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Cholinesterase Inhibition Activity and Molecular Docking Study of Eugenol Derivatives

(Perencatan Aktiviti Antikolinesterase dan Kajian Doking Molekul Terbitan Eugenol)

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ABSTRACT

*The study was conducted to explore the anticholinesterase inhibition property of eugenol derived molecules. Ten eugenol derivatives were synthesized and evaluated for the inhibitory activities against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) by Ellman's method. Most of the tested derivatives showed higher inhibition on BChE than AChE, however, their overall inhibitory activity was weak. In contrast, three derivatives (compounds **3**,**6**,**9**) showed higher and good AChE inhibitory activity of more than 50% inhibition at 10 µg/mL. Among them, compound **9** bearing a ethyl substituent at para position of the benzoyl ring showed the most potent AChE inhibition, with IC_{50} of 5.64 µg/mL. Ligand-protein docking simulation was also performed for the most active derived molecules (compounds **3**,**6**,**9**).*

Keywords: Acetylcholinesterase; butyrylcholinesterase; eugenol derivatives; molecular docking

ABSTRAK

*Kajian ini dijalankan untuk mengetahui sifat perencatan antikolinesterase bagi terbitan eugenol. Sepuluh terbitan eugenol telah disintesis dan dikaji untuk aktiviti perencatan terhadap asetilkolinesterase (AChE) dan butirilkolinesterase (BChE) melalui kaedah Ellman. Kebanyakan terbitan yang diuji menunjukkan aktiviti perencatan yang lebih tinggi terhadap BChE berbanding AChE, walau bagaimanapun, secara keseluruhannya aktiviti perencatan adalah lemah. Sebaliknya, tiga terbitan (sebatian **3**,**6**,**9**) menunjukkan aktiviti perencatan AChE yang tinggi dan bagus iaitu lebih daripada 50% perencatan pada 10 µg/mL. Antara sebatian tersebut, sebatian **9** yang mempunyai penukarganti etil pada kedudukan para benzena menunjukkan perencatan AChE yang paling kuat, dengan IC_{50} 5.64 µg/mL. Simulasi doking protein ligan juga dilakukan untuk molekul yang paling aktif (sebatian **3**,**6**,**9**).*

Kata kunci: Asetilkolinesterase; butirilkolinesterase; doking molekul; terbitan eugenol

INTRODUCTION

Alzheimer disease (AD) is an irreversible, chronic and progressive neurodegenerative disease characterized by memory loss and cognitive impairment (Thompson et al. 2012). According to the cholinesterase hypothesis, AD is associated with loss of cholinergic neurons in the brain and resulting a decrease inacetylcholine, which is a neurotransmitter (Lane et al. 2006). Cholinesterase inhibitors have been widely recognised as a gold standard for the management of AD. To date, six drugs have been approved by U.S. Food and Drug administration including galanthamine, donepezil, memantine, memantine combined with donepezil, tacrine (discontinued) and rivastigmine to treat mild to moderate AD. Due to the fact that the commercially available cholinesterase compounds were reported to possesses serious side effects (Ali et al. 2015), more studies are needed to discover potential compounds to treat AD.

Eugenol or 4-ally-2-methoxyphenol (**1**) (Figure 1) is one of the phenylpropanoids available in nature. It is the main constituent isolated from cloves, *Syzygiumaromaticum*, an aromatic plant belonging to family of *Myrtaceae* (Fichi et al. 2007). Eugenol and its derivatives have been shown to possess medicinal properties such as local antiseptic and analgesic (Markowitz et al. 1992), anesthetic (Goulet et al. 2010; Jirovetz et al. 2006),

anti-spasmodic (Wagner et al. 1979), antipyretic (Feng & Lipton 1987), anti-bacterial (da Silva et al 2018; Devi et al. 2010; Johny et al. 2010; Tippayatum & Chonhenchob 2007), anti-inflammatory (Maurya et al. 2018), antifungal (Olea et al. 2019) and antioxidant (Alqareer et al. 2006; da Silva et al. 2018; Jirovetz et al. 2006; Nassar et al. 2007) activities. Besides, eugenol also has vast applications in industrial products such as perfumes and flavoring agents. Eugenol also has repellent action (Kang et al. 2009; Zeringóta et al. 2013) and has been used as astabilizer (Li et al. 2015; Milczarek & Ciszewski 2012).

Hence, in continuation of our previous work on this molecule (Rahim et al. 2017), we report herein cholinesterase evaluation of a series of eugenol derivatives. Three compounds with the most potent and favorable properties as cholinesterase inhibitor was further deliberated for molecular docking studies.

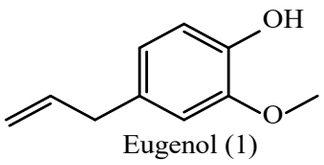


FIGURE 1. Chemical structure of Eugenol (**1**)

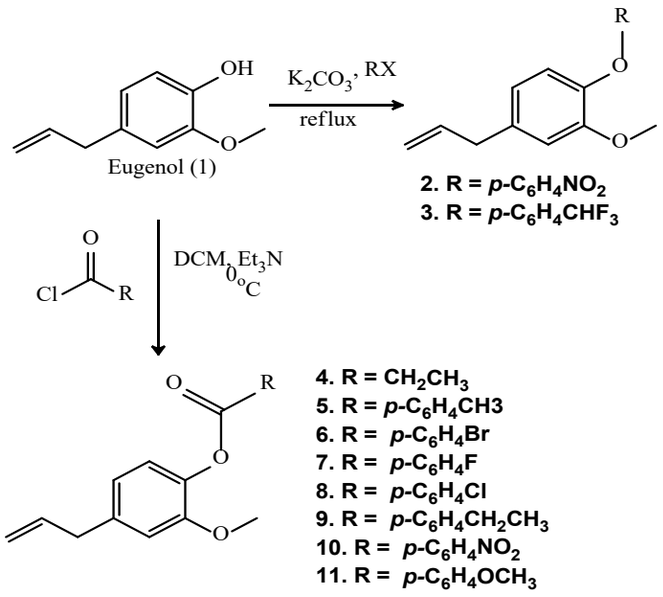
Materials and Methods

Materials

Acetylcholinesterase enzyme from electric eel (AChE), butyrylcholinesterase enzyme from equine serum (BChE), 5,5'-dithiobis[2-nitrobenzoic acid] (DTNB), acetylthiocholine iodide, S-butyrylthiocholine iodide, and physostigmine were purchased from Sigma Chemicals (St. Louis, MO, USA).

Synthesis and Structural Elucidation of Eugenol Derivatives

General synthetic routes for eugenol derivatives (**2-11**) are shown in Scheme 1. Synthesis methods, spectroscopy analyses were described in detail in the previous report (Rahim et al. 2017).



SCHEME 1. Synthetic route for the preparation of eugenol derivatives

4-Allyl-2-methoxy-1-(4-nitrobenzyloxy)-benzene (2): Yield: 49.95%; FTIR (KBr) ν_{max} 3081, 2903, 1639, 1512, 1342, 1231, 1038 cm^{-1}; UV-Vis (MeOH) λ_{max} (log **Ɛ**) 234 (4.03), 273 (4.12) nm; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ, 3.33 (d, *J* 6.8 Hz, 2H), 3.83 (s, 3H, OCH_3), 4.96-4.99 (m, 2H, CH_2), 5.24 (s, 2H), 5.90-6.00 (m, 1H), 6.68 (dd, *J* 2.2Hz, 8.0 Hz, 1H, CH_{ar}), 6.86 (d, *J* 2.0 Hz, 1H, CH_{ar}), 6.93 (d, *J* 8.0 Hz, 1H, CH_{ar}), 7.75 (d, *J* 8.8 Hz, 2H, CH_{ar}), 8.24 (d, *J* 8.8 Hz, 2H, CH_{ar}) ppm;^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 40.3, 56.1, 70.6, 113.7, 115.6, 115.9, 121.2, 124.2, 128.8, 134.8, 138.7, 146.5, 147.2, 148.3, 150.9 ppm. EIMS *m/z* $[M+2H]^+$ 300.14 ($C_{17}H_{17}NO_4$, 322.10); Anal. Calcd.for $C_{17}H_{17}NO_4$: C, 68.22; H, 5.72; N, 4.68; found: C, 69.22; H, 6.42; N, 4.70%.

4-Allyl-2-methoxy-1-(4-trifluoromethyl-benzyloxy) benzene (3): Yield: 57.10%; FTIR (KBr) ν_{max} 3011, 2935, 1641, 1515, 1252, 1113, 1067 cm^{-1}; UV-Vis (MeOH) λ_{max} (log **Ɛ**) 234 (4.25), 276 (4.22) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.24 (d, *J* 6.4 Hz, 2H), 3.80 (s, 3H, OCH_3), 4.96-5.02 (m, 2H, CH_2), 5.09 (s, 2H), 5.81-5.92 (m, 1H), 6.57-6.60 (dd, *J* 2.0 Hz, 8.4 Hz, 1H, CH_{ar}), 6.66 (d, *J* 2.0 Hz, 1H, CH_{ar}), 6.87 (d, *J* 8.0 Hz, 1H, CH_{ar}), 7.46 (d, *J* 8.4 Hz, 2H, CH_{ar}), 7.52 (d, *J* 8.4 Hz, 2H, CH_{ar}) ppm;^{13}C NMR (100 MHz, $CDCl_3$) δ 38.7, 54.8, 69.4, 111.4, 113.3, 114.7, 119.4, 124.4, 126.2, 128.7, 132.8, 136.4, 140.5, 145.1, 148.6 ppm. EIMS *m/z* $[M]^+$ 322 ($C_{18}H_{17}F_3O_2$, 322.32); Anal. Calcd. for $C_{18}H_{17}F_3O_2$: C, 67.07; H, 5.32; found: C, 68.14; H, 6.30%.

4-Allyl-2-methoxyphenyl propanoate (4): Yield: 74.54%; FTIR (KBr) ν_{max} 3001, 2938, 1759, 1602, 1509, 1266, 1032 cm^{-1}; UV-Vis (MeOH) λ_{max} (log **Ɛ**) ~sh 224 (4.01), 274 (3.53), 279 (3.50) nm; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.18 (t, *J* 7.4 Hz, 3H, CH_3), 2.52-2.58 (q, *J* 7.6 Hz, 2H), 3.36 (d, *J* 6.4 Hz, 2H), 3.77 (s, 3H, OCH_3), 5.01-5.05 (qd, *J* 1.2, 3.2,10.0 Hz, 1H), 5.06-5.12 (qd, *J* 1.6, 3.6,17.2 Hz, 1H), 5.92-6.02 (m, 1H, CH), 6.74-6.77 (dd, *J* 2.0 Hz, *J* 8.0 Hz, 1H,CH_{ar}), 6.92 (d, *J* 1.6 Hz, 1H, CH_{ar}), 6.93 (d, *J* 8.4 Hz, 1H, CH_{ar}) ppm; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 9.4, 27.5, 40.5, 56.0, 113.6, 116.0, 121.1, 123.3, 138.3, 139.2, 139.6, 152.0, 172.6 ppm; EIMS *m/z* $[M+H]^+$ 221 ($C_{13}H_{16}O_3$, 220.10).

4-Allyl-2-methoxyphenyl-4-methylbenzoate (5): Yield: 74.39%; FTIR (KBr) ν_{max} 3014, 2936, 1730, 1608, 1509, 1268, 1071 cm^{-1}; UV-Vis (MeOH) λ_{max} (log **Ɛ**) 241 (4.45), 274 (3.87) nm; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 2.44 (s, 3H,CH_3), 3.42 (d, *J* 6.8 Hz, 2H, CH_2), 3.78 (s, 3H, OCH_3), 504-5.08 (qd, *J* 1.2, 3.2, 10.0 Hz, 1H, CH_2), 510-5.16 (qd, *J* 1.6, 3.6, 17.2 Hz, 1H, CH_2), 5.96-6.06 (m, 1H, CH), 6.82-6.84 (dd, *J* 1.6 Hz, *J* 8.0 Hz, 1H, CH_{ar}), 6.99 (d, *J* 2.0 Hz, 1H, CH_{ar}), 7.09 (d, *J* 8.0 Hz, 1H, CH_{ar}), 7.38 (d, *J* 8.0 Hz, 2H, CH_{ar}), 8.04 (d, *J* 8.4 Hz, 2H, CH_{ar}) ppm; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 21.6, 40.5, 56.1, 113.7, 116.1, 121.2, 123.6, 127.7, 130.2, 130.8, 138.4, 139.2, 139.9, 145.2, 152.2, 165.0 ppm; EIMS *m/z* $[M]^+$ 282, ($C_{18}H_{18}O_3$, 282.10); Anal. Calcd. for $C_{18}H_{18}O_3$: C, 76.57; H, 6.43; found: C, 72.09; H, 6.02%.

4-Allyl-2-methoxyphenyl-4-bromobenzoate (6): Yield: 78.91%; IR (KBr) ν_{max} 3006, 2935, 1738, 1637, 1507, 1263, 1068, 1031 cm^{-1}; UV-Vis (MeOH) λ_{max} (log **Ɛ**) 246 (4.44), ~sh 272 (3.60) nm; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 3.41 (d, *J* 6.8 Hz, 2H, CH_2), 3.79 (s, 3H, OCH_3), 5.04-5.08 (qd, *J* 1.2, 3.2,10.0 Hz, 1H), 5.10-5.16 (qd, *J* 1.6, 3.6,17.2 Hz, 1H), 5.96-6.06 (m, 1H, CH), 6.83 (dd, *J* 1.2 Hz, *J* 8.0 Hz, 1H, CH_{ar}), 7.01 (d, *J* 1.6 Hz, 1H, CH_{ar}), 7.11 (d, *J* 8.0 Hz, 1H, CH_{ar}), 7.78 (d, *J* 8.8 Hz, 2H, CH_{ar}), 8.07 (d, *J* 8.8 Hz, 2H, CH_{ar}) ppm; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 40.5, 56.2, 113.8, 116.1, 121.3,123.5, 128.9, 132.0, 132.5, 132.9, 138.3, 139.1, 140.2, 152.1, 164.3 ppm; ESI-MS *m/z* $[M+CH_2]^+$ 360.19 ($C_{17}H_{15}BrO_3$,346.10); Anal. Calcd. for $C_{17}H_{15}BrO_3$, C, 58.81; H, 4.35; found: C, 53.10; H, 4.02%.

4-Allyl-2-methoxyphenyl-4-fluorobenzoate (7): Yield: 48.88%; FTIR (KBr) ν_{max}, 3017, 2939, 1736, 1603, 1508, 1265, 1238, 1149, 1068 cm^{-1}; UV-Vis (MeOH) λ_{max} (log Ɛ) 261 (4.18) nm; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 3.41 (d, *J* 6.8 Hz, 2H, CH_2), 3.79 (s, 3H, OCH_3), 5.04-5.08 (qd, *J* 2.0, 3.2, 10.0 Hz, 1H), 5.10-5.16 (qd, *J* 2.0, 3.6, 17.2 Hz, 1H), 5.98-6.05 (m, 1H, CH), 6.83-6.85 (dd, *J* 2.0 Hz,8.4 Hz, 1H, CH_{ar}), 7.01 (d, *J* 1.6 Hz, 1H, CH_{ar}), 7.11 (d, *J* 8.0 Hz, 1H, CH_{ar}), 7.33 (d, *J* 8.8 Hz, 2H, CH_{ar}), 8.22 (dd, *J* 5.6 Hz, *J* 8.8 Hz, 2H, CH_{ar}) ppm; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 40.5, 56.1, 113.8, 116.1, 116.7, 121.3, 123.5, 127.0, 133.5, 138.3, 139.1, 140.2, 152.2, 164.1, 165.6 ppm; EIMS *m/z* $[M+Na]^+$ 309 ($C_{17}H_{15}FO_3$, 286.90).

4-Allyl-2-methoxyphenyl-4-chlorobenzoate (8): Yield: 84.90%; FTIR (KBr) ν_{max} 3006, 2914, 1739, 1603, 1508, 1265, 1068, 1031, 751 cm^{-1}; UV-Vis (MeOH) λ_{max} (log Ɛ) 241 (4.32), 274 (3.60), ~sh 279 (3.55) nm; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 3.41 (d, *J* 6.8 Hz, 2H), 3.79 (s, 3H, OCH_3), 5.04 (qd, *J* 1.2, 3.2,10.0 Hz, 1H), 5.10 (qd, *J* 1.6, 3.2,17.2 Hz, 1H), 5.96-6.06 (m, 1H, CH), 6.83 (dd, *J* 1.6 Hz, *J* 8.0 Hz, 1H, CH_{ar}), 7.01 (d, *J* 1.6 Hz, 1H, CH_{ar}), 7.11 (d, *J* 8.0 Hz, 1H, CH_{ar}), 7.62 (d, *J* 8.8 Hz, 2H, CH_{ar}), 8.15 (d, *J* 8.4 Hz, 2H, CH_{ar}) ppm; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 40.6, 56.2, 113.7, 116.2, 121.3, 123.4, 129.2,

129.8, 132.4, 138.3, 139.0, 140.2, 152.1, 164.2 ppm; EIMS *m/z* $[M+]^+$ 140, $[M+2]^+$142, $[M]^+$ 302 ($C_{17}H_{15}ClO_3$, 302.75).

4-Allyl-2-methoxyphenyl-4-ethylbenzoate (9): Yield: 63.45%; FTIR (KBr) ν_{max} 3011, 2972, 17321, 1608, 1509, 1268, 1074 cm^{-1}; UV-Vis (MeOH) λ_{max} (log Ɛ) 240 (4.32), 273 (3.62) nm; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.26 (t, *J* 7.6 Hz, 3H, CH_3), 2.73-2.79 (q, *J* 7.6 Hz, 2H, CH_2), 3.41 (d, *J* 6.4 Hz, 2H), 3.78 (s, 3H, OCH_3), 5.04 (qd, *J* 1.2, 3.2, 10.0 Hz, 1H), 5.10 (qd, *J* 1.6, 3.6,17.2 Hz, 1H), 5.96-6.06 (m, 1H, CH), 6.83 (d, *J* 8.0 Hz, 1H, CH_{ar}), 6.99 (d, *J* 2.0 Hz, 1H, CH_{ar}), 7.09 (d, *J* 8.0 Hz, 1H, CH_{ar}), 7.42 (d, *J* 8.4 Hz, 2H, CH_{ar}), 8.07 (d, *J* 8.0 Hz, 2H, CH_{ar}) ppm; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 15.6, 29.2, 40.6, 56.1, 113.7, 116.1, 121.2, 123.6, 128.07, 129.0. 130.9, 138.4, 139.3, 139.9, 151.3, 152.2, 165.0 ppm; ESI-MS *m/z* $[M+Na]^+$ 319.13 ($C_{19}H_{20}O_3$, 296.10); Anal. Calcd. for $C_{19}H_{20}O_3$: C, 73.60; H, 6.79; found: C, 76.45; H, 7.61%.

4-Allyl-2-methoxyphenyl-4-nitrobenzoate (10): Yield: 57.90%; FTIR (KBr) ν_{max} 3003, 2939, 1746, 1604, 1530, 1508, 1344, 1264, 1075 cm^{-1}; UV-Vis (MeOH) λ_{max} (log Ɛ) 229 (4.28), 273 (3.54), ~sh 281 (3.46) nm; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 3.42 (d, *J* 6.8 Hz, 2H), 3.81 (s, 3H, OCH_3), 5.05 (qd, *J* 1.2 Hz, *J* 1.2 Hz, 3.2 Hz,10.0 Hz, 1H), 5.11 (qd, *J* 1.6, 3.2,16.8, 1H), 5.96-6.09 (m, 1H, CH), 6.85 (dd, *J* 2.0 Hz, 8.0 Hz, 1H, CH_{ar}), 7.03 (d, *J* 2.0 Hz, 1H, CH_{ar}), 7.16 (d, *J* 8.0 Hz, 1H, CH_{ar}), 8.39-8.44 (m, 4H, CH_{ar}) ppm; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 40.5, 56.2, 113.8, 116.2, 121.3, 123.3, 124.6, 132.1, 135.7, 138.2, 138.8, 140.5, 151.8, 151.9, 163.6 ppm; EIMS *m/z* $[M+CH_3]^+$ 328 ($C_{17}H_{15}NO_5$, 313.30); Anal. Calcd. for $C_{17}H_{15}NO_5$: C, 65.17; H, 4.83; N, 4.47; found: C, 64.34; H, 5.257; N, 4.66%.

4-Allyl-2-methoxyphenyl-4-methoxybenzoate (11): Yield: 97.03%; FTIR (KBr) ν_{max} 3010, 2941, 1728, 1606, 1513, 1264, 1029 cm^{-1}; UV-Vis (MeOH) λ_{max} (log Ɛ) 258 (4.41), ~sh 275 (4.27) nm; ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 3.41 (d, *J* 6.7 Hz, 2H), 3.78 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3),), 504 (qd, *J* 1.2, 3.2, 10.0 Hz, 1H), 5.10 (qd, *J* 1.6, 3.6, 17.2 Hz, 1H), 5.96-6.06 (m, 1H, CH), 6.82 (dd, *J* 2.0 Hz, *J* 8.0 Hz, 1H, CH_{ar}), 6.99 (d, *J* 2.0 Hz, 1H, CH_{ar}), 7.08 (d, *J* 5.6 Hz, 1H, CH_{ar}), 7.10 (d, *J* 6.8 Hz, 2H, CH_{ar}), 8.10 (d, *J* 8.8 Hz, 2H, CH_{ar}) ppm; ^{13}C NMR (100 MHz, $(CD_3)_2CO$) δ 40.6, 56.0, 56.1, 113.8, 114.8, 116.1, 121.2, 122.7, 123.7, 132.8, 139.4, 138.4, 139.8, 152.3, 164.7, 164.9 ppm; ESI-MS *m/z* $[M+H]^+$ 299.12, $[M+Na]^+$ 321.11 ($C_{18}H_{18}O_4$, 298.10); Anal. Calcd. for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08; found: C, 70.84; H, 6.67%.

CHOLINESTERASE INHIBITION ASSAY

The cholinesterase inhibitory activity of eugenol derivatives was evaluated following Ellman's method as described previously (Khaw et al. 2014). In brief, for AChE inhibitory assay, 140 µL of 0.1 M sodium phosphate buffer (pH 8) was first added to the 96-well microplate followed by 20 µL of the test sample (in 10 % methanol), 20 µL of 0.09 unit/mL AChE or BChE, 10 µL of 10 mM 5,5′-dithiobis(2-nitrobenzoic acid) was added into each well followed by 10 µL of 14 mM acetylthiocholine iodide or S-butyrylthiocholine chloride. The absorbance of the colored end-product was measured at 412 nm at designated intervals for 30 min after the initiation of enzymatic reaction by Tecan Infinite 200 ProMicroplate Spectrometer (Switzerland). Physostigmine was used as reference to compare the differences between the sample and standard drug. Each sample test was conducted in triplicate. Absorbance of the test sample was corrected by subtracting the absorbance of its respective blank. A set of five concentrations was used to estimate the 50% inhibitory concentration (IC_{50}) for the active compounds that showed more than 50% inhibition at 10 µg/mL. Data were analyzed by one-way analysis of variance (ANOVA) followed by tukey post-hoc test for the determination of statistically significant between samples and standard. *P* values of 0.05 or less were considered significant.

MOLECULAR DOCKING STUDY

Molecular docking was performed for the most potent inhibitors using BioSolveIT'sLeadIT software (LeadIT version 2.3.2; BioSolveIT GmbH, Sankt Augustin, Germany, 2017, www.biosolveit.de/LeadIT).

Results and Discussion

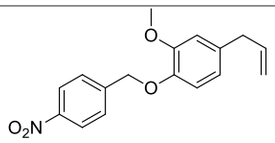
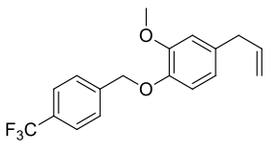
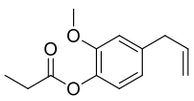
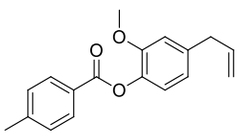
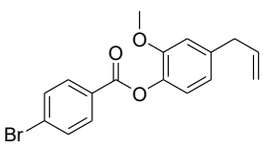
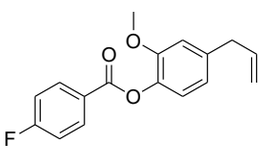
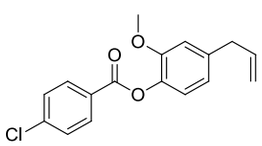
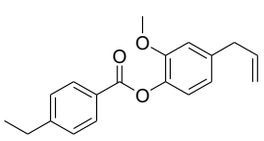
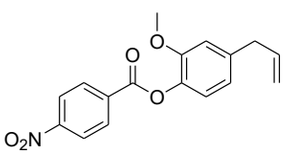
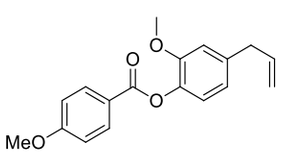
SYNTHESIS AND STRUCTURAL ELUCIDATION OF EUGENOL DERIVATIVES

Ten eugenol derivatives (compounds **2-11**) were synthesized, and their structures were confirmed with NMR, FTIR and MS, as reported previously (Rahim et al. 2017).

CHOLINESTERASE INHIBITION ASSAY

All compounds were examined for their AChE and BChE inhibitory activities by Ellman's assay. The cholinesterase inhibitory activity of the synthesized compounds is summarized in Table 1.

TABLE 1. Cholinesterase inhibitory activities of synthesized compounds

Compounds	Structure	Inhibition at 10 μg/mL (%)	
		AChE	BChE
2		12.79 ± 0.41	30.11 ± 1.10
3		55.13 ± 2.24	29.95 ± 1.45
4		No activity	18.52 ± 6.63
5		30.94 ± 2.39	30.47 ± 10.02
6		59.18 ± 2.77	22.70 ± 2.51
7		6.60 ± 4.5	10.00 ± 0.30
8		16.29 ± 1.18	28.41 ± 2.93
9		71.53 ± 27.64	22.15 ± 2.77
10		19.00 ± 6.72	36.73 ± 6.31
11		23.1 ± 0.10	38.41 3.76

All compounds were initially tested at 10 µg/mL on AChE and BChE enzymes. The eugenol derivatives showed inhibitory activity against the AChE in the range of 12.79 to 71.53%, while showing much weaker inhibition against BChE enzyme, in the range of 10.0 to 38.41%. Among them, compounds **4**, **2**, **7**, **8**, **10** and **11** had higher inhibition against BChE, while compounds **3**, **6**, **9** had higher inhibition against AChE. The substituents at the hyroxyl group of eugenols had variable effects on cholinesterase inhibition. Attachment of a benzoyl group resulted in better inhibitory activity as compared to aliphatic substituent (compound **4**). The para substituent of the benzoyl ring also affects the overall inhibitory activity. For instance, among the halogens, para substituted bromo derivative (compound **6**) had much higher AChE inhibitory activity as compared to fluorine and chlorine. Para substituted nitro and methoxy derivatives had relatively weaker inhibitory activity against AChE, while para substituted ethyl derivative had higher AChE inhibition than the para substituted methyl derivative.

DETERMINATION OF IC_{50}

Three eugenol derivatives (compounds **3**, **6**, **9**) demonstrated more than 50% inhibition on AChE were subjected for IC_{50} determination. The results are summarized in Table 2. Compound **9** showed most promising AChE inhibitory activity among the compounds tested with IC_{50} values of 5.64 ug/mL. Compounds **3, 6, 9** were statistical less significant than standard drug, physostigmine.

TABLE 2. IC50 on AChE for the active compounds

Compounds	IC_{50}	
	µg/mL	µM
3	12.23 ± 0.76***	
6	13.12 ± 1.33***	
9	5.64 ± 1.12***	
Physostigmine	0.044±0.003	0.16

Data are represented as mean ± SD (n=3). ***p<0.001 compared to physostigmine (standard)

MOLECULAR DOCKING STUDIES

Molecular docking studies were performed to provide a binding mode of eugenol derivatives within the cholinesterase enzymes. The crystal structure of human acetylcholinesterase (hAChE) was downloaded from the Protein Data Bank (PDB ID: 4M0E, 2.0 A) (Cheung et al. 2013). To validate the docking protocol, the ligand (Dihydrotanshinone I, a natural product and an AChE inhibitor) that had co-crystallized with the enzyme (4M0E) was docked against the same enzyme using BioSolveIT'sLeadIT software (LeadIT version 2.3.2; BioSolveIT GmbH, Sankt Augustin, Germany, 2017, www.biosolveit.de/LeadIT). The docking method was able to reproduce the experimentally observed conformation with a rmsd of 0.9 A. Three most potent AChE inhibitors (compound **9**; IC_{50} = 5.64 ± 1.12, compound **6**; IC_{50} = 13.12 ± 1.33, and compound **3** IC_{50} = 12.23 ± 0.76) were selected for docking studies.

Figure 2 shows most favorable docked conformation of compound **9**. The carbonyl oxygen was found to be making a hydrogen bond with Tyr124. One of the phenyl rings was making a Pi-Pi T-shaped interaction with Tyr337. The other phenyl group was making a pi-pi staked interaction with Ty341. The alkyl and allyl substituents on both phenyl rings were found to be making pi-alkyl interactions surrounding amino acids His447, Tro286, Phe295 and Val294.

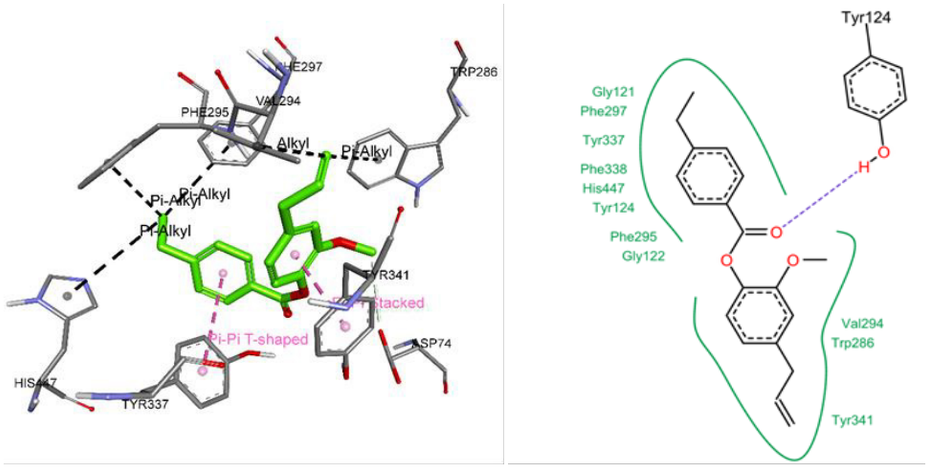


FIGURE 2. Most probable docked conformation of compound **9**

The most favorable docked conformation of second most potent inhibitor in the series (compound **6**), is given in Figure 3. Although compound **9** binds at the same place, it was found to have a slightly different binding conformation as compared to compound **9**, which may be due to the difference in bromo and bulky ethyl substituent on the phenyl ring. The carbonyl oxygen was making a hydrogen bond with Thr75. Additionally, pi-pi stacked interactions were observed between one of the phenyl rings and Trp286. The other phenyl ring was making a pi-alkyl interaction with Leu76.

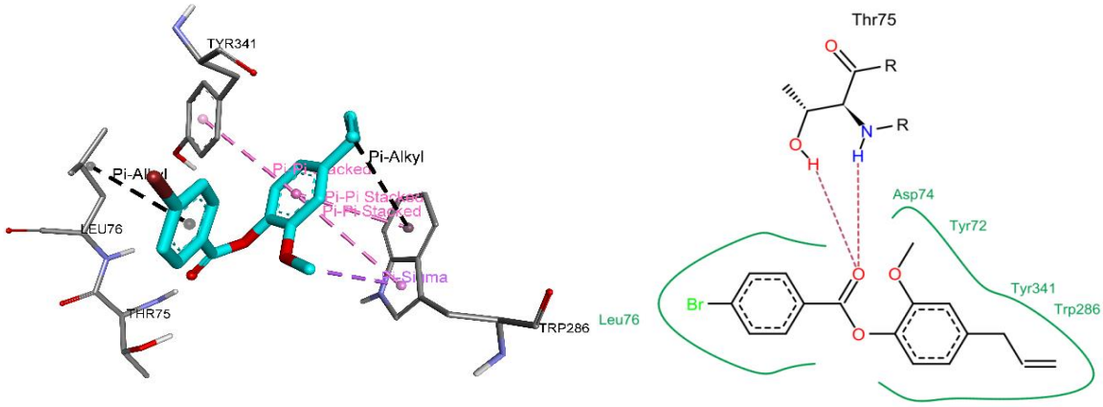


FIGURE 3. Most probable docked conformation of compound **6**

The docked conformation of compound **3** is given in Figure 4. The compound binds in the same binding site as that of co-crystallized ligand. A hydrogen bond was observed between the amino group of Phe295 and the fluorine atom of CF_3 group. The phenyl ring containing the CF_3 group was making pi-pi T-shaped and pi-pi stacked interactions with amino acids Phe297 and Tyr341. The other phenyl ring was making pi-pi T-shaped interaction with His447. Pi-alkyl interactions were observed for allyl group with amino acids His447 and Trp86. Pi-alkyl interactions were also observed between the CF_3 group and Trp286 and Val294.

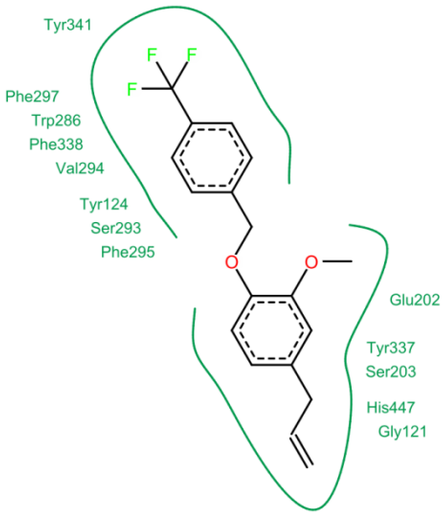


FIGURE 4. Most probable docked conformation of compound **3**

CONCLUSION

In this preliminary study, ten eugenol derivatives were prepared and evaluated for acetylcholinesterase and butyrylcholinesterase inhibition. Three derivatives (compounds **3**, **6**, **9**) showed higher and good AChE inhibitory activity of more than 50% inhibition at 10 µg/mL. Compound **9** which bore an ethyl substituent at para position of the benzoyl ring exhibited the strongest AChE inhibition with IC_{50} values of 5.64 ug/mL. However, these derivatives (**3**, **6**, **9**) were statistical less significant than standard drug, physostigmine. Further studies are necessary to investigate the potential of eugenol derived molecules with different substituents against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) for the development of new and effective synthetic anti-Alzheimer compounds to treat AD.

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REFERENCES

Ali, T.B., Schleret, T.R., Reilly, B.M., Chen, W.Y. & Abagyan, R. 2015. Adverse effects of cholinesterase inhibitors in dementia, according to the pharmacovigilance databases of the United-States and Canada. *PLoS ONE* 10(12): e0144337.

Alqareer, A., Alyahya, A. & Andersson, L. 2006. The effect of clove and benzocaine versus placebo as topical anesthetic. *Journal of Dentistry* 34(10): 747-750.

Cheung, J., Gary, E.N., Shiomi, K. & Rosenberry, T.L. 2013. Structures of human acetylcholinesterase bound to dihydrotanshinone I and territrem B show peripheral site flexibility. *ACS Medicinal Chemistry Letters* 4(11): 1091-1096.

da Silva, F.F.M., Monte, F.J.Q., de Lemos, T.L.G., Do Nascimento, P.G.G., de Medeiros Costa, A.K. & De Paiva, L.M.M. 2018. Eugenol derivatives: Synthesis, characterization, and evaluation of antibacterial and antioxidant activities. *Chemistry Central Journal* 12(1): 1-9.

Devi, K.P., Nisha, S.A., Sakthivel, R. & Pandian, S.K. 2010. Eugenol (an essential oil of clove) acts as an anti-bacterial agent against *Salmonella typhi* by disrupting the cellular membrane. *Journal of Ethnopharmacology* 130(1): 107-115.

Feng, J. & Lipton, J.M. 1987. Eugenol: Antipyretic activity in rabbits. *Neuropharmacology* 26(12): 1775-1778.

Fichi, G., Flamini, G., Giovanelli, F., Otranto, D. & Perucci, S. 2007. Efficacy of an essential oil of *Eugenia caryophllata* against *Psoroptes cuniculi. Experimental Parasitolog* 115(2): 168-172.

Goulet, F., Hélie, P. & Vachom, P. 2010. Eugenol anesthesia in African clawed frogs (*Xenopuslaevis*) of different body weights. *Journal of the American Association for Laboratory Animal Science* 49(4): 460-463.

Johny, A.K., Darre, M.J., Donoghue, A.M., Donoghue, D.J. & Venkitanarayanan, K. 2010. Antibacterial effect of trans-cinnamaldehyde, eugenol, carvacrol, and thymol on *Salmonella enteritidis* and *Campylobacter jejuni* in chicken cecal contents *in vitro*. *Journal of Applied Poultry Research* 19(3): 237-244.

Jirovetz, L., Buchbauer, G., Stoilova, I., Stoyanova, A., Krastanov, A. & Schmidt, E. 2006. Chemical composition and antioxidant properties of clove leaf essential oil. *Journal of Agriculture and Food Chemistry* 54(17): 6303-6307.

Kang, S.H., Kim, M.K., Seo, D.K., Noh, D.J., Yang, J.O., Yoon, C. & Kim, G.H. 2009. Comparative repellency of essential oils against *Culex pipiens pallens* (Diptera: Culicidae). *Journal of the Korean Society for Applied Biological Chemistry* 52(4): 353-359.

Khaw, K.Y., Choi, S.B., Tan, S.C., Wahab, H.A., Chan, K.L. & Murugaiyah, V. 2014. Prenylated xanthones from mangosteen as promising cholinesterase inhibitors and their molecular docking studies. *Phytomedicine* 21(11): 1303-1309.

Lane, R.M., Potkin, S.G. & Enz, A. 2006. Targeting acetylcholinesterase and butyrylcholinesterasein dementia. *International Journal of Neuropsychopharmacology* 9(1): 101-124.

Li, W., Chen, H., He, Z., Han, C., Liu, S. & Li, Y. 2015. Influence of surfactant and oil composition on the stability and anti-bacterial activity of eugenol nanoemulsions. *LWT-Food Science and Technology* 62(1): 39-47.

Markowitz, K., Moynihan, M., Liu, M. & Kim, S. 1992. Biological properties of eugenol and zinc oxide-eugenol: A clinical oriented review. *Oral Surgery, Oral Medical, Oral Pathology* 73(6): 729-737.

Maurya, A.K., Agarwal, K., Gupta, A.C., Saxena, A., Nooreen, Z., Tandon, S., Ahmad, A. & Bawankule, D.U. 2018. Synthesis of eugenol derivatives and its anti-inflammatory activity against skin inflammation. *Natural Product Research* 34(2): 251-260.

Milczarek, G. & Ciszewski, A. 2012. Functionalized gold nanoparticles and films stabilized by *in situ* formed polyeugenol. *Colloids and Surfaces B: Biointerfaces* 90: 53-57.

Nassar, M.I., Gaara, A.H., El-Ghorab, A.H., Farrag, A.R.H., Shen, H., Huq, E. & Mabry, T.J. 2007. Chemical constituents of clove (*Syzygiumaromaticum*, Fam. Myrtaceae) and their antioxidant activity. *Revista Latinoamericana de Química* 35(3): 47-57.

Olea, A.F., Bravo, A., Martínez, R., Thomas, M., Sedan, C., Espinoza, L., Zambrano, E., Carvajal, D., Silva-Moreno, E. & Carrasco, H. 2019. Antifungal activity of eugenol derivatives against *Botrytis cinerea*. *Molecules* 24(7): 1239.

Rahim, N.H.C.A., Asari, A., Ismail, N. & Osman, H. 2017. Synthesis and antibacterial study of eugenol derivatives. *Asian Journal of Chemistry* 29(1): 22-26.

Thompson, P.A., Wright, D.E., Counsell, C.E. & Zajicek, J. 2012. Statistical analysis, trial design and duration in Alzheimer's disease clinical trials: A review. *International Psychogeriatrics* 24(5): 689-697.

Tippayatum, P. & Chonhenchob, V. 2007. Antibacterial activities of thymol, eugenol and nisin against some food spoilage bacteria. *Agriculture and Natural Resources* 41(5): 319-323.

Wagner, H., Jurcic, K. & Deininger, R. 1979. Antispasmodic activity of eugenol-esters and eugenol-ethers. *Planta Medica* 37(1): 9-14.

Zeringóta, V., Senra, T.O.S., Calmon, F., Maturano, R., Faza, A.P., Catunda-Junior, F.E., Monteiro, C.M., de Carvalho, M.G. & Daemon, E. 2013. Repellent activity of eugenol on larvae of *Rhipicephalus microplus* and *Dermacentor nitens* (Acari: Ixodidae). *Parasitology Research* 112(7): 2675-2679.

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