SO_4 (2.67 mL, 2.82 mmol). The reaction mixture was stirred at 25 °C for 2 h, treated with morpholine (0.1 mL, 1 mmol) and stirred for a further 30 min,

then partitioned between EtOAc and water. The organic layer was washed with brine, dried ($MgSO_4$), filtered and concentrated. MeOH (20 mL) was added from which the product crystallised, affording **10a** (650 mg, 58%) as a white solid: ^{1}H NMR ($CDCl_3$) 0.96 (d, 3H, J = 7 Hz), 1.44 (s, 3H), 1.48 (s, 3H), 1.60–1.75 (m, 2H), 1.86 (q, 1H, J = 10 Hz), 1.98 (dd, 1H, J = 3,13 Hz), 2.29–2.37 (m, 2H), 2.64–2.73 (m, 1H), 3.27–3.34 (m, 2H), 3.75 (s, 3H), 5.37–5.54 (m, 1H), 6.27 (dd, 1H, J = 2,7 Hz), 6.48 (s, 1H), 6.52–6.54 (m, 1H), 6.56 (s, 1H), 7.17 (d, 1H, J = 4 Hz), 7.62 (s, 1H).

6.6. 9α-Chloro-6α-fluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11a)

HCl gas was bubbled through a solution of **10a** (550 mg, 1.14 mmol) in toluene (20 mL) for 5 min. The reaction flask was stoppered, then stirred at 25 °C for 18 h. The solid obtained after concentration of the reaction mixture was recrystallised from *i*-PrOH/MeOH to give **11a** (420 mg, 71%) as a white solid: mp 196–197 °C dec; Anal. HPLC t_R 7.20 min, 98%; ^{1}H NMR ($CDCl_3$) 1.03 (d, 3H, J = 7 Hz), 1.10 (s, 3H), 1.30–1.35 (m, 1H), 1.64 (s, 3H), 1.72 (dd, 1H, J = 3,15 Hz), 1.84–2.02 (m, 2H), 2.23–2.29 (m, 1H), 2.58–2.71 (m, 2H), 2.82 (dd, 1H, J = 3,15 Hz), 3.36–3.46 (m, 1H), 3.72 (s, 3H), 4.59 (br s, 1H), 5.29–5.46 (2m, 1H), 6.38 (dd, 1H, J = 2,9 Hz), 6.41 (s, 1H), 6.48 (m, 1H), 7.09 (s, 1H), 7.12 (m, 1H), 7.60 (s, 1H); MS(ES+) m/z 521 $(M+H)^+$; Anal. Calcd for $C_{27}H_{30}ClFO_7$: C, 62.25; H, 5.80. Found: C, 62.13; H, 5.78.

6.7. 9α-Chloro-6α-fluoro-17α-[(3-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11b)

Similarly to the procedure described for **11a**, the title compound was prepared starting from **8** and 3-furancarboxylic acid. Recrystallisation from *i*-PrOH afforded **11b** (24% yield for three steps from **8**) as a white solid: mp 197–201 °C; Anal. HPLC t_R 7.10 min, 97%; ^{1}H NMR ($CDCl_3$) 0.89 (d, 3H, J = 7 Hz), 0.97 (s, 3H), 1.16–1.22 (m, 1H), 1.52–1.58 (m, 5H), 1.68–1.91 (m, 2H), 2.11–2.19 (m, 1H), 2.46–2.56 (m, 2H), 2.66 (dd, 1H, J = 3,15), 3.24–3.29 (m, 1H), 3.63 (s, 3H), 4.47 (br s, 1H), 5.16–5.34 (2m, 1H), 6.26 (dd, 1H, J = 2,10 Hz), 6.31 (s, 1H), 6.57 (s, 1H), 6.98 (d, 1H, J = 10 Hz), 7.29 (s, 1H), 7.87 (s, 1H); MS(ES+) m/z 521 $(M+H)^+$; Anal. Calcd for $C_{27}H_{30}ClFO_7 \cdot 0.1H_2O$: C, 61.98; H, 5.80. Found: C, 61.93; H, 5.90.

6.8. 9α-Chloro-6α-fluoro-17α-[(2-furanylcarbonyl-4-methyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11c)

Similarly to the procedure described for **11a**, the title compound was prepared starting from **8** and 2-furan-4-methylcarboxylic acid.[18] Purification by column chromatography using EtOAc/hexane (1:2) as the eluent, afforded **11c** (47% yield for three steps from **8**) as a white solid: mp 188–190 °C dec; Anal. HPLC t_R 7.43 min, 98%; ^{1}H NMR (DMSO-d_6) 0.78 (d, 3H, J = 8 Hz), 0.92 (s, 3H), 1.12–1.18 (m, 1H), 1.55 (s, 3H), 1.55–1.72 (m, 2H), 1.86 (q, 1H, J = 12 Hz), 1.96 (s, 3H), 2.19–2.28 (m, 1H), 2.78 (t, 1H, J = 10 Hz), 3.23–3.50 (m, 2H), 3.71 (s, 3H), 4.53 (br s, 1H), 5.74–5.91 (2m, 2H), 6.34 (s, 1H), 6.55 (d, 1H, J = 11 Hz), 7.31 (s, 1H), 7.59 (d, 1H, J = 11 Hz), 8.08 (s, 1H); MS(ES+) m/z 535 $(M+H)^+$; Anal. Calcd for $C_{28}H_{32}ClFO_7 \cdot 0.3H_2O$: C, 62.18; H, 6.02. Found: C, 62.27; H, 6.05.

6.9. 9α-Chloro-6α-fluoro-17α-[(2-furanylcarbonyl-3-methyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11d)

Similarly to the procedure described for **11a**, the title compound was prepared starting from **8** and 3-methyl-2-furancarboxylic acid. Recrystallisation from *i*-Pr_2O/MeOH, followed by column chromatography eluting with CH_2Cl_2/MeOH (25:1) afforded **11d** (11% yield for three steps from **8**) as an off-white solid: mp 194 °C; Anal. HPLC t_R 7.21 min, 97%; ^{1}H NMR ($CDCl_3$) 0.90 (d, 3H, J = 7 Hz), 0.98 (s, 3H), 1.20–1.26 (m, 1H), 1.35 (s, 3H), 1.52–1.60 (m, 2H), 1.72–1.91 (m, 2H), 2.10–2.18 (m, 1H), 2.21 (s, 3H), 2.46–2.57 (m, 2H), 2.72 (dd, 1H, J = 3,15 Hz), 3.28–3.36 (m, 1H), 3.63 (s, 3H), 4.46 (br s, 1H), 5.17–5.33 (2m, 1H), 6.21 (s, 1H), 6.23 (dd, 1H, J = 2,10 Hz), 6.31 (s, 1H), 6.98 (d, 1H, J = 1 Hz), 7.29 (s, 1H); MS(ES+) m/z 535 $(M+H)^+$; Anal. Calcd for $C_{28}H_{32}ClFO_7 \cdot 0.8H_2O$: C, 61.16; H, 6.18. Found: C, 61.20; H, 6.21.

6.10. 9α-Chloro-6α-fluoro-17α-[(3-ethyl-2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11e)

General procedure for acylation of C-17α-hydroxyl group. Method B.

6.11. 9β,11β-Epoxy-17α-[(3-ethyl-2-furanylcarbonyl)-oxy]-6α-fluoro-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid (9e)

To a solution of 3-ethyl-2-furancarboxylic acid[19] (400 mg, 7.13 mmol) at 0 °C in DMF (2.5 mL) was added diisopropylethylamine 1.24 mL, 7.13 mmol), followed by portionwise addition of HATU (1.19 g, 3.13 mmol). The reaction mixture was stirred for 10 min after which time a solution of **8** (1.07 g, 2.85 mmol) in DMF (3 mL) was added. The reaction mixture was stirred for 2 h, allowing to warm slowly to room temperature. The reaction mixture was poured into water (30 mL), filtered to afford an off-white precipitate, which was washed with water (3×5 mL) and dried in vacuo. The crude material was purified by column chromatography on silica gel using 1:1 EtOAc/*n*-hexane as the eluent, finally eluting with EtOAc to give **9e** (540 mg, 38%) as an off-white glassy solid: ^{1}H NMR ($CDCl_3$) 0.99 (d, 3H, J = 7 Hz), 1.04 (s, 3H), 1.15 (t, 3H, J = 8 Hz), 1.44 (s, 3H), 1.60–1.77 (m, 2H), 1.91 (q, 2H, J = 8 Hz), 2.32–2.41 (m, 2H), 2.65–2.78 (m, 3H), 3.24–3.38 (m, 2H), 5.38–5.55 (m, 1H), 6.30 (dd, 1H, J = 2,8 Hz), 6.42 (d, 1H, J = 2 Hz), 6.51 (d, 1H, J = 2 Hz), 6.57 (d, 1H, J = 2 Hz), 7.50 (d, 1H, J = 2Hz).

6.12. 9β,11β-Epoxy-17α-[(3-ethyl-2-furanylcarbonyl)-oxy]-6α-fluoro-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (10e)

To a stirred solution of **9e** (121 mg, 1.24 mmol) in DMF (4 mL) at 0 °C, was added DBU (0.204 mL, 1.37 mmol), followed by Me_2SO_4 (0.129 mL, 1.37 mmol). The reaction mixture was stirred at 25 °C for 2 h, then poured into water (20 mL) and extracted with EtOAc (2×15 mL). The combined organic extracts were washed with water (2×15 mL), brine (10 mL), dried ($MgSO_4$), filtered and concentrated, affording **10e** (528 mg, 83%) as an off-white solid: ^{1}H NMR ($CDCl_3$) 0.99 (d, 3H, J=7 Hz), 1.15 (t, 3H, J=8 Hz), 1.46 (s, 3H), 1.59–1.76 (m, 5H), 1.89–2.04 (m, 2H), 2.66–2.72 (m, 2H), 2.74–2.79 (m, 3H), 3.32 (br s, 2H), 3.73 (s, 3H), 5.38–5.55 (m, 1H), 6.28 (dd, 1H, J=2,10 Hz), 6.40 (s, 1H), 6.50 (d, 1H, J=2 Hz), 6.58 (dd, 1H, J=2,10 Hz), 7.26 (d, 1H, J=2 Hz).

6.13. 9α-Chloro-6α-fluoro-17α-[(3-ethyl-2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11e)

HCl gas was bubbled through a solution of **10e** (509 mg, 0.99 mmol) in toluene (75 mL) over 5 min. The reaction flask was stoppered, then stirred at 25 °C for 18 h, prior to concentration to give a green syrup. Trituration with MeOH (8 mL) afforded a white solid in green supernatant liquid. The crude product was filtered, washed with MeOH (3×1 mL) and recrystallised from MeOH to afford **11e** (183 mg, 34%) as a white solid: mp 179–180 °C dec; Anal. HPLC t_R 7.60 min, 90%; ^{1}H NMR (DMSO-d_6) 0.88 (d, 3H, J=7 Hz), 1.01 (s, 3H), 1.08 (t, 3H, J=8 Hz), 1.23–1.30 (m, 1H), 1.57–1.76 (m, 5H), 1.92 (q, 1H, J=12 Hz), 2.21–2.28 (m, 1H), 2.39–2.47 (m, 2H), 2.66–2.84 (m, 3H), 3.64 (s, 3H), 4.39 (br s, 1H), 5.54–5.68 (m, 2H), 6.11 (s, 1H), 6.31 (d, 1H, J=10 Hz), 6.64 (s, 1H), 7.28 (d, 1H, J=10 Hz), 7.83 (s, 1H); MS(ES+) m/z 549 $(M+H)^+$; Anal. Calcd for $C_{29}H_{34}ClFO_7 \cdot 0.5H_2O$: C, 62.37; H, 6.14. Found: C, 62.44; H, 6.30.

6.14. 9α-Chloro-17α-[(3,5-dimethyl-2-furanylcarbonyl)oxy]-6α-fluoro-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11f)

Similarly to the procedure described for **11e**, the title compound was prepared starting from **8** and 3,5-dimethyl-2-furancarboxylic acid.[20] Column chromatography eluting with EtOAc/*n*-hexane (1:2) afforded **11f** (22% yield for three steps from **8**) as a pale green solid: mp 125–135 °C dec; Anal. HPLC t_R 7.68 min, 92%; ^{1}H NMR (DMSO-d_6) 0.89 (d, 3H, J=8 Hz), 1.01 (s, 3H), 1.24–1.29 (m, 1H), 1.61–1.78 (m, 5H), 1.90 (q, 1H, J=12 Hz), 2.20–2.30 (m, 7H), 2.40–2.54 (m, 2H), 2.76 (t, 1H, J=10 Hz), 3.64 (s, 3H), 4.39 (br s, 1H), 5.55–5.71 (m, 2H), 6.10 (s, 1H), 6.21 (s, 1H), 6.30 (dd, 1H, J=2,10 Hz), 7.28 (d, 1H, J=10 Hz); MS(ES+) m/z 549 $(M+H)^+$; Anal. Calcd for $C_{29}H_{34}ClFO_7 \cdot 0.8H_2O$: C, 61.77; H, 6.23. Found: C, 61.74; H, 6.41.

6.15. 9α-Chloro-6α-fluoro-17α-[(2-furanylcarbonyl-5-methyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11g)

Similarly to the procedure described for **11e**, the title compound was prepared starting from **8** and 5-methyl-2-furancarboxylic acid.[21] Trituration with MeOH afforded **11g** (32% yield for three steps from **8**) as a white solid: mp 193–195 °C; Anal. HPLC t_R 6.90 min, 94%; ^{1}H NMR (DMSO-d_6) 0.80 (d, 3H, J=7 Hz), 0.92 (s, 3H), 1.10–1.22 (m, 1H), 1.50–1.71 (m, 5H), 1.87 (q, 1H, J=12), 2.22 (m, 1H), 2.31 (s, 3H), 2.67–2.82 (m, 2H), 3.70 (s, 3H), 4.52 (br s, 1H), 5.74–5.92 (m, 2H), 6.34 (s, 1H), 6.53 (d, 1H, J=2 Hz), 6.56 (d, 1H, J=4 Hz), 7.34 (s, 1H), 7.58 (d, 1H, J=10 Hz); MS(ES+) m/z 535 $(M+H)^+$; Anal. Calcd for $C_{28}H_{32}ClFO_7 \cdot H_2O$: C, 60.76; H, 6.01. Found: C, 60.77; H, 6.30.

6.16. 9α-Chloro-17α-[(5-ethyl-2-furanylcarbonyl)oxy]-6α-fluoro-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11h)

Similarly to the procedure described for **11e**, the title compound was prepared starting from **8** and 5-ethyl-2-furancarboxylic acid.[22] Column chromatography eluting with EtOAc/*n*-hexane (1:2) afforded **11h** (14% yield for three steps from **8**) as a white solid: mp 182–183 °C dec; Anal. HPLC t_R 7.85 min, 97%; ^{1}H NMR (DMSO-d^6) 0.79 (d, 3H, J=8 Hz), 0.92 (s, 3H), 1.08 (t, 3H, J=8 Hz), 1.13–1.18 (m, 1H), 1.55–1.71 (m, 5H), 1.81–1.90 (q, 1H, J=13 Hz), 2.20–2.28 (m, 1H), 2.54 (s, 1H), 2.67 (q, 2H, J=8 Hz), 2.77 (t, 1H, J=11 Hz), 3.29–3.33 (m, 1H), 3.71 (s, 3H), 4.52 (br s, 1H), 5.74–5.92 (2m, 2H), 6.34 (s, 1H), 6.55 (d, 1H, J=11 Hz), 6.59 (d, 1H, J=3 Hz), 7.39 (d, 1H, J=3 Hz), 7.59 (d, 1H, J=11 Hz); MS(ES+) m/z 549 $(M+H)^+$; Anal. Calcd for $C_{29}H_{34}ClFO_7 \cdot 0.2H_2O$: C, 62.98; H, 6.19. Found: C, 63.01; H, 6.37.

6.17. 9α-Chloro-17α-[(5-chloro-2-furanylcarbonyl)oxy]-6-fluoro-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11i)

6.17.1. Preparation of 5-chloro-2-furancarboxylic acid.

To diisopropylamine (1.4 mL, 10 mmol) cooled to −50 °C, under argon, was added *n*-BuLi (2.5 M in hexanes; 4 mL, 10 mmol) followed by THF (20 mL). 2-Furoic acid (560 mg, 5 mmol) was then added dropwise as a solution in THF (10 mL). The reaction mixture was stirred at −50 °C for 30 min, then a dispersion of N-chlorosuccinimide (694 mg, 5.2 mmol) in THF (5 mL) was added. Once addition was complete, the reaction mixture was kept at −50 °C for 30 min then warmed up to 25 °C slowly over 16 h. The resultant suspension was filtered, the resultant solid was dissolved in H_2O, washed with Et_2O, then acidified to pH 1 with 1 M HCl. The product was extracted with Et_2O (2×25 mL), washed with brine, dried (Na_2SO_4), filtered and concentrated. The residue was repeatedly recrystallised from H_2O affording 5-chloro-2-furancarboxylic acid (60 mg, 8%): ^{1}H NMR ($CDCl_3$) 6.37 (d, 1H, J=4 Hz), 7.29 (d, 1H, J=3 Hz).

Similarly to the procedure described for **11e**, the title compound was prepared starting from **8** and 5-chloro-2-furancarboxylic acid. Trituration with MeOH afforded **11i** (20% yield for three steps from **8**) as a white solid: mp 194 °C; Anal. HPLC t_R 7.75 min, 99%; ^{1}H NMR ($CDCl_3$) 0.90 (d, 3H, J=7 Hz), 1.02 (s, 3H), 1.24–1.30 (m, 2H), 1.61–1.77 (m, 5H), 1.90 (q, 1H, J=12 Hz), 2.22–2.30 (m, 1H), 2.77 (t, 1H, J=10 Hz), 3.66 (s, 3H), 4.40 (br s, 1H), 5.55–5.71 (2m, 1H), 6.11 (s, 1H), 6.30 (dd, 1H, J=2,10 Hz), 6.75 (d, 1H, J=4 Hz), 7.26–7.30 (m, 2H); MS(ES+) m/z 555 $(M+H)^+$; Anal. Calcd for $C_{27}H_{29}Cl_2FO_7$: C, 58.39; H, 5.26. Found: C, 58.42; H, 5.10.

6.18. 9α-Chloro-6α-fluoro-11β-hydroxy-16α-methyl-17α-[(2-furanylcarbonyl-5-trifluoromethyl)oxy]-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11j)

Similarly to the procedure described for **11e**, the title compound was prepared starting from **8** and 5-trifluoromethyl-2-furancarboxylic acid. Column chromatography eluting with CH_2Cl_2/MeOH (50:1), followed by trituration with MeOH, afforded **11j** (4% yield for three steps from **8**) as a white solid: mp 196 °C; Anal. HPLC t_R 8.00 min, 97%; ^{1}H NMR (DMSO-d_6) 0.89 (d, 3H, J=7 Hz), 1.0 (s, 3H), 1.18–1.26 (m, 1H), 1.59–1.74 (m, 5H), 1.89 (q, 1H, J=12 Hz), 2.18–2.28 (m, 1H), 2.75 (t, 1H, J=8 Hz), 3.65 (s, 3H), 4.38 (br s, 1H), 5.52–5.70 (m, 2H), 6.09 (s, 1H), 6.28 (dd, 1H, J=2,10 Hz), 7.26 (d, 1H, J=10 Hz), 7.33 (d, 1H, J=3 Hz), 7.41 (d, 1H, J=3 Hz); MS(ES+) m/z 589 $(M+H)^+$; HR-TOFMS m/z $(M+H)^+$; HRMS Calcd for $C_{28}H_{29}ClF_4O_7$: 589.1616. Found: 589.1616.

6.19. 9α-Chloro-6α-fluoro-11β-hydroxy-17α-[(2-methyl-3-furanylcarbonyl)oxy]-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11k)

Similarly to the procedure described for **11a**, the title compound was prepared starting from **8** and 2-methyl-3-furancarboxylic acid.[20] Trituration with CH_2Cl_2 and MeOH afforded **11k** (61% yield for three steps from **8**) as a white solid: mp 195–196 °C; Anal. HPLC t_R 7.22 min, 96%; ^{1}H NMR (DMSO-d_6) 0.91 (d, 3H, J=7 Hz), 1.10 (s, 3H), 1.20–1.35 (m, 1H), 1.60–1.78 (m, 5H), 1.79–1.95 (m, 2H), 2.22–2.34 (m, 1H), 2.49 (s, 3H), 2.80 (t, 1H, J=10 Hz), 3.68 (s, 3H), 4.43 (br s, 1H), 5.58–5.71 (m, 2H), 6.14 (s, 1H), 6.32 (d, 1H, J=10 Hz), 6.61 (d, 1H, J=2 Hz), 7.31 (d, 1H, J=10 Hz), 7.62 (d, 1H, J=2 Hz); MS(ES+) m/z 535 $(M+H)^+$; Anal. Calcd for $C_{28}H_{32}ClFO_7 \cdot 0.6H_2O$: C, 61.56; H, 6.02. Found: C, 61.59; H, 6.19.

6.20. 9α-Chloro-17α-[(2,5-dimethyl-3-furanylcarbonyl)oxy]-6α-fluoro-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11l)

Similarly to the procedure described for **11a**, the title compound was prepared starting from **8** and 2,5-dimethyl-3-furancarboxylic acid. Column chromatography eluting with CH_2Cl_2/MeOH (50:1) afforded **11l** (25% yield for three steps from **8**) as a pale yellow solid: mp 173–175 °C dec; Anal. HPLC t_R 8.19 min, 89%; ^{1}H NMR (DMSO-d_6); 0.90 (d, 3H, J=7 Hz), 1.03 (s, 3H), 1.24–1.28 (m, 1H), 1.64–1.78 (m, 5H), 1.90 (q, 1H, J=12 Hz), 2.15–2.28 (m, 4H), 2.43–2.53 (m, 5H), 2.79 (t, 1H, J=10 Hz), 3.19–3.34 (m, 1H), 3.66 (s, 3H), 4.42 (s, 1H), 5.57–5.74 (2m, 2H), 6.14 (s, 1H), 6.21 (s, 1H), 6.33 (d, 1H, J=10 Hz), 7.31 (d, 1H, J=10 Hz); MS(ES+) m/z 549 $(M+H)^+$; Anal. Calcd for $C_{29}H_{34}ClFO_7 \cdot 0.1CH_2Cl_2$: C, 62.69; H, 6.18. Found: C, 62.39; H, 6.12.

6.21. 9α-Chloro-6α-fluoro-17α-[(3-furanylcarbonyl-4-methyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11m)

Similarly to the procedure described for **11e**, the title compound was prepared starting from **8** and 4-methyl-3-furancarboxylic acid.[22] Recrystallisation from MeOH afforded **11m** (28% yield for three steps from **8**) as a beige solid: mp 181–182 °C dec; Anal. HPLC t_R 7.72 min, 99%; ^{1}H NMR (DMSO-d_6) 0.90 (d, 3H, J=7 Hz), 1.01 (s, 3H), 1.22–1.28 (m, 1H), 1.61 (s, 3H), 1.66–1.78 (m, 2H), 1.90 (q, 1H, J=12 Hz), 2.10 (s, 3H), 2.21–2.29 (m, 1H), 2.40–2.48 (m, 2H), 2.74 (t, 1H, J=11 Hz), 3.23–3.30 (m, 1H), 3.65 (s, 3H), 4.39 (br s, 1H), 5.55–5.71 (m, 2H), 6.11 (s, 1H), 6.30 (d, 1H, J=10 Hz), 7.28 (d, 1H, J=11 Hz), 7.61 (s, 1H), 8.11 (d, 1H, J=2 Hz); MS(ES+) m/z 535 $(M+H)^+$; Anal. Calcd for $C_{28}H_{32}ClFO_7 \cdot 0.4H_2O$: C, 62.08; H, 6.02. Found: C, 62.07; H, 6.01.

6.22. 9α-Chloro-6α-fluoro-11β-hydroxy-17α-[(3-furanylcarbonyl-5-methyl)oxy]-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid, methyl ester (11n)

Similarly to the procedure described for **11e**, the title compound was prepared starting from **8** and 5-methyl-3-furancarboxylic acid.[21] Trituration with MeOH afforded **11n** (22% yield for three steps from **8**) as an off-white solid: mp 177–181 °C dec; Anal. HPLC t_R 7.29 min, 99%; ^{1}H NMR (DMSO-d_6) 0.79 (d, 3H, J=7 Hz), 0.91 (s, 3H), 1.10–1.17 (m, 2H), 1.55–1.72 (m, 5H), 1.82 (q, 1H, J=8 Hz), 2.24 (s, 3H), 2.49 (t, 1H, J=12 Hz), 2.97–3.22 (m, 3H), 3.70 (s, 3H), 4.53 (br s, 1H), 5.74–5.92 (2m, 2H), 6.34 (s, 1H), 6.54 (s, 1H), 6.56 (d, 1H, J=2 Hz), 7.58 (d, 1H, J=10 Hz), 8.43 (s, 1H); MS(ES+) m/z 535 $(M+H)^+$; Anal. Calcd for $C_{28}H_{32}ClFO_7$: C, 62.86; H, 6.03. Found: C, 63.12; H, 6.16.

6.23. 9α-Chloro-6α-fluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrost-4-ene-17β-carboxylic acid, methyl ester (12)

A suspension of **11a** (670 mg, 1.29 mmol) and tris(triphenylphosphine)chlororhodium (300 mg, 0.32 mmol) in EtOH (40 mL) and DMF (10 mL) was hydrogenated at 3.5 bar at 40 °C for 72 h. A further 0.2 equiv tris(triphenylphosphine)chlororhodium was added after 24 h to drive the reaction to completion. The reaction mixture was concentrated to dryness and the brown residue obtained was suspended in 50:1 CH_2Cl_2/MeOH, then eluted through a silica gel filter column. The filtrate obtained was concentrated to dryness, then successive trituration

with DMF (5 mL), MeOH (5 mL) and MeCN (5 mL) gave a white precipitate, which was filtered, washed with MeOH (10 mL) and dried in vacuo to afford **12** (185 mg, 28%) as a white solid: mp 169 °C; Anal. HPLC t_R 7.09 min, 96%; ^{1}H NMR ($CDCl_3$) 0.90 (d, 3H, J = 7 Hz), 0.94 (s, 3H), 1.18–1.23 (m, 1H), 1.35 (d, 1H, J = 3 Hz), 1.51–1.56 (m, 4H), 1.73 (q, 1H, J = 12 Hz), 1.83–1.99 (m, 2H), 2.07–2.14 (m, 1H), 2.32–2.49 (m, 3H), 2.52–2.60 (m, 1H), 2.65–2.75 (m, 2H), 3.24–3.32 (m, 1H), 3.77 (s, 3H), 4.38 (br s, 1H), 5.06–5.22 (m, 1H), 6.01 (s, 1H), 6.39 (d, 1H, J = 3 Hz), 7.04 (d, 1H, J = 3 Hz), 7.46 (s, 1H); MS(ES+) *m/z* 523 $(M+H)^+$; Anal. Calcd for $C_{27}H_{32}ClFO_7 \cdot 0.4H_2O$: C, 61.11; H, 6.11. Found: C, 61.11; H, 6.08.

6.24. 9α-Chloro-11β,17α-dihydroxy-6α-fluoro-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid (13)

HCl gas was bubbled through a solution of **8** (5.0 g, 13.3 mmol) in dioxane (250 mL) over 5 min. The reaction flask was stoppered, stirred at 25 °C for 18 h and concentrated to give a white residue. Trituration with EtOAc, followed by recrystallisation from MeOH afforded **13** (2.8 g, 51%) as a white solid: ^{1}H NMR (DMSO-d_6) 0.88 (d, 3H, J = 7 Hz), 1.0 (s, 3H), 1.05–1.11 (m, 1H), 1.52 (d, 1H, J = 13 Hz), 1.60–1.75 (m, 5H), 2.17–2.23 (m, 1H), 2.30–2.37 (m, 2H), 2.65 (t, 1H, J = 8 Hz), 2.82–2.87 (m, 1H), 3.32 (s, 1H), 4.33 (br s, 1H), 5.47–5.69 (m, 1H), 6.09 (s, 1H), 6.28 (dd, 1H, J = 2,10 Hz), 7.27 (d, 1H, J = 10 Hz).

6.25. 9α-Chloro-6α-fluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carboxylic acid (14)

To a stirred solution of **13** (500 mg, 1.21 mmol) in pyridine (2 mL) at 25 °C was added dropwise 2-furoyl chloride (0.166 g, 1.27 mmol). The reaction mixture was stirred at 25 °C for 1 h. The reaction mixture was then added dropwise to a vigorously stirred solution of 6 M HCl (10 mL). After 30 min, the resulting precipitate was filtered, washed with THF (10 mL), followed by diethyl ether (10 mL), then dried in vacuo to give **14** (613 mg, 98%) as an off-white solid: mp 175 °C; Anal. HPLC t_R 5.77 min, 94%; ^{1}H NMR (DMSO-d_6) 0.91 (d, 3H, J = Hz), 1.07 (s, 3H), 1.20–1.26 (m, 1H), 1.62–1.77 (m, 5H), 1.89 (q, 1H, J = 12 Hz), 2.23–2.30 (m, 1H), 2.78 (t, 1H, J = 10 Hz), 3.23–3.28 (m, 2H), 4.41 (br s, 1H), 5.56–5.72 (m, 2H), 6.12 (s, 1H), 6.31 (d, 1H, J = 10 Hz), 6.68 (d, 1H, J = 3 Hz), 7.16 (d, 1H, J = 3 Hz), 7.29 (d, 1H, J = 10 Hz), 7.98 (s, 1H); MS(ES+) *m/z* 507 $(M+H)^+$; Anal. Calcd for $C_{26}H_{28}ClFO_7 \cdot 0.7H_2O$: C, 60.16; H, 5.57. Found: C, 60.16; H, 5.66.

6.26. 9α-Chloro-11β,17α-dihydroxy-6α-fluoro-16α-methyl-3-oxoandrosta-1,4-diene-17β-carbothioic acid (15)

To a solution of **13** (500 mg, 1.21 mmol) in DMF (2 mL) was added carbonyl diimidazole (393 mg, 2.42 mmol) and the reaction mixture was stirred at 25 °C for 4 h. NaSH (272 mg, 4.85 mmol) was then added and stirring continued at 25 °C for 16 h. The reaction mixture was poured into a mixture of 2 M HCl and ice (50 mL). The resulting precipitate was filtered and dried in vacuo to give **15** (440 mg, 85%) as an off white solid: ^{1}H NMR (DMSO-d_6) 0.83 (d, 3H, J = 7 Hz), 0.97 (s, 3H), 1.07–1.13 (m, 1H), 1.58–1.81 (m, 6H), 2.17–2.24 (m, 1H), 2.30–2.49 (m, 2H), 2.64 (t, 1H, J = 9 Hz), 2.88–2.97 (m, 1H), 4.36 (br s, 1H), 5.53–5.70 (m, 2H), 6.10 (s, 1H), 6.29 (d, 1H, J = 10 Hz), 7.28 (d, 1H, J = 10 Hz).

6.27. 9α-Chloro-6α-fluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carbothioic acid, *S*-(chloromethyl) ester (17)

To a solution of **15** (2.0 g, 4.67 mmol) in pyridine (6 mL) was added dropwise at 25 °C 2-furoyl chloride (640 mg, 4.90 mmol). After 10 min, a solid formed and the reaction mixture was then partitioned between EtOAc (100 mL) and 1 M aq HCl (100 mL). The organic layer was washed with water, brine, dried ($MgSO_4$), then filtered and concentrated to yield **16** (1.3 g, 53%), which was used crude without further purification.

To a solution of **16** (1.0 g, 1.91 mmol) in dimethylacetamide (10 mL) was added $NaHCO_3$ (320 mg, 3.83 mmol), followed by bromochloromethane (990 mg, 7.66 mmol). The reaction mixture was stirred at 25 °C for 2 h, then diluted with EtOAc (100 mL), washed with satd $NaHCO_3$ solution, followed by water and brine then dried ($MgSO_4$). Filtration followed by concentration afforded **17** (0.95 g, 87%): ^{1}H NMR (DMSO-d_6) 0.96 (d, 3H, J = 7 Hz), 1.09 (s, 3H), 1.28–1.34 (m, 1H), 1.62 (s, 3H), 1.64–1.84 (m, 2H), 1.90 (q, 1H, J = 6 Hz), 2.23–2.30 (m, 1H), 2.53–2.61 (m, 2H), 2.80 (t, 1H, J = 9 Hz), 3.35–3.39 (m, 1H), 4.68 (br s, 1H), 5.20 (s, 2H), 5.55–5.71 (m, 1H), 5.75 (d, 1H, J = 4 Hz), 6.12 (s, 1H), 6.31 (d, 1H, J = 10 Hz), 6.71 (d, 1H, J = 3 Hz), 7.23 (d, 1H, J = 3 Hz), 7.28 (d, 1H, J = 11 Hz), 8.01 (s, 1H).

6.28. 9α-Chloro-6α-fluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carbothioic acid, *S*-(iodomethyl) ester (18)

To a solution of **17** (700 mg, 1.22 mmol) in acetone (20 mL) at 25 °C was added NaI (2.2 g, 14.69 mmol) and the reaction mixture was heated at reflux for 6 h. After this time the reaction mixture was diluted with EtOAc (100 mL), washed successively with water, 10% aq $Na_2S_2O_3$ solution, satd aq $NaHCO_3$ solution, water and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated. Trituration of the residue with MeOH afforded **17** (704 mg, 87%): ^{1}H NMR (DMSO-d_6) 0.95 (d, 3H, J = 7 Hz), 1.08 (s, 3H), 1.28–1.33 (m, 1H), 1.61 (s, 3H), 1.63–1.80 (m, 2H), 1.97 (q, 1H, J = 12 Hz), 2.22–2.29 (m, 1H), 2.47–2.59 (m, 2H), 2.79 (t, 1H, J = 9 Hz), 4.43 (br s, 1H), 4.55–4.58 (m, 2H), 5.55–5.71 (m, 1H), 5.79 (d, 1H, J = 5 Hz), 6.12 (s, 1H), 6.31 (d, 1H, J = 10 Hz), 6.71 (d, 1H, J = 3 Hz), 7.21 (d, 1H, J = 3 Hz), 7.28 (d, 1H, J = 10 Hz), 8.01 (s, 1H).

6.29. 9α-Chloro-6α-fluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxoandrosta-1,4-diene-17β-carbothioic acid, *S*-(fluoromethyl) ester (19)

Finely ground AgF (200 mg, 1.88 mmol) was added to a dispersion of **18** (250 mg, 0.38 mmol) in acetonitrile (10 mL). The reaction mixture was stirred at 25 °C for 2 h in the dark, then diluted with EtOAc (100 mL) and filtered through Celite™. The filtrate was concentrated and the residue purified by flash chromatography on silica gel using EtOAc/*n*-hexane 1:2 as the eluent. Trituration with *i*-Pr_2O afforded **19** (75 mg, 35%): mp 176–177 °C dec; Anal. HPLC t_R 7.35 min, 99%; ^1H NMR (DMSO-d_6) 0.95 (d, 3H, $J=7$ Hz), 1.07 (s, 3H), 1.28–1.33 (m, 1H), 1.61 (m, 3H), 1.63–1.77 (m, 1H), 1.84 (d, 1H, $J=14$ Hz), 1.97 (q, 1H, $J=12$ Hz), 2.23–2.29 (m, 1H), 2.50–2.67 (m, 2H), 2.76–2.81 (m, 1H), 3.31–3.42 (m, 1H), 4.44 (br s, 1H), 5.55–5.72 (2m, 1H), 5.76 (d, 1H, $J=5$ Hz), 5.89 (s, 1H), 6.02 (s, 1H), 6.12 (s, 1H), 6.31 (d, 1H, $J=10$ Hz), 6.72 (dd, 1H, $J=3,5$ Hz), 7.24 (d, 1H, $J=3$ Hz), 7.28 (d, 1H, $J=10$ Hz), 8.02 (s, 1H); MS(ES+) *m/z* 555 $(M+H)^+$; Anal. Calcd for $C_{27}H_{29}ClF_2O_6$ S: C, 58.43; H, 5.27; S, 5.78. Found: C, 58.33; H, 5.41; S, 5.57.

7. Determination of thermodynamic solubilities

Thermodynamic solubility was determined in pH 6.8 phosphate buffer using the saturation shake-flask method as described in the literature.[23]

8. Biological assay methods

8.1. In vitro methods

8.1.1. Inhibition of LPS induced TNFα release. Human macrophage cell line U937 was obtained from American Type Culture Collection (Rockville MD) and cultured in RPMI 1640 (Gibco UK) supplemented with 10% FCS (Gibco UK). Cell density was adjusted to 4×10^5 cells/mL and the cells were differentiated by adding PMA (20 ng/mL) for 4 h. The PMA was removed by washing and the adherent cells were incubated for a further 48 h at 37 °C in a humidified incubator with 5% CO_2. Differentiated U937 cells were removed using cell dissociation buffer (Gibco UK) and the cell density was adjusted to 1×10^6 cells/mL. 100 μL of the cell suspension was placed in 96 well culture plates and 50 μL of either medium or compound at the appropriate concentration in DMSO were added. After a preincubation of 20 min at 37 °C, the cells were stimulated with 10 ng/mL LPS (Sigma) and the supernatants were harvested after 24 h of incubation at 37 °C in a humidified incubator with 5% CO_2. Concentration of TNFα in the supernatants was determined by sandwich ELISA using two monoclonal antibodies recognising different epitopes of the cytokine (Pharmingen UK). Binding of the second antibody was analysed by stepwise incubation with streptavidin alkaline phosphatase conjugate (Sigma UK) and 4-nitrophenylphosphate disodium salt. Optical density was measured at 405 nm and cytokine concentration calculated based on results from serial diluations of standard recombinant TNFα. Curves were fitted and IC_{50} values calculated using Origin™ software.

8.1.2. Human glucocorticoid receptor (GR) binding assay. Recombinant human GR expressed in baculovirus–infected insect Sf-9 cells was obtained from Panvera (Madison, WI, USA), as was GR assay buffer and the proprietary fluorescent ligand Fluormone™-GS1 (200 nM methanol solution).

The assay was conducted in 384 well plates by sequential addition of a serially diluted DMSO solution of test compound in water (2 μL), Fluormone™-GS1 (2.2 nM in GR assay buffer, 10 μL) and GR solution (8.8 nM in GR assay buffer, 10 μL). The assay was incubated in the dark at room temperature for 1 h, prior to fluorescence polarisation measurement using an analyst multiwell instrument with 485 nm excitation and 530 nm emission filters. The concentration of test compound resulting in half-maximum shift in polarisation gave the IC_{50}. Curves were fitted using Origin™ software and K_i values were calculated using the Cheng–Prussoff equation.

8.1.3. Stability in plasma. A 10 μM solution of **11a** in rat or human plasma was incubated at 37 °C for 1 h. The sample was analysed by HPLC after precipitation by addition of acetonitrile.

8.2. In vivo methods

8.2.1. Preparation of dry powder blends. Approximately 100 mg of test compound was ball milled in a 1.5 mL agate grinding jar fitted with one 7 mm agate ball using a MM200 mixer mill (Glen Creston) for 20 min at 30 Hz. The milled powder was passed through a 212 μm sieve.

This material was then sandwiched between 2 aliquots of 325# lactose monohydrate (DMV) in a glass vessel. The mixture was tumble mixed (Turbula T2A) for 20 min at 50 rpm and subsequently sieved through a 212 μm sieve. This procedure was repeated a further two times and then aliquots (5×1 mg) removed and assayed by HPLC to establish blend uniformity.

8.2.2. Thymus involution studies. Male Sprague-Dawley CD1 rats (250–265 g body weight, $n=5$ to 8) were dosed once daily over 4 days either orally with compounds in Klucel suspension or i.t. as lactose blends under halothane anaesthesia. On day 5, animals were sacrificed by i.p. injection of pentobarbital and the thymus was removed and weighed. The percentage reduction in mean weights was calculated from the mean weights of vehicle treated animals. Significant differences were assessed by use of the SigmaStat™ package using the appropriate ANOVA analysis and appropriate subsequent analysis, which varied according to whether the data passed the Normality and Equal Variance tests.

8.2.3. Inhibition of OA-induced eosinophilia. Male Brown Norway rats (approximately 200 g body weight, $n=5$ to 8) were sensitised on day 1 with an i.p. injection of 0.5 mL of a mixture of ovalbumin (0.02 mg/ml) and

aluminium hydroxide (20 mg/mL), followed by Acellulare pertussis adsorbat vaccine (0.2 mL of a 1:4 dilution with 0.9% saline). The procedure was repeated on day 15 and day 21. On day 28, the test compound as a dry powder lactose blend was administered i.t. under isoflurane anaesthesia. Twenty four hours later, the sensitised dosed animals were exposed to an aerosol of ovalbumin (5 mg/mL) for 60 min and after a further 24 h they were sacrificed by i.p. injection of pentobarbital. The lungs were removed and after lavage with Hank's solution, eosinophil numbers in the recovered solution were quantified directly using a Corbas Helios 5Diff apparatus (Hoffman-LaRoche). Statistical significance was assessed with Student's *t*-test and the Hommel–Hochberg multiple comparison test.

8.2.4. Oral absorption study. Male Wistar rats (approx 230 g body weight, $n = 5$) were cannulated at the carotid artery under halothane anaesthesia. The test compound was dosed orally at 4.6 mg/kg, either a solution in 2% *N*-methyl pyrrolidinone in Placebo Neoral or a Klucel suspension. Blood samples were withdrawn at intervals for analysis up 6 h post dose and animals were sacrificed after the final sampling by i.p. injection of pentobarbital.

References and notes

1. *Lung Biology in Health and Disease*; Schleimer, R. P., O'Byrne, P. M., Szefler, S. J., Brattsand, R., Eds.; Marcel Dekker: New York, 2002; Vol. 163.
2. Buchman, A. L. *J. Clin. Gastroenterol.* **2001**, *33*, 289.
3. Rabe, K. F.; Schmidt, D. T. *Eur. Respir. J.* **2001**, *18*(Suppl 34), 34S.
4. Hoegger, P. *Curr. Med. Chem.: Anti-Inflammatory Anti-Allergy Agents* **2003**, *2*, 395–408.
5. Derendorf, H.; Hochhaus, G.; Meibohm, B.; Moellmann, H.; Barth, J. *J. Allergy Clin. Immunol.* **1998**, *101*, S440.
6. Staresinic, A. G.; Sorkness, C. A. *Exp. Opin. Pharmacother.* **2000**, *1*, 1227.
7. Angell, R. M.; Biggadike, K.; Farrell, R. M.; Flack, S. S.; Hancock, A. P.; Irving, W. R.; Lynn, S. M.; Procopiou, P. A. *J. Chem. Soc., Perkin Trans. 1* **2002**, 831.
8. Sharpe, M.; Jarvis, B. *Drugs* **2001**, *61*, 1325.
9. Bodor, N.; Buchwald, P. *Med. Res. Rev.* **2000**, *20*, 58.
10. Belvisi, M. G.; Hele, D. J. *Pulm. Pharmacol. Ther.* **2003**, *16*, 321.
11. Schmidlin, J. European Patent Application (Ciba-Geigy AG) 135476, 1985. *Chem. Abstr.* **1985**, *103*, 105215.
12. Kertesz, D. J.; Marx, M. *J. Org. Chem.* **1986**, *51*, 2315.
13. Sajjadi, F. G.; Takabayashi, K.; Foster, A. C.; Domingo, R. C.; Firestein, G. S. *J. Immunol.* **1996**, *156*, 3435.
14. Adcock, I. M. *Pulm. Pharmacol. Ther.* **2000**, *13*, 115.
15. Chung, K. F. In *Allergy and Allergic Diseases*; Kay, A. B., Ed.; Blackwell: London, 1997; pp 1068–1078.
16. *Burger's Medicinal Chemistry and Drug Discovery*, 6th ed.; Avery, M. A., Woolfrey, J. R., Abraham, D. J., Eds.; Wiley: New York, **2002**; Vol. 3, pp 747–853.
17. Chanoine, F.; Grenot, C.; Heidmann, P.; Junien, J. L. *Drug Metab. Disp.* **1991**, *19*, 546.
18. O'Hanlon, P. J.; Walker, G. European Patent Application (SmithKlineBeecham) 399645, 1990. *Chem. Abstr.* **1991**, *114*, 228892.
19. Datta, A.; Pooranchand, D.; Ila, H.; Junjappa, H. *Tetrahedron* **1989**, *45*, 7631.
20. Knight, D. W.; Nott, A. P. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1125.
21. Knight, D. W. *Tetrahedron Lett.* **1979**, *20*, 469.
22. Ichikawa, Y.; Naganawa, A.; Isobe, M. *Synlett* **1993**, *10*, 737.
23. Avdeef, A.; Berger, C. M.; Brownell, C. *Pharm. Res.* **2000**, *171*, 85.