The Thai Journal of Pharmaceutical Sciences

Volume 38
Issue 0 *supplement issue*

Article 2

1-1-2013

DISCOVERY OF NOVEL CHOLINESTERASE INHIBITOR (RKNU026) WITH p-AMYLOID AGGREGATION INHIBITORY ACTIVITY

Thippatai Hiranyaekaphap

Nitipol Srimongkolpithak

Thamrong Wongchang

Prayuth Poowaruttanawiwat

Kwanchai Rattanamanee

See next page for additional authors

DISCOVERY OF NOVEL CHOLINESTERASE INHIBITOR (RKNU026) WITH p-AMYLOID AGGREGATION INHIBITORY ACTIVITY

Authors

Thippatai Hiranyaekaphap, Nitipol Srimongkolpithak, Thamrong Wongchang, Prayuth Poowaruttanawiwat, Kwanchai Rattanamanee, and Ruengwit Kitbunnadaj

Thai J. Pharm. Sci. Vol. 38 (Suppl.) 2013

DISCOVERY OF NOVEL CHOLINESTERASE INHIBITOR (RKNU026) WITH β-AMYLOID AGGREGATION INHIBITORY ACTIVITY

Thippatai Hiranyaekaphap,[δ] Nitipol Srimongkolpithak,[δ] Thamrong Wongchang,[δ] Prayuth Poowaruttanawiwat,[φ] Kwanchai Rattanamanee,[φ] Ruengwit Kitbunnadaj[δ,†]

[δ]Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University Phitsanulok 65000, Thailand. [φ]Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand. [†]To whom correspondence should be addressed. Email: rkitbunna@hotmail.com

KEYWORDS: Alzheimer's disease, Cholinesterase enzyme, Cholinesterase inhibitors, Beta Amyloid aggregation, Coumarin

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia that caused by a reduction of the neurotransmitter, especially acetylcholine (ACh) which involves in memory and cognitive function in the brain. ACh, however, can be broken down by acetylcholinesterase (AChE). Many studies have indicated that butyrylcholinesterase (BuChE) activity increase continuously during progression. In addition, inhibitors binding to the peripheral anionic site (PAS) of the enzymes have hindered the amyloid β peptide (Aβ) formation. Such findings suggest that compound inhibiting AChE function, through an interaction with amino acid at PAS of the enzymes, might represent a new attractive candidate in Alzheimer's disease (AD) therapy.[1-2]

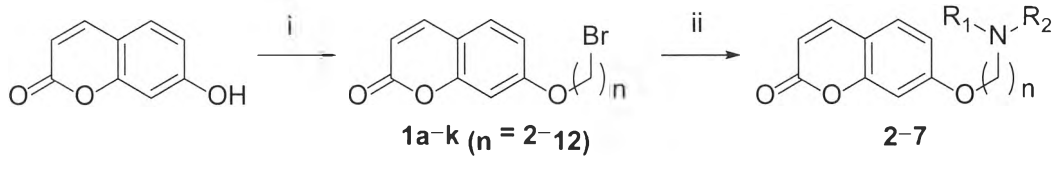
In this study, coumarin was chosen as core structure because its structure is similar to dihydroindenone ring of donepezil. Yet, coumarin and its metabolite, in particular 7-hydroxycoumarin, are known to be the safe chemicals for human. A series of coumarin derivatives are designed and synthesized by linking of coumarin moiety and amines with various spacer lengths. All targeted compounds will be tested for their inhibitory activities against AChE and BuChE. Some compounds, moreover are selected for further Aβ aggregation inhibitory activity. In order to identify binding pocket of the coumarin derivatives, a so-called computational modelling was applied.

MATERIALS AND METHODS

Experimental section Reagents were obtained from commercial suppliers and used without further purification. Solvents used were either commercial or AR grade. Thin-layer chromatography was carried out on Merck Kieselgel 60F254 and column chromatography was performed using Carlo erba Kieselgel (0.063-0.200 mm). Melting points were determined on a Buchi apparatus (Buchi535) an uncorrected ^{1}H NMR spectra were recorded on Bruker/avance at 400 MHz using the residual undeuterated solvent peak as reference. Mass spectrometry was performed on Applied Biosystems (API4000). Molecular docking was performed using Accelrys Discovery studio 3.1.

Methods Compounds (**1–7**) were accomplished as illustrated in scheme 1. The commercially available 7-hydroxy coumarin, 7-hydroxy-2H-chromen-2-one, was alkylated with dibromoalkanes in the presence of potassium carbonate to afford 7-bromoalkyloxy-2H-chromen-2-one (**1a–1k**). Next, the 7-bromoalkyloxy-2H-chromen-2one was treated with various amines, i.e., piperidine, diethylamine, morpholine, methylpiperazine, isoquinoline, dimethoxy, and isoquinoline, in acetone with the presence of potassium carbonate to give the targeted compounds (**2a–2k, 3a–3c, 4a–4c, 5a–5c, 6a–6c, and 7a–7c**).

Scheme 1 Synthetic pathway for coumarin derivatives.



Reagents and conditions (i) Br-$(CH_2)_n$-Br, acetone, K_2CO_3, Δ, 16 h; (ii) R_1-NH-R_2, acetone, K_2CO_3, Δ.

Pharmacological evaluation

Cholinesterase inhibition assay The inhibitory activities of all compounds against Cholinesterases(ChEs) were measured according to a method described previously by Ellman. The method employs 3.8 mM of acetylthiocholine (ATC) as a substrate for ChEs. ATC was broken down to thiocholine and acetate by ChEs. The resulting thiocholine was subsequently reacted with 3 mM of dithiobisnitrobenzoate (DTNB) to give a 2-nitrobenzoate-5-mercaptothiocholine and a yellowish 5-thio-2-nitrobenzoate. The quantity of yellow color which develops over time was a measurement of the activity of ChEs and can be determined using a UV-visible spectrophotometer. Upon UV-visible radiation condition, TNB undergone excitation and displays an absorption band with λmax of 405 nm. The positive control used in this study were neostigmine and galantamine.[3]

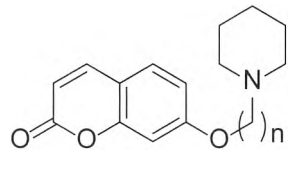
Amyloid-beta aggregation inhibition assay Thioflavin T (Th-T) fluorescence assay was used for the identification and quantification of amyloid (Aβ) fertilization. The mixtures of $A\beta_{1-42}$ peptide and AChE in presence or absence of the test inhibitors were incubated for 6 hours at 37 °C. A solution of Aβ (dissolved in DMSO and diluted sodium phosphate buffer, pH 8.0) and a solution AChE (dissolved in sodium phosphate buffer, pH 8.0) were added. After co-incubation, the mixture was added Th-T. To analyze co-aggregation inhibition, the fluorescence absorption and emission were measured at 450 nm and 490 nm, respectively.[4]

Molecular docking Docking of the various inhibitors into the *h*AChE active site (PDB4EY7) was performed using Accelrys Discovery studio 3.1. The default settings for Accelrys Discovery studio 3.1 were used. The initial structure for docking was the geometry optimized, whereas an active site of the protein was assigned from receptor cavity. Both ligand and protein were individually prepared by CHARMm force field. The CDOCKER algorithm optimization was then applied for the docking and energy minimization process of the ligand-protein complex. The energies for different conformations were calculated in term of CDOCKER energy.

RESULTS

Novel coumarin derivatives were designed and synthesized by linking of a 7-hydroxycoumarin core structure and a piperidine with various spacer lengths (2-12 methylene unit, **2a–k**). The result showed that the optimum spacer length was 7-9 methylene units. Within the series, compounds (**2f–2h**) possessed the highest inhibitory activities (Table 1). Thus, the 7-9 methylene unit spacer length was selected for the further study. A new series of compounds was designed and synthesized by replacement of the piperidine ring with various nitrogen-containing species. As shown in table 2, no such a compound exhibited higher inhibitor activity over its piperidine prototype. Hence, compound **2f**, RKNU026, was the most potent cholinesterase inhibitor with IC_{50} values of 0.3 and 15.8 μM against AChE and BuChE, respectively. Docking study of RKNU026-*h*AChE complex indicated that the coumarin moiety indeed interacts with Trp286 via a π-π interaction (Figure 1).

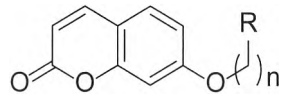
Table 1: Inhibitory of *h*AChE and *h*BuChE activities determined for the piperidine containing derivatives

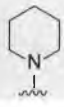
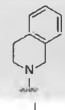
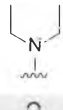
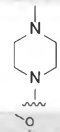
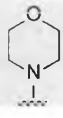
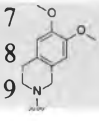


No	RKNU	n	*h*AChE IC_{50} (μM)	*h*BuChE IC_{50} (μM)	No	RKNU	n	*h*AChE IC_{50} (μM)	*h*BuChE IC_{50} (μM)
2a	021	2	3.3	56.6	**2h**	028	9	4.1	13.5
2b	022	3	6.0	> 100	**2i**	029	10	1.4	25.0
2c	023	4	1.1	29.1	**2j**	030	11	14.7	29.5
2d	024	5	1.3	37.0	**2k**	031	12	> 100	54.2
2e	025	6	4.1	16.9					
2f	026	7	0.3	15.8	**Neostigmine**			0.8	35.1
2g	027	8	0.7	6.8	**Galantamine**			0.4	18.6

The half maximum inhibitory concentration (IC_{50}, μM) values were measured using Ellman's method described previously. Results are presented as mean of at least three independent experiments.

Table 2: Inhibitory of *h*AChE and *h*BuChE activities determined for the coumarin derivatives bearing various nitrogen-containing species.



No	RKNU	n	R	*h*AChE IC_{50} (μM)	*h*BuChE IC_{50} (μM)	No	RKNU	n	R	*h*AChE IC_{50} (μM)	*h*BuChE IC_{50} (μM)
2f	026	7		0.3	15.8	**5a**	047	7		23.8	9.1
2g	027	8		0.7	6.8	**5b**	048	8		7.9	5.5
2h	028	9		4.1	13.5	**5c**	049	9		33.7	69.4
3a	059	7		0.7	> 100	**6a**	056	7		6.7	> 100
3b	060	8		1.6	> 100	**6b**	057	8		5.0	> 100
3c	061	9		2.6	> 100	**6c**	058	9		6.2	> 100
4a	050	7		20.9	79.7	**7a**	044	7		61.0	43.4
4b	051	8		4.9	28.6	**7b**	045	8		21.8	38.6
4c	052	9		16.7	> 100	**7c**	046	9		47.5	39.1
Neostigmine				0.8	35.1	**Galantamine**				0.4	18.6

The half maximum inhibitory concentration (IC_{50}, μM) values were measured using Ellman's method described previously. Results are presented as mean of at least three independent experiments.

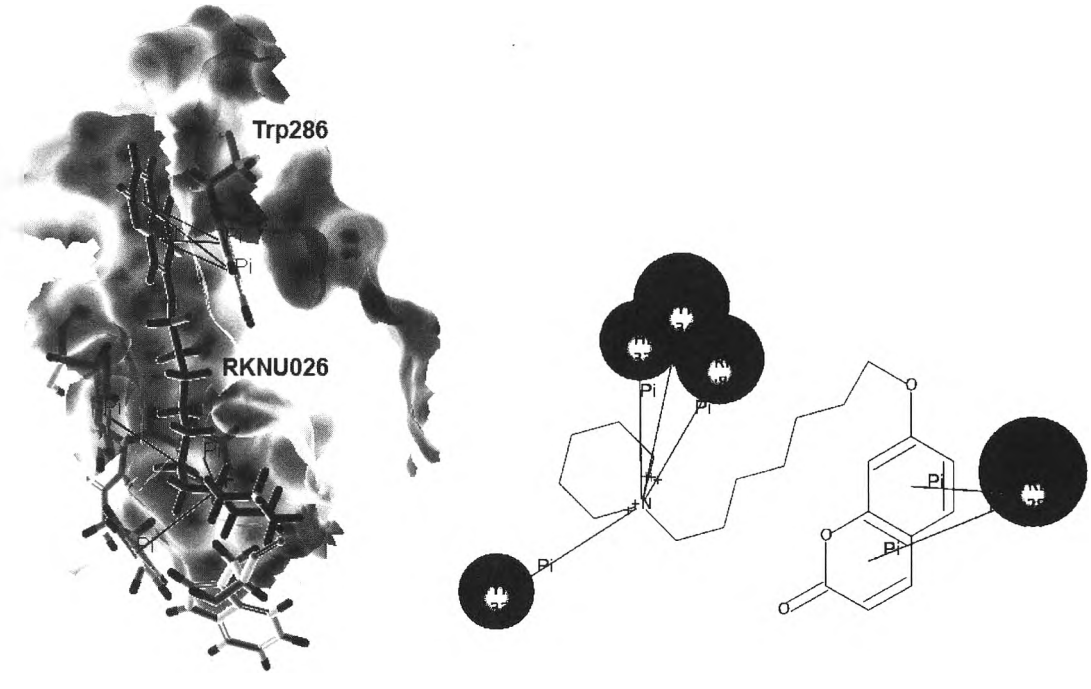


Figure 1 *In silico*, docking of RKNU026 into the active site of *h*AChE using CDOCKER algorithm on Accelrys Discovery studio 3.1. The RKNU026-AChE complex structure with the lowest energy is shown on the left panel, and the 2D diagram is depicted on the right panel.

DISCUSSION

In this study, a novel coumarin series was designed preliminarily by linking of coumarin moiety and a piperidine ring with various spacer lengths (2 to 12 methylene unit). The result showed that compound **2f** and **2g**, with spacer length of 7 and 8 methylene unit respectively, possessed the optimum inhibitory potency for both *h*AChE and *h*BuChE. Further study by replacement of the piperidine ring with various

nitrogen containing rings, i.e. dietyhlamine (**3a–c**), morpholine (**4a–c**), dietyhlamine (**5a–c**), 1,2,3,4-tetrahydroisoquinoline (**6a–c**), and 6,7-dimethoxy-1,2,3,4 tetrahydroisoquinoline (**7a–c**), showed that the bigger the ring the lower potency obtained. Compound **7a–c** display the lowest potency for *h*AChE in comparison with the others. In contrast to what was observed for *h*BuChE, compounds **3a–c** and **6a–c** with a small heterocyclic ring, i.e., dietyhlamine, and dietyhlamine, respective exhibited the lowest potency. However, in this study, compound **2f** (RKNU026) was the most potent AChE and BuChE inhibitor. The compound was further tested for Aβ aggregation inhibitory activity. In comparison with donepezil, a well-known potent AChE inhibitor currently used for AD treatment, RKNU026 exhibited an equal degree of Aβ aggregation inhibitory activity even with lower concentration; RKNU026 possessed the inhibitory activity for 19% at concentration of 5 μM whereas donepezil showed the inhibitory activity for 22% at concentration of 100 μM (Figure 2).[5]

In order to identify the binding interaction between RKNU026 and *h*AChE, computational docking was applied. CDOCKER algorithm allowed the ligand to align flexibly in the pocket of the enzyme. The complex structure with the lowest CDOCKER energy showed that the piperidine nitrogen of RKNU026 binds to Trp86, Tyr337, Phe338, and Ty341 *via* cation-π interactions. Interestingly, the coumarin ring indeed binds to Trp286 through a π-π interaction at PAS of the enzyme. This result was in agreement to what was reported earlier that compounds interacting to amino acid at PAS hinder the Aβ aggregation process.

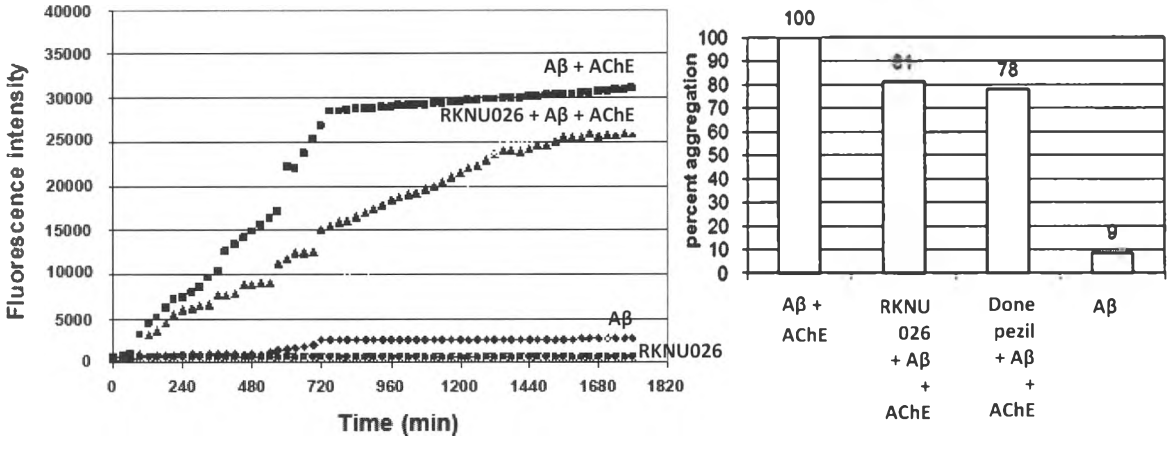


Figure 2 (Left panel) *In situ*, real time Th-T fluorescence assays of solutions of Aβ in the presence or absence of AChE and/or RKNU026. (Right panel) The maximum Aβ aggregation in the presence and absence of inhibitor (RKNU026, 5 μM or donepezil, 100 μM), % Aβ aggregation inhibition = 100 - the maximum Aβ aggregation. *The maximum Aβ aggregation value of donepezil was taken from reference 5.

CONCLUSION

In this study, a coumarin derivative RKNU026 (**2f**) was identified as novel potent cholinesterase inhibitor with IC_{50} of 0.3 μM for *h*AChE and 15.8 μM for *h*BuChE. The compound, moreover, inhibited Aβ aggregation (19% at concentration of 5 μM). Thus, RKNU026 might be a valuable candidate in the further development of drug for Alzheimer's disease.

ACKNOWLEDGMENTS

The First author would like to thank Faculty of Pharmaceutical Science, Naresuan University, Phitsanulok for some financial support and Dr. Nitra Nuengchamnong for valuable technical advice.

REFERENCES

1. Bourne, Y.; Taylor, P.; Radic, Z.; Marchot, P. Structural insights into ligand interaction at the acetylcholinesterase peripheral anionic site. EMBO J. 2003, 22, 1-12.
2. Bartolini, M.; Bertucci, C.; Cavrini, V.; Andrisano, V., β-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies. Biochemical Pharmacology 2003, 65 (3), 407-416.
3. Ellman, G. L.; Courtney, D. K.; Andres, V.; Featherstone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 1961, 7, 88.
4. Biancalana, M.; Koide, S. Molecular Mechanism of Thioflavin-T Binding to Amyloid Fibrils. Biochem. Biophys. Acta. 2010, 1804, 1405-1412.
5. Mishra, N.; Sasmal, D.; Singh, K. K. Attenuation Aβ1-42-induced toxicology by a novel acetylcholinesterase inhibitor. Neurosci. 2013, 250, 309-319.