

3D molecular descriptors important for clinical success

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Table of contents:

Sheet S1. Percent Inhibition data of compound 12 and experimental details

Sheet S2. Percent Inhibition data of compound 16 and experimental details.

Sheet S3. Descriptors investigated in these studies.

S4. Summary statistics of relevant descriptors and their distributions.

Table S1. Binding Binding affinity (K_i) of exemplified nicotinic compounds at human neuronal

$\alpha 4\beta 2$, $\alpha 3\beta 4$ and $\alpha 7$ nAChR subtypes.

S5. nAChRs binding assays.

References.

Sheets S1 and S2. See accompanying Excel sheets. Screening assays, designed to test for inhibition of binding or enzyme activity, provide information about the biological activity and selectivity of compounds tested. Values are expressed as the percent inhibition of specific binding and represent the average of replicate tubes at the concentration tested. The standard baseline range runs from -20% to +20% inhibition of binding or enzyme activity. Compounds exhibiting results in this range are considered inactive at those receptor sites, while those showing 50% inhibition or greater are considered as actives.

S5. nAChRs binding assay.

Binding to nAChRs was conducted by modification of standard methods.^{66, 67} The human epithelial cells, SH-EP1, and SH-SY5Y cells expressing human $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nAChRs, were used as membrane sources, respectively. [^3H]nicotine and [^3H]epibatidine were used as radioligands at $\alpha 4\beta 2$ and $\alpha 3\beta 4$, respectively. For human $\alpha 7$ nAChR, the HEK_Human $\alpha 7$ /RIC3 cell line and [^3H]epibatidine were used as membrane source and radioligand, respectively. The HEK_human $\alpha 7$ /RIC3 cells were obtained from Jon Lindstrom (University of Pennsylvania, Philadelphia, PA). Both [^3H]nicotine and [^3H]epibatidine were purchased from Perkin Elmer (Waltham, MA). For rat $\alpha 4\beta 2$ binding assay, membranes from the cortex of female Sprague-

Dawley rats were used. The radioligand concentration for $\alpha 4\beta 2$, $\alpha 3\beta 4$, and $\alpha 7$ binding assays were 2, 1, and 10 nM, respectively. In brief, membranes were freshly reconstituted from their frozen stocks and incubated for 2 h on ice in 150 μ l assay buffer containing (in mM) Tris (50), NaCl (154), pH 7.4, in the presence of test compound (10 pM to 100 μ M) and radioligand. Non-specific binding was determined in the presence of 100 μ M nicotine. Incubation was terminated by rapid filtration on a 96-channel manifold tissue harvester (Brandel, Gaithersburg, MD), using glass-fiber filters presoaked in 0.33% (w/v) polyethyleneimine. Filters were washed three times with ice-cold assay buffer and the retained radioactivity was determined by liquid scintillation counting. Data were analyzed with GraphPad Prism 5.0 (GraphPad Software, Inc., CA) fitting them to the built-in non-linear least-square equation for IC₅₀ determination and K_i calculation. For the Cheng-Prusoff equation the K_d value of 0.7 nM for [³H] nicotine, 0.3 nM and 6.3 nM for [³H] epibatidine were used for $\alpha 4\beta 2$, $\alpha 3\beta 4$ and $\alpha 7$, respectively.⁶⁸

Table S1. Binding affinity (K_i) of exemplified nicotinic compounds at human neuronal $\alpha 4\beta 2$, $\alpha 3\beta 4$ and $\alpha 7$ nAChR subtypes.

Compound	nAChR Subtype			
	Human $\alpha 4\beta 2$	Human $\alpha 7$	Human $\alpha 3\beta 4$	Rat $\alpha 4\beta 2$
	K _i value (mean \pm S.E.M.), nM			
1	670 \pm 420(3)	340 \pm 96(4)	3600 \pm 771.24(4)	150 \pm 70
2	1100 \pm 170(2)	NT	NT	10000
3	NT	NT	NT	10000
4	1100(1)	NT	NT	NT
5	0.18 \pm 0.08(5)	1.3 \pm 0.74(3)	23 \pm 8.3(4)	0.16 \pm 0.06(4)

6	12±3.1(2)	4700±1100(2)	8600(1)	22±6(2)
7	0.38(1)	10(1)	21(1)	0.22±0.1(5)
8	12±7.2(3)	> 10000	6600±1100(2)	5.5±1.5(4)
9	27±7.9(6)	> 10000	> 10000	65±18.64
10	3±0.7(12)	623.1±95.1(7)	269.9±63.3 (8)	2.3±1.0(9)
11	0.3±0.02(402)	1.2±0.04(258)	3.8±0.18(174)	0.2±0.07(207)
12	0.92±0.18(3)	750±330(3)	180±28 (3)	0.91±0.13(3)
13	NT	NT	NT	26
14	36±9.1(7)	5300±1810(5)	> 10000	140±40 (6)
15	1.8(1)	120(1)	77(1)	1.1±0.83(2)
16	0.14±0.04(3)	290±34(5)	160±84(3)	0.13±(3)
17	NT	NT	NT	3.1±0.45(2)
18	1.8±0.38(3)	76±17(3)	790±260(3)	1.3±0.55(3)
19	9.4±2 (3)	70±4.6(4)	1800±510(4)	12±1.9(3)
20	0.4(1)	> 10000	NT	87±29(2)
21	33±8.56(6)	40000±29877(3)	5285.94±1811.6(6)	53±14.76(5)
22	20.1±8.51(5)	NT	5950.83±4170.37(3)	28.97±4.46(4)
23	5.28±0.75(9)	6204.1	3451.81±1845.59(5)	6.68±2.34(5)
24	13.59	NT	NT	23.58
25	8.39±0.89(6)	21817.24±8240.4(4)	4653.03±1439.38(4)	59.72±12.49(5)
26	653.01±152.54(7)	31.49±13.96(6)	66497.96±49752.42(3)	2173±752.4(5)
27	1737.42±799.56(9)	1.4±0.35(5)	NT	1463.12±372.24(7)
28	8037.25±2262.22(5)	413.7±79.72(4)	NT	24276.6±7318.27(3)
29	335.51±86.36(11)	2.72±0.45(8)	9385.86±4028.17(4)	360.43±84.05(6)

30	472.07±192.28(5)	56.32±27.37(4))	NT	530.1±98.86(3)
31	1213.78±292.06(6)	2.99±1.08(19)	6578±935.21(4)	4165.46±1851.38(3)
32	1221.23±290.44(7)	0.94±0.16(6)	44338.38±808.62(3)	1044.6±246.84(6)
33	1032.14±289.91(3)	3.84±0.86(4)	NT	767.39±114.13(3)
34	466.5±124.5(7)	2.08±0.28(8)	8445.65±1757.56(3)	1087.74±480.68(4)

K_i values ± standard error of mean for the exemplified nicotinic ligands tested in radioactive displacement assays, to measure binding to nAChRs. The number of replicates is listed beside each result in brackets. [³H]nicotine was used as the radioligand at human $\alpha 4\beta 2$ heterologously expressed in SH-EP1 cells and rat $\alpha 4\beta 2$ in rat cortex. [³H]epibatidine was used as radioligand at $\alpha 3\beta 4$, the ganglion-type nicotinic receptors on SH-SY5Y cellular membranes and human $\alpha 7$ nAChR in the HEK_Human $\alpha 7$ /RIC3 cell line. See Experimental Section S2 for details. NT = not tested.

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