Journal Pre-proofs

The development of highly potent and selective small molecule correctors of Z α_1-antitrypsin misfolding

John Liddle, Andrew C. Pearce, Christopher Arico-Muendel, Svetlana Belyanskaya, Andrew Brewster, Murray Brown, Chun-wa Chung, Alexis Denis, Nerina Dodic, Anthony Dossang, Peter Eddershaw, Diana Klimaszewska, Imran Haq, Duncan S. Holmes, Alistair Jagger, Toral Jakhria, Emilie Jigorel, Ken Lind, Jeff Messer, Margaret Neu, Allison Olszewski, Riccardo Ronzoni, James Rowedder, Martin Rüdiger, Steve Skinner, Kathrine J. Smith, Lionel Trottet, Iain Uings, Zhengrong Zhu, James A. Irving, David A. Lomas

PII: S0960-894X(21)00199-2
DOI: https://doi.org/10.1016/j.bmcl.2021.127973
Reference: BMCL 127973

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 2 December 2020
Revised Date: 5 March 2021
Accepted Date: 13 March 2021

Please cite this article as: Liddle, J., Pearce, A.C., Arico-Muendel, C., Belyanskaya, S., Brewster, A., Brown, M., Chung, C-w., Denis, A., Dodic, N., Dossang, A., Eddershaw, P., Klimaszewska, D., Haq, I., Holmes, D.S., Jagger, A., Jakhria, T., Jigorel, E., Lind, K., Messer, J., Neu, M., Olszewski, A., Ronzoni, R., Rowedder, J., Rüdiger, M., Skinner, S., Smith, K.J., Trottet, L., Uings, I., Zhu, Z., Irving, J.A., Lomas, D.A., The development of highly potent and selective small molecule correctors of Z α_1-antitrypsin misfolding, *Bioorganic & Medicinal Chemistry Letters* (2021), doi: https://doi.org/10.1016/j.bmcl.2021.127973

The development of highly potent and selective small molecule correctors of Z α_1-antitrypsin misfolding

John Liddle [a,*], Andrew C. Pearce [a,*], Christopher Arico-Muendel [b], Svetlana Belyanskaya [b], Andrew Brewster [a], Murray Brown [a], Chun-wa Chung [a], Alexis Denis [c], Nerina Dodic [c], Anthony Dossang [a], Peter Eddershaw [a], Diana Klimaszewska [a], Imran Haq [d], Duncan S. Holmes [a], Alistair Jagger [d], Toral Jakhria [a], Emilie Jigorel [c], Ken Lind [b], Jeff Messer [b], Margaret Neu [a], Allison Olszewski [b], Riccardo Ronzoni [d], James Rowedder [a], Martin Rüdiger [a], Steve Skinner [b], Kathrine J. Smith [a], Lionel Trottet [c], Iain Uings [a], Zhengrong Zhu [b], James A. Irving [d] and David A. Lomas [d].

[a] GlaxoSmithKline, Gunnels Wood Road, Stevenage, Herts. SG1 2NY.

[b] GlaxoSmithKline, Cambridge Park Drive, 6th floor, Cambridge, MA 02140.

[c] GlaxoSmithKline, Avenue du Quebec, Paris 91140.

[d] UCL Respiratory, Rayne Institute, University College London, London. WC1E 6JF

*These authors contributed equally

Address for correspondence: Prof David Lomas, UCL Respiratory, Rayne Institute, University College London, London WC1E 6JF E-mail: d.lomas@ucl.ac.uk and Dr Andy Pearce, GlaxoSmithKline, Gunnels Wood Road, Stevenage, Herts. SG1 2NY. E-mail: andrew.c.pearce@gsk.com

Declarations of interest: Kate Smith, Alexis Denis, Nerina Dodic, John Liddle and David Lomas are inventors on patent PCT/GB2019/051761.

Keywords: α1-antitrypsin, misfolding, 2-oxindole.

Abstract

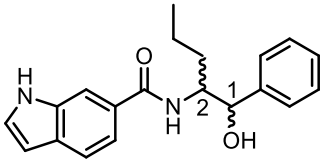
α1-antitrypsin deficiency is characterised by the misfolding and intracellular polymerisation of mutant α1-antitrypsin protein within the endoplasmic reticulum (ER) of hepatocytes. Small molecules that bind and stabilise Z α_1-antitrypsin were identified via a DNA-encoded library screen. A subsequent structure based optimisation led to a series of highly potent, selective and cellular active α1-antitrypsin correctors.

α1-Antitrypsin deficiency is characterised by the misfolding and intracellular polymerisation of mutant α1-antitrypsin protein within the endoplasmic reticulum (ER) of hepatocytes.[1,2] The retention of polymeric mutant protein causes hepatic damage and cirrhosis whilst the lack of an important circulating protease inhibitor predisposes the individuals with severe α1-antitrypsin deficiency to early onset emphysema. α1-antitrypsin deficiency is one of the most common genetic disorders with the severe Z deficiency allele (Glu342Lys) being present in 1:25 of the North European Caucasian population of whom 1:2000 are homozygotes.[1] α1-Antitrypsin is a 394 residue, 52kDa member of the serpin superfamily. It is a major circulating protease inhibitor and its key function is regulation of the proteolytic effects of neutrophil elastase within the lung. There is no specific treatment for the liver disease associated with α1-antitrypsin deficiency, which accounts for 3.5% and 1.1% of paediatric and adult liver transplants in the UK respectively.

The aim of the project was to develop small molecule correctors of Z α_1-antitrypsin folding that block the formation of polymers within the endoplasmic reticulum of hepatocytes and would be amenable to oral dosing as a potential treatment for α_1-antitrypsin deficiency. To that end, a DNA encoded library technology (ELT)[3] screen was performed with a nominal diversity of 2×10^{12} unique components to identify small molecules that bind to monomeric Z α_1-antitrypsin. The most attractive hit, a diastereomeric mixture of indoles **1,** demonstrated functional activity in an antibody-based time-resolved fluorescence resonance energy transfer (TR-FRET) assay monitoring the polymerisation of 5nM Z α_1-antitrypsin. **1** inhibited Z α_1-antitrypsin polymerisation with a half maximum concentration (IC_{50}) of approximately 300nM.

To establish the preferred stereochemistry of **1**, the individual homochiral analogues were prepared, revealing the most potent isomer **1a** to have the 1S,2R configuration (Table 1). The stereochemistry of the pendent propyl group at the 2-position had a major impact on potency with R-stereochemistry required for good inhibition. Analogues with 2S confirmation, **1c** and **1d,** were either inactive or weakly active in in the TR-FRET polymerisation assay. Thus **1a** represents a highly ligand efficient (LE: 0.38) hit molecule found directly from the DNA encoded library.

Table 1: *In vitro* potency of 1 and corresponding homochiral diastereoisomers.



Compound	Stereochemistry	TR-FRET pIC_{50} (n)
1	Diastereoisomeric mixture	6.5 (2)
1a	1S,2R	6.5 (7)
1b	1R,2R	5.5 (3)
1c	1S,2S	4.1 (3)
1d	1R,2S	<4.0 (7)*

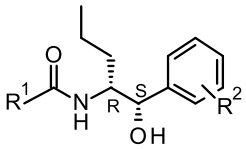
* inactive in 6 out of 7 test occasions. pIC_{50} is the negative log of the IC_{50} in molar concentration

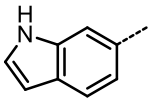
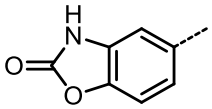
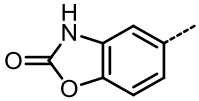
The cellular activity of **1a** to block Z α_1-antitrypsin polymerisation in the ER during folding was assessed in CHO-TET-ON-Z-α1AT CHO cells with simultaneous induction of Z α_1-antitrypsin expression using doxycycline.[4] Encouragingly, **1a** showed robust inhibition of polymerisation in CHO cells, albeit with modest activity (pIC_{50} 4.5, Table 2).

To establish the structure activity relationships (SAR) around the ligand-efficient ELT hit **1a,** with the aim of lowering lipophilicity and increasing cellular activity, initial exploration involved indole replacements and limited substitution on the phenyl ring. Replacement of the indole ring by a benzoxazolone, **2,** led to comparable inhibition in the TR-FRET assay but importantly was accompanied by a ten-fold drop in lipophilicity. **2** showed no significant cellular activity, likely due to a drop in permeability driven by the lower lipophilicity. Substitution in the 2, 3 and 4-position of the phenyl ring with small lipophilic groups led to a marginal increase in activity,

as exemplified by the 2-methyl compound **3**, (TR-FRET pIC_{50} 6.9) and a regain in measurable cellular activity (pIC_{50} 4.5).

Table 2: SAR of compound 1a.



Cmpd	R^1	R^2	TR-FRET pIC_{50} (n)	Cellular pIC_{50} (n)	LE[5]	Chrom[6] LogD	HT Sol[7] (µM)
1a		H	6.5 (7)	4.5 (8)	0.38	4.3	≥439
2		H	6.3 (3)	<4.0 (4)	0.35	3.2	299
3		2-CH_3	7.1 (3)	4.3 (6)	0.37	3.9	≥420

Cellular inhibition: inhibition of Z α_1-antitrypsin polymerisation in CHO cells. LE: Ligand Efficiency. HT Sol: high throughput kinetic solubility measurement. pIC_{50} is the negative log of the IC_{50} in molar concentration.

A high resolution co-crystal structure of **3** bound to apo α_1-antitrypsin was solved and revealed that interaction with the inhibitors induces the formation of a cryptic binding site that is not evident in apo structures, at the top of β-sheet-A behind strand 5 (Figure 1; supplementary data).[8] The phenyl ring and the propyl chain occupy two highly hydrophobic pockets. The central portion of the **3** forms a precise network of H-bonds to the protein. The hydroxyl group H-bonds to Leu291 backbone, the amide carbonyl forms a H-bond to Tyr244 OH and the amide NH H-bonds to the Pro289 carbonyl. The benzoxazolone bicycle stacks with the indole ring of Trp194 and the NH makes a water-bridged interaction with the backbone NH of Trp194. The crystal structure suggested that increased affinity may be achievable by substituting the 2 and 3-positions of the phenyl ring with small lipophilic groups. Modulation of the physicochemical properties by introduction of polarity appeared suited to the benzoxazolone portion of the molecule. Initial SAR confirmed that the hydroxyl, propyl group and amide central portion of the molecule was optimal and hence chemistry focused on optimising the phenyl ring and the bicycle interactions with Trp194. This led to the key breakthrough

identification of the corresponding 2-oxoindole derivative **4** (Table 3), a highly ligand efficient (LE: 0.44) corrector of Z α_1-antitrypsin misfolding. Compared to its benzoxazolone analogue **3, 4** was approximately 300-times more potent in the TR-FRET assay and this increased activity translated into superior cell activity. **4** showed good inhibition of Z α_1-antitrypsin polymerisation in CHO cells (pIC_{50} 5.6), was marginally less lipophilic and maintained good aqueous solubility. **4** had good *in vitro* metabolic stability and when incubated with human hepatocytes (0.3 ml/min/g tissue).

Figure 1: crystal structure of **3** (yellow) bound to apo α_1-antitrypsin

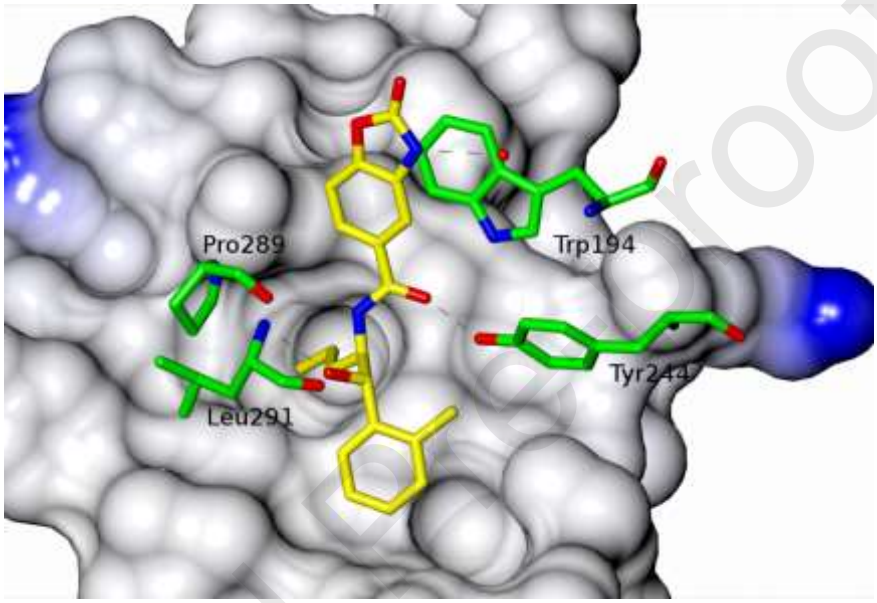
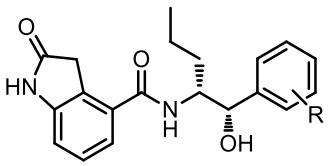


Table 3: *in vitro* profile for substitution of the phenyl ring



Cmpd	R	TR-FRET pIC_{50}	Cellular pIC_{50}	Chrom[5] LogD	HT sol[7] (μM)	Hu *in vitro* Cl (ml/min/g)
4	2-CH_3	8.3	5.6	3.4	≥456	0.3
5	2-CH_2CH_3	8.3	6.0	4.1	≥316	1.6
6	2-Cl	8.1	5.7	3.6	≥356	0.4
7	2-CF_3	7.3	4.9	3.7	≥420	0.2

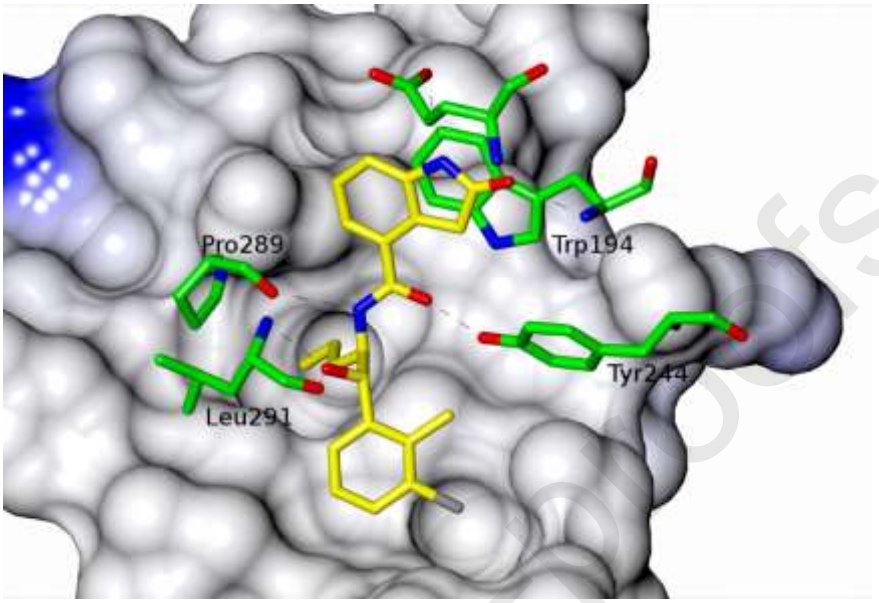
8	2-OCH_3	7.8	5.1	3.4	≥299	<0.8
9	3-CH_3	8.1	5.7	3.6	19	ND
10	3-Cl	8.4	6.0	3.7	52	ND
11	2-CH_3,3-Cl	8.6	6.7	4.3	≥450	0.4
12	2-CH_3,5-Cl	8.6	5.3	4.3	≥408	ND
13	2-CH_3,3-F	8.3	6.3	3.8	≥528	0.3
14	2-Cl,3-F	8.2	6.2	3.7	≥504	<0.4

Cellular inhibition: inhibition of Z α_1-antitrypsin polymerisation in CHO cells. Hu in vitro: in vitro clearance in human liver hepatocytes. ND : not determined. pIC_{50} is the negative log of the IC_{50} in molar concentration.

The greater lipophilicity of the corresponding 2-ethyl compound **5** led to improved cellular permeability and good cell activity (pIC_{50} 6.1) but also introduced high intrinsic clearance in human hepatocytes. A range of alternative 2-substituents were evaluated but none offered a superior profile to the methyl analogue **4.** The chloro derivative **6** had a comparable profile. 3 and 4-substitution were similarly tolerated and delivered compounds with good cellular activity but surprisingly caused a significant drop in aqueous solubility, suggesting that the 2-position substituent was disrupting a stable lattice conformation favouring good aqueous solubility. Combining the optimal 2 and 3-substituents in adjacent positions on the phenyl ring produced an additive effect and an improvement over mono-substitution in cellular activity. The 2-methyl-3-chloro analogue **11** was one of the most cellular active compounds (pIC_{50} 6.7) prepared in this series. However, the combination of two lipophilic groups raised the lipophilicity (ChromlogD 4.4) beyond good physicochemical space and consequently introduced a range of undesirable off-target activity (data not shown). The corresponding analogue with the substituents on opposite sides of the phenyl ring, 2-methyl-5-chloro **12**, had much weaker cellular activity. The 3-fluoro analogues **13** and **14** were less lipophilic and, although weaker in the cell assay, both maintained good activity and had a superior selectivity profile compared to **11**.

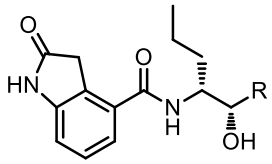
A crystal structure of α_1-antitrypsin complexed with compound **11** (Figure 2), showed the 2-oxoindole ring stacking with the side chain of Trp194 whilst the carbonyl group forming a hydrogen bond with Trp194.[9] The chlorine and the methyl groups are occupying the hydrophobic space above Met374. These improved interactions explain the observed increased potency compared to **3**. N-methylation of the 2-oxindole had negligible impact on activity (data not shown) but had a significant increase in lipophilicity taking the series beyond typical good physicochemical space.

Figure 2: crystal structure of **11** (yellow) bound to apo α_1-antitrypsin

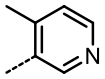
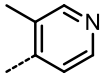


To retain the good cell activity of **11** and remove the undesirable off-target activities, we sought to identify positions on the molecule to introduce polarity and hence reduce lipophilicity. A series of 2-methyl pyridinyl analogues **15-17** (table 4) were prepared. All analogues showed a significant decrease in activity in the TR-FRET polymerisation assay and no or weak cell activity, confirming that polar interactions in this region are poorly tolerated due to the high hydrophobicity in this area of the Z α_1-antitrypsin binding site.

Table 4: *in vitro* profile of selected pyridinyl analogues



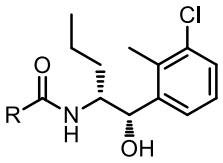
Cmpd	R	TR-FRET pIC_{50}	Cellular pIC_{50}	Chrom[5] LogD	HT Sol[7] (µM)
4		8.3	5.6	3.5	≥395

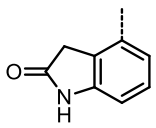
15		6.6	4.4	3.2	≥392
16		5.9	<4	1.9	≥346
17		6.5	<4	1.6	300

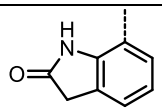
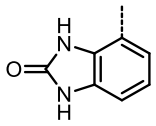
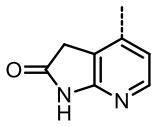
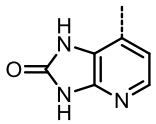
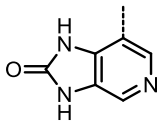
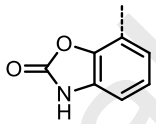
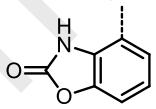
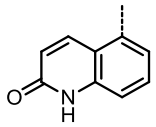
Cellular inhibition: inhibition of Z α_1-antitrypsin polymerisation in CHO cells.
pIC_{50} is the negative log of the IC_{50} in molar concentration

Similarly, we investigated alternative bicyclic heterocycles to the 2-oxindole which were able to retain the hydrogen bond interaction with Trp 194 of **11** with a view to reducing logD whilst maintaining good cellular activity and good aqueous solubility (table 5). The alternative 2-oxindole isomer **18** was less active in the cell assay but was significantly more lipophilic than **11** presumably because the isomer **18** can form an intramolecular hydrogen bond between the NH of the oxindole and the amide carbonyl. The increased lipophilicity was mirrored with an increase in human *in vitro* clearance in hepatocytes.

Table 5: *In vitro* profile for selected oxindole replacements



Cmpd	R	TR-FRET pIC_{50}	Cellular pIC_{50}	Chrom[5] LogD	HT Sol[7] (µM)	Hu Clint (ml/min/g)
11		8.6	6.7	4.4	≥450	0.4

18		8.6	6.3	5.2	128	3.7
19		8.5	6.5	4.4	307	1.8
20		8.1	5.9	3.9	≥314	0.9
21		8.0	5.5	3.9	200	ND
22		7.4	4.2	3.4	≥227	ND
23		8.0	5.4	5.1	326	ND
24		8.2	5.1	5.2	233	ND
25		7.7	4.8	3.7	309	ND

Cellular inhibition: inhibition of Z α_1-antitrypsin polymerisation in CHO cells.

pIC_{50} is the negative log of the IC_{50} in molar concentration

The 2-oxoimidazole derivative **19** had a similar profile to **11** with comparable *in vitro* potency but marginally higher human *in vitro* clearance. Efforts to reduce the lipophilicity by introduction of a nitrogen atom into the benzo-fused ring had a detrimental effect on the *in*

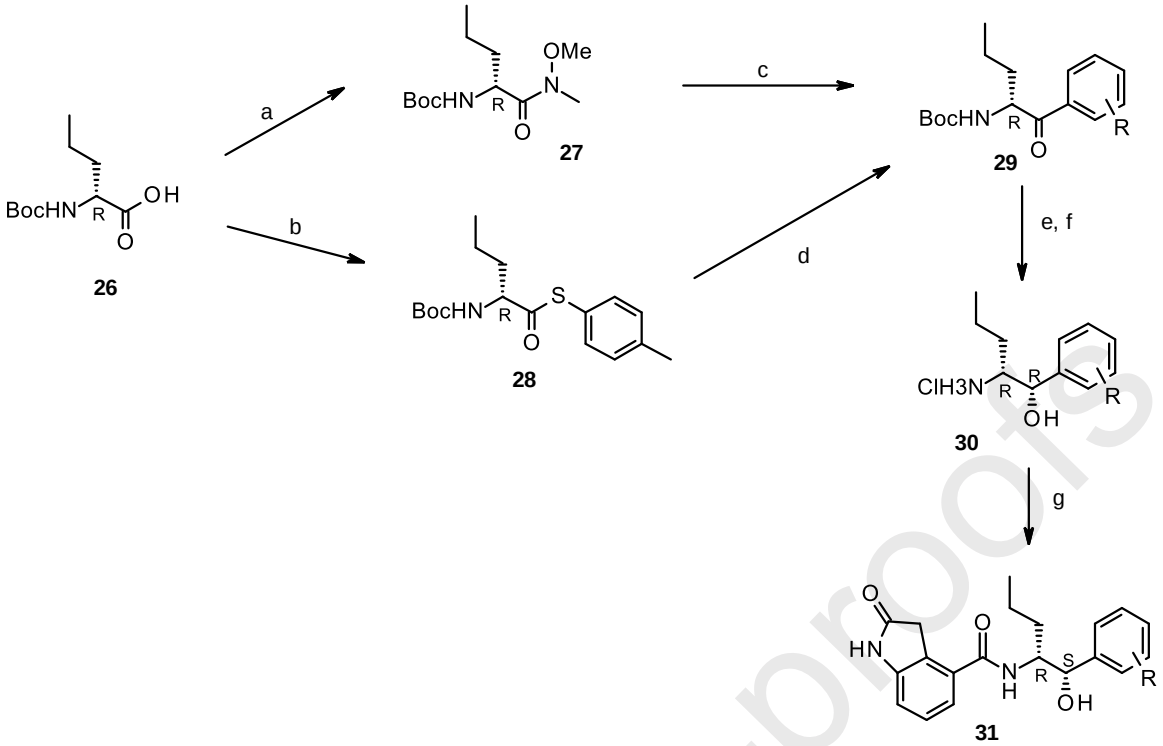
vitro potency in all cases **20-22**. Revisiting the benzoxazolone isomers **23-24** saw a significant drop in cell activity. A range of 6,6-fused heterocycles were also evaluated as exemplified by 2-quinolinone **25** but all were inferior to the 2-oxindole **11**.

Compound **13** offered the optimal combination of cellular activity, physicochemical properties and human metabolic clearance and was therefore profiled further both *in vitro* and *in vivo*.[10] **13** binds to Z α1-antitrypsin with a high-affinity (mean pKD 8.5) and demonstrates a 50-fold lower affinity for plasma purified wild-type M α1-antitrypsin.[10] Importantly **13** was able to increase secretion of Z α_1-antitrypsin approximately 3-fold compared to vehicle control in CHO cells (pEC_{50} of 6.2) with comparable potency to inhibition of polymerisation supporting the hypothesis that these effects are caused by the same pharmacological mode of action.

Furthermore **13** showed an excellent selectivity profile with negligible activity against a panel of over 50 unrelated proteins (date not shown). Amorphous **13** had good solubility in fasted simulated intestinal fluids (969μg/ml). **13** had low binding to human serum albumin (84%) but very high binding in human plasma and blood (>98%) reflecting the specific binding to α_1-antitrypsin. **13** was found to be 81% bound in the presence of rat plasma. **13** exhibited low metabolic clearance in human hepatocytes (0.31 ml/min/g tissue) but clearance was high in rat hepatocytes (7.4 mL/min/g tissue) and consistent with that observed *in vivo*. Following intravenous infusion to male Wistar Han rats at 1mg/kg, the mean *in vivo* blood clearance was high (69mL/min/kg, n=2) and volume of distribution was moderate (2.3L/kg), resulting in a short elimination half-life (0.5h). Oral bioavailability following a single dose of **13** (suspension in 1% aqueous methylcellulose) at 3mg/kg was moderate (48%).

All compounds were prepared via the homochiral ketone **29**. **29** was prepared via 3 similar synthetic routes, dependent on the phenyl substituents. The Weinreb amide **27**, derived from N-(tert-butoxycarbonyl)norvaline **26**, was treated with either the appropriate aryl lithium species or Grignard reagent. Alternatively, the thioester **28**, also derived from **26**, was treated with the appropriately substituted phenyl boronic acid using Liebeskind-Sgroll coupling conditions. Stereoselective reduction of the ketone **29**, using Meerwein-Ponndorf-Verley conditions followed by deprotection led to the homochiral alcohol **30** which was subsequently acylated with the corresponding carboxylic acid to provide the desired products **31**.

Scheme 1: Synthetic route



Reagents and conditions: (a) Me-NH-OMe, HOBT, EDCI, NEt_3, DMF, rt; (b) 4-Methyl-thiophenol, DCC, EtOAc, 0°C to rt; (c) R_2-PhMgBr, THF, 0°C to rt or BuLi, R_2-PhBr, THF, -78°C (d) R_2-Ph-$B(OH)_2$, $P(OEt)_3$, Copper (I) 2-thiophenecarboxylate, $Pd_2(dba)_3$, dioxane, rt or 50°C; (e) $Al(OiPr)_3$, 2-propanol, toluene, 50°C; (f) HCl/dioxane; (g) R_1-COOH, HATU, NEt_3, DMF, rt.

In summary, we report the optimisation of small molecule drug-like correctors of Z α_1-antitrypsin folding. An initial DNA encoded library technology screen identified a highly ligand-efficient hit molecule which was able to inhibit Z α_1-antitrypsin polymerisation. A disciplined medicinal chemistry strategy, exploiting structural knowledge from crystal structures of lead molecules in complex with α1-antitrypsin, led to a series of highly potent, cellular active and selective α1-antitrypsin correctors. Compound **13** represents a high quality tool molecule, with an excellent *in vitro* profile, moderate bioavailability in rat and importantly is predicted to have high oral bioavailability in human.

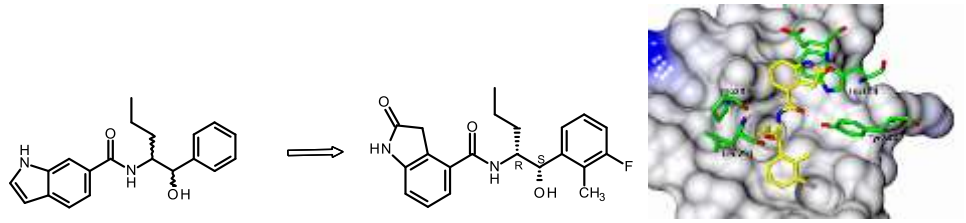
Acknowledgements

This research was supported by a Discovery Partnership with Academia (DPAc) grant from GSK to D.A.L.

References and Notes

1. Lomas, D. A.; Hurst, J. R. Bibek Gooptu, B. *Journal of Hepatology* **2016,** 65, 413.
2. Lomas, D. A.; Evans, D. L.; Finch, J. T. ; Carrell, R. W. *Nature* **1992**; 357, 605
3. Clark, M. A. et al ; *Nat. Chem. Biol*. **2009**, 5, 647
4. Ordóñez, A.; Snapp, E. L.; Tan, L.; Miranda, E.; Marciniak, S. J.; Lomas, D. A. *Hepatology*, 2013 ; 57(5) 1.
5. Abadzapatero, C.; Metz, J. *Drug Discovery Today* **2005**, 10 (7): 464–469
6. Young, R. J.; Green, D. V. S.; Luscombe, C. N.; Hill, A. P. *Drug Discovery Today*, **2011**, 16, 822-830.
7. Bhattachar S. N.; Wesley J. A.; Seadeek C. Evaluation of the chemiluminescent nitrogen detector for solubility determinations to support drug discovery, *J. Pharm. Biomed. Anal*. 41, **2006**, 152-157.
8. The coordinates and structure factors have been deposited in the Protein Data Bank under the accession code 7NPK.pdb.
9. The coordinates and structure factors have been deposited in the Protein Data Bank under the accession code 7NPL.pdb
10. Lomas, D. A.; James A Irving, J. A.; Arico-Muendel, C.; Belyanskaya, S.; Brewster, A. ; Brown, M.; Chung, C.; Dave, H.; Denis, A.; Dodic, N.; Dossang, A.; Eddershaw, P.; Klimaszewska, D.; Haq, I. ; Holmes, D. S.; Hutchinson, J. P.; Jagger, A. M.; Jakhria, T.; Jigorel, E.; Liddle, J.; Lind, K.; Marciniak, S. J.; Messer, J.; Neu, M.; Olszewski, A.; Ordonez, A.; Ronzoni, R.; Rowedder, J.; Rudiger, M.; Skinner, S.; Smith, K. J.; Terry, R.; Trottet, L.; Uings, I.; Wilson, S.; Zhu, Z.; Pearce, A. C. *EMBO Mol. Med*. 2021, 13 : e13167. DOI : 10.1101/2020.07.26.217661v1.

Development of highly potent and selective small molecule correctors of Z α_1-antitrypsin misfolding



DNA Encoded Library Hit **13**

pIC_{50}: 6.5 pIC_{50}: 8.3 Cell pIC_{50} : 6.3