Supplementary Information

SUPPLEMENTARY INFROMATION

SI1. Dose deciding pilot study

As shown in Fig. S2, the test compounds (**13**, **17** and **70**) were unable to induce significant effects in MWM test at an equivalent dose corresponding to donepezil (5 mg/kg, p.o.) i.e. the results obtained as escape latency time (ELT) and platform area crossings in MWM test were not significant. Later, the compounds (**13**, **17** and **70**) were evaluated at relatively higher dose (10 mg/kg, p.o.) where they showed significant improvement of scopolamine-induced impaired spatial learning and memory in MWM test (Fig. 2). The dose (10 mg/kg, p.o.) was then continued for further *in vivo* experiments. Thus the dose of **13**, **17** and **70** has been decided on the basis of this study.

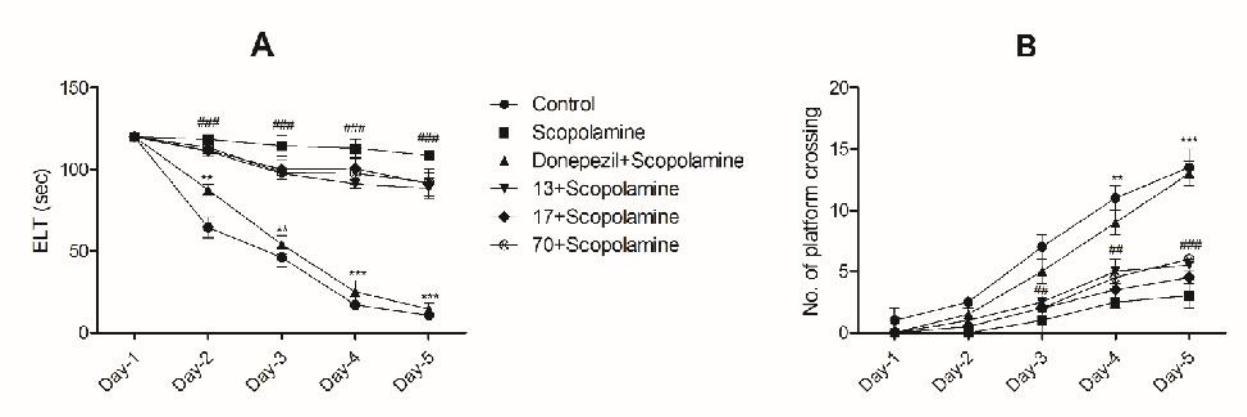

Fig. S1: Test compounds (**13**, **17** and **70**) did not significantly improve spatial learning and memory impairment in scopolamine induced amnesic mice in MWM test at 5 mg/kg, p.o. dose. Scopolamine treatment (1.4 mg/kg, i.p.) increased the ELT during probe trial sessions (A), and reduced the number of platform area crossings (B) as compared to the vehicle-treated control mice. Donepezil (5 mg/kg, p.o.) significantly shortened ELT (A) and increased number of platform area crossings (B) as compared to the scopolamine-treated control group. Compounds (**13**, **17** and **70)** did not significantly improve the spatial learning and memory impairment (A, B) in MWM test at relatively lower dose (5 mg/kg, p.o.). Data are expressed as mean ± SEM (n=6). $^{###}$ p<0.001, $^{##}$ p<0.01 *vs.* vehicle-treated control group. *** p<0.001, ** p<0.01, *vs.* scopolamine-treated control group.

Supplementary Information

SI2. *In vitro* blood-brain barrier permeation assay

Table S1: Permeability (P_e 10^{-6} cm/s) of nine commercial quality standards in the PAMPA-BBB assay.

Commercial drugs	Reported value[a]	Experimental value[b]
Diazepam	16	21.2±1.8
Verapamil	13	15.1±1.2
Progesterone	9.3	12.2±1.1
Clonidine	6.2	9.4±0.4
Corticosterone	5.1	8.2±0.5
Lomefloxacin	1.1	2.3±0.2
Ofoxacin	0.8	1.5±0.3
Atenolol	0.8	3.2±0.4
Dopamine	0.2	1.8±0.2

[a]Taken from reference [1]. [b]Determined using PAMPA-BBB assay. Data are expressed as mean ± SEM of three independent experiments.

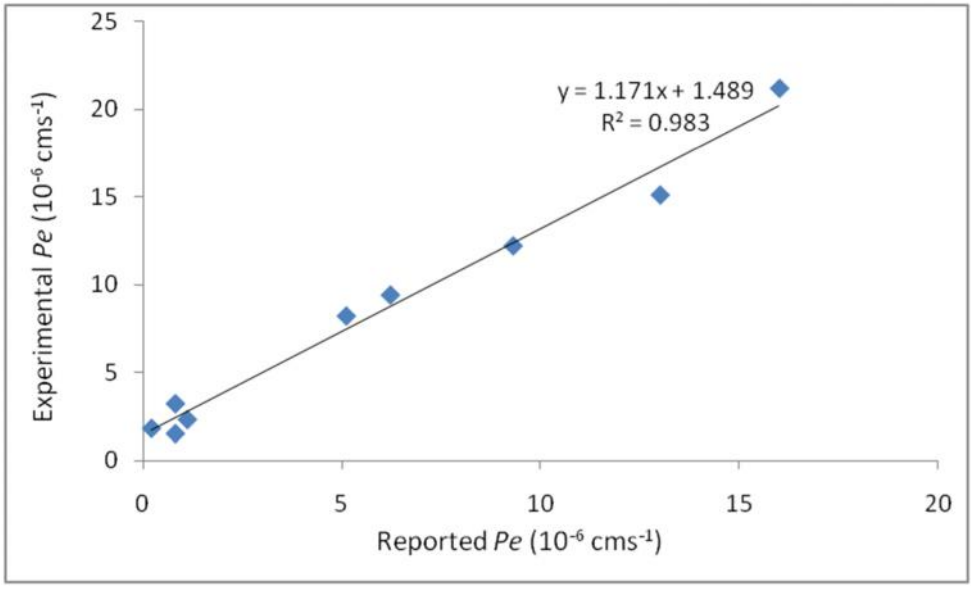

Fig S2: Linear correlation between experimental and reported permeability values of nine commercial drugs in the PAMPA-BBB assay. P_e(Exp.)= 1.171P_e(Ref.) + 1.489 (R^2 = 0.983)

Supplementary Information

Table S2: Ranges of permeability (P_e, 10^{-6} cm/s) in the PAMPA-BBB assay.

	Permeability (P_e, 10^{-6} cm/s)
Compounds of high BBB permeation (CNS+)	$P_e > 6.2$
Compounds of uncertain BBB permeation (CNS+/-)	$6.2 > P_e > 3.8$
Compounds of low BBB permeation (CNS-)	$P_e < 3.8$

REFERENCE

1. Di L, Kerns EH, Fan K, McConnell OJ and Carter GT. High throughput artificial membrane ermeability assay for blood–brain barrier. *Eur J Med Chem*, **2003**. 38: p. 223-232.