

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 in acetylcholine, 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 possess serious side effects (Ali et al. 2015), more studies are needed to discover potential compounds to treat AD.

Eugenol or 4-allyl-2-methoxyphenol (**1**) (Figure 1) is one of the phenylpropanoids available in nature. It is the main constituent isolated from cloves, *Syzygium aromaticum*, 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.

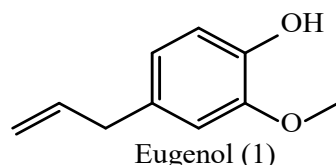


FIGURE 1. Chemical structure of Eugenol (1)

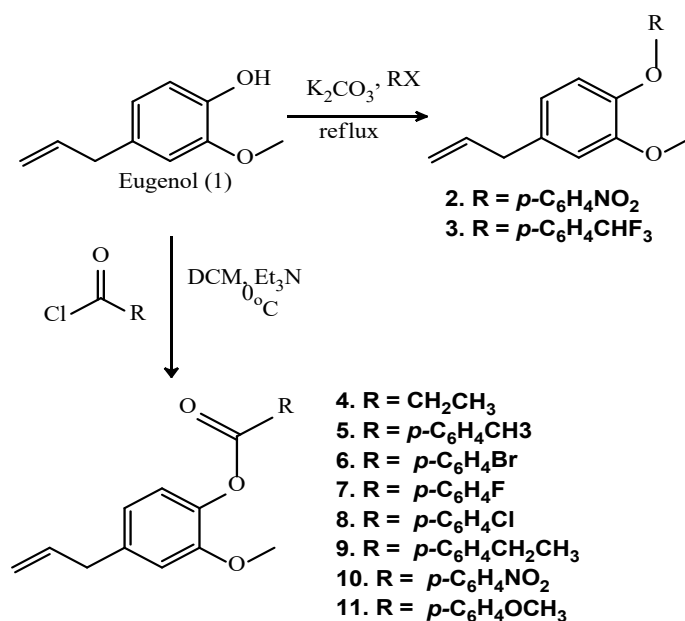
MATERIALS AND METHODS

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 ϵ) 234 (4.03), 273 (4.12) nm; ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$) 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 2.2 Hz, 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, $(\text{CD}_3)_2\text{CO}$) 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 $[\text{M}+2\text{H}]^+$ 300.14 ($\text{C}_{17}\text{H}_{17}\text{NO}_4$, 322.10); Anal. Calcd. for $\text{C}_{17}\text{H}_{17}\text{NO}_4$: 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 ϵ) 234 (4.25), 276 (4.22) nm; ^1H 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 $[\text{M}]^+$ 322 ($\text{C}_{18}\text{H}_{17}\text{F}_3\text{O}_2$, 322.32); Anal. Calcd. for $\text{C}_{18}\text{H}_{17}\text{F}_3\text{O}_2$: 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 ϵ) \sim sh 224 (4.01), 274 (3.53), 279 (3.50) nm; ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$) 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, $(\text{CD}_3)_2\text{CO}$) 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 $[\text{M}+\text{H}]^+$ 221 ($\text{C}_{13}\text{H}_{16}\text{O}_3$, 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 ϵ) 241 (4.45), 274 (3.87) nm; ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$) δ 2.44 (s, 3H, CH_3), 3.42 (d, J 6.8 Hz, 2H, CH_2), 3.78 (s, 3H, OCH_3), 5.04-5.08 (qd, J 1.2, 3.2, 10.0 Hz, 1H, CH_2), 5.10-5.16 (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, $(\text{CD}_3)_2\text{CO}$) 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 $[\text{M}]^+$ 282, ($\text{C}_{18}\text{H}_{18}\text{O}_3$, 282.10); Anal. Calcd. for $\text{C}_{18}\text{H}_{18}\text{O}_3$: 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 ϵ) 246 (4.44), \sim sh 272 (3.60) nm; ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$) 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, $(\text{CD}_3)_2\text{CO}$) 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 $[\text{M}+\text{CH}_2]^+$ 360.19 ($\text{C}_{17}\text{H}_{15}\text{BrO}_3$, 346.10); Anal. Calcd. for $\text{C}_{17}\text{H}_{15}\text{BrO}_3$: 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 ϵ) 261 (4.18) nm; ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$) 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, $(\text{CD}_3)_2\text{CO}$) 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 $[\text{M}+\text{Na}]^+$ 309 ($\text{C}_{17}\text{H}_{15}\text{FO}_3$, 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 ϵ) 241 (4.32), 274 (3.60), \sim sh 279 (3.55) nm; ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$) 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, $(\text{CD}_3)_2\text{CO}$) 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 ϵ) 240 (4.32), 273 (3.62) nm; ¹H 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; ¹³C 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 ϵ) 229 (4.28), 273 (3.54), ~sh 281 (3.46) nm; ¹H 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; ¹³C 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 ϵ) 258 (4.41), ~sh 275 (4.27) nm; ¹H 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), 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), 6.82 (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; ¹³C 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 μ L of 0.1 M sodium phosphate buffer (pH 8) was first added to the 96-well microplate followed by 20 μ L of the test sample (in 10 % methanol), 20 μ L of 0.09 unit/mL AChE or BChE, 10 μ L of 10 mM 5,5'-dithiobis(2-nitrobenzoic acid) was added into each well followed by 10 μ L 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 Pro Microplate 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 μ g/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's LeadIT 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 $\mu\text{g/mL}$ (%)	
		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 hydroxyl 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 IC₅₀

Three eugenol derivatives (compounds **3**, **6**, **9**) demonstrated more than 50% inhibition on AChE were subjected for IC₅₀ determination. The results are summarized in Table 2. Compound **9** showed most promising AChE inhibitory activity among the compounds tested with IC₅₀ values of 5.64 µg/mL. Compounds **3**, **6**, **9** were statistically 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 Å) (Cheung et al. 2013). To validate the docking protocol, the ligand (Dihydroxanthinone I, a natural product and an AChE inhibitor) that had co-crystallized with the enzyme (4M0E) was docked against the same enzyme using BioSolveIT's LeadIT 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 Å. Three most potent AChE inhibitors (compound **9**; IC₅₀ = 5.64 ± 1.12, compound **6**; IC₅₀ = 13.12 ± 1.33, and compound **3** IC₅₀ = 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 stacked 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.

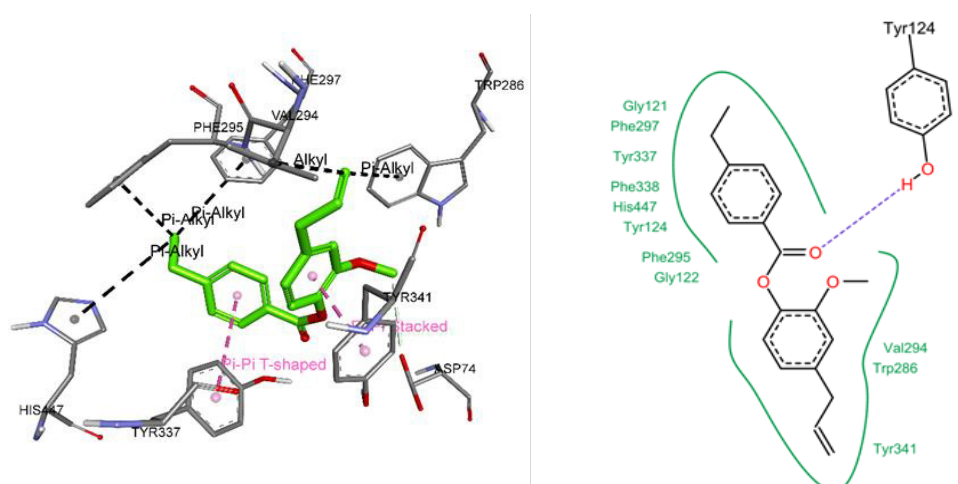


FIGURE 2. Most probable docked conformation of compound **9**

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.

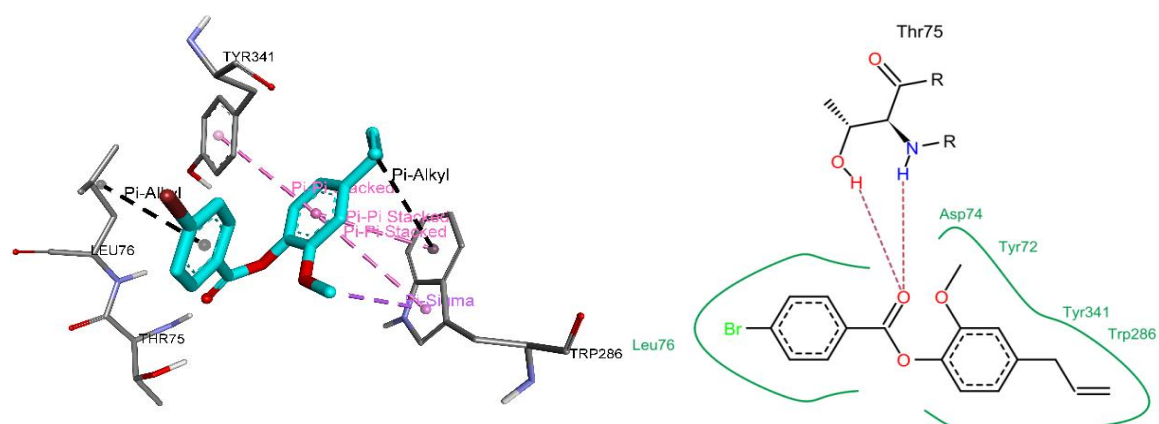


FIGURE 3. Most probable docked conformation of compound **6**

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.

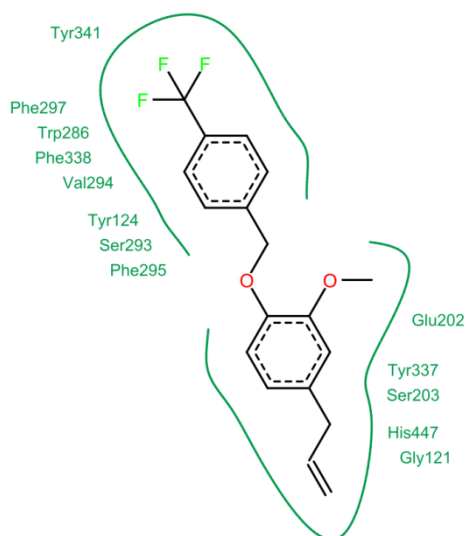


FIGURE 4. Most probable docked conformation of compound **3**

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 $\mu\text{g}/\text{mL}$. 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 $\mu\text{g}/\text{mL}$. 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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