

Bis-benzimidazole hits against *Naegleria fowleri* discovered with new high throughput screens

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RUNNING TITLE: Bis-benzimidazole analogs for *N. fowleri*

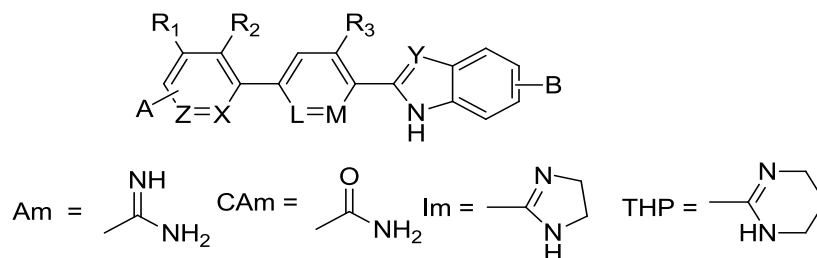
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Table S1. *In vitro* activity of Furamide derivatives against *Naegleria fowleri*.

Code	X	A	B	C	D	R ₁	R ₂	<i>N. f.</i> IC50 (μ M)
DB75		CH	CH	CH	CH	Am	Am	69.8
DB103		CH	CH	CH	CH	THP	THP	47.4
DB569		CH	CH	CH	CH	<i>N</i> -Ph-Am	<i>N</i> -Ph-Am	46.9
DB568		CH	CH	CH	CH	<i>N</i> -4-MeOPh-Am	<i>N</i> -4-MeOPh-Am	83.3
DB573		CH	CH	CH	CH	<i>N</i> -Me <i>N'</i> -4-MeOPh-Am	<i>N</i> -Me <i>N'</i> -4-MeOPh-Am	31.7
DB574		CH	CH	CH	CH	<i>N</i> -4-MePh-Am	<i>N</i> -4-MePh-Am	32.6
DB820		CH	N	CH	CH	Am	Am	84.2
DB829		CH	N	N	CH	Am	Am	>50
DB351		CH	CH	CH	CH	Am	Am	68.6
DB1213		CH	CH	CH	CH	Am	Am	74.9
DB1237		CH	N	N	CH	Am	Am	62.5
DB1404		CH	CH	CH	CH	Am	Am	55.7
DB1210		CH	CH	CH	CH	Am	Am	77.1
DB1044		CH	CH	CH	CH	Am	Am	45.8

Table S2. *In vitro* activity of Linear Benzimidazole Types against *Naegleria fowleri*.

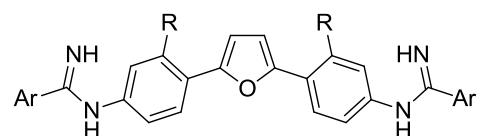


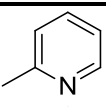
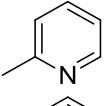
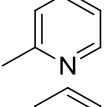
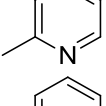
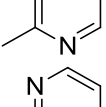
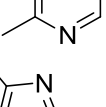
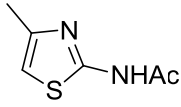
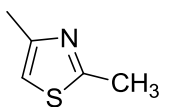
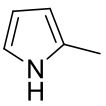
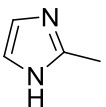
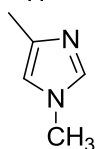
Code	X	Y	Z	L	M	R ₁	R ₂	R ₃	A	B	<i>N. f.</i> IC ₅₀ (μM)
DB921	CH	N	CH	CH	CH	H	H	H	<i>p</i> -Am	3-Am	58.7
DB1055	CH	N	CH	CH	CH	H	H	H	<i>m</i> -Am	3-Am	41.6
DB1963	CH	N	CH	CH	CH	H	H	H	<i>p</i> -CAm	3-Am	78.5
DB1964	CH	N	CH	CH	CH	H	H	H	<i>p</i> -CAm	3-Im	ND
DB988	CH	N	CH	CH	CH	H	Me	H	<i>p</i> -Am	3-Am	13.4
DB1236	CH	N	CH	CH	CH	H	H	F	<i>p</i> -Am	3-Am	59.4
DB1883	CH	CH	CH	CH	CH	H	H	H	<i>p</i> -Am	3-Am	61.8
DB1944	CH	CH	CH	CH	CH	H	H	H	H	3-Am	55.5
DB1803	CH	CH	CH	CH	CH	H	H	H	<i>p</i> -Im	3-Im	55.7
DB1804	CH	CH	CH	CH	CH	H	H	H	<i>p</i> -THP	3-THP	17.3
DB1798	CH	CH	CH	CH	CH	H	H	H	<i>p</i> -Am	4-Am	36.6
DB1780	CH	CH	CH	CH	CH	H	H	H	<i>p</i> -Im	4-Im	66.5
DB1781	CH	CH	CH	CH	CH	H	H	H	<i>p</i> -THP	4-THP	8.66

Table S3. *In vitro* activity of central indole ring diamidines against *Naegleria fowleri*.

Code	L	Ar	<i>N. f.</i> IC50 (μ M)
DB1884			43.2
DB1902			50.8
DB1885			>50
DB1887			10.3
DB1476			25.5
DB2285			>50

Table S4. *In vitro* activity of arylimidamide derivatives against *Naegleria fowleri*.



Code	R	Ar	<i>N. f.</i> IC ₅₀ (μM)
DB745	OEt		18.6
DB766 ^a	<i>O-i</i> -Pr		12.5
DB1960 ^b	<i>O-i</i> -Pr		22.7
DB1852 ^a	<i>O-c</i> -pentyl		25.7
DB1955 ^b	<i>O-c</i> -pentyl		37.9
DB1831	<i>O-i</i> -Pr		>50
DB1931	<i>O-i</i> -Pr		>50
DB1850	<i>O-i</i> -Pr		47.5
DB1858	<i>O-i</i> -Pr		>50
DB1857	<i>O-i</i> -Pr		57.6
DB1848	<i>O-i</i> -Pr		50.6

a) Hydrochloride salt; b) mesylate salt

Table S5. *In vitro* activity of additional arylimidamide derivatives against *Naegleria fowleri*.

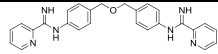
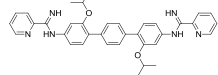
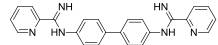
Code	Structure	<i>N. f.</i> IC ₅₀ (μM)
DB1835		>50
DB1969		>50
DB1832		>50

Figure S1. Optimization of alamarBlue and CTG assays for *Naegleria fowleri* drug discovery. A,B) First the optimal seeding density of amoebae/well was determined in 96 and 384 well plates. C,D) The alamarBlue colorimetric readout was robust and reproducible. E,F) There was a strong correlation between luminescence (RLUs) in CTG assay with number of amoebae/well.

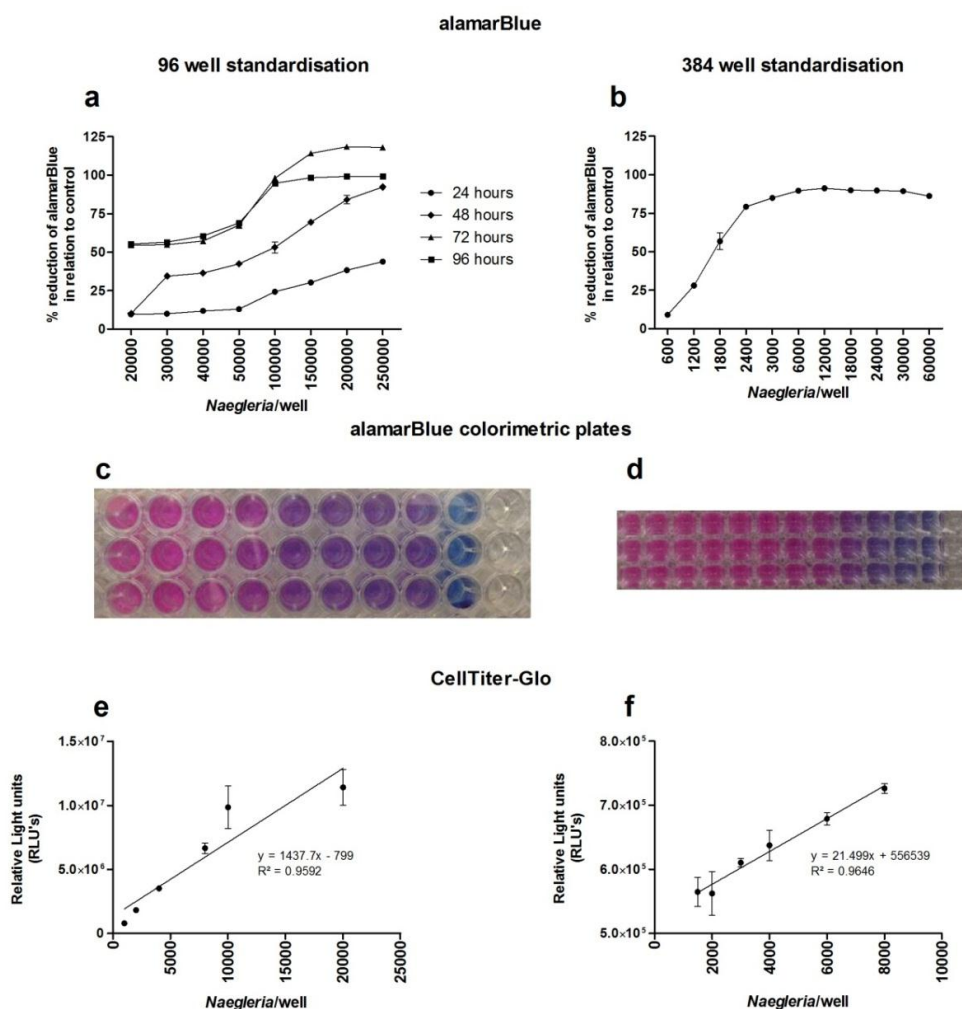


Table S6. Comparative potency and cytotoxicity of amidino derivatives against *Naegleria fowleri*, bacteria, fungi, and Rat myoblast *in vitro*.

Compounds*	Current Study			Bacteria (MIC, µg/ml)			Fungi (IC50, µg/ml)		Parasites (IC50, µg/ml)		Rat Myoblast	Ref.	
	<i>N. f.</i> IC50 (µM)*	J774 IC50 (µM)*	SI	<i>Staphylococcus aureus</i> ATCC 29213	<i>Staphylococcus pneumoniae</i> ATCC 6301	<i>E. faecalis</i> ATCC 51575	<i>Candida albicans</i>	<i>Cryptococcus neoformans</i>	<i>Trypanosoma brucei</i>	<i>Plasmodium falciparum</i>	L6 IC50 (µg/ml)		
Hits	DB173	0.177	7.89	44.6								46	
	DB183	0.458	6.47	14.2								38,46	
	DB210	0.795	64.9	81.6								38,46	
	DB1766	0.43	6.92	16.1	1	0.12	0.25	3.12	1.56	0.017	0.013	4.2	25
Marginal Hits	HG17	0.318	2.91	9.2				25		0.06	0.008	>90	38
	HG24	0.462	2.54	9.15				3.12		0.036	0.015	29.4	38
	DB1684	1.04	9.58	9.2	1	0.12	.5						25
	DB1734	0.385	3.22	8.4	0.25	≤0.06	0.25						23
	DB1736	1.035	10.2	9.8	0.25	≤0.06	0.12						25
Compounds not active against <i>Nf</i>	DB1754	10.2	15.5	1.5	0.25	0.12	0.5	4					23
	DB1767	7.06	37.1	5.3	0.5	0.25	0.5	16					23
	DB1768	9.44	24.2	2.6	0.25	0.12	0.5	4					23
	HK11	17.2	31.8	1.84				25		0.33	0.015	29.8	38
	HK12	41.3	23.9	0.58				1.56		1.06	0.031	13	38
	HK16	>88.2	>88.2	~1				50		0.59	0.017	25.5	38
	HK23	>80.0	28.4	~0.35				25		0.708	0.028	46	38

* Hits are defined as compounds having an IC50 < 1 µM for *Naegleria fowleri* (*N.f.*) and a selectivity index (SI) ≥ 10; Marginal hits are defined as having an IC50 ~1 for *N.f.* and a SI > 8 and < 10; Inactive compounds have an IC50 > 1 µM for *N.f.*