SYNTHESIS, IN VITRO INHIBITION EFFECT AND STRUCTURE-ACTIVITY RELATIONSHIP OF NEW THYMOL DERIVATIVES ON CHOLINESTERASES

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common form of dementia that affects aged people. Acetylcholinesterase is a hydrolase involved in the termination of impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter ACh in the central and peripheral nervous system. Thymol is a main bioactive monoterpene isolated from many medicinal herbs, such as Thymus vulgaris, Monarda punctate and Origanum vulgare spp. Thymol has been widely used as an active anti-inflammatory ingredient, which can inhibit the isoproterenol induced inflammation in myocardial infarcted rats. Moreover, many studies have reported that thymol has various bioactivities, such as anticancer, anti-bacterial, and antioxidant properties. In this paper, a series of 12 novel thymol substituted carbamate derivatives (**2a-l**) was synthesized and their inhibitory activities on AChE and BuChE were evaluated. Among them, **2k** exhibited the strongest inhibition against AChE with an IC_{50} value of 2.498 μM, which was 183-fold more than that of thymol (IC_{50} = 458.23 μM). The structure-activity relationship were also investigated.

Keywords: Thymol, Cholinesterases, Inhibitor

Introduction

Thymol is a main bioactive monoterpene isolated from many medicinal herbs, such as Thymus vulgaris, Monarda punctate and Origanum vulgare spp. Thymol has been widely used as an active anti-inflammatory ingredient, which can inhibit the isoproterenol induced inflammation in myocardial infarcted rats, attenuate collagenase-induced osteoarthritis, and alleviate allergic airway inflammation in ovalbumin-induced Mouse asthma. Moreover, studies have reported that thymol has various bioactivities, such as anticancer, anti-bacterial, and antioxidant properties. Recent studies focusing on the neuroprotective activities of thymol have shown that it attenuates amyloid or scopolamine induced cognitive impairment in rats (Deng, 2015)

Numerous plants and their constituents have been reputed in traditional practices of medicine to enhance the cognitive function and to alleviate other symptoms of AD, including depression. Besides the *Salvia* species are known as a remedy for cognitive disorders, research on *Thymus vulgaris* essential oil also indicates their neuroprotective effects. Due to their small molecular size and lipophilicity, volatile constituents of essential oils and liberated volatile aglycones from glycosides are likely to readily cross the blood–brain barrier (Jukic, 2007). Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common form of dementia that affects aged people (Ruiz, 2005). Acetylcholinesterase (AChE; EC 3.1.1.7) is a hydrolase involved in the termination of impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter ACh in the central and peripheral nervous system. AChE inhibitors (AChEI) inhibit the hydrolysis of ACh improving both the level and of duration of neurotransmitter (Citron, 2004). Another cholinesterase, butyrylcholinesterase (BuChE; EC 3.1.1.8), primarily localized in plasma, liver and muscle tissues, able of hydrolyzing ACh and other acylcholines differs from AChE for tissue distribution and sensitivity to substrates and inhibitors (Wu, 2012). AChE inhibitors such as galantamine, rivastigmine, and donepezil are the main stay drugs for the clinical management of AD in the early to moderate stage (Catto, 2013).

In this study, a novel series of 12 thymol derivatives (**2a-l**) was synthesized and their inhibitory effects on AChE and BuChE were evaluated. Structure-activity relationship was also investigated.

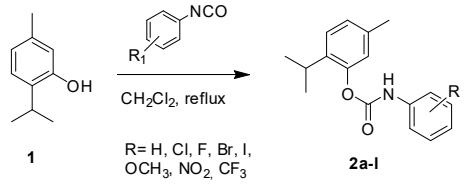
Materials and Methods

Synthesis of thymol derivatives: Thymol (1 mmol) was dissolved in CH_2Cl_2, then Et_3N (1 mmol) and isocyanate derivatives (1.25 mmol) were added to the solution, respectively. The mixture was refluxed for overnight, cooled and washed with water. The organic layer was dried over Na_2SO_4, and concentrated in vacuo. *2-isopropyl-5-methylphenyl-4-nitrophenylcarbamate (**2k**)*: ^{1}H NMR ($CDCl_3$, 300 MHz) δ/ppm: 1.21 (6H, d, J=7.0 Hz), 2.33 (3H, s), 3.05-3.10 (1H, m), 6.92 (1H, s), 7.07 (1H, d, J=8.0 Hz), 7.23 (1H, d, J=8.0 Hz), 7.53 (1H, s, NH), 7.59 (2H, d, J=9.4 Hz), 8.19 (2H, d, J=9.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ/ppm: 21.1, 23.4, 27.3, 118.3, 122.9, 125.4, 126.9, 127.9, 137.1, 137.6, 143.5, 143.8, 147.4, 151.8.

Anticholinesterase activity assays: Acetyl- (AChE) and butyryl-cholinesterase (BuChE) inhibitory activities of the synthesized compounds were determined according to Ellman's method (Ellman, 1961). The IC_{50} was determined by constructing an absorbance and/or inhibition (%) curve and examining the effect of five different concentrations. IC_{50} values were calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. The substrates of the reaction were acetylthiocholine iodide and butyrylthiocholine iodide. 5,5'-dithio-bis(2-nitrobenzoic) acid (DTNB) was used to measure anticholinesterase activity. Stock solutions of the compounds and galanthamine in methanol were prepared at a concentration of 4000 µg/mL. Aliquots of 150 µL of 100 mM phosphate buffer (pH 8.0), 10 µL of sample solution and 20 µL AChE ($2.476x10^{-4}$ U/µL) (or $3.1813x10^{-4}$ U/µL BuChE) solution were mixed and incubated for 15 min at 25ºC. 10 µL of DTNB solution was prepared by adding 2.0 mL of pH 7.0 and 4.0 mL of pH 8.0 phosphate buffers to a mixture of 1.0 mL of 16 mg/mL DTNB and 7.5 mg/mL $NaHCO_3$ in pH 7.0 phosphate buffers. The reaction was initiated by the addition of 10 µL (7.1 mM) acetylthiocholine iodide (or 0.79 mM butyrylthiocholine iodide). In this method, the activity was measured by following the yellow colour produced as a result of the thio anion produced by reacting the enzymatic hydrolysis of the substrate with DTNB. Also, methanol was used as a control solvent. The hydrolysis of the substrates was monitored using a BioTek Power Wave XS at 412 nm.

Results and Discussion

The synthetic procedures to obtain the target compounds **2a-l** are depicted in Scheme 1.



Scheme 1. Synthesis of novel thymol derivatives

The IC_{50} values for AChE and BuChE inhibitions are summarized in Table 1. IC_{50} values against AChE ranges from 2.498 µM to 288,46 µM. Compound **2k** exhibited the strongest inhibition against AChE with an IC_{50} value of 2.498 µM, which was 183-fold more than that of thymol (IC_{50} = 458.23 µM). Also, it approached that of galantamine (IC_{50} = 2.051 µM). Furthermore, most of the synthesized carbamate derivatives (**2a**, **2c**, **2d**, **2e**, **2f**, **2g**, **2h**, **2i**, **2j**, **2k**, and **2l**) exhibited better AChE inhibition (IC_{50} = 2.498 µM – 288,46 µM) than thymol, by 0.6-183 fold. All of the synthesized compounds inhibited BuChE. Compound **2a** exhibited the strongest inhibition against BuChE with an IC_{50} value of 1.65 µM, which was 137-fold more than that of thymol (IC_{50} = 226.13 µM).

The following conclusions should be noted regarding the ChEs inhibitory data of Table 1: (i) Moving the fluorine atom at the phenyl ring from the meta-position to the para-position led to a major decline of the inhibitory activity for both ChEs (compared **2c** (R=3-F, IC_{50} = 88.52 and 3.85 µM for AChE and BuChE, respectively) and **2d** (R=4-F, IC_{50} = 288.46 and 405.15 µM for AChE and BuChE, respectively)). (ii) The same decrease of the inhibitory activities of **2a-b** was indicated against both ChEs with moving methoxy group from the meta-position to the para-position (compared **2a** (R=3-OMe, IC_{50} = 154.32 and 1.65 µM for AChE and BuChE, respectively) and **2b** (R=4-OMe, IC_{50} = n.a and 59.96 µM for AChE and BuChE, respectively)). (iii) The presence of chlorine atom at the meta-position of phenyl ring led to an increase of the inhibitory activity for both ChEs (compared **2e** (R=3,4-diCl, IC_{50} = 89.53 and 2.54 µM for AChE and BuChE, respectively) with **2f** (R=4-Cl, IC_{50} = 269.8 and 137.41 µM for AChE and BuChE, respectively)).

(iv) Moving the nitro group at the phenyl ring from the meta-position to the para-position led to a major decrease of the inhibitory activity for AChE (compared **2j** (R=3-NO_2, IC_{50} = 82.88 µM) and **2k** (R=4-NO_2, IC_{50} = 2.498 µM)). The opposite effect was observed for inhibition of BuChE (compared **2j** (R=3-NO_2, IC_{50} = 3.31 µM) and **2k** (R=4-NO_2, IC_{50} = 37.71 µM)) (v) Electron-donating groups (methoxy group) at the meta- position of the phenyl

ring exhibited lower inhibitory activity than halogens and electron-withdrawing group for AChE (compared **2a** (R=3-OMe, IC_{50} = 154.32 µM) with **2c** (R=3-F, IC_{50} = 88.52 µM) and **2e** (R=3,4-diCl, IC_{50} = 89.53 µM) and **2j** (R=3-NO_2, IC_{50} = 82.88 µM)). On the contrary, methoxy group at the meta position increased the BuChE inhibitory activity (compared **2a** (R=3-OMe, IC_{50} = 1.65 µM) with **2c** (R=3-F, IC_{50} = 3.85 µM) and **2e** (R=3,4-diCl, IC_{50} = 2.54 µM) and **2j** (R=3-NO_2, IC_{50} = 3.31 µM)). (v) The inhibitor activity on both ChEs seems to be strongly dependent on the size and polarizability of the halogen substituent at the para-position of the phenyl ring (for size and polarizability, I > Br > Cl > F; for AChE inhibitory activity, **2h** (R=4-I, IC_{50} = 31.61 µM) > **2g** (R=4-Br, IC_{50} = 98.56 µM) > **2f** (R=4-Cl, IC_{50} = 269.80 µM) > **2d** (R=4-F, IC_{50} = 288.46 µM); for BuChE inhibitory activity, **2h** (R=4-I, IC_{50} = 4.77 µM) > **2g** (R=4-Br, IC_{50} = 107.22 µM) > **2f** (R=4-Cl, IC_{50} = 137.41 µM) > **2d** (R=4-F, IC_{50} = 405.15 µM)).

Table 1. In vitro inhibition IC_{50} values (µM) and selectivity of compounds **6a-l** for AChE and BuChE.

Compound	R	Yield (%)	Mp (°C)	AChE (IC_{50}, µM)[a]	BuChE (IC_{50}, µM)[a]	Selectivity index[b]
2a	**3-OCH_3**	42	182.6	154,32±1,745	**1,65±0,014**	0.01
2b	4-OCH_3	60	136.1	na	59,96±1,542	-
2c	3-F	73	111.3	88,52±1,253	3,85±0,457	0.04
2d	4-F	72	119.0	288,46±1,124	405,15±1,452	1.40
2e	3,4-diCl	74	113.5	89,53±1,248	2,54±0,568	0.03
2f	4-Cl	81	163.0	269,8±1,560	137,41±2,014	0.51
2g	4-Br	76	176.2	98,56±1,263	107,22±1,785	1.09
2h	4-I	74	179.6	31,61±0,952	4,77±0,748	0.15
2i	H	54	75.2	197,99±1,542	5,02±0,879	0.03
2j	3-NO_2	38	177.5	82,88±1,224	3,31±0,564	0.04
2k	**4-NO_2**	54	152.8	**2,498±0,012**	37,71±1,022	15.10
2l	4-CF_3	89	156.1	98,53±1,114	147,85±1,567	1.50
Thymol	**-**	**-**	**-**	**458,23±1,745**	**226,13±1,214**	**0.49**
Donezepil[c]	-	-	-	0.03±0.0005	4.66±0.503	155.30
Galantamine	-	-	-	2,051±0,011	18,13±0,457	8.84
Rivastigmine[c]	-	-	-	12,4±1,011	1,00±0,251	0.08

[a] IC_{50} values represent the means ± S.E.M. of three parallel measurements ($p < 0.05$).
[b] Selectivity index = IC_{50} (BuChE) / IC_{50} (AChE).
[C] (Kurt et al., Eur. J. Med. Chem., 2015)

Conclusion

A series of 12 novel thymol substituted carbamate derivatives (**2a-l**) was synthesized and their inhibitory activities on AChE and BuChE were evaluated. Among them, **2k** exhibited the strongest inhibition against AChE with an IC_{50} value of 2.498 µM, which was 183-fold more than that of thymol (IC_{50} = 458.23 µM). Additionally, **2a** exhibited the strongest inhibition against BuChE with an IC_{50} value of 1.65 µM, which was 137-fold more than that of thymol (IC_{50} = 226.13 µM).Generally, the presence of carbamate moiety at the thymol increased the both ChEs inhibition. This finding can provide guidance for researches to design new efficient ChEs inhibitors in the future works. The SAR revealed that the inhibitory activity of the synthesized compounds could also be affected by the type and position of the halogen and electron-donating and electron-withdrawing groups substituent on the phenyl ring. Overall these derivatives could be recommended as new chemotypes to develop new ChEIs for the treatment of AD disease by suitably modulating the substitution pattern also in the perspective of multifunctional anti AD agents.

Acknowledgments

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