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Synthesis of Quinoline Derivatives: Discovery of a Potent and Selective Phosphodiesterase 5 Inhibitor for the Treatment of Alzheimer's disease

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Abstract

Phosphodiesterase type 5 (PDE5) mediates the degradation of cGMP in a variety of tissues including brain. Recent studies have demonstrated the importance of the nitric oxide/cGMP/cAMP-responsive element-binding protein (CREB) pathway to the process of learning and memory. Thus, PDE5 inhibitors (PDE5Is) are thought to be promising new therapeutic agents for the treatment of Alzheimer's disease (AD), a neurodegenerative disorder characterized by memory loss. To explore this possibility, a series of quinoline derivatives were synthesized and evaluated. We found that compound **7a** selectively inhibits PDE5 with an IC₅₀ of 0.27 nM and readily crosses the blood brain barrier. In an *in vivo* mouse model of AD, compound **7a** rescues synaptic and memory defects. Quinoline-based, CNS-permeant PDE5Is have potential for AD therapeutic development.

Keywords

PDE5 inhibitor; Alzheimer's disease; quinoline derivative; cGMP; memory; long-term potentiation

1. Introduction

Phosphodiesterases (PDEs) are a superfamily of enzymes that degrade the intracellular second messengers, cyclic adenine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), leading to the modulation of several biological processes, such as intracellular calcium level via Ca²⁺-Calmodulin signaling pathway [1]. The PDEs superfamily is classified into 11 families (PDE1 to PDE11) on the basis of tissue distribution, amino acid sequences, regulatory properties, substrate specificities and pharmacological properties [1–3]. Some PDEs specifically bind to cAMP (PDE4, PDE7,

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and PDE8); PDE5, PDE6, and PDE9 are highly specific for cGMP while the remaining PDEs have mixed specificity (PDE1, PDE2, PDE3, PDE10, and PDE11) [2, 3]. Among all PDEs, PDE5 is widely expressed in a variety of tissues, such as brain, smooth muscle, lung, platelets and kidney (see Gene Logic's ASCENTA System and [4–7]). Due to the PDE5 smooth muscle localization, inhibitors of this enzyme were initially developed for the treatment of erectile dysfunction (Viagra®, Cialis®, and Levitra®) and subsequently pulmonary hypertension [8, 9]. Recently, a number of studies have shown a growing interest in PDE5 as a promising target for the treatment of other diseases, including Alzheimer's disease (AD) [2, 10, 11].

AD is the most common form of dementia and is characterized by aggregation of amyloid β peptides ($A\beta$) into fibrillar plaques that lead to memory loss [12]. The nitric oxide/cGMP/cAMP-responsive element-binding protein (CREB) pathway has been demonstrated to play a crucial role in memory, neurotransmission, as well as hippocampal long-term potentiation (LTP), a type of synaptic plasticity thought to underline learning and memory [11, 13–15]. In fact, impairment of CREB phosphorylation has been shown to occur as a result of $A\beta$ overproduction and accumulation [15]. Based on these findings, elevation of cGMP levels through the inhibition of PDE5 represents an alternative strategy for improving learning and memory [4, 16–18]. In this regard, the PDE5 inhibitor (PDE5I) sildenafil (Viagra®) has been previously demonstrated to re-establish normal synaptic and cognitive function in AD mouse models with a beneficial effect that outlasts the presence of the drug in the blood stream by several months [4].

None of the PDE5Is were developed for the central nervous system (CNS) penetration with the selectivity necessary for chronic administration. Among the commercial PDE5Is, likely due to PDE6 inhibition, sildenafil and vardenafil cause moderate-severe visual disturbances, [19] whereas tadalafil induces back pain as a side effect, which is likely to be a concern for chronic use in senile population (incidentally, it is not clear whether this side effect is due to the inhibition of PDE11 for which the compound is not selective or other targets) [20–23]. This prompted us to develop novel selective molecules customized for the CNS. As a strategy, we first searched for a valid scaffold from a pool of compounds known to possess PDE5 inhibitory activity. Next, we modified its structure in order to obtain potent and selective PDE5Is. Most importantly, during the process of designing new compounds, we considered physicochemical properties that are important for molecules to penetrate the blood-brain barrier (BBB), such as molecular weight, polar surface area, and number of hydrogen-bond donors and acceptors [24].

An extensive literature analysis shows a large variety of chemical structures as PDE5Is that can be grouped, on the basis of their structural similarity, as follows: 1) cGMP-based derivatives, represented by sildenafil and vardenafil (Fig. 1A); 2) β -carboline-derived molecules, represented by tadalafil (Fig. 1B); 3) pyrazolopyridine, phthalazine, pyrazolopyridopyridazine and quinoline derivatives (Fig. 1C); 4) isoquinolinone, naphthyridinone and pyridopyrazinone derivatives (Fig. 1D) [25–30]. Among them, compounds based on a quinoline structure have demonstrated to be potent inhibitors with a selectivity for PDE1 through 6 and unknown selectivity for the remaining PDEs [28]. Therefore, we decided to use the quinoline scaffold to continue the development of potent and selective PDE5Is for the treatment of AD. Additionally, we decided to maintain the cyano group at the C-7 position, the hydroxymethyl group at the C-3 position, and the benzylamino moiety of the quinoline ring because they have been shown to be important for PDE5 potency and selectivity [28]. We, in turn, focused on the modification of the C-8 position of the quinoline ring in order to evaluate the influence of a variety of substituents on PDE5 activity.

In this study we report the synthesis and PDE activity of a series of quinoline derivatives. Pharmacokinetic (PK) studies of compound **7a** (the most potent PDE5I of our quinoline analogs) were also performed. Further, we investigated the effect of **7a** on synaptic dysfunction and cognitive abnormalities in two mouse models of AD, a transgenic model (the APP/PS1 mouse) and a non-transgenic model in which amyloid- β is infused into dorsal hippocampi.

2. Results and discussion

2.1. Chemistry

The quinoline derivatives **7a–f** were synthesized from commercially available 4-amino-3-bromobenzonitrile **1**, which was condensed with ethoxymethylenemalonate by refluxing in toluene to yield cluster **2** (Scheme 1). Cyclization of **2** in refluxing diphenylether afforded oxoquinoline **3**. The chlorination of **3** with POCl₃ provided 4-chloroquinoline **4** in good yield. The reaction of 4-chloroquinoline **4** with (3-chloro-4-methoxyphenyl)methanamine hydrochloride in the presence of DIEA afforded the quinoline ester **5**. Organometallic reactions in the presence of palladium were used to obtain quinolines **6a–f**. In particular, the Suzuki coupling reaction of **5** with cyclopropylboronic acid, in the presence of Cs₂CO₃ and a catalytic amount of Pd(PPh₃)₄ yielded **6a**. Quinolines **6b–f** were synthesized by Buchwald-Hartwig coupling with Pd(OAc)₂, (*R*)-BINAP and Cs₂CO₃, under refluxing condition. Finally, the reduction of quinoline esters **6a–f** with lithium tri(*tert*-butoxy)aluminum hydride afforded 3-hydroxymethyl derivatives **7a–f**.

2.2 Biological analysis

2.2.1 PDE5 inhibitory activity—The PDE5 inhibitory activity of all compounds was determined by *in vitro* enzymatic assay (PBS PDE assay kits). All substituents at C-8 position provided potent PDE5Is (**7a–f**) with potency in the low nanomolar range (Table 1). Compounds **7a**, **7b**, and **7f** were the most potent, exceeding sildenafil, vardenafil and tadalafil, with the least active within two orders of magnitude. The two most potent compounds, **7a** and **7b** showed an excellent selectivity against a panel of all eleven PDEs (Table 2). Importantly, the selectivity of **7a** and **7b** against PDE6 and PDE11 was much improved compared to sildenafil, vardenafil and tadalafil. Encouraged by these *in vitro* results, **7a** was chosen for further evaluation including assessment of *in vivo* activity, PK evaluation, electrophysiological analysis and behavioral studies.

2.2.2 Hippocampal cGMP levels—To confirm the *in vitro* data, we measured cGMP levels in adult mice after treatment with compound **7a**. In a series of preliminary experiments we demonstrated that foot shock induces an immediate increase in cGMP levels in the hippocampus (Fig. 2). Compound **7a** (3 mg/kg, i.p., 30 minutes prior to electric shock) further enhanced cGMP levels at different time points (10 sec, 1 min, and 3 min) (0.83 ± 0.059 , 0.69 ± 0.055 , and 0.89 ± 0.0786 after 10 sec, 60 sec and 3 min, respectively) as compared to vehicle (0.318 ± 0.0145 , 0.41 ± 0.028 , and 0.46 ± 0.0385 after 10 sec, 60 sec and 3 min, respectively) (Fig. 2).

2.2.3 Pharmacokinetics—The PK study of compound **7a** (50mg/kg, p.o.) was conducted in male BALB/c mice. The plasma and brain concentrations of compound **7a** at different time points are shown in Fig. 3. The data in Table 3 indicates that **7a** is rapidly absorbed as illustrated by the peak plasma concentration occurring at 0.5 hrs after dosing. Moreover, the T_{max} values in the brain and plasma were similar, indicating that the distribution of **7a** to the brain is also fast. Finally, the amount of **7a** in the brain was lower than that in the plasma with an AUC_{0–t} ratio of 0.41 and the elimination half-lives of **7a** in the brain and plasma were 1.04 and 1.33, respectively.

2.2.4 Electrophysiological and Behavioral studies—Natural oligomers of human A β ₄₂, in the absence of monomers and fibrils, markedly reduce memory and LTP [31–36]. We therefore determined whether compound **7a** could attenuate LTP and memory defects following elevation of A β ₄₂ in the presence of oligomeric A β ₄₂, or vehicle. For LTP, 200 nM A β ₄₂ or vehicle were perfused through the bath solution for 20 min prior to the tetanus. For memory, 200 nM A β ₄₂ (in a final volume of 1 l over 1 min) or vehicle were bilaterally infused, 15 min prior to fear memory induction through a foot shock, into the hippocampus of the mouse that had been pre-implanted with cannulas the week before. A β ₄₂ reduced LTP (144.65 ± 9.99 potentiation in A β ₄₂-treated slices vs. 212.35 ± 18.62 in vehicle-treated slices) and contextual memory (~10% freezing in A β ₄₂-infused mice vs. ~30% in vehicle-treated mice) (Fig. 4 A–B). In turn, compound **7a** (50 nM, for 10 min prior to the θ -burst in the LTP experiments; 3 or 10 mg/Kg, p.o., immediately after training in the behavioral experiments) rescued the LTP (192.63 ± 8.91 potentiation; $F_{(1,11)}=6.073$, $p=0.0314$ comparing A β ₄₂-treated slices vs. A β ₄₂ + **7a** treated slices) and behavioral defects (~30% freezing in mice treated with both 3 and 10 mg/Kg **7a** + A β ₄₂; $p=0.113$ or $p=0.018$ comparing 3 and 10 mg/Kg **7a** + A β ₄₂ treated mice vs. A β ₄₂-treated mice, respectively; Fig. 4 A–B). Collectively, these experiments suggest that **7a** can rescue the damage to synaptic plasticity and memory caused by A β ₄₂ elevation.

Our next goal was to determine whether compound **7a** was capable of rescuing synaptic and memory defects in APP/PS1 mouse model, an amyloid-depositing animal that represents a more “physiological” approach than exogenous application of A β ₄₂. We induced LTP or fear memory in 3–4 month old transgenic animals and their wild-type (WT) littermates either treated with **7a** or vehicle. As previously shown [37, 38], vehicle-treated APP/PS1 mice showed a reduction in LTP and contextual memory (Fig. 5A and B). Compound **7a** (50 nM, through the bath perfusion, for 10 min prior to the tetanus for LTP; 3 mg/kg, i.p, for 3 weeks in behavioral experiments), in turn, rescued the defect in LTP and memory in the transgenic animals. We found 180.85 ± 11.46 potentiation in slices from APP/PS1 mice treated with compound vs. 145.84 ± 7.78 in slices from APP/PS1 mice treated with vehicle ($F_{(1,13)}=8.958$, $p=0.0104$, Fig. 5A). Transgenic mice treated with the compound froze ~30% of the time when they were exposed to the same context in which they had received an electric shock compared to ~10% in vehicle-treated APP/PS1 mice ($p=0.011$, Fig. 5B). Additionally, the beneficial effect of compound **7a** could be extended to spatial short-term memory, a type of memory that can be assessed in transgenic mice using the 2-day radial arm water maze (RAWM) [39]. APP/PS1 mice treated with the compound made less than 2 errors in the 2-day RAWM test vs. ~3 errors in vehicle-treated APP/PS1 mice ($F_{(1,28)}=12.21$, $p=0.002$, Fig. 5C). Thus, findings obtained with the A β preparation are valid also in transgenic mice.

Our preliminary studies with sildenafil have demonstrated that PDE5 inhibition has a prolonged beneficial effect on synaptic and cognitive abnormalities in APP/PS1 mice that persists beyond the administration of the inhibitor. This finding suggests the possibility of using this class of compounds to interfere with the progression of the memory deficits. Does this important therapeutic possibility occur with **7a**? In these experiments, both APP/PS1 and WT mice of 3 months of age were i.p. injected with 3 mg/kg/day **7a** for 3 weeks, then the treatment was stopped for 9–12 weeks prior to testing. Mice were next subjected to fear conditioning and 2-day RAWM. Finally, they were sacrificed for electrophysiological and biochemical studies. We found that slices from transgenic mice treated with the compound had 180.90 ± 17.91 potentiation compared to 112.29 ± 9.12 in slices from vehicle-treated transgenic mice ($F_{(1,14)}=12.32$, $p=0.0035$, Fig. 5D). In the contextual memory experiments, transgenic mice treated with the compound froze ~30% of the time compared to ~10% in vehicle-treated transgenic mice ($p=0.067$, Fig. 5E) and made ~1 error in the 2-day RAWM test compared to ~4 in vehicle-treated transgenic mice ($F_{(1,26)}=4.454$, $p=0.045$, Fig. 5F).

Thus, synaptic and cognitive improvements persist beyond the administration of the inhibitor.

3 Conclusions

A series of quinoline derivatives were synthesized and evaluated for PDE5 inhibitory activity. Among the synthesized derivatives, compound **7a** showed to possess higher potency and selectivity compared to sildenafil, vardenafil and tadalafil. Compound **7a** increased the level of cGMP in mouse hippocampus and rescued the defects in synaptic plasticity and memory. The *in vivo* activity of **7a**, along with its good pharmacokinetics profile, supports the potential of PDE5Is for the treatment of AD and encourages us to pursue the drug optimization of this class of molecules.

4. Experimental Section

4.1 Chemistry

4.1.1 Materials and methods—Solvents and reagents were obtained from commercial suppliers and were used without further purification. Flash chromatography purification was performed on a Merck silica gel 60 0.040–0.063 mm (230–400 mesh) stationary phase. ¹H NMR and ¹³C NMR spectra were recorded using Varian INOVA (300 MHz for ¹H and 75 MHz for ¹³C) and Agilent-NMR-vnmrs400 (400 MHz for ¹H) spectrometers in CDCl₃, DMSO-d₆ or Acetone-d₆. TMS was used as an internal standard. Coupling constants (*J*) are reported in hertz. Thin-layer chromatography (TLC) was performed on silica gel plates with a fluorescence indicator of F254 (0.2 mm, Merck); the spots were visualized by UV light. The mass spectra were recorded on Shimadzu LCMS-2010A Liquid Chromatography Mass Spectrometer. Elemental analyses were obtained by Atlantic Microlab (www.atlanticmicrolab.com).

4.1.2 Synthesis of compounds 2–5 and 6a

4.1.2.1 Diethyl 2-[(2-bromo-4-cyanophenylamino)methylene]malonate (2): Diethyl ethoxymethylenemalonate (8.23 g, 38.1 mmol) was added to a solution of **1** (5.00 g, 25.4 mmol) in 30 mL of toluene. The mixture was then heated to reflux overnight with the condenser open to the air. The resulting solution was cooled down to room temperature and poured into 100 mL of hexanes. The white precipitate was collected and washed with hexanes (3×30 mL) to yield 11.9 g of an off-white solid as the desired product. MS ESI (*m/z*) 367 (*M*+1)⁺; ¹H NMR (CDCl₃, 300 MHz): δ 11.44 (d, 1H, *J*=12.6 Hz), 8.44 (d, 1H, *J*=12.9 Hz), 7.86 (d, 1H, *J*=1.8 Hz), 7.63 (dd, 1H, *J*₁=1.8 Hz, *J*₂=8.7 Hz), 7.33 (d, 1H, *J*=8.4 Hz), 4.35 (q, 2H, *J*=6.9 Hz), 4.27 (q, 2H, *J*=6.9 Hz), 1.38 (t, 3H, *J*=6.9 Hz), 1.33 (t, 3H, *J*=6.9 Hz).

4.1.2.2 Ethyl 8-bromo-6-cyano-4-hydroxyquinoline-3-carboxylate (3): 100 mL of diphenyl ether was heated to reflux followed by addition of **2** (5.00 g, 13.6 mmol) in portions over 30 minutes. The resulting brown solution was refluxed for another hour and then cooled down to room temperature. The precipitate was collected and washed with hexanes (3×15 mL) to give 5.69 g of a light brown solid as the desired product. MS ESI (*m/z*) 321 (*M*+1)⁺; ¹H NMR (DMSO-d₆, 300 MHz): δ 11.9 (s, 1H), 8.52 (s, 1H), 8.45 (s, 1H), 8.42 (s, 1H), 4.21 (q, 2H), 1.25 (t, 3H).

4.1.2.3 Ethyl 8-bromo-4-chloro-6-cyanoquinoline-3-carboxylate (4): The mixture of **3** (4.0 g, 12.45 mmol) and 50 mL of POCl₃ was heated to reflux for 48 hrs. The solvent was removed in vacuum and co-distilled with CHCl₃ (50 mL) and toluene (2×50 mL). The resulting dark brown syrup was dissolved in 50 mL of CH₂Cl₂ and treated with Et₃N until

pH >10. The dark-red solution was then allowed to go through a silica gel pad (3 cmx4 cm). The silica pad was washed with 100 mL of CH₂Cl₂. The filtrates were collected and concentrated to obtain an off-white solid as the desired product. MS ESI (m/z) 321 (M+1)⁺; ¹H NMR (CDCl₃, 300 MHz): δ 9.41 (s, 1H), 8.78 (s, 1H), 8.31 (s, 1H), 4.52 (q, 2H, J=6.9 Hz), 1.47 (t, 3H, J=6.9 Hz).

4.1.2.4 Ethyl 8-bromo-4-[(3-chloro-4-methoxybenzyl)amino]-6-cyanoquinoline-3-carboxylate (5): Compound **4** (4.5 g, 13.2 mmol), 3.12 g of (3-chloro-4-methoxyphenyl)methanamine hydrochloride (15 mmol), and 7.74 g of diisopropylethylamine were dissolved in 50 mL of n-propanol. The resulting mixture was refluxed for 2.5 hrs and then poured to 100 ml of ice-water. The precipitate was collected by filtration and washed with H₂O (2x30 mL) and ethanol (2x30 mL) to give 5.0 g of a yellow solid as the title compound. MS ESI (m/z) 474 (M+1)⁺, ¹H NMR (CDCl₃, 300 MHz): δ 9.89 (bs, 1H), 9.32 (s, 1H), 8.49 (d, 1H, J=1.5 Hz), 8.15 (d, 1H, J=1.8 Hz), 7.39 (d, 1H, J=2.1 Hz), 7.25 (dd, 1H, J₁=2.1 Hz, J₂=8.1 Hz), 6.97 (d, 1H, J=8.7 Hz), 4.87 (d, 2H, J=5.4 Hz), 4.38 (q, 2H, J=7.2 Hz), 3.92 (s, 3H), 1.40 (t, 3H, J=7.2 Hz).

4.1.2.5 Ethyl 4-[(3-chloro-4-methoxybenzyl)amino]-6-cyano-8-cyclopropylquinoline-3-carboxylate (6a): To a mixture of **5** (475 mg, 1 mmol) in 5 mL of toluene anhydrous 129 mg of cyclopropylboronic acid (1.5 mmol), 58 mg of tetrakis(triphenylphosphine)palladium(0) (0.05 mmol) and 815 mg of Cs₂CO₃ (2.5 mmol) were added. The mixture was refluxed overnight under nitrogen and then the precipitate was removed by filtration. The filtrate was concentrated and purified by flash chromatography (Hex:AcOEt 4:1) to yield 366 mg of a yellow solid as the desired compound. MS ESI (m/z) 436 (M+1)⁺, ¹H NMR (CDCl₃, 400 MHz): δ 9.56 (t, 1H, J=5.6 Hz), 9.27 (s, 1H), 8.30 (d, 1H, J=1.6 Hz), 7.38 (d, 1H, J=2.0 Hz), 7.24–7.22 (m, 2H), 6.95 (d, 1H, J=8.4 Hz), 4.83 (d, 2H, J=5.6 Hz), 4.37 (q, 2H, J=7.2 Hz), 3.91 (s, 3H), 3.12–3.05 (m, 1H), 1.39 (t, 3H, J=7.2 Hz), 1.21–1.16 (m, 2H), 0.82–0.77 (m, 2H).

4.1.3 General synthetic procedure for Buchwald-Hartwing coupling reaction (6b–6f)—To a solution of quinoline bromide **5** (1.0 mmol) in toluene anhydrous was added palladium (II) acetate (0.02 mmol), (*R*)-BINAP (0.01 mmol), Cs₂CO₃ (0.5 mmol), and the amine (0.5 mmol). After the mixture was refluxed overnight and under nitrogen, the precipitate was removed by filtration. The filtrate was concentrated and purified by flash chromatography.

4.1.3.1 Ethyl 4-[(3-chloro-4-methoxybenzyl)amino]-6-cyano-8-(*N,N*-dimethylamino)quinoline-3-carboxylate (6b): Flash chromatography (Hex:AcOEt 2:1); yellow solid, yield: 32%; MS ESI (m/z) 439 (M+1)⁺; ¹H NMR (CDCl₃, 300 MHz): δ 9.42 (t, 1H, J=5.4 Hz), 9.16 (s, 1H), 8.00 (d, 1H, J=1.5 Hz), 7.38 (d, 1H, J=2.1 Hz), 7.27–7.23 (m, 1H), 7.12 (d, 1H, J=1.8 Hz), 6.96 (d, 1H, J=8.4 Hz), 4.81 (d, 2H, J=5.7 Hz), 4.38 (q, 2H, J=7.2 Hz), 3.92 (s, 3H), 3.09 (s, 6H), 1.41 (t, 3H, J=7.2 Hz).

4.1.3.2 Ethyl 4-[(3-chloro-4-methoxybenzyl)amino]-6-cyano-8-(*N,N*-dimethylethane-1,2-diamino)quinoline-3-carboxylate (6c): Flash chromatography (AcOEt:MeOH 10:1); yellow solid, yield: 60%; MS ESI (m/z) 482 (M+1)⁺; ¹H NMR (CDCl₃, 400 MHz): δ 9.47 (t, 1H, J=5.2 Hz), 9.06 (s, 1H), 7.69 (d, 1H, J=1.6 Hz), 7.37 (d, 1H, J=2.4 Hz), 7.24–7.22 (m, 1H), 6.94 (d, 1H, J=8.4 Hz), 6.69 (t, 1H, J=4.8 Hz), 6.66 (d, 1H, J=1.6 Hz), 4.85 (d, 2H, J=5.2 Hz), 4.34 (q, 2H, J=7.2 Hz), 3.90 (s, 3H), 3.30 (q, 2H, J=5.8 Hz), 2.67 (t, 2H, J=6.2 Hz), 2.31 (s, 6H), 1.38 (t, 3H, J=7.2 Hz).

4.1.3.3 Ethyl 4-[(3-chloro-4-methoxybenzyl)amino]-6-cyano-8-(ethylamino)quinoline-3-carboxylate (6d): Flash chromatography (Hex:AcOEt 2:1); yellow solid, yield: 76%; MS ESI (m/z) 439 (M+1)⁺; ¹H NMR (CDCl₃, 300 MHz): δ 9.50 (t, 1H, *J*=5.4 Hz), 9.02 (s, 1H), 7.68 (d, 1H, *J*=1.5 Hz), 7.38 (d, 1H, *J*=2.4 Hz), 7.23 (d, 1H, *J*=2.1 Hz), 6.95 (d, 1H, *J*=8.4 Hz), 6.66 (s, 1H), 6.29 (t, 1H, *J*=5.1 Hz), 4.85 (d, 2H, *J*=5.7 Hz), 4.36 (q, 2H, *J*=7.2 Hz), 3.92 (s, 3H), 3.28 (m, 2H), 1.39 (t, 6H, *J*=7.2 Hz).

4.1.3.4 Ethyl 4-[(3-chloro-4-methoxybenzyl)amino]-6-cyano-8-(N-cyclopropylamino)quinoline-3-carboxylate (6e): Flash chromatography (Hex:AcOEt 8:2); yellow solid, yield: 80%; MS ESI (m/z) 451 (M+1)⁺; ¹H NMR (CDCl₃, 300 MHz): δ 9.53 (t, 1H, *J*=5.1 Hz), 8.99 (s, 1H), 7.74 (s, 1H), 7.38 (d, 1H, *J*=1.8 Hz), 7.23 (s, 1H), 7.12 (s, 1H), 6.95 (d, 1H, *J*=8.4 Hz), 6.59 (s, 1H), 4.85 (d, 2H, *J*=5.4 Hz), 4.35 (q, 2H, *J*=7.2 Hz), 3.91 (s, 3H), 2.53–2.50 (m, 1H), 1.39 (3H, *J*=7.2 Hz), 0.89–0.83 (m, 2H), 0.66–0.61 (m, 2H).

4.1.3.5 Ethyl 4-[(3-chloro-4-methoxybenzyl)amino]-6-cyano-8-(morpholin-4-yl)quinoline-3-carboxylate (6f): Flash Chromatography (Hex:AcOEt 8:2); yellow solid; yield: 70%; MS ESI (m/z) 481(M+1)⁺; ¹H NMR (CDCl₃, 300 MHz): δ 9.49 (t, 1H, *J*=5.1 Hz), 9.15 (s, 1H), 8.08 (s, 1H), 7.36 (s, 1H, *J*=2.4 Hz), 7.22 (dd, 1H, *J*₁=2.4 Hz, *J*₂=8.7 Hz), 7.15 (s, 1H), 6.95 (d, 1H, *J*=8.7 Hz), 4.80 (d, 2H, *J*=5.4 Hz), 4.35 (q, 2H, *J*=7.2 Hz), 3.99 (s, 4H), 3.90 (s, 3H), 3.35 (s, 4H), 1.38 (t, 3H, *J*=7.2 Hz).

4.1.4 General procedure for the reduction of 3-ethylquinoline ester (7a–7f)—

Lithium tri(*tert*-butoxy) aluminum hydride 1M sol. in THF (2.2 mmol) was added to a solution of 3-ethyl-quinoline ester (0.43 mmol) in THF anhydrous. The resulting solution was refluxed overnight and then quenched with 1 mL of methanol and stirred for 30 minutes at room temperature. The mixture was poured into a separatory funnel, followed by addition of 150 mL of CH₂Cl₂ and 50 mL of 1N NaOH, the organic layer was separated, washed with 1N NaOH (50 mL) and dried over MgSO₄. The solid was filtered off and the filtrate was concentrated to give the final compound.

4.1.4.1 4-[(3-chloro-4-methoxybenzyl)amino]-8-cyclopropyl-3-(hydroxymethyl)quinoline-6-carbonitrile (7a): White solid, yield: 62%; MS ESI (m/z) 394 (M+1); ¹H NMR (DMSO-d₆, 300 MHz): δ 8.69 (d, 1H, *J*=1.2), 8.48 (s, 1H), 7.42 (t, 1H, *J*=7 Hz), 7.37 (d, 1H, *J*=2.1 Hz), 7.33 (d, 1H, *J*=1.2 Hz), 7.21 (dd, 1H, *J*₁=8.4 Hz, *J*₂=2.1 Hz), 7.08 (d, 1H, *J*=8.4 Hz), 5.38 (t, 1H, *J*=5.1 Hz), 4.79 (d, 2H, *J*=7 Hz), 4.43 (d, 2H, *J*=5.1 Hz), 3.79 (s, 3H), 3.09–3.14 (m, 1H), 1.02–1.08 (m, 2H), 0.72–0.87 (m, 2H); ¹³C NMR (CDCl₃, 75 MHz) 154.01, 150.61, 149.30, 148.67, 145.00, 134.76, 128.44, 126.72, 123.09, 121.62, 120.26, 120.09, 116.56, 113.56, 109.98, 107.05, 107.05, 56.88, 51.45, 48.40, 10.97. Anal. Calcd. for C₂₂H₂₀ClN₃O₂: C, 67.09; H, 5.12; N, 10.67. Found: C, 66.92; H, 5.06; N, 10.58.

4.1.4.2 4-[(3-chloro-4-methoxybenzyl)amino]-8-(N,N-dimethylamino)-3-(hydroxymethyl)quinoline-6-carbonitrile (7b): Yellow solid, yield: 27%; MS ESI (m/z) 397 (M+1); ¹H NMR (DMSO-d₆, 400 MHz): δ 8.37 (s, 1H), 8.28 (d, 1H, *J*=1.2 Hz), 7.34 (d, 1H, *J*=2.4 Hz), 7.20–7.15 (m, 2H), 7.07–7.05 (m, 2H), 5.31 (t, 1H, *J*=5.2 Hz), 4.72 (d, 2H, *J*=6.8 Hz), 4.41 (d, 2H, *J*=5.2 Hz), 3.77 (s, 3H), 2.99 (s, 6H). Anal. Calcd. for C₂₁H₂₁ClN₄O₂: C, 63.55; H, 5.33; N, 14.12. Found: C, 63.42; H, 5.41; N, 13.55.

4.1.4.3 4-[(3-chloro-4-methoxybenzyl)amino]-8-(N,N-dimethylethane-1,2-diamino)-3-(hydroxymethyl)quinoline-6-carbonitrile (7c): Yellow solid, yield: 50%; MS ESI (m/z) 440 (M+1)⁺; ¹H NMR (DMSO-d₆, 400 MHz): δ 8.28 (s, 1H), 7.93 (s, 1H), 7.32 (d, 1H, *J*=2.0 Hz), 7.22 (t, 1H, *J*=7.0 Hz), 7.17 (dd, 1H, *J*₁=2.2 Hz, *J*₂=8.6 Hz), 7.05 (d, 1H, *J*=8.4 Hz), 6.72 (t, 1H, *J*=5.2 Hz), 6.67 (s, 1H), 5.30 (t, 1H, *J*=5.0 Hz), 4.74 (d, 2H, *J*=6.8 Hz), 4.37

(d, 2H, $J=5.2$ Hz), 3.76 (s, 3H), 3.23 (q, 2H, $J=5.6$ Hz), 2.51 (t, 2H, $J=6.2$ Hz), 2.16 (s, 6H). Anal. Calcd. for $C_{23}H_{26}ClN_5O_2 \cdot \frac{1}{2} H_2O$: C, 61.53; H, 6.06; N, 15.60. Found: C, 61.57; H, 6.02; N, 14.95.

4.1.4.4 4-[(3-chloro-4-methoxybenzyl)amino]-8-ethylamino-3-(hydroxymethyl)quinoline-6-carbonitrile (7d): Yellow solid, yield: 72%; MS ESI (m/z) 497 ($M+1$)⁺; ¹H NMR (DMSO- d_6 , 400 MHz): δ 8.27 (s, 1H), 7.91 (d, 1H, $J=1.2$ Hz), 7.32 (d, 1H, $J=2.4$ Hz), 7.22–7.15 (m, 2H), 7.05 (d, 1H, $J=8.8$ Hz), 6.64 (d, 1H, $J=1.2$ Hz), 6.57 (t, 1H, $J=5.6$ Hz), 5.30 (t, 1H, $J=4.8$ Hz), 4.73 (d, 2H, $J=7.2$ Hz), 4.38 (d, 2H, $J=5.2$ Hz), 3.76 (s, 3H), 3.22 (m, 2H), 1.99 (t, 3H, $J=7.2$ Hz). Anal. Calcd. for $C_{21}H_{21}ClN_4O_2 \cdot \frac{1}{2} H_2O$: C, 63.55; H, 5.33; N, 14.12. Found: C, 62.75; H, 5.28; N, 13.43.

4.1.4.5 4-[(3-chloro-4-methoxybenzyl)amino]-8-(N-cyclopropylamino)-3-(hydroxymethyl)quinoline-6-carbonitrile (7e): Yellow solid, yield: 85%; MS ESI (m/z) 409 ($M+1$)⁺; ¹H NMR ((CD_3)₂CO, 400 MHz) δ 8.38 (s, 1H), 7.87 (s, 1H), 7.44 (d, 1H, $J=2.4$ Hz), 7.33–7.30 (m, 1H), 7.07 (d, 1H, $J=8.8$ Hz), 7.01 (s, 1H), 6.77 (br s, 1H), 6.59 (t, 1H, $J=6.8$ Hz), 4.91 (d, 2H, $J=7.2$ Hz), 4.68 (d, 2H, $J=5.6$ Hz), 4.45 (t, 1H, $J=5.6$ Hz), 3.87 (s, 3H), 2.61–2.56 (m, 1H), 0.90–0.86 (m, 2H), 0.63–0.60 (m, 2H). Anal. Calcd. for $C_{22}H_{21}ClN_4O_2$: C, 64.62; H, 5.18; N, 13.70. Found: C, 64.08; H, 5.44; N, 13.72.

4.1.4.6 4-[(3-chloro-4-methoxybenzyl)amino]-8-(morpholin-4-yl)-3-(hydroxymethyl)quinoline-6-carbonitrile (7f): Yellow solid, yield: 70%; MS ESI (m/z) 439 ($M+1$)⁺; ¹H NMR (DMSO- d_6 , 400 MHz): δ 8.40 (s, 1H), 8.37 (s, 1H), 7.33 (d, 1H, $J=2.0$ Hz), 7.25 (t, 1H, $J=6.6$ Hz), 7.18–7.14 (m, 2H), 7.05 (d, 1H, $J=8.8$ Hz), 5.31 (t, 1H, $J=5.0$ Hz), 4.72 (d, 2H, $J=6.8$ Hz), 4.38 (d, 2H, $J=5.2$ Hz), 3.76 (s, 7H), 3.30–3.29 (m, 4H). Anal. Calcd. for $C_{23}H_{23}ClN_4O_3$: C, 62.94; H, 5.28; N, 12.77. Found: C, 62.95; H, 5.50; N, 12.10.

4.2 PDE Enzyme Assay Procedure

The enzymatic reactions were conducted at room temperature for 60 minutes in a 50 μ l volume containing PDE assay buffer, 100 nM FAM-cGMP or FAM-cAMP substrate, 0.125 ng PDE, and the test compound. After the enzymatic reaction, 100 μ l of a binding solution (1:100 dilution of the binding agent with the binding agent diluent) was added to each sample. After 60 minutes at room temperature fluorescence intensity was measured at an excitation of 485 nm and an emission of 528 nm using a Tecan Infinite M1000 microplate reader.

4.3 Hippocampal cGMP levels

2–3 month old male and female mice (20–25 g; C57Bl6 mice) were injected with **7a** (3 mg/kg, 2% DMSO & 2% Tween, i.p.) or Vehicle (2% DMSO & 2% Tween, i.p.). 30 min after administration of vehicle or **7a**, mice were subjected to foot shock and sacrificed 10 sec, 1 min and 3 min after shock by cervical dislocation and decapitation. The hippocampal samples were extracted and snap frozen in liquid nitrogen. Levels of cGMP were quantitated by Enzyme Immunoassay procedure (Cayman Chemical Company, Item no. 581021) following the manufacturer's guidelines in duplicate. cGMP levels were normalized with the protein concentration calculated using BCA Protein Assay Reagent (Thermo Scientific).

4.4 Pharmacokinetics

To determine the time course of compound **7a** action in the brain, we investigated the plasma pharmacokinetics and BBB penetration capability of the inhibitor. In these experiments, **7a** was administered to mice p.o. at a dosage of 50 mg/kg. Blood and brain samples were collected at six time points (0, 0.25, 0.5, 1.0, 2.0, and 4.0 h) from three

animals at each time point. For plasma measurements, blood (approximately 250 μ l) was collected via retro-orbital puncture into tubes containing sodium heparin anticoagulant. Plasma was separated via centrifugation (4°C, 3500 rpm, 10 min) and stored at -80°C. At the time of measurement, frozen plasma samples were thawed at room temperature and vortexed thoroughly. Plasma (25 μ l) was transferred into a 1.5 ml Eppendorf tube. To each sample, 25 μ l of methanol and 25 μ l of the internal standard were added, followed by the addition of 100 μ l of methanol. The sample mixture was vortexed for approximately 1 min. After centrifugation at 11,000 g for 5 min, the upper organic layer was transferred to a glass tube and evaporated at 40°C under a gentle stream of nitrogen. Residues were dissolved in 150 μ l of the mobile phase and mixed using a Vortex mixer. A 20 μ l aliquot of the resulting solution was injected onto the liquid chromatography/tandem mass spectrometry (LC/MS/MS) system for analysis. For measurement of brain concentrations, mice were killed by cervical dislocation after blood harvest. Brains were immediately excised, weighed, and rinsed by cold saline and then frozen at -80°C until further processing for LC/MS/MS analysis. On the day of the assay, frozen tissue samples were thawed unassisted at room temperature. When completely thawed, each tissue sample of 200 mg was weighed and placed into a plastic tube. Methanol (1.0 ml) was added and homogenization conducted using a Fluko F6/10 superfine homogenizer for approximately 1 min. Then, the samples were vortexed for 1 min and a 25 μ l aliquot was transferred into an Eppendorf tube. To each sample, 25 μ l of methanol and 25 μ l of the internal standard were added and the samples centrifuged at 11,000 g for 5 min. A 20 μ l aliquot of the supernatants was diluted to 60 μ l with the mobile phase, and a 10 μ l aliquot was injected onto the LC/MS/MS system for analysis. Quantification of the drug concentration in each aliquot was achieved by the internal standard method using peak area ratios of the analyte to the internal standard in plasma and brain. Concentrations were calculated using a weighted least-squares linear regression ($W = 1/\times 2$).

4.5 Cannula Infusion techniques

Following anaesthesia with 20 mg/kg Avertin, mice were implanted with 26-gauge guide cannulas into the dorsal part of the hippocampi (coordinates: P=2.46 mm, L=1.50 mm to a depth of 1.30 mm) [40]. The cannulas were fixed to the skull with acrylic dental cement (made from Paladur powder). After 6–8 days, mice were bilaterally infused with A β (200 nM) or vehicle in a final volume of 1 μ l over 1 min with a microsyringe connected to the cannulas via polyethylene tubing.

4.6 Electrophysiological studies

Transverse hippocampal slices (400 μ m) were cut with a tissue chopper (EMS, PA) and maintained in an interface chamber at 29 °C for 90 min prior to recording, as previously described [14]. The extracellular bath solution consisted of 124.0 mM NaCl, 4.4 mM KCl, 1.0 mM Na₂HPO₄, 25.0 mM NaHCO₃, 2.0 mM CaCl₂, 2.0 mM MgSO₄, and 10.0 mM glucose, continuously aerated with 95% O₂/5% CO₂ to a final pH of 7.4. Field extracellular postsynaptic responses (fEPSPs) were recorded by placing the stimulating and recording electrodes in CA1 stratum radiatum. A bipolar tungsten electrode (FHC, Bowdoin, ME) was used as a stimulating electrode, and a glass pipette filled with bath solution was used as a recording electrode. Basal synaptic transmission was first assessed by plotting the stimulus voltages (V) against slopes of fEPSP to generate input-output relations. A 15 min baseline was first recorded every minute at an intensity that evoked a response at approximately 35% of the maximum evoked response. LTP was induced using a theta-burst stimulation (4 pulses at 100 Hz, with the bursts repeated at 5 Hz, and each tetanus consisting of 3 ten-burst trains separated by 15 sec). Responses were measured as fEPSP slopes expressed as percentage of baseline.

4.7 Behavioral Studies

Fear conditioning was assessed as previously described [37, 38, 41]. First, sensory perception of electric foot shock was examined in different groups of mice through the threshold assessment test. Briefly, animals were placed in the conditioning chamber and the electric current (0.1 mA for 1 sec) was increased at 30 s intervals from 0.1 mA to 0.7 mA. Threshold to flinching (first visible response to shock), jumping (first extreme motor response), and vocalized response were quantified for each animal by averaging the shock intensity at which each animal showed the behavioral response to that type of shock. Training of fear conditioning was performed by placing the mouse in a conditioning chamber for 2 min before the onset of a tone (Conditioned Stimulus (CS), 30 sec, 85 dB sound at 2800 Hz). In the last 2 sec of the CS, mice were given a 2 sec, 0.7 mA mild foot shock (Unconditioned Stimulus, (US)) through the bars of the floor. After the US, the mice were left in the chamber for another 30 s. Freezing behavior, defined as the absence of movements except for respiratory excursions, was scored using Freezeview software (Med Associates, St. Albans, VT). Contextual fear learning was evaluated 24 hrs after training by measuring freezing responses for 5 min in the same chamber where the mice were trained. Cued fear learning was evaluated 24 hrs after contextual testing. The mice were placed in a novel context for 2 min (pre-CS test), after which they were given a CS for 3 min (CS test), and freezing behavior was measured during the first 30 sec that mimic the CS-US conditioning and the remaining 2.5 min.

The radial arm water maze task, a hybrid of the classic Morris Water Maze and the radial arm land maze, was performed as previously described [39]. The mouse had to swim in 6 alleys (arms) radiating from a central area until it found a hidden (submerged) platform at the end of one of the arms, based on visual cues placed in the room. The first day of the protocol was a training day on which mice were trained to identify the platform location by alternating between a visible and a hidden platform in a goal arm. The final 3 trials on that day and all 15 trials on day 2 used a hidden escape platform to force mice to use spatial cues to identify the location of the goal arm. To avoid learning limitations imposed by exhausting practice and to avoid fatigue that may result from consecutive trials, spaced practice training was established by running the mice in cohorts of 4 and alternating different cohorts through the 15 training trials over 3-hour testing periods each day. The number of incorrect arm entries (entries to arms with no platform) was counted. If the animal entered the incorrect arm it was gently pulled back to the start arm. Failure to select an arm after 15 sec was counted as an error and the mouse was returned to the start arm. Each trial lasted up to 1 min. After 1 min, if the platform had not been located, the mouse was guided gently through the water by placing a hand behind it to direct it towards the platform. The mouse rested on the platform for 15 sec. The goal platform location was different for each mouse. On day 2, the same procedure was repeated as on day 1 for all 15 trials using only the hidden platform. For data analysis, averages for each mouse were calculated using blocks of 3 trials.

4.8 Statistical Analyses

Experiments were performed in blind. Results were expressed as Standard Error of the Mean (SEM). Level of significance was set for $p < 0.05$. Results were analyzed by student t-test/2-way ANOVA for repeated measures. Planned comparisons were used for post-hoc analysis.

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- Synthesis and PDE5 inhibitory activity of a series of quinoline derivatives.
- Compound 7a showed an excellent activity and selectivity on PDE5.
- Compound 7a rescues synaptic and memory defects in mouse models of Alzheimer's disease.

Figure 1. PDE5 inhibitors: A) cGMP-based derivatives; B) β -carbolines-derived molecules; C) pyrazolopyridine, phthalazine, pyrazolopyridopyridazine and quinoline derivatives; D) isoquinolinone, naphthyridinone and pyridopyrazinone derivatives.

Figure 2. Hippocampal cGMP levels measurement. Concentration of cGMP was measured by enzyme immunoassay. Basal represents cGMP levels without foot shock. Values are the mean of duplicate determinations. Error bars show S.E.M. (n=3 per group); *p<0.01.

Figure 3.
Concentration-Time curve of **7a** in mouse brain tissue and plasma (n=3 mice per group).

Figure 4. Beneficial effect of compound **7a** on Aβ₄₂-induced synaptic and cognitive dysfunction. **A)** **7a** (50 nM through the bath solution for 10 min prior to the θ-burst) ameliorates the LTP deficit in Aβ₄₂-treated slices. The graph represents the average of the last 5 min of recording at 60 min after the tetanus; n represents the number of slices per group. **B)** **7a** (3 or 10mg/Kg, p.o., immediately after the electric shock in the behavioral experiments) ameliorates the contextual fear memory deficit in Aβ₄₂-infused mice; n represents the number of mice per group.

Figure 5.

Beneficial effect of compound **7a** on synaptic and cognitive deficits in APP/PS1 mice. **A)** Residual potentiation recorded during the last 5 minutes of a 2 hr recording following tetanic stimulation of the Schaffer collateral fibers at the CA3-CA1 connection. Compound **7a** (50 nM through the bath solution for 10 min prior to the θ -burst) rescued the LTP defect in transgenic slices, whereas had no effect onto WT slices. **B)** Daily treatment with compound **7a** (3mg/kg, i.p.) for 3 weeks at the age of 3–4 months re-established normal freezing in a test for contextual fear memory. **C)** Daily treatment with compound **7a** (3mg/kg, i.p.) for 3 weeks at the age of 3–4 months reduced the number of errors with the 2-day radial arm water maze. **D)** Residual potentiation recorded during the last 5 minutes of a 2 hr recording following tetanic stimulation of the Schaffer collateral fibers at the CA3-CA1 connection. Daily treatment with compound **7a** (3mg/kg, i.p.) for 3 weeks at the age of 3–4 months re-established normal potentiation when slices were recorded at 6–7 months of age. **E)** Daily treatment with compound **7a** (3mg/kg, i.p.) for 3 weeks at the age of 3–4 months re-established normal freezing in a test for contextual fear memory when mice were examined at 6–7 months of age. **F)** Daily treatment with compound **7a** (3mg/kg, i.p.) for 3 weeks at the age of 3–4 months reduced the number of errors with the 2-day radial arm water maze when mice were examined at 6–7 months of age.

Scheme 1.

Synthesis of compounds **7a–f**. Reagents and conditions: (i) diethyl ethoxymethylenemalonate, toluene, reflux, overnight; (ii) Ph_2O , reflux, 2 h; (iii) POCl_3 , reflux, 48 h; (iv) (3-chloro-4-methoxyphenyl)methanamine hydrochloride, DIPEA, n-propanol, 2.5 h; (v) cyclopropylboronic acid, $\text{Pd(PPh}_3)_4$, Cs_2CO_3 , toluene, reflux, overnight; or amines, Pd(OAc)_2 , (*R*)-BINAP, Cs_2CO_3 , toluene, reflux, overnight; (vi) $\text{LiAlH(O}^t\text{Bu)}_3$ in THF, reflux, overnight.

Table 1PDE5 inhibitory activity of compounds **7a–f** compared to sildenafil.

Compd	R8	PDE5 IC ₅₀ (nM)
7a		0.27
7b		0.40
7c		4.3
7d		15.0
7e		4.1
7f		0.63
Sildenafil		3.4

Table 2

PDE5 selectivity of compounds **7a** and **7b** compared to sildenafil, vardenafil and tadalafil.

Compd	PDE1	PDE2	PDE3	PDE4	PDE5	PDE6	PDE7	PDE8	PDE9	PDE10	PDE11
7a ^a	IC50 (nM)	>10 ⁴	>10 ⁴	>10 ⁴	0.27	339	>10 ⁴	>10 ⁴	>10 ⁴	>10 ⁴	>10 ⁴
	PDEX/PDE5	>10 ⁴	>10 ⁴	>10 ⁴	1	1256	>10 ⁴	>10 ⁴	>10 ⁴	>10 ⁴	>10 ⁴
7b ^a	IC50 (nM)	>10 ⁴	>10 ⁴	>10 ⁴	0.40	5100	>10 ⁴	>10 ⁴	>10 ⁴	>10 ⁴	>10 ⁴
	PDEX/PDE5	>10 ⁴	>10 ⁴	>10 ⁴	1	12750	>10 ⁴	>10 ⁴	>10 ⁴	>10 ⁴	>10 ⁴
Sildenafil ^b	IC50 (nM)	1500	>10 ⁴	>10 ⁴	2.20	9.5 ^d	>10 ⁴	>10 ⁴	5600	6800	6100
	PDEX/PDE5	682	>10 ⁴	>10 ⁴	1	4	>10 ⁴	>10 ⁴	2545	3091	2773
Vardenafil ^b	IC50 (nM)	300	3100	580	1.0	11.0 ^c	1900	>10 ⁴	680	880	240
	PDEX/PDE5	300	3100	580	1	11	1900	>10 ⁴	680	880	240
Tadalafil ^b	IC50 (nM)	>10 ⁴	>10 ⁴	>10 ⁴	1.2	5200 ^d	>10 ⁴	>10 ⁴	>10 ⁴	>10 ⁴	10
	PDEX/PDE5	>10 ⁴	>10 ⁴	>10 ⁴	1	4333	>10 ⁴	>10 ⁴	>10 ⁴	>10 ⁴	8

^aData obtained by BPS Bioscience, CA, USA;

^bG.L Card *et al.* Structural Basis for the Activity of Drugs that Inhibit Phosphodiesterases, Structure 12 (2004) 2233–2247;

^cI. Saenz de Tejada *et al.* The phosphodiesterase inhibitory selectivity and the in vitro and in vivo potency of the new PDE5 inhibitor vardenafil, Intern. J. Impot. Res. 13 (2001) 282–290;

^dA. Daugan *et al.* The Discovery of Tadalafil, A Novel and Highly Selective PDE5 Inhibitor. 2: 2,3,6,7,12,12a-hexahydropyrazino[1',2':1,6]pyrido[3,4-b]indole-1,4-dione Analogues, J. Med. Chem. 46 (2003)4533–4542.

Table 3Pharmacokinetic parameters of compound **7a** in mouse brain tissue and plasma.

Parameters		Compound 7a		
		Brain	Plasma	Ratio [*]
T_{max}	(h)	0.5	0.5	-
C_{max}	(ng/mL or ng/g)	385	1022	0.38
AUC_{0-t}	(ng·h/mL or ng·h/g)	418	1014	0.41
AUC_{0-∞}	(ng·h/mL or ng·h/g)	420	1133	0.37
T_{1/2}	(h)	1.04	1.33	-
MRT	(h)	1.66	1.61	-

^{*} Ratio= brain/plasma; 50 mg/kg, p.o.; vehicle for **7a** is 0.05% methylcellulose aqueous solution; AUC: Area under Curve; MRT: Mean Residence Time