Bioorganic & Medicinal Chemistry Letters xxx (2013) xxx-xxx



Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters



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

Design, synthesis and structure–activity relationships of substituted oxazole–benzamide antibacterial inhibitors of FtsZ

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ARTICLE INFO

Article history: Received 2 October 2013 Revised 1 November 2013 Accepted 4 November 2013 Available online xxxx

Keywords: Antibacterial FtsZ Cell division 3-Methoxybenzamide MRSA

ABSTRACT

The design, synthesis and structure–activity relationships of a series of oxazole–benzamide inhibitors of the essential bacterial cell division protein FtsZ are described. Compounds had potent *anti*-staphylococcul activity and inhibited the cytokinesis of the clinically-significant bacterial pathogen *Staphylococcus aureus*. Selected analogues possessing a 5-halo oxazole also inhibited a strain of *S. aureus* harbouring the glycine-to-alanine amino acid substitution at residue 196 of FtsZ which conferred resistance to previously reported inhibitors in the series. Substitutions to the pseudo-benzylic carbon of the scaffold improved the pharmacokinetic properties by increasing metabolic stability and provided a mechanism for creating pro-drugs. Combining multiple substitutions based on the findings reported in this study has provided small-molecule inhibitors of FtsZ with enhanced in vitro and in vivo antibacterial efficacy. © 2013 Elsevier Ltd. All rights reserved.

Small-molecule inhibitors of the essential and conserved prokaryotic cell division protein FtsZ offer promise as candidate new antibacterial agents for the treatment of drug-resistant infections.¹⁻⁶ In particular, synthetic analogues of 3-methoxybenzamide⁷ **1** (3-MBA, Fig. 1), such as PC190723 (**2**), have demonstrated the potential to inhibit methicillin-resistant *Staphylococcus aureus* in vitro and in relevant animal models of infection.⁸⁻¹¹ Although no FtsZ inhibitors have yet progressed through clinical development, compound **2** is the best-characterised lead compound of this novel class. These authors recently reported another substituted derivative of 3-MBA, **3**, and its succinate pro-drug, **4** (Fig. 1).¹² Benzamides **3** and **4** exhibited a number of superior in vitro and

0960-894X/\$ - see front matter \circledcirc 2013 Elsevier Ltd. All rights reserved. http://dx.doi.org/10.1016/j.bmcl.2013.11.002 in vivo properties over **2**. The most significant improvements were whole-cell potency against the FtsZ G196A amino acid substitution that renders *S. aureus* non-susceptible to **2**, enhanced pharmacokinetic properties and improved in vivo efficacy.

Compounds **3** and **4** have two key structural differences relative to **2**: (i) the presence of a phenyl-substituted oxazole heterocycle in place of the thiazolopyridine; and (ii) substitutions at the pseudo-benzylic position of the linker between the benzamide and oxazole moieties. These novel improvements were discovered by means of an iterative medicinal chemistry and screening effort. This Letter describes the design and synthesis of a substantial number of novel analogues of compounds 3 and 4 prepared during this programme and reports their biological properties, along with the structure-activity relationships (SAR) of the oxazole chemotype and the effects of substitutions on the benzamide linker. Synthesized compounds were initially evaluated in minimal inhibitory concentration (MIC) assays with S. aureus strain ATCC 29213, or a derivative carrying the previously-characterised⁸ G196A amino acid substitution in FtsZ, using the broth microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute.¹³ As with earlier studies the cell division inhibition assay was used to monitor the on-target action of these analogues.⁸⁻¹² This assay provides a convenient confirmation of

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Figure 1. Chemical structures of 1 (3-methoxybenzamide), 2 (PC190723), 3 and 4.

whole-cell activity based on the well-understood phenotypic response to chemical or genetic inhibition of FtsZ in cocci.^{14,15} In each case the lowest concentration of compound at which inhibition of cell division in *S. aureus* was observed is reported. Selected compounds were also evaluated for their in vivo pharmacokinetic behaviour in mice as well as their stability in vitro in various chemical and biological matrices.^{16,17}

Our initial investigations into the oxazole series began with the synthesis and characterization of phenyl oxazole 5. Synthesis of this target commenced, with a Blümlein-Lewy oxazole-forming reaction between 2-benzyloxyacetamide and phenacyl bromide as shown in Scheme 1. Subsequent treatment with boron tribromide resulted in concomitant debenzylation/bromination and the resulting alkyl halide was then used to alkylate 2,6-difluoro-3hydroxybenzamide¹⁸ and yield benzamide **5**. Three other 2,4-substituted oxazoles (6-8) were also prepared as outlined in Scheme 1. Compound 5 inhibited wild-type S. aureus with an MIC of 16 μ g/mL (Table 1) and was also observed to begin inhibiting cell division at around the same concentration. This is consistent with 5 exerting its antibacterial effect through the disruption of FtsZ function. Small substituents at the 4-position of the phenyl ring improved the antibacterial potency. For the 4-hydroxy analogue (6), potency was improved twofold. In the

case of the 4-chloro analogue (**7**), potency was increased fourfold. The greatest uplift in antibacterial potency was measured with the 4-methoxy analogue (**8**) for which an eightfold decrease in MIC was observed.

A feature of the oxazole chemotype is the option for substitutions at the C5 position of the heterocycle. In silico modeling using the crystal structure of 2 bound to $FtsZ^{8,19}$ indicated that when compound **5** was docked into the putative binding site there was space to extend substitutions from the C5 position (data not shown). To this end, trisubstituted oxazoles 9-28 were prepared as outlined in Scheme 1, combining substitutions to both the phenyl moiety and the C5 position of the oxazole.²⁰ Combining substitutions in this way produced compounds with markedly enhanced potencies against wild-type S. aureus, with MICs as low as 0.03 µg/ mL recorded (Table 1). Again, the inhibition of cell division phenotype tracked potency. A wide range of alkyl and aryl substitutions at the C5 position were tolerated and retained activity. Analogues with amino (11) and nitro (12) groups were found to be inactive. Unexpectedly, with certain combinations of substitutions, inhibition of the S. aureus G196A mutant was also observed. Compounds 10, 23, 25, and 26 that harbour halogen substitutions (specifically a bromine or chlorine atom) at the C5 position had single-digit $\mu g/$ mL MICs against the mutant strain and still acted by blocking cell division.12

Selected compounds with potent in vitro anti-staphylococcal activity were evaluated for their in vivo pharmacokinetic properties. The pharmacokinetic parameters for the representative compounds 16 and 19 are shown in Table 2. With clearance rates of $\geq 137 \text{ mL/min/kg}$ and the area under the curve (AUC) $\leq 0.4 \,\mu g \cdot h/mL$ the pharmacokinetic properties of these compounds were considered to be unsuitable for demonstrating efficacy in animal models of infection. However, data with the non-oxazole chemotypes depicted in Figure 2 indicated that substitutions at the pseudo-benzylic carbon position could improve the pharmacokinetic properties of benzamide derivatives. For example, a methyl-substituted analogue (30) of the oxadiazole-benzamide showed improvements in its pharmacokinetic profile over the unsubstituted oxadiazole- benzamide (29) (Fig. 3). The same effect was also observed for the substituted benzothiazole-benzamide (32) relative to the unsubstituted benzothiazole-benzamide (31) (Fig. 3). This learning was then applied to the oxazole-benzamide chemotype producing analogues with improved pharmacokinetic profiles. The potentially labile pseudo-benzylic positions of 23, 25 and 26 were blocked by the introduction of a methyl group to produce compounds 33, 34 and 35, respectively. The pharmacokinetic parameters for these three analogues are also presented in Table 2. In each case compounds containing the methyl substitution showed good AUCs, half-lives and clearance rates. The pharmacokinetic profile was even better in the case of the hydroxymethyl-substituted analogue of 23, that is, compound 36 (Table 2).

The in vitro stability of compounds **16**, **19**, **35** and **36** was evaluated in a range of chemical and biological matrices. Data are presented in Table 3. All four compounds were stable over a 24 h period in phosphate-buffered saline at pH 7.4. All of the compounds were also stable in mouse plasma. In the presence of mouse microsomes or mouse hepatocytes, the half-lives of compounds **35** and **36**, which have a methyl and hydroxymethyl substitution, respectively, at the carbon adjacent to the 2-oxazolyl position, were higher than the half-lives of compounds **16** and **19**, which have no substitution at the pseudo-benzylic position. These data indicate that such substitutions improve metabolic stability, which is a critical pharmacokinetic parameter of compounds for use in staphylococcal infections.

Compound **36** and a variety of other pseudo-benzylic substituted examples were prepared by means illustrated in Scheme 2.

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R' = Et (for 15)

Scheme 1. Reagents and conditions: (a) 2-benzyloxyacetamide, DMF/130 °C, 6 h; (b) BBr₃, dichloromethane (DCM)/–78 °C to 25 °C, 2 h; (c) 2,6-difluoro-3-hydroxybenzamide, K₂CO₃, DMF/rt, 6 h; (d) acetamide, DMF/130 °C, 6 h; (e) NBS, acetic acid/rt, 2 h; (f) azobisisobutyronitrile (AIBN), NBS, CCl₄/reflux, 3 h; (g) 2-benzyloxyacetyl chloride, tetraethylammonium (TEA), CH₂Cl₂/0 °C to 25 °C; (h) POCl₃, benzene/reflux; (j) (i) H₂, Pd/C, MeOH/rt/1 atm, 1 h, (ii) SOCl₂, CH₂Cl₂; (k) HNO₃, H₂SO₄; (m) (i) SeO₂, xylene/ reflux, (ii) NaBH₄, MeOH/reflux, 2 h, (iii) AcCl, TEA, CH₂Cl₂/0 °C to rt, 0.5 h; (n) Zn, AcOH/120 °C, 1 h; (o) (i) methyl acrylate, Pd(OAc)₂, PPh₃, dimethylacetamide (DMA) (Heck coupling), (ii) H₂, Pd/C, tetrahydrofuran (THF), (iii) lithium aluminium hydride (LAH), THF; (p) CuCN, pyridine, microwave heating; (q) SnCl₂·2H₂O, EtOH; (r) K₂CO₃, MeOH/rt 1 h.

Table 1

SAR of substitutions at the C4- and C5-positions of the oxazole chemotype



Compound	\mathbb{R}^4	R ⁵	S. aureus ATCC 29213		FtsZ G196A
			MIC (µg/mL)	Phenotype ^a (µg/mL)	MIC (µg/mL)
2	N/A	N/A	1	0.5	>64
5	Ph	Н	16	16	>64
6	4-OH Ph	Н	8	8	>64
7	4-Cl Ph	Н	4	4	>64
8	4-OMe Ph	Н	2	2	>64
9	4-Cl Ph	Me	0.5	0.5	>64
10	4-Cl Ph	Br	0.06	0.03	2
11	4-Cl Ph	NH ₂	>64	> 64	>64
12	4-Cl Ph	NO ₂	>64	> 64	>64
13	4-Cl Ph	OMe	0.5	0.5	n.d.
14	4-Cl Ph	CH ₂ OH	8	8	> 64
15	4-Cl Ph	OEt	0.06	0.03	> 64
16	4-Cl Ph	Propyl	0.03	0.015	n.d.
17	4-Cl Ph	Allyl	0.06	0.06	>64
18	4-Cl Ph	3-OH propyl	2	2	>64
19	4-OMe Ph	Et	0.12	0.12	>64
20	4-OMe Ph	Cyclopropyl	0.12	0.12	>64
21	4-OMe Ph	Ph	0.12	0.12	>64
22	4-OMe Ph	Thiazol-5-yl	2	2	n.d.
23	4-CF ₃ Ph	Br	0.12	0.12	4
24	4-CF ₃ Ph	CN	2	4	>64

(continued on next page)

Please cite this article in press as: Stokes, N. R.; et al. Bioorg. Med. Chem. Lett. (2013), http://dx.doi.org/10.1016/j.bmcl.2013.11.002

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Table 1 (continued)

Compound	\mathbb{R}^4	R ⁵	S. aure	FtsZ G196A	
			MIC (µg/mL)	Phenotype ^a (µg/mL)	MIC (µg/mL)
25	4-OCF ₃ Ph	Cl	0.12	0.12	8
26	4-OCF₃ Ph	Br	0.06	0.03	2
27	4-OCF ₃ Ph	Ι	0.06	0.06	>64
28	4-OCF ₃ Ph	CF ₃	0.5	0.5	>64
Linezolid	N/A	N/A	2	N/A	n.d.
Vancomycin	N/A	N/A	1	N/A	n.d.

N/A, not applicable.

n.d., not determined.

^a Lowest concentration at which an inhibition of cell division phenotype was measured.

Table 2

In	vivo	pharmacokinetic	parameters	of	compounds	16,	19,	33,	34,	35,	and	36
foll	owin	g a 3 mg/kg IV do	se in mice									

Parameter (unit)	16	19	33	34	35	36
$C_0 (\mu g/mL)$	1.2	1.1	2.0	2.1	2.5	4.2
AUC (µg·h/mL)	0.4	0.2	2.2	1.6	1.4	6.7
$t_{1/2}$ (min)	25	10	63	79	53	127
CL (mL/min/kg)	137	319	22	31	36	7
Volume of distribution (L/kg)	5.0	4.3	2.0	3.5	2.7	1.3

















Figure 2. Chemical structures of 29, 30, 31 and 32.

The biological data indicated that a diverse range of substitutions were tolerated, including extended alkyls, alcohols, amines and five- and six-membered heterocycles (Table 4). The inactivity of



Figure 3. Pharmacokinetic profiles of **29**, **30**, **31** and **32** in mice following a single IV administration. Compound **29** (open squares) and compound **30** (solid squares) were dosed individually at 3 mg/kg. Compound **31** (open circles) and compound **32** (solid circles) were dosed in a cassette at 2 mg/kg. Samples were taken after 5, 15, 30, 60, 120, 240 and 480 min and total plasma concentrations of the compounds were determined by LC-MS/MS.

Table 3

In vitro stability of selected compounds in various chemical and biological matrices

Parameter (unit)	16	19	35	36
Chemical stability (% remaining after 24 h)	98	97	96	102
Plasma stability (% remaining after 24 h)	101	100	104	100
Microsomal stability (Half-life, min)	68.2	71.6	>120	>120
Hepatocyte stability (Half-life, min)	17.9	23.4	101.2	71.5

the gem dimethyl analogue, **37**, was striking and pointed to the likely requirement for a specific chiral configuration due to steric hindrance at the ligand binding site.

Up to this stage, the pseudo benzylic-substituted analogues had been prepared as racemic mixtures. Investigations were therefore undertaken to determine whether one or both enantiomers were active. To this end, a batch of compound **36** was resolved by chiral chromatography resulting in pure (>99%) enantiomers **48** and **3**.²¹ Circular Dichroism (CD) and Optical Rotatory Dispersion (ORD) analyses revealed compound **48** to be levorotatory and compound **3** dextrorotatory.²² Each of the two separate enantiomers was then tested for biological activity (Table 5) and the results indicated that



Please cite this article in press as: Stokes, N. R.; et al. Bioorg. Med. Chem. Lett. (2013), http://dx.doi.org/10.1016/j.bmcl.2013.11.002

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Scheme 2. Reagents and conditions: (a) DMF/130 °C, 6 h; (b) NBS, acetic acid/rt, 2 h; (c) AIBN, NBS, CCl₄/reflux, 3 h; (d) 2,6-difluoro-3-hydroxybenzamide, K₂CO₃, DMF/rt, 6 h; (e) K₂CO₃, MeOH/rt, 1 h; (f) NaBH₄, MeOH/reflux, 2 h; (g) AcCl, TEA, CH₂Cl₂/0 °C to rt, 0.5 h; (h) (i) PBr₃, toluene/reflux, 0.5 h, (ii) NMe₂, THF/rt, 16 h; (j) Jones Reagent, acetone/ 0 °C to rt, 16 h; (k) (i) acetic hydrazide, EDC, HOBt, DMAP, DMF/rt, (ii) POCl₃, toluene/reflux. Additionally, 5-chloro-oxazole **34** was prepared by the same route as shown for bromide **35** using NCS in place of NBS. Diol **45** and pyridyl derivative **40** were prepared as described in WO2012/142671 A1, 2012 (see representative example 11, page 51).

Table 4

SAR of benzylic substitutions of the oxazole chemotype

 H_2N F F R^4 R^4 R^4 R^4

Compound	\mathbb{R}^4	R ⁵	R ^α	S. aureus ATCC 29213	
				MIC (µg/mL)	Phenotype ^a (µg/mL)
33	4-CF ₃ Ph	Br	Me	0.06	0.06
34	4-OCF ₃ Ph	Cl	Me	0.12	0.25
35	4-OCF ₃ Ph	Br	Me	0.12	0.12
36	4-CF ₃ Ph	Br	CH ₂ OH	0.25	0.5
37	4-Cl Ph	Br	Gem-dimethyl	>64	_
38	4-Cl Ph	Br	Et	2	1
39	4-Cl Ph	Br	CH ₂ OH	0.25	0.25
40	4-Cl Ph	Br	Pyrid-3-yl	4	4
41	$4-CF_3$ Ph	Br	CH ₂ CH ₂ OH	0.5	0.5
42	$4-CF_3$ Ph	Br	$CH_2CH_2N(Me)_2$	8	16
43	$4-CF_3$ Ph	Br	CH ₂ CH ₂ CH ₂ OH	1	1
44	$4-CF_3$ Ph	Br	CH ₂ CH ₂ CO ₂ H	8	8
45	$4-CF_3$ Ph	Br	CH ₂ CH(OH)CH ₂ OH	0.5	0.5
46	4-CF ₃ Ph	Br	CH ₂ OAc	0.5	0.5
47	4-CF ₃ Ph	Br	(5-Methyl-1,3,4-oxadiazol-2-yl)methyl	8	16

^a Lowest concentration at which an inhibition of cell division phenotype was measured.

Table 5

	Biological	activity	of com	pound	36 and	purified	enantiomers	48	and	3
--	------------	----------	--------	-------	---------------	----------	-------------	----	-----	---

Compound	Stereo	S. aureus	ATCC 29213	FtsZ G196A		
	assignment MIC (µg/mL)		Phenotype ^a (µg/mL)	MIC (µg/mL)		
36	Racemic	0.25	0.5	8		
48	(-)	16	16	64		
3	(+)	0.12	0.25	4		

^a Lowest concentration at which an inhibition of cell division phenotype was measured.

the *anti*-staphylococcal and cell division inhibitory activity resided primarily with the (+) form. A stereoselective synthetic route was subsequently developed using commercially available *N*-Boc-(OBn)-serine as the chiral source (Scheme 3) and this served to establish the absolute configuration of the required stereoisomer. Key steps in the synthetic sequence included diazotization/ substitution of the amino acid with overall retention of configuration and a subsequent Mitsunobu coupling with chiral inversion to give the R enantiomer of the target product. This fortuitously proved to be the desired more active configuration (**3**), as confirmed by *S. aureus* susceptibility testing (Table 5). The less

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Scheme 3. Reagents and conditions: (a) (i) TFA/rt, 1 h, (ii) NaNO₂, water, acetate buffer (pH 4), acetone/5 °C to rt, 16 h, (iii) H₂SO₄, trimethylorthoformate, MeOH/reflux, 16 h; (b) PPh₃, diethyl azodicarboxylate (DEAD), 2,6-difluoro-3-hydroxybenzamide, TEA, THF/0 °C to rt, 48 h; (c) H₂, Pd(OH)₂/C, THF/rt/1 atm, 1 h; (d) Cs₂CO₃, water, acetonitrile/ 55 °C, 4 h; (e) 2-bromo-1-(4-trifluoromethylphenyl)ethanone, DMF/rt, 1 h; (f) BF₃:Et₂O, acetamide, xylenes/reflux, 2 h; (g) Br₂, CH₂Cl₂/rt, 16 h.

active enantiomer (**48**) was prepared by a slight modification to the sequence, using Mitsunobu esterification with 4-nitrobenzoic acid and then hydrolysis to invert the chirality of the secondary alcohol immediately prior to step (b). This ultimately yielded the S configuration of the final compound and a 128-fold reduction of biological activity compared to the more active enantiomer **3** was observed by *S. aureus* susceptibility testing (Table 5).

As well as providing enhanced metabolic stability, substitutions at the pseudo-benzylic position also offered the prospect of enhancing the solubility of the compounds either by increasing the intrinsic solubility of the parent molecule or by providing a handle to introduce pro-drug functionality via an ester linkage. At neutral pH the kinetic solubility²³ of compound **36** was 100 μ g/mL, compared to 12.5 μ g/mL of the unsubstituted analogue, compound 23 (Table 6). A number of other substituted analogues also had improved solubility over compound 23 (Table 6). To explore this opportunity, a library of derivatives encompassing ester 50 and carbamates 51-56 was prepared by modification of alcohol 36 according to methods described elsewhere.²⁴ This library was evaluated for solubility (Table 7). Using this strategy, improvements in the solubility were observed with various substitutions, such as a succinate (50), piperazine (51), and amino tetrazole (56).

Table 6

In vitro kinetic solubility at pH 7.4 of selected compounds



Compound	R^4	R ⁵	R ^α	Solubility (µg/mL)
23	4-CF ₃ Ph	Br	Н	12.5
36	4-CF ₃ Ph	Br	CH ₂ OH	100
43	4-CF ₃ Ph	Br	CH ₂ CH ₂ CH ₂ OH	50
44	4-CF ₃ Ph	Br	CH ₂ CH ₂ CO ₂ H	400
45	4-CF ₃ Ph	Br	CH ₂ CH(OH)CH ₂ OH	100
46	4-CF ₃ Ph	Br	CH ₂ OAc	25
49	4-CF ₃ Ph	Br	CH ₂ OPO(OH) ₂	>800

Table 7

In vitro kinetic solubility of selected ester and carbamate derivatives at pH 7.4



	8	
Compound	R	Solubility (µg/mL)
50	CH ₂ CH ₂ CO ₂ H	200
51	Piperazin-1-yl	200
52	NHCH ₂ CO ₂ H	800
53	NHCH ₂ CH ₂ NHMe	200
54	2-Pyrrolidin-1-ylethylamino	400
55	2-(1H-Imidazol-4-yl)ethylamino	100
56	1H-Tetrazol-5-ylamino	800

Having established the significance of a halogen-substituted oxazole for potency against the FtsZ G196A mutant, the importance of a substituted pseudo-benzylic position for metabolic stability and a pro-drug strategy for enhancing solubility, these three chemical features were combined to produce the advanced lead compound **3** and its succinate pro-drug **4**. As reported previously, the improved in vitro properties of **3** and **4** translated into enhanced in vivo pharmacokinetics and efficacy in the murine thigh model of staphylococcal infection.¹²

In summary, the SAR of advanced benzamide-derived FtsZ inhibitors has been presented. The activity of the oxazole-benzamide chemotype has been described and the importance of 5-halo substitutions to the oxazole group in driving potency against the mutant encoding the FtsZ G196A amino acid substitution has been reported. In parallel, the significance of substitutions to the pseudo-benzylic position for driving improvements in the pharmacokinetic properties by increasing metabolic stability and by providing a means to enhance solubility has been established.

Acknowledgements

This work was funded in part by an investment from The Wellcome Trust under the Seeding Drug Discovery Initiative. We thank Steve Ruston and Simon Tucker for their encouragement during the execution of this work. Circular Dichroism and Optical Rotatory Dispersion spectroscopy were performed by Chiralabs Ltd (Begbroke, United Kingdom). Compounds are the subject of international patent application numbers WO2007/107758 and WO2012/142671. The authors have been paid for the work presented and some of the Biota authors are inventors on patents and hold shares in Biota Pharmaceuticals Inc.

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- 16. Groups of healthy adult male Balb/c mice received compounds by intravenous (IV) or oral (PO) administration at a dose level of 3 mg/kg. In all cases the dose volume was 5 mL/kg. Compounds were formulated in 10% dimethyl sulfoxide (DMSO), 40% tetraethylene glycol (TEG), and 20% 2-hydroxypropyl-βcyclodextrin (HPβCD) in water (final). At various time points, samples of blood were collected for analysis. Three animals were used for each time point. Total plasma concentrations of compounds were measured by LC-MS/MS and pharmacokinetic parameters were calculated using Win-Nonlin.
- 17. The chemical and plasma stability of a 10 μM solution of compound in phosphate buffered saline (PBS, pH 7.4) or neat mouse plasma (Sera Laboratories International Ltd, Haywards Heath, UK) was determined following 24 h incubation at 37 °C. The final concentration of DMSO was 5% v/v. Microsomal stability was assessed at 10 μM with a final concentration of

0.1% v/v DMSO. Microsomes were added to a final protein concentration of 1 mg/mL and reactions were started by the addition of NADPH to a concentration of 1 mM. Reactions were stopped at 0, 10, 30 and 60 min by the addition of 100 μ L of DMSO. For the hepatocyte stability assay, compounds were tested in duplicate at 10 μ M with a final DMSO concentration of 0.5% v/v and incubated with 5×10^4 cryopreserved CD-1 mouse hepatocytes (CellzDirect, Durham, NC) in a 100 μ L volume. The assay was stopped by the addition of 100 μ L DMSO at seven time points up to 120 min. In all stability experiments, samples were extracted using acetonitrile and centrifugation, and the supernatants were analyzed by LC-MS.

- 2,6-Difluoro-3-hydroxybenzamide was prepared from commercially available 2,6-difluoro-3-methoxybenzamide by treatment with BBr₃ under nitrogen in CH₂Cl₂ at ambient temperature for 48 h.
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- 20. In addition to the steps shown explicitly, phenyl oxazole 21 was prepared via Suzuki coupling of the appropriate oxazolyl bromide. Compounds 16, 17, 19 and 22 were prepared from the corresponding oxazolyl bromide by means of Stille coupling and, where required, subsequent Pd-catalysed hydrogenation. 5-Chloro- and 5-iodo-oxazoles 25 and 27 were synthesised in a manner analogous to 26 using N-chlorosuccinimide (NCS) or N-iodosuccinimide (NIS) in place of N-bromosuccinimide (NBS). Trifluoromethyl derivative 28 was prepared by treatment of the appropriate oxazolyl iodide intermediate with methyl 2,2-difluoro-2-(fluorosulfonyl)acetate and copper(1) iodide in HPMA and N,N-dimethylformamide (DMF).
- 21. Enantiomers 48 and 3 were separated from a 1 g sample of the racemic mixture 36 using a CHIRALPAK[®] AD-H, (250 × 30) mm, 5 μm 5 122 column with a *n*-hexane/EtOH/MeOH (80:10:10 v/v/v) mobile phase.
- 22. For the CD analysis acetonitrile solutions of the compounds were prepared and dispensed into pre-cleaned Quartz Suprasil cylindrical cells. CD spectra were acquired on a Jasco J720 spectrometer. Instrument parameters were: wavelength range, 200–360 nm; spectral bandwidth, 1 nm; scan speed, 10 nm/min; response time, 4 s; accumulations, 4; data interval, 0.1 nm; nominal cell length, 1.0 cm; temperature, 20 ± 0.1 °C. In each case a CD spectrum and UV absorption spectrum were simultaneously acquired. Spectra were corrected by solvent baseline subtraction, followed by normalization for molar concentration and pathlength. For the ORD analysis tetrahydrofuran solutions of the compounds were prepared and dispensed into pre-cleaned polarimetry cells. Optical rotations were determined using a specially converted [600 spectropolarimeter. Instrument parameters were: wavelength range, 320-800 nm; spectral bandwidth, 1 nm; scan speed, 50 nm/min; response time, 1 s; accumulations, 4; data interval, 0.1 nm; nominal cell length, 1.0 cm; temperature, 20 ± 0.1 °C. In each case an ORD spectrum and UV absorption spectrum were simultaneously acquired. Spectra were corrected by solvent baseline subtraction, followed by normalization for concentration and pathlength.
- 23. The kinetic solubility of the compounds was measured using a turbidimetric method. A series of doubling dilutions of compounds were prepared in neat DMSO. Five microlitre samples were diluted twenty-fold into 95 µL volumes of phosphate buffered saline (PBS, pH 7.4) in microtiter assay plates and allowed to reach equilibrium at room temperature for 24 h. The absorbance within each well of the plate was read spectrophotometrically at a wavelength of 620 nm. A precipitate forms when the maximum aqueous solubility level is reached. The value quoted is the highest concentration where the compound was in solution, that is, where no measurable precipitate was present.
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