Supporting Information

Discovery of novel *Trypanosoma brucei* phosphodiesterase B1 inhibitors by virtual screening against the unliganded TbrPDEB1 crystal structure

Chimed Jansen,[1,‡] Huanchen Wang,[2,‡] Albert J. Kooistra,[1] Chris de Graaf,[1] Kristina Orrling,[1] Hermann Tenor,[3] Thomas Seebeck,[4] David Bailey,[5] Iwan J.P. de Esch,[1] Hengming Ke [2,] and Rob Leurs[1,*]*

[1]Division of Medicinal Chemistry, Faculty of Sciences, Amsterdam Institute of Molecules, Medicines and Systems (AIMMS), VU University Amsterdam, The Netherlands.

[2]Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC, USA.

[3]Department of Biochemistry, Nycomed GmbH, Konstanz, Germany.

[4]Institute of Cell Biology, University of Bern, Baltzerstrasse 4, 3012 Bern, Switzerland.

[5]IOTA Pharmaceuticals, St. John's Innovation Centre, Cowley Road, Cambridge CB4 0WS, United Kingdom.

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Supplementary Tables and Figures

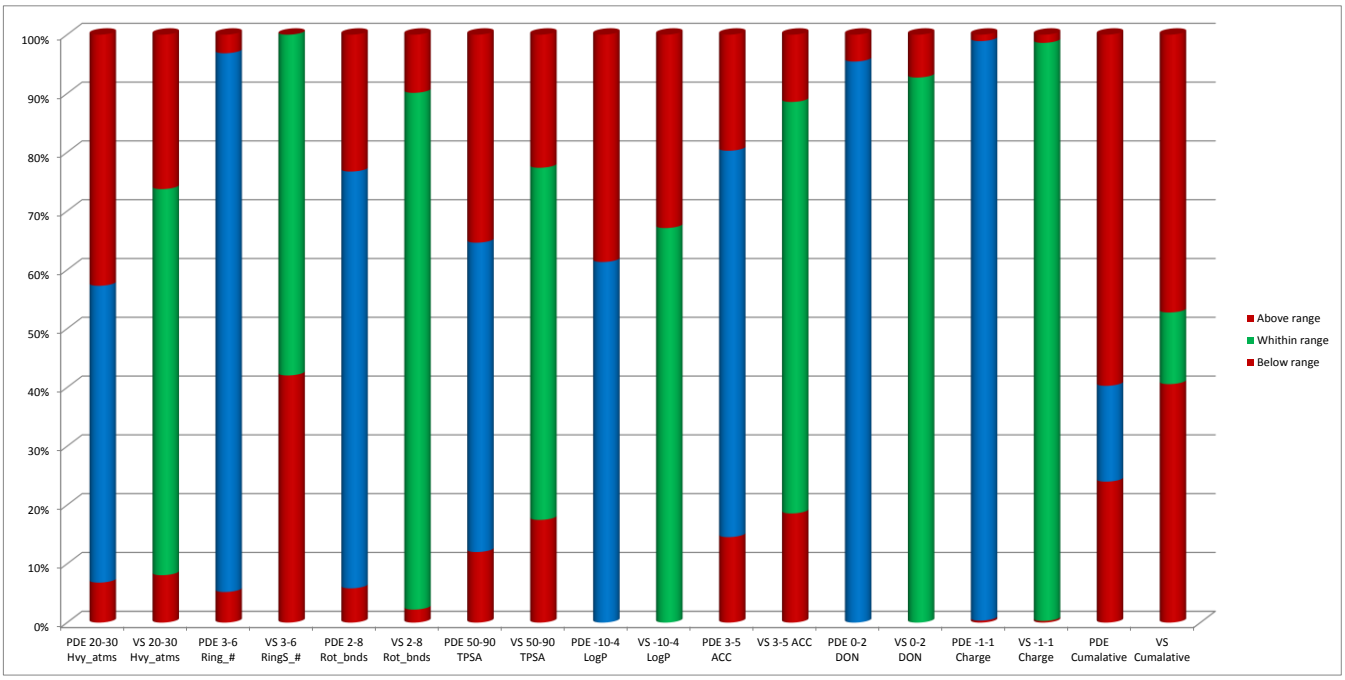
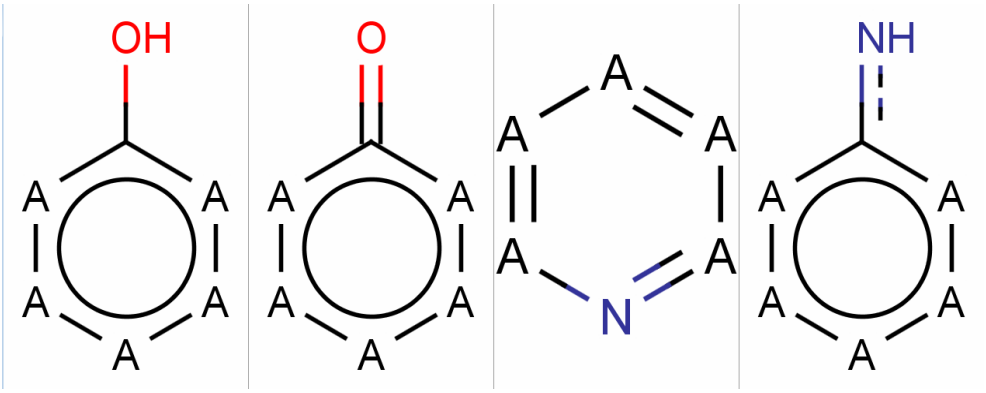


Figure S1. The physical filters as applied to the set of known PDE inhibitors used to select filter ranges for the virtual screening study are shown as blue bars (passed filter). The unique ZINC compounds from select suppliers are shown as green bars (passed filter). The red bars indicate compounds which fell above or below the filter range. The final two bars show the cumulative effect of all filters on the original databases.



A B C D

Figure S2. Substructure filter applied to the compound library where A is any atomtype. Ligands were required to contain at least one of the four systems, A, B, C or D. Note that the software does not distinguish aromatic rings from conjugated ring systems.

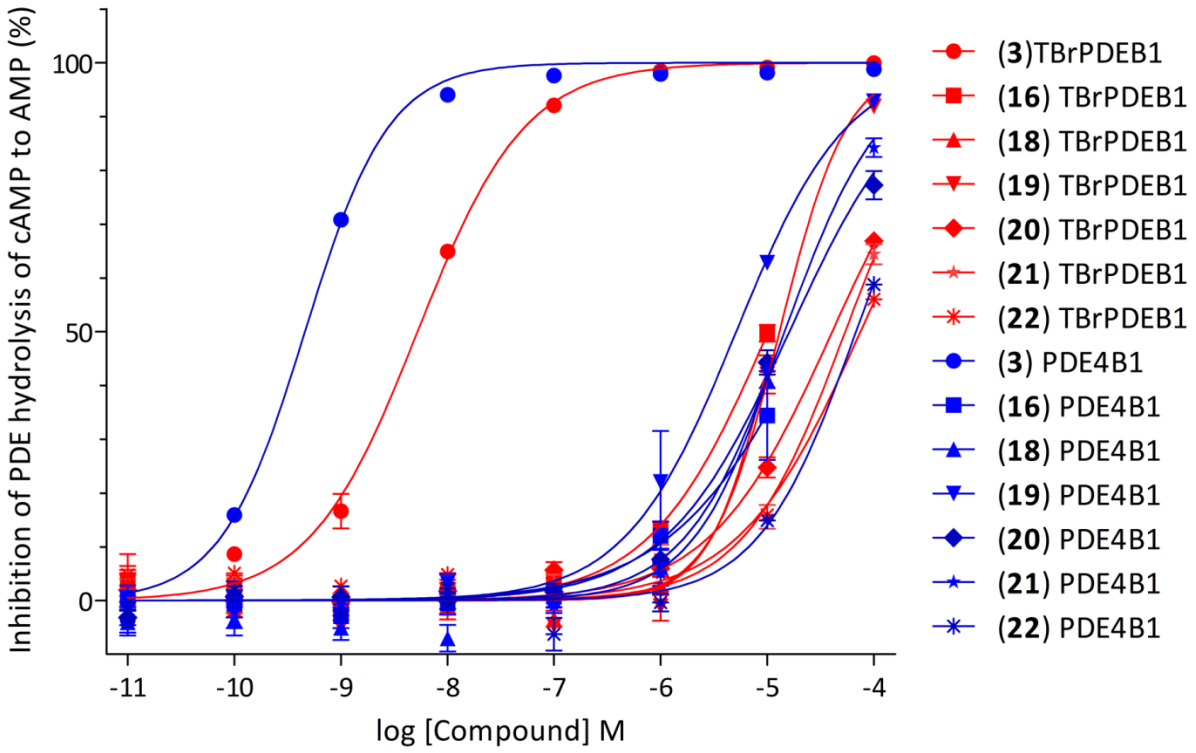
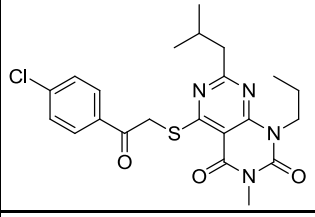
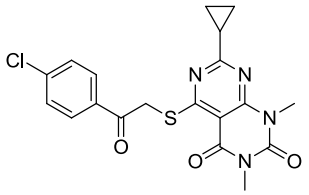
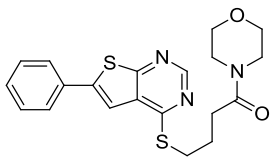
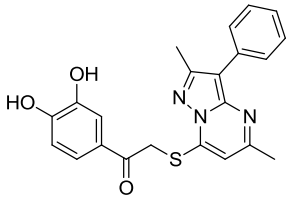
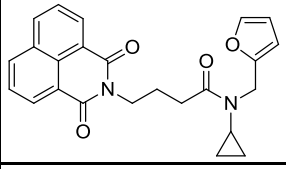
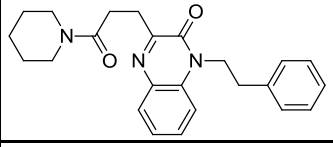
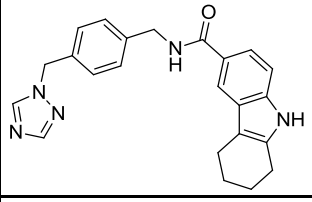
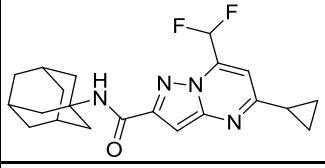
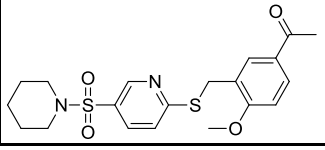
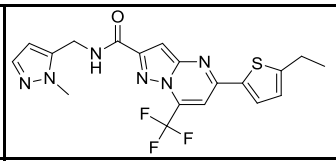
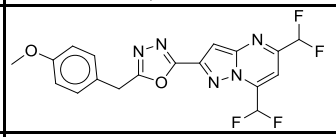
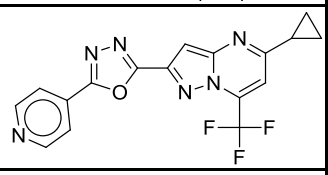
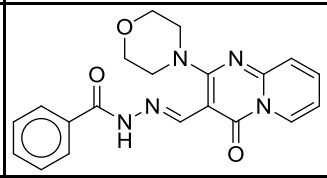
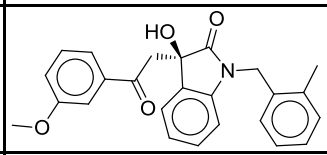
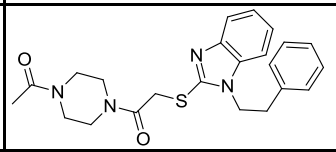
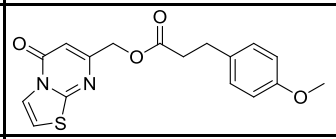
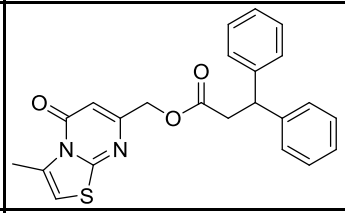
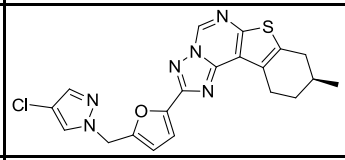
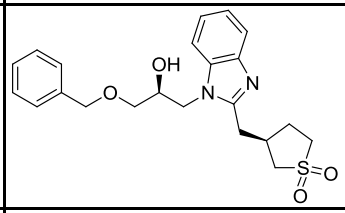
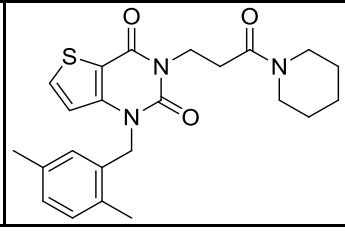
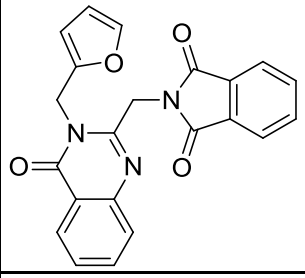
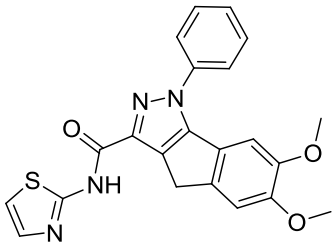
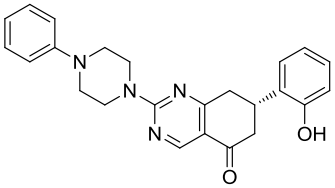
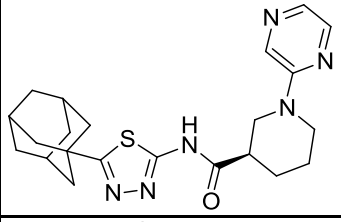
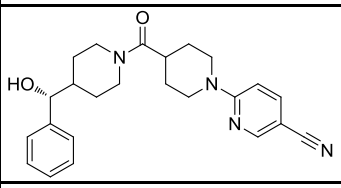
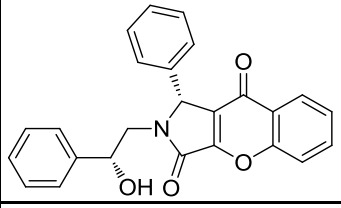
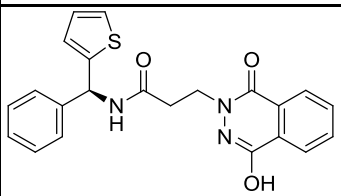
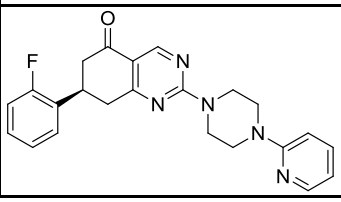


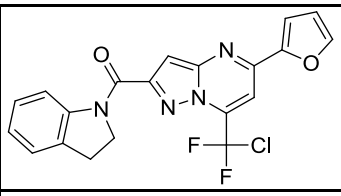
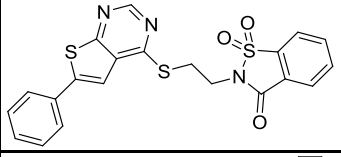
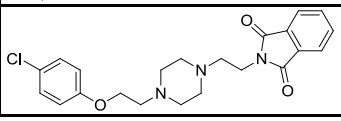
Figure S3. Inhibition of the hydrolysis of [^{3}H]-5'-cAMP to [3H]-5'-AMP by TbrPDEB1 or hPDE4 in the presence of an inhibitor, as measured using SPA yttrium silicate beads in the presence of zinc sulfate. Inhibition data are provided for novel TbrPDEB1 inhibitors **16-22** and compound **3**, used as a reference in this assay. Not all compounds were soluble at concentrations above 10 μM. Short error bars are concealed by the symbols in the graph. For inhibitors, which did not reach 100% inhibition at the highest concentration measured, inhibition curves were fitted to derive IC_{50} values with the assumption that the inhibition curves plateau at 100% inhibition, since the inhibitors are expected to bind competitively to the PDE substrate binding pocket.

Table S1. The activity of purchased compounds on TbrPDEB1 and hPDE4. Displayed with the virtual screening scores of the binding mode used to select each compound for purchase. The similarity of the structures to known TbrPDEB1 inhibitors is provided.

cpd	Purchased Compounds	TbrPDEB1 IC_{50} in µM	IFP1 (rank)	IFP2 (rank)	Plants (rank)	ROCS (closest inhibitor)	ECFP4 (closest inhibitor)	hPDE4B IC_{50} in µM
16		**10**	0.80	0.63	-102.8	1.11 (**15**)	0.18 (**6** - NEU222)	26
17			**0.79** (33)	0.61	-94.7	1.21 (**15**)	0.18 (**6**)	
18		**12**	**0.75** (44)	0.70	-93.8	1.07 (**15**)	0.20 (**2**/**4** - 8d)	14
19		**13**	0.53	**0.75** (14)	-101.3	1.02 (**12**)	0.21 (**5**)	4.9
20		**40**	0.65	**0.75** (6)	-103.6	0.95 (**15**)	0.16 (**2**/**4** - 16b)	15
21		**60**	**0.75** (24)	0.63	-95.3	1.10 (**6** - NEU227)	0.24 (**2**/**4** - 8d)	15
22		**78**	0.45	0.55	**-109.1** (9)	0.92 (**2**/**4** - 13b)	0.21 (**6** - NEU432)	71
23		>100	0.75 (66)	0.70	-92.9	1.23 (**6** – NEU432)	0.16 (**6**)	4
24		>100	**0.75** (28)	0.67	-94.9	1.03 (**14**)	0.30 (**6** – NEU222)	6

25		>100	0.65	**0.75** (22)	-100.6	1.10 (**6** – NEU227)	0.22 (**14**)	10
26		>100	0.69	0.78 (75)	-97.7	1.03 (**2**/**4** – 15a)	0.22 (**2**/**4** – 15a)	28
27		>100	0.65	0.75 (508)	-91.0	1.08 (**6** – NEU432)	0.14 (**10**)	34
28		>100	0.71	0.52	-97.2	1.00 (**14** – 26a)	0.15 (**9**)	50
29[a]		>100	0.5	0.50	-107.7[b]	1.09 (**6** – NEU227)	0.25 (**5**)	56
30		>100	0.59	0.60	-100.6	1.03 (**6** – NEU227)	0.22 (**2**/**4** – 8d)	74
31		>100				1.17 (**2**/**4** – 14a)	0.25 (**2**/**4** – 15a)	94
32			0.63	0.42	**-106.6** (39)	1.01 (**2**/**4** - 8b)	0.20 (**6** – NEU227)	
33[a]		>100	**0.75** (16)	0.67	-96.8	1.17 (**2**/**4** - 6b)	0.17 (**3**)	>100
34[a]		>100	**0.77** (27)	0.48	-95.1	1.00 (**2**/**4** - 8b)	0.22 (**2**/**4** – 8d)	>100
35		>100	**0.86** (36)	0.54	-94.4	1.07 (**6** – NEU227)	0.18 (**2**/**4** – 18b)	>100

36		>100	**0.77** (39)	0.42	-94.1	1.01 (**6**)	0.17 (**14** - 7)	>100
37		>100	**0.80** (48)	0.58	-93.5	1.10 (**10**)	0.27 (**6** – NEU227)	>100
38[a]		>100	0.63	**0.76** (2)	-104.9	1.09 (**2**/**4** – 8d)	0.20 (**15**)	>100
39[a]		>100	0.43	0.52	**-108.8** (10)	1.04 (**2**/**4** – 8d)	0.20 (**6**)	>100
40[a]		>100	0.39	0.52	**-107.1** (24)	0.97 (**2**/**4** - 13b)	0.21 (**2**/**4** – 13b)	>100
41[a]		>100	0.39	0.42	**-106.9** (31)	0.95 (**6**)	0.16 (**2**/**4** – 8d)	>100
42[a]		>100	0.53	0.58	**-106.4** (45)	0.92 (**6**)	0.17 (**2**/**4** – 8c)	>100
43[a]		>100	0.50	0.61	**-107.6** (20)	1.10 (**2**/**4** – 8d)	0.19 (**15**)	>100

44		>100	0.67	0.75[b]	-97.2	1.09 (**6** – NEU227)	0.14 (**11**)	>100
45		>100	0.75 (82)	0.58	-92.1	1.10 (**15**)	0.20 (**2/4** – 8b)	>100
46		>100	0.56	0.50	**-106.5** (42)	0.97 (**6** – NEU227)	0.23 (**2/4** – 20b)	>100

[a]All compounds were purchased as racemates and diastereomeric mixtures where chirality was present in the structures.

[b]These compounds were filtered out during the filtering of the Diverse Top 50 sets from the selection routes B and C, they have been included in route D.

Table S2. Purity data and mass identification of purchased compounds.

cpd	ZINC #	Supplier	Supplier code	Purity (% by LC-MS)[a]	Recorded M/Z	Exact mass
16	(Analogue of 17) ZINC14077623	Enamine	T5795068	96	460.95	460.13
17	(VS Hit) ZINC04139100					
18	ZINC09336923	Enamine	T5692782	98	399.90	399.11
19	ZINC05532912	Enamine	T6353379	99	405.95	405.11
20	ZINC12910984	Enamine	T6108077	99[+]	403.00	402.16
21	ZINC08704885	Enamine	T5794109	99	390.05	389.21
22	ZINC40024665	Enamine	T6555037	97	386.00	385.19
23	ZINC06828559	Vitas-M	STK303337	99	387.00	386.19
24	ZINC08194149	Enamine	T5604160	95	420.95	420.12
25	ZINC02794126	Vitas-M	STK307638	95	434.95	434.12
26	ZINC02803730	Vitas-M	STK309957	99	407.90	407.10
27	ZINC02803366	Vitas-M	STK309884	99	372.85	372.09
28	ZINC04070223	Vitas-M	STK821967	95	377.95	377.15
29	ZINC04784334	Vitas-M	STK214384	98	402.00	401.16
30	ZINC05264706	Enamine	T5332365	93[b]	423.00	422.18
31	(Analogue of 32) ZINC07201382	Enamine	T6367699	95	344.90	344.08
32	(VS Hit) ZINC11398873					
33	ZINC02789699	Vitas-M	STK306257	96	424.95	424.09
34	ZINC32907350	Enamine	T6378025	95	415.00	414.16
35	ZINC06756443	Vitas-M	STK605276	99[+]	425.95	425.18
36	ZINC00978529	Vitas-M	STK860151	99	386.00	385.10
37	ZINC20735167	Vitas-M	STK644500	98	418.95	418.11
38	ZINC05274263	Vitas-M	STK871199	98	401.05	400.19
39	ZINC40071327	Enamine	T6550918	95	425.05	424.20
40	ZINC48309056	Enamine	T6644883	97	405.05	404.22
41	ZINC02343899	Vitas-M	STK852257	99	397.95	397.13
42	ZINC12270605	Enamine	T6031050	97	405.90	405.11
43	ZINC18223039	Vitas-M	STK235219	98	404.00	403.18
44	ZINC02503785	Vitas-M	STK768883	98	414.95	414.07
45	ZINC09634941	Enamine	T5481576	96	453.90	453.03
46	ZINC32679558	Enamine	T6273443	99	413.95	413.15

[a] Analytical HPLC-MS analyses were conducted using a Shimadzu LC-20AD liquid chromatograph pump system with a Shimadzu SPD-M20A diode array detector. MS detection was performed with a Shimadzu LCMS-2010 EV liquid chromatograph mass spectrometer.

The analyses were performed using the following conditions; Xbridge (C18) 5 μm column (50 mm × 4.6 mm) with solvent A (acetonitrile with 0.1% formic acid) and B (water with 0.1% formic acid), flow rate of 1.0 mL/min, start 5% A, linear gradient to 90% A in 4.5 min, then 1.5 min at 90% A, then a linear gradient to 5% A in 0.5 min, then 1.5 min at 5% A, total run time of 8.0 min. Compound purities were calculated as the percentage peak area of the analyzed compound by UV detection at 230 nm.

[b] This compound was reported to be 95% pure according to the supplier Enamine.

Table S3. Structure databases downloaded from ZINC.

Companies
Acros
Apollo
Asinex
Chembridge
Chembridge BB (building blocks)
Enamine[a]
Enamine BB
Fluorochem
IBScreen
IBScreen BB
IBScreen drugs
Labotest
Labotest BB
Matrix
Maybridge
Maybridge BB
Sigma-Aldrich
Specs
Specs BB
Timtec
Timtec BB
VitasM[a]
VitasM BB

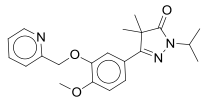
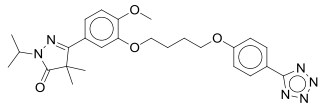
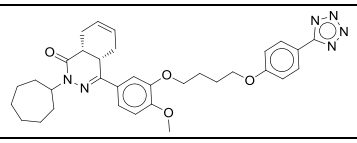
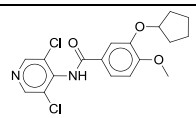
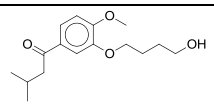
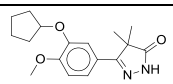
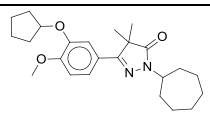
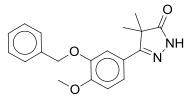
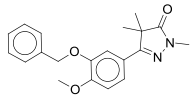
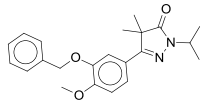
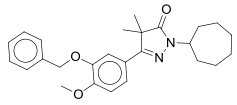
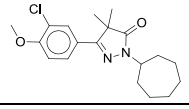
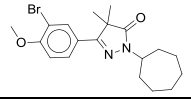
[a] Compounds were eventually purchased from just Enamine and VitasM due to the number of compounds in the final selection sets available from these companies.

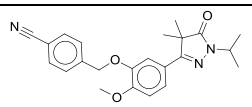
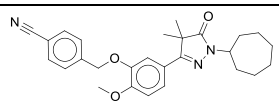
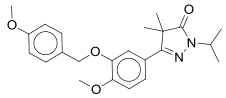
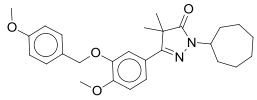
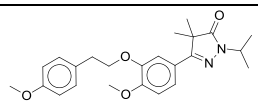
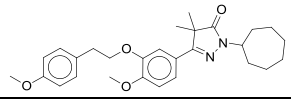
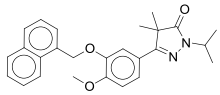
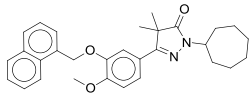
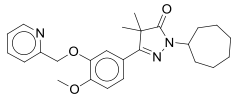
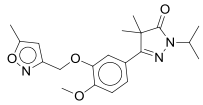
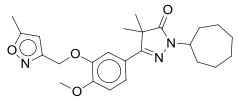
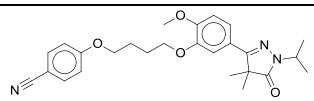
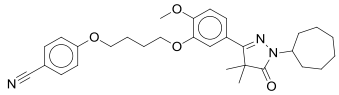
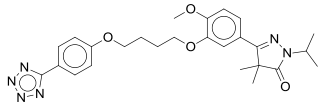
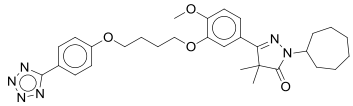
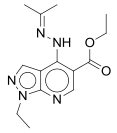
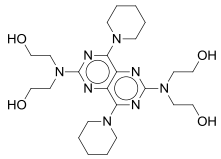
Table S4. The top 50 diverse compounds selected using routes A-D ranked by PLANTS scores.

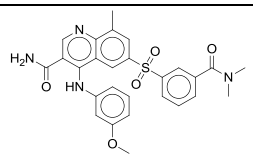
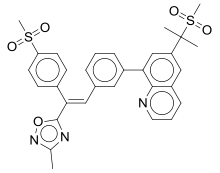
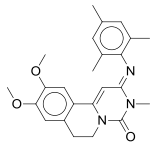
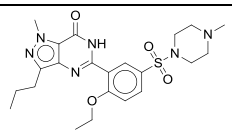
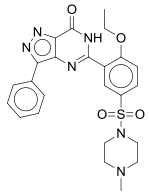
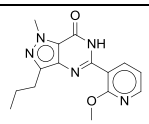
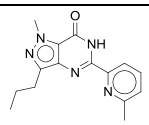
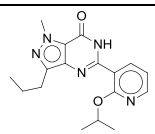
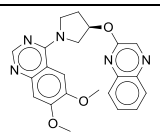
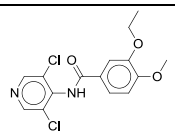
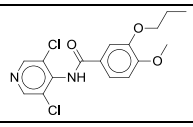
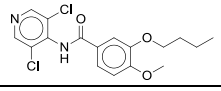
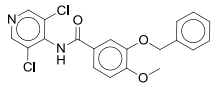
PLANTS score rank	IFP1 Diverse Top 50	IFP2 Diverse Top 50	PLANTS Diverse Top 50	Visual Top 50
1	ZINC04859189	ZINC44427189	ZINC35702500	ZINC10256687
2	ZINC07341961	ZINC05274263 (**38**)	ZINC10654376	ZINC04784334 (**29**)
3	ZINC40049185	ZINC20589454	ZINC10256761	ZINC44426989
4	ZINC02302046	ZINC07985044	ZINC32854262	ZINC34756294
5	ZINC05369829	ZINC18223137	ZINC04940098	ZINC35435996
6	ZINC07007212	ZINC12910984 (**20**)	ZINC26404382	ZINC05264706 (**30**)
7	ZINC19286607	ZINC11969980	ZINC36059048	ZINC11953787
8	ZINC32754938	ZINC20589325	ZINC44427179	ZINC13082922
9	ZINC00825537	ZINC12050083	ZINC40024665 (**22**)	ZINC33451317
10	ZINC05626395	ZINC55099474	ZINC40071327 (**39**)	ZINC12975890
11	ZINC24055184	ZINC20589507	ZINC14883991	ZINC09410355
12	ZINC35919898	ZINC04816090	ZINC19136671	ZINC13626803
13	ZINC18194984	ZINC38530038	ZINC34738807	ZINC09634941 (**45**)
14	ZINC00969099	ZINC05532912 (**19**)	ZINC11937966	ZINC06710214
15	ZINC04420715	ZINC23316779	ZINC38531189	ZINC06828559 (**23**)
16	ZINC02789699 (**33**)	ZINC14733669	ZINC20599963	ZINC02803730 (**26**)
17	ZINC25150965	ZINC12373726	ZINC44920958	ZINC00931312
18	ZINC12145740	ZINC12436366	ZINC05354996	ZINC12606885
19	ZINC55344232	ZINC19459711	ZINC11874105	ZINC02503785 (**44**)
20	ZINC32754134	ZINC12148703	ZINC18223039 (**43**)	ZINC04070223 (**28**)
21	ZINC09409634	ZINC12145452	ZINC36062296	ZINC12442274
22	ZINC31807311	ZINC02794126 (**25**)	ZINC25812935	ZINC58186226
23	ZINC19574501	ZINC35769460	ZINC19520544	ZINC13082899
24	ZINC08704885 (**21**)	ZINC15070214	ZINC48309056 (**40**)	ZINC07201382
25	ZINC20602439	ZINC37396949	ZINC11937720	ZINC07625095
26	ZINC20455928	ZINC05274303	ZINC35702460	ZINC01140858
27	ZINC32907350 (**34**)	ZINC07105645	ZINC23382374	ZINC35895715
28	ZINC08194149 (**24**)	ZINC44200937	ZINC14979951	ZINC17207060
29	ZINC10913577	ZINC09135087	ZINC04250137	ZINC11973006
30	ZINC35958590	ZINC02326460	ZINC44428673	ZINC22322128
31	ZINC00918167	ZINC09699404	ZINC02343899 (**41**)	ZINC24427178
32	ZINC08395277	ZINC12206356	ZINC36740043	ZINC08015074
33	ZINC04139100 (**17**)	ZINC06834043	ZINC38530049	ZINC19626952
34	ZINC16524521	ZINC11753735	ZINC18223137	ZINC11665980
35	ZINC19839878	ZINC58225567	ZINC34739521	ZINC44440630
36	ZINC06756443 (**35**)	ZINC36621353	ZINC32908432	ZINC19524964
37	ZINC32815479	ZINC35819779	ZINC04924922	ZINC04859238
38	ZINC12192368	ZINC12116467	ZINC40135218	ZINC04898188
39	ZINC00978529 (**36**)	ZINC12449018	ZINC11398873 (**31**)	ZINC04859129
40	ZINC04162297	ZINC14953639	ZINC14749896	ZINC09517533
41	ZINC12508767	ZINC35702601	ZINC09731579	ZINC34711584
42	ZINC04272446	ZINC24082910	ZINC32679558 (**46**)	ZINC13284516
43	ZINC09522374	ZINC23427858	ZINC35556172	ZINC04375111

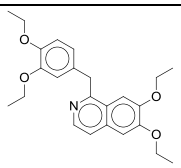
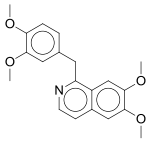
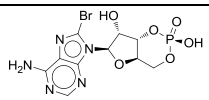
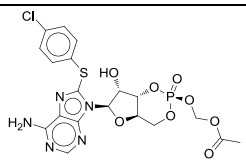
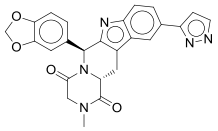
44	ZINC09336923 (**18**)	ZINC20591464	ZINC24754510	ZINC05215818
45	ZINC32930758	ZINC12653560	ZINC12270605 (**42**)	ZINC35842211
46	ZINC20920532	ZINC20602438	ZINC12185356	ZINC12233593
47	ZINC00674064	ZINC47013587	ZINC24479982	ZINC34915770
48	ZINC20735167 (**37**)	ZINC20602481	ZINC36058970	ZINC02803366 (**27**)
49	ZINC02793272	ZINC15597290	ZINC49586919	ZINC01350577
50	ZINC12652768	ZINC38530026	ZINC08664904	ZINC52686128

Table S5. Published TbrPDEB1 inhibitors. Only inhibitors with IC_{50} values under 100μM were included, single point measurements were accepted where inhibition was over 50% at 10μM or at 100μM. Compound structures are shown with their number, name as published, IC_{50} value in μM or percent inhibition for TbrPDEB1 and the human PDE for which they are most potent according to literature, and PLANTS docking scores.

Compound	Name in source	Structure	**TbrPDEB1 IC_{50} in μM**	**Human PDE IC_{50} in μM**	**TbrPDEB1 PLANTS**
1	VUF11851		2.5[1]		-85
2	VUF13525		0.4[1]		-91
3	PPS54019		0.004[2]	0.0006[2, b]	-104
4	piclamilast		4.7[3]	0.001[4, b]	-69
5	1-(3-(4-hydroxybutoxy)-4-methoxyphenyl)-3-methylbutan-1-one		4.6[5]		-75
48	6a		12[1]		-73
49	6b		0.41[1]		-84
50	8a		6.3[1]		-77
51	8b		10[1]		-86
52	8c		1.0[1]		-87
53	8d		0.5[1]		-91
54	9c		2.0[1]		-75
55	10c		2.5[1]		-76

56	13a		2.0[1]		-88
57	13b		1.0[1]		-89
58	14a		2.0[1]		-87
59	14b		0.79[1]		-95
60	15a		0.83[1]		-91
61	15b		0.63[1]		-98
62	16a		0.79[1]		-89
63	16b		0.25[1]		-99
64	17b		0.50[1]		-92
65	18a		5.0[1]		-84
66	18b		0.63[1]		-90
67	19b		5.0[1]		-91
68	19b		0.16[1]		-103
69	19a		0.40[1]		-91
70	20b		0.049[1]	0.0012[1, b]	-95
71	etazolate		31[6]	1.1[7, b]	-71
72	dipyridamole		15[6]	0.4[8, d]	-88

73	GSK256066		53% inh/10 μM[3]	0.000003[9, b]	-77
74	L-454560		8.8[3]	0.0005[10, b]	-70
75	trequinsin		13[6]	0.4[11, b]	-75
76	sildenafil		42[6]	0.01[12, c]	-95
77	7		71% inh/100 μM[13]		-87
78	26c		77% inh/100 μM[13]		-75
79	24a		72% inh/100 μM[13]		-74
80	26a		54% inh/100 μM[13]		-76
81	PFE-PDE10-2		55% inh/100 μM[3]	0.004[14, e]	-81
82	NEU432		17[3]		-63
83	NEU222		14[3]		-65
84	NEU230		8[3]		-71
85	NEU227		13[3]		-75

86	ethaverine		27[6]	0.4[15, b]	-89
87	papaverine		30[16]	1.1[15, b]	-88
88	8-Br-cAMP		37[17]	14[18, a]	-82
89	8-CPT-cAMP		1.2[17]	0.9[18, c]	-94
90	tadalafil		72% inh/10 μM[19]	0.001[20, c]	-103

[a] hPDE3 IC_{50} value, [b] hPDE4 IC_{50} value, [c] hPDE5 IC_{50} value, [d] hPDE6 IC_{50} value, [e] hPDE10 IC_{50} value

Table S6. Protein-ligand interactions of the docking poses of the reference compounds and those used to select novel inhibitors. The IFPs are encoded as seven bits per residue as follows: 1. Apolar; 2. Aromatic face-to-face; 3. Aromatic edge-to-face; 4. H-bond donor (protein) – H-bond acceptor (ligand); 5. H-bond donor (ligand) – H-bond acceptor (protein); 6. Ionic interaction positive (protein) – negative (ligand); 7. Ionic interaction positive (ligand) – negative (protein).

Compd	TYR668	HIS669	HIS673	HIS709	ASP710	HIS713	LEU716
1	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
2	**1 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
16	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
17	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
18	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
19	**1 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
20	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
21	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
22	**1 0 1 0 0 0 0**	1 0 0 0 0 0 0	**1 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
23	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
24	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
25	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
26	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
27	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
28	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
29	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
30	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**
32	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**

Compd	ASN717	ASN718	SER719	THR783	MET785	ALA786	GLY789
1	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	1 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
2	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	1 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
16	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	1 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
17	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
18	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	1 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
19	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
20	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
21	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	1 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
22	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
23	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	1 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
24	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	1 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
25	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	1 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
26	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
27	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	1 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
28	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
29	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
30	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0
32	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0	**0 0 0 0 0 0 0**	0 0 0 0 0 0 0

Compd	ASP822	ILE823	ASN825	VAL826	SER833	TRP836	ALA837
1	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
2	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
16	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
17	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
18	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
19	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0
20	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
21	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
22	1 0 0 0 1 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
23	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0
24	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
25	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0
26	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
27	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0
28	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
29	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
30	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
32	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0

Compd	VAL840	THR841	GLU843	PHE844	TYR845	LEU859	PRO860
1	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
2	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 1 1 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
16	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 1 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
17	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
18	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
19	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 1 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
20	1 0 0 0 0 0 0	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 1 1 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
21	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 1 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
22	1 0 0 0 0 0 0	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 0 1 0 0 0 0	1 0 1 1 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
23	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
24	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 1 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
25	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 1 0 0 0 0	1 0 1 1 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
26	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 1 1 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
27	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 1 0 0 0 0	1 0 1 1 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
28	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
29	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
30	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 1 0 0 0 0	1 0 0 1 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
32	1 0 0 0 0 0 0	0 0 0 1 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0

Compd	MET861	PHE862	ASN867	MET868	GLU869	LEU870	GLY873
1	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
2	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
16	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
17	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
18	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
19	1 0 0 0 1 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
20	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
21	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
22	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0
23	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
24	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
25	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
26	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
27	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0
28	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
29	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 1 0 0
30	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0
32	1 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0	1 0 0 0 0 0 0	0 0 0 0 0 0 0	1 0 0 0 0 0 0

Compd	GLN874	GLY876	PHE877	ILE878	PHE880	VAL881	ALA882
1	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
2	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
16	1 0 0 1 0 0 0	0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
17	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
18	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
19	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
20	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
21	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
22	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
23	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
24	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
25	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
26	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
27	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
28	0 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
29	0 0 0 1 0 0 0	0 0 0 0 0 0 0	1 0 1 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
30	1 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0
32	0 0 0 1 0 0 0	0 0 0 0 0 0 0	1 1 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0

References

(1) Orrling, K. M.; Jansen, C.; Vu, X. L.; Balmer, V.; Bregy, P.; Shanmugham, A.; England, P.; Cos, P.; Maes, L.; Adams, E.; van de Bogaart, E.; Chatelain, E.; Ioset, J.-R.; Stolpe, A.; Zorg, S.; Veerman, J.; Seebeck, T.; Sterk, G. J.; de Esch, I. J. P.; Leurs, R., Catechol Pyrazolinones as Trypanocidals: Fragment-Based Design, Synthesis and Pharmacological Evaluation of Nanomolar Inhibitors of Trypanosomal Phosphodiesterase B1. *J. Med. Chem.* **2012**.

(2) de Koning, H. P.; Gould, M. K.; Sterk, G. J.; Tenor, H.; Kunz, S.; Luginbuehl, E.; Seebeck, T., Pharmacological Validation of *Trypanosoma brucei* Phosphodiesterases as Novel Drug Targets. *J. Infect. Dis.* **2012,** *206*, 229-237.

(3) Bland, N. D.; Wang, C.; Tallman, C.; Gustafson, A. E.; Wang, Z.; Ashton, T. D.; Ochiana, S. O.; McAllister, G.; Cotter, K.; Fang, A. P.; Gechijian, L.; Garceau, N.; Gangurde, R.; Ortenberg, R.; Ondrechen, M. J.; Campbell, R. K.; Pollastri, M. P., Pharmacological Validation of *Trypanosoma brucei* Phosphodiesterases B1 and B2 as Druggable Targets for African Sleeping Sickness. *J. Med. Chem.* **2011,** *54*, 8188-8194.

(4) Napoletano, M.; Norcini, G.; Pellacini, F.; Marchini, F.; Morazzoni, G.; Fattori, R.; Ferlenga, P.; Pradella, L., Phthalazine PDE4 inhibitors. Part 3: The synthesis and in vitro evaluation of derivatives with a hydrogen bond acceptor. *Bioorg. Med. Chem. Lett.* **2002,** *12*, 5-8.

(5) Seebeck, T.; Sterk, G. J.; Ke, H., Phosphodiesterase inhibitors as a new generation of antiprotozoan drugs: exploiting the benefit of enzymes that are highly conserved between host and parasite. *Future Med. Chem.* **2011,** *3*, 1289-1306.

(6) Zoraghi, R.; Seebeck, T., The cAMP-specific phosphodiesterase TbPDE2C is an essential enzyme in bloodstream form *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. U.S.A.* **2002,** *99*, 4343-4348.

(7) Wang, H.; Liu, Y.; Chen, Y.; Robinson, H.; Ke, H., Multiple Elements Jointly Determine Inhibitor Selectivity of Cyclic Nucleotide Phosphodiesterases 4 and 7. *J. Biol. Chem.* **2005,** *280*, 30949-30955.

(8) Fawcett, L.; Baxendale, R.; Stacey, P.; McGrouther, C.; Harrow, I.; Soderling, S.; Hetman, J.; Beavo, J. A.; Phillips, S. C., Molecular cloning and characterization of a distinct human phosphodiesterase gene family: PDE11A. *Proc. Natl. Acad. Sci. U.S.A.* **2000,** *97*, 3702-3707.

(9) Tralau-Stewart, C. J.; Williamson, R. A.; Nials, A. T.; Gascoigne, M.; Dawson, J.; Hart, G. J.; Angell, A. D. R.; Solanke, Y. E.; Lucas, F. S.; Wiseman, J.; Ward, P.; Ranshaw, L. E.; Knowles, R. G., GSK256066, an Exceptionally High-Affinity and Selective Inhibitor of Phosphodiesterase 4 Suitable for Administration by Inhalation: In Vitro, Kinetic, and In Vivo Characterization. *J. Pharmacol. Exp. Ther.* **2011,** *337*, 145-154.

(10) Aspiotis, R.; Deschênes, D.; Dubé, D.; Girard, Y.; Huang, Z.; Laliberté, F.; Liu, S.; Papp, R.; Nicholson, D. W.; Young, R. N., The discovery and synthesis of highly potent subtype selective phosphodiesterase 4D inhibitors. *Bioorg. Med. Chem. Lett.* **2010,** *20*, 5502-5505.

(11) Marivet, M. C.; Bourguignon, J. J.; Lugnier, C.; Mann, A.; Stoclet, J. C.; Wermuth, C. G., Inhibition of cyclic adenosine-3',5'-monophosphate phosphodiesterase from vascular smooth muscle by rolipram analogs. *J. Med. Chem.* **1989,** *32*, 1450-1457.

(12) Kempson, J.; Pitts, W. J.; Barbosa, J.; Guo, J.; Omotoso, O.; Watson, A.; Stebbins, K.; Starling, G. C.; Dodd, J. H.; Barrish, J. C.; Felix, R.; Fischer, K., Fused pyrimidine based inhibitors of phosphodiesterase 7 (PDE7): synthesis and initial structure–activity relationships. *Bioorg. Med. Chem. Lett.* **2005,** *15*, 1829-1833.

(13) Wang, C.; Ashton, T. D.; Gustafson, A.; Bland, N. D.; Ochiana, S. O.; Campbell, R. K.; Pollastri, M. P., Synthesis and evaluation of human phosphodiesterases (PDE) 5 inhibitor analogs as trypanosomal PDE inhibitors. Part 1. Sildenafil analogs. *Bioorg. Med. Chem. Lett.* **2012,** *22*, 2579-2581.

(14) Chappie, T. A.; Humphrey, J. M.; Allen, M. P.; Estep, K. G.; Fox, C. B.; Lebel, L. A.; Liras, S.; Marr, E. S.; Menniti, F. S.; Pandit, J.; Schmidt, C. J.; Tu, M.; Williams, R. D.; Yang, F. V., Discovery of a Series of 6,7-Dimethoxy-4-pyrrolidylquinazoline PDE10A Inhibitors†. *J. Med. Chem.* **2006,** *50*, 182-185.

(15) Bihel, F. J. J.; Justiniano, H.; Schmitt, M.; Hellal, M.; Ibrahim, M. A.; Lugnier, C.; Bourguignon, J.-J., New PDE4 inhibitors based on pharmacophoric similarity between papaverine and tofisopam. *Bioorg. Med. Chem. Lett.* **2011,** *21*, 6567-6572.

(16) Kunz, S.; Kloeckner, T.; Essen, L.-O.; Seebeck, T.; Boshart, M., TbPDE1, a novel class I phosphodiesterase of *Trypanosoma brucei*. *Eur. J. Biochem.* **2004,** *271*, 637-647.

(17) Oberholzer, M.; Marti, G.; Baresic, M.; Kunz, S.; Hemphill, A.; Seebeck, T., The *Trypanosoma brucei* cAMP phosphodiesterases TbrPDEB1 and TbrPDEB2: flagellar enzymes that are essential for parasite virulence. *FASEB J.* **2007,** *21*, 720-731.

(18) Connolly, B. J.; Willits, P. B.; Warrington, B. H.; Murray, K. J., 8-(4-Chlorophenyl)thio-cyclic AMP is a potent inhibitor of the cyclic GMP-specific phosphodiesterase (PDE VA). *Biochem. Pharmacol.* **1992,** *44*, 2303-2306.

(19) Ochiana, S. O.; Gustafson, A.; Bland, N. D.; Wang, C.; Russo, M. J.; Campbell, R. K.; Pollastri, M. P., Synthesis and evaluation of human phosphodiesterases (PDE) 5 inhibitor analogs as trypanosomal PDE inhibitors. Part 2. Tadalafil analogs. *Bioorg. Med. Chem. Lett.* **2012,** *22*, 2582-2584.

(20) Yu, G.; Mason, H.; Wu, X.; Wang, J.; Chong, S.; Beyer, B.; Henwood, A.; Pongrac, R.; Seliger, L.; He, B.; Normandin, D.; Ferrer, P.; Zhang, R.; Adam, L.; Humphrey, W. G.; Krupinski, J.; Macor, J. E., Substituted Pyrazolopyridopyridazines as Orally Bioavailable Potent and Selective PDE5 Inhibitors: Potential Agents for Treatment of Erectile Dysfunction. *J. Med. Chem.* **2003,** *46*, 457-460.