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Design and synthesis of long acting inhaled corticosteroids for the treatment of asthma

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ABSTRACT

In this Letter we present data for a novel series of ICS for the treatment of asthma. 'Inhalation by design' principles have been applied to a series of highly potent steroidal GR agonists, with a focus on optimising the potential therapeutic index in human. Pharmacokinetic properties were tuned with high intrinsic clearance and low oral bioavailability in mind, to minimise systemic exposure and reduce systemically driven adverse events. High CYP mediated clearance as well as glucuronidation were targeted to achieve high intrinsic clearance coupled with multiple routes of clearance to minimise drug-drug interactions. Furthermore, pharmaceutical properties such as stability, crystallinity and solubility were considered to ensure compatibility with a dry powder inhaler. This work culminated in the identification of the clinical candidate **15**, which demonstrates preclinically the desired efficacy and safety profiles confirming its potential as an inhaled agent for the treatment of asthma.

Inhaled corticosteroids (ICS) as stand alone agents, or in combination with long acting β2-agonists (LABA) are highly effective treatments for asthma. The worldwide prevalence of this chronic disease is substantial with estimates of over 300 million people affected, with reports of this number rising.[1] The commercial market for ICS is therefore extremely large, with inhaled agents such as fluticasone propionate **1** and budesonide **2** (Fig. 1) achieving multi-billion dollar sales annually, either as stand alone agents, or as part of LABA combinations. The next generation ICS fluticasone furoate **3**, which is in phase 3 clinical trials as a combination with the LABA vilanterol, is also expected to capture a substantial section of the market.[2] ICS and ICS/LABA combinations are also extensively used in chronic obstructive pulmonary disease (COPD), but with steroid resistance believed to limit the efficacy of ICS in most patients.[3] The lack of significant clinical progression of novel anti-inflammatory mechansims in recent years suggests the market dominance of either ICS or ICS/LABA will not be significantly challenged in the next 10 years, and therefore ICS will remain as a necessary treatment for chronic respiratory diseases.

While ICS are generally regarded as offering a good risk/benefit profile, there exists a general discomfort with steroid safety, particularly in paediatric use, and at the higher doses generally used in COPD, for long term systemic safety. Corticosteroid efficacy in asthma is dose limited due to mechanism related adverse events such as bone metabolism, growth retardation in children, skin thinning and adrenal suppression.[4] There is therefore remaining scope for improvement by offering an ICS with a better safety/tolerability profile, and hence a greater therapeutic index (TI). Moreover, achieving once daily dosing across all severities of asthma could lead to greater patient compliance, and hence better pharmacotherapeutic control of the disease (all ICS are dosed twice daily in more severe patients).[1]

All currently available ICS have some bioavailability to the systemic circulation, whether it be through absorption through the lung, or via oral absorption from a swallowed component of the inhaled dose.[1] If the systemic exposure could be further reduced through improved pharmacokinetics, alongside greater potency and a longer duration of action (DoA), then an ICS could be pro-

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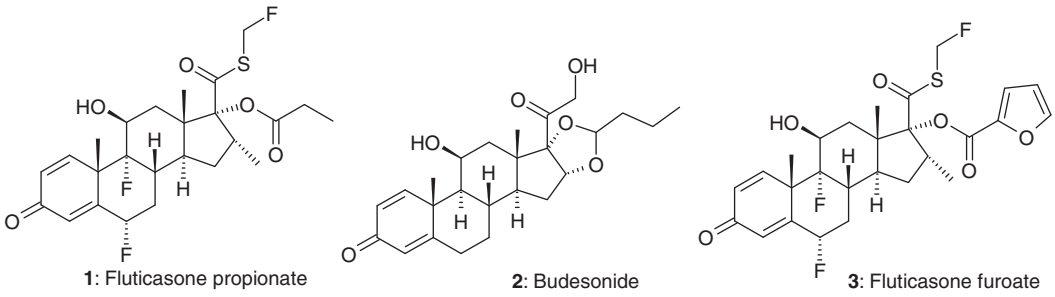


duced with a superior TI. A greater TI creates the opportunity to develop a safer ICS at the same level of efficacy as current agents, or alternatively to dose escalate and generate greater efficacy without compromising safety in comparison with current agents. This paper will detail our efforts to design a potent, long acting ICS with a superior safety profile, through improved pharmacokinetics, relative to the marketed agents.

We decided to restrict the design of a novel ICS series to the conventional steroid scaffold and utilise the D ring of the structure to engineer the desired pharmacokinetic and pharmaceutical properties required for inhalation. In order to design a successful ICS series we focussed on the 'inhalation by design' principles that have been detailed in a previous publication.[5] These include (1) high potency to achieve a low inhaled dose ($\leqslant$1 mg drug product); (2) duration of action (DoA) for a therapeutically relevant period; (3) high clearance and low oral absorption of any swallowed dose to minimise systemic exposure and therefore potential for adverse events; (4) multiple routes of clearance to minimise drug-drug interactions (DDIs); (5) physical form characteristics suitable for a dry powder inhaler including high crystallinity and compatability with lactose which is the commonly used excipient.

These design principles suggest maintaining or actually increasing the lipophilicity of a novel series, relative to marketed agents, could potentially deliver a compound with higher clearance, as well as a slower rate of absorption of drug from the lung to the systemic circulation, through a reduction in permeability and solubility. The latter concept could conceivably lead to increasing the DoA of the inhibitor at the site of action, by retaining the compound in the lung. We also hypothesised that by simply increasing affinity for the glucocorticoid receptor may also increase residency time on the receptor through a slower k_{off}.

Towards these goals, we began working on an acetal series of ICS compounds **4–15** which were prepared from a common steroidal scaffold **16** (Table 1). The commercially available acetonide **17** was first reacted with tetrafluoroboric acid to reveal the precursor **16**, which was then reacted with various aromatic aldehydes **18** under acidic dehydrating conditions to afford target compounds **4–15** (Scheme 1).[6]

Many of the aromatic aldehydes **18** were not commercially available and were therefore synthesised. These were produced by either a suzuki cross coupling reaction to produce the biaryl aldehydes leading to **4**, **6** and **9** (X = direct bond, or CH_2), or by nucleophlic aromatic substitution to afford aldehydes which went on to produce **5**, **8** and **10** (X = O, S). Aldehydes that produced **11–15** (X = SCH_2, or CH_2S) were synthesised by nucleophilic displacement of a benzylic halide with a thiophenol.

Generally this series of ICS compounds demonstrated excellent in vitro potency, whether it be in our GR agonist reporter assay or in a whole cell PBMC assay whereby the inhibition of TNFα release was measured. Initial starting points in **4** and **5** were highly potent in both the GR agonist reporter assay as well as the PBMC assay, with similar potency to the marketed agent fluticasone propionate **1**, and more potent than budesonide **2**. **6** dropped off in potency somewhat, relative to the starting points in **4** and **5**, and therefore we did not pursue biaryl analogues further. **7** demonstrated that the terminal ring system could be saturated in the form of a morpholine ring system, albeit with weaker activity in our GR reporter assay. The highly lipophilic **8** also retained excellent potency suggesting that physicochemical properties could be modulated without loss of significant potency.

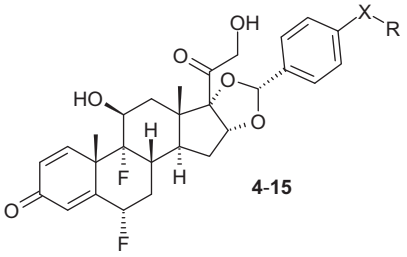
We also measured the potency of several analogues against inhibition of TNFα release from human whole blood to assess the impact of blood binding on the potency, relative to the isolated human PBMC assay. Fluticase propionate **1** and fluticasone furoate **2** showed a 93 and 169 fold shift in IC_{50}, respectively, from the PBMC to the human whole blood assay which gave an indication of the extent of binding. Compounds **4** and **8** showed an even greater shift of 252 and 474 fold, respectively, from isolated cell to whole blood suggesting even higher binding. We considered high blood binding, in combination with high unbound clearance, as another way to minimise unbound systemic exposure.

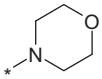
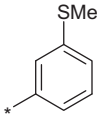
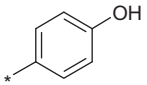
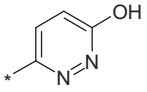
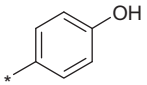
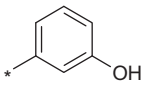
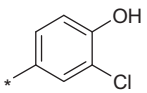
It became apparent that achieving potency in this series would not be difficult and therefore we focussed our attention on optimising the series for inhalation. As mentioned previously we were keen to follow our 'inhalation by design' principles to achieve an inhaled agent with minimal systemic exposure and hence reduce systemically driven adverse events and therefore achieving high metabolic clearance was important.[5] Furthermore, we were also keen to identify compounds that would be cleared by multiple routes so that becoming a victim to cytochrome P450 (CYP) mediated DDIs would be less likely to occur. To this end we investigated the introduction of a phenol group onto our inhibitor series to see if phase II mediated metabolism through glucuronidation could provide a second route of metabolism. Glucuronidation is well documented as a phase II metabolic pathway that has high capacity and is a major route of drug inactivation.[9]

Gratifyingly, the introduction of a phenol in **9** was well tolerated from a potency standpoint. However, the introduction of a more polar heterocycle into the terminal aromatic ring of the acetal in **10** led to a significant loss of potency in the GR reporter assay but interestingly was still potent in the PBMC assay. The reasons for this are not clear. Compounds **11** and **12** suggested that introduction of a second linking atom between the aromatic rings of the acetal was tolerated in terms of potency and therefore the phenols **13** and **14** were designed and synthesised which were also highly potent. Chloro phenol **15** was also highly potent in the GR reporter assay, as well as the PBMC assay. Furthermore, the shift from PBMC to whole blood of 291 fold suggested that **15** was much more highly bound relative to fluticasone propionate **1**. With a series of potent phenols now in hand, we sought to understand the in vitro and in vivo pharmacokinetic profile of lead compounds to investigate their potential as optimal inhaled agents with low systemic expoure, and reduced potential for CYP mediated DDI.

To understand the in vitro intrinsic clearance for CYP mediated metabolism we utilised a human liver microsome system, which could also be set up to measure the intrinsic clearance of glucuron-

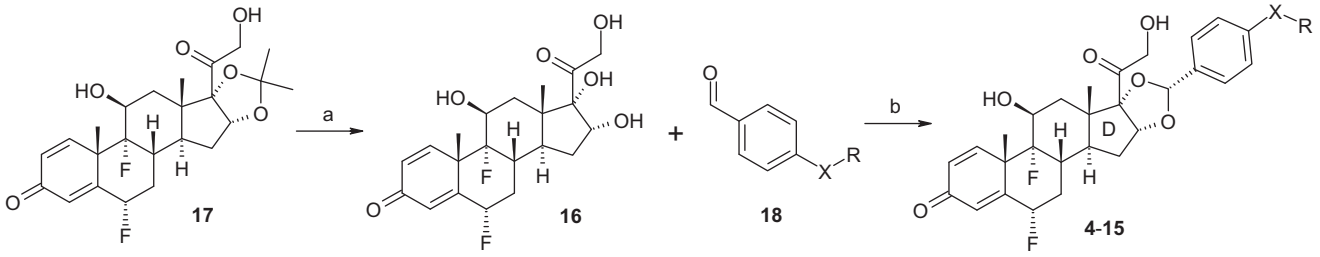
Table 1
GR agonist activity[a], inhibition of TNFα release from human PBMC[b], and whole blood[c] for target compounds **4–15**



Compound	X	R	*c* Log *P*	GR agonist[a] EC_{50} (nM)	PBMC[b] IC_{50} (nM)	Whole blood[c] IC_{50} (nM)
1, Fluticasone propionate			3.8	1.09	0.10	9.3
2, Budesonide			2.9	12.40	0.96	
3, Fluticasone furoate			4.3	0.49	0.05	8.46
4	CH_2		4.8	0.78	0.17	43
5	O		4.9	0.43	0.09	
6	Direct bond		4.7	8.78	0.41	
7	CH_2		2.5	0.71	1.81	
8	S		5.7	3.93	0.10	47.4
9	CH_2		4.2	0.38	0.04	
10	O		2.9	21.10	0.12	
11	SCH_2		4.9	7.35	0.16	
12	CH_2S		4.9	1.75	0.22	
13	CH_2S		4.2	1.87	0.06	
14	CH_2S		4.2	2.82	0.15	
15	CH_2S		4.9	2.97	0.03	8.73

[a] Glucocorticoid receptor (GR) agonist activity was determined using a reporter assay, see ref. 7 for details. Differences of <2-fold should not be considered significant.
[b] Inhibition of TNFα release from human PBMC was determined by an ELISA assay, see ref. 8 for details. Differences of <2-fold should not be considered significant.
[c] Inhibition of TNFα release from human whole blood was determined by an ELISA assay, see ref. 8 for details. Differences of <2-fold should not be considered significant.

idation. Both fluticasone propionate **1**, and lead **8**, showed high in vitro intrinsic clearance, as well as high in vivo clearance (unbound) in rat, leading to a short half life and negligible oral bioavailability. However, both showed little if any glucuronidation in the HLM UGT experiment, suggesting a potential for a CYP mediated DDI. Little glucuronidation would be expected as both



Scheme 1. General synthesis of target compounds **4–15**. Reagents and conditions: (a) 48% aq HBF_4, rt, 7 h; (b) CF_3SO_3H, $MgSO_4$, 1,4-dioxane, rt, 24 h.

fluticasone propionate **1** and **8** do not contain a phenol, although incorporation of a phenol does not always lead to high levels of glucuronidation. Phenol containing **15**, however, demonstrated high levels of glucuronidation, alongside liver blood flow clearance in the rat and therefore this compound immediately became a very interesting lead (Table 2). Metabolite identification studies from human and rat hepatocytes suggested that the major metabolite for compound **15** was indeed a glucuronide, which is likely to have much weaker agonist potency against the GR receptor. Gratifyingly when hepatocyte incubations were carried out in the absence or presence of ketoconazole (CYP 3A4 inhibitor) there was not a significant difference in intrinsic clearance observed, suggesting a reduced potential for a CYP mediated DDI. Importantly, the unbound clearance was significantly higher than that for fluticasone propionate **1** suggesting that **15** has the potential to have significantly lower unbound drug concentrations in the human systemic circulation, relative to fluticasone propionate and therefore may offer an improved TI.

With lead compound **15** suggesting it had favourable pharmacokinetic properties we were interested to further investigate the in vitro pharmacology and selectivity in relation to other GR agonists, such as fluticasone propionate **1**. Compound **15** was profiled against a number of other human nuclear hormone receptors including mineralocorticoid (MR), progesterone (PR), estrogen (ER) and androgen (AR) receptors in both agonist and antagonist format to determine selectivity as a GR agonist. **15** was more than 10 fold selective for GR agonism over PR agonism and was superior to fluticasone propionate **1** (4.1-fold). Compound **15** was a partial agonist for MR with selectivity of 4.1 fold over MR antagonism which was comparable to fluticasone propionate **1** (3.7-fold). **15** did not function as an agonist or antagonist of AR or ER. As mentioned above we were also interested in identifying a compound with slow dissociation kinetics from GR to enable a long DoA, and therefore we sought to investigate the dissociation half-life from GR of **15** relative to fluticasone propionate **1** using a competition kinetic binding methodology. **15** exhibited a slower dissociation half-life from the human receptor (1284 min) in comparison to fluticasone propionate **1** (544 min) suggesting a potential for a longer DoA in vivo.[10]

The intriguing pharmacological and pharmacokinetic properties of **15** prompted us to evaluate the in vivo potency and DoA of the compound. The PBMC potency of **15** and fluticasone propionate **1** were similar in rat and human and therefore experiments in rat were deemed suitable. Bacterial lipopolysaccharide (LPS) challenge to the lungs of rats results in acute pulmonary neutrophilia. The use of this model has been extensively cited in the literature to characterise anti-inflammatory compounds, including ICS.[11] Efficacy and DoA studies were dosed via the inhaled route to replicate the intended human delivery route. In two independent studies, **15** was dosed as an aqueous solution (containing 0.5% Tween-20) intra-tracheally 4 h prior to stimulus, which dose dependently inhibited LPS induced lung neutrophilia with an ED_{50} of 3 μg and 4.7 μg (Fig. 1). The same experiment was conducted for fluticasone propionate **1** resulting in an ED_{50} of 36 μg, suggesting **15** was indeed significantly more potent in vivo.

To investigate the in vivo DoA, **15** and fluticasone propionate **1** were dosed by intratracheal administration at various times before LPS challenge. The dose levels were chosen using ED_{50} values derived from studies dosed 1 h pre-LPS challenge for fluticasone pro-

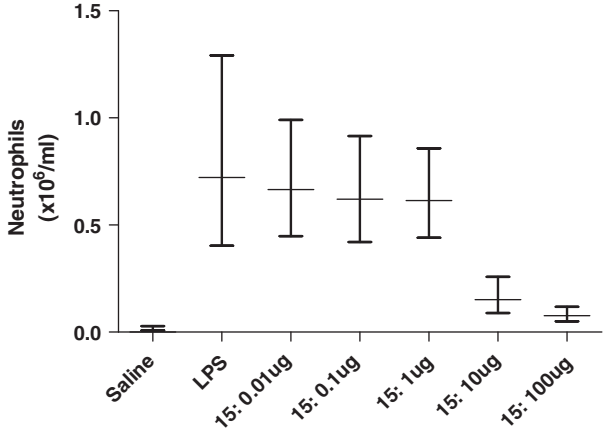


Figure 1. Dose dependent inhibition of LPS induced neutrophilia by **15**.

Table 2
In vitro and in vivo pharmacokinetic properties for compounds **1**, **8**, and **15**

Compound	HLM Clint (Clintu) [a] (μL/min/mg)	HLM UGT[b] (μL/min/mg)	Rat IV Clb (Clu)[c] (mL/min/Kg)	Rat PO F[d] F (%)	$T_{1/2}$ (h)
1 Fluticasone propionate	51 (300)	<3.5	56 (1800)	<2	1.1
8	154 (>3080)	<3.5	26 (>18000)	<0.5	1.9
15	122 (121000)	100	84 (>59000)	<1	2.8

[a] Compounds were assessed for metabolic stability in human liver microsomes (HLM), and the unbound Clint (Clintu) also determined by first determining the microsomal binding by equilibrium dialysis.
[b] Compounds were assessed for phase II glucuronidation in HLM by including the co-factor UDPGA, and excluding the cytochrome P450 dependent co-factor NADPH.
[c] Intravenous (IV) pharmacokinetics were determined in rats from a 0.5 mg/Kg dose. The unbound clearance was calculated after measuring the plasma protein binding, and blood binding.
[d] Oral (PO) pharmacokinetics were determined from a 2 mg/Kg dose.

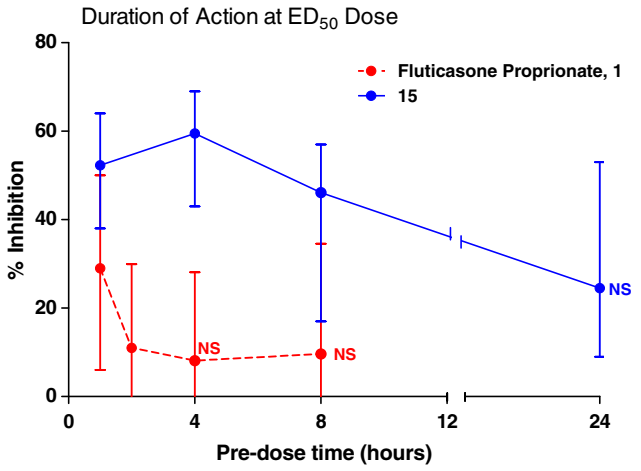


Figure 2. Duration of action of **15**, and fluticasone propionate **1** in rat LPS neutrophilia model (NS-not statistically significant from vehicle).

pionate **1** (10 μg) and 4 h pre-LPS challenge for **15** (4.3 μg). Fluticasone propionate **1** inhibited neutrophilia by 29% at 1 h, but declined to 11%, 8% and 10% at 2, 4 and 8 h, respectively. **15**, however, inhibited neutrophilia by 52% at 1 h, 59% at 4 h, 46% at 8 h, and 24% at 24 h suggesting that **15** has the potential to have a significantly increased DoA relative to fluticasone propionate **1** (Fig. 2).

Compound **15** has also demonstrated suitable pharmaceutical properties, such as crystallinity, stability on lactose (commonly used inhaled excipient) and solubility. The simulated lung fluid solubility[12] of **15** is 0.36 μg/ml, which is comparable to fluticasone propionate **1** (0.32 μg/ml). The preclinical safety profile also suggests **15** is suitable to take forward to human trials.

In summary, we have described our approaches toward the identification of a novel series of GR agonists, which were optimised for potency, and pharmacokinetic properties suitable for an inhaled drug. Compound **15** was subsequently identified and shown to possess high potency for the GR receptor, both in vitro and in vivo, and non-inferior selectivity against a panel of human nuclear hormone receptors, relative to the marketed agent fluticasone propionate **1**. The slow in vitro dissociation kinetics against the human receptor was shown to translate into a relatively long DoA in an in vivo experiment, when compared to fluticasone propionate **1**. The in vitro and in vivo pharmacokinetic profile suggests the compound will display low oral bioavailability in humans following any swallowed dose, coupled with very high intrinsic clearance such that any lung absorbed drug will be rapidly metabolised to less potent metabolites. Moreover, **15** is metabolised by both phase I and II pathways and therefore should not be a victim or a perpetrator of any DDIs. The pharmaceutical properties of **15** are suitable for combination with lactose in a dry powder inhaler, and finally the compound has demonstrated a preclinical safety profile suitable to move forward into human trials. This promising overall profile has led to the nomination of compound **15** as a development candidate for the treatment of asthma. Further information relating to the research and development of compound **15** will be reported in due course.

Acknowledgments

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7. The potency of marketed agents **1–3** and test compounds **4–15** as a GR agonist was assessed using a human cell-line (SW1353, a human bone carcinoma cell-line) expressing endogenous GR and stably expressing an MMTV-luciferase reporter construct. GR agonism was assessed by the ability of marketed agents **1–3** and test compounds **4–15** to bind the endogenous GR, transactivate the MMTV promoter and drive expression of luciferase. At least $n = 2$ determinants were used to quote an EC_{50}.
8. The potency and efficacy of marketed agents **1–3** and test compounds **4–15** was assessed by measuring the inhibition of TNFα release from primary human peripheral blood mononuclear cells (PBMC) and human whole blood (WB) following LPS stimulation, where stated. The cellular composition of human PBMC preparations was typically: lymphocytes (79.3% ± 1.3), monocytes (19.2% ± 1.2), neutrophils (2.1% ± 0.3) and basophils (0.4% ± 0.0). The potency and efficacy of marketed agents **1–3** and test compounds **4–15** was also assessed in rat PBMC and WB to support translation to in vivo models, where stated. At least $n = 3$ determinants were used to quote an IC_{50}.
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10. Binding kinetics were determined by a competition assay with radio-labelled ligand. [^{3}H]-Dexamethasone at 5 nM was incubated together with recombinant GR ligand-binding domain and three different concentrations of test compound for various time points. The association of the radio-ligand was examined over time in the presence and absence of the test compound. Experiments were performed at 4 °C to maintain protein stability and carried out over 3 days. The altered profile of the ligand association in the presence of the test compound allowed the kinetics (k_{on} and k_{off}) of the test compound to be determined. The $T_{½}$ for each compound was then calculated as ln (2)/k_{off}. Because of assay to assay variation in determining k_{on} and k_{off} for [^{3}H]-dexamethasone, non-labelled dexamethasone was always tested as a reference standard so that ratios for $T_{½}$ could be calculated, thus standardising data. All statistical tests were performed on this ratio data.
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12. The simulated lung fluid solubility is determined using an aqueous solution containing 0.02%w/v DPPC and 5% v/v dog plasma protein and assessed by the dialysis bag method.