Accepted Manuscript

2-Benzoyl-6-benzylidenecyclohexanone analogs as potent dual inhibitors of acetylcholinesterase and butyrylcholinesterase

Sze Wei Leong, Faridah Abas, Kok Wai Lam, Khozirah Shaari, Nordin H. Lajis

PII: S0968-0896(16)30439-4
DOI: http://dx.doi.org/10.1016/j.bmc.2016.06.016
Reference: BMC 13072

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 28 March 2016
Revised Date: 26 May 2016
Accepted Date: 9 June 2016

Please cite this article as: Leong, S.W., Abas, F., Lam, K.W., Shaari, K., Lajis, N.H., 2-Benzoyl-6-benzylidenecyclohexanone analogs as potent dual inhibitors of acetylcholinesterase and butyrylcholinesterase, *Bioorganic & Medicinal Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bmc.2016.06.016

2-Benzoyl-6-benzylidenecyclohexanone analogs as potent dual inhibitors of acetylcholinesterase and butyrylcholinesterase

Sze Wei Leong [a], Faridah Abas [a,b*], Kok Wai Lam [c], Khozirah Shaari [a,d], Nordin H. Lajis [a,*]

[a]*Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.*

[b]*Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.*

[c]*Drug and Herbal Research Centre Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abd. Aziz, 50300 Kuala Lumpur, Malaysia.*

[d]*Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.*

*Corresponding authors

Tel +603 89471491; Fax: +603 89423552

E-mail addresses: faridah_abas@upm.edu.my (Abas. F), nordinlajis@gmail.com (NHLajis)

Abstract

In the present study, a series of 2-benzoyl-6-benzylidenecyclohexanone analogs have been synthesized and evaluated for their anti-cholinesterase activity. Among the forty-one analogs, four compounds (**38**, **39**, **40** and **41**) have been identified as lead compounds due to their highest inhibition on both AChE and BChE activities. Compounds **39** and **40** in particular exhibited highest inhibition on both AChE and BChE with IC_{50} values of 1.6 μM and 0.6 μM, respectively. Further structure-activity relationship study suggested that presence of a long-chain heterocyclic in one of the rings played a critical role in the dual enzymes' inhibition. The Lineweaver-Burk plots and docking results suggest that both compounds could simultaneously bind to the PAS and CAS regions of the enzyme. ADMET analysis further confirmed the therapeutic potential of both compounds based upon their high BBB-penetrating. Thus, 2-benzoyl-6-benzylidenecyclohexanone containing long-chain heterocyclic amine analogs represent a new class of cholinesterase inhibitor, which deserve further investigation for their development into therapeutic agents for cognitive diseases such as Alzheimer.

Keywords:

Acetylcholinesterase; Butyrylcholinesterase; 2-Benzoyl-6-benzylidenecyclohexanone; Kinetic studies; Molecular docking

1. Introduction

Acetylcholinesterase and butyrylcholinesterase are important enzymes that catalyze the degradation of acetylcholine, an important neurotransmitter involved in memory and cognition.[1-3] These enzymes have been closely associated to Alzheimer's disease (AD), a progressive and irreversible neurodegenerative brain disorder characterized by permanent memory loss, cognitive impairment, disorientation, confusion and language deficits.[4] It is the most common type of age-related dementia which affects more than 46 million elderly globally and the number is expected to be triple by the year 2050.[5] Cholinesterase enzymes have also been found to promote the aggregation of neurotoxic *β*-amyloid which responsible for neuronal cell apoptosis.[6, 7] Therefore, targeting AChE and BChE may be one of the most promising approaches in treating AD.

To date, several cholinesterase inhibitors such as tacrine, donepezil, rivastigmine and galantamine have been approved for the treatments of AD. Although they offered some improvement in AD, several adverse effects including nausea, diarrhea, dizziness and vomiting, have also been observed.[8-10] These unpleasant side effects may not only affect the patients' health but also reduce their quality of life. New safer cholinesterase inhibitors with minimal side effects are therefore urgently warranted.

Curcumin is a well-known chemical constituent abundantly found in *Curcuma longa*, which has been extensively studied for centuries due to its valuable medicinal properties, especially the anti-oxidant and anti-inflammatory activities.[11-14] Recent studies showed that curcumin could be an ideal agent for treating AD upon its excellent safety profile and distinctive interference on several AD-related pathological pathways including acetylcholine degradation, *β*-amyloid aggregation, *tau* protein accumulation and neuronal cell destruction.[15-19] However, the therapeutic potential of curcumin is constrained by its poor absorbability and stability, resulting in low oral bioavailability, which therefore diminishes its usefulness in clinical trials.[20]

Diarylpentanoids, a bioactive group of compounds derived from curcumin has received increasing attention for its multiple medicinal properties. Diarylpentanoids have been found to exhibit excellent anti-oxidant and anti-inflammatory properties based on their distinctive suppressing effects on numerous free radicals and pro-inflammatory cytokines such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, superoxide radical, hydroxyl radical, tumor necrosis factor alpha (TNF-α), and interleukins.[21-24] Recent studies showed that diarylpentanoids could be the most impactful candidate to substitute curcumin as therapeutic agent for AD due to its better stability and anti-Alzheimer properties including anti-cholinesterase and anti-*β*-amyloid aggregation activities.[25, 26] On the basis of this, we therefore further derivatize and investigate the anti-

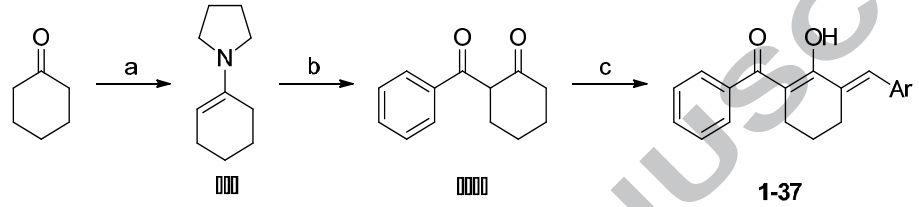
cholinesterase potential of our novel cyclohexanone containing diarylpentenedione series, which has also been shown to exhibit anti-inflammatory properties with excellent metabolic stability.[27]

2. Results and discussion

2.1. Chemistry

Synthesis of target compounds was performed according to the reaction sequence outlined in **Scheme 1** and **2**.

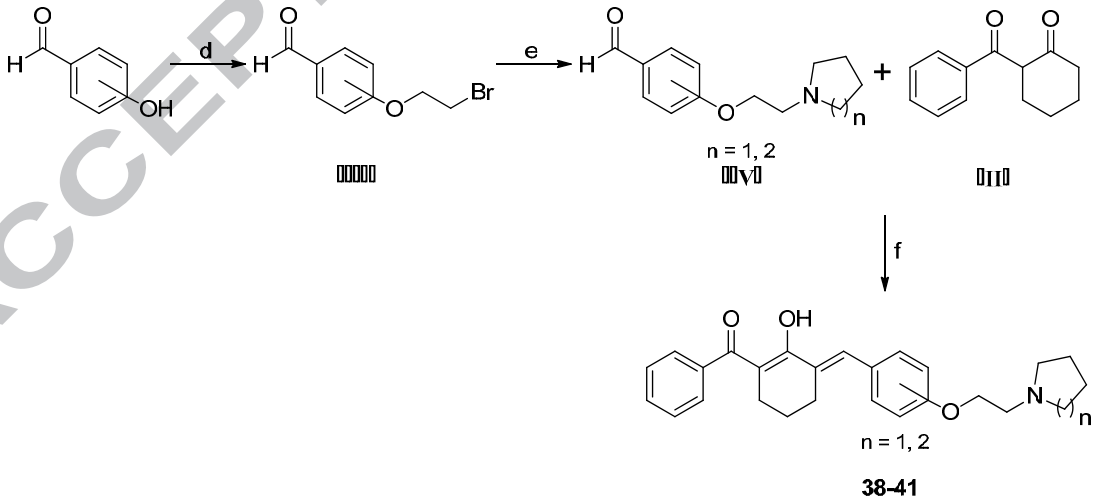
Scheme 1. General synthetic steps for compounds **1-37**[x]



[x]Reagents and conditions: (a) pyrrolidine, *p*-toluene-sulphonic acid, toluene, reflux (2h); (b) benzoic anhydride, RT (24h); (c) H_2O, reflux (0.5h); (h) benzaldehyde, acetic acid, H_2SO_4, RT (overnight).

As shown in **Scheme 1**, cyclohexanone was first reacted with pyrrolidine to prepare the enamine intermediate **I**. The reaction was carried out using Dean-Stark trap to remove water by product in order to prevent the hydrolysis of the desired enamine. Enamine **I** was then reacted with a benzoic anhydride to afford **II**, the key intermediate in the synthesis of the target compounds. The formation of diketone intermediate **II** was confirmed by the detection of a triplet at 4.38 ppm in the proton NMR spectra (data not shown). The purified intermediate **II** was further reacted with commercially available aromatic aldehydes to achieve compounds **1-37** with appreciable yield, ranging from 35-70 %.

Scheme 2. General synthetic steps for compounds **38-41**[x]



[x]Reagents and conditions: (d) 1,2-dibromoethane, K_2CO_3, DMF, 80ºC (6h); (e) pyrrolidine or piperidine, K_2CO_3, DMF, reflux (8h); (f) acetic acid, H_2SO_4, RT (overnight).

To prepare compounds **38-41** (**Scheme 2**), hydroxylated benzaldehydes were first reacted with 1,2-dibromoethane to form alkoxylated benzaldehyde **III**. The reactions were carried out in DMF with the presence of K_2CO_3. Excellent yield (>95%) was obtained for both the 3'- and 4'-hydroxybenzaldehydes after six hours of reaction time. The product **III** was then reacted with appropriate heterocyclic amines to form aminated aldehyde **IV**. Finally, aminated aldehydes were further reacted with previously synthesized diketone intermediate **II** to afford the target compounds **38-41**. All synthesized compounds were purified by column chromatography and characterized by ^{1}H-NMR, ^{13}C-NMR, and high-resolution electron impact-mass spectrometry. All purified compounds were of 95–99% purity based on their respective HPLC profiles (refer to Supplementary data).

2.2. In-vitro AChE and BChE inhibitory activity

The *in-vitro* AChE and BChE inhibitory activities of the compounds were determined by Ellman's method and the preliminary screening results for synthesized compounds are presented in **Figure 1**.

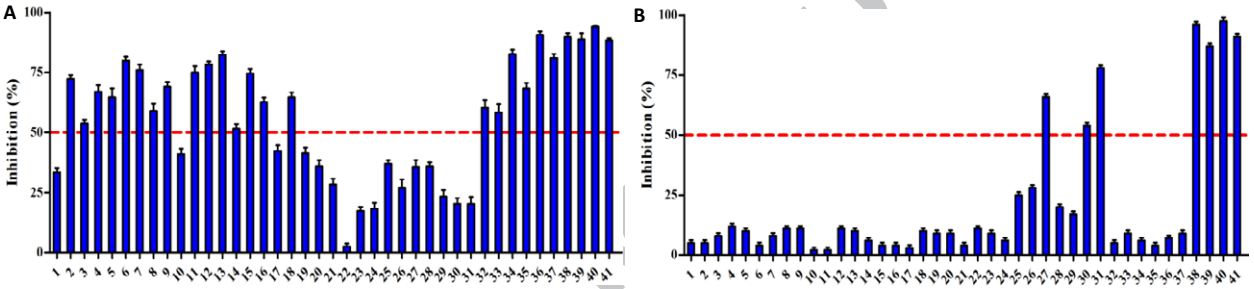
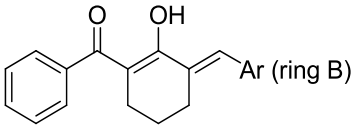


Figure 1. AChE **(A)** and BChE **(B)** inhibitory effects of compounds **1-41** at a testing concentration of 10 µM.

As depicted in **Figure 1**, twenty-five compounds were found to inhibit AChE activity as compared to only seven compounds that showed reasonable inhibition on BChE activity. These results suggested that cyclohexanone containing diarylpentenedione scaffold is generally more selective towards AChE. The IC_{50} values of the compounds are listed in **Table 1**.

Table 1. AChE and BChE inhibitory activities of compounds **1-41**.



Compounds	Ar (Ring B)	AChE IC_{50} (µM)	BChE IC_{50} (µM)
1	Phenyl	ND	ND
2	2-fluorophenyl	6.6 ± 0.5	ND
3	3-fluorophenyl	9.7 ± 1.1	ND
4	4-fluorophenyl	6.6 ± 0.3	ND
5	3,4-difluorophenyl	8.7 ± 0.8	ND
6	2-bromophenyl	8.4 ± 0.6	ND
7	4-bromophenyl	8.7 ± 0.7	ND
8	3,4-dibromophenyl	9.0 ± 0.4	ND
9	2-chlorophenyl	8.2 ± 0.9	ND
10	3-chlorophenyl	ND	ND
11	4-chlorophenyl	7.6 ± 0.4	ND

12	2,3-dichlorophenyl	5.9 ± 0.6	ND
13	2,4-dichlorophenyl	5.4 ± 0.2	ND
14	3,4-dichlorophenyl	9.8 ± 0.2	ND
15	3-methylphenyl	8.6 ± 0.6	ND
16	4-methylphenyl	8.5 ± 0.2	ND
17	2-methoxyphenyl	ND	ND
18	3-methoxyphenyl	7.4 ± 0.5	ND
19	4-methoxyphenyl	ND	ND
20	2,3-dimethoxyphenyl	ND	ND
21	2,5-dimethoxyphenyl	ND	ND
22	3,4-dimethoxyphenyl	ND	ND
23	2,3,4-trimethoxyphenyl	ND	ND
24	3,4,5-trimethoxyphenyl	ND	ND
25	3-hydroxyphenyl	ND	ND
26	4-hydroxyphenyl	ND	ND
27	3,4-dihydroxyphenyl	ND	6.5 ± 0.6
28	3-hydroxy-4-methoxyphenyl	ND	ND
29	4-hydroxy-3-methoxyphenyl	ND	ND
30	3-chloro-4-hydroxyphenyl	ND	10.6 ± 0.3
31	3-bromo-4-hydroxyphenyl	ND	4.7 ± 0.2
32	naphthalen-1-yl	7.6 ± 0.1	ND
33	naphthalen-2-yl	8.3 ± 1.5	ND
34	thiophen-2-yl	5.1 ± 0.3	ND
35	5-methylthiophen-2-yl	5.9 ± 0.5	ND
36	3-benzylphenyl	7.8 ± 0.7	ND
37	4-benzylphenyl	8.4 ± 0.7	ND
38	3-(2-(piperidin-1-yl)ethoxy)phenyl	3.1 ± 0.4	1.4 ± 0.4
39	4-(2-(piperidin-1-yl)ethoxy)phenyl	1.6 ± 0.2	2.7 ± 0.6
40	3-(2-(pyrrolidin-1-yl)ethoxy)phenyl	3.5 ± 0.3	0.6 ± 0.1
41	4-(2-(pyrrolidin-1-yl)ethoxy)phenyl	2.0 ± 0.3	2.3 ± 0.2
Tacrine	-	0.095 ± 0.011	0.010 ± 0.004

ND = Not determine

Based on the IC_{50} values of diarylpentenedione analogs against AChE, four compounds were found to exhibit significant anti-AChE activity with IC_{50} values ranging from 1.6 to 3.5 µM. Compound **39** was identified as the most potent due to its strong inhibition profile with IC_{50} value of 1.6 µM. The overall results suggested that heterocyclic amine is particularly important for AChE inhibitory activity as all compounds bearing pyrrolidine or piperidine fragments exhibited strong AChE inhibition. With respect to the substitution pattern of heterocyclic amines on aryl ring, *para*- substitution is preferable since compounds **39** and **41** exhibited approximately 2-fold higher inhibition than their respective *meta*-aminated analogs, **38** and **40**. On the other hand, the presence of thiophene ring in place of aryl (ring B) was found to contribute moderately to AChE inhibition on the basis that compounds **34** and **35** achieved the IC_{50} values of 5.1 and 5.9 µM, respectively. In contrast, presence of any halogen group in aryl ring B was not favorable since analogs **2-14** generally displayed weaker inhibition. Besides, methoxy and hydroxy groups (**17-31**) are considered to be undesirable due to their poor AChE inhibition.

The BChE was strongly inhibited by compounds **38-41** with the IC_{50} values ranging from 0.6 to 2.7 µM. Compound **40** demonstrated the strongest inhibitory activity with IC_{50} value of 0.6 µM. This observation suggested that heterocyclic amine is not only important for AChE inhibition but is also critical for BChE inhibitory effect. Unlike the SAR trend exhibited by AChE, the presence of heterocyclic amine at *meta*-position is more preferable for BChE inhibition activity, as shown by analogs **38** and **40**, which were 2- to 4-fold more active than the *para*-aminated analogs **39** and **41**. In addition, pyrrolidine fragment appeared to be a better choice than piperidine, on account that compound **40** exhibited 2.3-fold better activity than **38**. Apart from heterocyclic amine, the only other contributing factor for BChE inhibitory activity was the hydroxyl

group. This is clearly implied based on the stronger BChE inhibition of compounds **25-31** (Figure 1) at 10 µM testing concentration. However, only three compounds, **27**, **30** and **31** achieved greater than 50% BChE inhibition. Further IC_{50} determination showed that they are moderate BChE inhibitors with IC_{50} values of 6.5, 10.6 and 4.7 µM, respectively.

Interestingly, it was reported that BChE activity is dramatically increased when AChE is inhibited, which implied that dual inhibitors are a better choice for treating Alzheimer's disease.[28] Thus, compounds **38**-**41** with strong inhibitory effects on both AChE and BChE activity could be potential candidates, which deserve further investigation towards a new anti-Alzheimer agent.

2.3. Kinetic analysis of AChE and BChE inhibition

To determine the inhibition type and inhibition constant, K_i of the compounds, enzyme kinetic studies were carried out on compounds **39** and **40** using AChE and BChE. The analysis was carried out by investigating the effects of inhibitors' concentration on enzyme's activity at different substrate concentrations. The data resulted from cholinesterase inhibition was further analyzed with Lineweaver-Burk method followed by dissociating constant (Ki) calculation from Michaellis-menten using GraphPad prism 5. The calculated results suggest that both compounds exhibited mixed-type inhibition with K_i values of 1.9 and 0.2 µM, respectively. The dual character (competitive and uncompetitive) of compounds **39** and **40** suggests that both of them could simultaneously bind to catalytically active site (CAS) and peripheral anionic site (PAS) of the respective enzymes. The Lineweaver-Burk double reciprocal plots of compounds **39** and **40** are depicted in **Figures 2A** and **2B**, respectively.

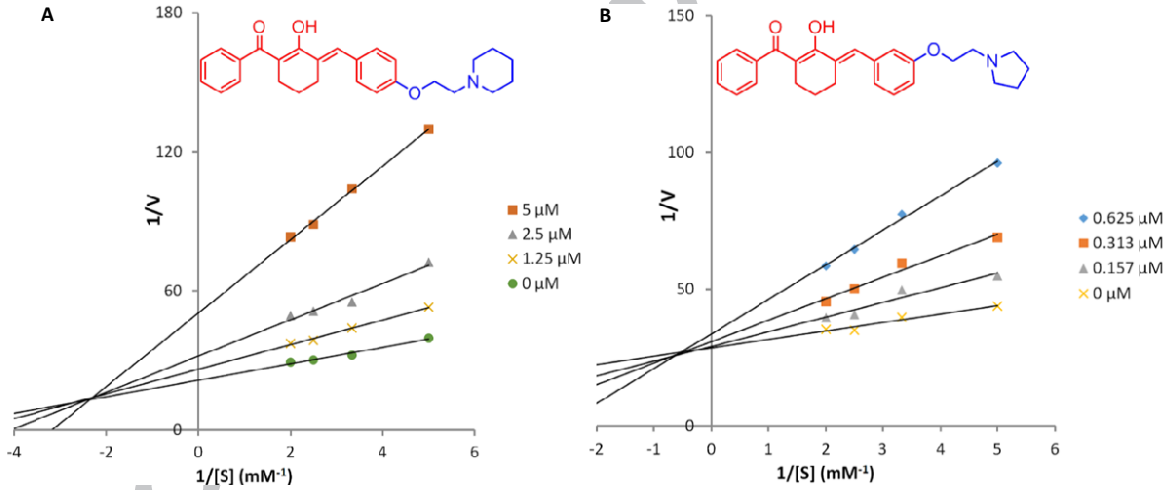


Figure 2. Lineweaver–Burk double reciprocal plots resulting from substrate-velocity curves of AChE activity of compound **39** (A) and BuChE activity of compound **40** (B).

2.4.Molecular modeling

In order to gain functional and structural insights into the binding mode of compounds **39** and **40**, flexible docking was performed using Discovery Studio 3.1. The docking parameters were first validated by re-docking of co-crystallized ligands into the active site of the enzymes. The parameters are considered successful if the Root Mean Square Deviation (RMSD) values of the re-docked ligands are less than 1.5 Å, in comparison with their original conformation in the crystal structure.[29] As depicted in **Figure 3**, both the re-docked donepezil and tacrine (red) were found to bind in a similar manner as their respective crystallographic conformations (blue), indicating that the selected docking parameters are acceptable. The RMSD values for donepezil and tacrine conformations are 1.06 Å and 0.89 Å, respectively.

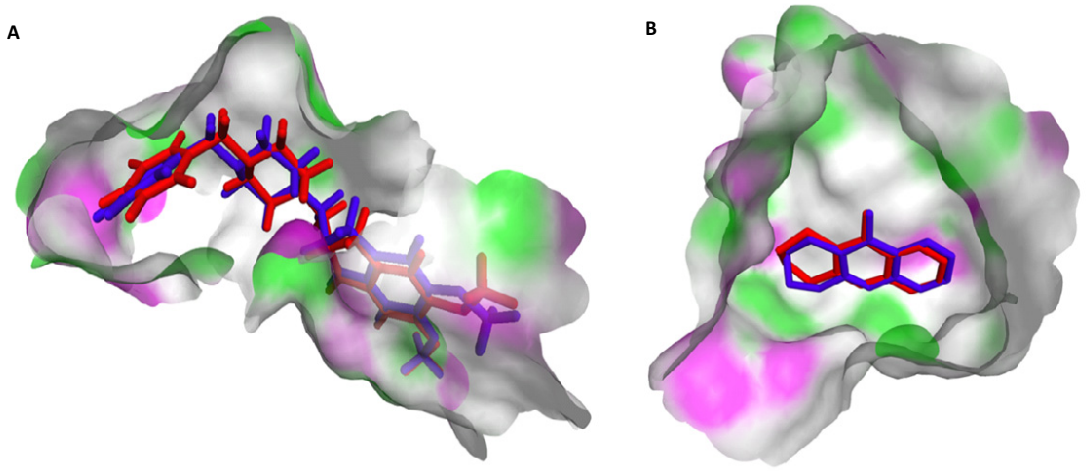


Figure 3. Overlay of re-docked (red) and crystallographic (blue) conformations of donepezil in AChE (A) and tacrine in BChE (B), respectively.

The optimized parameters were then used for the docking of compounds **39** and **40** in AChE and BChE active sites, respectively**.** Based on the docking result (**Figure 4**), compound **39** was found to simultaneously interact with both the PAS and CAS of the AChE. This observation further supported the mixed type inhibition mechanism of compound **39**, which has been suggested in our previous kinetic study.

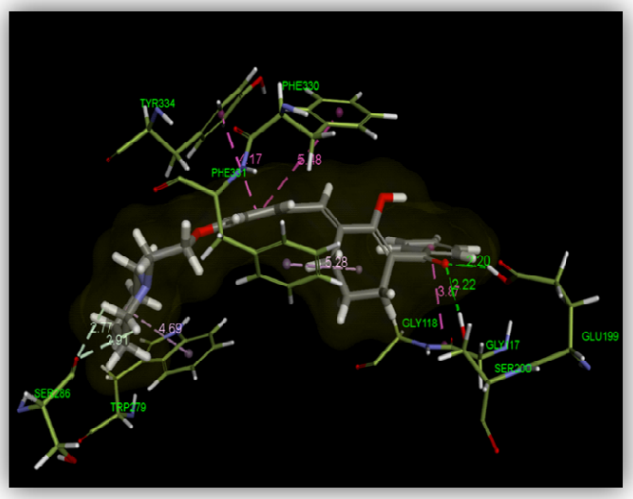


Figure 4. Binding interactions of compound **39** with the active site residues of TcAChE receptor.

The piperidine moiety of compound **39** showed a hydrophobic interaction with Trp-279 residue in PAS (**Figure 4**). This interaction is particularly important as several studies have reported the promoting role of Trp-279 in β-amyloid deposition.[30, 31] Apart from Trp-279, Tyr-334 residue of PAS also interacted with the alkoxylated phenyl ring of compound **39** through a π-π stacking. Besides, a similar interaction was also been observed in CAS in which the alkoxylated phenyl ring stacked against Phe-330 residue with a distance of 5.48 Å. It is worth noting that the cyclohexyl ring of the respective compound displayed a hydrophobic contact with Phe-331 residue located in the middle of the active site. Interestingly, the benzoyl moiety of compound **39** was found to bind to the oxyanion site and catalytic triad of TcAChE with multiple interactions. The carbonyl group of benzoyl fragment forms two hydrogen bonds with the hydroxyl group of Ser-200 and Glu-199 residues in the oxyanion site. Meanwhile, the phenyl ring of the same fragment interacts with Gly-118 residue of catalytic triad by an amide-π interaction.

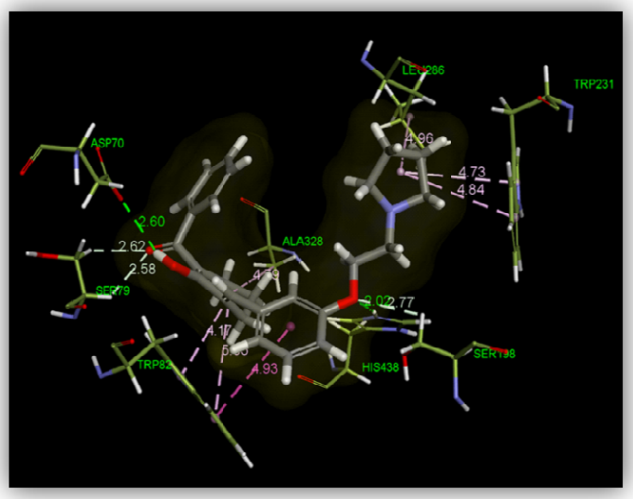
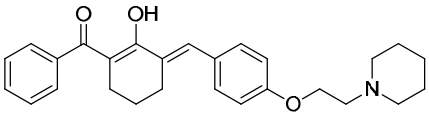
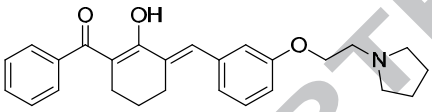


Figure 5. Binding interactions of compound **40** with the active site residues of hBChE receptor.

Similar to compound **39**, compound **40** was also found to be a mixed type inhibitor based upon its double reciprocal plots in kinetic study and dual binding character in molecular docking. As presented in **Figure 5**, compound **40** binds to the PAS of BChE by hydrogen bonding interaction with both Asp-70 and Ser-79 residues. Meanwhile, the cyclohexyl moiety of compound **40** is aligned with the choline binding site and simultaneously interacts with Trp-82 and Ala-328 residues through hydrophobic bonds. The Trp-82 interaction is further enhanced by an additional π-π stacking system between the alkoxylated phenyl moiety of compound **40** and the indole ring of the respective residue. On the other hand, compound **40** also displayed hydrogen bonding with catalytic triad through the interaction of oxygen atom of the alkoxylated phenyl ring on both the His-438 and Ser-198 residues. Interestingly, the pyrrolidine fragment was found to fit into the acyl binding pocket by interacting with both the Trp-231 and Leu-286 residues through hydrophobic contacts, resulting in the binding enhancement of compound **40** on BChE. The flexible docking results of compounds **39** and **40** are summarized in **Table 2**.

Table 2. Data resulted from the flexible docking of compounds **39** and **40** in active site gorge of TcAChE and hBChE receptors.

Compound Structure	Enzyme	Interacting site	Amino acid residue	Bond type	Bonding distance (Å)
 Compound **39**	TcAChE	PAS	Ser-286	Hydrogen bonding	2.77, 2.91
			Trp-279	Hydrophobic	4.69
			Try-334	π- π stacking	4.17
		Anionic site	Phe-330	π- π stacking	5.48
			Phe-331	Hydrophobic	5.28
		Catalytic triad	Gly-118	Amide - π stacking	3.87
		Oxyanion site	Ser-200	Hydrogen bonding	2.22
			Glu-199	Hydrogen bonding	2.20
 Compound **40**	hBChE	PAS	Asp-70	Hydrogen bonding	2.60
			Ser-79	Hydrogen bonding	2.62, 2.58
		Cation – π site	Trp-82	Hydrophonic; π- π stacking	4.17, 5.35; 4.93
			Ala-328	Hydrophobic	4.79
		Catalytic triad	His-438	Hydrogen bonding	2.02
			Ser-198	Hydrogen bonding	2.77
		Acyl binding site	Leu-286	Hydrophobic	4.96
			Trp-231	Hydrophobic	4.84, 4.73

2.5. ADMET analysis

Apart from strong cholinesterase inhibitory activity, the most fundamental condition for a promising cholinesterase inhibitor is to exhibit high blood brain barrier (BBB) penetration. Therefore, ADMET analysis was carried out, specifically to predict the BBB penetration of compounds **39** and **40**. The ADMET plot of compounds **39** and **40** is presented in **Figure 6**.

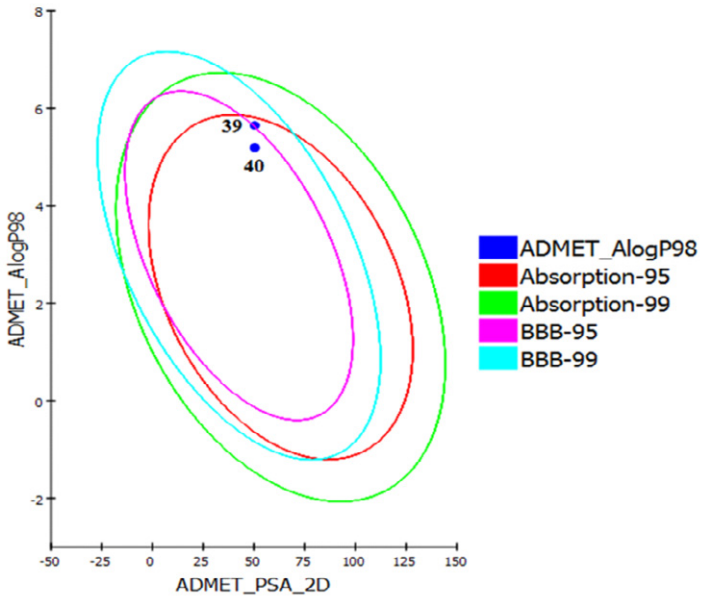


Figure 6. ADMET plot of compounds **39** and **40**

Compounds **39** and **40** are within the ellipses of 95 and 99% blood brain barrier (BBB) confidence region, indicating that both compounds could be highly BBB-penetrating agents (**Figure 6**). This observation further supports the promising potential of compounds **39** and **40** as cholinesterase inhibitors. Apart from BBB-penetration prediction, ADMET analysis was also used to predict their aqueous solubility (AS), human intestinal absorption (HIA), cytochrome P450 2D6 (CYP2D6) inhibition, plasma protein binding (PPB), and hepatotoxicity (HT). Based on the results in **Table 3**, both compounds are non-hepatotoxic with good human intestinal absorption. This implies that they have huge potential to serve as oral drugs. Poor water solubility of compounds **39** and **40** is the only possible drawback in developing the practical use of them. However, the presence of nitrogen atom in both compounds could allow the formation of salt which therefore improve their solubility.[32] On the other hand, these compounds were also found to bind to plasma protein with no interaction with cytochrome enzymes, indicating that higher dose may be required to achieve therapeutic concentration in treatments.

Table 3. Results of ADMET predictions on six important parameters.

Compounds	AS	HIA	BBB	CYP2D6	PPB	HT
39	Low	Good	High	Non-inhibit	Bound	Non-hepatotoxin
40	Low	Good	High	Non-inhibit	Bound	Non-hepatotoxin

Notes: AS = Aqueous Solubility; HIA = Human Intestinal Absorption; BBB = Blood Brain Barrier; CYP2D6 = cytochrome P450 2D6; PPB = Plasma Protein Binding; HT = Hepatotoxicity.

3. Conclusion

In summary, forty-one analogs of cyclohexanone containing diarylpentenedione have been synthesized and evaluated for their AChE and BChE inhibitory activities. Compounds **39** and **40** appeared to be the most active analogs based on the determined IC_{50} values for both AChE and BChE inhibition, respectively. Further kinetic analyses and docking studies revealed that these compounds exhibited mixed-type inhibition. The overall findings suggested that the diarylpentenedione core structure is selective towards AChE inhibition, while the long chain heterocyclic moieties are critical for both AChE and BChE inhibitory activities. ADMET analysis further confirmed the potential development of both compounds on the basis of their high blood brain barrier penetration. As a conclusion, we believe that diarylpentenedione containing a long chain heterocyclic amines is a promising class, which deserve further investigation for their development of novel cholinesterase inhibitors.

4. Experimental Section

4.1. Chemistry

Starting materials and chemical reagents were purchased from Sigma-Aldrich and Merck, and were used without purification. Solvents, which were purchased from common commercial suppliers, were dried and distilled before use. The chemical reactions were routinely checked on 0.20 mm Merck TLC plate silica gel 60 F254 in every reaction step. Purification procedures were conducted using column chromatography on Merck silica gel 60 (mesh 70-230). Melting points were determined using Fisher-Johns melting point apparatus and were uncorrected. Mass spectra were measured by GCMS-QP5050A (Shimadzu) Mass Spectrometer. High-resolution electron ionization-mass spectrometry (HREI-MS) was determined using a DFS high resolution GC/MS (Thermo Scientific, San Jose, CA, USA). Nuclear Magnetic Resonance Spectra were recorded on Varian 500 MHz NMR Spectrometer.

*4.1.1. General procedure for the synthesis of **I** and **II***

A toluene solution (100 mL) containing cyclohexanone (20 mmol), pyrrolidine (20 mmol) and a catalytic amount of *p*-toluenesulphonic acid (0.1 g) was heated under Dean-Stark condition for 2 hours to obtain **I**. Then, the reaction mixture was cooled to room temperature and further stirred with 20 mmol of benzoic anhydride for 24 hours. Upon completion, the reaction mixture was added with 10 mL of distilled water and refluxed for 30 minutes. The resulting reaction mixture was extracted thrice with 100 mL HCl (3M) and once with 20 mL water. The toluene layer was dried over anhydrous magnesium sulphate and concentrated in vacuo to give a crude product of 2-benzoylcyclohexanone (**II**). The resulting crude product was purified by column chromatography.

*4.1.2. General procedure for the synthesis of compounds **1-37***

One millimole of 2-benzoylcyclohenxanone (**II**) was reacted with an equivalent amount of appropriate aromatic aldehyde in acetic acid (30 mL) in the presence of sulfuric acid as catalyst. The reaction mixture was stirred at room temperature for overnight. Upon completion, the reaction mixture was poured into 100 mL of distilled water and stirred for 10 minutes, followed by extraction with 100 mL of ethyl acetate. The organic layer was then washed with 10% sodium bicarbonate solution and dried over anhydrous magnesium sulphate. The further evaporation in vacuo gave crude of targeted compounds **1-37**. The resulting crude product was purified by column chromatography.

2-benzoyl-6-benzylidenecyclohexen-1-ol (1). Yellow; m.p.: 126-127ºC; Mass calculated: 290.1307; Mass found: 290.1324. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.68 (quin, *J*=6.04 Hz, 2 H) 2.50 - 2.57 (m, 2 H) 2.75 - 2.82 (m, 2 H) 7.29 - 7.35 (m, 1 H) 7.38 - 7.49 (m, 7 H) 7.59 (dd, *J*=7.86, 1.46 Hz, 2 H) 7.78 (s, 1 H) 16.78 (s, 1 H).^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 27.2, 27.6, 108.4, 127.6, 128.1, 128.2, 128.4, 130.1, 130.6, 132.6, 133.4, 136.3, 138.3, 176.3, 195.0

2-benzoyl-6-(2-fluorobenzylidene)cyclohexen-1-ol (2). Yellow; m.p.: 124-125ºC; Mass calculated: 308.1213; Mass found: 308.1218. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.67 (m, 2 H) 2.54 (t, *J*=6.12 Hz, 2 H) 2.62 - 2.68 (m, 2 H) 7.08 - 7.13 (m, 1 H) 7.14 - 7.19 (m, 1 H) 7.28 - 7.34 (m, 1 H) 7.36 - 7.41 (m, 1 H) 7.41 - 7.50 (m, 3 H) 7.58 (d, *J*=6.99 Hz, 2 H) 7.78 (br. s., 1 H) 16.56 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.5, 27.3, 27.5, 108.5, 115.8, 123.7, 124.2, 125.8, 127.5, 128.1, 129.9, 130.6, 134.6, 138.4, 161.6, 174.8, 196.5

2-benzoyl-6-(3-fluorobenzylidene)cyclohexen-1-ol (3). Yellow; m.p.: 121-122ºC; Mass calculated: 308.1213; Mass found: 308.1228. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.68 (quin, *J*=6.12 Hz, 2 H) 2.51 - 2.58 (m, 2 H) 2.73 - 2.79 (m, 2 H) 7.02 (td, *J*=8.45, 1.75 Hz, 1 H) 7.14 (d, *J*=10.19 Hz, 1 H) 7.21 (d, *J*=7.57 Hz, 1 H) 7.36 (td, *J*=8.01, 6.12 Hz, 1 H) 7.41 - 7.51 (m, 3 H) 7.55 - 7.61 (m, 2 H) 7.70 (s, 1 H) 16.64 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.5, 27.2, 27.5, 108.7, 115.0, 116.5, 125.9, 127.6, 128.2, 129.9, 130.7, 131.8, 133.6, 138.2, 138.5, 163.6, 175.3, 196.1

2-benzoyl-6-(4-fluorobenzylidene)cyclohexen-1-ol (4). Yellow; m.p.: 127-128ºC; Mass calculated: 308.1213; Mass found: 308.1218. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.68 (m, 2 H) 2.54 (t, *J*=6.12 Hz, 2 H) 2.71 - 2.76 (m, 2 H) 7.05 - 7.12 (m, 2 H) 7.39 - 7.50 (m, 5 H) 7.55 - 7.59 (m, 2 H) 7.71 (s, 1 H) 16.74 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.5, 27.1, 27.5, 108.3, 115.5, 127.6, 128.1, 130.6, 131.9, 132.1, 132.3, 132.4, 138.2, 163.4, 176.1, 195.4

2-benzoyl-6-(3,4-difluorobenzylidene)cyclohexen-1-ol (5). Yellow; m.p.: 97-98ºC; Mass calculated: 326.1118; Mass found: 326.1123. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.69 (quin, *J*=6.12 Hz, 2 H) 2.51 - 2.57 (m, 2 H) 2.68 - 2.76 (m, 2 H) 7.16 (d, *J*=8.45 Hz, 1 H) 7.25 (d, *J*=8.45 Hz, 1 H) 7.41 - 7.50 (m, 4 H) 7.58 (d, *J*=8.25 Hz, 2 H) 7.63 (s, 1 H) 16.62 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.4, 27.1, 27.4, 108.7, 117.2, 118.4, 126.5, 126.6, 127.6, 128.1, 130.7, 130.8, 133.3, 138.2, 147.1, 148.3, 175.2, 196.0

2-benzoyl-6-(2-bromobenzylidene)cyclohexen-1-ol (6). Yellow; m.p.: 107-109ºC; Mass calculated: 368.0412; Mass found: 368.0427. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.64 - 1.70 (m, 2 H) 2.54 (t, *J*=6.12 Hz, 2 H) 2.57 - 2.62 (m, 2 H) 7.13 - 7.21 (m, 1 H) 7.33 (d, *J*=4.66 Hz, 2 H) 7.39 - 7.50 (m, 3 H) 7.57 (d, *J*=6.99 Hz, 2 H) 7.64 (d, *J*=8.15 Hz, 1 H) 7.78 (s, 1 H) 16.54 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 27.2, 27.3, 108.6, 124.9, 126.8, 127.5, 128.1, 129.3, 130.5, 130.6, 132.4, 132.9, 133.9, 136.6, 138.4, 174.6, 196.7

2-benzoyl-6-(4-bromobenzylidene)cyclohexen-1-ol (7). Yellow; m.p.: 140-141ºC; Mass calculated: 368.0412; Mass found: 368.0417. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.68 (dt, *J*=12.23, 6.12 Hz, 2 H) 2.54 (t, *J*=5.82 Hz, 2 H) 2.70 - 2.75 (m, 2 H) 7.30 (d, *J*=8.15 Hz, 2 H) 7.41 - 7.49 (m, 3 H) 7.52 (d, *J*=8.74 Hz, 2 H) 7.57 (d, *J*=6.99 Hz, 2 H) 7.67 (s, 1 H) 16.65 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.5, 27.1, 27.5, 108.5, 122.3, 127.6, 128.1, 130.6, 131.5, 131.5, 131.9, 13325, 135.2, 138.2, 175.5, 195.9

2-benzoyl-6-(3,4-dibromobenzylidene)cyclohexen-1-ol (8). Yellow; m.p.: 125-126ºC; Mass calculated: 445.9517; Mass found: 445.9561. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.68 (dt, *J*=12.23, 6.12 Hz, 2 H) 2.54 (t, *J*=6.12 Hz, 2 H) 2.70 (t, *J*=5.82 Hz, 2 H) 7.21 (d, *J*=8.15 Hz, 1 H) 7.42 - 7.50 (m, 3 H) 7.55 - 7.60 (m, 3 H) 7.63 (d, *J*=8.74 Hz, 1 H) 7.68 (s, 1 H) 16.54 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.4, 27.1, 27.4, 108.8, 124.3, 124.8, 127.6, 128.1, 129.8, 130.2, 130.8, 133.5, 134.3, 134.6, 137.1, 138.2, 174.6, 196.5

2-benzoyl-6-(2-chlorobenzylidene)cyclohexen-1-ol (9). Yellow; m.p.: 92-94ºC; Mass calculated: 324.0917; Mass found: 324.0932. ^{1}H NMR ($CDCl_3$) δ: 1.67 (quin, *J*=6.12 Hz, 2 H) 2.54 (t, *J*=5.97 Hz, 2 H) 2.59 - 2.64 (m, 2 H) 7.23 - 7.30 (m, 2 H) 7.35 (dd, *J*=6.99, 2.04 Hz, 1 H) 7.41 - 7.50 (m, 4 H) 7.58 (dd, *J*=8.15, 1.46 Hz, 2 H) 7.85 (s, 1 H) 16.56 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 27.3, 27.4, 108.7, 126.2, 127.5, 128.1, 129.2, 129.7, 130.1, 130.5, 130.7, 134.2, 134.7, 134.8, 138.4, 174.7, 196.7

2-benzoyl-6-(3-chlorobenzylidene)cyclohexen-1-ol (10). Yellow; m.p.: 97-99ºC; Mass calculated: 324.0917; Mass found: 324.0928. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.68 (quin, *J*=6.04 Hz, 2 H) 2.54 (t, *J*=5.97 Hz, 2 H) 2.71 - 2.78 (m, 2 H) 7.27 - 7.35 (m, 3 H) 7.40 - 7.51 (m, 4 H) 7.58 (dd, *J*=8.01, 1.31 Hz, 2 H) 7.67 (s, 1 H) 16.62 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$ $CDCl_3$) δ: 23.5, 27.2, 27.5, 108.7, 127.6, 128.0, 128.2, 128.2, 129.6, 129.6, 130.7, 131.5, 133.8, 134.3, 138.14, 138.2, 175.2, 196.2

2-benzoyl-6-(4-chlorobenzylidene)cyclohexen-1-ol (11). Yellow; m.p.: 129-130ºC; Mass calculated: 324.0917; Mass found: 324.0925. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.68 (quin, *J*=6.12 Hz, 2 H) 2.50 - 2.57 (m, 2 H) 2.70 - 2.76 (m, 2 H) 7.37 (s, 4 H) 7.42 - 7.50 (m, 3 H) 7.58 (dd, *J*=8.01, 1.31 Hz, 2 H) 7.69 (s, 1 H) 16.69 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.5, 27.2, 27.5, 108.6, 127.6, 128.1, 128.6, 130.7, 131.3, 131.9, 133.1, 134.0, 134.8, 138.2, 175.6, 195.8

2-benzoyl-6-(2,3-dichlorobenzylidene)cyclohexen-1-ol (12). Yellow; m.p.: 131-132ºC; Mass calculated: 358.0527; Mass found: 358.0545. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.66 (dt, *J*=12.23, 6.12 Hz, 2 H) 2.51 - 2.62 (m, 4 H) 7.19 - 7.25 (m, 2 H) 7.41 - 7.50 (m, 4 H) 7.57 (d, *J*=6.99 Hz, 2 H) 7.79 (s, 1 H) 16.47 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.5, 27.2, 27.3, 108.8, 126.7, 127.5, 128.1, 128.6, 129.7, 129.8, 130.7, 132.6, 133.5, 134.8, 137.1, 138.3, 174.0, 197.1

2-benzoyl-6-(2,4-dichlorobenzylidene)cyclohexen-1-ol (13). Yellow; m.p.: 135-136ºC; Mass calculated: 358.0527; Mass found: 358.0537. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.67 (dt, *J*=12.23, 6.12 Hz, 2 H) 2.54 (t, *J*=5.82 Hz, 2 H) 2.56 - 2.61 (m, 2 H) 7.24 - 7.30 (m, 2 H) 7.41 - 7.50 (m, 4 H) 7.57 (d, *J*=6.99 Hz, 2 H) 7.76 (s, 1 H) 16.48 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.5, 27.3, 27.3, 108.7, 126.6, 127.5, 128.1, 128.8, 129.6, 130.7, 131.2, 133.3, 134.2, 134.7, 135.4, 138.3, 174.1, 197.0

2-benzoyl-6-(3,4-dichlorobenzylidene)cyclohexen-1-ol (14). Yellow; m.p.: 133-135ºC; Mass calculated: 358.0527; Mass found: 358.0544. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.68 (dt, *J*=12.23, 6.12 Hz, 2 H) 2.53 - 2.56 (m, 2 H) 2.69 - 2.73 (m, 2 H) 7.25 (dt, *J*=7.3, 2.3 Hz, 1 H) 7.42 - 7.49 (m, 4 H) 7.52 (d, *J*=2.33 Hz, 1 H) 7.57 (d, *J*=6.99 Hz, 2 H) 7.61 (s, 1 H) 16.56 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.4, 27.1, 27.5, 108.8, 127.6, 128.1, 129.2, 130.3, 130.3, 130.8, 131.4, 132.0, 132.5, 134.1, 136.3, 138.2, 174.7, 196.4

2-benzoyl-6-(3-methylbenzylidene)cyclohexen-1-ol (15). Yellow; m.p.: 84-86ºC; Mass calculated: 304.1463; Mass found: 304.1470. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.67 (dt, *J*=12.23, 6.12 Hz, 2 H) 2.54 (t, *J*=5.82 Hz, 2 H) 2.76 - 2.80 (m, 2 H) 7.14 (d, *J*=6.99 Hz, 1 H) 7.25 - 7.31 (m, 3 H) 7.41 - 7.49 (m, 3 H) 7.58 (dd, *J*=6.99, 1.16 Hz, 2 H) 7.75 (s, 1 H) 16.79 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 21.5, 23.6, 27.2, 27.6, 108.3, 127.1, 127.6, 128.1, 128.2, 129.0, 130.6, 130.8, 132.3, 133.6, 136.2, 137.9, 138.3, 176.4, 195.4,

2-benzoyl-6-(4-methylbenzylidene)cyclohexen-1-ol (16). Yellow; m.p.: 121-122ºC; Mass calculated: 304.1463; Mass found: 304.1468. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.67 (quin, *J*=6.12 Hz, 2 H) 2.51 - 2.56 (m, 2 H) 2.76 - 2.80 (m, 2 H) 7.22 (d, *J*=8.15 Hz, 2 H) 7.37 (d, *J*=8.15 Hz, 2 H) 7.41 - 7.50 (m, 3 H) 7.58 (d, *J*=6.99 Hz, 2 H) 7.75 (s, 1 H) 16.84 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 21.4, 23.6, 27.2, 27.7, 108.2, 127.6, 128.1, 129.1, 130.2, 130.5, 131.7, 133.4, 133.5, 138.3, 138.4, 176.8, 195.0,

2-benzoyl-6-(2-methoxybenzylidene)cyclohexen-1-ol (17). Yellow; m.p.: 127-128ºC; Mass calculated: 320.1412; Mass found: 320.1422. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.66 (dt, *J*=11.43, 5.79 Hz, 2 H) 2.53 (t, *J*=5.68 Hz, 2 H) 2.70 (t, *J*=5.82 Hz, 2 H) 3.88 (s, 3 H) 6.93 (d, *J*=8.15 Hz, 1 H) 6.98 (t, *J*=7.43 Hz, 1 H) 7.29 - 7.37 (m, 2 H) 7.41 - 7.48 (m, 3 H) 7.54 - 7.61 (m, 2 H) 7.96 (s, 1 H) 16.77 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.7, 27.4, 27.6, 55.5, 108.1, 110.6, 119.9, 125.3, 127.6, 128.1, 129.2, 129.7, 130.2, 130.1, 132.4, 138.5, 158.0, 176.4, 195.5

2-benzoyl-6-(3-methoxybenzylidene)cyclohexen-1-ol (18) . Yellow; m.p.: 95-97ºC; Mass calculated: 320.1412 ; Mass found: 320.1424. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.67 (quin, *J*=6.12 Hz, 2 H) 2.51 - 2.56 (m, 2 H) 2.75 - 2.81 (m, 2 H) 3.84 (s, 3 H) 6.88 (dd, *J*=8.15, 2.04 Hz, 1 H) 6.99 (s, 1 H) 7.05 (d, *J*=7.57 Hz, 1 H) 7.32 (t, *J*=7.86 Hz, 1 H) 7.41 - 7.50 (m, 3 H) 7.58 (dd, *J*=8.01, 1.60 Hz, 2 H) 7.74 (s, 1 H) 16.75 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 27.2, 27.6, 55.3, 108.5, 113.8, 115.5, 122.6, 127.6, 128.1, 129.3, 130.6, 132.8, 133.2, 137.7, 138.3, 159.5, 176.2, 195.5

2-benzoyl-6-(4-methoxybenzylidene)cyclohexen-1-ol (19). Yellow; m.p.: 120-121ºC; Mass calculated: 320.1412; Mass found: 320.1421. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.68 (quin, *J*=6.04 Hz, 2 H) 2.54 (t, *J*=5.97 Hz, 2 H) 2.78 (t, *J*=5.39 Hz, 2 H) 3.85 (s, 3 H) 6.94 (d, *J*=8.74 Hz, 2 H) 7.40 - 7.47 (m, 5 H) 7.58 (dd, *J*=7.72, 1.60 Hz, 2 H) 7.74 (s, 1 H) 16.93 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ : 23.6, 27.1, 27.7, 55.4, 108.0, 113.9, 127.7, 128.1, 129.0, 130.5, 130.6, 131.9, 133.4, 138.3, 159.7, 177.5, 194.3

2-benzoyl-6-(2,3-dimethoxybenzylidene)cyclohexen-1-ol (20). Yellow; m.p.: 69-71ºC; Mass calculated: 350.1518; Mass found: 350.1535. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.65 (dt, *J*=11.87, 6.15 Hz, 2 H) 2.53 (t, *J*=5.82 Hz, 2 H) 2.66 (t, *J*=5.39 Hz, 2 H) 3.84 (s, 3 H) 3.89 (s, 3 H) 6.93 (dd, *J*=16.02, 7.86 Hz, 2 H) 7.04 - 7.09 (m, 1 H) 7.41 - 7.49 (m, 3 H) 7.56 - 7.59 (m, 2 H) 7.89 (s, 1 H) 16.69 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 27.3, 27.6, 55.9, 61.1, 108.2, 112.4, 122.1, 123.4, 127.5, 128.1, 128.9, 130.5, 130.7, 133.5, 138.4, 148.0, 152.8, 175.9, 195.9

2-benzoyl-6-(2,5-dimethoxybenzylidene)cyclohexen-1-ol (21). Yellow; m.p.: 128-129ºC; Mass calculated: 350.1518; Mass found: 350.1545. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.66 (quin, *J*=6.04 Hz, 2 H) 2.53 (t, *J*=5.82 Hz, 2 H) 2.71 (t, *J*=5.39 Hz, 2 H) 3.80 (s, 3 H) 3.84 (s, 3 H) 6.85 (d, *J*=1.46 Hz, 2 H) 6.91 (s, 1 H) 7.40 - 7.50 (m, 3 H) 7.55 - 7.59 (m, 2 H) 7.91 (s, 1 H) 16.71 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 27.3, 27.6, 55.8, 56.1, 108.2, 111.5, 114.0, 116.4, 126.1, 127.6, 128.1, 128.9, 130.5, 132.7, 138.5, 152.4, 152.9, 176.1, 195.6

2-benzoyl-6-(3,4-dimethoxybenzylidene)cyclohexen-1-ol (22). Yellow; m.p.: 152-153ºC; Mass calculated: 350.1518; Mass found: 350.1542 ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.69 (quin, *J*=6.04 Hz, 2 H) 2.52 - 2.57 (m, 2 H) 2.76 - 2.84 (m, 2 H) 3.92 (s, 3 H) 3.92 (s, 3 H) 6.91 (d, *J*=8.45 Hz, 1 H) 7.00 (d, *J*=1.75 Hz, 1 H) 7.09 (dd, *J*=8.30, 1.60 Hz, 1 H) 7.41 - 7.49 (m, 3 H) 7.58 (dd, *J*=7.72, 1.60 Hz, 2 H) 7.72 (s, 1 H) 16.89 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 27.1, 27.7, 55.9, 56.0, 108.1, 110.9, 113.5, 123.5, 127.7, 128.1, 129.3, 130.5, 130.8, 133.5, 138.3, 148.7, 149.3, 177.2, 194.4

2-benzoyl-6-(2,3,4,-trimethoxybenzylidene)cyclohexen-1-ol (23). Yellow; m.p.: 140-141ºC; Mass calculated: 380.1624; Mass found: 380.1643. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.63 - 1.69 (m, 2 H) 2.53 (t, *J*=5.82 Hz, 2 H) 2.69 (t, *J*=5.24 Hz, 2 H) 3.90 (s, 6 H) 3.91 (s, 3 H) 6.69 (d, *J*=8.74 Hz, 1 H) 7.09 (d, *J*=8.74 Hz, 1 H) 7.40 - 7.48 (m, 3 H) 7.55 - 7.59 (m, 2 H) 7.88 (s, 1 H) 16.80 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.7, 27.3, 27.7, 56.1, 61.0, 61.5, 106.7, 107.9, 123.3, 125.0, 127.6, 128.1, 128.7, 130.4, 131.8, 138.5, 142.3, 153.1, 154.1, 176.8, 195.1

2-benzoyl-6-(3,4,5-trimethoxybenzylidene)cyclohexen-1-ol (24). Yellow; m.p.: 150-151ºC; Mass calculated: 380.1624; Mass found: 380.1631. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.69 (t, *J*=5.68 Hz, 2 H) 2.54 (t, *J*=5.97 Hz, 2 H) 2.80 (t, *J*=5.24 Hz, 2 H) 3.89 (s, 6 H) 3.89 (s, 3 H) 6.69 (s, 2 H) 7.42 - 7.48 (m, 3 H) 7.58 (dd, *J*=7.86, 1.46 Hz, 2 H) 7.69 (s, 1 H) 16.79 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 27.1, 27.7, 56.2, 56.2, 61.0, 107.6, 108.3, 127.6, 128.1, 130.6, 131.8, 131.9, 131.9, 133.5, 138.2, 138.4, 153.0, 176.4, 195.2

2-benzoyl-6-(3-hydroxybenzylidene)cyclohexen-1-ol (25). Yellow; m.p.: 120-121ºC; Mass calculated: 306.1256; Mass found: 306.1258. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.66 (quin, *J*=6.04 Hz, 2 H) 2.53 (t, *J*=5.97 Hz, 2 H) 2.76 (t, *J*=5.53 Hz, 2 H) 5.39 (br. s., 1 H) 6.80 (dd, *J*=8.01, 2.18 Hz, 1 H) 6.91 (s, 1 H) 7.02 (d, *J*=7.86 Hz, 1 H) 7.22 - 7.29 (m, 1 H) 7.40 - 7.50 (m, 3 H) 7.58 (d, *J*=7.28 Hz, 2 H) 7.70 (s, 1 H) 16.71 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.5, 27.2, 27.6, 108.6, 115.4, 116.7, 122.7, 127.6, 128.2, 129.5, 130.7, 132.8, 133.1, 137.8, 138.2, 155.5, 176.2, 195.8

2-benzoyl-6-(4-hydroxybenzylidene)cyclohexen-1-ol (26). Yellow; m.p.: 158-160ºC; Mass calculated: 306.1256; Mass found: 306.1268. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.68 (quin, *J*=5.97 Hz, 2 H) 2.53 (t, *J*=5.82 Hz, 2 H) 2.77 (t, *J*=5.53 Hz, 2 H) 6.87 (d, *J*=8.45 Hz, 2 H) 7.38 (d, *J*=8.15 Hz, 2 H) 7.41 - 7.49 (m, 3 H) 7.57 (d, *J*=7.28 Hz, 2 H) 7.71 (s, 1 H) 16.87 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 27.1, 27.7, 108.1, 115.4, 127.6, 128.1, 129.1, 130.5, 130.6, 132.1, 133.4, 138.2, 155.8, 177.5, 194.4

2-benzoyl-6-(3,4-dihydroxybenzylidene)cyclohexen-1-ol (27). Yellow; m.p.: 212-213ºC; Mass calculated: 322.1205; Mass found: 322.1215. ^{1}H NMR (500 MHz, acetone) δ: 1.67 (quin, *J*=5.61 Hz, 2 H) 2.54 (t, *J*=5.24 Hz, 2 H) 2.79 (t, *J*=5.97 Hz, 2 H) 6.87 - 7.00 (m, 2 H) 7.09 (s, 1 H) 7.44 - 7.56 (m, 3 H) 7.58 - 7.68 (m, 3 H) 8.22 (br. s., 2 H) 17.12 (s, 1 H). ^{13}C NMR (125 MHz, acetone) δ: 23.4, 26.7, 27.5, 107.8, 115.4, 117.2, 123.4, 127.6, 128.1, 128.3, 129.9, 130.5, 133.7, 138.2, 144.9, 146.1, 177.8, 193.8

2-benzoyl-6-(3-hydroxyl-4-methoxybenzylidene)cyclohexen-1-ol (28). Yellow; m.p.: 152-153ºC; Mass calculated: 336.1362; Mass found: 336.1375. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.69 (quin, *J*=6.12 Hz, 2 H) 2.50 - 2.56 (m, 2 H) 2.77 - 2.81 (m, 2 H) 3.92 (s, 3 H) 5.83 (s, 1 H) 6.95 (d, *J*=8.44 Hz, 1 H) 6.98 (d, *J*=2.04 Hz, 1 H) 7.06 (dd, *J*=8.15, 2.04 Hz, 1 H) 7.42 - 7.47 (m, 3 H) 7.58 (dd, *J*=7.86, 1.75 Hz, 2 H) 7.71 (s, 1 H) 16.92 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 27.1, 27.7, 56.00, 108.1, 113.0, 114.4, 124.1, 127.7, 128.1, 128.8, 130.5, 130.6, 133.7, 138.2, 146.1, 146.3, 177.4, 194.3

2-benzoyl-6-(4-hydroxyl-3-methoxybenzylidene)cyclohexen-1-ol (29). Yellow; m.p.: 153-154ºC; Mass calculated: 336.1362; Mass found: 336.1382. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.67 (quin, *J*=5.97 Hz, 2 H) 2.53 (t, *J*=5.82 Hz, 2 H) 2.78 (t, *J*=5.39 Hz, 2 H) 3.93 (s, 3 H) 5.67 (s, 1 H) 6.89 (d, *J*=8.44 Hz, 1 H) 7.00 (d, *J*=8.45 Hz, 1 H) 7.10 (s, 1 H) 7.39 - 7.49 (m, 3 H) 7.58 (d, *J*=6.70 Hz, 2 H) 7.68 (s, 1 H) 16.88 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.5, 27.1, 27.7, 56.0, 108.1, 110.4, 115.9, 123.4, 127.7, 128.1, 129.8, 130.5, 131.0, 133.4, 138.3, 145.3, 146.8, 177.3, 194.4

2-benzoyl-6-(3-chloro-4-hydroxybenzylidene)cyclohexen-1-ol (30). Yellow; m.p.: 164-165ºC; Mass calculated: 340.0866; Mass found: 340.0878. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.69 (quin, *J*=6.04 Hz, 2 H) 2.51 - 2.56 (m, 2 H) 2.69 - 2.78 (m, 2 H) 5.74 (s, 1 H) 7.05 (d, *J*=8.44 Hz, 1 H) 7.30 (dd, *J*=8.59, 1.89 Hz, 1 H) 7.42 - 7.50 (m, 4 H) 7.56 - 7.60 (m, 2 H) 7.63 (s, 1 H) 16.75 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.5, 27.1, 27.6, 108.4, 116.2, 120.0, 127.6, 128.1, 130.1, 130.5, 130.6, 130.7, 131.7, 131.8, 138.2, 151.3, 176.3, 195.2

2-benzoyl-6-(3-bromo-4-hydroxybenzylidene)cyclohexen-1-ol (31). Yellow; m.p.: 172-173ºC; Mass calculated: 384.0361; Mass found: 384.0379. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.69 (quin, *J*=6.04 Hz, 2 H) 2.50 - 2.57 (m, 2 H) 2.71 - 2.78 (m, 2 H) 5.70 (br. s., 1 H) 7.05 (d, *J*=8.45 Hz, 1 H) 7.34 (dd, *J*=8.45, 2.04 Hz, 1 H) 7.41 - 7.50 (m, 3 H) 7.55 - 7.61 (m, 3 H) 7.63 (s, 1 H) 16.74 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.5, 27.1, 27.6, 108.3, 110.3, 116.0, 127.6, 128.1, 130.5, 130.6, 131.4, 131.5, 131.9, 133.6, 138.2, 152.2, 176.2, 195.2

2-benzoyl-6-(1-naphthalenylmethylene)cyclohexen-1-ol (32). Yellow; m.p.: 109-110ºC; Mass calculated: 340.1463; Mass found: 340.147. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.64 (quin, *J*=5.97 Hz, 2 H) 2.56 (t, *J*=5.82 Hz, 2 H) 2.63 (t, *J*=5.39 Hz, 2 H) 7.43 - 7.56 (m, 7 H) 7.59 - 7.64 (m, 2 H) 7.84 (d, *J*=8.15 Hz, 1 H) 7.87 - 7.91 (m, 1 H) 8.03 - 8.08 (m, 1 H) 8.31 (s, 1 H) 16.74 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.7, 27.5, 27.6, 108.4, 124.9, 125.1, 126.1, 126.4, 126.9, 127.6, 128.2, 128.5, 128.5, 130.6, 131.4, 131.9, 133.5, 133.5, 134.4, 138.5, 175.5, 196.4

2-benzoyl-6-(2-naphthalenylmethylene)cyclohexen-1-ol (33). Yellow; m.p.: 114-115ºC; Mass calculated: 340.1463; Mass found: 340.1488. ^{1}H NMR (500 MHz, $CDCl_3$) δ:1.71 (quin, *J*=5.82 Hz, 2 H) 2.57 (t, *J*=5.68 Hz, 2 H) 2.89 (t, *J*=5.53 Hz, 2 H) 7.42 - 7.53 (m, 5 H) 7.55 - 7.64 (m, 3 H) 7.81 - 7.89 (m, 3 H) 7.93 (br. s., 2 H) 16.80 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 27.2, 27.7, 108.5, 126.4, 126.7, 127.6, 127.6, 127.9, 128.1, 128.3, 129.7, 130.6, 132.8, 132.9, 133.2, 133.4, 133.9, 138.3, 176.2, 195.5

2-benzoyl-6-(thien-2-ylmethylene)cyclohexen-1-ol (34). Orange; m.p.: 110-111ºC; Mass calculated: 296.0871; Mass found: 296.0896. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.77 (dt, *J*=12.09, 6.19 Hz, 2 H) 2.52 - 2.58 (m, 2 H) 2.75 - 2.82 (m, 2 H) 7.14 (dd, *J*=4.95, 3.79 Hz, 1 H) 7.33 (d, *J*=3.49 Hz, 1 H) 7.43 - 7.48 (m, 3 H) 7.51 (d, *J*=4.95 Hz, 1 H) 7.58 (dd, *J*=7.86, 1.46 Hz, 2 H) 7.95 (s, 1 H) 16.87 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.0, 26.8, 27.7, 108.4, 126.5, 127.6, 127.7, 128.1, 129.3, 129.4, 130.5, 131.9, 138.1, 139.9, 177.3, 193.8

2-benzoyl-6-(5-methyl-2-thienylmethylene)cyclohexen-1-ol (35). Yellow; m.p.: 100-102ºC; Mass calculated: 310.1028; Mass found: 310.1035. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.75 (quin, *J*=6.19 Hz, 2 H) 2.49 - 2.58 (m, 5 H) 2.75 (t, *J*=5.39 Hz, 2 H) 6.79 - 6.82 (m, 1 H) 7.14 (d, *J*=3.49 Hz, 1 H) 7.39 - 7.48 (m, 3 H) 7.57 (dd, *J*=7.86, 1.46 Hz, 2 H) 7.86 (s, 1 H) 16.96 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 15.7, 23.0, 26.8, 27.6, 108.1, 126.1, 127.2, 127.8, 128.0, 128.1, 130.4, 132.5, 138.0, 138.1, 144.9, 178.0, 193.0

2-benzoyl-6-(3-benzyloxybenzylidene)cyclohexen-1-ol (36). Yellow; m.p.: 97-98ºC; Mass calculated: 396.1725; Mass found: 396.1735. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.64 (dt, *J*=12.23, 6.12 Hz, 2 H) 2.53 (t, *J*=6.12 Hz, 2 H) 2.68 - 2.72 (m, 2 H) 5.11 (s, 2 H) 6.95 (dd, *J*=7.57, 1.16 Hz, 1 H) 7.02 - 7.07 (m, 2 H) 7.29 - 7.36 (m, 2 H) 7.37 - 7.50 (m, 7 H) 7.58 (d, *J*=7.57 Hz, 2 H) 7.72 (s, 1 H) 16.74 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.5, 27.2, 27.6, 70.1, 108.5, 114.8, 116.3, 123.0, 127.4, 127.6, 128.0, 128.1, 128.6, 129.3, 130.6, 132.8, 133.1, 136.8, 137.6, 158.6, 176.1, 195.6

2-benzoyl-6-(4-benzyloxybenzylidene)cyclohexen-1-ol (37). Yellow; m.p.: 133-135ºC; Mass calculated: 396.1725; Mass found: 396.1738. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.68 (quin, *J*=6.12 Hz, 2 H) 2.52 - 2.55 (m, 2 H) 2.75 - 2.80 (m, 2 H) 5.11 (s, 2 H) 7.01 (d, *J*=8.74 Hz, 2 H) 7.32 - 7.37 (m, 1 H) 7.38 - 7.48 (m, 9 H) 7.58 (dd, *J*=6.99, 1.16 Hz, 2 H) 7.73 (s, 1 H) 16.91 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 27.1, 27.7, 70.0, 114.8, 127.5, 127.7, 128.1, 128.1, 128.7, 129.2, 130.5, 130.6, 131.9, 133.3, 136.6, 138.2, 158.8, 177.4, 194.4

*4.1.3. General procedure for the synthesis of **III***

A DMF solution (100 mL) containing hydroxylated benzaldehyde (20 mmol), 1,2-dibromoethane (60 mmol) and anhydrous potassium carbonate (40 mmol) was heat at 70-80˚C for 6 hours to obtain **III**. Upon completion, the reaction mixture was poured into 200 mL of distilled water and stirred for 10 minutes, followed by extraction with 100 mL of ethyl acetate. The organic layer was then washed twice with 100 mL of distilled water and dried over anhydrous magnesium sulphate. Further evaporation *in vacuo* gave crude product of the targeted intermediate (**III**). The resulting crude product was purified by column chromatography.

*4.1.4. General procedure for the synthesis of **IV***

A DMF solution (50 mL) containing intermediate **III** (2 mmol), appropriate secondary amine (3 mmol) and anhydrous potassium carbonate (4 mmol) was heated under reflux for 8 hours to obtain **IV**. Upon completion, the reaction mixture was poured into 200 mL of distilled water and stirred for 10 minutes, followed by extraction with 100 mL of ethyl acetate. The organic layer was then washed twice with 100 mL of distilled water and dried over anhydrous magnesium sulphate. Further evaporation *in vacuo* gave crude product of the targeted intermediate (**IV**). The resulting crude product was purified by column chromatography.

*4.1.5. General procedure for the synthesis of compounds **38-41***

One milimole of 2-benzoylcyclohenxanone (**II**) was reacted with equivalent amount of aminated benzaldehyde in acetic acid (30 mL), in the presence of sulfuric acid as catalyst. The reaction mixture was stirred at room temperature for overnight. Upon completion, the reaction mixture was poured into 100 mL of distilled water and stirred for 10 minutes, followed by extraction with 100 mL of ethyl acetate. The organic layer was then washed with 10% sodium bicarbonate solution and dried over anhydrous magnesium sulphate. Further evaporation *in vacuo* gave crude product of the targeted compounds **38-41**. The resulting crude product was purified by column chromatography.

2-benzoyl-6-(3-(2-(piperidin-1-yl)ethoxy)benzylidene)cyclohexen-1-ol (38). Yellow; m.p.: - ; Mass calculated: 417.2304; Mass found: 417.2344. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.41 - 1.51 (m, 2 H) 1.57 - 1.71 (m, 6 H) 2.50 - 2.58 (m, 6 H) 2.74 - 2.84 (m, 4 H) 4.14 (td, *J*=6.26, 1.46 Hz, 2 H) 6.88 (d, *J*=8.15 Hz, 1 H) 6.98 (s, 1 H) 7.03 (d, *J*=7.57 Hz, 1 H) 7.28 - 7.33 (m, 1 H) 7.41 - 7.49 (m, 3 H) 7.58 (d, *J*=7.57 Hz, 2 H) 7.72 (s, 1 H) 16.71 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 24.2, 25.9, 27.2, 27.6, 55.0, 57.9, 66.0, 108.41, 114.5, 116.1, 122.7, 127.1, 127.6, 128.2, 129.3, 130.6, 132.7, 133.2, 137.6, 138.3, 176.1, 195.5

2-benzoyl-6-(4-(2-(piperidin-1-yl)ethoxy)benzylidene)cyclohexen-1-ol (39). Yellow; m.p.: 102-104ºC; Mass calculated: 417.2304; Mass found: 417.2321. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.42 - 1.49 (m, 2 H) 1.62 (quin, *J*=5.68 Hz, 4 H) 1.67 (dt, *J*=12.23, 6.12 Hz, 2 H) 2.48 - 2.58 (m, 6 H) 2.73 - 2.84 (m, 4 H) 4.11 - 4.18 (m, 2 H) 6.93 (d, *J*=8.74 Hz, 2 H) 7.38 - 7.49 (m, 5 H) 7.57 (dd, *J*=7.57, 1.16 Hz, 2 H) 7.72 (s, 1 H) 16.91 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.6, 24.2, 25.9, 27.1, 27.7, 55.0, 57.8, 66.0, 108.0, 114.5, 127.7, 128.1, 129.0, 130.5, 130.5, 131.9, 133.4, 138.2, 158.9, 177.5, 194.3

2-benzoyl-6-(3-(2-(pyrrolidin-1-yl)ethoxy)benzylidene)cyclohexen-1-ol (40). Yellow; m.p.: 78-80ºC; Mass calculated: 403.2147; Mass found: 403.2161. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.59 - 1.70 (m, 2 H) 1.80 - 1.85 (m, 4 H) 2.53 (t, *J*=5.82 Hz, 2 H) 2.65 (t, *J*=6.41 Hz, 4 H) 2.73 - 2.80 (m, 2 H) 2.93 (t, *J*=6.12 Hz, 2 H) 4.14 (t, *J*=6.12 Hz, 2 H) 6.89 (dd, *J*=8.15, 2.91 Hz, 1 H) 6.99 (s, 1 H) 7.03 (d, *J*=7.57 Hz, 1 H) 7.30 (t, *J*=7.86 Hz, 1 H) 7.39 - 7.49 (m, 3 H) 7.57 (d, *J*=7.57 Hz, 2 H) 7.71 (s, 1 H) 16.71 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.4, 23.5, 27.2, 27.6, 54.7, 55.0, 67.1, 107.9, 114.5, 116.1, 122.7, 127.6, 127.8, 128.1, 129.2, 130.5, 132.7, 133.2, 137.6, 176.1, 193.2, 195.5

2-benzoyl-6-(4-(2-(pyrrolidin-1-yl)ethoxy)benzylidene)cyclohexen-1-ol (41). Yellow; m.p.: 99-101ºC; Mass calculated: 403.2147; Mass found: 403.2171. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.67 (quin, *J*=6.12 Hz, 2 H) 1.78 - 1.86 (m, 4 H) 2.51 - 2.55 (m, 2 H) 2.64 (t, *J*=6.41 Hz, 4 H) 2.74 - 2.80 (m, 2 H) 2.92 (t, *J*=5.82 Hz, 2 H) 4.15 (t, *J*=6.12 Hz, 2 H) 6.95 (d, *J*=8.74 Hz, 2 H) 7.39 - 7.47 (m, 5 H) 7.57 (dd, *J*=7.86, 1.46 Hz, 2 H) 7.72 (s, 1 H) 16.91 (s, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 23.5, 23.6, 27.1, 27.7, 54.7, 55.0, 67.1, 108.00, 114.5, 127.7, 128.1, 130.5, 130.5, 131.9, 133.4, 138.2, 158.9, 177.5, 194.3

4.2. In-vitro AChE and BuChE inhibition assay

AChE and BChE inhibitory activity of the compounds were evaluated using Ellman's microplate assay following the conditions described by A. Basiri *et al*. with slight modifications. Electric eel AChE (type V-S, Sigma Chemical Co. code: C2888) and equine serum BChE (Sigma Chemical Co. code: C7512) were used as the sources of cholinesterases, while acetylthiocholine iodide (Sigma Chemical Co. [ATCI] code: A5751) and S-butyrylthiocholine iodide (Sigma Chemical Co. [BTCI] code: 20820) were used as the sources of substrates. The enzymes were prepared in 0.1M sodium phosphate buffer (pH 7.4) with the concentration of 0.25 units/mL while the substrates were prepared in water with the concentration of 0.25 mM.

For cholinesterase inhibitory assays, 190 µL of 0.15 mM of 5,5-dithiobis-2-nitrobenzoic acid (DTNB) (Sigma Chemical Co. Code: D21200, 99%) in sodium phosphate buffer (0.1M, pH 7.4) was first added to 96-well microplate followed by the addition of 20 µL of AChE/BChE solution and 20 µL of test compounds. The mixture was incubated at 37 ºC for 15 minutes. Then, 20 µL of ATCI/BTCI was added to initiate the enzyme reaction and the reaction mixture was further incubated for 30 minutes at 37 ºC. The absorbance of the colored end-product was measured at 412 nm using SpectraMax Plus 384 Microplate Reader (Molecular Devices LLC, Sunnyvale, CA, USA). All reactions were carried out in triplicate. The IC_{50} values were calculated in µM using graph Pad software.

4.3. Kinetic studies of AChE and BChE inhibition

The kinetic studies were performed as described in the above Section (4.2.) using different concentrations of substrate and compounds. Compound **39** with concentrations of 5, 2.5, 1.25 and 0 µM were used for AChE kinetic analysis while compound **40** with concentrations of 0.625, 0.313, 0.157 and 0 µM were used for BChE kinetic analysis. Meanwhile, the concentrations of substrate for both assays were set at 0.2, 0.3, 0.4 and 0.5 mM. Lineweaver-Burk plots were derived from the resulting data and the Ki values of both compounds were calculated using GraphPad Prism 5.

4.4. Molecular modeling

Molecular docking studies were carried out using Discovery Studio 3.1 (Accelrys, San Diego, USA) on an Intel® (TM)2 Quad CPU Q8200 @2.33 GHz running under a Windows XP Professional environment.

4.4.1. Receptors preparation

The crystal structure of AChE from Torpedo californica (TcAChE; Code ID: 1EVE) and BChE from Homo sapiens (hBChE; Code ID: 4BDS) were obtained from the Protein Data Bank. Then, all water molecules and co-crystalized ligands were removed followed by protein preparation protocol with CHARMm force field. The

crystal structures of TcAChE and hBChE were used for docking studies due to their sequential similarity with EeAChE and equine serum BChE, respectively.

4.4.2. Ligands preparation

Compounds **39** and **40**, as well as co-crystallized ligands (donepezil and tacrine) were drawn with ChemDraw Ultra 12.0.Then, the structures were imported to the Discovery Studio 3.1 followed by ligands preparation protocol with the default setting, as recommended by Accelrys. The prepared ligands were then subjected to ligands minimization with CHARMm force field before being used for docking analyses.

4.4.3. Flexible docking

Minimized co-crystallized ligands were re-docked into their respective enzymes with several sets of amino acids as flexible residues. The top ranked conformations resulted from the docking experiment were compared to their original crystallographic confirmation in terms of RMSD. The parameters with lowest RMSD values were selected for the flexible docking of compounds **39** and **40**. The flexible docking results were analyzed using Discovery Studio Visualizer v4.1.0.14169 (Accelrys, San Diego, USA).

4.5. ADMET analysis

Compounds **39** and **40** were selected for Discovery Studio 3.1 ADMET analysis based on their best performance in cholinesterase inhibitory activity. The ADMET analysis performed was on their aqueous solubility (AS), human intestinal absorption (HIA), blood brain barrier (BBB), cytochrome P450 2D6 (CYP2D6), plasma protein binding (PPB), and hepatotoxicity (HT) descriptors.

Acknowledgments

The authors thank the Ministry of Education (MOE) of Malaysia and Universiti Putra Malaysia for financial support under the project ERGS/1/11/STG/UPM/01/24. The first author acknowledges the support from the Malaysian Ministry of Science, Technology and Innovation (MOSTI) for a scholarship under the National Science Foundation (NSF).

References

1. Geula, C., M. M. Mesulam, *Alzheimer Dis. Assoc. Disord.*, **1995**, *9 Suppl 2* 23-28.
2. Babic, T., *J. Neurol. Neurosurg. Psychiatry.*, **1999**, *67* 558.
3. Ballard, C. G., N. H. Greig, A. L. Guillozet-Bongaarts, A. Enz, S. Darvesh, *Curr Alzheimer Res.*, **2005**, *2* 307-318.
4. Lindsay, J., D. Laurin, R. Verreault, R. Hebert, B. Helliwell, G. B. Hill, I. McDowell, *Am. J. Epidemiol.*, **2002**, *156* 445-453.
5. Prince, M., A. Wimo, M. Guerchet, G.-C. Ali, Y.-T. Wu, M. Prina, *World Alzheimer's Soceity*, **2015**.
6. Inestrosa, N. C., A. Alvarez, C. A. Perez, R. D. Moreno, M. Vicente, C. Linker, O. I. Casanueva, C. Soto, J. Garrido, *Neuron*, **1996**, *16* 881-891.
7. Bashir, M., A. A. Parray, R. A. Baba, H. F. Bhat, S. S. Bhat, U. Mushtaq, K. I. Andrabi, F. A. Khanday, *Neuromolecular Med.*, **2014**, *16* 137-149.
8. Anand, P., B. Singh, *Arch. Pharm. Res.*, **2013**, *36* 375-399.
9. Scarpini, E., P. Scheltens, H. Feldman, *Lancet Neurol*, **2003**, *2* 539-547.
10. Forsyth, D. R., D. J. Surmon, R. A. Morgan, G. K. Wilcock, *Age Ageing*, **1989**, *18* 223-229.
11. Naik, S. R., V. N. Thakare, S. R. Patil, *Exp. Toxicol. Pathol.*, **2011**, *63* 419-431.

12. Bisht, K., K. H. Wagner, A. C. Bulmer, *Toxicology*, **2010**, *278* 88-100.
13. Ak, T., I. Gulcin, *Chem. Biol. Interact.*, **2008**, *174* 27-37.
14. Ben, P., J. Liu, C. Lu, Y. Xu, Y. Xin, J. Fu, H. Huang, Z. Zhang, Y. Gao, L. Luo, Z. Yin, *Int Immunopharmacol.*, **2011**, *11* 179-186.
15. Ono, K., K. Hasegawa, H. Naiki, M. Yamada, *J. Neurosci. Res.*, **2004**, *75* 742-750.
16. Ahmed, T., A. H. Gilani, *Pharmacol. Biochem. Behav.*, **2009**, *91* 554-559.
17. Cheng, A. L., C. H. Hsu, J. K. Lin, M. M. Hsu, Y. F. Ho, T. S. Shen, J. Y. Ko, J. T. Lin, B. R. Lin, W. Ming-Shiang, H. S. Yu, S. H. Jee, G. S. Chen, T. M. Chen, C. A. Chen, M. K. Lai, Y. S. Pu, M. H. Pan, Y. J. Wang, C. C. Tsai, C. Y. Hsieh, *Anticancer Res.*, **2001**, *21* 2895-2900.
18. Park, S. Y., H. S. Kim, E. K. Cho, B. Y. Kwon, S. Phark, K. W. Hwang, D. Sul, *Food Chem. Toxicol.*, **2008**, *46* 2881-2887.
19. Lee, H. S., K. K. Jung, J. Y. Cho, M. H. Rhee, S. Hong, M. Kwon, S. H. Kim, S. Y. Kang, *Pharmazie*, **2007**, *62* 937-942.
20. Anand, P., A. B. Kunnumakkara, R. A. Newman, B. B. Aggarwal, *Mol. Pharm.*, **2007**, *4* 807-818.
21. Liang, G., X. Li, L. Chen, S. Yang, X. Wu, E. Studer, E. Gurley, P. B. Hylemon, F. Ye, Y. Li, H. Zhou, *Bioorg. Med. Chem. Lett.*, **2008**, *18* 1525-1529.
22. Zhao, C., J. Yang, Y. Wang, D. Liang, X. Yang, X. Li, J. Wu, X. Wu, S. Yang, X. Li, G. Liang, *Bioorg. Med. Chem.*, **2010**, *18* 2388-2393.
23. Lee, K. H., F. H. Ab Aziz, A. Syahida, F. Abas, K. Shaari, D. A. Israf, N. H. Lajis, *Eur. J. Med. Chem.*, **2009**, *44* 3195-3200.
24. Liang, G., S. Yang, H. Zhou, L. Shao, K. Huang, J. Xiao, Z. Huang, X. Li, *Eur. J. Med. Chem.*, **2009**, *44* 915-919.
25. Zhang, Y., C. Zhao, W. He, Z. Wang, Q. Fang, B. Xiao, Z. Liu, G. Liang, S. Yang, *Drug. Des. Devel. Ther.*, **2014**, *8* 373-382.
26. Bukhari, S. N., I. Jantan, V. H. Masand, D. T. Mahajan, M. Sher, M. Naeem-ul-Hassan, M. W. Amjad, *Eur. J. Med. Chem.*, **2014**, *83* 355-365.
27. Leong, S. W., S. M. Mohd Faudzi, F. Abas, M. F. Mohd Aluwi, K. Rullah, K. W. Lam, M. N. Abdul Bahari, S. Ahmad, C. L. Tham, K. Shaari, N. H. Lajis, *Bioorg. Med. Chem. Lett.*, **2015**, *25* 3330-3337.
28. Mesulam, M. M., A. Guillozet, P. Shaw, A. Levey, E. G. Duysen, O. Lockridge, *Neuroscience*, **2002**, *110* 627-639.
29. Hevener, K. E., W. Zhao, D. M. Ball, K. Babaoglu, J. Qi, S. W. White, R. E. Lee, *J. Chem. Inf. Model*, **2009**, *49* 444-460.
30. Pakaski, M., Z. Rakonczay, P. Kasa, *Neurochem. Int.*, **2001**, *38* 219-226.
31. Castro, A., A. Martinez, *Mini Rev. Med. Chem.*, **2001**, *1* 267-272.
32. Serajuddin, A. T., *Adv. Drug. Deliv. Rev.*, **2007**, *59* 603-616.

Graphical abstract