

Heteroaryl ether analogues of an antileishmanial 7-substituted 2-nitroimidazooxazine lead afford attenuated hERG risk: *in vitro* and *in vivo* appraisal

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Abstract

Previous investigation of the potent antileishmanial properties of antitubercular 7-substituted 2-nitroimidazo[2,1-*b*][1,3]oxazines with biaryl side chains led to our development of a new clinical candidate for visceral leishmaniasis (DNDI-0690). Within a collaborative backup program, a racemic monoaryl lead (**3**) possessing comparable activity in mice but a greater hERG liability formed the starting point for our pursuit of efficacious second generation analogues having good solubility and safety. Asymmetric synthesis and appraisal of its enantiomers first established that chiral preferences for *in vivo* efficacy were species dependent and that neither form afforded a reduced hERG risk. However, in line with our findings in a structurally related series, less lipophilic heteroaryl ethers provided significant solubility enhancements (up to 16-fold) and concomitantly attenuated hERG inhibition. One promising pyridine derivative (**49**) displayed 100% oral bioavailability in mice and delivered a 96% parasite burden reduction when dosed at 50 mg/kg in a *Leishmania donovani* mouse model of visceral leishmaniasis.

Keywords: Pretomanid, leishmaniasis, *in vivo* efficacy, pharmacokinetics, hERG inhibition, Chagas disease

Abbreviations

NTD, Neglected tropical disease; *L. don*, *Leishmania donovani*; *L. inf*, *Leishmania infantum*; VL, visceral leishmaniasis; TB, tuberculosis; PK, pharmacokinetic; DMPK, drug metabolism and pharmacokinetic; MEK, methyl ethyl ketone (2-butanone); *T. cruzi*, *Trypanosoma cruzi*; *T. brucei*, *Trypanosoma brucei*; SD, standard deviation; *M. tb*, *Mycobacterium tuberculosis*; HRESIMS, high resolution electrospray ionization mass spectrometry; APCI MS, atmospheric pressure chemical ionization mass spectrometry.

1. Introduction

Neglected tropical diseases (NTDs) have been dubbed the burden of the “bottom billion”, based on their prevalence amongst the poorest people in developing regions of Africa, Asia and South America [1]. One of the most concerning NTDs is leishmaniasis, with approximately one million new cases and an estimated 30,000 deaths each year [2]. Two parasite species, *Leishmania donovani* (*L. don.*, on the Indian subcontinent and East Africa) and *Leishmania infantum* (*L. inf.*, in Latin America and the Mediterranean region) are the causative agents for the severe visceral form of the disease (VL; named kala-azar or black fever), which is characterised by progressive damage to the internal organs, especially spleen, liver, bone marrow and lymph nodes [2,3]. Domestic dogs, asymptomatic carriers, and untreated post kala-azar dermal leishmaniasis cases may all serve as disease reservoirs for the sandfly vector that carries these parasites [3-6]. Because vector control can be challenging to sustain, a vaccine for VL has long been sought. However, while canine and human vaccines continue to be studied, the prospect of an effective vaccine still appears distant [7-9]. Furthermore, chemotherapeutic options are inadequate, with the four front-line VL drugs all suffering from major limitations, such as parenteral administration (except miltefosine **1**; see Fig. 1), high cost, variable efficacy within and between regions, significant toxicities (some leading to ongoing health problems) and growing resistance [10-12]. Combination regimens with these agents are not universally effective [13] and the long-term outcomes for patients co-infected with HIV are frequently unsatisfactory [14,15]. Thus, there is a pressing need for the discovery of new low-cost short-course oral drugs with better safety and efficacy [16,17].

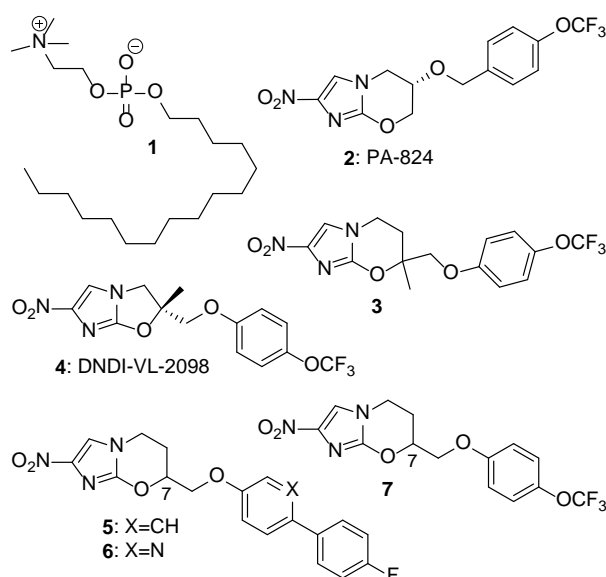


Fig. 1. Structures of antitubercular or antileishmanial agents.

Pretomanid (PA-824, **2**) is an orally active drug for tuberculosis (TB) that was recently FDA-approved (in combination with bedaquiline and linezolid) to treat highly challenging cases [18]. As part of our extensive SAR investigations of **2** with the TB Alliance [19], we had successfully applied a scaffold hopping approach to design a potent novel class of 7-substituted 2-nitroimidazooxazines (e.g., **3**) [20]. Phenotypic screening of some early examples against kinetoplastid diseases by the Drugs for Neglected Diseases *initiative* (DNDi) unexpectedly revealed their potent antileishmanial effects, alongside those of some closely related 2-substituted 6-nitroimidazooxazoles [21]; the biological mechanism involves activation by a novel leishmanial nitroreductase (NTR2) [22]. Following the identification of

oxazole **4** as an early preclinical lead [23], we further explored our new class for VL, in collaboration with DNDi. We have recently described [20] initial studies around an *in vivo*-active biphenyl lead (**5**) that culminated in the development of a new VL clinical candidate, DNDI-0690 (**7R-6**), having comparable efficacy but improved safety compared to **4**. In that report [20], we noted that the monoaryl analogue **3** displayed *in vivo* activity similar to that of biphenyl lead **5** in mouse and hamster VL models and showed a better pharmacokinetic (PK) profile. However, **3** posed some safety concerns due to moderately potent hERG inhibition, dissuading its further advance, while the 7-H analogue of **3** (**7**) had inferior PK and efficacy.

Physical properties, such as aqueous solubility, are an important consideration in the design of successful drugs [24-27]. Therefore, lead selection criteria for infectious disease drug discovery recommend that the aspiration to achieve high potency not be at the expense of inadequate physicochemical properties or DMPK characteristics [28]. As part of our VL lead optimisation program with DNDi, we aimed to identify several efficacious backup candidates having diverse product profiles to mitigate development risks [29]. Our previous SAR investigations of **2** and **4** had demonstrated that replacement of the side chain aryl ring by a nitrogen-containing heteroaryl ring was a promising strategy to increase aqueous solubility, without incurring inordinate activity loss [23,30]. For example, 2-pyridinyl ether **9** was just as efficacious as its phenyl ether counterpart **8** in two animal models of VL (Fig. 2), despite having a 2-fold lower *in vitro* potency against *L. inf*, due to its much better aqueous solubility and significantly improved PK data (greater oral exposure) [30]. We now report the results of a follow-up SAR study of 7-substituted 2-nitroimidazooxazine **3** in which we incorporate new aryl or heteroaryl side chains, seeking additional lead compounds for VL that possess improved solubility and safety profiles, together with suitable efficacy in animal models.

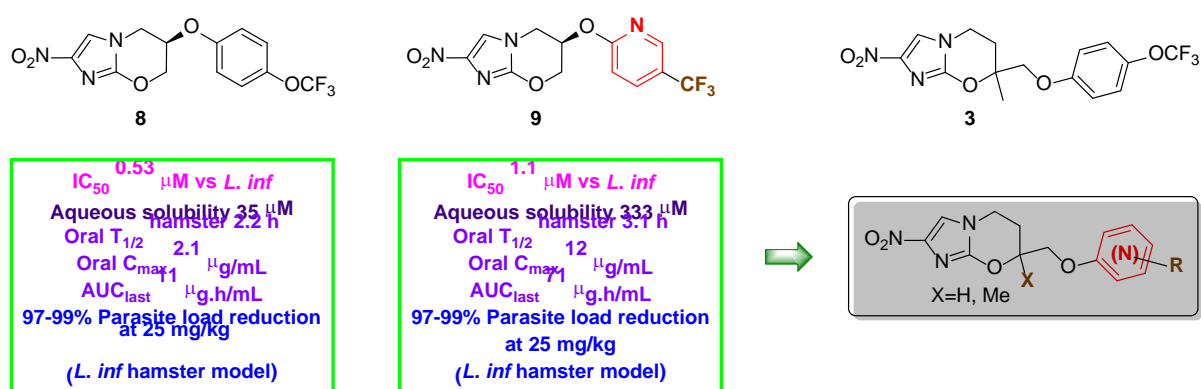


Fig. 2. Key data supporting the design of new heteroaryl ether analogues of early lead **3**.

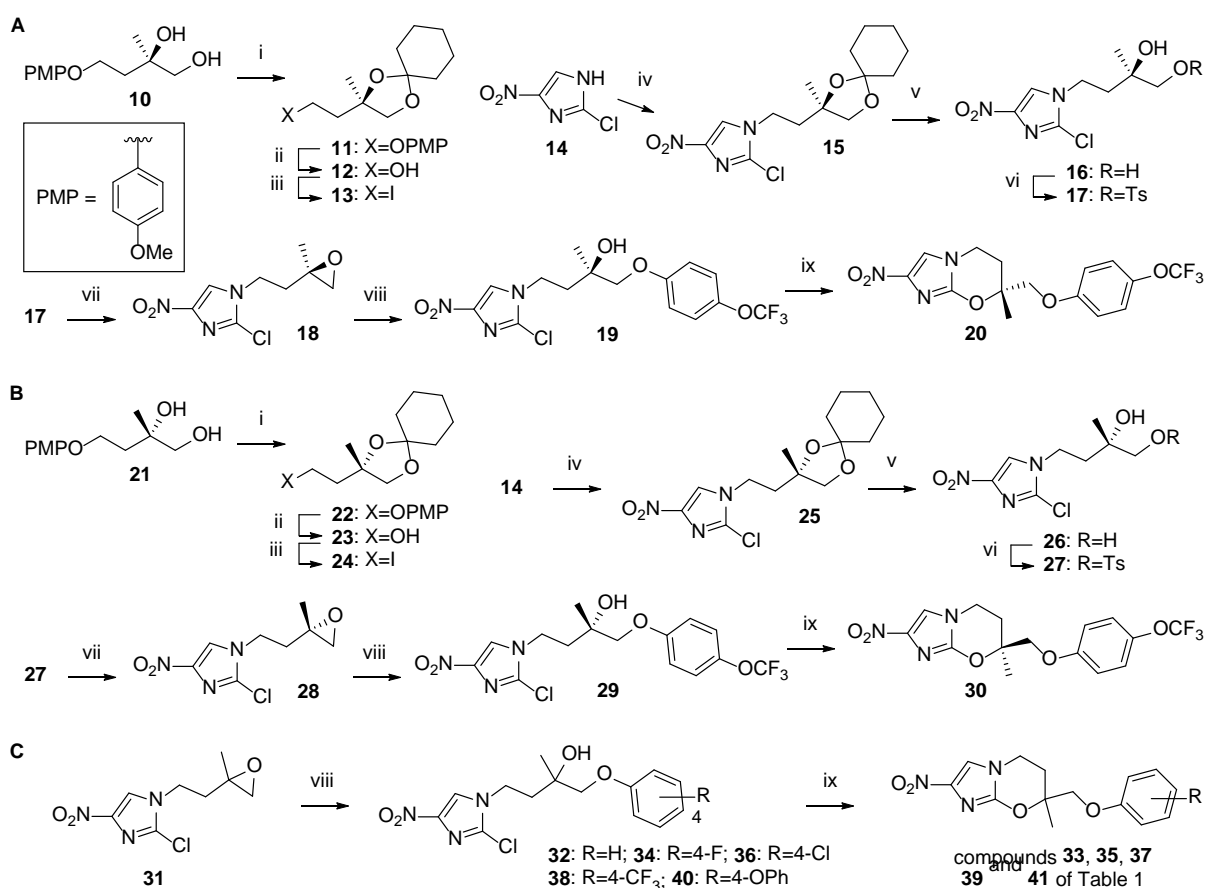
2. Results and discussion

2.1 Strategies for synthesis of the new analogues

Our first targets were the enantiomers of lead **3** (**20** and **30**), which could be achieved in nine steps from the known diols **10** [31] and **21** [32] (Scheme 1A,B). These diols were nicely accessible in high purity (>96% ee) by Sharpless asymmetric dihydroxylation of 4-methoxyphenyl-protected 3-methylbut-3-en-1-ol (the racemic diol [32] was also made as an HPLC standard). Ketalization with cyclohexanone, followed by oxidative cleavage of the 4-methoxyphenyl ether (CAN), generated alcohols **12** [31] and **23**, from which the key iodo derivatives (**13** and **24**) could be obtained directly, in almost quantitative yield. These iodides were further elaborated via successive alkylation of 2-chloro-4-nitroimidazole (**14**), acetal cleavage, tosylation, and base-assisted cyclization (DBU) to provide the requisite epoxides **18**

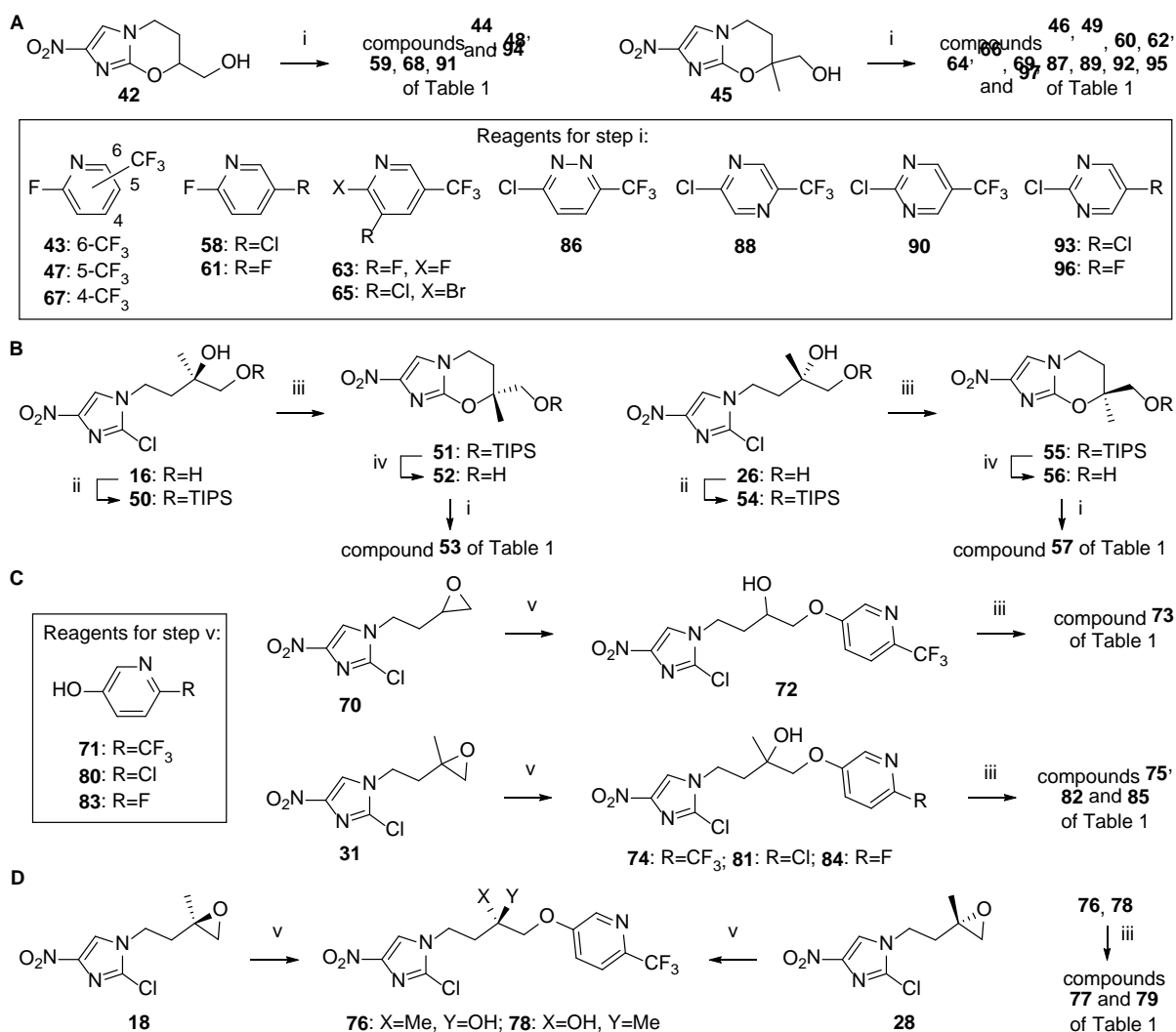
and **28**. Reaction of the latter with 4-(trifluoromethoxy)phenol (K_2CO_3 , MEK, 82 °C) and sodium hydride-induced ring closure of the resulting alcohol intermediates then furnished the desired enantiomers of **3**.

Several aryl (**33**, **35**, **37**, **39** and **41**) or 3-pyridinyl (**73**, **75**, **82** and **85**) analogues of **3** were efficiently secured by applying the above chemistry to racemic epoxides **31** [20] and **70** [20], using a variety of phenols (Scheme 1C) or 6-substituted pyridin-3-ols (Scheme 2C). Likewise, we prepared the enantiomers of the early 3-pyridine lead **75** (**77** and **79**) by employing epoxides **18** and **28** (Scheme 2D). Alternatively, sodium hydride-catalysed S_NAr reactions [23] of alcohols **42** [20] and **45** [20] with various haloheterocycles (Scheme 2A) led to a more extensive range of heteroaryl ether targets (**44**, **46**, **48**, **49**, **59**, **60**, **62**, **64**, **66**, **68**, **69**, **87**, **89**, **91**, **92**, **94**, **95** and **97**). To access the enantiomers of the leading 2-pyridinyl ether **49** (**53** and **57**), chiral diols **16** and **26** were converted into the pure *7R* and *7S* forms of alcohol **45** (Scheme 2B). This involved selective TIPS-protection of the primary hydroxyl group, ring closure with sodium hydride, and acid-catalysed desilylation [33]. These chiral alcohols (**52** and **56**) [20] were then reacted with fluoropyridine **47**, as before.



Scheme 1. Synthesis of the enantiomers of **3** and various aryl analogues.

Reagents and conditions: (i) cyclohexanone, TsOH, 20 °C, 0.5-1 h (98-100%); (ii) CAN, CH₃CN, H₂O, 0 °C, 10-20 min (90-93%); (iii) I₂, PPh₃, imidazole, CH₂Cl₂, 20 °C, 16-18 h (91-97%); (iv) **13** or **24**, K₂CO₃, DMF, 63-72 °C, 56-79 h (73-90%); (v) TsOH, MeOH, 20 °C, 73-78 h (81-89%); (vi) TsCl, pyridine, 0-20 °C, 21-30 h (100%); (vii) DBU, CH₂Cl₂, 20 °C, 2.5-3 h (96-97%); (viii) 4-OCF₃PhOH or RPhOH, K₂CO₃, MEK, 70-82 °C, 14-21 h (35-79%); (ix) NaH, DMF, 20 °C, 2.8-3 h, or 40-60 °C, 15-45 min (28-72%).



Scheme 2. Synthesis of new heteroaryl analogues of **3**.

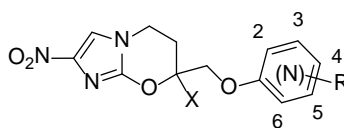
Reagents and conditions: (i) haloheterocycle (**43**, **47**, **58**, **61**, **63**, **65**, **67**, **86**, **88**, **90**, **93**, or **96**), NaH, DMF, 0-20 °C, 0.5-4 h (26-95%); (ii) TIPSCl, imidazole, DMF, 0-20 °C, 6.5-7.5 d (97%); (iii) NaH, DMF, 0-60 °C (or 80 °C), 0.25-3.5 h (36-85%); (iv) 1% HCl in 95% EtOH, 45-47 °C, 3.5-4 d (87%); (v) 6-substituted pyridin-3-ol (**71**, **80**, or **83**), K₂CO₃, MEK, 80-83 °C, 16-72 h (49-96%).

2.2 Biological assessment of new analogues

2.2.1 Overview of dataset and screening paradigm

In Table 1, we present the structures, calculated lipophilicities, and *in vitro* growth inhibition data for 33 new analogues of the 7-substituted 2-nitroimidazooxazine **3**. As in our previous studies [20,23,30], most of these were tested only once against *L. don* amastigotes in mouse macrophages, using a luciferase assay (CDRI, India) [34], with promising leads then being followed up through microsomal stability and *in vivo* PK/efficacy assessments in mice and hamsters. However, retrospective testing of the entire dataset against three more parasites (*L. inf*, *T. cruzi*, and *T. brucei*) and versus human lung fibroblasts (MRC-5) in replicate assays performed at the University of Antwerp (LMPH) [35] facilitated a much more definitive understanding of the *in vitro* activity trends and the possible utility of such compounds against up to three crucial NTDs (VL, Chagas disease and human African trypanosomiasis).

Table 1. *In vitro* antiparasitic and antitubercular activities of 7-(aryl or heteroaryl)oxymethyl 2-nitroimidazooxazines



compd	X	aza	R	CLogP ^a	IC ₅₀ ^{b,c} (μM)				Sel. Index ^d	MIC ^{c,e} (μM)	
					<i>L. don</i>	<i>L. inf</i>	<i>T. cruzi</i>	MRC-5		MABA	LORA
7^f	H		4-OCF ₃	3.21	0.04	0.047	0.061	>64	>1362	5.2	4.7
3^f	Me		4-OCF ₃	3.73	0.10	0.13	0.14	>64	>492	0.94	6.8
20	Me ^g		4-OCF ₃	3.73	0.06	0.098	0.052	>64	>653	0.59	1.4
30	Me ^h		4-OCF ₃	3.73	0.37	0.17	0.34	>64	>376	3.6	18
33	Me		4-H	2.53	0.30	0.52	0.59	>64	>123	1.3	8.1
35	Me		4-F	2.81	0.29	0.45	0.45	>64	>142	1.3	5.0
37	Me		4-Cl	3.38	0.05	0.13	0.18	>64	>492	0.86	7.0
39	Me		4-CF ₃	3.66	0.16	0.16	0.15	>64	>400	0.80	4.1
41	Me		4-OPh	4.63	0.17	0.17	0.25	>64	>376	0.70	2.7
44	H	2	3-CF ₃	2.39		>64	0.53	>64		(3.8)	(26)
46	Me	2	3-CF ₃	2.91		0.58	0.44	>64	>110	(2.0)	(14)
48	H	2	4-CF ₃	2.39	0.08	>64	1.0	>64		2.6	20
49	Me	2	4-CF ₃	2.91	0.21	0.27	0.29	>64	>237	0.60	8.9
53	Me ^g	2	4-CF ₃	2.91		0.29	0.34	>64	>221	0.16	3.8
57	Me ^h	2	4-CF ₃	2.91		0.75	0.98	>64	>85	(6.5)	(57)
59	H	2	4-Cl	2.17	0.10	>64	0.94	>64		(7.2)	(53)
60	Me	2	4-Cl	2.69	0.15	0.38	0.63	>64	>168	2.0	8.7
62	Me	2	4-F	2.12	0.26	1.6	3.3	>64	>40	2.3	19
64	Me	2	4-CF ₃ , 6-F	2.87		0.47	0.73	>64	>136	(1.9)	(16)
66	Me	2	4-CF ₃ , 6-Cl	3.41		0.26	0.29	>64	>246	(2.7)	(15)
68	H	2	5-CF ₃	2.39		>64	1.0	>64		(12)	(23)
69	Me	2	5-CF ₃	2.91		0.45	0.86	>64	>142	(2.3)	(12)
73	H	3	4-CF ₃	1.99	0.11	>64	1.1	>64		(30)	(49)
75	Me	3	4-CF ₃	2.51	0.09	1.9	0.78	>64	>34	66	33
77	Me ^g	3	4-CF ₃	2.51	0.44	1.3	0.90	>64	>49	(16)	>128
79	Me ^h	3	4-CF ₃	2.51	0.74	2.3	1.8	>64	>28	>128	>128
82	Me	3	4-Cl	2.29	0.86	2.8	0.93	>64	>23	(11)	(28)
85	Me	3	4-F	1.72	0.60	7.1	4.3	>64	>9	(28)	(54)
87	Me	2,3	4-CF ₃	1.48		>64	3.8	>64		(30)	>128
89	Me	2,5	4-CF ₃	2.02		2.2	0.94	>64	>29	(5.1)	(48)
91	H	2,6	4-CF ₃	1.50		>64	3.3	>64		(7.7)	(106)
92	Me	2,6	4-CF ₃	2.02		>64	3.1	>64		(7.8)	(117)
94	H	2,6	4-Cl	1.31	0.12	>64	39	>64		(13)	>128
95	Me	2,6	4-Cl	1.83	0.32	>64	3.7	>64		(30)	(16)
97	Me	2,6	4-F	1.26	0.67	>64	14	>64		(27)	(21)

^aCalculated log P values from ChemDraw v18.1. ^bIC₅₀ values for inhibition of the growth of *Leishmania donovani* and *Leishmania infantum* (in mouse macrophages) and *Trypanosoma cruzi* (on MRC-5 cells), or for cytotoxicity toward human lung fibroblasts (MRC-5 cells). ^cEach value (except the single test *L. don* data and MIC data in parentheses) is the mean of at least two independent determinations. For full results (mean ± SD), see the Supplementary data. ^dSelectivity index: ratio of MRC-5 to *L. inf* IC₅₀ values. ^eMinimum inhibitory concentration against *M. tb*, run under aerobic (MABA) [36] or hypoxic (LORA) [37] conditions. ^fRef 20. ^g(*R*)-Enantiomer. ^h(*S*)-Enantiomer.

Finally, the submicromolar MIC potencies of several derivatives from this class against *Mycobacterium tuberculosis* (*M. tb*) [20] motivated us to counter-screen our new analogues in MABA [36] (aerobic) and LORA [37] (anaerobic) assays. For better clarity, these results and the antitrypanosomal data will be briefly discussed in a separate final section.

2.2.2 Evaluation of the enantiomers of **3** for VL and SAR for new phenyl ring substituents

To begin this study, we elected to first investigate the enantiomers of **3**, in an attempt to gain greater insight into chiral preferences for activity, metabolic stability and safety in the 7-methyl series. In line with findings for the enantiomers of **6** [20], the 7*R* form **20** was ca. 6-more potent than its 7*S* counterpart **30** against *L. don* (IC₅₀s 0.06 vs 0.37 μM; Table 1). This trend was maintained for *L. inf*, although the difference was only approximately 2-fold (IC₅₀s 0.098 vs 0.17 μM). Moreover, 7*R* form **20** was also found to be more resistant than **30** toward *in vitro* metabolism, as determined by incubations with liver microsomes from three species (human, mouse and hamster); e.g., mouse microsomes: 65% parent compound remained after 1 h for **20** vs 36% for **30** (Table 2). As expected, their aqueous solubility values were similar.

Table 2. Aqueous solubility, microsomal stability, hERG inhibitory activity, and *in vivo* (mouse) antileishmanial efficacy data for selected analogues

compd	aq solubility ^a (μg/mL)		microsomal stability ^b [% remaining at 1 (0.5) h]			hERG IC ₅₀ (μM)	<i>in vivo</i> efficacy against <i>L. don</i> (% load redn at dose in mg/kg) ^d				
	pH=7	pH=1	H	M	Ham	(or %) ^c	50	25	12.5	6.25	3.13
4^e	2.4		(92)	(89)	18 (54)	10.5			>99	>99	83
7^f	4.0		85	57	(23)			87			
3^f	2.3		58	50	2.1	3.8		100	100	83	25
20	3.0		63	65	1.9	2.6		>99	98	49	51
30	2.2		50	36	1.2	1.4		66	42	56	18
48	37	32	83	71 (86)	5.5 (31)	(4)	46				
49	19	12	100	77 (86)	3.0 (17)	(18)	96				
53	26		73	61	0.4		84				
57	21		70	52	1.8		38				
59	18			(87)	(33)	(17)					
60	23		(100)	(88)	(5)	(12)	14				
64	13		64	44	0.4		56				
66	11		23	14	0		0				
73	29		(100)	(91)	(85)	(4)			0		
75	7.3		(92)	(80)	(78)	(17)	42		23		
77	12		86	89	57		72				
79	15		89	90	55		46				
87	10		90	89	58		0				
89	17		91	80	36		17				
92	9.3		88	86	40		35				
94	6.6		(99)	(100)	(90)	(12)			0		

^aKinetic solubility (μg/mL) in water (pH 7) or 0.1 M HCl (pH 1) at 20 °C, determined by HPLC (see the Experimental Section). ^bPooled human (H), CD-1 mouse (M), or hamster (Ham) liver microsomes; data in parentheses are the percentage parent compound remaining following a 30 min incubation. ^cPercent inhibition at 10 μM. ^dDosing was orally, once daily for 5 days consecutively; data are the mean percentage reduction of parasite burden in the liver. ^eData from ref 23. ^fData from ref 20.

These results translated into superior efficacy for the 7*R* enantiomer **20** in the mouse model of acute *L. don* infection (Table 2). Hence, once daily oral dosing of **20** at 25 mg/kg for five days gave essentially complete elimination of the parasites in mouse livers, compared to a 66% reduction in parasite load for **30** in the same assay. Dose-response testing confirmed this conclusion and furnished ED₅₀ values of 3.8 and 11 mg/kg for **20** and **30**, respectively. Finally, both enantiomers were evaluated in the stringent *L. inf* early curative hamster model. This chronic infection *in vivo* assay is widely considered to be a more authentic experimental model for the progressive human VL disease [38]. Unexpectedly, in this case, the efficacy data marginally favoured the 7*S* enantiomer **30**, which provided parasite burden reductions of 91-97% in liver, spleen and bone marrow following oral dosing twice daily for 5 days at 25 mg/kg, and 99.7-100% at 50 mg/kg (Table 3 and Fig. 3). In comparison, **20** gave parasite load reductions of 82-94% at 25 mg/kg and 97-99.6% at 50 mg/kg in the same experiment.

Table 3. *In vivo* efficacy data for selected compounds in the *L. inf* hamster model

compd	dose ^a (mg/kg)	% load redn in target organs ^b		
		liver	spleen	bone marrow
1	40	98.5	99.7	94.9
3^c	50	100	99.7	99.5
20	25	92.2	91.1	82.5
	50	99.6	98.0	96.7
	25	93.6	83.0	81.5
30	12.5	67.8	59.5	42.5
	50	100	99.9	99.7
	25	97.2	92.3	91.1
53	12.5	47.3	44.4	0.0
	50	51.8	47.1	54.0
57	50	46.5	52.3	37.4

^aAll test compounds were dosed orally, twice daily for 5 days consecutively; miltefosine (**1**) was dosed once daily for the same period. ^bData are the mean percentage reduction of parasite burden in target organs. ^cLow dose data from ref 20.

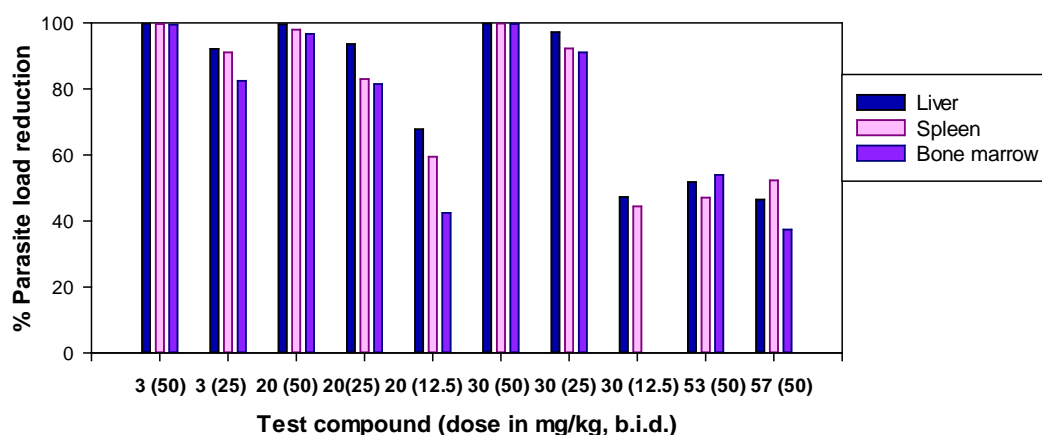


Fig. 3. Comparative *in vivo* efficacy against *L. inf* in the VL hamster model (LMPH).

To better understand the origins of this variance, we measured *in vivo* PK data for **20** and **30** in mice and hamsters (Table 4). In both cases, the 7*S* enantiomer **30** provided greater oral exposure (see also Figures S1 and S3 in the Supplementary data), and the intravenous PK data in hamsters further revealed that **20** had a shorter half-life and slightly higher rate of

clearance than **30**. Given that these two enantiomers exhibit more comparable activity against *L. inf* (the species used in the hamster model) than against *L. don* (the species used in the mouse model), it is not hard to see how divergent potency and PK advantages for one form over the other might lead to differing outcomes in the two *in vivo* assays. This also highlights one of the challenges of working with chiral lead compounds, as the decision about which enantiomer to progress toward human trials often hinges on data from various animal models.

Unfortunately, in terms of safety, both **20** and **30** were found to be fairly potent inhibitors of the hERG channel (IC₅₀s 2.6 and 1.4 μM vs 10.5 μM for **4**; Table 2), conferring a significant risk of QT prolongation [39]. Recommended strategies to address hERG inhibition include reducing CLogP by the incorporation of heteroatoms or polar groups, as well as employing electron-withdrawing groups on aryl rings wherever possible [40]. With this in mind, we examined the influence of the phenyl ring substituent in racemic lead **3** via a small set of new analogues (**33**, **35**, **37**, **39**, and **41**). Overall, the most lipophilic groups (4-OCF₃, 4-Cl, 4-CF₃ and 4-OPh) led to superior activity (*L. inf* IC₅₀s 0.13-0.17 μM, Table 1; about 3-fold better than with 4-H and 4-F). Given that trifluoromethoxy-substituted heteroaryl intermediates are not readily accessible (either commercially or synthetically), these data pointed toward the potential value of chloro- or trifluoromethyl-substituted heteroaryl ether side chains.

Table 4. Pharmacokinetic parameters for selected compounds in mice and hamsters

compd	intravenous (1-2 mg/kg) ^a					oral (12.5-50 mg/kg) ^a				
	C ₀ (μg/mL)	CL (mL/ min/kg)	V _{dss} (L/kg)	t _{1/2} (h)	AUC _{last} ^b (μg·h/mL)	C _{max} (μg/mL)	T _{max} (h)	t _{1/2} (h)	AUC _{last} ^b (μg·h/mL)	F ^c (%)
<i>Mice</i>										
7 ^d	0.36	48	3.2	1.1	0.341	1.3	0.50		3.86	45
3 ^d	0.79	12	2.5	2.8	1.31	1.4	3.0		11.5	35
20						5.6	6.7		59.5	
30						12	4.7	3.1	85.7	
49	0.81	31	1.3	0.85	0.544	15	0.50		57.1	100
53						9.5	3.7	1.5	39.6	
57						33	2.0	1.5	183	
60	2.6	8.3	0.50	0.65	2.01	11	1.0		57.8	100
73	2.3	5.4	0.47	1.1	3.10	5.8	0.25		15.1	39
75	1.6	4.1	0.46	1.5	4.00	11	1.0		41.8	84
77	1.1	6.6	0.91	1.3	2.52	7.3	2.0		37.2	59
79	1.9	6.0	0.75	1.2	2.76	8.0	2.0		37.3	54
94	1.9	7.3	0.37	0.58	2.07	0.76	2.0		3.83	15
<i>Hamsters</i>										
3	0.48	172	5.7	0.48	0.174	1.4	2.0	1.4	3.86	95
20	0.57	240	6.2	0.38	0.125	0.60	2.0	0.94	1.86	72
30	0.43	181	8.2	0.71	0.180	0.63	4.0	- ^e	2.45	61
53						0.045	0.75	0.72	0.072	
57						0.31	4.3	- ^e	0.924	

^aThe intravenous dose was 1 mg/kg for mice and 2 mg/kg for hamsters. The oral dose in mice was 25 mg/kg, except for **53** and **57** (45 and 51 mg/kg, respectively) and **73**, **75** and **94** (12.5 mg/kg), while the oral dose in hamsters was 41-51 mg/kg. ^bArea under the curve calculated to the last time point (8 or 24 h). ^cOral bioavailability, determined using dose normalised AUC_{last} values. ^dData from ref 20. ^eNot calculable.

2.2.3 Evaluation of new heteroaryl ether analogues of **3** for VL

The effectiveness of trifluoromethylpyridine as a substitute for 4-(trifluoromethoxy)phenyl has already been demonstrated in our previous VL and TB studies [19,20,23,30] (e.g., **9**). Therefore, for the present work, we probed a broad set of both substituted pyridinyl ethers and pyrimidinyl, pyrazinyl and pyridazinyl ethers (Table 1). Initial screening of several 2-pyridinyl and 3-pyridinyl examples against *L. don* *in vitro* indicated that high potency could be maintained (e.g., **48**, **49**, **59**, **60**, **73** and **75**; IC₅₀s 0.08-0.21 μM *cf.* 0.04 and 0.10 μM for **7** and **3**); likewise, a 2,6-pyrimidinyl ether congener (**94**) was similarly active (IC₅₀ 0.12 μM). The early SAR appeared to track with the results for aryl ethers, with 4-chloro and 4-trifluoromethyl substituents being generally preferred (except in the case of the less active 3-pyridinyl ether **82**), and 7-H analogues being about 2-fold more potent than the corresponding 7-Me derivatives. Consistent with their lower predicted lipophilicity values in comparison to **3** (by 0.8-2.5 log units), these first heteroaryl ether examples were up to 16-fold more water soluble (e.g., 7-H pyridine **48**: 37 μg/mL; Table 2). Importantly, these compounds also demonstrated high microsomal stability (>80% parent remaining after 30 min) and only a modest interaction with the hERG channel (<20% inhibition at 10 μM; Table 2).

In view of these very encouraging findings, the enantiomers of 7-Me pyridines **49** and **75** were prepared, and three promising compounds (**73**, **75** and **94**) were further evaluated in the *L. don* mouse model, dosing at 12.5 mg/kg, once daily for five days. However, of these three candidates, only **75** displayed any measurable efficacy at this overly-optimistic dose level (23% parasite load reduction). A parallel investigation of their mouse PK data (Table 4) revealed that 7-H pyrimidine **94** had very poor oral exposure, coupled with low oral bioavailability (15%) and a short half-life (35 min). Similarly, the PK profile of 7-H pyridine **73** was markedly inferior to that of the 7-Me derivative **75**, with large differences being observed in total oral exposure and oral bioavailability (Table 4 and Supplementary data, Figure S2), despite the former compound (**73**) being 4-fold more soluble in water. These findings recapitulated some of the variances seen in analogous data for 7-H aryl ether **7** and its 7-Me counterpart **3**, such as the better solubility but shorter half-life and smaller area under the curve recorded for **7** [20].

Another factor to consider in the unsatisfactory *in vivo* efficacy outcomes for **73**, **75** and **94** was the unforeseen *in vitro* potency data that was retrospectively measured against *L. inf* (Table 1). Surprisingly, only the 7-Me pyridine **75** (*L. inf* IC₅₀ 1.9 μM) exhibited any significant activity in this assay. Disparate *in vitro* results from the *L. don* and *L. inf* assays had been occasionally noticed in our previous studies [20,23,30], particularly for more hydrophilic heterobiaryl side chain analogues (CLogP <2.5) in this 7-H subclass of 7-substituted 2-nitroimidazooxazines [20]. In general, we discovered that potency against *L. inf* was more discriminating and tended to better correlate with observed *in vivo* outcomes. Therefore, we will focus the remaining discussion of *in vitro* activity on these new assay data.

A reanalysis of the *in vitro* SAR based on *L. inf* potencies across the entire dataset of heteroaryl ether analogues of **3** led to the following conclusions. First, all 7-H compounds and all pyrimidinyl ethers displayed negligible activity (IC₅₀s >64 μM); pyridazinyl ether **87** was also inactive but pyrazinyl ether **89** showed modest activity (IC₅₀ 2.2 μM). Second, the optimal position for ring substitution was *para* to the ether linkage (position 4, as labelled in the Table 1 drawing), with both **46** (3-CF₃) and **69** (5-CF₃) being less effective than **49** (4-CF₃) (IC₅₀s 0.58, 0.45 and 0.27 μM, respectively). Third, 7*R* enantiomers (**53**, **77**) were about 2-fold more potent than 7*S* counterparts (**57**, **79**), as seen for **20** and **30**. Fourth, both chloro and trifluoromethyl substituents were consistently preferred over fluoro; this was also true

when **49** (4-CF₃) was additionally substituted with a 6-halide (compounds **64** and **66**), as 6-chloro derivative **66** was the most active one. Fifth, 3-pyridinyl ethers (**75**, **82**, and **85**) were 4- to 7-fold less effective than the corresponding 2-pyridinyl ethers (**49**, **60**, and **62**). Overall, the most potent new heteroaryl ether analogues were **49**, **53**, and **66** and there were no cytotoxicity concerns for any compounds (MRC-5 IC₅₀s all >64 μM, leading to selectivity indices exceeding 100 in many cases).

In an attempt to better understand the potential utility of these new derivatives, a much larger set of compounds (**48**, **49**, **53**, **57**, **60**, **64**, **66**, **75**, **77**, **79**, **87**, **89** and **92**) was assessed in the same VL mouse model, employing the higher dosage of 50 mg/kg. Here, the 7-H 2-pyridinyl ether **48** (4-CF₃) was only weakly efficacious (46% load reduction), but its 7-Me counterpart **49** exhibited excellent activity (96% reduction in liver parasite burden; Table 2 and Fig. 4). Comparing the two enantiomers of **49**, the 7*R* enantiomer **53** was indeed the more active form (84% reduction vs 38% for **57**). However, the 4-chloro analogue of **49** (**60**) was ineffective (14%), despite these compounds having very similar *in vitro* potency and aqueous solubility profiles (**60** and **49**: *L. inf* IC₅₀s 0.38 and 0.27 μM, solubility values 23 and 19 μg/mL). This result was much less predictable, as **60** displayed excellent mouse microsomal stability (88% parent remaining after 30 min), an oral exposure in mice equal to that of **49**, and an identical oral bioavailability of 100% (Table 4 and Supplementary data, Figure S2). Addition of a second aryl ring substituent to **49** (**64** and **66**) unfortunately led to more rapid metabolism and lower *in vivo* efficacy (56% and 0%, respectively). Of the remaining compounds in this set, only the 7*R* enantiomer of **75** (**77**: 72% load reduction) showed reasonable activity in the VL mouse model. Hence, 2-pyridinyl ether **49** was identified as the most active lead in this study.

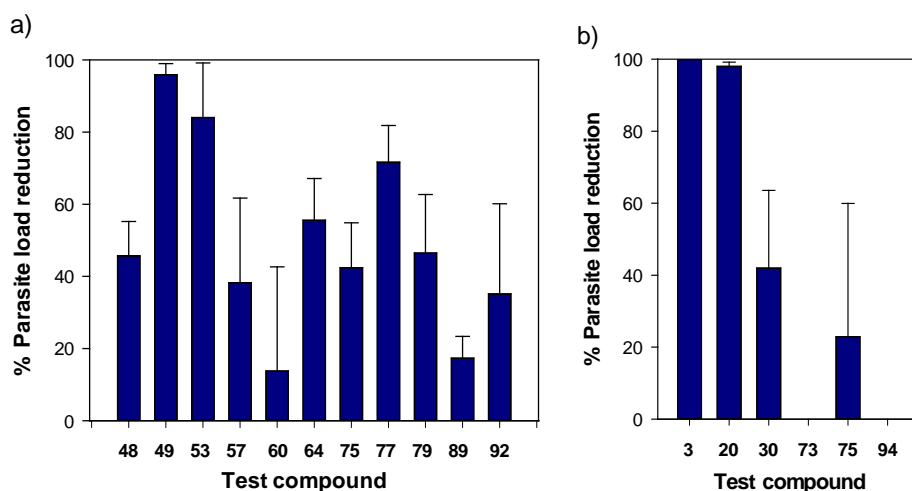


Fig. 4. Comparative *in vivo* efficacy against *L. don* in the VL mouse model: (a) 50 mg/kg and (b) 12.5 mg/kg.

The final stage in this investigation of heteroaryl ethers was to evaluate the enantiomers of **49** (**53** and **57**) in the more stringent hamster model of VL. The compounds were dosed orally, twice daily for five days at 50 mg/kg, but in this case the percentage reduction of amastigote burden in target organs was inadequate (47-54% for **53** and 37-52% for **57**; Table 3). A follow-up PK study indicated that the oral exposure of 7*R* enantiomer **53** in hamsters was exceptionally poor, about 13-fold inferior to data for the less potent 7*S* enantiomer **57** (Table 4 and Supplementary data, Figure S3). Given that both enantiomers were about 10-fold more soluble than **3** and the formulations for dosing were clear solutions, it appears likely that the underlying issue for **53** was very rapid first-pass metabolism, as suggested by the hamster microsomal stability data (Table 2), where **53** had a half-life of just 7 min. Rapid rates of

metabolism in hamsters were also observed for **3** and its enantiomers (**20** and **30**), as well as for the other 2-pyridinyl ethers assessed here.

Current target product profiles of a new chemical entity for the treatment of VL call for clinical efficacy of at least 95% through either once daily oral dosing for 10 days, or (less preferably) twice daily oral dosing for less than 10 days [41]. In weighing up the possible usefulness of new leads **49** and **53**, it is important to note that while their *in vivo* efficacies may be somewhat diminished in comparison to results for **3-8**, their aqueous solubility values are significantly better (53-73 μM), and finding the right balance of properties is an important consideration in drug development, as discussed above [24,27,28]. Both compounds exhibit greater efficacy than the 6*R* enantiomer of pretomanid (**2**), which has equivalent aqueous solubility and was previously touted as a suitable development candidate for VL [42]. In this context, it is also worthwhile to reference the profile of a new drug candidate for VL, GSK3186899 (DDD853651), which is currently in phase I clinical trials [43]. This compound has 7-fold weaker activity than **49** in macrophages (*L. don* IC₅₀ 1.4 μM) and requires twice daily oral dosing at 50 mg/kg for five days to achieve a 96% reduction in parasite load in the VL mouse model (or once daily dosing at 50 mg/kg for ten days to see a 95% inhibitory effect) [44]. The plasma half-life of this clinical candidate in mice, following intravenous administration, is approximately 18 minutes, and its solubility in simulated physiological media (pH 6.5) is 17-25 $\mu\text{g/mL}$ (35-51 μM) [44]. Therefore, a quick comparison of some key attributes of the molecules as potential antileishmanial drugs suggests that our new leads **49** and **53** hold significant promise when benchmarked against equally soluble compounds, and may offer an important safety advantage in comparison to earlier leads, such as **3** and **4**.

2.2.4 SAR of new analogues of **3** for Chagas disease and tuberculosis

While the major focus of this present work was the discovery of new lead candidates to treat VL, we were also interested to know whether these new compounds had any wider potential as therapies for trypanosomal infections. 2-Nitroimidazooxazines have typically [20,30] displayed weak or poor inhibitory potencies against *T. brucei* (the primary screen for human African trypanosomiasis) and this pattern continued here, with no compounds showing any significant activity (all IC₅₀s >64 μM ; see Table S1 in the Supplementary data). In contrast, this class has previously [20] demonstrated meaningful (submicromolar) potencies against *T. cruzi*, the causative agent for Chagas disease, and this was also evident for the majority of examples in Table 1. Further analysis revealed that the 7*R* enantiomer of **3** (**20**: IC₅₀ 0.052 μM) was the standout compound, while the 2-pyridinyl ethers **49** and **66** (IC₅₀s 0.29 μM) and 7*R* enantiomer **53** (IC₅₀ 0.34 μM) were the best of the heteroaryl derivatives. As shown for *L. inf*, activity against *T. cruzi* was nicely maintained for close aryl analogues of **3** (**37**, **39** and **41**; IC₅₀s 0.15-0.25 μM) but tended to fall away for 3-pyridinyl and diaza heteroaryl ether congeners. However, unlike the *L. inf* dataset, all compounds had measurable *T. cruzi* activity and only two IC₅₀ values were greater than 5 μM .

Finally, because the original design and exemplification of this 7-substituted oxazine class was for TB [20], the antitubercular activities of any new compounds have also remained a focus of ongoing interest. As shown for both *L. inf* and *T. cruzi*, the three more lipophilic aryl analogues of **3** (**37**, **39** and **41**) retained potencies at least comparable to the parent in both MIC assays, and 7*R* enantiomer **20** (MABA 0.59 μM , LORA 1.4 μM) was the preferred chiral form of **3**. Moreover, the 2-pyridinyl ether **49** and its 7*R* enantiomer **53** (MABA MICs 0.60 and 0.16 μM) were again the most useful heteroaryl derivatives, in this case slightly

better than **3** and **20**, respectively. Conversely, 3-pyridinyl and diaza heteroaryl ethers yielded consistently poor results. Therefore, the preferred lead for TB from this study was **53**.

3. Conclusions

Currently available treatments for VL are inadequate to meet all of the medical needs, but new chemical entities are now beginning to emerge and repopulate the VL drug candidate pipeline [45]. Nevertheless, recent clinical trial failures with sitamaquine and fexinidazole (for VL) [45,46] have emphasised the major challenges faced in gaining successful approval of even one new therapy, suggesting the prudence of generating multiple backup candidates with diverse product profiles to mitigate development risks. In this new study of the promising 7-substituted 2-nitroimidazooxazine class for VL, we first evaluated the enantiomers of an earlier lead (**3**) to determine whether better efficacy and safety lay with one chiral form. However, efficacy preferences were model (species) dependent and neither enantiomer could successfully reduce the significant hERG risk encountered with **3**.

A limited study of phenyl substitution effects then identified a clear potency advantage for more lipophilic substituents (4-OCF₃, 4-Cl, 4-CF₃, and 4-OPh) and this was applied to our wider investigation of heteroaryl ethers, where trifluoromethoxy substitution was not readily accessible. Here, the incorporation of one or two aza atoms into the side chain aryl ring reduced overall lipophilicity of the compounds in comparison to **3** (CLogP decreases of 0.8-2.5 log units), leading to aqueous solubility enhancements of up to 16-fold and markedly reduced hERG inhibition. In terms of activity against *L. inf*, 2-pyridinyl ethers were strongly preferred over 3-pyridinyl or other heteroaryl ethers and a 7-methyl group was mandatory. Overall, **49** was the best analogue, displaying 100% oral bioavailability in mice and excellent efficacy (96% parasite load reduction with once daily oral dosing for five days at 50 mg/kg) in the *L. don* mouse model. An assessment of its two enantiomers further revealed that 7*R* enantiomer **53** was the most active chiral form, albeit, the oral PK profile of **53** was less favourable than that of the racemate **49** or its 7*S* counterpart **57**. Finally, counter-screening of the new compounds against both *T. cruzi* and *M. tb* revealed a surprisingly high degree of overlap in the SAR for VL, Chagas disease and TB, as noted in our earlier report [20], such that **53** was also the most potent TB lead. Collectively, these findings provide further critical insight into the effectiveness of heteroaryl groups (notably trifluoromethylpyridine) as substitutes for a 4-(trifluoromethoxy)phenyl ring, as well as the potential opportunity to develop new 7-substituted 2-nitroimidazooxazine-based drug candidates for several important neglected diseases.

4. Experimental section

4.1. General information

Combustion analyses were performed by the Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined using an Electrothermal IA9100 melting point apparatus and are as read. NMR spectra were measured on a Bruker Avance 400 spectrometer at 400 MHz for ¹H and 100 MHz for ¹³C and were referenced to Me₄Si or solvent resonances. Chemical shifts and coupling constants were recorded in units of ppm and hertz, respectively. High-resolution electrospray ionisation mass spectrometry (HRESIMS) was conducted on a Bruker micrOTOF-Q II mass spectrometer. Low-resolution atmospheric pressure chemical ionisation (APCI) mass spectra were obtained for organic solutions using a ThermoFinnigan Surveyor MSQ mass spectrometer connected to a Gilson autosampler. Optical rotations were measured on a Schmidt + Haensch

Polartronic NH8 polarimeter. Column chromatography was performed on silica gel (Merck 230-400 mesh). Thin-layer chromatography was carried out on aluminium-backed silica gel plates (Merck 60 F₂₅₄), with visualization of components by UV light (254 nm), I₂, or KMnO₄ staining. Tested compounds (including batches screened *in vivo*) were ≥95% pure, as determined by combustion analysis and/or by HPLC conducted on an Agilent 1100 system with diode array detection, using a 150 mm x 3.2 mm Altima 5 μm reversed phase C8 or C18 column. Chiral purity was assessed by HPLC carried out on a Gilson Unipoint system (322-H pump, 156 UV/vis detector), employing a 250 mm x 4.6 mm CHIRALPAK IA 5.0 μm analytical column. Combustion analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values.

4.2 Syntheses of aryl ethers **20**, **30**, **33**, **35**, **37**, **39** and **41**

4.2.1 General procedure for preparing cyclohexylidene acetals **11** and **22**

A mixture of the chiral diol (**10** or **21**, 6.8 g, 1.0 equiv) and 4-methylbenzenesulfonic acid monohydrate (0.22 equiv) in cyclohexanone (90 mL) was stirred at 20 °C for 1 h. The resulting mixture was added to saturated aq NaHCO₃ (200 mL) and extracted with EtOAc (4 x 200 mL). The combined extracts were evaporated to dryness under reduced pressure (at 30 °C) and the residue was chromatographed on silica gel (0-5% Et₂O/petroleum ether) to afford the required products.

4.2.1.1 (2*R*)-2-[2-(4-Methoxyphenoxy)ethyl]-2-methyl-1,4-dioxaspiro[4.5]decane (**11**)

The title compound was obtained from (2*R*)-4-(4-methoxyphenoxy)-2-methylbutane-1,2-diol [31] (**10**). Colourless oil (100% yield); ¹H NMR (CDCl₃) δ 6.83 (s, 4 H), 4.08 (dt, *J* = 9.5, 6.3 Hz, 1 H), 4.02 (dt, *J* = 9.5, 7.1 Hz, 1 H), 3.94 (d, *J* = 8.5 Hz, 1 H), 3.77 (s, 3 H), 3.75 (d, *J* = 8.5 Hz, 1 H), 2.12-1.99 (m, 2 H), 1.72-1.51 (m, 8 H), 1.49-1.27 (m, 5 H); HRESIMS calcd for C₁₈H₂₆NaO₄ *m/z* [M + Na]⁺ 329.1723, found 329.1724.

4.2.1.2 (2*S*)-2-[2-(4-Methoxyphenoxy)ethyl]-2-methyl-1,4-dioxaspiro[4.5]decane (**22**)

The title compound was obtained from (2*S*)-4-(4-methoxyphenoxy)-2-methylbutane-1,2-diol [32] (**21**), using the above procedure for 30 min. Colourless oil (98% yield); ¹H NMR (CDCl₃) δ 6.83 (s, 4 H), 4.08 (dt, *J* = 9.5, 6.2 Hz, 1 H), 4.02 (dt, *J* = 9.5, 7.1 Hz, 1 H), 3.94 (d, *J* = 8.5 Hz, 1 H), 3.77 (s, 3 H), 3.75 (d, *J* = 8.5 Hz, 1 H), 2.12-1.99 (m, 2 H), 1.72-1.52 (m, 8 H), 1.49-1.28 (m, 5 H); HRESIMS calcd for C₁₈H₂₆NaO₄ *m/z* [M + Na]⁺ 329.1723, found 329.1717.

4.2.2 General procedure for preparing alcohols **12** and **23**

A solution of the PMP ether (**11** or **22**, 9.4 g, 1.0 equiv) in acetonitrile (240 mL) and water (60 mL) at 0 °C was treated with CAN (2.1 equiv). The mixture was stirred at 0 °C for 20 min and then added to saturated aq NaHCO₃ (200 mL) and extracted with Et₂O (6 x 200 mL). The extracts were washed with brine (150 mL) and then concentrated under reduced pressure (at 25 °C) and the residue was chromatographed on silica gel (0-30% Et₂O/petroleum ether) to afford the required products.

4.2.2.1 2-[(2*R*)-2-Methyl-1,4-dioxaspiro[4.5]decan-2-yl]ethan-1-ol (**12**)

The title compound was obtained from **11**. Pale red oil (90% yield); ¹H NMR (CDCl₃) δ 3.91 (ddt, *J* = 11.4, 8.7, 3.7 Hz, 1 H), 3.84 (d, *J* = 8.4 Hz, 1 H), 3.81-3.72 (m, 2 H), 2.84 (dd, *J* = 7.1, 3.9 Hz, 1 H), 1.92 (ddd, *J* = 14.4, 8.8, 4.5 Hz, 1 H), 1.73 (ddd, *J* = 14.4, 5.8, 3.8 Hz, 1 H), 1.70-1.49 (m, 8 H), 1.48-1.30 (m, 5 H); HRESIMS calcd for C₁₁H₂₀NaO₃ *m/z* [M + Na]⁺ 223.1305, found 223.1308; [α]²⁵_D 10.7 (*c* 1.03, CHCl₃) [lit. [31] [α]²⁰_D 11.2 (*c* 0.66, CHCl₃)].

4.2.2.2 2-[(2*S*)-2-Methyl-1,4-dioxaspiro[4.5]decan-2-yl]ethan-1-ol (**23**)

The title compound was obtained from **22**, using the above procedure for 10 min. Pale orange oil (93% yield); ¹H NMR (CDCl₃) δ 3.91 (ddt, *J* = 11.4, 8.7, 3.8 Hz, 1 H), 3.84 (d, *J* = 8.4 Hz, 1 H), 3.81-3.72 (m, 2 H), 2.83 (dd, *J* = 7.1, 3.9 Hz, 1 H), 1.92 (ddd, *J* = 14.4, 8.7, 4.5 Hz, 1 H), 1.73 (ddd, *J* = 14.4, 5.8, 3.8 Hz, 1 H), 1.70-1.50 (m, 8 H), 1.48-1.30 (m, 5 H); HRESIMS calcd for C₁₁H₂₀NaO₃ *m/z* [M + Na]⁺ 223.1305, found 223.1296; [α]²⁷_D -12.2 (c 1.064, CHCl₃).

4.2.3 General procedure for preparing iodides **13** and **24**

A solution of iodine (1.4 equiv) in anhydrous CH₂Cl₂ (24 mL/g of iodine) was added dropwise over 1 h (with water bath cooling) to a stirred mixture of the alcohol (**12** or **23**, 1.0 equiv), imidazole (2.6 equiv) and triphenylphosphine (1.3 equiv) in anhydrous CH₂Cl₂ (7 mL/g of alcohol) under N₂. After being stirred at 20 °C for 16 h, the mixture was concentrated under reduced pressure (at 25 °C) to about 20 mL and then added to excess petroleum ether (150 mL) at the top of a silica gel column (40 g in petroleum ether), rinsing residues onto the column with minimal additional CH₂Cl₂ (4 x 3 mL). Elution of this mixture and then 10% Et₂O/pentane afforded the required products.

4.2.3.1 (2*R*)-2-(2-Iodoethyl)-2-methyl-1,4-dioxaspiro[4.5]decane (**13**)

The title compound was obtained from alcohol **12**. Colourless oil (97% yield); ¹H NMR (CDCl₃) δ 3.79 (d, *J* = 8.5 Hz, 1 H), 3.69 (d, *J* = 8.5 Hz, 1 H), 3.23 (ddd, *J* = 11.5, 9.5, 5.7 Hz, 1 H), 3.18 (ddd, *J* = 11.3, 9.5, 6.0 Hz, 1 H), 2.23 (ddd, *J* = 14.0, 11.6, 5.8 Hz, 1 H), 2.17 (ddd, *J* = 13.9, 11.6, 6.1 Hz, 1 H), 1.69-1.50 (m, 8 H), 1.48-1.21 (m, 5 H); HRESIMS calcd for C₁₁H₁₉INaO₂ *m/z* [M + Na]⁺ 333.0322, found 333.0324.

4.2.3.2 (2*S*)-2-(2-Iodoethyl)-2-methyl-1,4-dioxaspiro[4.5]decane (**24**)

The title compound was obtained from alcohol **23**, using the above procedure for 18 h. Colourless oil (91% yield); ¹H NMR (CDCl₃) δ 3.79 (d, *J* = 8.5 Hz, 1 H), 3.69 (d, *J* = 8.5 Hz, 1 H), 3.23 (ddd, *J* = 11.5, 9.5, 5.7 Hz, 1 H), 3.18 (ddd, *J* = 11.3, 9.5, 6.0 Hz, 1 H), 2.24 (ddd, *J* = 13.8, 11.4, 5.6 Hz, 1 H), 2.17 (ddd, *J* = 13.8, 11.5, 6.0 Hz, 1 H), 1.69-1.51 (m, 8 H), 1.48-1.23 (m, 5 H); HRESIMS calcd for C₁₁H₁₉INaO₂ *m/z* [M + Na]⁺ 333.0322, found 333.0311.

4.2.4 General procedure for preparing acetals **15** and **25**

A mixture of the iodide (**13** or **24**, 1.0 equiv), 2-chloro-4-nitro-1*H*-imidazole (**14**) (1.07 equiv) and powdered K₂CO₃ (1.2 equiv) in anhydrous DMF (6 mL/g of iodide) under N₂ was stirred at 72 °C for 79 h. The resulting cooled mixture was added to ice/aq NaHCO₃ (100 mL) and extracted with EtOAc (5 x 100 mL). The extracts were washed with brine (100 mL) and then evaporated to dryness under reduced pressure (at 30 °C) and the residue was chromatographed twice on silica gel (firstly with 0-50% Et₂O/petroleum ether and secondly with 50% CH₂Cl₂/petroleum ether and 0-5% Et₂O/CH₂Cl₂) to afford the required products.

4.2.4.1 2-Chloro-1-{2-[(2*R*)-2-methyl-1,4-dioxaspiro[4.5]decan-2-yl]ethyl}-4-nitro-1*H*-imidazole (**15**)

The title compound was obtained from iodide **13**. Cream solid (73% yield): mp (pentane) 54-56 °C; ¹H NMR (CDCl₃) δ 7.78 (s, 1 H), 4.22 (ddd, *J* = 14.2, 9.8, 6.2 Hz, 1 H), 4.15 (ddd, *J* = 14.2, 9.7, 6.3 Hz, 1 H), 3.83 (d, *J* = 8.7 Hz, 1 H), 3.80 (d, *J* = 8.7 Hz, 1 H), 2.06 (ddd, *J* = 13.6, 9.8, 6.2 Hz, 1 H), 2.00 (ddd, *J* = 13.5, 9.8, 6.3 Hz, 1 H), 1.70-1.51 (m, 8 H), 1.49-1.31 (m, 5 H); [α]²⁵_D -5.95 (c 1.008, CHCl₃). Anal. (C₁₄H₂₀ClN₃O₄) C, H, N.

4.2.4.2 2-Chloro-1-{2-[(2S)-2-methyl-1,4-dioxaspiro[4.5]decan-2-yl]ethyl}-4-nitro-1H-imidazole (**25**)

The title compound was obtained from iodide **24**, using the above procedure at 63-68 °C for 56 h. Pale yellow oil (90% yield); ¹H NMR (CDCl₃) δ 7.78 (s, 1 H), 4.22 (ddd, *J* = 14.2, 9.8, 6.2 Hz, 1 H), 4.15 (ddd, *J* = 14.2, 9.7, 6.3 Hz, 1 H), 3.83 (d, *J* = 8.7 Hz, 1 H), 3.80 (d, *J* = 8.7 Hz, 1 H), 2.06 (ddd, *J* = 13.6, 9.8, 6.2 Hz, 1 H), 2.00 (ddd, *J* = 13.6, 9.8, 6.3 Hz, 1 H), 1.70-1.51 (m, 8 H), 1.50-1.29 (m, 5 H); HRESIMS calcd for C₁₄H₂₁ClN₃O₄ *m/z* [M + H]⁺ 332.1191, 330.1215, found 332.1199, 330.1221; [α]_D²⁴ 7.93 (*c* 1.009, CHCl₃).

4.2.5 General procedure for preparing diols **16** and **26**

A mixture of the acetal (**15** or **25**, 1.0 equiv) and 4-methylbenzenesulfonic acid monohydrate (1.1 equiv) in MeOH (67 mL/g of acetal) was stirred at 20 °C for 73 h and then NaHCO₃ (1.4 equiv) and water (4 mL/g of NaHCO₃) were added. The solvents were removed under reduced pressure (at 30 °C) and the residue was chromatographed on silica gel (0-50% EtOAc/petroleum ether then EtOAc) to afford the required products.

4.2.5.1 (2R)-4-(2-Chloro-4-nitro-1H-imidazol-1-yl)-2-methylbutane-1,2-diol (**16**)

The title compound was obtained from acetal **15**. White solid (89% yield): mp (Et₂O/pentane) 106-108 °C; ¹H NMR [(CD₃)₂SO] δ 8.58 (s, 1 H), 4.73 (t, *J* = 5.7 Hz, 1 H), 4.45 (s, 1 H), 4.19-4.08 (m, 2 H), 3.23 (dd, *J* = 10.7, 5.5 Hz, 1 H), 3.17 (dd, *J* = 10.6, 5.8 Hz, 1 H), 1.89 (dt, *J* = 13.4, 8.1 Hz, 1 H), 1.81 (dt, *J* = 13.4, 8.2 Hz, 1 H), 1.08 (s, 3 H); [α]_D²⁶ -8.33 (*c* 3.002, DMF). Anal. (C₈H₁₂ClN₃O₄) C, H, N.

4.2.5.2 (2S)-4-(2-Chloro-4-nitro-1H-imidazol-1-yl)-2-methylbutane-1,2-diol (**26**)

The title compound was obtained from acetal **25**, using the above procedure for 78 h. Cream solid (81% yield): mp (Et₂O/hexane) 105-106 °C; ¹H NMR [(CD₃)₂SO] δ 8.58 (s, 1 H), 4.72 (br t, *J* = 5.4 Hz, 1 H), 4.44 (br s, 1 H), 4.19-4.08 (m, 2 H), 3.24 (dd, *J* = 10.6, 5.5 Hz, 1 H), 3.17 (dd, *J* = 10.6, 5.7 Hz, 1 H), 1.89 (dt, *J* = 13.5, 8.1 Hz, 1 H), 1.82 (dt, *J* = 13.6, 8.1 Hz, 1 H), 1.08 (s, 3 H); HRESIMS calcd for C₈H₁₂ClN₃NaO₄ *m/z* [M + Na]⁺ 274.0382, 272.0409, found 274.0380, 272.0409; [α]_D²⁶ 7.64 (*c* 3.009, DMF).

4.2.6 General procedure for preparing tosylates **17** and **27**

A solution of 4-methylbenzenesulfonyl chloride (1.3 equiv) in anhydrous pyridine (8 mL/g of TsCl) was added dropwise to a stirred solution of the diol (**16** or **26**, 1.0 equiv) in anhydrous pyridine (6 mL/g of diol) under N₂ at 0 °C. The mixture was stirred at 0-20 °C for 12 h and then cooled to 0 °C and treated dropwise with additional 4-methylbenzenesulfonyl chloride (0.1 equiv) in anhydrous pyridine. The resulting mixture was stirred at 0-20 °C for 9 h and then poured onto ice (100 mL) and extracted with CH₂Cl₂ (4 x 100 mL). The combined extracts were evaporated to dryness under reduced pressure (at 30 °C) and the residue was chromatographed on silica gel (0-20% Et₂O/CH₂Cl₂) to afford the required products.

4.2.6.1 (2R)-4-(2-Chloro-4-nitro-1H-imidazol-1-yl)-2-hydroxy-2-methylbutyl 4-methylbenzene-1-sulfonate (**17**)

The title compound was obtained from diol **16**. Colourless oil (100% yield); ¹H NMR (CDCl₃) δ 7.80 (br d, *J* = 8.4 Hz, 2 H), 7.75 (s, 1 H), 7.38 (br d, *J* = 8.6 Hz, 2 H), 4.23-4.11 (m, 2 H), 3.92 (d, *J* = 10.1 Hz, 1 H), 3.86 (d, *J* = 10.1 Hz, 1 H), 2.47 (s, 3 H), 2.14 (s, 1 H), 2.03 (ddd, *J* = 13.9, 9.1, 6.7 Hz, 1 H), 1.90 (ddd, *J* = 13.9, 9.3, 6.8 Hz, 1 H), 1.29 (s, 3 H); HRESIMS calcd for C₁₅H₁₉ClN₃O₆S *m/z* [M + H]⁺ 406.0652, 404.0678, found 406.0651, 404.0682.

4.2.6.2 (2S)-4-(2-Chloro-4-nitro-1H-imidazol-1-yl)-2-hydroxy-2-methylbutyl 4-methylbenzene-1-sulfonate (**27**)

The title compound was obtained from diol **26**, using the above procedure but with 1.2 equiv of TsCl for 12 h and then an additional 0.2 equiv of TsCl for 18 h. Pale yellow oil (100% yield); ¹H NMR (CDCl₃) δ 7.80 (br d, *J* = 8.3 Hz, 2 H), 7.75 (s, 1 H), 7.38 (br d, *J* = 8.0 Hz, 2 H), 4.23-4.11 (m, 2 H), 3.92 (d, *J* = 10.1 Hz, 1 H), 3.86 (d, *J* = 10.1 Hz, 1 H), 2.47 (s, 3 H), 2.14 (s, 1 H), 2.03 (ddd, *J* = 13.9, 9.1, 6.7 Hz, 1 H), 1.90 (ddd, *J* = 13.9, 9.3, 6.8 Hz, 1 H), 1.29 (s, 3 H); HRESIMS calcd for C₁₅H₁₈ClN₃NaO₆S *m/z* [M + Na]⁺ 428.0472, 426.0497, found 428.0470, 426.0499.

4.2.7 General procedure for preparing epoxides **18** and **28**

A stirred solution of the tosylate (**17** or **27**, 1.0 equiv) in anhydrous CH₂Cl₂ (8 mL/g of tosylate) under N₂ was treated dropwise with DBU (1.15 equiv). The mixture was stirred at 20 °C for 3 h and then added to brine (100 mL) and extracted with CH₂Cl₂ (4 x 100 mL). The combined extracts were evaporated to dryness under reduced pressure (at 30 °C) and the residue was chromatographed on silica gel (CH₂Cl₂) to afford the required products.

4.2.7.1 2-Chloro-1-{2-[(2R)-2-methyloxiran-2-yl]ethyl}-4-nitro-1H-imidazole (**18**)

The title compound was obtained from tosylate **17**. Cream solid (96% yield): mp (CH₂Cl₂/pentane) 80-82 °C; ¹H NMR (CDCl₃) δ 7.79 (s, 1 H), 4.13 (t, *J* = 7.6 Hz, 2 H), 2.67 (br d, *J* = 4.4 Hz, 1 H), 2.62 (br d, *J* = 4.5 Hz, 1 H), 2.19 (dt, *J* = 14.3, 7.7 Hz, 1 H), 2.04 (dt, *J* = 14.3, 7.5 Hz, 1 H), 1.40 (s, 3 H); [α]_D²⁶ 7.47 (*c* 2.007, CHCl₃). Anal. (C₈H₁₀ClN₃O₃) C, H, N.

4.2.7.2 2-Chloro-1-{2-[(2S)-2-methyloxiran-2-yl]ethyl}-4-nitro-1H-imidazole (**28**)

The title compound was obtained from tosylate **27**, using the above procedure for 2.5 h. Cream solid (97% yield): mp (Et₂O/pentane) 81-82 °C; ¹H NMR (CDCl₃) δ 7.79 (s, 1 H), 4.13 (t, *J* = 7.6 Hz, 2 H), 2.67 (br d, *J* = 4.4 Hz, 1 H), 2.62 (br d, *J* = 4.4 Hz, 1 H), 2.19 (dt, *J* = 14.3, 7.7 Hz, 1 H), 2.04 (dt, *J* = 14.3, 7.4 Hz, 1 H), 1.40 (s, 3 H); HRESIMS calcd for C₈H₁₁ClN₃O₃ *m/z* [M + H]⁺ 234.0456, 232.0483, found 234.0458, 232.0489; [α]_D²⁵ -8.0 (*c* 1.001, CHCl₃).

4.2.8 General procedure for preparing aryloxy alcohols **19**, **29**, **32**, **34**, **36**, **38** and **40**

A mixture of the epoxide (**18**, **28** or **31**, 1.0 equiv), powdered K₂CO₃ (3.0 equiv) and the appropriately substituted phenol (3.0 equiv) in anhydrous 2-butanone (1 mL/100 mg of epoxide) was stirred in a sealed vial at 82 °C for 16 h. The resulting cooled mixture was added to water (50 mL) and extracted with CH₂Cl₂ (6 x 50 mL). The combined extracts were evaporated to dryness under reduced pressure (at 30 °C) and the residue was chromatographed on silica gel (0-5% EtOAc/CH₂Cl₂ or 1:1 EtOAc/petroleum ether or 0-2% MeOH/CH₂Cl₂) to afford the required products.

4.2.8.1 (2R)-4-(2-Chloro-4-nitro-1H-imidazol-1-yl)-2-methyl-1-[4-(trifluoromethoxy)phenoxy]butan-2-ol (**19**)

The title compound was obtained from epoxide **18** and 4-(trifluoromethoxy)phenol. Pale yellow oil (70% yield); ¹H NMR (CDCl₃) δ 7.82 (s, 1 H), 7.18 (br d, *J* = 9.0 Hz, 2 H), 6.91 (br d, *J* = 9.2 Hz, 2 H), 4.34-4.20 (m, 2 H), 3.85 (d, *J* = 8.9 Hz, 1 H), 3.82 (d, *J* = 8.9 Hz, 1 H), 2.24 (s, 1 H), 2.24 (ddd, *J* = 13.7, 9.5, 6.3 Hz, 1 H), 2.04 (ddd, *J* = 13.8, 9.7, 6.5 Hz, 1 H), 1.41 (s, 3 H); HRESIMS calcd for C₁₅H₁₆ClF₃N₃O₅ *m/z* [M + H]⁺ 412.0701, 410.0725, found 412.0690, 410.0715.

4.2.8.2 (2*S*)-4-(2-Chloro-4-nitro-1*H*-imidazol-1-yl)-2-methyl-1-[4-(trifluoromethoxy)phenoxy]butan-2-ol (**29**)

The title compound was obtained from epoxide **28** and 4-(trifluoromethoxy)phenol, using the above procedure at 80 °C for 17 h. Pale yellow oil (79% yield); ¹H NMR (CDCl₃) δ 7.82 (s, 1 H), 7.17 (br d, *J* = 9.0 Hz, 2 H), 6.91 (br d, *J* = 9.1 Hz, 2 H), 4.34-4.20 (m, 2 H), 3.85 (d, *J* = 8.9 Hz, 1 H), 3.82 (d, *J* = 8.9 Hz, 1 H), 2.25 (s, 1 H), 2.24 (ddd, *J* = 13.6, 9.5, 6.3 Hz, 1 H), 2.04 (ddd, *J* = 13.8, 9.7, 6.5 Hz, 1 H), 1.41 (s, 3 H); HRESIMS calcd for C₁₅H₁₆ClF₃N₃O₅ *m/z* [M + H]⁺ 412.0701, 410.0725, found 412.0703, 410.0724.

4.2.8.3 4-(2-Chloro-4-nitro-1*H*-imidazol-1-yl)-2-methyl-1-phenoxybutan-2-ol (**32**)

The title compound was obtained from 2-chloro-1-[2-(2-methyloxiran-2-yl)ethyl]-4-nitro-1*H*-imidazole [20] (**31**), powdered K₂CO₃ (3.5 equiv) and phenol (2.5 equiv), using the above procedure at 70 °C. Pale yellow oil (52% yield); ¹H NMR (CDCl₃) δ 7.81 (s, 1 H), 7.31 (br dd, *J* = 8.7, 7.4 Hz, 2 H), 7.01 (tt, *J* = 7.4, 1.0 Hz, 1 H), 6.91 (br dd, *J* = 8.8, 1.0 Hz, 2 H), 4.34-4.20 (m, 2 H), 3.87 (d, *J* = 9.0 Hz, 1 H), 3.83 (d, *J* = 9.0 Hz, 1 H), 2.33 (s, 1 H), 2.25 (ddd, *J* = 13.7, 9.7, 6.2 Hz, 1 H), 2.04 (ddd, *J* = 13.7, 9.7, 6.5 Hz, 1 H), 1.40 (s, 3 H); APCI MS calcd for C₁₄H₁₇ClN₃O₄ *m/z* [M + H]⁺ 328, 326, found 328, 326.

4.2.8.4 4-(2-Chloro-4-nitro-1*H*-imidazol-1-yl)-1-(4-fluorophenoxy)-2-methylbutan-2-ol (**34**)

The title compound was obtained from epoxide **31**, powdered K₂CO₃ (3.5 equiv) and 4-fluorophenol (2.5 equiv), using the above procedure at 70 °C for 14 h. Pale yellow oil (60% yield); ¹H NMR (CDCl₃) δ 7.82 (s, 1 H), 7.00 (br dd, *J* = 9.2, 8.1 Hz, 2 H), 6.85 (br dd, *J* = 9.2, 4.3 Hz, 2 H), 4.34-4.20 (m, 2 H), 3.83 (d, *J* = 8.9 Hz, 1 H), 3.79 (d, *J* = 9.0 Hz, 1 H), 2.34 (br s, 1 H), 2.24 (ddd, *J* = 13.7, 9.7, 6.2 Hz, 1 H), 2.04 (ddd, *J* = 13.8, 9.8, 6.5 Hz, 1 H), 1.40 (s, 3 H); APCI MS calcd for C₁₄H₁₆ClFN₃O₄ *m/z* [M + H]⁺ 346, 344, found 346, 344.

4.2.8.5 4-(2-Chloro-4-nitro-1*H*-imidazol-1-yl)-1-(4-chlorophenoxy)-2-methylbutan-2-ol (**36**)

The title compound was obtained from epoxide **31**, powdered K₂CO₃ (3.5 equiv) and 4-chlorophenol (2.5 equiv), using the above procedure at 70 °C. Pale yellow oil (52% yield); ¹H NMR (CDCl₃) δ 7.81 (s, 1 H), 7.26 (br d, *J* = 9.1 Hz, 2 H), 6.84 (br d, *J* = 9.1 Hz, 2 H), 4.35-4.19 (m, 2 H), 3.84 (d, *J* = 9.0 Hz, 1 H), 3.80 (d, *J* = 9.0 Hz, 1 H), 2.27 (s, 1 H), 2.23 (ddd, *J* = 13.8, 9.7, 6.2 Hz, 1 H), 2.03 (ddd, *J* = 13.8, 9.7, 6.5 Hz, 1 H), 1.40 (s, 3 H); APCI MS calcd for C₁₄H₁₆Cl₂N₃O₄ *m/z* [M + H]⁺ 362, 360, found 362, 360.

4.2.8.6 4-(2-Chloro-4-nitro-1*H*-imidazol-1-yl)-2-methyl-1-[4-(trifluoromethyl)phenoxy]butan-2-ol (**38**)

The title compound was obtained from epoxide **31**, powdered K₂CO₃ (3.5 equiv) and 4-(trifluoromethyl)phenol (2.5 equiv), using the above procedure at 70 °C. Pale yellow oil (35% yield); ¹H NMR (CDCl₃) δ 7.83 (s, 1 H), 7.58 (br d, *J* = 8.5 Hz, 2 H), 6.98 (br d, *J* = 8.5 Hz, 2 H), 4.35-4.21 (m, 2 H), 3.91 (d, *J* = 9.0 Hz, 1 H), 3.88 (d, *J* = 9.0 Hz, 1 H), 2.28 (s, 1 H), 2.25 (ddd, *J* = 13.8, 9.5, 6.3 Hz, 1 H), 2.11-2.01 (m, 1 H), 1.42 (s, 3 H); APCI MS calcd for C₁₅H₁₆ClF₃N₃O₄ *m/z* [M + H]⁺ 396, 394, found 396, 394.

4.2.8.7 4-(2-Chloro-4-nitro-1*H*-imidazol-1-yl)-2-methyl-1-(4-phenoxyphenoxy)butan-2-ol (**40**)

The title compound was obtained from epoxide **31**, powdered K₂CO₃ (2.6 equiv) and 4-phenoxyphenol (2.5 equiv), using the above procedure for 21 h. Pale yellow oil (76% yield); ¹H NMR (CDCl₃) δ 7.82 (s, 1 H), 7.31 (br dd, *J* = 8.6, 7.4 Hz, 2 H), 7.07 (tt, *J* = 7.4, 1.1 Hz, 1 H), 6.99 (br d, *J* = 9.1 Hz, 2 H), 6.95 (br dd, *J* = 8.7, 1.0 Hz, 2 H), 6.89 (br d, *J* = 9.1 Hz, 2 H), 4.35-4.20 (m, 2 H), 3.85 (d, *J* = 9.0 Hz, 1 H), 3.81 (d, *J* = 9.0 Hz, 1 H), 2.31 (s, 1 H),

2.25 (ddd, $J = 13.8, 9.7, 6.2$ Hz, 1 H), 2.04 (ddd, $J = 13.7, 9.8, 6.4$ Hz, 1 H), 1.40 (s, 3 H); HRESIMS calcd for $C_{20}H_{20}ClN_3NaO_5$ m/z $[M + Na]^+$ 442.0963, 440.0984, found 442.0960, 440.0984.

4.2.9 General procedure for preparing aryl ethers **20**, **30**, **33**, **35**, **37**, **39** and **41**

Sodium hydride (60% in mineral oil, 1.5 equiv) was added to a solution of the alcohol (**19**, **29**, **32**, **34**, **36**, **38** or **40**, 1.0 equiv) in anhydrous DMF (1 mL/60 mg of alcohol) under N_2 at 20 °C. The mixture was immediately degassed and resealed under N_2 , cooled in an ice bath for 3 min and then stirred at 20 °C for 170 min. The resulting mixture was rapidly cooled (CO_2 /acetone), quenched with ice/aqueous $NaHCO_3$ (10 mL), added to brine (100 mL), and extracted with CH_2Cl_2 (8 x 100 mL). The combined extracts were evaporated to dryness under reduced pressure (at 30 °C) and the residue was chromatographed on silica gel (CH_2Cl_2 or 0-2% MeOH/ CH_2Cl_2 or 0-50% EtOAc/petroleum ether) to afford the required products.

4.2.9.1 (7R)-7-Methyl-2-nitro-7-{[4-(trifluoromethoxy)phenoxy]methyl}-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**20**)

The title compound was obtained from alcohol **19**. Light yellow solid (72% yield): mp (CH_2Cl_2 /hexane) 164-166 °C; 1H NMR ($CDCl_3$) δ 7.46 (s, 1 H), 7.16 (br d, $J = 9.1$ Hz, 2 H), 6.87 (br d, $J = 9.2$ Hz, 2 H), 4.17 (dt, $J = 12.7, 6.2$ Hz, 1 H), 4.12 (ddd, $J = 12.7, 7.5, 5.8$ Hz, 1 H), 4.10 (d, $J = 9.6$ Hz, 1 H), 4.05 (d, $J = 9.6$ Hz, 1 H), 2.51 (ddd, $J = 14.6, 7.4, 6.0$ Hz, 1 H), 2.26 (ddd, $J = 14.6, 6.3, 6.0$ Hz, 1 H), 1.60 (s, 3 H); $[\alpha]^{25}_D$ 25.0 (c 1.001, $CHCl_3$). Anal. ($C_{15}H_{14}F_3N_3O_5$) C, H, N. HPLC purity: 100%.

Chiral HPLC (using a CHIRALPAK IA analytical column and eluting with 30% EtOH/hexane at 0.7 mL/min) determined that the ee of **20** was >99%.

4.2.9.2 (7S)-7-Methyl-2-nitro-7-{[4-(trifluoromethoxy)phenoxy]methyl}-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**30**)

The title compound was obtained from alcohol **29**. Light yellow solid (70% yield): mp (CH_2Cl_2 /hexane) 163-164 °C; 1H NMR [$(CD_3)_2SO$] δ 8.10 (s, 1 H), 7.31 (br d, $J = 8.5$ Hz, 2 H), 7.07 (br d, $J = 9.2$ Hz, 2 H), 4.20 (s, 2 H), 4.19 (dt, $J = 12.7, 6.1$ Hz, 1 H), 4.13 (ddd, $J = 13.2, 8.1, 5.7$ Hz, 1 H), 2.38 (ddd, $J = 14.5, 7.9, 6.2$ Hz, 1 H), 2.18 (dt, $J = 14.4, 5.8$ Hz, 1 H), 1.49 (s, 3 H); ^{13}C NMR [$(CD_3)_2SO$] δ 157.0, 147.2, 142.2, 142.1 (q, $J_{C-F} = 2.0$ Hz), 122.5 (2 C), 120.1 (q, $J_{C-F} = 255.2$ Hz), 117.7, 115.9 (2 C), 80.4, 72.4, 39.5, 27.0, 21.3; $[\alpha]^{25}_D$ -24.0 (c 1.001, $CHCl_3$). Anal. ($C_{15}H_{14}F_3N_3O_5$) C, H, N. HPLC purity: 100%.

Chiral HPLC (using a CHIRALPAK IA analytical column and eluting with 30% EtOH/hexane at 0.7 mL/min) determined that the ee of **30** was 95.0%.

4.2.9.3 7-Methyl-2-nitro-7-(phoxymethyl)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**33**)

The title compound was obtained from alcohol **32**, using the above procedure at 60 °C for 45 min. Cream solid (31% yield): mp (Et_2O triturate) 146-148 °C; 1H NMR ($CDCl_3$) δ 7.46 (s, 1 H), 7.30 (br dd, $J = 8.7, 7.4$ Hz, 2 H), 7.00 (tt, $J = 7.4, 1.0$ Hz, 1 H), 6.86 (br dd, $J = 8.7, 1.0$ Hz, 2 H), 4.17 (ddd, $J = 12.7, 7.0, 5.8$ Hz, 1 H), 4.10 (ddd, $J = 12.6, 7.0, 5.7$ Hz, 1 H), 4.10 (d, $J = 9.5$ Hz, 1 H), 4.06 (d, $J = 9.5$ Hz, 1 H), 2.52 (ddd, $J = 14.5, 7.0, 6.0$ Hz, 1 H), 2.27 (ddd, $J = 14.5, 7.0, 5.9$ Hz, 1 H), 1.60 (s, 3 H). Anal. ($C_{14}H_{15}N_3O_4$) C, H, N.

4.2.9.4 7-[(4-Fluorophenoxy)methyl]-7-methyl-2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**35**)

The title compound was obtained from alcohol **34** and NaH (1.8 equiv), using the above procedure at 40 °C for 15 min. Yellow-orange solid (28% yield): mp (Et_2O triturate) 130-132 °C; 1H NMR [$(CD_3)_2SO$] δ 8.10 (s, 1 H), 7.13 (br dd, $J = 9.1, 8.6$ Hz, 2 H), 6.98 (dd, $J = 9.2,$

4.4 Hz, 2 H), 4.18 (dt, $J = 13.1, 6.0$ Hz, 1 H), 4.15 (s, 2 H), 4.13 (ddd, $J = 13.3, 8.1, 5.6$ Hz, 1 H), 2.38 (ddd, $J = 14.5, 7.9, 6.2$ Hz, 1 H), 2.17 (dt, $J = 14.4, 5.8$ Hz, 1 H), 1.48 (s, 3 H); ^{13}C NMR [(CD₃)₂SO] δ 156.7 (d, $J_{\text{C-F}} = 236.4$ Hz), 154.5 (d, $J_{\text{C-F}} = 2.0$ Hz), 147.3, 142.2, 117.7, 116.0 (d, $J_{\text{C-F}} = 8.0$ Hz, 2 C), 115.8 (d, $J_{\text{C-F}} = 22.9$ Hz, 2 C), 80.5, 72.6, 39.5, 27.0, 21.3. Anal. (C₁₄H₁₄FN₃O₄) C, H, N.

4.2.9.5 7-[(4-Chlorophenoxy)methyl]-7-methyl-2-nitro-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (37)

The title compound was obtained from alcohol **36**, using the above procedure at 60 °C for 45 min. Pale yellow solid (49% yield): mp (Et₂O triturate) 173-175 °C; ^1H NMR (CDCl₃) δ 7.46 (s, 1 H), 7.25 (br d, $J = 9.1$ Hz, 2 H), 6.80 (br d, $J = 9.1$ Hz, 2 H), 4.17 (dt, $J = 12.7, 6.3$ Hz, 1 H), 4.11 (ddd, $J = 12.7, 7.4, 5.8$ Hz, 1 H), 4.08 (d, $J = 9.6$ Hz, 1 H), 4.03 (d, $J = 9.6$ Hz, 1 H), 2.50 (ddd, $J = 14.5, 7.4, 6.0$ Hz, 1 H), 2.25 (ddd, $J = 14.6, 6.4, 6.0$ Hz, 1 H), 1.59 (s, 3 H). Anal. (C₁₄H₁₄ClN₃O₄) C, H, N.

4.2.9.6 7-Methyl-2-nitro-7-[[4-(trifluoromethyl)phenoxy]methyl]-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (39)

The title compound was obtained from alcohol **38** and NaH (2.4 equiv), using the above procedure at 60 °C for 45 min. Cream solid (35% yield): mp (Et₂O triturate) 147-149 °C; ^1H NMR (CDCl₃) δ 7.57 (br d, $J = 8.5$ Hz, 2 H), 7.47 (s, 1 H), 6.95 (br d, $J = 8.5$ Hz, 2 H), 4.18 (dt, $J = 12.7, 6.3$ Hz, 1 H), 4.16 (d, $J = 9.5$ Hz, 1 H), 4.13 (ddd, $J = 12.9, 7.5, 5.8$ Hz, 1 H), 4.10 (d, $J = 9.6$ Hz, 1 H), 2.53 (ddd, $J = 14.5, 7.6, 6.1$ Hz, 1 H), 2.27 (dt, $J = 14.5, 6.0$ Hz, 1 H), 1.61 (s, 3 H). Anal. (C₁₅H₁₄F₃N₃O₄) C, H, N.

4.2.9.7 7-Methyl-2-nitro-7-[(4-phenoxyphenoxy)methyl]-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (41)

The title compound was obtained from alcohol **40**, using the above procedure for 3 h. Cream solid (54% yield): mp (CH₂Cl₂/pentane) 148-150 °C; ^1H NMR (CDCl₃) δ 7.46 (s, 1 H), 7.31 (br dd, $J = 8.6, 7.4$ Hz, 2 H), 7.06 (tt, $J = 7.4, 1.0$ Hz, 1 H), 6.97 (br d, $J = 9.1$ Hz, 2 H), 6.94 (br dd, $J = 8.7, 1.0$ Hz, 2 H), 6.84 (br d, $J = 9.1$ Hz, 2 H), 4.19 (ddd, $J = 12.7, 6.8, 5.9$ Hz, 1 H), 4.11 (ddd, $J = 12.7, 7.0, 5.7$ Hz, 1 H), 4.09 (d, $J = 9.6$ Hz, 1 H), 4.04 (d, $J = 9.6$ Hz, 1 H), 2.52 (ddd, $J = 14.4, 7.2, 5.9$ Hz, 1 H), 2.27 (ddd, $J = 14.5, 6.8, 5.9$ Hz, 1 H), 1.60 (s, 3 H). Anal. (C₂₀H₁₉N₃O₅) C, H, N.

4.3 General procedure for the syntheses of 2-pyridinyl ethers **44**, **46**, **48**, **49**, **59**, **60**, **62**, **64**, **66**, **68** and **69**

Sodium hydride (60% in mineral oil, 1.5-1.9 equiv) was added to a mixture of the alcohol (**42** or **45**, 60 mg, 1.0 equiv) and the halopyridine (2.6 equiv) in anhydrous DMF (1 mL) under N₂ at 20 °C. The mixture was immediately degassed and resealed under N₂ and then stirred at 20 °C for 2.5 h. The resulting mixture was rapidly cooled (CO₂/acetone), quenched with ice/aqueous NaHCO₃ (5 mL), added to brine (40 mL), and extracted with CH₂Cl₂ (5 x 50 mL). The combined extracts were evaporated to dryness under reduced pressure (at 30 °C) and the residue was chromatographed on silica gel (0-5% EtOAc/CH₂Cl₂ or 0-2% MeOH/CH₂Cl₂ or 10-50% EtOAc/petroleum ether) to afford the required products.

4.3.1 2-Nitro-7-([6-(trifluoromethyl)pyridin-2-yl]oxy)methyl)-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (44)

The title compound was obtained from (2-nitro-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazin-7-yl)methanol [20] (**42**), 2-fluoro-6-(trifluoromethyl)pyridine (**43**) and NaH (1.9 equiv). Cream solid (76% yield): mp (CH₂Cl₂/pentane) 128-130 °C; ^1H NMR [(CD₃)₂SO] δ 8.08 (s, 1 H),

8.05-7.98 (m, 1 H), 7.53 (br d, $J = 7.3$ Hz, 1 H), 7.23 (br d, $J = 8.4$ Hz, 1 H), 4.99-4.89 (m, 1 H), 4.62 (dd, $J = 12.0, 3.4$ Hz, 1 H), 4.58 (dd, $J = 12.1, 5.8$ Hz, 1 H), 4.19 (ddd, $J = 12.6, 5.8, 2.8$ Hz, 1 H), 4.10 (ddd, $J = 12.5, 11.1, 5.2$ Hz, 1 H), 2.37-2.28 (m, 1 H), 2.28-2.14 (m, 1 H); ^{13}C NMR [(CD₃)₂SO] δ 162.8, 147.7, 143.7 (q, $J_{\text{C-F}} = 34.2$ Hz), 142.0, 141.4, 121.3 (q, $J_{\text{C-F}} = 273.7$ Hz), 117.8, 115.3, 114.4 (q, $J_{\text{C-F}} = 3.2$ Hz), 75.8, 66.8, 41.7, 22.3. Anal. (C₁₃H₁₁F₃N₄O₄) C, H, N.

4.3.2 7-Methyl-2-nitro-7-([6-(trifluoromethyl)pyridin-2-yl]oxy)methyl)-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (**46**)

The title compound was obtained from (7-methyl-2-nitro-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazin-7-yl)methanol [20] (**45**), 2-fluoro-6-(trifluoromethyl)pyridine (**43**, 2.5 equiv) and NaH (1.6 equiv), using the above procedure for 30 min. Pale yellow solid: mp (Et₂O/hexane) 109-111 °C; ^1H NMR (CDCl₃) δ 7.75 (br t, $J = 7.9$ Hz, 1 H), 7.46 (s, 1 H), 7.32 (d, $J = 7.3$ Hz, 1 H), 6.92 (d, $J = 8.4$ Hz, 1 H), 4.58 (d, $J = 11.7$ Hz, 1 H), 4.52 (d, $J = 11.6$ Hz, 1 H), 4.21 (dt, $J = 12.6, 6.2$ Hz, 1 H), 4.11 (ddd, $J = 12.7, 7.8, 5.7$ Hz, 1 H), 2.47 (ddd, $J = 14.5, 7.8, 5.9$ Hz, 1 H), 2.19 (dt, $J = 14.5, 6.1$ Hz, 1 H), 1.59 (s, 3 H). Anal. (C₁₄H₁₃F₃N₄O₄) C, H, N.

4.3.3 2-Nitro-7-([5-(trifluoromethyl)pyridin-2-yl]oxy)methyl)-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (**48**)

The title compound was obtained from alcohol **42**, 2-fluoro-5-(trifluoromethyl)pyridine (**47**, 3.0 equiv) and NaH (1.5 equiv), using the above procedure for 40 min. Cream solid (74% yield): mp (*i*-Pr₂O triturate) 142-143 °C; ^1H NMR [(CD₃)₂SO] δ 8.64-8.58 (m, 1 H), 8.12 (br dd, $J = 9.0, 2.5$ Hz, 1 H), 8.09 (s, 1 H), 7.11 (d, $J = 8.7$ Hz, 1 H), 5.00-4.90 (m, 1 H), 4.69 (dd, $J = 12.0, 3.2$ Hz, 1 H), 4.63 (dd, $J = 12.0, 6.1$ Hz, 1 H), 4.19 (ddd, $J = 12.6, 5.8, 2.7$ Hz, 1 H), 4.10 (ddd, $J = 12.5, 11.1, 5.1$ Hz, 1 H), 2.37-2.27 (m, 1 H), 2.26-2.12 (m, 1 H). Anal. (C₁₃H₁₁F₃N₄O₄) C, H, N.

4.3.4 7-Methyl-2-nitro-7-([5-(trifluoromethyl)pyridin-2-yl]oxy)methyl)-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (**49**)

The title compound was obtained from alcohol **45**, 2-fluoro-5-(trifluoromethyl)pyridine (**47**, 1.5 equiv) and NaH (1.5 equiv), using the above procedure for 40 min. Cream solid (83% yield): mp (*i*-Pr₂O triturate) 130-131 °C; ^1H NMR [(CD₃)₂SO] δ 8.61-8.55 (m, 1 H), 8.11 (br dd, $J = 8.8, 2.5$ Hz, 1 H), 8.09 (s, 1 H), 7.07 (d, $J = 8.8$ Hz, 1 H), 4.57 (s, 2 H), 4.20 (dt, $J = 13.0, 5.8$ Hz, 1 H), 4.14 (ddd, $J = 13.1, 8.7, 5.6$ Hz, 1 H), 2.38 (ddd, $J = 14.5, 8.5, 6.2$ Hz, 1 H), 2.19 (dt, $J = 14.4, 5.4$ Hz, 1 H), 1.49 (s, 3 H); ^{13}C NMR [(CD₃)₂SO] δ 165.1, 147.2, 144.8 (q, $J_{\text{C-F}} = 4.4$ Hz), 142.2, 136.8 (q, $J_{\text{C-F}} = 3.0$ Hz), 124.0 (q, $J_{\text{C-F}} = 271.5$ Hz), 119.3 (q, $J_{\text{C-F}} = 32.5$ Hz), 117.7, 111.5, 80.3, 70.0, 39.5, 26.8, 21.1. Anal. (C₁₄H₁₃F₃N₄O₄) C, H, N.

4.3.5 7-[(5-Chloropyridin-2-yl)oxy)methyl]-2-nitro-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (**59**)

The title compound was obtained from alcohol **42**, 5-chloro-2-fluoropyridine (**58**, 6 equiv) and NaH (1.5 equiv), using the above procedure for 40 min. Pale yellow solid (70% yield): mp (*i*-Pr₂O triturate) 171-172 °C; ^1H NMR [(CD₃)₂SO] δ 8.23 (br d, $J = 2.7$ Hz, 1 H), 8.08 (s, 1 H), 7.86 (dd, $J = 8.8, 2.7$ Hz, 1 H), 6.96 (br d, $J = 8.9$ Hz, 1 H), 4.95-4.87 (m, 1 H), 4.58 (dd, $J = 12.0, 3.3$ Hz, 1 H), 4.52 (dd, $J = 12.0, 6.1$ Hz, 1 H), 4.17 (ddd, $J = 12.6, 5.8, 2.8$ Hz, 1 H), 4.08 (ddd, $J = 12.5, 11.1, 5.1$ Hz, 1 H), 2.35-2.25 (m, 1 H), 2.23-2.10 (m, 1 H). Anal. (C₁₂H₁₁ClN₄O₄) C, H, N.

4.3.6 7-[(5-Chloropyridin-2-yl)oxy)methyl]-7-methyl-2-nitro-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (**60**)

The title compound was obtained from alcohol **45**, 5-chloro-2-fluoropyridine (**58**, 6 equiv) and NaH (1.5 equiv), using the above procedure for 40 min. Cream solid (60% yield): mp (*i*-Pr₂O triturate) 132-133 °C; ¹H NMR [(CD₃)₂SO] δ 8.21 (br d, *J* = 2.7 Hz, 1 H), 8.09 (s, 1 H), 7.84 (dd, *J* = 8.8, 2.7 Hz, 1 H), 6.92 (br d, *J* = 8.9 Hz, 1 H), 4.46 (s, 2 H), 4.18 (dt, *J* = 13.1, 5.8 Hz, 1 H), 4.13 (ddd, *J* = 13.1, 8.6, 5.6 Hz, 1 H), 2.36 (ddd, *J* = 14.5, 8.4, 6.1 Hz, 1 H), 2.17 (dt, *J* = 14.4, 5.5 Hz, 1 H), 1.48 (s, 3 H); ¹³C NMR [(CD₃)₂SO] δ 161.4, 147.2, 144.8, 142.2, 139.4, 123.9, 117.7, 112.3, 80.4, 69.8, 39.5, 26.9, 21.1. Anal. (C₁₃H₁₃ClN₄O₄) C, H, N.

4.3.7 7-((5-Fluoropyridin-2-yl)oxy)methyl)-7-methyl-2-nitro-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (**62**)

The title compound was obtained from alcohol **45**, 2,5-difluoropyridine (**61**, 9.9 equiv) and NaH (1.6 equiv), using the above procedure for 4 h. Cream solid (33% yield): mp (MeOH/CH₂Cl₂/pentane) 101-103 °C; ¹H NMR [(CD₃)₂SO] δ 8.14 (d, *J* = 3.1 Hz, 1 H), 8.08 (s, 1 H), 7.72 (ddd, *J* = 9.0, 8.1, 3.1 Hz, 1 H), 6.91 (dd, *J* = 9.1, 3.6 Hz, 1 H), 4.43 (s, 2 H), 4.18 (dt, *J* = 13.0, 5.9 Hz, 1 H), 4.12 (ddd, *J* = 13.1, 8.6, 5.6 Hz, 1 H), 2.36 (ddd, *J* = 14.5, 8.4, 6.1 Hz, 1 H), 2.17 (dt, *J* = 14.4, 5.5 Hz, 1 H), 1.47 (s, 3 H). Anal. (C₁₃H₁₃FN₄O₄) C, H, N.

4.3.8 7-((3-Fluoro-5-(trifluoromethyl)pyridin-2-yl)oxy)methyl)-7-methyl-2-nitro-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (**64**)

The title compound was obtained from alcohol **45**, 2,3-difluoro-5-(trifluoromethyl)pyridine (**63**, 1.9 equiv) and NaH (1.6 equiv), using the above procedure for 2 h. Cream solid (85% yield): mp (CH₂Cl₂/pentane) 122-124 °C; ¹H NMR (CDCl₃) δ 8.24-8.18 (m, 1 H), 7.57 (dd, *J* = 9.4, 1.9 Hz, 1 H), 7.47 (s, 1 H), 4.69 (d, *J* = 11.4 Hz, 1 H), 4.53 (d, *J* = 11.5 Hz, 1 H), 4.24 (ddd, *J* = 12.6, 6.6, 6.0 Hz, 1 H), 4.13 (ddd, *J* = 12.7, 7.5, 5.8 Hz, 1 H), 2.51 (ddd, *J* = 14.5, 7.5, 5.9 Hz, 1 H), 2.26 (dt, *J* = 14.5, 6.3 Hz, 1 H), 1.62 (s, 3 H). Anal. (C₁₄H₁₂F₄N₄O₄) C, H, N.

4.3.9 7-((3-Chloro-5-(trifluoromethyl)pyridin-2-yl)oxy)methyl)-7-methyl-2-nitro-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (**66**)

The title compound was obtained from alcohol **45**, 2-bromo-3-chloro-5-(trifluoromethyl)pyridine (**65**, 2.5 equiv) and NaH (1.7 equiv), using the above procedure for 140 min. Cream solid (88% yield): mp (CH₂Cl₂/pentane) 111-112 °C; ¹H NMR [(CD₃)₂SO] δ 8.56 (dq, *J* = 2.1, 1.0 Hz, 1 H), 8.43 (br d, *J* = 2.2 Hz, 1 H), 8.11 (s, 1 H), 4.65 (s, 2 H), 4.24 (dt, *J* = 13.2, 6.1 Hz, 1 H), 4.15 (ddd, *J* = 13.1, 7.6, 5.9 Hz, 1 H), 2.41 (ddd, *J* = 14.5, 7.5, 6.0 Hz, 1 H), 2.24 (dt, *J* = 14.4, 6.1 Hz, 1 H), 1.52 (s, 3 H); ¹³C NMR [(CD₃)₂SO] δ 160.2, 147.2, 142.8 (q, *J*_{C-F} = 4.5 Hz), 142.1, 136.2 (q, *J*_{C-F} = 3.0 Hz), 123.1 (q, *J*_{C-F} = 272.0 Hz), 120.3 (q, *J*_{C-F} = 33.2 Hz), 117.9, 117.8, 80.0, 71.4, 39.6, 27.2, 21.5. Anal. (C₁₄H₁₂ClF₃N₄O₄) C, H, N.

4.3.10 2-Nitro-7-((4-(trifluoromethyl)pyridin-2-yl)oxy)methyl)-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (**68**)

The title compound was obtained from alcohol **42**, 2-fluoro-4-(trifluoromethyl)pyridine (**67**) and NaH (1.7 equiv), using the above procedure for 160 min. White solid (85% yield): mp (CH₂Cl₂/pentane) 153-155 °C; ¹H NMR (CDCl₃) δ 8.30 (d, *J* = 5.3 Hz, 1 H), 7.45 (s, 1 H), 7.15 (br d, *J* = 5.3 Hz, 1 H), 7.04-7.00 (m, 1 H), 4.85-4.78 (m, 1 H), 4.72 (dd, *J* = 11.7, 5.0 Hz, 1 H), 4.65 (dd, *J* = 11.8, 4.9 Hz, 1 H), 4.20 (ddd, *J* = 12.4, 5.8, 3.3 Hz, 1 H), 4.13 (ddd, *J* = 12.4, 10.3, 5.6 Hz, 1 H), 2.47-2.29 (m, 2 H). Anal. (C₁₃H₁₁F₃N₄O₄) C, H, N.

4.3.11 7-Methyl-2-nitro-7-((4-(trifluoromethyl)pyridin-2-yl)oxy)methyl)-6,7-dihydro-5H-imidazo[2,1-*b*][1,3]oxazine (**69**)

The title compound was obtained from alcohol **45**, 2-fluoro-4-(trifluoromethyl)pyridine (**67**, 2.5 equiv) and NaH (1.6 equiv), using the above procedure for 30 min. Pale yellow solid (60% yield): mp (Et₂O/hexane) 103-106 °C; ¹H NMR [(CD₃)₂SO] δ 8.43 (d, *J* = 5.3 Hz, 1 H), 8.09 (s, 1 H), 7.37 (dd, *J* = 5.3, 1.0 Hz, 1 H), 7.26 (br s, 1 H), 4.57 (d, *J* = 11.7 Hz, 1 H), 4.54 (d, *J* = 11.7 Hz, 1 H), 4.21 (dt, *J* = 13.1, 5.9 Hz, 1 H), 4.14 (ddd, *J* = 13.1, 8.6, 5.5 Hz, 1 H), 2.39 (ddd, *J* = 14.4, 8.5, 6.1 Hz, 1 H), 2.19 (dt, *J* = 14.4, 5.5 Hz, 1 H), 1.50 (s, 3 H); ¹³C NMR [(CD₃)₂SO] δ 163.2, 148.9, 147.2, 142.2, 139.8 (q, *J*_{C-F} = 33.4 Hz), 122.6 (q, *J*_{C-F} = 273.3 Hz), 117.7, 113.0 (q, *J*_{C-F} = 3.3 Hz), 107.3 (q, *J*_{C-F} = 4.0 Hz), 80.4, 69.9, 39.5, 26.8, 21.1. Anal. (C₁₄H₁₃F₃N₄O₄) C, H, N.

4.4 Syntheses of chiral 2-pyridinyl ethers **53** and **57**

4.4.1 General procedure for preparing silyl ether alcohols **50** and **54**

Chlorotriisopropylsilane (1.1 equiv) was added to a stirred solution of the diol (**16** or **26**, 1.0 g, 1.0 equiv) and imidazole (2.2 equiv) in anhydrous DMF (10 mL) under N₂ at 0 °C. The mixture was stirred at 20 °C for 4 d and then additional chlorotriisopropylsilane (0.33 equiv) was added. The resulting mixture was stirred at 20 °C for 3.5 d and then added to ice-water (100 mL) and extracted with 50% EtOAc/petroleum ether (4 x 100 mL). The combined extracts were evaporated to dryness under reduced pressure (at 30 °C) and the remaining oil was chromatographed on silica gel (0-20% EtOAc/petroleum ether) to afford the required products.

4.4.1.1 (2*R*)-4-(2-Chloro-4-nitro-1*H*-imidazol-1-yl)-2-methyl-1-[(triisopropylsilyl)oxy]butan-2-ol (**50**)

The title compound was obtained from diol **16**. White solid (97% yield): mp (CH₂Cl₂/pentane) 50-52 °C; ¹H NMR (CDCl₃) δ 7.79 (s, 1 H), 4.25 (ddd, *J* = 14.1, 10.2, 5.7 Hz, 1 H), 4.18 (ddd, *J* = 14.1, 10.2, 6.0 Hz, 1 H), 3.54 (s, 2 H), 2.52 (s, 1 H), 2.09 (ddd, *J* = 13.6, 10.3, 5.8 Hz, 1 H), 1.85 (ddd, *J* = 13.6, 10.2, 6.0 Hz, 1 H), 1.23 (s, 3 H), 1.19-1.02 (m, 21 H); [α]²⁶_D -0.66 (*c* 3.008, CHCl₃). Anal. (C₁₇H₃₂ClN₃O₄Si) C, H, N.

4.4.1.2 (2*S*)-4-(2-Chloro-4-nitro-1*H*-imidazol-1-yl)-2-methyl-1-[(triisopropylsilyl)oxy]butan-2-ol (**54**)

The title compound was obtained from diol **26**, using the above procedure for 6.5 d. White solid (97% yield): mp (CH₂Cl₂/pentane) 49-50 °C; ¹H NMR (CDCl₃) δ 7.79 (s, 1 H), 4.25 (ddd, *J* = 14.1, 10.2, 5.8 Hz, 1 H), 4.18 (ddd, *J* = 14.0, 10.2, 6.0 Hz, 1 H), 3.54 (s, 2 H), 2.51 (s, 1 H), 2.09 (ddd, *J* = 13.6, 10.2, 5.8 Hz, 1 H), 1.85 (ddd, *J* = 13.6, 10.2, 6.0 Hz, 1 H), 1.23 (s, 3 H), 1.19-1.02 (m, 21 H); [α]²⁶_D 0.67 (*c* 3.006, CHCl₃). Anal. (C₁₇H₃₂ClN₃O₄Si) C, H, N.

4.4.2 Syntheses of ring closed silyl ethers **51** and **55**

4.4.2.1 (7*R*)-7-Methyl-2-nitro-7-[[triisopropylsilyl]oxy]methyl}-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine (**51**)

The title compound was obtained from alcohol **50** and NaH, using the procedure described in 4.2.9 at 0-20 °C for 3 h and then at 55 °C for 3 h, and following chromatography on silica gel (0-25% EtOAc/petroleum ether). Light yellow solid (80% yield): mp (CH₂Cl₂/pentane) 114-116 °C; ¹H NMR (CDCl₃) δ 7.42 (s, 1 H), 4.15 (ddd, *J* = 12.5, 6.9, 5.7 Hz, 1 H), 4.04 (ddd, *J* = 12.4, 7.3, 5.8 Hz, 1 H), 3.84 (d, *J* = 10.2 Hz, 1 H), 3.77 (d, *J* = 10.1 Hz, 1 H), 2.37 (ddd, *J* = 14.4, 7.3, 5.8 Hz, 1 H), 2.11 (ddd, *J* = 14.4, 6.9, 5.8 Hz, 1 H), 1.45 (s, 3 H), 1.16-0.97 (m, 21 H); [α]²⁵_D 4.66 (*c* 3.007, CHCl₃). Anal. (C₁₇H₃₁N₃O₄Si) C, H, N.

4.4.2.2 (7S)-7-Methyl-2-nitro-7-[[triiisopropylsilyl]oxy]methyl]-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**55**)

The title compound was obtained from alcohol **54** and NaH, using the procedure described in 4.2.9 at 0-20 °C for 4.5 h and then at 50-57 °C for 3.5 h, and following chromatography on silica gel (0-25% EtOAc/petroleum ether). Light yellow solid (85% yield): mp (CH₂Cl₂/pentane) 117-120 °C; ¹H NMR (CDCl₃) δ 7.42 (s, 1 H), 4.15 (ddd, *J* = 12.5, 6.9, 5.7 Hz, 1 H), 4.04 (ddd, *J* = 12.5, 7.3, 5.8 Hz, 1 H), 3.84 (d, *J* = 10.2 Hz, 1 H), 3.77 (d, *J* = 10.1 Hz, 1 H), 2.37 (ddd, *J* = 14.4, 7.2, 5.8 Hz, 1 H), 2.11 (ddd, *J* = 14.4, 6.9, 5.8 Hz, 1 H), 1.45 (s, 3 H), 1.16-0.97 (m, 21 H); [α]²⁵_D -4.32 (*c* 3.011, CHCl₃). Anal. (C₁₇H₃₁N₃O₄Si) C, H, N.

4.4.3 General procedure for preparing alcohols **52** and **56**

The silyl ether (**51** or **55**, 1.1 g, 1.0 equiv) was suspended in a solution of 1% HCl in 95% EtOH [33] (39 mL, 3.2 equiv). The mixture was stirred at 47 °C for 85 h and then rapidly cooled (CO₂/acetone) and neutralised with a solution of ammonia in MeOH (2.0 mL of 7 M). The resulting mixture was evaporated to dryness under reduced pressure (at 30 °C) and the residue was chromatographed on silica gel (0-1.5% MeOH/CH₂Cl₂) to afford the required products.

4.4.3.1 [(7R)-7-Methyl-2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazin-7-yl]methanol (**52**)

The title compound was obtained from silyl ether **51**. Light yellow solid (87% yield): mp (MeOH/CH₂Cl₂/hexane) 220-222 °C; ¹H NMR [(CD₃)₂SO] δ 8.05 (s, 1 H), 5.25 (t, *J* = 5.8 Hz, 1 H), 4.12 (dt, *J* = 12.9, 6.0 Hz, 1 H), 4.05 (ddd, *J* = 13.0, 8.1, 5.6 Hz, 1 H), 3.53 (dd, *J* = 11.6, 5.6 Hz, 1 H), 3.47 (dd, *J* = 11.6, 5.9 Hz, 1 H), 2.21 (ddd, *J* = 14.4, 8.1, 5.9 Hz, 1 H), 2.00 (dt, *J* = 14.4, 5.8 Hz, 1 H), 1.31 (s, 3 H); [α]²⁵_D -17.0 (*c* 2.004, DMF). Anal. (C₈H₁₁N₃O₄) C, H, N.

4.4.3.2 [(7S)-7-Methyl-2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazin-7-yl]methanol (**56**)

The title compound was obtained from silyl ether **55**, using the above procedure at 45 °C for 4 d. Light yellow solid (87% yield): mp (MeOH/CH₂Cl₂/hexane) 220-222 °C; ¹H NMR [(CD₃)₂SO] δ 8.05 (s, 1 H), 5.25 (t, *J* = 5.8 Hz, 1 H), 4.12 (dt, *J* = 12.9, 6.0 Hz, 1 H), 4.05 (ddd, *J* = 13.0, 8.2, 5.6 Hz, 1 H), 3.53 (dd, *J* = 11.6, 5.6 Hz, 1 H), 3.47 (dd, *J* = 11.6, 5.9 Hz, 1 H), 2.21 (ddd, *J* = 14.4, 8.1, 5.9 Hz, 1 H), 2.00 (dt, *J* = 14.4, 5.8 Hz, 1 H), 1.31 (s, 3 H); [α]²⁵_D 16.5 (*c* 2.006, DMF). Anal. (C₈H₁₁N₃O₄) C, H, N.

4.4.4 General procedure for preparing 2-pyridinyl ethers **53** and **57**

Sodium hydride (60% in mineral oil, 1.2 equiv) was added to a mixture of the alcohol (**52** or **56**, 650 mg, 1.0 equiv) and 2-fluoro-5-(trifluoromethyl)pyridine (**47**, 1.5 equiv) in anhydrous DMF (12 mL) under N₂ at 20 °C. The mixture was stirred at 20 °C for 30 min and then quenched with ice/aqueous NaHCO₃ (200 mL). The precipitate was collected by filtration, washed with water and hexane, dried, and then chromatographed on silica gel (30% EtOAc/petroleum ether and EtOAc) to afford the required products.

4.4.4.1 (7R)-7-Methyl-2-nitro-7-([5-(trifluoromethyl)pyridin-2-yl]oxy)methyl]-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**53**)

The title compound was obtained from alcohol **52**. Light yellow solid (70% yield): mp (Et₂O/hexane) 153-154 °C; ¹H NMR [(CD₃)₂SO] δ 8.61-8.56 (m, 1 H), 8.11 (br dd, *J* = 9.0, 2.5 Hz, 1 H), 8.09 (s, 1 H), 7.07 (d, *J* = 8.8 Hz, 1 H), 4.57 (s, 2 H), 4.19 (dt, *J* = 13.1, 5.8 Hz, 1 H), 4.14 (ddd, *J* = 13.1, 8.7, 5.5 Hz, 1 H), 2.38 (ddd, *J* = 14.4, 8.5, 6.2 Hz, 1 H), 2.19 (dt, *J*

= 14.4, 5.4 Hz, 1 H), 1.49 (s, 3 H); $[\alpha]^{26}_D$ 25.8 (*c* 1.008, CHCl₃). Anal. (C₁₄H₁₃F₃N₄O₄) C, H, N.

Chiral HPLC (using a CHIRALPAK IA analytical column and eluting with 30% EtOH/hexane at 0.6 mL/min) determined that the ee of **53** was >99%.

4.4.4.2 (7*S*)-7-Methyl-2-nitro-7-([5-(trifluoromethyl)pyridin-2-yl]oxy)methyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine (**57**)

The title compound was obtained from alcohol **56**. Light yellow solid (64% yield): mp (Et₂O/hexane) 157-158 °C; ¹H NMR [(CD₃)₂SO] δ 8.61-8.56 (m, 1 H), 8.11 (br dd, *J* = 9.0, 2.6 Hz, 1 H), 8.09 (s, 1 H), 7.07 (d, *J* = 8.8 Hz, 1 H), 4.57 (s, 2 H), 4.19 (dt, *J* = 13.0, 5.7 Hz, 1 H), 4.14 (ddd, *J* = 13.2, 8.7, 5.5 Hz, 1 H), 2.38 (ddd, *J* = 14.4, 8.6, 6.2 Hz, 1 H), 2.19 (dt, *J* = 14.4, 5.5 Hz, 1 H), 1.49 (s, 3 H); $[\alpha]^{26}_D$ -27.9 (*c* 1.005, CHCl₃). Anal. (C₁₄H₁₃F₃N₄O₄) C, H, N.

Chiral HPLC (using a CHIRALPAK IA analytical column and eluting with 30% EtOH/hexane at 0.6 mL/min) determined that the ee of **57** was >99%.

4.5 Syntheses of 3-pyridinyl ethers **73**, **75**, **77**, **79**, **82** and **85**

4.5.1 General procedure for preparing 3-pyridinyloxy alcohols **72**, **74**, **76**, **78**, **81** and **84**

A mixture of the epoxide (**18**, **28**, **31** or **70**, 1.0 equiv), powdered K₂CO₃ (1.0 equiv) and the appropriately substituted pyridin-3-ol (**71**, **80** or **83**, 2.5 equiv) in anhydrous 2-butanone (1 mL/100 mg of epoxide) was stirred in a sealed tube at 80 °C for 40 h. The resulting cooled mixture was diluted with EtOAc (20 mL) and filtered through Celite, washing with EtOAc, and the filtrate was evaporated to dryness under reduced pressure. The residue was chromatographed on silica gel (1:1 EtOAc/petroleum ether and then 2% MeOH/CH₂Cl₂) to afford the required products.

4.5.1.1 4-(2-Chloro-4-nitro-1*H*-imidazol-1-yl)-1-([6-(trifluoromethyl)pyridin-3-yl]oxy)butan-2-ol (**72**)

The title compound was obtained from 2-chloro-4-nitro-1-[2-(oxiran-2-yl)ethyl]-1*H*-imidazole [20] (**70**) and 6-(trifluoromethyl)pyridin-3-ol (**71**). Pale yellow oil (56% yield); ¹H NMR (CDCl₃) δ 8.36 (d, *J* = 2.8 Hz, 1 H), 7.89 (s, 1 H), 7.64 (d, *J* = 8.7 Hz, 1 H), 7.31 (dd, *J* = 8.6, 2.6 Hz, 1 H), 4.41-4.26 (m, 2 H), 4.13-3.99 (m, 3 H), 3.01-2.94 (m, 1 H), 2.19-2.01 (m, 2 H); APCI MS calcd for C₁₃H₁₃ClF₃N₄O₄ *m/z* [M + H]⁺ 383, 381, found 383, 381.

4.5.1.2 4-(2-Chloro-4-nitro-1*H*-imidazol-1-yl)-2-methyl-1-([6-(trifluoromethyl)pyridin-3-yl]oxy)butan-2-ol (**74**)

The title compound was obtained from epoxide **31**, powdered K₂CO₃ (3.5 equiv) and 6-(trifluoromethyl)pyridin-3-ol (**71**), using the above procedure at 83 °C for 16 h. Pale yellow oil (58% yield); ¹H NMR (CDCl₃) δ 8.41 (d, *J* = 2.8 Hz, 1 H), 7.85 (s, 1 H), 7.66 (d, *J* = 8.7 Hz, 1 H), 7.33 (dd, *J* = 8.7, 2.8 Hz, 1 H), 4.37-4.22 (m, 2 H), 3.98 (d, *J* = 9.0 Hz, 1 H), 3.95 (d, *J* = 9.0 Hz, 1 H), 2.40 (br s, 1 H), 2.26 (ddd, *J* = 13.9, 9.3, 6.3 Hz, 1 H), 2.08 (ddd, *J* = 13.9, 9.5, 6.6 Hz, 1 H), 1.45 (s, 3 H); APCI MS calcd for C₁₄H₁₅ClF₃N₄O₄ *m/z* [M + H]⁺ 397, 395, found 397, 395.

4.5.1.3 (2*R*)-4-(2-Chloro-4-nitro-1*H*-imidazol-1-yl)-2-methyl-1-([6-(trifluoromethyl)pyridin-3-yl]oxy)butan-2-ol (**76**)

The title compound was obtained from epoxide **18**, powdered K₂CO₃ (0.94 equiv) and 6-(trifluoromethyl)pyridin-3-ol (**71**, 2.9 equiv). Pale yellow oil (67% yield); ¹H NMR (CDCl₃) δ 8.42 (d, *J* = 2.8 Hz, 1 H), 7.83 (s, 1 H), 7.66 (d, *J* = 8.7 Hz, 1 H), 7.32 (dd, *J* = 8.6, 2.7 Hz,

1 H), 4.36-4.22 (m, 2 H), 3.97 (d, $J = 9.0$ Hz, 1 H), 3.94 (d, $J = 8.9$ Hz, 1 H), 2.25 (ddd, $J = 13.8, 9.3, 6.3$ Hz, 1 H), 2.22 (s, 1 H), 2.07 (ddd, $J = 13.8, 9.4, 6.5$ Hz, 1 H), 1.45 (s, 3 H); APCI MS calcd for $C_{14}H_{15}ClF_3N_4O_4$ m/z $[M + H]^+$ 397, 395, found 397, 395.

4.5.1.4 (2S)-4-(2-Chloro-4-nitro-1H-imidazol-1-yl)-2-methyl-1-[[6-(trifluoromethyl)pyridin-3-yl]oxy]butan-2-ol (**78**)

The title compound was obtained from epoxide **28**, powdered K_2CO_3 (1.2 equiv) and 6-(trifluoromethyl)pyridin-3-ol (**71**, 2.9 equiv), using the above procedure for 48 h. Pale yellow oil (96% yield); 1H NMR ($CDCl_3$) δ 8.41 (d, $J = 2.8$ Hz, 1 H), 7.84 (s, 1 H), 7.66 (d, $J = 8.7$ Hz, 1 H), 7.33 (dd, $J = 8.7, 2.6$ Hz, 1 H), 4.37-4.23 (m, 2 H), 3.98 (d, $J = 9.0$ Hz, 1 H), 3.95 (d, $J = 9.0$ Hz, 1 H), 2.35 (br s, 1 H), 2.26 (ddd, $J = 13.9, 9.3, 6.4$ Hz, 1 H), 2.08 (ddd, $J = 13.9, 9.6, 6.5$ Hz, 1 H), 1.45 (s, 3 H); APCI MS calcd for $C_{14}H_{15}ClF_3N_4O_4$ m/z $[M + H]^+$ 397, 395, found 397, 395.

4.5.1.5 4-(2-Chloro-4-nitro-1H-imidazol-1-yl)-1-[(6-chloropyridin-3-yl)oxy]-2-methylbutan-2-ol (**81**)

The title compound was obtained from epoxide **31**, powdered K_2CO_3 (1.2 equiv) and 6-chloropyridin-3-ol (**80**, 1.5 equiv), using the above procedure at 83 °C for 24 h. Pale yellow oil (49% yield); 1H NMR ($CDCl_3$) δ 8.09 (br d, $J = 3.0$ Hz, 1 H), 7.82 (s, 1 H), 7.30-7.25 (m, 1 H), 7.22 (dd, $J = 8.7, 3.1$ Hz, 1 H), 4.35-4.21 (m, 2 H), 3.90 (d, $J = 8.9$ Hz, 1 H), 3.87 (d, $J = 8.9$ Hz, 1 H), 2.23 (ddd, $J = 13.8, 9.3, 6.5$ Hz, 1 H), 2.21 (br s, 1 H), 2.05 (ddd, $J = 13.8, 9.6, 6.6$ Hz, 1 H), 1.42 (s, 3 H); APCI MS calcd for $C_{13}H_{15}Cl_2N_4O_4$ m/z $[M + H]^+$ 363, 361, found 363, 361.

4.5.1.6 4-(2-Chloro-4-nitro-1H-imidazol-1-yl)-1-[(6-fluoropyridin-3-yl)oxy]-2-methylbutan-2-ol (**84**)

The title compound was obtained from epoxide **31** and 6-fluoropyridin-3-ol (**83**, 1.5 equiv), using the above procedure for 3 d. Pale yellow oil (61% yield); 1H NMR ($CDCl_3$) δ 7.85 (s, 1 H), 7.84 (dd, $J = 2.9, 1.8$ Hz, 1 H), 7.37 (ddd, $J = 8.9, 6.4, 3.2$ Hz, 1 H), 6.90 (dd, $J = 8.9, 3.3$ Hz, 1 H), 4.36-4.22 (m, 2 H), 3.91 (d, $J = 8.9$ Hz, 1 H), 3.87 (d, $J = 8.9$ Hz, 1 H), 2.53 (s, 1 H), 2.25 (ddd, $J = 13.8, 9.4, 6.3$ Hz, 1 H), 2.06 (ddd, $J = 13.8, 9.7, 6.4$ Hz, 1 H), 1.43 (s, 3 H); APCI MS calcd for $C_{13}H_{15}ClFN_4O_4$ m/z $[M + H]^+$ 347, 345, found 347, 345.

4.5.2 General procedure for preparing 3-pyridinyl ethers **73**, **75**, **77**, **79**, **82** and **85**

Sodium hydride (60% in mineral oil, 1.5 equiv) was added to a solution of the alcohol (**72**, **74**, **76**, **78**, **81** or **84**, 1.0 equiv) in anhydrous DMF (1 mL/40 mg of alcohol) under N_2 at 20 °C. The mixture was stirred at 80 °C for 3.5 h, cooled to 20 °C, quenched with aqueous ammonium chloride (10 mL), diluted with water (90 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The combined extracts were evaporated to dryness under reduced pressure and the residue was chromatographed on silica gel (0-2% MeOH/ CH_2Cl_2) to afford the required products.

4.5.2.1 2-Nitro-7-([6-(trifluoromethyl)pyridin-3-yl]oxy)methyl)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**73**)

The title compound was obtained from alcohol **72**. Cream solid (67% yield): mp (*i*-Pr₂O triturate) 180-181 °C; 1H NMR [$(CD_3)_2SO$] δ 8.52 (d, $J = 2.8$ Hz, 1 H), 8.10 (s, 1 H), 7.89 (d, $J = 8.7$ Hz, 1 H), 7.69 (dd, $J = 8.7, 2.7$ Hz, 1 H), 5.02-4.92 (m, 1 H), 4.54 (dd, $J = 11.2, 3.1$ Hz, 1 H), 4.48 (dd, $J = 11.2, 5.9$ Hz, 1 H), 4.20 (ddd, $J = 12.5, 5.7, 2.9$ Hz, 1 H), 4.11 (ddd, $J = 12.5, 11.0, 5.2$ Hz, 1 H), 2.38-2.28 (m, 1 H), 2.28-2.15 (m, 1 H); ^{13}C NMR [$(CD_3)_2SO$] δ

156.6, 147.6, 142.0, 138.8 (q, $J_{C-F} = 34.3$ Hz), 138.8, 122.0, 121.9, 121.9 (q, $J_{C-F} = 272.8$ Hz), 117.8, 75.8, 69.3, 41.7, 22.2. Anal. (C₁₃H₁₁F₃N₄O₄) C, H, N.

4.5.2.2 7-Methyl-2-nitro-7-([6-(trifluoromethyl)pyridin-3-yl]oxy)methyl)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (75)

The title compound was obtained from alcohol **74** and NaH (1.8 equiv), using the above procedure for 15 min. Cream solid (54% yield): mp (*i*-Pr₂O triturate) 198-200 °C; ¹H NMR [(CD₃)₂SO] δ 8.49 (d, $J = 2.8$ Hz, 1 H), 8.11 (s, 1 H), 7.88 (d, $J = 8.7$ Hz, 1 H), 7.67 (dd, $J = 8.6, 2.7$ Hz, 1 H), 4.39 (s, 2 H), 4.20 (dt, $J = 13.1, 5.9$ Hz, 1 H), 4.15 (ddd, $J = 13.2, 8.3, 5.6$ Hz, 1 H), 2.40 (ddd, $J = 14.4, 8.3, 6.1$ Hz, 1 H), 2.20 (dt, $J = 14.4, 5.6$ Hz, 1 H), 1.52 (s, 3 H); ¹³C NMR [(CD₃)₂SO] δ 156.7, 147.1, 142.2, 138.9 (q, $J_{C-F} = 34.3$ Hz), 138.8, 122.0, 121.9 (q, $J_{C-F} = 272.9$ Hz), 121.9 (q, $J_{C-F} = 2.6$ Hz), 117.8, 80.4, 72.4, 39.4, 26.8, 21.1. Anal. (C₁₄H₁₃F₃N₄O₄) C, H, N.

4.5.2.3 (7R)-7-Methyl-2-nitro-7-([6-(trifluoromethyl)pyridin-3-yl]oxy)methyl)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (77)

The title compound was obtained from alcohol **76**, using the above procedure at 50 °C for 2 h. Light yellow solid (67% yield): mp (*i*-Pr₂O triturate) 202-204 °C; ¹H NMR [(CD₃)₂SO] δ 8.49 (d, $J = 2.8$ Hz, 1 H), 8.11 (s, 1 H), 7.88 (d, $J = 8.7$ Hz, 1 H), 7.67 (dd, $J = 8.6, 2.7$ Hz, 1 H), 4.39 (s, 2 H), 4.20 (dt, $J = 13.1, 5.9$ Hz, 1 H), 4.14 (ddd, $J = 13.2, 8.3, 5.6$ Hz, 1 H), 2.40 (ddd, $J = 14.4, 8.2, 6.1$ Hz, 1 H), 2.20 (dt, $J = 14.4, 5.6$ Hz, 1 H), 1.52 (s, 3 H); [α]²⁶_D 6.97 (*c* 1.005, DMF). Anal. (C₁₄H₁₃F₃N₄O₄) C, H, N.

4.5.2.4 (7S)-7-Methyl-2-nitro-7-([6-(trifluoromethyl)pyridin-3-yl]oxy)methyl)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (79)

The title compound was obtained from alcohol **78**, using the above procedure at 50 °C for 2 h. Light yellow-orange solid (62% yield): mp (*i*-Pr₂O triturate) 200-202 °C; ¹H NMR [(CD₃)₂SO] δ 8.49 (d, $J = 2.8$ Hz, 1 H), 8.11 (s, 1 H), 7.88 (d, $J = 8.8$ Hz, 1 H), 7.67 (dd, $J = 8.7, 2.8$ Hz, 1 H), 4.39 (s, 2 H), 4.20 (dt, $J = 13.1, 5.9$ Hz, 1 H), 4.14 (ddd, $J = 13.2, 8.3, 5.6$ Hz, 1 H), 2.40 (ddd, $J = 14.4, 8.2, 6.2$ Hz, 1 H), 2.20 (dt, $J = 14.4, 5.6$ Hz, 1 H), 1.51 (s, 3 H); [α]²⁶_D -6.97 (*c* 1.004, DMF). Anal. (C₁₄H₁₃F₃N₄O₄) C, H, N.

4.5.2.5 7-[(6-Chloropyridin-3-yl)oxy]methyl)-7-methyl-2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (82)

The title compound was obtained from alcohol **81** and NaH (1.6 equiv), using the above procedure at 50 °C for 1.5 h. Pale yellow solid (54% yield): mp (*i*-Pr₂O triturate) 171-173 °C; ¹H NMR [(CD₃)₂SO] δ 8.17 (d, $J = 2.9$ Hz, 1 H), 8.10 (s, 1 H), 7.54 (dd, $J = 8.8, 3.1$ Hz, 1 H), 7.46 (d, $J = 8.6$ Hz, 1 H), 4.29 (s, 2 H), 4.19 (dt, $J = 13.1, 5.9$ Hz, 1 H), 4.13 (ddd, $J = 13.1, 8.2, 5.5$ Hz, 1 H), 2.37 (ddd, $J = 14.5, 8.2, 6.2$ Hz, 1 H), 2.18 (dt, $J = 14.4, 5.7$ Hz, 1 H), 1.49 (s, 3 H). Anal. (C₁₃H₁₃ClN₄O₄) C, H, N.

4.5.2.6 7-[(6-Fluoropyridin-3-yl)oxy]methyl)-7-methyl-2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (85)

The title compound was obtained from alcohol **84** and NaH (1.9 equiv). Light yellow solid (36% yield): mp (*i*-Pr₂O triturate) 157-159 °C; ¹H NMR [(CD₃)₂SO] δ 8.10 (s, 1 H), 7.94 (dd, $J = 2.9, 1.8$ Hz, 1 H), 7.65 (ddd, $J = 9.0, 6.6, 3.2$ Hz, 1 H), 7.15 (dd, $J = 9.0, 3.3$ Hz, 1 H), 4.27 (s, 2 H), 4.19 (dt, $J = 13.1, 6.0$ Hz, 1 H), 4.13 (ddd, $J = 13.1, 8.2, 5.6$ Hz, 1 H), 2.38 (ddd, $J = 14.4, 8.1, 6.1$ Hz, 1 H), 2.18 (dt, $J = 14.4, 5.7$ Hz, 1 H), 1.49 (s, 3 H). Anal. (C₁₃H₁₃FN₄O₄) C, H, N.

4.6 Syntheses of heterocyclic ethers **87**, **89**, **91**, **92**, **94**, **95** and **97**

4.6.1 7-Methyl-2-nitro-7-({[6-(trifluoromethyl)pyridazin-3-yl]oxy}methyl)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**87**)

The title compound was obtained from alcohol **45**, 3-chloro-6-(trifluoromethyl)pyridazine (**86**, 2.0 equiv) and NaH (1.5 equiv), using the procedure described in 4.3 for 1 h. White solid (92% yield): mp (MeOH) 213-215 °C; ¹H NMR [(CD₃)₂SO] δ 8.19 (d, *J* = 9.3 Hz, 1 H), 8.10 (s, 1 H), 7.58 (d, *J* = 9.1 Hz, 1 H), 4.79 (d, *J* = 11.8 Hz, 1 H), 4.76 (d, *J* = 11.8 Hz, 1 H), 4.22 (dt, *J* = 13.1, 5.6 Hz, 1 H), 4.15 (ddd, *J* = 13.1, 8.8, 5.5 Hz, 1 H), 2.42 (ddd, *J* = 14.5, 8.8, 6.2 Hz, 1 H), 2.22 (dt, *J* = 14.4, 5.4 Hz, 1 H), 1.54 (s, 3 H). Anal. (C₁₃H₁₂F₃N₅O₄) C, H, N.

4.6.2 7-Methyl-2-nitro-7-({[5-(trifluoromethyl)pyrazin-2-yl]oxy}methyl)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**89**)

The title compound was obtained from alcohol **45**, 2-chloro-5-(trifluoromethyl)pyrazine (**88**, 2.0 equiv) and NaH (1.6 equiv), using the procedure described in 4.3 at 0-20 °C. Cream solid (89% yield): mp (CH₂Cl₂/pentane) 170-171 °C; ¹H NMR [(CD₃)₂SO] δ 8.78-8.73 (m, 1 H), 8.55 (d, *J* = 1.0 Hz, 1 H), 8.10 (s, 1 H), 4.63 (s, 2 H), 4.21 (dt, *J* = 13.0, 5.6 Hz, 1 H), 4.15 (ddd, *J* = 13.2, 8.8, 5.5 Hz, 1 H), 2.40 (ddd, *J* = 14.4, 8.8, 6.1 Hz, 1 H), 2.20 (dt, *J* = 14.4, 5.4 Hz, 1 H), 1.52 (s, 3 H); ¹³C NMR [(CD₃)₂SO] δ 161.00, 147.0, 142.2, 138.9 (q, *J*_{C-F} = 3.4 Hz), 136.2, 134.9 (q, *J*_{C-F} = 34.8 Hz), 121.8 (q, *J*_{C-F} = 272.5 Hz), 117.8, 80.2, 70.3, 39.4, 26.7, 20.9. Anal. (C₁₃H₁₂F₃N₅O₄) C, H, N.

4.6.3 2-Nitro-7-({[5-(trifluoromethyl)pyrimidin-2-yl]oxy}methyl)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**91**)

The title compound was obtained from alcohol **42**, 2-chloro-5-(trifluoromethyl)pyrimidine (**90**, 1.2 equiv) and NaH (1.6 equiv), using the procedure described in 4.3 for 130 min. Cream solid (93% yield): mp (MeOH/CH₂Cl₂/hexane) 244-246 °C; ¹H NMR [(CD₃)₂SO] δ 9.11 (br s, 2 H), 8.10 (s, 1 H), 5.02-4.94 (m, 1 H), 4.75 (dd, *J* = 12.1, 3.2 Hz, 1 H), 4.69 (dd, *J* = 12.1, 6.1 Hz, 1 H), 4.19 (ddd, *J* = 12.6, 5.8, 2.7 Hz, 1 H), 4.10 (ddd, *J* = 12.5, 11.2, 5.1 Hz, 1 H), 2.37-2.28 (m, 1 H), 2.27-2.14 (m, 1 H). Anal. (C₁₂H₁₀F₃N₅O₄) C, H, N.

4.6.4 7-Methyl-2-nitro-7-({[5-(trifluoromethyl)pyrimidin-2-yl]oxy}methyl)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**92**)

The title compound was obtained from alcohol **45**, 2-chloro-5-(trifluoromethyl)pyrimidine (**90**, 1.2 equiv) and NaH (1.5 equiv), using the procedure described in 4.3 for 2 h. Cream solid (95% yield): mp (Et₂O/pentane) 220-222 °C; ¹H NMR [(CD₃)₂SO] δ 9.08 (s, 2 H), 8.10 (s, 1 H), 4.63 (s, 2 H), 4.21 (dt, *J* = 13.0, 5.6 Hz, 1 H), 4.14 (ddd, *J* = 13.2, 8.4, 5.6 Hz, 1 H), 2.39 (ddd, *J* = 14.3, 8.1, 6.3 Hz, 1 H), 2.21 (ddd, *J* = 14.3, 5.5, 5.1 Hz, 1 H), 1.51 (s, 3 H); ¹³C NMR [(CD₃)₂SO] δ 166.0, 157.9 (q, *J*_{C-F} = 3.4 Hz, 2 C), 147.1, 142.2, 123.4 (q, *J*_{C-F} = 271.1 Hz), 118.3 (q, *J*_{C-F} = 33.7 Hz), 117.8, 80.1, 71.4, 39.4, 26.8, 21.0. Anal. (C₁₃H₁₂F₃N₅O₄) C, H, N.

4.6.5 7-{{[5-Chloropyrimidin-2-yl]oxy}methyl}-2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**94**)

The title compound was obtained from alcohol **42**, 2,5-dichloropyrimidine (**93**, 3.0 equiv) and NaH (1.5 equiv), using the procedure described in 4.3 for 40 min. Cream solid (71% yield): mp (*i*-Pr₂O triturate) 249 °C (dec); ¹H NMR [(CD₃)₂SO] δ 8.76 (s, 2 H), 8.09 (s, 1 H), 4.99-4.90 (m, 1 H), 4.64 (dd, *J* = 12.0, 3.2 Hz, 1 H), 4.58 (dd, *J* = 12.1, 6.1 Hz, 1 H), 4.18 (ddd, *J* = 12.6, 5.8, 2.8 Hz, 1 H), 4.09 (ddd, *J* = 12.5, 11.1, 5.1 Hz, 1 H), 2.36-2.26 (m, 1 H), 2.25-

2.11 (m, 1 H); ¹³C NMR [(CD₃)₂SO] δ 162.7, 157.9 (2 C), 147.7, 142.0, 123.9, 117.8, 75.6, 68.2, 41.7, 22.3. Anal. (C₁₁H₁₀ClN₅O₄) C, H, N.

4.6.6 7-[[5-(5-Chloropyrimidin-2-yl)oxy]methyl]-7-methyl-2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**95**)

The title compound was obtained from alcohol **45**, 2,5-dichloropyrimidine (**93**, 3.0 equiv) and NaH (1.5 equiv), using the procedure described in 4.3 for 40 min. Cream solid (59% yield): mp (*i*-Pr₂O triturate) 220-221 °C; ¹H NMR [(CD₃)₂SO] δ 8.74 (s, 2 H), 8.10 (s, 1 H), 4.52 (s, 2 H), 4.19 (dt, *J* = 13.0, 5.7 Hz, 1 H), 4.13 (ddd, *J* = 13.1, 8.7, 5.5 Hz, 1 H), 2.37 (ddd, *J* = 14.4, 8.7, 6.1 Hz, 1 H), 2.19 (dt, *J* = 14.4, 5.4 Hz, 1 H), 1.49 (s, 3 H). Anal. (C₁₂H₁₂ClN₅O₄) C, H, N.

4.6.7 7-[[5-(5-Fluoropyrimidin-2-yl)oxy]methyl]-7-methyl-2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (**97**)

The title compound was obtained from alcohol **45**, 2-chloro-5-fluoropyrimidine (**96**, 3.0 equiv) and NaH (1.5 equiv), using the procedure described in 4.3 for 3 h. Cream solid (26% yield): mp (*i*-Pr₂O triturate) 202-205 °C; ¹H NMR (CDCl₃) δ 8.38 (s, 2 H), 7.46 (s, 1 H), 4.56 (d, *J* = 11.3 Hz, 1 H), 4.44 (d, *J* = 11.3 Hz, 1 H), 4.20 (ddd, *J* = 12.7, 6.5, 6.1 Hz, 1 H), 4.11 (ddd, *J* = 12.7, 7.7, 5.7 Hz, 1 H), 2.55 (ddd, *J* = 14.6, 7.7, 5.9 Hz, 1 H), 2.25 (ddd, *J* = 14.6, 6.4, 5.8 Hz, 1 H), 1.61 (s, 3 H). Anal. (C₁₂H₁₂FN₅O₄) C, H, N.

4.7 Biological and physicochemical assays

4.7.1 *In vitro* parasite growth inhibition and cytotoxicity assays

The activity of test compounds against the amastigote stage of the *L. don* parasite was measured at CDRi using a mouse macrophage-based luciferase assay, carried out according to the published procedures [34]. Replicate assays quantifying the growth inhibitory action of compounds against *L. inf.*, *T. cruzi*, and *T. brucei* and assessing any cytotoxic effects on human lung fibroblasts (MRC-5 cells) were conducted at the University of Antwerp (LMPH), as previously described [35]. Results in Table 1 are the mean of at least two independent determinations (SD data are given in Supplementary data, Table S1).

4.7.2 Minimum inhibitory concentration assays (MABA and LORA)

These assays against *M. tb* (strain H37Rv) were performed according to the reported procedures [36,37]. The MIC was defined as the lowest compound concentration effecting a growth inhibition of at least 90% relative to the growth of drug-free controls. Results in Table 1 are the mean of two or three independent determinations, unless otherwise noted (SD data are given in Supplementary data, Table S1).

4.7.3 Microsomal stability assays

Studies of compounds **48**, **49**, **59**, **60**, **73**, **75**, and **94** (Table 2) were run by Advinus Therapeutics Ltd., Bangalore, India, using a published procedure [47] in which the compound concentration was 0.5 μM and the incubation time was 30 min. Additional analyses of compounds **20**, **30**, **48**, **49**, **53**, **57**, **64**, **66**, **77**, **79**, **87**, **89**, and **92** were performed by WuXi AppTec (Shanghai) Co., Ltd., Shanghai, China, via a reported method [23]; the compound concentration was 1 μM, and the incubation time was 1 h.

4.7.4 hERG assay

The effects of compounds **20** and **30** on cloned hERG potassium channels expressed in Chinese hamster ovary cells were assessed by WuXi AppTec (Shanghai) Co., Ltd., using the automated patch clamp method. Six concentrations (0.12, 0.37, 1.11, 3.33, 10, and 30 μM)

were tested (at room temperature), and at least three replicates were obtained for each. Compounds **48**, **49**, **59**, **60**, **73**, **75**, and **94** were also screened in duplicate (at 10 μ M) against hERG channels expressed in HEK-293 cells by Ricerca Biosciences LLC, Taipei, Taiwan, using a literature protocol [48].

4.7.5 Solubility measurements

The solid compound sample was mixed with water or 0.1 M HCl (enough to make a 2 mM solution) in an Eppendorf tube, and the suspension was sonicated for 15 min and then centrifuged at 13000 rpm for 6 min. An aliquot of the clear supernatant was diluted 2-fold with water (or 0.1 M HCl), and then HPLC was implemented. The kinetic solubility was calculated by comparing the peak area obtained with that from a standard solution of the compound in DMSO (after allowing for varying dilution factors and injection volumes).

4.7.6 Ethics statement for animal experiments

All animal experiments were performed according to institutional ethical guidelines for animal care. All LSHTM animal work was carried out under a UK Home Office project licence according to the Animal (Scientific Procedures) Act 1986 and the new European Directive 2010/63/EU. The project licence (70/8427) was reviewed by the LSHTM Animal Welfare and Ethical Review Board prior to submission and consequent approval by the UK Home Office. Hamster studies (LMPH) were approved by the ethical committee of the University of Antwerp (UA-ECD 2010-17).

4.7.7 Mouse model for acute *L. don* infection (London)

Test compounds were orally dosed once per day for 5 days consecutively to groups of five female BALB/c mice infected with 2×10^7 *L. don* amastigotes, with treatment commencing 1 week postinfection, as described [34]. Miltefosine (**1**) and AmBisome were positive controls, and parasite burdens were determined from impression smears of liver sections. Efficacy was expressed as the mean percentage reduction in parasite load for treated mice in comparison to untreated (vehicle-only) controls.

4.7.8 Hamster model for chronic *L. inf* infection (Antwerp)

Golden hamsters (weighing 75-80 g) were infected with 2×10^7 *L. inf* amastigotes, and 21 days post-infection, treatment groups of 6 animals each were dosed orally twice per day with test compounds (formulated in PEG-400) for 5 days consecutively. Parasite burdens in three target organs (liver, spleen, and bone marrow) were determined by microscopic evaluation of impression smears (stained with Giemsa), and efficacy was expressed as the mean percentage parasite load reduction for treated hamsters in comparison to untreated (vehicle-only) controls. Miltefosine (**1**) was included as a reference drug in all experiments.

4.7.9 Mouse pharmacokinetics

Studies of compounds **49**, **60**, **73**, **75**, **77**, **79**, and **94** were conducted by Advinus Therapeutics Ltd., Bangalore, India, according to a published protocol [47]. Briefly, compounds were administered to groups of male Swiss Albino mice; intravenous dosing (at 1 mg/kg) employed a solution vehicle comprising 10% NMP, 10% Cremophor EL, and either 10% PEG-300 or 10-20% propylene glycol in water, while oral dosing (at 12.5 or 25 mg/kg) was as a suspension in 7% Tween 80 and 3% EtOH in water. Samples derived from plasma (at 0.083 for iv only, 0.25, 0.5, 1, 2, 4, 6, 10, and 24 h) were centrifuged prior to analysis by LC-MS/MS, and the PK parameters were determined using Phoenix WinNonlin software (version 5.2). The remaining compounds (**20**, **30**, **53**, and **57**) were evaluated by WuXi AppTec (Shanghai) Co., Ltd.; in this case, oral dosing of female BALB/c mice was at 25-51

mg/kg in PEG-400 (sampling at 0.25, 1, 2, 4, 8, and 24 h), and the PK data were obtained using WinNonlin software (version 6.2) following similar LC-MS/MS analysis.

4.7.10 Hamster pharmacokinetics

All studies were conducted in fasted animals (female golden Syrian hamsters) by WuXi AppTec (Shanghai) Co., Ltd. Intravenous dosing (at 2 mg/kg) utilised a solution formulation of 20% NMP and 40% PEG-400 in citrate buffer (pH 3), whereas PEG-400 was the vehicle employed for oral dosing (at 41-51 mg/kg). Plasma samples (at 0.083 for iv only, 0.25, 0.5, 1, 2, 4, 8, and 24 h) were analysed by LC-MS/MS, and the PK parameters were calculated using Phoenix WinNonlin software (version 6.2).

Declaration of competing interest

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2020.*

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