

Supplemental Material for: Evaluation of Arylimidamides DB1955 and DB1960 as Candidates against Visceral Leishmaniasis and Chagas Disease – In Vivo Efficacy, Acute Toxicity, Pharmacokinetics and Toxicology Studies

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Preparation and characterization of DB1955 and DB1960

2,5-Bis[2-cyclopentyloxy-4-(2-pyridylimino)aminophenyl]-furan methanesulfonate (DB 1955). The free base of DB1852 (1) (1.06 g, 1.7 mmol) was suspended in dry dichloromethane (40 mL) and cooled to 0 °C in an ice bath. Freshly distilled methanesulfonic acid (0.36 mL, 3.74 mmol) was added to the suspension and the mixture was stirred at room temperature for 0.5 h. The resulting red solution was concentrated under reduced pressure. The red crude solid was crystallized from dry dichloromethane and dry ether and filtered to give a red orange solid. Yield: 0.9 g (61%), mp > 300 °C dec. ¹H NMR (DMSO-*d*₆): δ 11.90 (s, 2H), 10.06 (s, 2H), 9.34 (s, 2H), 8.93 (s, 2H), 8.38 (d, *J* = 7.2 Hz, 2H), 8.27 (d, *J* = 7.2 Hz, 2H), 8.17 (d, *J* = 7.2 Hz, 2H), 7.88 (br s, 2H), 7.30 (s, 2H), 7.17-7.15 (m, 4H), 5.06 (s, 2H), 2.31 (s, 6H), 2.02-1.98 (m, 8H), 1.81 (br s, 2H), 1.69 (br s, 2H). ¹³C NMR (DMSO-*d*₆): δ 159.9, 154.6, 150.3, 148.5, 144.9, 138.8, 134.6, 129.0, 127.0, 124.4, 119.4, 118.1, 113.3, 111.5, 80.4, 38, 32.7, 24.1; ESI-MS: *m/z* calcd. for C₃₈H₃₈N₆O₃: 626.74; found: 627.20 (base M⁺ +1). Analysis calcd. for C₃₈H₃₈N₆O₃·2.0 CH₃SO₃H· 2.75 H₂O: C, 55.32; H, 5.98; N, 9.68; found: C, 55.08; H, 6.09; N, 9.52.

2,5-Bis[2-*i*-propyloxy-4-(2-pyridylimino)aminophenyl]-furan methanesulfonate (DB 1960).

To a suspension of the free base of DB766 (2) (4.85 g, 8.45 mmol) in EtOH (60 mL) was added methanesulfonic acid (1.64 g, 17.1 mmol) and the mixture was stirred briefly to yield a clear solution. The solution was then treated with a large volume of diethyl ether to precipitate the dimesylate salt as a yellow solid, which was filtered and dried in vacuum. Yield: 6.07 g (94%), mp 245-250 °C dec. ¹H NMR (DMSO-*d*₆): 11.70 (br s, 2H), 10.3 (br s, 2H), 9.32 (br s, 2H), 8.90 (m, 2H), 8.35 (d, *J* = 8.0 Hz, 2H), 8.25 (t, 2H), 8.14 (d, *J* = 8.3 Hz, 2H), 7.84-7.88 (m, 2H), 7.31 (d, *J* = 1.2 Hz, 2H), 7.19 (s, 2H), 7.14 (dd, *J* = 8.4, 1.5 Hz, 2H), 4.83 (m, 2H), 2.29 (s, 6H), 1.42

(d, 12H). ^{13}C NMR (DMSO- d_6): 159.0, 153.5, 149.3, 147.6, 144.0, 137.9, 133.7, 128.0, 126.0, 123.4, 118.7, 117.2, 112.6, 110.7, 70.0, 21.8, 21.4. ESI-MS: m/z calculated for $\text{C}_{34}\text{H}_{34}\text{N}_6\text{O}_3$: 574.67; found: 575.27 (base $\text{M}^+ + 1$). Analysis calcd for $\text{C}_{34}\text{H}_{34}\text{N}_6\text{O}_3 \cdot 2\text{CH}_3\text{SO}_3\text{H} \cdot 1.0 \text{H}_2\text{O}$: C, 55.09; H, 5.65; N, 10.71. Found: C, 55.39; H, 5.59; N, 10.54.

Table S1. Five-day repeat dose study design

Group	Treatment	Dose (mg/kg)	Dose conc. (mg/ml)	Volume (ml/kg)	No. of animals		
					Main necropsy (day 6)	Recovery necropsy (day 12)	Toxicokinetic satellite (day 1 and 5)
1	Vehicle	0	0	10	5	3	0
2	DB1955	100	10	10	5	3	15
3	DB1955	200	20	10	5	3	15
4	DB1955	500	50	10	5	3	15
5	DB1960	100	10	10	5	3	15
6	DB1960	200	20	10	5	3	15
7	DB1960	500	50	10	5	3	15

Table S2. Summary of body weight by treatment group (in grams)

Drug	Dose (mg/kg/day)	Study Day	Body weight		
			N	Mean	SD
Vehicle	0	1	8	18.44	0.498
		6	8	18.56	0.55
		12	3	19.17	0.473
DB1955	100	1	8	18.06	0.787
		6	8	17.63	0.349
		12	3	18.63	0.577
	200	1	8	18.93	0.972
		6	8	18.83	1.096
		12	3	19.07	1.25
	500	1	8	17.75	0.661
		6	8	17.99	0.948
		12	3	16.53*	1.155
DB1960	100	1	8	17.94	0.765
		6	8	17.86	0.682
		12	3	18.97	0.503
	200	1	8	17.85	1.768
		6	8	17.4	1.865
		12	3	20	1.48
	500	1	8	18.16	0.585
		6	8	14.65**	1.042
		12	3	NA ^a	NA

^a NA: value not available since animals in this group were euthanized in moribund condition earlier in the study.

* $p < 0.05$, compared with vehicle control; ** $p < 0.01$, compared with vehicle control

Table S3. Toxicokinetic parameters for DB1955 and DB1960 after oral administration in female BALB/c mice

Drug	Dose Level (mg/kg)	Study Day	C _{max} (µg/ml)	T _{max} (h)	AUC _{0 to 6 hr} (h·µg/ml)	AUC _{0 to 24 hr} (h·µg/ml)	t _{1/2} (h)
DB1955	100	1	2.03 ± 0.23	1	6.94 ± 0.64	NA ^a	NA
		5	1.24 ± 0.23	4	5.94 ± 1.15	NA	NA
	200	1	2.32 ± 0.31	1	9.05 ± 1.06	NA	NA
		5	2.67 ± 1.00	6	10.27 ± 1.30	NA	NA
	500	1	4.42 ± 1.10	1	17.92 ± 3.22	NA	NA
		5	4.41 ± 0.17	1	17.37 ± 1.71	36.81 ± 6.67	6.1
DB1960	100	1	2.94 ± 0.62	0.5	9.52 ± 0.58	NA	NA
		5	2.20 ± 0.23	0.5	8.69 ± 0.62	27.75 ± 1.51	18.4
	200	1	3.13 ± 0.09	1	14.23 ± 0.32	NA	NA
		5	2.95 ± 0.87	0.5	13.17 ± 1.57	52.08 ± 2.48	31.3
	500	1	4.36 ± 0.44	1	21.55 ± 1.77	NA	NA
		5	5.19 ± 1.24	1	27.09 ± 2.49	99.15 ± 9.31	29.5

^a NA: value not available because there were insufficient data points to calculate this parameter

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