Bioorganic & Medicinal Chemistry Letters 19 (2009) 4846–4850

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Highly tractable, sub-nanomolar non-steroidal glucocorticoid receptor agonists

Keith Biggadike, Matilde Caivano, Margaret Clackers, Diane M. Coe, George W. Hardy, Davina Humphreys, Haydn T. Jones, David House, Annette Miles-Williams, Philip A. Skone, Iain Uings, Vicki Weller, Iain M. McLay, Simon J. F. Macdonald *

Medicines Research Centre, Respiratory CEDD, GlaxoSmithKline, Gunnels Wood Road, Stevenage SG1 2NY, United Kingdom

ARTICLE INFO

Article history:
Received 14 April 2009
Revised 3 June 2009
Accepted 5 June 2009
Available online 13 June 2009

Keywords:
Glucocorticoid
Agonist
Non-steroidal
Tractable

ABSTRACT

Starting from a non-steroidal glucocorticoid agonist aryl pyrazole derivative, the NFκB agonist activity was optimised in an iterative process from pIC_{50} 7.5 (for **7**), to pIC_{50} 10.1 (for **38E1**). An explanation for the SAR observed based is presented along with a proposed docking of **38E1** into the active site of the glucocorticoid receptor.

Glucocorticoid receptor (GR) agonists have been used for many years as anti-inflammatory agents for treating a whole spectrum of conditions including asthma and rheumatoid arthritis.[1] Whilst steroids such as prednisolone and dexamethasone are used orally, fluticasone propionate **1** is commonly used in the clinic as an inhaled treatment for asthma and rhinitis.[2] Fluticasone furoate (Veramyst® or Avamys®) **2** is a new GR agonist approved by the FDA in 2007 for the once daily intranasal therapy for seasonal allergic rhinitis.[3] Fluticasone propionate sales for intranasal and inhaled treatments in 2007 totalled over £800 million.[4] Fluticasone propionate is also a component of Advair® (or Seretide®) as an inhaled treatment for asthma and chronic obstructive pulmonary disease sales of which totalled £3.4 billion in 2007.[4] Recent reports for new inhaled corticosteroids (steroidal in structure) include ciclesonide (approved by the FDA in 2008 and marketed as Alvesco®),[5] androstadiene C-17 esters from Sandham at Novartis[6] and other androstene derivatives from GSK,[7] but the principle research focus has been the search for non-steroidal GR agonists which maintain anti-inflammatory activity whilst having reduced side effects.[8]

Fluticasone furoate **2** is a fully efficacious sub-nanomolar GR agonists in a cellular NFκB assay with pIC_{50} potency of 10.4 (108%) (cf. dexamethasone pIC_{50} 9.0 (102%)). This extremely high potency means that only very small doses of drug are used in the clinical setting (e.g., a total of 110 μg per is administered once daily for adults).[3] One aspect of our programme of research activities into glucocorticoid agonists is to investigate whether agonists of similar potency could be developed with non-steroidal structures. Such compounds might form the basis for future inhaled or intranasal therapies for respiratory disease. We describe here a series of compounds with sub-nanomolar potency which, as far as we are aware, are among the most potent non-steroidal agonists described in the literature so far.

Previously we have described the evolution of a highly tractable series of GR agonists with lower lipophilicity. These originated from a tetrahydronaphthalene aryl pyrazole series[9] represented by **3** (NFkB pIC_{50} 8.8 (108%)) (Fig. 1). With the aid of modelling, we successfully discovered a tractable replacement for the tetrahydronaphthalene with increased polarity as represented by the ethyl benzene sulfonamide **4** which proved the starting point for the development of orally active agonists.[10,11]

As previously described,[9] examination of the hydrogen-bonding interactions of the amino-pyrazole **3** showed that that there was a hydrogen bond between one of the hydrogen atoms on the amino group and the oxygen of the proximal carbonyl of the amide forming a six membered ring. We were intrigued as to whether replacing the hydrogen bond with bicyclic systems such as the pyrazolopyrimidine **5** or indazole **6** would show functional activity (and potentially increased activity) particularly when combined with the new sulfonamide left hand side.

The pyrazolopyrimidine sulfonamide **13** was prepared by initially protecting the known aminopyrazolopyrimidine[12] as its *t*-butyl carbamate **9**. Deprotonation and reaction with the epoxytosylate[13] **10** gave the epoxide **11** which was opened with ethylamine and the resultant secondary amine sulfonylated to give **13** (Scheme 1).

* Corresponding author.
E-mail address: simon.jf.macdonald@gsk.com (S.J.F. Macdonald).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.06.020

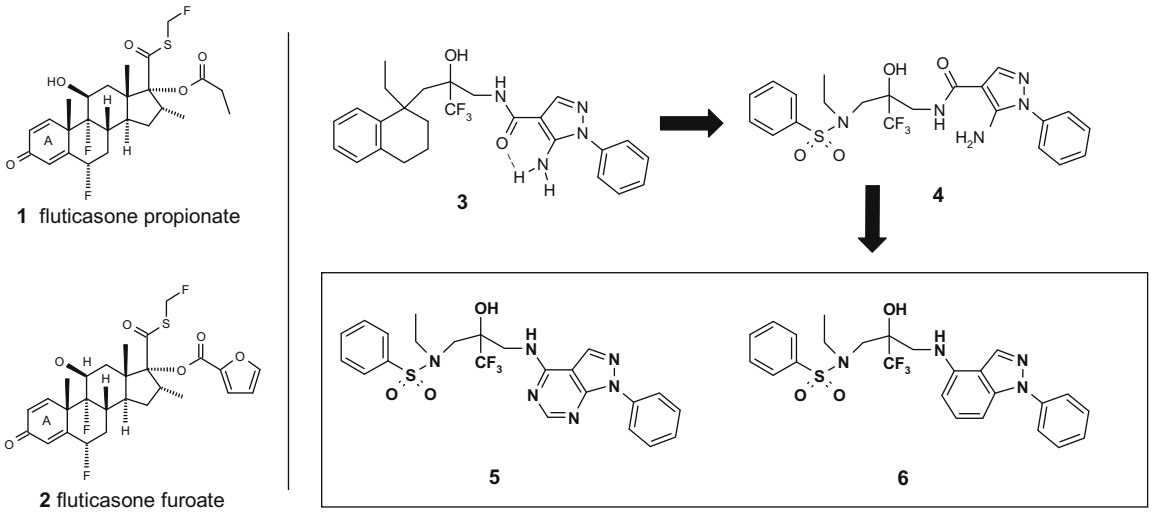
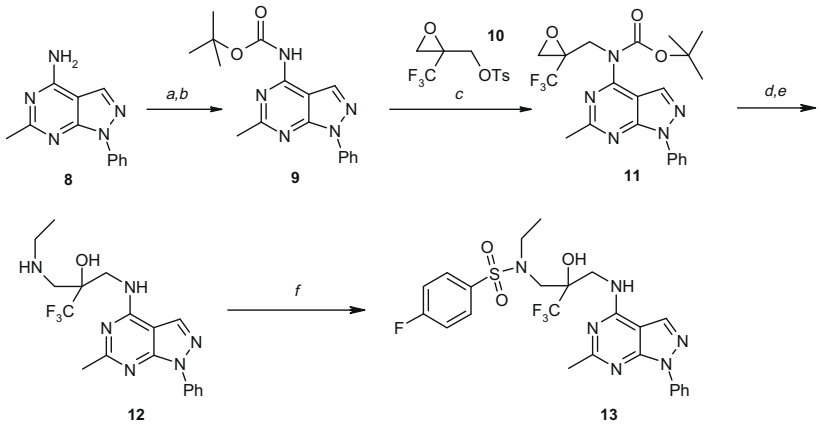
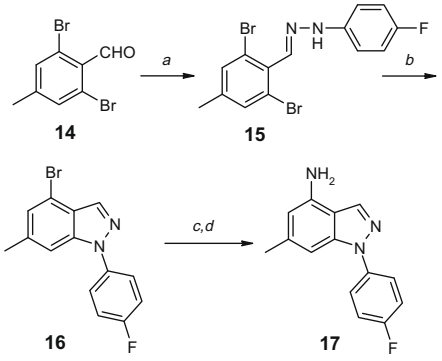


Figure 1. Steroidal (**1**–**2**) and non-steroidal glucorticoid agonists (**3**–**6**).



Scheme 1. Reagents: (a) $(^tBuO_2C)_2O$ (2 equiv), 4-dimethylaminopyridine, THF; (b) aqueous KOH or silica gel, 80% over two steps; (c) **10**, NaH, DMF, 35%; (d) $EtNH_2$, MeCN; (e) CF_3CO_2H, CH_2Cl_2; (f) $FC_6H_4SO_2Cl$, $^iPr_2NEt$, CH_2Cl_2, 32% yield over three steps.

4-Amino-6-methyl-*N*-aryl indazoles (and related analogues which are not commercially available) were made according to the routed exemplified in Scheme 2. Methyldibromobenzaldehyde[14] **14** was converted to the arylhydrazone **15** prior to a palladium catalysed cyclisation and then a palladium catalysed amination to afford **17**.[15]



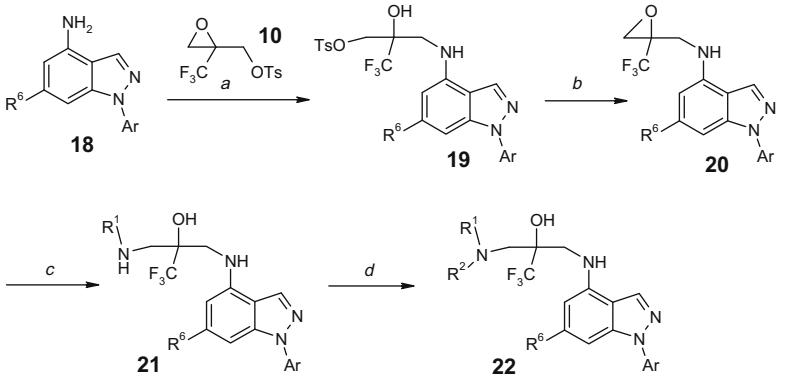
Scheme 2. Reagents: (a) 4-$FC_6H_4NHNH_2$, NaOAc, MeOH, 85%; (b) racemic 1,1′-[1,1′-binaphthalene]-2,2′-diylbis[1,1-diphenylphosphine (BINAP), tris(dibenzylideneacetone)dipalladium ($Pd_2(dba)_3$), PhMe, 39%; (c) NaO^{t}Bu, racemic BINAP, Pd_2dba_3, PhC(NH)Ph, PhMe; (d) aqueous HCl, THF, 79% over two steps.

The aryl indazoles were then reacted with the epoxytosylate **10** mediated by bismuth(III) chloride to give the tosylate **19** which was converted into the corresponding epoxides **20** using polymer supported carbonate resin. Epoxide opening with a primary amine was followed by acylation or sulfonylation to give the target compounds represented by **22** (Scheme 3).

A functional GR agonist assay was carried out using human A549 lung epithelial cells.[9] This assay allows determination of the ability of compounds to repress transcription (i.e., transpression). Efficacy is expressed as a percentage of the dexamethasone response.[16]

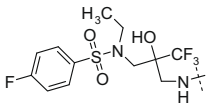
Based on the 5-aminopyrazole derivative **7** it was decided to initially replace the intramolecular hydrogen bond between the pyrazole NH_2 and the carbonyl of the amide with a bicyclic system such as the pyrazolopyrimidine **13** (the 6-methyl group being included for ease of synthesis). In the transrepression NFκB assay both **7** and **13** have equivalent potency and efficacy (Table 1).[16] The isosteric indazole **23** showed a half log unit increase in activity to NFκB pIC_{50} 8.1, and given measured lipophilicity values of pyrazolopyrimidines and indazoles are similar, and given also the increased tractability of the indazoles, we switched predominantly to this series.

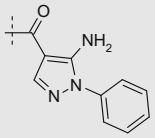
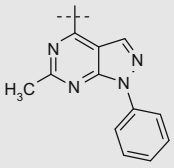
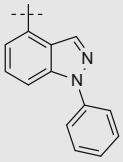
Attention was then focussed on the sulfonamide linker group in the indazoles and the nature of the N-substituent referred to as the



Scheme 3. Reagents: (a) **1**, $BiCl_3$, CH_2Cl_2; (b) polymer supported carbonate resin, CH_2Cl_2; (c) R^1NH_2, MeCN; (d) R^2CO_2H, 1-[bis(dimethylamino)methylene]-, 3-oxide 1*H*-1,2,3-triazolo[4,5-*b*]pyridinium hexafluorophosphate (HATU), $^iPr_2NEt$, DMF or R^2COCl, $^iPr_2NEt$, THF, or R^2SO_2Cl, $^iPr_2NEt$, CH_2Cl_2.

Table 1
Comparison of a phenyl pyrazole with its analogous pyrazolopyrimidine and indazole (all compounds racemic unless otherwise stated)



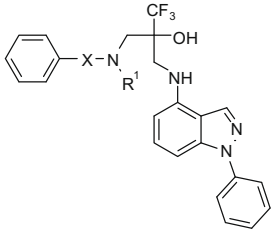
	Structure	NFκB[a]		
		pIC_{50}	% Max	*n*[b]
7		7.5 ± 0.1	86 ± 6	8
13		7.6 ± 0.1	91 ± 3	8
23		8.1 ± 0.1	94 ± 6	8

[a] The efficacy maximal responses are quoted as a percentage of the maximum of dexamethasone set at 100%.
[b] *n* = number of repeats.

'R^1 agonist trigger' due to its profound ability to control the level of agonist (NFκB) potency[17] (Table 2). Replacing the sulfonamide with an amide results in a 0.5 log unit jump in agonist potency; this together with the greater commercial availability of acids and the reduced lipophilicity of amides compared with sulfonamides (clog *P* values are ca. 0.5 units lower) led to switching to the amide series. Comparing methyl, ethyl and *n*-propyl, the most potent agonist trigger proved to be ethyl (as in **24**)—this trigger combined with an amide linker gave dexamethasone like NFκB potency with a pIC_{50} 9.1 (107%).[10,18]

Some simple substitutions on the phenyl indazole whilst keeping the left hand aryl amide as either the phenyl amide or the *o*-fluoroaryl amide both with an R^1 ethyl agonist trigger were then investigated (Table 3). Previous SAR suggested the 6-position of the indazole should be investigated whilst previous literature[19] suggested inclusion of a *para*-fluoro on the aryl ring attached to

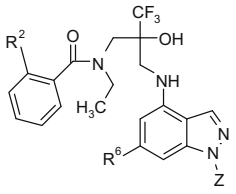
Table 2
Comparison of indazole sulfonamides with indazole amides and trigger (R^1) groups (all compounds racemic unless otherwise stated)[a]



	X	R^1	NFkB[a]		
			pIC_{50}	% Max	*n*
6	SO_2	Et	8.6 ± 0.4	98 ± 8	6
24	CO	Et	9.1 ± 0.3	107 ± 2	4
25	CO	Me	8.3 ± 0.2	98 ± 3	7
26	CO	^{n}Pr	8.5 ± 0.1	105 ± 6	8

[a] See Table 1 for notes.

Table 3
Comparison of the indazole substituents (all compounds racemic unless otherwise stated)[a]



	R^2	R^6	Z	NFkB[a]		
				pIC_{50}	% Max	*N*
24	H	H	Ph	9.1 ± 0.3	107 ± 2	4
27	H	Me	Ph	9.4 ± 0.2	108 ± 5	5
28	H	H	4-FC_6H_4–	9.1 ± 0.2	104 ± 5	8
29	H	Me	4-FC_6H_4–	9.4 ± 0.3	105 ± 5	8
30	F	H	Ph	9.5 ± 0.2	102 ± 6	8
31	F	Me	Ph	9.6 ± 0.3	106 ± 3	8
32	F	H	4-FC_6H_4–	9.7 ± 0.2	106 ± 4	6
33	F	Me	4-FC_6H_4–	9.7 ± 0.4	106 ± 6	8
32E1[b]	F	H	4-FC_6H_4–	9.9 ± 0.1	105 ± 2	8
32E2[b]	F	H	4-FC_6H_4–	7.5 ± 0.2	91 ± 6	8

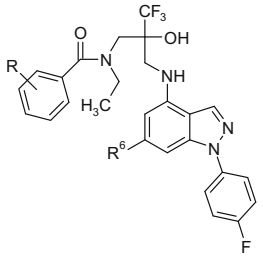
[a] See Table 1 for notes.
[b] E1 and E2 indicate enantiomer 1 and 2 of the racemate respectively.

the indazole N1. Whilst none of these changes alone make a significant difference, in combination, potency does increase. Thus introduction of a *para*-fluoro in the aryl group attached to N1 of the indazole has almost no effect on the potency (compare **24** NFκB pIC_{50} 9.1 with **28** NFκB pIC_{50} 9.1 or compare **31** NFκB pIC_{50} 9.6 with **33** NFκB pIC_{50} 9.7). On replacing the R^6 indazole hydrogen with a methyl group there is a trend for slight increase in potency with the *less* potent compounds. For example, compare **24** NFκB pIC_{50} 9.1 and **27** NFκB pIC_{50} 9.4 and then **32** NFκB pIC_{50} 9.7 with **33** NFκB pIC_{50} 9.7. In fact the R^2 substituent on the benzamide probably contributes most to potency with a trend of around a 0.3 increase in activity when an *ortho*-fluorine is incorporated. For example, compare **24** NFκB pIC_{50} 9.1 with **30** NFκB pIC_{50} 9.5 or **29** NFκB pIC_{50} 9.4 with **33** NFκB pIC_{50} 9.7. The most potent compounds are **32** and **33** both with pIC_{50} potencies of 9.7. Separation of the enantiomers[20] of **32** gave **32E1** (enantiomer 1) with an NFκB pIC_{50} 9.9 and with the second enantiomer **32E2** still active but significantly less potent with an NFκB pIC_{50} 7.5. These findings led to a final round of optimisation where substitution of the benzamide was explored.

Optimisation of benzamide substitution was carried out with the *p*-fluoroarylindazole with R^6 = H (as in **32**) given the similarities in potency with R^6 = methyl (as in **33**). A wide range of benzamide substituents were prepared (Table 4 shows a selection). *Meta* and *para* substituents whilst tolerated, are several orders of magnitude less potent (for example *m*-CF_3 and *p*-Et have pIC_{50} 7.6 (106%) and 7.7 (104%) respectively). Similarly polar *ortho* substituents, in a related series,[10] whilst active are much less potent. A variety of lipophilic *ortho* substituents are much preferred such as **34** methyl (pIC_{50} 9.5) or **35** chloro (pIC_{50} 9.6). Di-*ortho* substitution such as **36** 2,6-dimethyl (pIC_{50} 9.3), **37** 2,6-difluoro (pIC_{50} 9.5) and **38** 2,6-dichloro (pIC_{50} 9.9) are similarly active to the mono-substituents as racemates. These di-chloro compounds were separated into their enantiomers[20] with the most potent enantiomer being **38E1** the 2,6-dichloro analogue with NFκB pIC_{50} 10.1 (105%)—in the same potency range as fluticasone furoate (pIC_{50} 10.4). This represents one of the most potent non-steroidal glucocorticoid agonist reported to date.

The *aryl pyrazole* **39** (a direct analogue of the *indazole* **38E1**) has been crystallised in the GR ligand binding domain and has been reported recently.[21] This structure (Fig. 2a) clearly shows the positioning of the arylpyrazole, the central trifluoromethyl and hydroxyl groups, the terminal benzamide moiety and three hydrogen bonds holding the ligand in the site. The possible impact of cyclisation was explored through docking experiments using the protein from the pyrazole crystal structure and docking into this protein the directly equivalent structure from the indazole series (**38**). Docking was carried out using Flo+[21] and allowed all the side chains in the active site the freedom to move. It can be seen (Fig. 2a–c) that cyclisation has minimal impact on the placement of the ligand in the site with all portions of the molecule assuming similar positions and the three hydrogen bonds retained. This is perhaps unsurprising given the 6-membered ring caused by intramolecular hydrogen-bonding between the NH_2 group on the pyrazole and the carbonyl of the amide.[20]

Table 4
Optimisation of benzamide substituents (all compounds racemic unless otherwise stated)[a]



	R	R^6	NFkB[a]		
			pIC_{50}	% Max	*n*
32	2-F	H	9.7 ± 0.2	106 ± 4	6
34	2-Me	H	9.5 ± 0.2	108 ± 6	6
35	2-Cl	H	9.6 ± 0.3	103 ± 4	8
36	2-Me, 6Me	H	9.3 ± 0.4	109 ± 6	8
37	2-F, 6-F	H	9.5 ± 0.3	109 ± 7	8
38	2-Cl, 6-Cl	H	9.9 ± 0.2	105 ± 2	4
38E1[b]	2-Cl, 6-Cl	H	10.1 ± 0.1	105 ± 5	16
38E2[b]	2-Cl, 6-Cl	H	8.0 ± 0.2	98 ± 7	15

[a] See Table 1 for notes.
[b] E1 and E2 indicate enantiomer 1 and 2 of the racemate respectively.

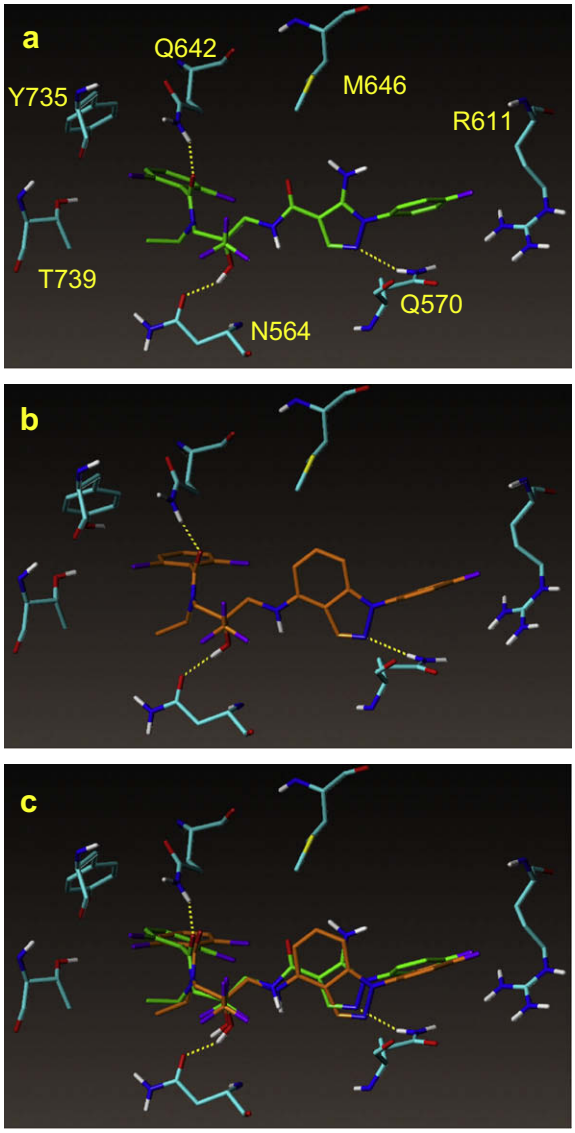


Figure 2. (a) Crystal structure ligand orientation for aryl pyrazole **39** (in green). Hydrogen bonds are shown as dotted yellow lines. (b) Docking pose for **38** (in orange) in the crystal structure protein; note the minimal side chain movement from crystal structure form; (c) Structures for **38** and **39** superimposed (for clarity side chains for crystal form only shown).

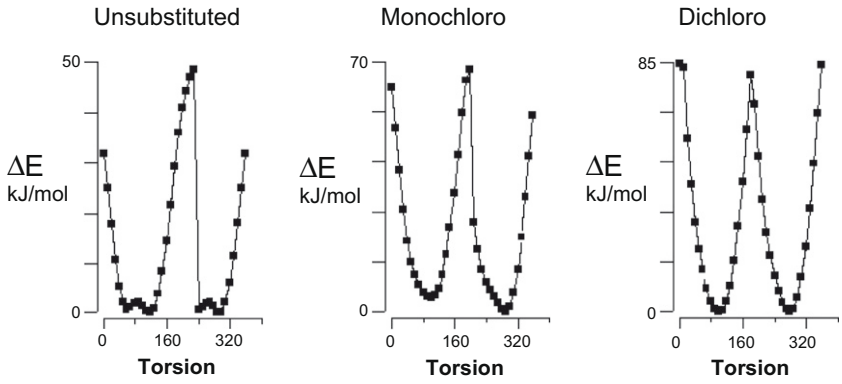


Figure 3. Changes in energy against torsional scans for the unsubstituted **28**, mono-chloro **35** and dichloro **38** indazole derivatives around the benzamide phenyl to carbonyl bond. Increments of 10° were used for the scan and for each step in the scan the structures were allowed to minimise whilst holding the torsion fixed. Energies are relative to the lowest value found in the scan.

It was noted that *ortho* substitution of the benzamide phenyl ring had a positive effect on the agonist activity of the indazoles. Conformational analysis was performed to explore the effect of *ortho* substitution. Torsional scans were performed around the phenyl to carbonyl bond for the unsubstituted **28**, *o*-chloro **35** and *o*-dichloro **38** analogues using Macromodel and the OPLS 2005 force field. The results are presented as torsional plots (Fig. 3). For the more potent compounds (mono-*ortho* **35** and di-*ortho* **38**) it is clear that the preferred conformation has the phenyl ring orthogonal—twisted at ~90°—to the plane of the carbonyl, which is also the conformation found in the crystal structure (Fig. 2).[21] The conformational preferences are the same for both, but the energy well for the dichloro derivative is deeper. The unsubstituted derivative shows a rather different pattern. The same orthogonal twisted conformations are available, but the well is much broader with many more additional conformations accessible. This greater conformational freedom could be the cause of the lower activity seen with the unsubstituted compounds.

In summary, this paper describes the discovery of highly potent non-steroidal glucocorticoid agonists. The dichloro derivative **38E1** has a profile consistent with inhaled steroidal glucocorticoid drugs—it is extremely potent, has high lipophilicity and molecular weight. This physicochemical profile, whilst unsuitable for drugs dosed by the oral route, are in fact frequently found in inhaled steroidal glucocorticoids on the market today.[22]

References and notes

1. Schimmer, B. P.; Parker, K. L. In *Goodman s The Pharmacological Basis of Therapeutics*; Hardman, J. G., Limbird, L. E., Gilman, A. G., Eds., 10th ed.; McGraw-Hill: New York, 2001; p 1649.
2. Hughes, J. *Rev. Contemp. Pharmacother.* **1998**, *9*, 569.
3. McCormack, P.L.; Scott, L.J. *Drugs* **2007**, *67*, 1905; Anonymous, *The Medical Letter* **2007**, *49*, 90.
4. GlaxoSmithKline 2007 Annual Report http://www.gsk.com/investors/annual-reports.htm.
5. Humbert, M. *Exp. Opin. Invest. Drugs* **2004**, *13*, 1349.
6. Sandham, D. A.; Barker, L.; Beattie, D.; Beer, D.; Bidlake, L.; Bentley, D.; Butler, K. D.; Craig, S.; Farr, D.; Ffoulkes-Jones, C.; Fozard, J. R.; Haberthuer, S.; Howes, C.; Hynx, D.; Jeffers, S.; Keller, T. H.; Kirkham, P. A.; Maas, J. C.; Mazzoni, L.; Nicholls, A.; Pilgrim, G. E.; Schaebulin, E.; Spooner, G. M.; Stringer, R.; Tranter, P.; Turner, K. L.; Tweed, M. F.; Walker, C.; Watson, S. J.; Cuenoud, B. M. *Bioorg. Med. Chem.* **2004**, *12*, 5213.
7. Biggadike, K.; Needham, D. PCT Int. Appl., WO 2005005452, 2005.
8. Schacke, H.; Berger, M.; Hansson, T. G.; McKerrecher, D.; Rehwinkel, H. *Exp. Opin. Ther. Patents* **2008**, *18*, 339; Takaashi, H.; Razavi, H.; Thomson, D. *Curr. Top. Med. Chem.* **2008**, *8*, 521; Mohler, M. L.; He, Y.; Wu, Z.; Hong, S.-S.; Miller, D. D. *Exp. Opin. Ther. Patents* **2007**, *17*, 37. These papers provide succinct reviews of the non-steroidal GR agonist and contain many leading references.
9. Clackers, M.; Coe, D. M.; Demaine, D. A.; Hardy, G. W.; Humphreys, D.; Inglis, G. G. A.; Johnston, M. J.; Jones, H. T.; House, D.; Loiseau, R.; Minick, D. J.; Skone, P. A.; Uings, I.; McLay, I. M.; Macdonald, S. J. F. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4737.
10. Barnett, H. A.; Coe, D. M.; Cooper, A. W. J.; Jack, T. I.; Jones, H. T.; Macdonald, S. J. F.; McLay, I. M.; Rayner, N.; Sasse, R. Z.; Shipley, T. S.; Skone, P. A.; Somers, G. I.; Taylor, S.; Uings, I. J.; Woolven, J. M.; Weingarten, G. G. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 158.
11. Akouvi, O.; Heather Barnett, H.; Biggadike, K.; Coe, D. M.; Cubbage, K.; Diallo, H.; Evans, D.; Hardy, G. W.; Humphreys, D.; Jones, H. T.; Macdonald, S. J. F.; McLay, I. M.; Rayner, N.; Shipley, T.; Skone, P. A.; Uings I.;Weingarten, G. G. *Bioorg. Med. Chem. Lett.*, manuscript in preparation.
12. Biggadike, K.; House, D.; Inglis, G. G. A.; Macdonald, S. J. F.; McLay, I. M.; Skone, P. A. PCT Int. Appl. WO2007054294, 2007.
13. Keeling, S. P.; Campbell, I. B.; Coe, D. M.; Cooper, T. W. J.; Hardy, G. W.; Jack, T. I.; Jones, H. T.; Needham, D.; Shipley, T. J.; Skone, P. A.; Sutton, P. W.; Weingarten, G. A.; Macdonald, S. J. F. *Tetrahedron Lett.* **2008**, *49*, 5101.
14. Lulinski, S.; Serwatowski, J. *J. Org. Chem.* **2003**, *68*, 5384.
15. Eldred, C. D.; House, D.; Inglis, G. G. A.; Macdonald, S. J. F.; Skone, P. A. PCT Int. Appl. WO 2006108699, 2006.
16. The compounds were tested for their ability to bind to GR using competition experiments with fluorescent-labelled dexamethasone (see Ref. 9). The tight binding limit of the assay is about pIC_{50} 8.5. pIC_{50} values of all the analogues described here are between 7.5 and 8.5. All compounds described show excellent selectivity over other nuclear receptors (androgen, progesterone and estrogen receptors) with pIC_{50} values in the respective binding assays showing 50–1000 fold selectivity over GR. All the compounds described are full agonists in a transactivation assay (see Ref. 9).
17. Barker, M.; Clackers, M.; Copley, R.; Demaine, D. A.; Humphreys, D.; Inglis, G. G. A.; Johnston, M. J.; Jones, H. T.; Haase, M. V.; House, D.; Loiseau, R.; Nisbet, L.; Pacquet, F.; Skone, P. A.; Shanahan, S. E.; Tape, D.; Vinader, V. M.; Washington, M.; Uings, I.; Upton, R.; McLay, I. M.; Macdonald, S. J. F. *J. Med. Chem.* **2006**, *49*, 4216.
18. A similar pattern of agonist trigger potency was observed in the aryl pyrazole series (see Ref. 9).
19. Hannah, J.; Kelly, K.; Patchett, A. A.; Steelman, S. L.; Morgan, E. R. *J. Med. Chem.* **1975**, *18*, 168.
20. Compound **32** was separated into its enantiomers **32E1** and **32E2** on a 25 cm Chiralpak AD column eluting at 1 mL/min with 50% ethanol in heptane containing 0.1% trifluoroacetic acid over 30 min. **32E1** eluted around 9.0 min and **32E2** eluted around 16.3 min. **38** was separated into its enantiomers **38E1** and **38E2** on a 25 cm Chiralpak AD column eluting at 1 mL/min with 40% ethanol in heptane over 30 min. **38E1** eluted around 5.4 min and **38E2** eluted around 9.1 min.
21. Madauss, K. P.; Bledsoe, R. K.; Mclay, I.; Stewart, E. L.; Uings, I. J.; Weingarten, G.; Williams, S. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6097.
22. Ritchie, T.; Luscombe, C. N.; Macdonald, S. J. F. *J. Chem. Inf. Model.* **2009**, *49*, 1025.