Supporting information

Pentamidine analogs as inhibitors of [^{3}H]MK-801 and [^{3}H]ifenprodil binding to rat brain NMDA receptors

Michael L. Berger[a,], Dorota Maciejewska[b], Jean Jacques Vanden Eynde[c], Madhusoodanan Mottamal[d], Jerzy Żabiński[b], Paweł Kaźmierczak[b], Mateusz Rezler[b], Ivana Jarak[e], Ivo Piantanida[e], Grace Karminski-Zamola[e], Annie Mayence[d], Patrick Rebernik[a], Arvind Kumar[f], Mohamed A Ismail[f], David W. Boykin[f], Tien L. Huang[d]*

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Table S1. List of compounds with references

	ref		ref		ref		ref		ref
1	a	**15**	b	**32**	15	**46**	32	**63**	34
2	23	**16**	15	**33**	29	**47**	33	**64**	34
3	26	**17**	30	**34**	31	**48**	33	**65**	34
4	26	**18**	30	**35**	31	**49**	33	**66**	34
5	27	**19**	30	**36**	31	**50**	33	**67**	34
6	23	**20**	30	**37**	31	**51**	33	**68**	a
7	28	**21**	30	**38**	31	**52**	26	**69**	35
8	24	**22**	15	**39**	28	**53**	26	**70**	35
9a	b	**23**	30	**40a**	b	**54**	26	**71a**	b
9	b	**24**	15	**40**	b	**55**	26	**71**	b
10	23	**25**	29	**41**	28	**56**	32	**72**	39
11a	b	**26**	29	**42**	28	**57**	32	**73**	36
11	b	**27**	29	**43a**	b	**58**	32	**74**	37
12	26	**28**	29	**43**	b	**59**	32	**75**	38
13	26	**29**	29	**44a**	b	**60**	32	**76b**	b
14	25	**30**	29	**44**	b	**61**	32	**76a**	b
15a	b	**31**	29	**45**	32	**62**	32	**76**	b

a, commercially available; b, described in this article.

^{13}C NMR of most new compounds

9a: ^{13}C NMR (75.40 MHz, $CDCl_3$) δ: 24.7 (Y=CH_2), 29.0 (C-9,-9'), 42.8 (C-8,-8'), 55.8 (2x R_P=OMe), 97.2 (C-1,-1'), 108.4 (C-5,-5'), 111.6 (C-2,-2'), 120.9 (C-7,-7'), 127.4 (C-6,-6'), 142.2 (C-4,-4'), 145.8 (C-3,-3').

9: ^{13}C NMR (75.40 MHz, DMSO-d_6) δ: 24.0 (Y=CH_2), 28.1 (C-9,-9'), 41.9 (C-8,-8'), 55.7 (2 x R_P=OMe), 107.4 (C-5,-5'), 108.6 (C-2,-2'), 111.4 (C-1,-1'), 123.2 (C-6,-6'), 143.4 (C-4,-4'), 145.3 (C-3,-3'), 164.2 (C-7,-7').

11a: ^{13}C NMR (75.40 MHz, DMSO-d_6) δ: 39.1 (2 x R_N=CH_3), 52.2 (C-8,-8'), 69.0 (C-9,-9'), 97.8 (C-1,-1'), 111.6 (C-5,-3,-5',-3'), 120.7 (C-7,-7'), 133.6 (C-6,-2,-6',-2'), 151.8 (C-4,-4').

11: ^{13}C NMR (75.40 MHz, DMSO-d_6) δ: 38.6 (2 x R_N=CH_3), 51.1 (C-8,-8'), 68.1 (C-9,-9'), 111.0 (C-5,-3,-5',-3'), 112.0 (C-1,-1'), 129.6 (C-6,-2,-6',-2'), 152.9 (C-4,-4'), 164.0 (C-7,-7').

15a: ^{13}C NMR (100.61 MHz, $CDCl_3$) δ: 21.7 (C-11), 49.9 (C-9,-9'), 56.1 (C-10,-10'), 68.3 (C-8,-8'), 104.6 (C-1,-1'), 112.7 (C-5,-5'), 114.4 (C-2,-2'), 119.2 (C-7,-7'), 126.5 (C-6,-6'), 127.4 (C-2'',-6''), 130.1 (C-3'',-5''), 136.0 (C-1''), 144.1 (C-4''), 149.5 (C-3,-3'), 151.6 (C-4,-4').

15: ^{13}C NMR (75.40 MHz, DMSO-d_6) δ: 20.9 (C-11), 48.5 (C-9,-9'), 55.9 (C-10,-10'), 67.6 (C-8,-8'), 111.4 (C-2,-2'), 112.1 (C-5,-5'), 119.6 (C-1,-1'), 121.8 (C-6,-6'), 126.9 (C-2'',-6''), 129.9 (C-3'',-5''), 135.6 (C-1''), 143.5 (C-4''), 148.5 (C-3,-3'), 152.0 (C-4,-4'), 164.6 (C-7,-7').

71a: ^{13}C NMR (100.0 MHz, DMSO-d_6) δ: 142.6, 140.1, 135.0, 128.0, 125.2, 113.8, 106.8.

71: ^{13}C NMR (100.0 MHz, DMSO-d_6/50 °C) δ:158.3, 142.8, 135.7, 135.6, 128.2, 127.5, 126.0.

76b: ^{13}C NMR (100.0 MHz, DMSO-d_6) δ: 152.6, 150.2, 141.4, 139.7, 132.4, 127.0, 122.5, 120.1, 118.3, 111.6.

76a: ^{13}C NMR (100.0 MHz, DMSO-d_6) δ: 153.4, 147.6, 142.0, 135.1, 132.3, 131.5, 126.9, 120.8, 118.2, 111.4.

76: ^{13}C NMR (100.0 MHz, DMSO-d_6) δ: 165.8, 154.3, 147.7, 142.9, 136.8, 132.3, 129.0, 128.6, 127.8, 122.3.

Table S2. Data from Tables 1-4 with single values for n = 2

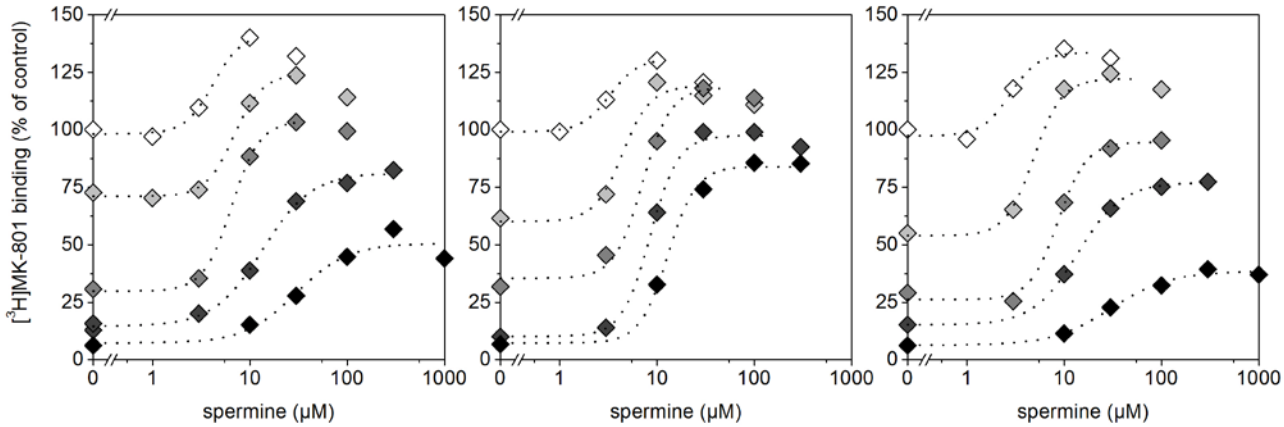
	[^{3}H]MK-801			[^{3}H]ifenprodil
	no spm	30 μM spm	ratio	no spm
arcaine	4.60±1.71 (4)	203, 189 (2)	35.0, 32.1 (2)	23.1, 25.7 (2)
N12N	7.05±2.38 (11)	245±30 (5)	32.9±6.3 (5)	749, 1171 (2)
N4T8N	0.52±0.32 (46)	6.10±1.43 (24)	15.1±7.1 (24)	53.2, 45.4 (2)
1	2.68±0.65 (9)	24.9±5.7 (7)	9.1±2.5 (7)	33.3, 31.7 (2)
2	3.41±0.52 (4)	148±22 (3)	40.6±4.2 (3)	68.3, 59.2 (2)

3	10.6±3.9 (4)	343±27 (3)	33.1±11.7 (3)	44.9, 42.4 (2)
4	2.20±0.32 (3)	26.8, 31.5 (2)	10.9, 13.7 (2)	20.3, 18.0 (2)
5	1.22±0.23 (3)	23.8±2.7 (3)	20.2±6.1 (3)	n.d.
6	317±20 (3)	553, 1018 (2)	1.73, 3.45 (2)	n.d.
7	3.10±0.11 (3)	42.3±1.9 (3)	13.7±0.6 (3)	20.6±5.4 (3)
8	0.56±0.08 (4)	5.62±2.88 (4)	9.2±3.5 (4)	16.0, 17.2 (2)
9	3.00, 2.76 (2)	23.0, 18.4 (2)	7.67, 6.66 (2)	4.54±1.43 (3)
10	0.85±0.23 (7)	38.5±10.4 (6)	50.6±19.7 (6)	11.4±3.7 (3)
11	0.76±0.15 (4)	12.5±1.8 (3)	15.2±2.2 (3)	21.9±5.2 (3)
12	21.2±5.1 (3)	136, 150 (2)	8.90, 6.41 (2)	n.d.
13	0.23±0.06 (4)	2.13±0.17 (3)	10.4±1.56 (3)	1.47±0.55 (3)
14	0.24±0.08 (5)	1.32±0.23 (4)	6.28±1.18 (4)	0.41±0.13 (3)
15	3.77±1.37 (4)	28.7±4.4 (3)	8.11±2.91 (3)	0.84±0.48 (4)
16	3.08±0.59 (4)	69.4, 72.1 (2)	31.5, 20.9 (2)	20.0, 14.1 (2)
17	21.4±5.2 (4)	544±34 (3)	32.9±5.8 (3)	n.d.
18	19.4±7.4 (4)	387, 544 (2)	23.1, 20.4 (2)	n.d.
19	15.9±4.9 (4)	277, 465 (2)	21.8, 21.7 (2)	n.d.
20	10.3±3.6 (5)	139±32 (4)	16.0±3.2 (4)	n.d.
21	9.25±3.85 (5)	159±59 (4)	19.3±6.4 (4)	n.d.
22	6.43±1.64 (4)	106±20 (3)	15.1±4.1 (3)	n.d.
23	23.8±8.9 (5)	264±26 (3)	13.6±5.2 (3)	n.d.
24	0.25±0.07 (14)	5.47±1.42 (6)	20.4±7.3 (6)	21.6, 16.1 (2)
25	17.7±4.8 (4)	389±85 (3)	25.3±10.5 (3)	n.d.
26	11.2±2.9 (4)	212±32 (4)	19.9±5.7 (4)	n.d.
27	34.6±4.1 (3)	603, 521 (2)	16.1, 17.4 (2)	n.d.
28	9.94±3.72 (3)	103±3 (3)	11.2±3.3 (3)	n.d.
29	8.13±0.29 (3)	85.7±7.8 (3)	10.6±1.2 (3)	n.d.
30	9.44±2.70 (6)	324±34 (5)	35.7±6.9 (5)	n.d.
31	41.2± 13.2 (4)	498, 356 (2)	8.34, 11.1 (2)	n.d.
32	3.03±0.21 (3)	80.2, 104 (2)	28.2, 32.1 (2)	n.d.
33	12.8±4.3 (3)	143±15 (3)	11.7±2.5 (3)	n.d.

34	2.01±0.76 (6)	50.7±13.8 (5)	30.2±10.4 (5)	0.76±0.05 (3)
35	3.18±0.60 (3)	78.3, 92.3 (2)	24.4, 36.1 (2)	6.88, 7.39 (2)
36	2.61±0.55 (4)	48.1±6.5 (3)	19.3±7.8 (3)	13.9, 12.2 (2)
37	2.71±0.41 (3)	54.5, 54.5 (2)	17.7, 24.1 (2)	14.5, 19.4 (2)
38	1.14±0.19 (3)	14.2, 13.4 (2)	10.5, 13.1 (2)	8.86, 9.46 (2)
39	10.0±2.3 (3)	148, 148 (2)	18.0, 16.1 (2)	87.5, 68.0 (2)
40	1.84±0.65 (4)	24.7±0.8 (3)	14.7±5.3 (3)	25.8, 36.6 (2)
41	4.55±1.74 (3)	93.2, 75.2 (2)	14.2, 20.2 (2)	n.d.
42	4.37±0.32 (3)	23.9, 23.8 (2)	5.12, 5.41 (2)	n.d.
43	4.67±0.38 (3)	23.9, 27.0 (2)	5.45, 5.29 (2)	n.d.
44	8.24, 6.27 (2)	30.6, 31.6 (2)	3.71, 5.03 (2)	n.d.
45	230±97 (4)	675±131 (3)	2.81±0.76 (3)	54.5, 65.6 (2)
46	165±68 (3)	483, 391 (2)	3.29, 3.63 (2)	n.d.
47	>1000	>1000	n.d.	n.d.
48	22.9±6.7 (4)	170±15 (3)	7.25±1.95 (3)	n.d.
49	26.5±2.6 (3)	111±13 (3)	4.25±0.94 (3)	n.d.
50	15.7±2.2 (3)	62.6±4.8 (3)	4.07±0.96 (3)	n.d.
51	9.63±1.45 (3)	27.7±6.9 (3)	2.98±0.58 (3)	n.d.
52	>1000	>1000	n.d.	n.d.
53	>1000	>1000	n.d.	n.d.
54	688 (2)	>1000	n.d.	n.d.
55	302, 230 (2)	1270, 1147 (2)	4.20, 4.99 (2)	n.d.
56	2.19±0.85 (5)	18.4±3.2 (4)	10.1±3.2 (4)	7.29±2.85 (4)
57	1.46±0.60 (4)	5.54±1.23 (3)	4.00±0.93 (3)	1.42, 2.05 (2)
58	0.79±0.32 (4)	6.70±0.89 (4)	9.04±1.93 (4)	3.97±1.59 (3)
59	5.24±1.12 (4)	25.7±0.9 (3)	5.25±1.29 (3)	10.8, 16.8 (2)
60	10.8±1.7 (3)	44.2, 49.1 (2)	3.62, 4.33 (2)	38.6, 27.0 (2)
61	42.3±6.8 (4)	42.6, 30.9 (2)	1.01, 0.94 (2)	n.d.
62	53.9±2.4 (3)	53.3, 52.5 (2)	0.97, 0.94 (2)	n.d.
63	152±10 (3)	153±7 (3)	1.01±0.02 (3)	n.d.
64	10.6±1.3 (3)	174±45 (3)	16.9±6.0 (3)	n.d.
65	56.2, 60.2 (2)	67.9, 74.0 (2)	1.21, 1.23 (2)	>100

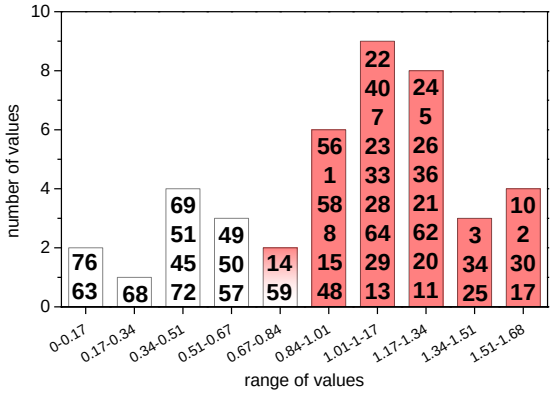
66	6.94±1.22 (3)	75±13 (3)	10.8±1.2 (3)	50.1, 49.4 (2)
67	119, 132 (2)	210, 255 (2)	1.77, 194 (2)	n.d.
68	4.23±2.47 (4)	7.48±2.51 (4)	2.37±1.69 (4)	n.d.
69	6.97±2.15 (3)	24.0±19.2 (3)	3.47±2.20 (3)	n.d.
70	16.4, 7.95 (2)	40.1, 21.2 (2)	2.45, 2.67 (2)	n.d.
71	3.08, 7.60 (2)	6.32, 7.20 (2)	2.05, 0.95 (2)	n.d.
72	6.68±1.25 (4)	17.7±6.3 (4)	2.61±0.61 (4)	n.d.
73	9.9±4.4 (3)	39.0, 29.9 (2)	4.67, 4.59 (2)	n.d.
74	3.55, 2.86 (2)	15.9, 10.8 (2)	4.49, 3.78 (2)	n.d.
75	5.01±3.28 (3)	14.3, 9.21 (2)	2.66, 5.87 (2)	n.d.
76	26.8±13.1 (3)	30.0±6.7 (3)	1.24±0.38 (3)	n.d.

Fig. S1. Influence of 1, 10 and 13 on spermine-stimulation of [^{3}H]MK-801 binding



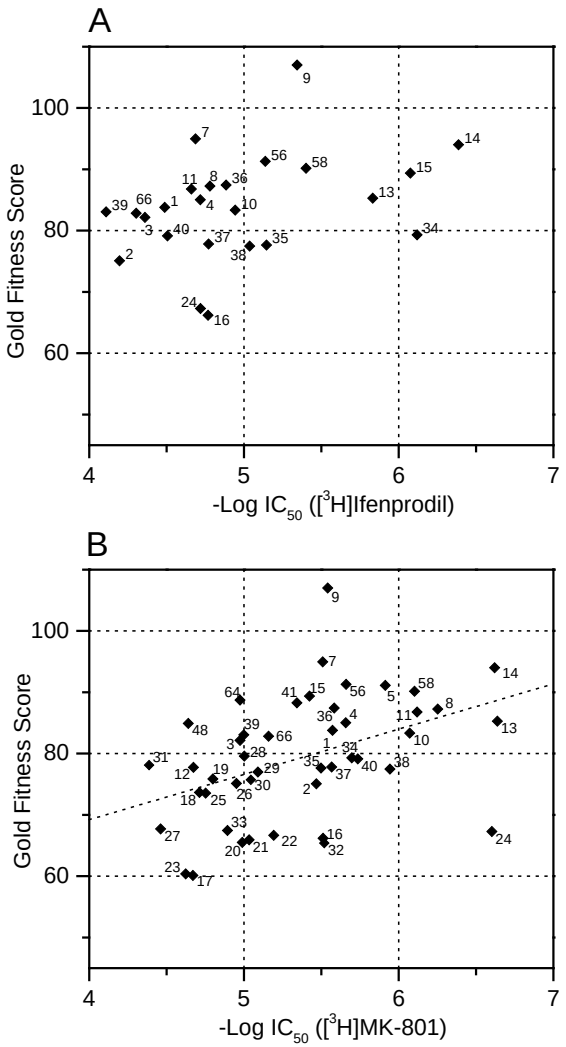
[^{3}H]MK-801 binding was stimulated by spermine in presence of increasing concentrations of pentamidine (0, 3, 10, 30, 100 µM; left panel); of the 'diaza-γ-oxa' analog **10** (0, 1, 3, 10, 30 µM; middle panel); and of the γ-*N*-benzenesulfonyl analog **13** (0, 0.3, 1, 3, 10 µM; right panel). Although dose/response curves were shifted to the right and inhibitions were partly reversed by spermine, inhibitions were not reversed completely. Data pooled from 8 experiments performed with triplicates (SDs around 3%), dotted lines follow saturation functions with n_H 1-3.

Fig. S2. Frequency distribution of spermine-sensitivities



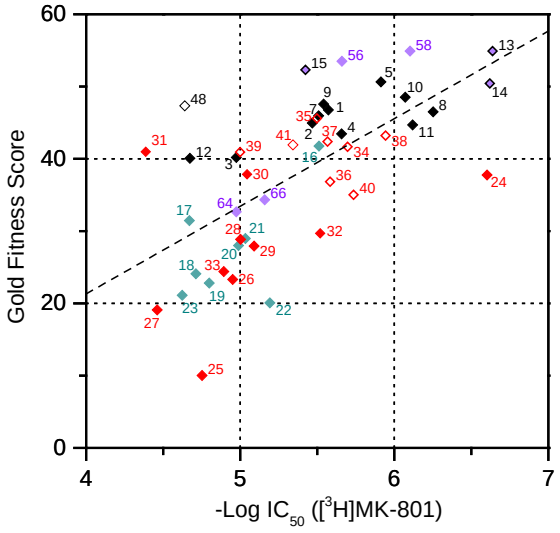
Inhibition of [^{3}H]MK-801 binding to rat brain membranes by bisbenzamidines was attenuated by 30 µM spermine. The logarithm of this attenuation factor (IC_{50} in presence / IC_{50} in absence of spermine) ranged from 0 (no influence) to 1.68 for compound **10** (factor 50.6; note that 1.68 is not the logarithm of 50.6, but the mean of 6 logarithms). Only mean values from at least 3 independent experiments are shown in this distribution. Compound numbers with sensitivities in the respective range are inscribed to the columns. Docking calculations were based on 31 compounds as sensitive as or more sensitive than **14** (in red); 14 additional compounds with sensitivity higher than **14** were also included in docking calculations (just 2 experiments; not shown in this distribution).

Fig. S3. Docking to ifenprodil site



Relationship between goodness of fit to an ifenprodil site modeled at the GluN1/2B interface and potency to inhibit the binding of [^{3}H]ifenprodil (A) and of [^{3}H]MK-801 (B) to native rat brain membranes. Only for the channel ligand (B), significant correlation (r^2 = 0.18, dotted line) was observed ($p<0.01$).

Fig. S4. Docking of structural classes to GluN1



Docking of bisbenzamidines to the acidic domain of GluN1, with colors indicating structural classes. Filled black symbols refer to pentamidine (**1**) and close isosters **2-12**. The piperazines **16-23** are fully cyan, and the homopiperazines **24-33** fully red. Analogs with short bridge **34-41** are open red. Violet indicates an aromatic ring inserted into or attached to the bridge connecting the benzamidine moieties; among them, **13-15** are structurally close to **1** (black envelop), but not **56**, **58**, **64** & **66**. The most extended bisbenzamidine **48** does not fit into any of these classes (open black symbol). Note that the lead homopiperazine **24** is far off the correlation line and thus its high potency is not explained by the model.