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Novel PDE5 inhibitors derived from rutaecarpine for the treatment of Alzheimer's disease

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ABSTRACT

A series of novel rutaecarpine derivatives were synthesized and subjected to pharmacological evaluation as PDE5 inhibitors. The structure–activity relationships were discussed and their binding conformation and simultaneous interaction mode were further clarified by the molecular docking studies. Among the 25 analogues, compound **8i** exhibited most potent PDE5 inhibition with IC_{50} values about 0.086 μM. Moreover, it also produced good effects against scopolamine-induced cognitive impairment *in vivo*. These results might bring significant instruction for further development of potential PDE5 inhibitors derived from rutaecarpine as a good candidate drug for the treatment of Alzheimer's disease.

Alzheimer's disease (AD), being a long-term and incurable neurodegenerative brain disorder, poses a major health problem worldwide.[1] As the most common form of dementia, AD is characterized by aggregation of amyloid β peptides (Aβ) into fibrillar plaques that lead to memory loss.[2,3] Currently, many hypotheses, including low levels of acetylcholine, the deposition of Aβ plaques and the aggregation of tau protein, etc., have been proposed to explain the pathophysiology of AD.[4] However, the pathogenic mechanism of AD is still not fully understood. Consequently, there is no ideal drugs for the treatment of AD, and only a few anti-AD drugs such as acetylcholine esterase inhibitors (donepezil, rivastigmine, and galantamine), NMDA (*N*-methyl-D-aspartate) receptor antagonists (memantine) have been used clinically, which only achieve limited treatment effects accompanied by a lot of side effects in AD patients.[5] Thus, the search for AD drugs remains an urgent issue in the pharmaceutical community.

Phosphodiesterases (PDEs), being responsible for the hydrolysis of two second messengers, cyclic AMP (cAMP) and cyclic GMP (cGMP), can affect neuronal cell survival, and they may play a vital part in neurodegenerative diseases, such as AD, Parkinson's disease and Huntington's disease, etc.[6] It has been reported that different inhibitors of PDE subtypes such as PDE3, PDE4, PDE5 and PDE9 showed significant memory-enhancing effects *in vitro* and *in vivo*.[7–11] Of all the PDE subtypes, PDE5 specifically hydrolyzes cGMP, which is widely distributed in vascular smooth muscle cells, lungs, platelets, and corpus cavernosum. PDE5 inhibitors are used to regulate many biological processes.[12] In fact, several PDE5 inhibitors have been approved as therapeutics for the treatment of various diseases. Currently, various chemical structures of PDE5 inhibitors including cGMP-based derivatives, β-carboline-derived molecules, quinoline derivatives, isoquinolinone derivatives and pyridopyrazinone derivatives have been reported.[13–17] The most well-known drugs are sildenafil, vardenafil, tadalafil and avanafil, which were approved by the FDA for the treatment of male erectile dysfunction (Viagra) and pulmonary hypertension (Revatio).[18] However, an upregulation of PDE5 expression was also found in the brains of mild AD patients, and recent studies have demonstrated that several PDE5 inhibitors possess a potential therapeutic effect on AD by reversing cognitive impairment and improving learning and memory.[19–23]

Rutaecarpine [Fig. 1], a major quinazolinocarboline alkaloid isolated from Evodia rutaecarpa, exerts extensive biological and pharmacological activities such as vasodilation, anti-inflammation and neuroprotectiveeffects.[24,25] Considering quinoline structures have demonstrated to be potent PDE5 inhibitors, we decided to use rutaecarpine to develop PDE5 inhibitors for the treatment of AD. In this study, a series of novel PDE5 inhibitors based on rutaecarpine were synthesized, and tested with PDE5 inhibitory activities and memory and cognitive function *in vivo*.

The preparation of a series of rutaecarpine derivatives **6a**-**6e**, **7a**-**7j**

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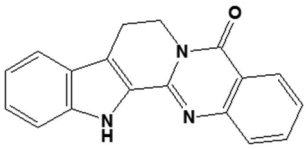


Fig. 1. The structure of rutaecarpine.

and **8a**-**8j** was accomplished using the general methods outlined in Scheme 1..[26,27] The reaction of anthranilamide (**1**) with 5-chlorovaleryl chloride in CH_2Cl_2 at ice water furnished the corresponding acid amide **2**, which reacted with two equivalents of potassium *tert*-butoxide in THF led to two steps of cyclisation and afford **3** and **4**, respectively. The mackinazolinone **4** reacted with in situ generated diazonium salts of substituted benzenamines at −5 to 5 °C and gave the corresponding hydrazone **5**. The Fischer-indole reaction of hydrazone **5** in refluxing glacial acetic acid yielded compounds **6a**, **7a**, and **7f**, which was reacted with alkyl bromide in DMF to afford **6b**-**6e**, **7b**-**7e and 7g**-**7f**, respectively. Compounds **8a**-**8j** were obtained by the further demethylation of **7a**-**7j** using BBr_3.

Rutaecarpine and its derivatives (25 compounds) were evaluated for the PDE5 inhibitory activities. The inhibitory activities were presented as IC_{50} (μM) and the results were summarized in Table 1. The well known PDE5 inhibitor tadalafil was used as positive reference. Rutaecarpine in this study exhibited PDE5 inhibition with IC_{50} value about 1.23 μM, manifesting it is a promising candidate as PDE5 inhibitors. The following results showed that the introduction of substituted group such as methoxy and hydroxyl on phenyl ring (R^1 and R^2) of rutaecarpine could significantly influence the inhibitory activity of the derivatives. According to the screening data, we found that the OH group substitution can increase their inhibitory activity. Especially, compound **8i** showed an IC_{50} of 86 nM. On the contrary, compounds with CH_3O groups remarkably decreased the PDE5 inhibitory activities. For example, compounds **7f**-**7j** with two CH_3O groups only exhibited weak activities (IC_{50} values: 18.23–47.32 μM). The influence of substituent group on N atom (R^3) on PDE5 inhibitory activity was also evaluated. It seems that these compounds with *N*-butyl substituents and isopentenyl compounds exhibited more potent PDE5 inhibitory activities than other compounds with small substituent group. In brief, the bulk of R^3 substituents deeply influenced PDE5 inhibitory activities. Large R substituents were favorable for the activity, while small R substituents were adverse. The most potent compound **8i** was also evaluated for the PDE5 inhibitory selectivity over other PDEs (PDE2, 4, 5, 9 and 10) [Table 2]. The compound displayed > 5000-fold selectivity with IC_{50} > 500 μM against PDE2, 4, 9, and inhibited moderately PDE6 and 10 with IC_{50} values of 43.8 and 32.1 μM, respectively. It was noticed

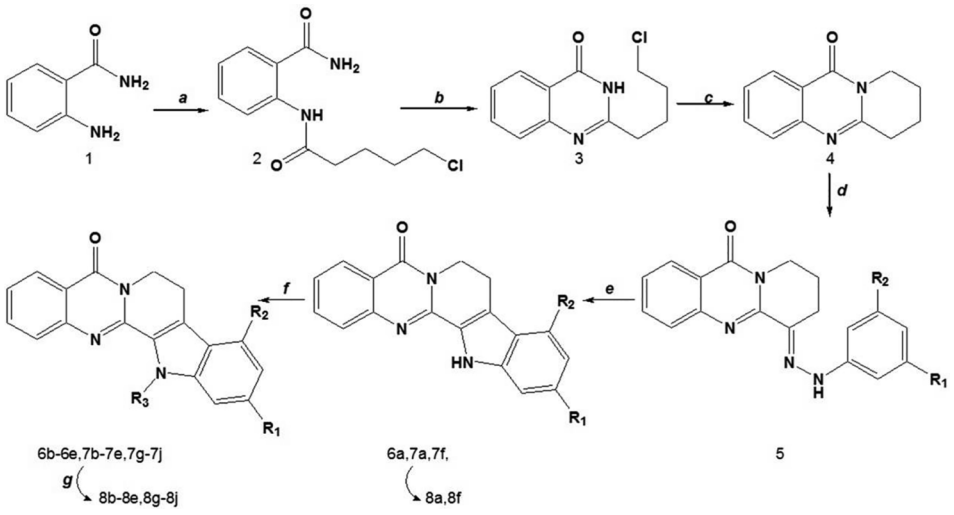
Table 1
In vitro PDE5 inhibitory activity of rutaecarpine derivatives.

Compounds	R^1	R^2	R^3	PDE5A (μM)[a]	CLogP
6a	H	H	H	1.23 ± 0.13	3.20
6b	H	H	CH_2CH_3	1.12 ± 0.09	4.09
6c	H	H	$(CH_2)_2CH_3$	1.7 ± 0.15	4.62
6d	H	H	$(CH_2)_2CH(CH_3)_2$	0.61 ± 0.07	5.54
6e	H	H	$(CH_2)_3CH_3$	1.12 ± 0.11	5.14
7a	CH_3O	H	H	22.05 ± 3.52	3.15
7b	CH_3O	H	CH_2CH_3	11.63 ± 2.02	4.12
7c	CH_3O	H	$(CH_2)_2CH_3$	12.12 ± 1.97	4.65
7d	CH_3O	H	$(CH_2)_2CH(CH_3)_2$	6.17 ± 0.77	5.58
7e	CH_3O	H	$(CH_2)_3CH_3$	9.19 ± 1.05	5.18
7f	CH_3O	CH3O	H	45.62 ± 5.84	3.13
7g	CH_3O	CH3O	CH_2CH_3	47.32 ± 4.49	4.13
7h	CH_3O	CH3O	$(CH_2)_2CH_3$	19.76 ± 3.12	4.66
7i	CH_3O	CH3O	$(CH_2)_2CH(CH_3)_2$	18.23 ± 2.59	5.59
7j	CH_3O	CH3O	$(CH_2)_3CH_3$	20.63 ± 3.76	5.19
8a	OH	H	H	1.36 ± 0.18	2.54
8b	OH	H	CH_2CH_3	0.97 ± 0.07	3.66
8c	OH	H	$(CH_2)_2CH_3$	1.33 ± 0.15	4.18
8d	OH	H	$(CH_2)_2CH(CH_3)_2$	0.11 ± 0.02	5.11
8e	OH	H	$(CH_2)_3CH_3$	0.63 ± 0.07	4.71
8f	OH	OH	H	0.79 ± 0.08	1.87
8g	OH	OH	CH_2CH_3	0.97 ± 0.11	3.06
8h	OH	OH	$(CH_2)_2CH_3$	0.33 ± 0.05	3.59
8i	OH	OH	$(CH_2)_2CH(CH_3)_2$	0.086 ± 0.009	4.52
8j	OH	OH	$(CH_2)_3CH_3$	0.26 ± 0.03	4.12
Tadalafil				0.005 ± 0.0001	
Donepezil				> 100	

[a] Results are expressed as the mean of at least three experiments.

that compound **8i** showed a very excellent selectivity over PDE6 (> 500-fold), while sildenafil has only 10-fold selectivity over PDE6, which is the main reason for its side effect of visual disturbance.[28] Furthermore, most of the derivatives have appropriate values of CLogP (2.0–5.0), indicating favorable lipophilicity that may allow blood brain barrier penetration.

To explore the interaction mode of the optimal compound **8i** with PDE5, molecular docking simulation was performed using discovery studio 2017 software based on the crystal structure of hPDE5A complexed tadalafil (PDB ID: 1UDU). The predicted binding mode of compound **8i** within the active site pockets of hPDE5A is presented in Fig. 2. In general, compound **8i** contacts with Gln817, Phe820, Met816, Gln775, Tyr 612, Leu804 and Phe786 of hPDE5A *via* three hydrogen bonds and hydrophobic interactions. In detail, the indole fragment of **8i** interacts with the phenyl ring of Phe820 through face-to-face π-π stacking interactions and the OH of indole fragment interacts with Gln817, Gln775 and Tyr 612 *via* hydrogen bond interactions. Moreover,



Scheme 1. Synthesis of rutaecarpine derivatives. Reagents and conditions: (a) $N(C_2H_5)_3$, 5-chlorovaleryl chloride, CH_2Cl_2, 0 °C to r.t., 2 h; (b) potassium *tert*-butoxide, THF, 0 °C, 2 h; (c) potassium *tert*-butoxide, THF, r.t., 5 h; (d) amines, $NaNO_2$, 20% HCl, CH_3COOH, 0 °C; (e) $ZnCl_2$, CH_3COOH, reflux; (f) R^3Br, K_2CO_3, DMF, 37–80 °C; (g) BBr_3, CH_2Cl_2, 0 °C to r.t.

Table 2
PDE selectivity of compounds **8i.**

Compounds	PDE IC_{50} (μM)[a]					
	PDE5	PDE2	PDE4	PDE6	PDE9	PDE10
8i	0.086 ± 0.007	> 500	> 500	43.8 ± 2.7	> 500	32.1 ± 2.9

[a] Results are expressed as the mean of at least three experiments.

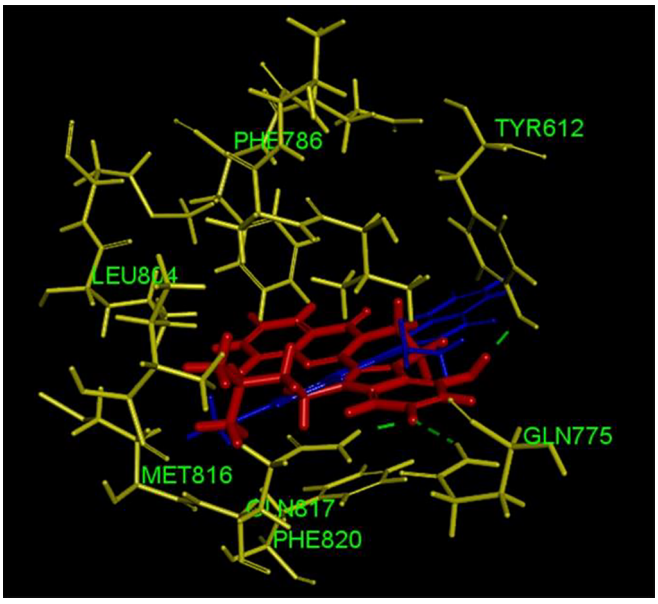


Fig. 2. Binding mode of compounds **8i** (red) and **7a** (blue) within the active pocket of PDE5A (PDB ID: 1UDU). Hydrogen bonds are represented by dashed lines (green).

quinazolin-4(3H)-one of **8i** were also found to form hydrophobic interactions with the phenyl ring of Phe786. The side chain on N atom of **8i** occupies the hydrophobic pocket of hPDE5A, forming hydrophobic interactions with Met816, Leu804 and Phe786. By contrast, compound **7a**, exhibiting weak PDE5A inhibitory activity, was accommodated into the active pocket with a slightly different behaviour. Although its indole fragment also interacts with the phenyl ring of Phe820 through a face-to-face π-π stacking, no hydrogen bonds were observed in this mode. On the other hand, its methoxy substituted at the indole ring fits into the cavity toward the hydrophobic Met816, Leu804 and Phe786, which is different with compound **8i**. Overall, the above docking studies provided an explanation for enzyme assay results that both substitution on aromatic ring and side chain on N atom could evidently influence the inhibitory activity of these derivatives.

To evaluate the *in vivo* effect of compound **8i** on cognitive improvement, the passageway water maze experiment was performed on a scopolamine (Scop)-induced cognitive deficit mouse model with donepezil (Don) as a positive control (Ctrl). As shown in Fig. 3, the Scop group (model group) exhibited a longer escape latency and more frequent errors than the Ctrl group ($^{***}p < 0.001$). The groups treated with 5 and 10 mg/kg compound **8i** demonstrated shorter escape latencies and less frequent errors than the Scop group [$F(4, 40) = 9.65$, $^{\#}p < 0.05$; $F(4, 40) = 13.69$, $^{\#\#}p < 0.01$], confirming that the compound could relieve the Scop-induced learning and memory deficits at dosages of 5 and 10 mg/kg, which was comparable to donepezil.

In conclusion, a series of novel rutaecarpine derivatives with excellent PDE5 inhibitory activities were designed and synthesized, the SARs of the 25 compounds were explicit and provided some insights into the structural modification of effective PDE5 inhibitors based on rutaecarpine. Of all the rutaecarpine derivatives, compounds **8i** showed high inhibitory activities with the IC_{50} values of 86 nM, and good selectivity over PDEs 2, 4, 6, 9 and 10. Furthermore, The *in vivo* study suggested that compound **8i** could improve cognitive dysfunction in an AD mouse model. Our study provided a basis for the rational design of novel rutaecarpine derivatives as PDE5 inhibitors, and subsequent efforts on further optimization of this structural class would lead to more potent and selective PDE5 inhibitors as a good candidate drug for the treatment of AD.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

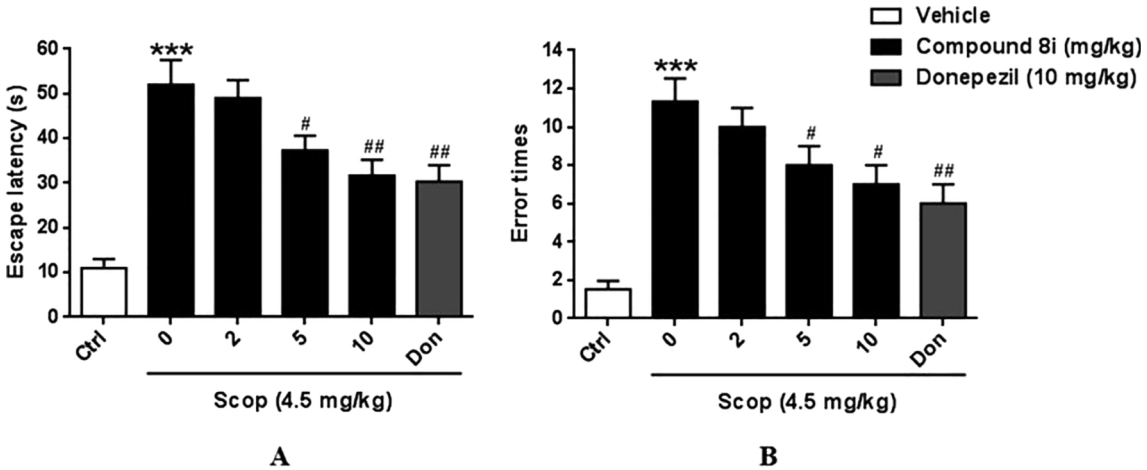


Fig. 3. Effects of compound **8i** (5 and 10 mg/kg) on scopolamine-induced cognitive deficits in mice. Donepezil (10 mg/kg) as a reference. (A) The escape latency of mice in the passageway water maze. (B) The number of errors of mice in the passageway water maze. Data are shown a s the mean ± SEM, $^{***}p < 0.001$ vs control group, $^{\#}p < 0.05$ and $^{\#\#}p < 0.05$ vs Scop group (one-way ANOVA followed by post hoc Dunnett's test).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127097.

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