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Novel Linker Variants of Antileishmanial/Antitubercular 7-Substituted 2-Nitroimidazooxazines Offer Enhanced Solubility

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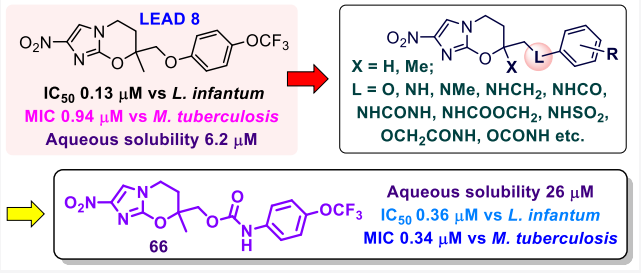
ABSTRACT: Antitubercular 7-substituted 2-nitroimidazo[2,1-*b*]-[1,3]oxazines were previously shown to exhibit potent anti-leishmanial and antitrypanosomal activities, culminating in a new clinical investigational drug for visceral leishmaniasis (DNDI-0690). To offset development risks, we continued to seek further leads with divergent candidate profiles, especially analogues possessing greater aqueous solubility. Starting from an efficacious monoaryl derivative, replacement of the side chain ether linkage by novel amine, amide, and urea functionality was first explored; the former substitution was well-tolerated *in vitro* and *in vivo* but elicited marginal alterations to solubility (except through a less stable benzylamine), whereas the latter groups resulted in significant solubility improvements (up to 53-fold) but an antileishmanial potency reduction of at least 10-fold. Ultimately, we discovered that *O*-carbamate **66** offered a more optimal balance of increased solubility, suitable metabolic stability, excellent oral bioavailability (100%), and strong *in vivo* efficacy in a visceral leishmaniasis mouse model (97% parasite load reduction at 25 mg/kg).



KEYWORDS: *pretomanid, leishmaniasis, tuberculosis, Chagas disease, pharmacokinetics, in vivo efficacy*

Leishmaniasis comprises a family of four diseases that are caused by *Leishmania* parasites and spread by infected sandflies.[1] The most prevalent malady, cutaneous leishmaniasis (CL), leads to deep skin ulcers, whereas visceral leishmaniasis (VL) describes a condition where *L. donovani* (*L. don*) or *L. infantum* (*L. inf*) engulf critical organs, e.g., the spleen and liver, eventually causing death (without chemotherapy). Current options to treat VL include just four drugs (only one oral; miltefosine, **1**; Figure 1) whose success varies greatly inside and among different regions.[2] The World Health Organization recognizes that achievement of its 2030 target to "eliminate VL as a public health problem in 85% of countries" will need "critical action to develop more effective and user-friendly treatment and diagnostics, especially for East Africa".[3]

Historically, drug discovery for leishmaniasis has relied heavily upon the repurposing of approved medicines.[4] Today, there are still few well-validated drug targets for VL and CL, as the "biological pathways essential for survival and disease progression" are still being unraveled.[5] Nevertheless, recent extensive investment in phenotypic screening of large compound collections and lead optimization activities[4] has now produced five new VL drug candidates in phase I clinical trials (**2**-**6**) and another (DNDI-6174) in preclinical studies.[5−8] Importantly, these agents possess diverse modes of action, providing the opportunity for future combination therapy; **2** (GSK3186899)[9] is a CRK-12 inhibitor,[2] both **3** (LXE408)[8] and **6** (GSK3494245)[10] are proteasome inhibitors, **4** (DNDI-6148)[5] may inhibit mRNA maturation by binding to the enzyme CPSF3 (based on studies of other benzoxaboroles in *T. brucei* and target site identity in *Leishmania*[11]), and **5** (DNDI-0690)[12] is activated by a novel nitroreductase, NTR2.[13] Compound **5** originated from our VL lead optimization program with DND*i* around analogues of the newly approved[14] tuberculosis (TB) drug pretomanid (PA-824, **7**). It exhibits very rapid leishmanicidal activity against *L. don* and *L. inf* without cytotoxicity and displays remarkable efficacy in mouse and hamster models of VL.[12,15] Candidates **4** and **5** also demonstrate excellent oral activity in mouse models of CL,[16,17] while **5** additionally retains the strong antitubercular properties of **7**.[12]

Despite such progress, the failures of sitamaquine and fexinidazole in previous VL clinical trials[2,12] and an unexpected recent suspension to the phase I study of **2**[18] provide a timely

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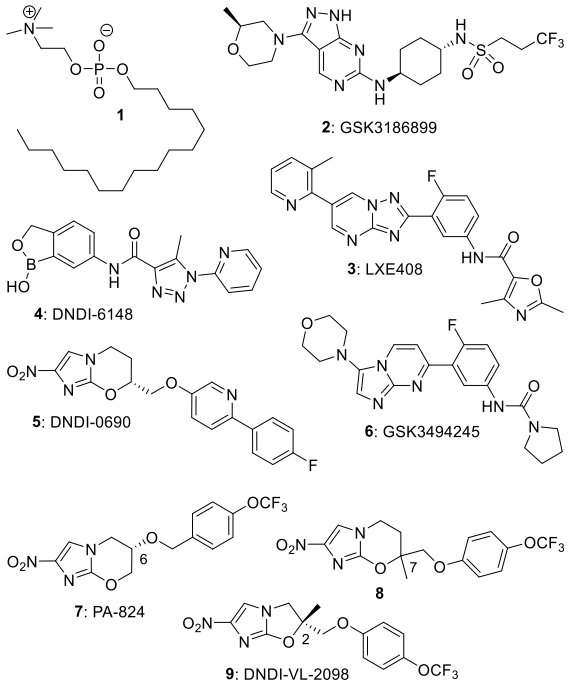


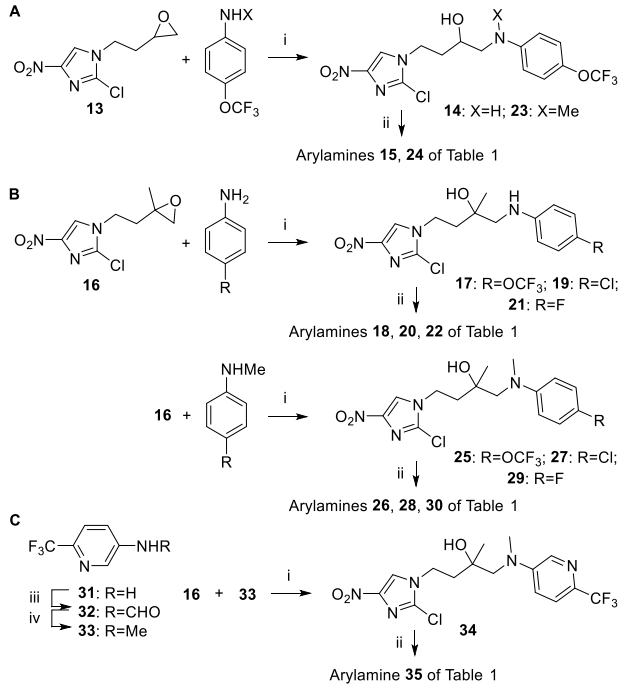
Figure 1. Various oral agents against VL (and/or tuberculosis).

reminder regarding the high attrition rate (~90%) for small molecules in clinical development.[19] To offset this risk, we aimed to discover novel backup candidates to **5** having greater aqueous solubility.[20] An excellent starting point for new SAR exploration was analogue **8** (*L. inf* IC_{50} 0.13 μM), which displayed *in vivo* activity comparable to that of **5** in a VL mouse model but had inferior safety.[12] In previous SAR investigations on pretomanid (**7**), we demonstrated that replacement of the oxygen atom at C-6 by nitrogen-based linkers (viz. amines, amides, ureas, and N-carbamates) or extension of the O-linkage with additional polar functionality (e.g., acetamide, O-carbamate) were effective strategies to reduce compound lipophilicity, improve aqueous solubility, and modulate PK properties.[20,21] An NH- (rather than O−)linkage to the phenyl ring of *rac*-**9** also secured good potency and enhanced solubility (6-fold) in the 6-nitroimidazooxazole class.[22]

Our first targets encompassed several novel amino- and methylamino-linked analogues of **8**. Reaction of known[12] epoxides **13** and **16** with various anilines (catalyzed by anhydrous cobaltous chloride[22]) gave the uncyclized β-anilino alcohols, which were ring closed in low to moderate yield by careful treatment with sodium hydride[22] (1.4−1.6 equiv; Scheme 1A, B). One pyridine congener (**35**) was also prepared, although here the epoxide-opening step was inefficient (17%) due to the poor nucleophilicity of methylaminopyridine **33** (Scheme 1C).

Additional N-linked targets were obtained through azide intermediates **37** and **42**, generated from epoxide **16** (via ring opening with sodium azide[23] and ring closure as above) and alcohol **41**[12] (via Mitsunobu reaction with diphenylphosphoryl azide), respectively (Scheme 2A, B). Reduction of **37** to amine **38** using 1:1 propane-1,3-dithiol/triethylamine[20] was complicated by low solubility and side product formation (acylation then gave amide **45** in just 38% yield; Scheme 2B). Therefore, we turned to a one-pot amidation method involving modified Staudinger conditions (triphenylphosphine added to a mixture of the azide and acid chloride),[24] which furnished the

Scheme 1. Synthesis of Novel Arylamino Analogues of 8[a]



[a]Reagents and conditions: (i) $CoCl_2$, CH_3CN, 65−75 °C, 1−3 d (17−97%); (ii) NaH, DMF, 0−20 °C, 2−4.3 h (or 50−70 °C, 2−3 h) (9−63%); (iii) HCOOH/Ac_2O, THF, 20 °C, 23 h (97%); (iv) $Me_2S{\cdot}BH_3$, THF, 0−20 °C, 0.5 h, then 65 °C, 3.5 h (72%).

carboxamides (**44** and **46**−**48**) directly and in high yield (73−84%; Scheme 2B). Carbamoylation (urea **51**) was also achieved through another Staudinger approach[25] (triphenylphosphine added to a mixture of **37** and the appropriate aniline in the presence of 2 M triethylammonium bicarbonate; Scheme 2C), albeit, these conditions seemed to favor the formation of symmetrical ureas (**52**). The same chemistry was applied to 7H azide **42** to give amide **43** and urea **49** (Scheme 2B, C).

In related methodology, the Boc derivative **56** was obtained from azide **37** using triphenylphosphine and Boc-ON[26] [2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile] (Scheme 2E), but purification of this compound was not straightforward. Removal of the Boc group (TFA/CH_2Cl_2) and basification gave hygroscopic material (after chromatography), so this was elaborated immediately to sulfonamides **57** and **58**. Alternatively, treatment of **37** with triphenylphosphine alone (in aqueous dioxane) generated a ca. 4:3 mixture of amine **38** and iminophosphorane **39**. Although **39** resisted mild hydrolysis, some pure **38** could be separated out as a stable, nonhygroscopic solid, enabling the syntheses of benzylamine **40** (Scheme 2A) and urea **53** (Scheme 2D) via standard methods,[20] and N-carbamate **55** (via aminolysis of the novel 4-nitrophenyl carbonate **54**; Scheme 2D). One ether-linked amide (**64**) was also accessed in meager yield (4%) by coupling alcohol **63**[12] and iodoacetamide **62** (Scheme 3A). Finally, several O-carbamate derivatives (**65**, **66**, and **68**−**74**) were formed by Cu(I)-induced reaction[20] of alcohols **41**, **63**, **67**, and *ent*-**67**[12,27] with aryl isocyanates (Scheme 3B).

The 32 new analogues were screened for activity against *L. inf* (Table 1), two *Trypanosoma* parasites, and *Mycobacterium tuberculosis* as well as for cytotoxicity toward MRC-5 cells (see Table S1 in the Supporting Information for additional data).

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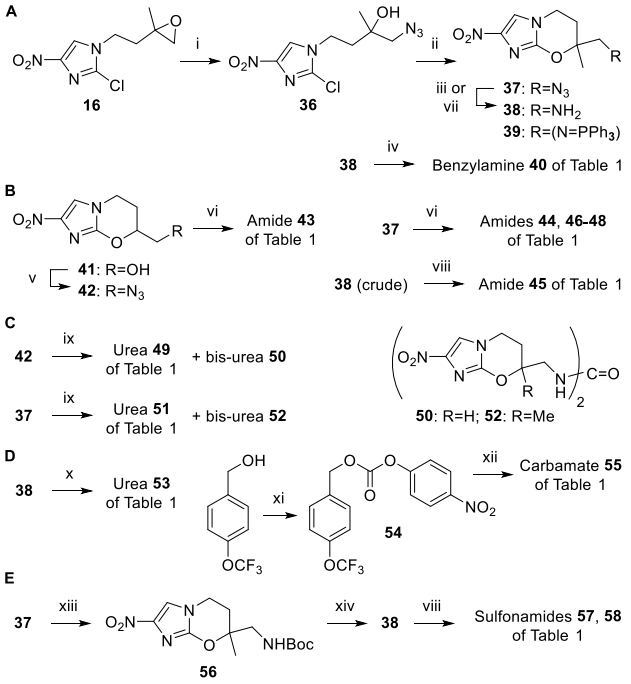
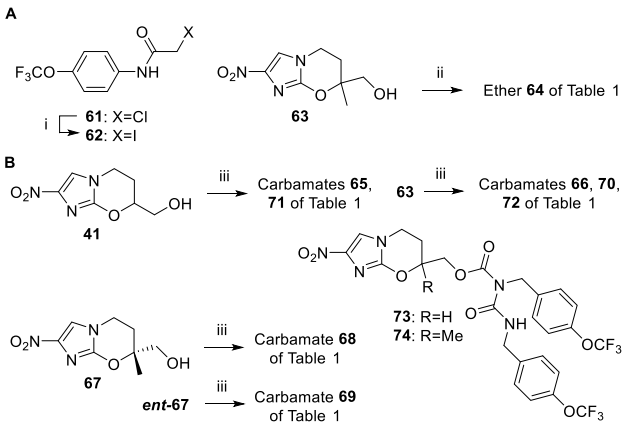
Scheme 2. Synthesis of Further N-Linked Analogues of 8[a]



[a]Reagents and conditions: (i) NaN_3, CTAB, MeOH, 20 °C, 45 min, then 40 °C, 17 h (73%); (ii) NaH, DMF, 0−20 °C, 2.5 h (70%); (iii) PPh_3, aq dioxane, 12−20 °C, 1 d (**38**: 48%, **39**: 32%); (iv) 4-OCF_3PhCHO, $NaBH_3CN$, AcOH, DMF, 0−20 °C, 21 h (50%); (v) PPh_3, DEAD, DPPA, DMF, 0−20 °C, 45 h (83%); (vi) PPh_3, ArCOCl (or 4-OCF_3PhOCH_2COCl), CH_2Cl_2, 20 °C, 1.5−2.2 h (51−84%); (vii) $HS(CH_2)_3SH$, Et_3N, MeOH, CH_2Cl_2, 15−20 °C, 12 h (83%); (viii) 3-OCF_3PhCOCl or $RPhSO_2Cl$, DIPEA, DMF, 0−20 °C, 3−19 h (46−81%); (ix) PPh_3, 4-OCF_3PhNH_2, 2 M TEAB, dioxane, 12−20 °C, 35 h (14−17%); (x) 4-OCF_3BnNCO, DIPEA, $Bu_2Sn(OAc)_2$, DMF, 20 °C, 16 h (93%); (xi) 4-NO_2PhOCOCl, pyridine, CH_2Cl_2, 0−20 °C, 20 h (98%); (xii) **38**, DMAP, DIPEA, DMF, 20 °C, 44 h (71%); (xiii) PPh_3, Boc-ON, CH_2Cl_2, 0−20 °C, 1 d (67%); (xiv) TFA, CH_2Cl_2, 20 °C, 7 h (100%).

Scheme 3. Synthesis of New O-Linked Analogues of 8[a]



[a]Reagents and conditions: (i) NaI, acetone, 56 °C, 2 h, then 20 °C, 15 h (96%); (ii) **62**, NaH, DMF, 0−20 °C, 80 min (4%); (iii) ArNCO, CuCl, DMF, 20 °C, 32−52 h (44−98%).

All compounds were classified as nontoxic (MRC-5 IC_{50}s ≥ 40 μM) with many providing VL selectivity indices of >100. For substituted phenyl side chains, good anti-VL activity was maintained across both the amino (NH) and methylamino (NMe) linker series (**15**−**30**). Compared to known ether-linked counterparts (**8**, **10**−**12**), potency was similar or ∼2-fold lower for the new 7-Me analogues (*L. inf* IC_{50}s 0.18 and 0.27 μM for **18** and **26**), and 3- to 5-fold lower for 7H congeners **15** and **24** (*L. inf* IC_{50}s 0.15 and 0.22 μM). In both NH and NMe linker series, the best phenyl substituent was 4-trifluoromethoxy, followed by 4-chloro, whereas the more hydrophilic 4-fluoro derivatives displayed weaker inhibition. The trifluoromethylpyridine analogue **35** was ∼8-fold less potent than **26**.

Two promising examples (**18** and **26**) showed modest (2- to 4-fold) solubility improvements over **8** at low pH only (Table 2), consistent with small lipophilicity changes (ΔCLogP −0.4 and +0.2 units, respectively). The NH-linked compound (**18**) also demonstrated reasonable stability toward mouse liver microsomes (MLM) (45% parent remaining vs 50% for **8**; Table 2) but its NMe-linked derivative **26** was metabolized more quickly (an MLM half-life of 17 min). However, because **26** was considerably easier to synthesize than **18**, we elected to evaluate both compounds in the *L. don* mouse model.[12] Here, once daily oral dosing of **18** for 5 days gave essentially complete parasite clearance at 50 mg/kg (99.7%; Tables 2 and S3), but **26** was only moderately effective (58% at 50 mg/kg). Further testing of **18** at 6.25 mg/kg suggested that it was ∼2-fold less dose-potent than **8**.

In subsequent work, we prepared a methylene homologue of **18**, benzylamine **40**, which retained the excellent potency of **8** (*L. inf* IC_{50} 0.13 μM) and exhibited markedly better aqueous solubility (54- to 14 300-fold at pH 7 and pH 1, respectively). However, inferior MLM stability (36%) discouraged any *in vivo* appraisal.

Incorporation of an amide (**43**−**48**), urea (**49**, **51**, **53**) or sulfonamide (**57**, **58**) linkage led to much larger lipophilicity changes over **8** and **10** (ΔCLogP −0.8 to −1.2 units) and greater aqueous solubility (e.g., **44** and **51**: 78 and 121 μg/mL, respectively). Nevertheless, much weaker potencies were observed in all cases (*L. inf* IC_{50}s generally 1−3 μM). The most useful compounds were benzamides **44** and **45** and phenylurea **51** (*L. inf* IC_{50}s 1.3−1.4 μM). Linker extension (**47**, **48**, **53**) led to reduced potency, while aryl sulfonamides **57** and **58** were also less impressive (*L. inf* IC_{50}s ∼ 2 μM). Stability testing of three molecules in MLM (**44**, **45**, **51**) revealed that benzamide **44** had the best profile (65 vs 39% for urea **51**). This translated into stronger efficacy for **44** over **51** in the *L. don* mouse model (71 vs 36% parasite burden reduction at 50 mg/kg), albeit, this level of activity was considered only borderline according to published lead selection criteria.[28]

To complete our investigation of acylamino linkers, N-linked benzyl carbamate **55** was prepared (ΔCLogP −0.5 units vs **8**). Unexpectedly, this compound afforded excellent potency (*L. inf* IC_{50} 0.16 μM) and 12-fold better aqueous solubility than **8**, but was much less stable toward MLM (22 vs 50%, respectively). These findings reinforced the difficulty of simultaneously attaining high potency, solubility, and stability in one molecule.

Extension of the ether linkage of **8** by including an amide functionality (**64**) also gave a considerable lipophilicity reduction (ΔCLogP −1.1 units) but correspondingly weaker potency (4-fold vs **8**), so we switched our focus to O-carbamates (**65**−**72**). These new analogues provided more moderate lipophilicity changes over **8** and **10** (ΔCLogP −0.5 units) and generally displayed potencies in a more acceptable

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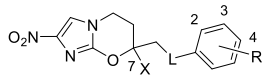
Table 1. Structures, Calculated Lipophilicities, and Antileishmanial Activities of New Linker Analogues



compound	X	linker (L)	R	CLogP[a]	IC_{50} (μM)[b]	compound	X	linker (L)	R	CLogP[a]	IC_{50} (μM)[b]
10[c]	H	O	4-OCF_3	3.21	0.047	**48**	Me	$NHCOCH_2O$	4-OCF_3	2.85	1.7
8[c]	Me	O	4-OCF_3	3.73	0.13	**49**	H	NHCONH	4-OCF_3	2.36	3.3
11[d]	Me	O	4-Cl	3.38	0.13	**51**	Me	NHCONH	4-OCF_3	2.88	1.4
12[d]	Me	O	4-F	2.81	0.45	**53**	Me	$NHCONHCH_2$	4-OCF_3	2.91	1.8
15	H	NH	4-OCF_3	2.80	0.15	**55**	Me	$NHCOOCH_2$	4-OCF_3	3.21	0.16
18	Me	NH	4-OCF_3	3.32	0.18	**57**	Me	$NHSO_2$	4-OCF_3	2.92	2.2
20	Me	NH	4-Cl	3.12	0.29	**58**	Me	$NHSO_2$	3-OCF_3	2.92	2.0
22	Me	NH	4-F	2.55	0.93	**59**[c]	H	OCH_2	4-OCF_3	2.84	0.12
24	H	NMe	4-OCF_3	3.39	0.22	**60**[c]	Me	OCH_2	4-OCF_3	3.36	0.30
26	Me	NMe	4-OCF_3	3.91	0.27	**64**	Me	OCH_2CONH	4-OCF_3	2.63	0.49
28	Me	NMe	4-Cl	3.66	0.31	**65**	H	OCONH	4-OCF_3	2.71	0.36
30	Me	NMe	4-F	3.09	0.48	**66**	Me	OCONH	4-OCF_3	3.23	0.36
35	Me	NMe	3-aza, 4-CF_3	2.86	2.2	**68**	Me[e]	OCONH	4-OCF_3	3.23	0.14
40	Me	$NHCH_2$	4-OCF_3	3.13	0.13	**69**	Me[f]	OCONH	4-OCF_3	3.23	0.20
43	H	NHCO	4-OCF_3	2.28	1.5	**70**	Me	OCONH	2-OCF_3	3.23	0.64
44	Me	NHCO	4-OCF_3	2.80	1.4	**71**	H	$OCONHCH_2$	4-OCF_3	2.69	0.18
45	Me	NHCO	3-OCF_3	2.80	1.3	**72**	Me	$OCONHCH_2$	4-OCF_3	3.21	0.32
46	Me	NHCO	2-OCF_3	2.73	9.9	**73**	H	$OCONZCH_2$[g]	4-OCF_3	6.09	0.79
47	Me	$NHCOCH_2$	4-OCF_3	2.50	2.0	**74**	Me	$OCONZCH_2$[g]	4-OCF_3	6.61	1.3

[a]Calculated log P values from ChemDraw v19.1. [b]Values for 50% growth inhibition of *L. inf* (in mouse macrophages); all data are averages from two or more independent experiments (for standard deviations, see the Supporting Information). [c]Ref 12. [d]Ref 27. [e](7*R*)-Enantiomer. [f](7*S*)-Enantiomer. [g]Z = $CONHCH_2$(4-OCF_3Ph).

Table 2. Aqueous Solubility, Microsomal Stability, and *In Vivo* Efficacy Data for Selected Compounds

	aqueous solubility (μg/mL)[a]		stability in liver microsomes (% parent remaining at 1 h)		efficacy in *L. don* mouse model (% parasite burden reduction at oral dose in mg/kg)[b]				
compound	pH 7	pH 1	human	mouse	50	25	12.5	6.25	3.13
8[c]	2.3		58	50		100	100	83	25
10[c]	4.0		85	57		87			
18	3.0	10	47	45	>99			29	
26	1.5	4.6	8.0	9.2	58				
40	124	33 000	56	36					
44	78		67	65	71				
45	14		58	36					
51	121		54	39	36				
55	28		44	22					
65	4.5		93	58					
66	11		68	46	>99	97	7		
68	6.3		95	51		89	40		
69	7.9		78	55		51	31		
72	117		45	8.7					

[a]Solubility in water (pH 7) or 0.1 M HCl (pH 1). [b]Dosing was once daily for 5 days (see the Supporting Information for all protocols). [c]Ref 12.

Table 3. Mouse Pharmacokinetic Parameters for Selected Compounds

	intravenous (1 mg/kg)					oral (25 mg/kg)				
compound	C_0 (μg/mL)	CL (mL/min/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (h)	AUC_{last}[a] (μg·h/mL)	C_{max} (μg/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{last}[a] (μg·h/mL)	F[b] (%)
10[c]	0.36	48	3.2	1.1	0.341	1.3	0.50		3.86	45
8[c]	0.79	12	2.5	2.8	1.31	1.4	3.0		11.5	35
65	0.74	14	1.6	1.2	1.17	9.6	1.0	1.6	35.4	100
66	0.80	8.5	1.2	1.3	2.07	13.5	0.75	1.7	57.8	100

[a]Area under the curve calculated to the last time point. [b]Oral bioavailability, determined using dose normalized AUC_{last} values. [c]Ref 12.

range (*L. inf* IC_{50}s 0.18−0.36 μM). In terms of both solubility and microsomal stability, the 7H phenyl carbamate **65** was not significantly superior to its more active ether-linked counterpart **10**, whereas the 7-Me congener **66** was 5-fold more soluble than **8** and provided better stability toward human liver microsomes (HLM; 68% parent for **66** vs 58% for **8**). Benzyl

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homologue **72** demonstrated ~11-fold greater solubility than **66** (117 μg/mL) but, like **26**, this molecule was rapidly metabolized by MLM (a half-life of 17 min).

Previous VL mouse model efficacy studies for this 7-substituted 2-nitroimidazooxazine class have identified different preferences for 7H or 7-Me derivatives, depending upon the length of the side chain.[12,27] With PK effects being largely responsible, we elected to measure mouse PK data on both **65** and **66**. Compared to their ether-linked counterparts (**10**, **8**), both carbamate derivatives afforded slower clearance rates and much greater oral absorption, leading to improved exposure levels and oral bioavailability values of 100% (Table 3 and Supporting Information, Figures S2 and S3; no *in vitro* or *in vivo* metabolite identification studies were performed on any compounds).

Overall, 7-Me congener **66** delivered better oral exposure (Figure 2) and was advanced to efficacy testing. At 50 mg/kg,

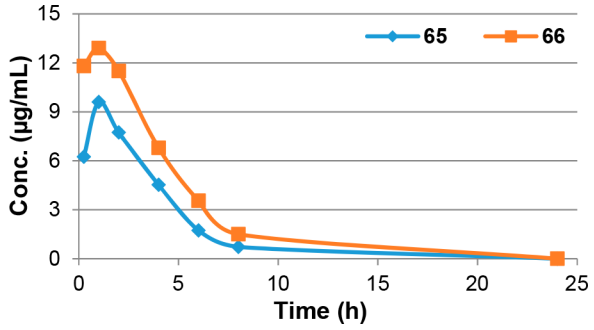

Figure 2. Plasma concentration−time profiles for **65** and **66**, dosed orally at 25 mg/kg in BALB/c mice.

66 gave essentially complete clearance of parasites (liver: >99.9%, spleen: 100%); it also showed strong efficacy at 25 mg/kg (liver: 97%, spleen: 96%). The ED_{50} (liver) was 16 mg/kg, ~4-fold lower than the value obtained for **8** but still very promising; hence, the enantiomers of **66** (**68**, **69**) were prepared and assessed. Compared to the reported enantiomers of **8** (**75** and **76** in Table S2),[27] these chiral carbamates were only marginally less effective *in vitro* (1.2- to 1.4-fold) with the 7*R* form (**68**) being slightly more potent than the 7*S* form (**69**) (*L. inf* IC_{50}s 0.14 and 0.20 μM, respectively). Moreover, **68** exhibited strikingly improved HLM stability (95% parent, cf. 68% for **66**, 78% for **69**, 58% for **5**,[12] and 63% for the 7*R* enantiomer of **8**[27]). Finally, **68** delivered significantly greater efficacy than **69** in the *L. don* mouse model (89% parasite burden reduction at 25 mg/kg for **68** vs 51% for **69**). Thus, the present investigation of novel linker analogues of **8** has identified 7*R* carbamate **68** as a preferred new lead for VL.

In addition to their antileishmanial effects, the new compounds of Table 1 also displayed low- or submicromolar activities against TB and Chagas disease (see the Supporting Information, Table S1). In both cases, amino-linked analogues were favored, but a broader range of linking groups was tolerated for TB. The best TB lead was 7*R* carbamate **68** (MIC_{90}s 0.085 and 1.0 μM under aerobic and hypoxic conditions, respectively). However, across all 7-Me linker derivatives, the association between VL potency (as pIC_{50}) and effectiveness against TB (as pMIC in the aerobic MABA assay) was only moderate (R^2 = 0.54; Figure 3), and neither activity correlated well with CLogP data (see the Supporting Information, Figure S4). These results were consistent with

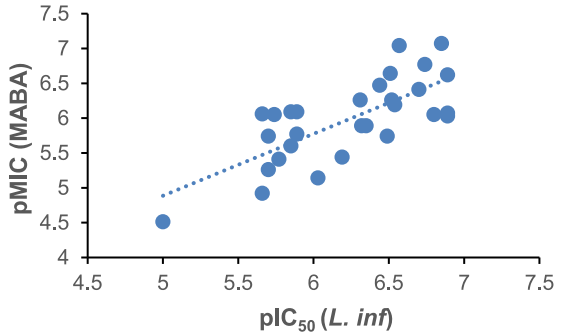

Figure 3. Similarity of SAR against VL and TB for 7-Me linker analogues.

the involvement of unrelated nitroreductases in the activation mechanisms of 2-nitroimidazooxazines against VL and TB.[13,29]

In summary, we prepared and evaluated 32 novel linker analogues of **8** with the objective of identifying efficacious backup leads to clinical candidate **5** that were more soluble. While amino, amide, urea and N-carbamate linkages all provided some solubility improvements (up to 54-fold over **8**), O-carbamate **66** offered the best overall balance of drug-like properties, displaying 100% oral bioavailability in mice and excellent *in vivo* efficacy in a VL mouse model. Appraisal of its enantiomers pinpointed 7*R* carbamate **68** as superior, having notably improved HLM stability and 14-fold better solubility than **5**.[12] This molecule was also a potent lead against TB, suggesting that further assessments are warranted. Overall, these results have illustrated the utility of linker replacement as a lipophilicity reduction strategy (to enhance aqueous solubility), facilitating the discovery of a potential new drug candidate from this very promising 7-substituted 2-nitroimidazooxazine class.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.0c00649.

Additional biological and pharmacokinetic data and methods, experimental section, combustion analytical data, and NMR spectra for key compounds (PDF)

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Notes

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■ ABBREVIATIONS

CL	cutaneous leishmaniasis;
VL	visceral leishmaniasis
L. don	*Leishmania donovani*
L. inf	*Leishmania infantum*
CTAB	cetyltrimethylammonium bromide
TEAB	triethylammonium bicarbonate
DPPA	diphenylphosphoryl azide
Boc-ON	2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile
DIPEA	*N*,*N*-diisopropylethylamine
MLM	mouse liver microsomes
HLM	human liver microsomes.

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