

Synthesis and Biological Evaluation of Flavonoids as Antiacetylcholinesterase Agent

Saleh Nazifi Ibrahim, Farediah Ahmad*

Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia

*Corresponding author: farediah@kimia.fs.utm.my

Article history

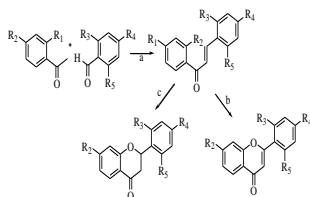
Received :28 January 2014

Received in revised form :

13 April 2014

Accepted :15 June 2014

Graphical abstract



Abstract

A series of chalcones, a flavone and one flavanone were synthesized and elucidated structurally by IR and ¹H NMR spectroscopies. The synthetic compounds were then screened for acetylcholinesterase inhibitory activity using thin layer chromatography (TLC) and microplate assays. In the TLC assay, only 2'-hydroxy-4-methoxychalcone and 2'-hydroxy-4'-O-prenyl-2,6-dichlorochalcone were found to show moderate and weak activity respectively against acetylcholinesterase (AChE) at 0.1 mM concentration compared to the control galanthamine. 4'-Hydroxy-2,6-dichlorochalcone, 2'-hydroxy-4-nitrochalcone, 2'-hydroxy-4-(dimethyl)aminochalcone and 2'-hydroxy-4-methoxychalcone showed moderate AChE inhibitory activity with percentage inhibition of 54.24, 46.14 and 49.32 % respectively in the microplate assay.

Keywords: Flavonoids; chalcone; flavone; flavanone; acetylcholinesterase

Abstrak

Satu siri kalkon, satu flavon dan flavanon telah disintesis dan strukturnya dikenal pasti melalui spektroskopi IR dan ¹H NMR. Semua sebatian yang telah disintesis disaring untuk cerakin rencatan asetilkolinesterase menggunakan kromatografi lapisan nipis (TLC) dan plat mikro. Dalam cerakin TLC, hanya 2'-hidroksi-4-metoksikalkon dan 2'-hidroksi-4'-O-prenil-2,6-diklorokalkon masing-masing mempamerkan aktiviti sederhana dan lemah terhadap asetilkolinesterase (AChE) pada kepekatan 0.1 mM berbanding kawalan galantamina. 4'-Hidroksi-2,6-diklorokalkon, 2'-hidroksi-4-nitrokalkon, 2'-hidroksi-4-(dimetil)aminokalkon dan 2'-hidroksi-4-metoksikalkon menunjukkan rencatan AChE sederhana masing-masing dengan peratus rencatan 54.24, 46.14 dan 49.32% dalam cerakin plat mikro.

Kata kunci: Flavonoid; kalkon; flavon; flavanon; asetilkolinesterase

© 2014 Penerbit UTM Press. All rights reserved.

1.0 INTRODUCTION

Flavonoids are polyphenolic compounds that are abundant in nature and over 8,150 flavonoids have been identified thus far [1]. Scientific studies involving pharmaceutical drug potencies of flavonoids are extensively examined in the past several years and are increasing [2]. Recent studies show that flavonoids possess antimicrobial, antioxidant, antimalarial, cytotoxic, antidepressant, anti-inflammatory, anti HIV and anticancer activities [2-8]. Chalcones are considered as the precursors of various flavonoids and isoflavonoids as well as many biologically important heterocycles such as 1,4-diketones and pyrazolines. The presence of a reactive α,β -unsaturated carbonyl group in chalcones is found to be responsible for their antimicrobial activity, which may be altered depending on the type and position of the substituent(s) on the aromatic rings [9]. Alzheimer's disease is considered a progressive neurological disorder that is leading to memory and behavioral disorders [10]. For the treatment of Alzheimer's disease,

inhibition of the acetylcholinesterase, which is responsible for the hydrolysis of acetylcholine, is the most effective approach. Herein, we report the synthesis of known chalcones, a flavone and flavanone and their acetylcholinesterase inhibitory activities. This is the first report of the acetylcholinesterase inhibitory of the targeted compounds.

2.0 EXPERIMENTAL

2.1 Measurements

The melting points of the synthesized compounds were measured and recorded using Leica Galen III Kofler micro melting points apparatus and were uncorrected. The ¹H (400 MHz) NMR experiments were recorded on a Bruker Avance spectrometer with CDCl₃ as the solvent. The Infrared (IR) spectra were recorded on Perkin Elmer 1650 FTIR spectrophotometer as thin film for liquid

and KBr pellet for solid samples. The reaction pathway is summarized in Scheme 1.

2.2 Synthesis

2.2.1 General Procedure for the Preparation of Chalcones

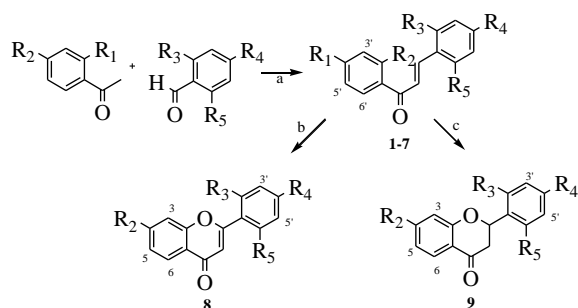
A mixture of acetophenone (0.01 mole) and benzaldehyde (0.01 mole) was stirred in ethanol. Sodium hydroxide solution (50 %) was added until highly turbid solution was obtained. The mixture was kept overnight at room temperature it was then poured into crushed ice and neutralized with dilute hydrochloric acid (10 %). The precipitate was washed with EtOH and purified by recrystallization and chromatographic technique [11].

2.2.2 General Procedure for the Preparation of Flavone

A mixture of chalcone (0.01 mole) and I₂ (0.0078 mmole) in DMSO (6 ml) was refluxed for 45 min and monitored using TLC. Water was added to the mixture and extracted with EtOAc. The organic extract was then washed with saturated Na₂O₃ solution followed by water and brine. It was dried over anhydrous MgSO₄, filtered and evaporated under reduced pressure. The resulting solid was then recrystallized from EtOH [12].

2.2.3 General Procedure for the Preparation of Flavanone

A mixture of chalcone (0.01 mole), MeOH (30 ml) and 10% HCl (5 ml) were refluxed for 60 min. NaOAc (0.5 g) was added to the mixture and further refluxed for 48 hrs. The progress of the reaction was monitored by TLC. Water was added and the organic layer was extracted with EtOAc, dried using anhydrous MgSO₄, filtered and evaporated under reduced pressure. The residual yellow oil was purified using silica gel column chromatography to afford flavanone [13].



1. R₁= OH, R₂=R₄=H, R₃=R₅=Cl, 2. R₁= R₄=H, R₂=OH, R₃=R₅=Cl, 3. R₁= R₃=R₅=H, R₂=OH, R₄=Cl, 4. R₁= R₃=R₅=H, R₂=OH, R₄=NO₂, 5. R₁=R₃=R₅=H, R₂=OH, R₄=NMe₂, 6. R₁R₃=R₅=H, R₄=OMe, 7. R₁=*O*-prenyl, R₂=OH, R₃=R₅=Cl, R₄=H, 8. R₂=R₄=H, R₃=R₅=Cl, 9. R₂=R₄=H, R₃=R₅=Cl

Scheme 1 Synthesis of chalcones, flavone and flavanone derivative. (a): NaOH, EtOH, stir 24 hrs; (b): I₂, DMSO, reflux 2 hrs and (c): (i): 10% HCl, MeOH, reflux 60 mins; (ii): NaOAc, reflux 48 hrs

2.3 Characterization of the Synthesized Compounds

2.3.1 4'-Hydroxy-2,6-dichlorochalcone (1) [9]

IR (ν cm⁻¹): 3340 (–OH), 1654 (C=O), 1613 (C=C alkene), 1599 and 1566 (C=C aromatic), 1226 (C–O) and 770 (C–Cl); ¹H NMR

(400 MHz, CDCl₃): δ 6.96 (2H, d, *J*=8.4 Hz, H–3' and H–5'), 7.23 (1H, t, *J*=8.0 Hz, H–4), 7.42 (2H, d, *J*=8.0 Hz, H–3 and H–5), 7.66 (1H, d, *J*=16.0 Hz, H–α), 7.85 (1H, d, *J*=16.0 Hz, H–β), 8.02 (2H, d, *J*=8.4 Hz, H–2' and H–6').

2.3.2 2'-Hydroxy-2,6-dichlorochalcone (2) [6]

IR (ν cm⁻¹): 3435 (–OH), 1686 (C=O), 1645 (C=C alkene), 1580 and 1437 (C=C aromatic), 1307 (C–O) and 775 (C–Cl); ¹H NMR (400 MHz, CDCl₃): δ 6.96 (1H, ddd, *J*=2.0, 8.0 and 8.0 Hz, H–5'), 7.08 (1H, dd, *J*=2.0 and 8.0 Hz, H–3'), 7.26 (1H, dd, *J*=8.0 and 8.0 Hz, H–4), 7.43 (2H, d, *J*=8.0 Hz, H–3 and H–5), 7.55 (1H, ddd, *J*=2.0, 8.0 and 8.0 Hz, H–4'), 7.89 (1H, dd, *J*=2.0 and 8.0 Hz, H–6'), 7.85 (1H, d, *J*=16.0 Hz, H–α), 8.00 (1H, d, *J*=16.0 Hz, H–β), and 12.65 (1H, s, –OH).

2.3.3 2'-Hydroxy-4-chlorochalcone (3) [14]

IR (ν cm⁻¹): 3446 (–OH), 1639 (C=O), 1579 (C=C alkene), 1564 and 1487 (C=C aromatic), 1205 (C–O alcohol) and 760 (C–Cl); ¹H NMR (400 MHz, CDCl₃): δ 6.98 (1H, ddd, *J*=1.6, 8 and 8 Hz, H–5'), 7.06 (1H, dd, *J*=1.6 and 8 Hz, H–3'), 7.45 (2H, d, *J*=8 Hz, H–3 and H–5), 7.54 (1H, ddd, *J*=1.6, 8 and 8 Hz, H–4'), 7.63 (2H, d, *J*=8 Hz, H–2 and H–6), 7.68 (1H, d, *J*=15.6 Hz, H–α), 8.00 (1H, d, *J*=15.6 Hz, H–β), 7.95 (1H, dd, *J*=1.6 and 8 Hz, H–6') and 12.78 (1H, s, –OH).

2.3.4 2'-Hydroxy-4-nitrochalcone (4) [6]

IR (ν cm⁻¹): 3447 (–OH), 1704 (C=O), 1645 (C=C alkene), 1607 and 1442 (C=C aromatic), 1541 and 1345 (N=O), 1197 (C–N) and 1104 (C–O); ¹H NMR (400 MHz, CDCl₃): δ 7.01 (1H, ddd, *J*=1.6, 8.0 and 8.0 Hz, H–5'), 7.09 (1H, dd, *J*=1.6 and 8.0 Hz, H–3'), 7.58 (1H, ddd, *J*=1.6, 8.0 and 8.0 Hz, H–4'), 7.79 (1H, d, *J*=15.6 Hz, H–α), 7.85 (2H, d, *J*=8.0 Hz, H–2 and H–6), 7.94 (1H, d, *J*=15.6 Hz, H–β), 7.96 (1H, dd, *J*=1.6 and 8.0 Hz, H–6'), 8.32 (2H, d, *J*=8.0 Hz, H–3 and H–5), and 12.62 (1H, s, –OH).

2.3.5 2'-Hydroxy-4-(dimethyl)aminochalcone (5) [15]

IR (ν cm⁻¹): 3435 (–OH), 2919 (C–H *sp*³), 1622 (C=O), 1599 (C=C alkene), 1520 and 1487 (C=C aromatic), 1177 (C–O) and 1035 (C–N); ¹H NMR (400 MHz, CDCl₃): δ 3.09 (6H, s, 2 x CH₃), 6.74 (2H, d, *J*=8.8 Hz, H–3 and H–5), 6.95 (1H, ddd, *J*=1.6, 8.0 and 8.0 Hz, H–5'), 7.03 (1H, dd, *J*=1.6 and 8.0 Hz, H–3'), 7.47 (1H, ddd, *J*=1.6, 8.0 and 8.0 Hz, H–4'), 7.52 (1H, d, *J*=16.0 Hz, H–α), 7.62 (2H, d, *J*=8.8 Hz, H–2 and H–6), 7.93 (1H, d, *J*=16.0 Hz, H–β), 7.95 (1H, dd, *J*=1.6 and 8.0 Hz, H–6'), and 13.23 (1H, s, –OH).

2.3.6 2'-Hydroxy-4-methoxychalcone (6) [16]

IR (ν cm⁻¹): 3422 (–OH), 1688 (C=O), 1622 (C=C alkene), 1623 and 1462 (C=C aromatic) and 1134 (C–O); ¹H NMR (400 MHz, CDCl₃): δ 3.89 (3H, s, OCH₃), 6.94 (1H, ddd, *J*=1.6, 8.0 and 8.0 Hz, H–4'), 6.97 (2H, d, *J*=8.8 Hz, H–3 and H–5), 7.03 (1H, dd, *J*=2.0 and 8.0 Hz, H–3'), 7.49 (1H, ddd, *J*=1.6, 8.0 and 8.0 Hz, H–5'), 7.56 (1H, d, *J*=15.2 Hz, H–α), 7.65 (2H, d, *J*=8.8 Hz, H–2 and H–6), 7.91 (1H, d, *J*=15.2 Hz, H–β), 7.94 (1H, dd, *J*=2.0 and 8.0 Hz, H–6') and 12.97 (1H, s, –OH).

2.3.7 2'-Hydroxy-4'-*O*-prenyl-2,6-dichlorochalcone (7) [17]

IR (ν cm⁻¹): 3445 (–OH), 3098 (C–H *sp*²), 2952 (C–H *sp*³), 1644 (C=O), 1598 (C=C alkene), 1506 and 1467 (C=C aromatic), 1232 (C–O) and 777 (C–Cl); ¹H NMR (400 MHz, CDCl₃): δ 1.78 (3H,

s, H-4''), 1.83 (3H, s, H-5''), 4.58 (2H, d, $J=6.8$ Hz, H-1''), 5.48 (1H, t, $J=6.8$ Hz, H-2''), 6.50 (1H, d, $J=2.4$ and 8.8 Hz, H-5'), 6.52 (1H, d, $J=2.4$ Hz, H-3'), 7.22 (1H, dd, $J=8.0$ and 8.0 Hz, H-4), 7.41 (2H, d, $J=8.0$ Hz, H-3 and H-5), 7.76 (1H, d, $J=8.8$ Hz, H-6'), 7.79 (1H, d, $J=15.6$, H- α), 7.94 (1H, d, $J=15.6$ Hz, H- β) and 13.27 (1H, s, -OH).

2.3.8 2',6'-Dichloroflavone (8) [18]

IR (ν cm^{-1}): 1686 (C=O), 1645 (C=C alkene), 1681 and 1438 (C=C aromatic), 1311 (C-O) and 776 (C-Cl); ^1H NMR (400 MHz, CDCl_3): δ 6.42 (1H, s, H-3), 7.29 (1H, dd, $J=1.6$ and 8.0 Hz, H-3), 7.42 (2H, d, $J=8.0$ Hz, H-3' and H-5'), 7.46 (1H, ddd, $J=2.0$, 8.0 and 8.0 Hz, H-5), 7.55 (1H, dd, $J=8.0$ and 8.0 Hz, H-4'), 7.73 (1H, ddd, $J=2.0$, 8.0 and 8.0 Hz, H-4) and 8.32 (1H, dd, $J=1.6$ and 8.0 Hz, H-6).

2.3.9 2',6'-Dichloroflavanone (9) [19]

IR (ν cm^{-1}): 2863 (C-H sp^3), 1693 (C=O), 1610 and 1463 (C=C aromatic), 1283 (C-O) and 762 (C-Cl); ^1H NMR (400 MHz, CDCl_3): δ 3.60 (1H, dd, $J=2.4$ and 12.8 Hz, vicinal H-2), 5.49 (1H, dd, $J=2.4$ and 15.6 Hz, geminal H-3a), 6.09 (1H, dd, $J=12.8$ and 15.6 Hz, geminal H-3b), 7.06 (1H, dd, $J=1.6$ and 8 Hz, H-3), 7.12 (1H, ddd, $J=1.6$, 8 and 8 Hz, H-5), 7.29 (1H, dd, $J=8.0$ and 8.0 Hz, H-4'), 7.43 (2H, d, $J=8.0$ Hz, H-3' and H-5'), 7.57 (1H, ddd, $J=1.6$, 8.0 and 8.0 Hz, H-4) and 7.95 1H, dd, $J=1.6$ and 8.0 Hz, H-6).

2.4 Acetylcholinesterase (Ache) Inhibitory Activity

2.4.1 Preparation of Reagents

The buffers used in the analysis were; A: 50 mM tris-hydrochloride (pH 8), B: buffer A containing 0.1% bovin serum albumin (BSA) and C: buffer A containing 0.1 M NaCl and 0.02 M $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$. Acetylcholinesterase from electric eel was purchased from Sigma. The enzyme was dissolved in buffer A to produce a stock solution of 1000 units/ml, and diluted with buffer A to 3 units/ml for TLC assay or to 0.22 units/ml for microplate assay [20, 21]. Acetylthiocholine iodide (ATCI) and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma Aldrich and were used at concentration of 1 mM in buffer A for TLC assay and at 3 mM for microplate assay.

2.4.2 TLC Assay

The enzyme inhibitory activities of the samples on the TLC plates were detected by spraying the substrate with the dye and enzyme following Ellman's method [20]. Aliquots (10 μl each) of various concentrations of the samples (0.1–100 mM in methanol) were applied to the TLC plates. The plates was then sprayed with DTNB/ATCI reagent (1 mM DTNB and 1 mM ATCI in buffer A) until the layer was saturated with the solvent. The plate was then allowed to dry for 5 min after which the enzyme solution was sprayed on the plates. A white spot appeared to show the inhibition after 10 min. The white spot was quickly observed and recorded as it disappeared within 15 min. The results were then compared to that of galanthamine which was used as a reference.

2.4.3 Microplate Assay

The microplate assay was also performed for AchE inhibitory activities. The AchE inhibitory activities were determined by a 96-well microplate reader using acetylthiocholine as a substrate as described in Ellman's method [16, 17, 20, 21]. In the 96-well

plates, 25 μl of 15 mM ATCI in water, 125 μl of 3 mM DTNB in buffer C, 50 μl of buffer B and 25 μl of sample (0.1 and 1 mM in MeOH) were added and the absorbance was measured at 405 nm 10 times every 47 s. After adding a solution of 0.22 units/ml of enzyme, the absorbance was read again 10 times every 47 s. The absorbance was measured using Epoch Biotekmicroplate reader. Percentage inhibition was calculated by comparing the absorbance rates for the sample to the blank (MeOH). Galanthamine was used as the positive control. The mean of two measurements for each concentration was determined.

3.0 RESULTS AND DISCUSSION

3.1 Chemistry

The synthetic approach of the target compounds is illustrated in Scheme 1. A high concentration of NaOH was used for the Claisen-Schmidt condensation reaction of acetophenone and benzaldehyde derivatives [11]. Chalcones were obtained by neutralization of the reaction mixture followed by washing with ethanol and chromatographic purification. In the synthesis of flavone, the respective chalcone was subjected to oxidative cyclisation using I_2 in DMSO. Flavanone was synthesized by treating the chalcone with NaOAc in the presence of methanolic HCl [13]. The structures of compounds (**1-9**) were ascertained by spectral analysis (IR and NMR) and identical to the earlier reported compounds [6, 9, 15-19]. **Table 1** summarized the percentage yield and the physical properties of the synthesized compounds. Compound **1** displayed the highest percentage yield (87.4%) followed by **5** (76%), **4** (75.1%), **7** (74.5%), **3** (65.9%), **2** (62.1%) and **6** (61.8%). The IR spectrum of 2'-hydroxy-4-chlorochalcone **3** showed broad stretching band at 3446 cm^{-1} attributed to chelated O-H. The presence of a strong stretching absorption at 1639 cm^{-1} proved the existence of C=O. Conjugation of the carbonyl group with α,β -unsaturated double bond resulted in a shift of the normal stretching band to a frequency 1579 cm^{-1} which leads to increasing the single bond character. The C=C aromatic stretching was observed at 1564 cm^{-1} and 1487 cm^{-1} , followed by the C-O absorption band at 1205 cm^{-1} . The absorption band for C-Cl was observed at 760 cm^{-1} . The ^1H NMR showed the presence of chelated O-H group at δ 12.78. The *trans* olefinic protons, H- α and H- β were represented by two doublet signals each resonated at δ 7.68 ($J=15.6$ Hz, H- α) and 8.00 ($J=15.6$ Hz, H- β). The aromatic protons of ring A were also observed at δ 6.98 (ddd, $J=1.6$, 8.0 and 8.0 Hz), 7.06 (dd, $J=1.6$ and 8.0 Hz), 7.54 (ddd, $J=1.6$, 8.0 and 8.0 Hz), 7.95 (dd, $J=1.6$ and 8.0 Hz) corresponding to H-5', H-3', H-4' and H-6' respectively. Moreover, the aromatic protons of ring B were resonated at δ 7.45 (2H, d, $J=8.0$ Hz) and 7.63 (2H, d, $J=8.0$ Hz) which were attributed to H-3 and H-5, H-2 and H-6 respectively thus confirming the structure of the targeted compound **3**. The IR and ^1H NMR data of compound **3** were identical with similar compound reported by Kamboj *et al.* [14]. Thus compound **3** was characterized as 2'-hydroxy-4-chlorochalcone.

3.2 Ache Inhibitory Activity

There is no studies on AchE inhibitory reported against the synthesized compounds. The AchE inhibitory mechanism was initiated by the hydrolysis of acetylthiocholine (ATCI) with AchE to form thiocholine. The thiocholine reacted with the Ellman's reagent (DTNB) to produce 2-nitrobenzoic-5-mercaptopthiocholine and a yellow colour 5-thio-2-nitrobenzoic acid which may be detected at 405 nm. In the TLC assay, compound **6** and **7** appeared as white spots at various concentrations when sprayed with solution

of AchE (**Figure 1**). The remaining compounds did not show any white spots which indicated to be inactive. The detection limits of the compounds were higher (0.1 mM) than that of galanthamine (1.0 mM) and the overall results are shown in **Table 2**. The microplate analysis was carried out to confirm the results of AchE inhibition by TLC method. Addition of samples and control inhibited the action of AchE against formation of thiocholine. Compound **1** was found to have the highest activity in both

concentrations (1.0 and 0.1 mM) compared to **4**, **5** and **6**. The remaining compounds displayed very weak or no AchE inhibitory activity as tabulated in **Table 3**. The activity shown by compound **4** might be due to the presence of highly electron withdrawing group (-NO₂) at the *para*-position of ring B as suggested by Yoon *et al.*, [22] in the cholinesterase inhibitors of benzimidazole derivatives.

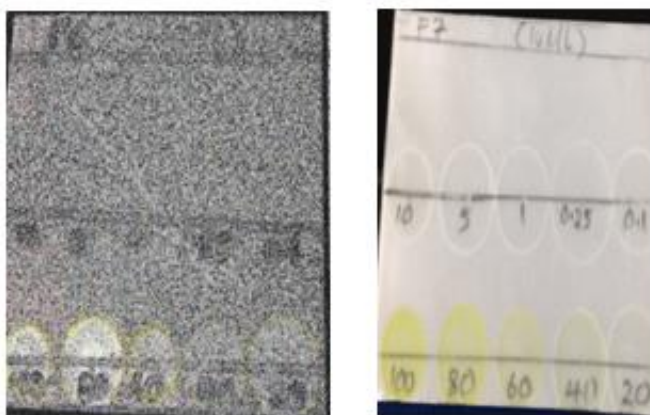


Figure 1 TLC spots of compound 6 and 7 after spraying with solution of AchE

Table 1 Physical data of the synthesized chalcones, flavone and flavanone

Compound Codes	m. p. (°C)	m. p. [Lit.]	Yield (%)	R _f	Color
1	124-126	190-192 [9]	87.4	0.62	Yellow
2	66-68	68-70 [6]	62.1	0.79	Yellow
3	144-146	149-150 [14]	65.9	0.72	Yellow
4	102-104	104-106 [6]	75.1	0.81	Yellow
5	58-60	55 [15]	76.0	0.59	Orange
6	86-90	92-93 [16]	61.8	0.60	Yellow
7	96-98	101-102 [17]	74.5	0.83	Yellow
8	142-144	144-146 [18]	64.9	0.75	White
9	144-146	148-149 [19]	56.9	0.60	White

Table 2 TLC assay of AchE inhibition at various concentrations

Compound	Concentration (mM)									
	100	80	60	40	20	10	5	1	0	0.1
1	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-
6	++	++	+	+	+	+	+	+	+	+
7	-	-	+	+	+	+	+	+	+	+
8	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-
G								+	+	+

Note: - = no activity detected; += white spot detected indicating activity =Galanthamine

Table 3 Microplate assay of AchE inhibitory activity of the targeted compounds

Compound codes	AchE inhibition (%)	
	1.0 mM	0.1mM
1	54.24	44.22
2	0	0
3	21.08	30.08
4	52.39	0
5	46.36	40.14
6	49.32	19.79
7	46.33	0
8	21.23	15.55
9	11.23	0
Gаланthamine	85.95	

4.0 CONCLUSION

In the present work, a flavone, flavanone and a series of chalcones were successfully synthesized and characterized by spectral studies. The synthesized compounds were evaluated for their acetylcholinesterase inhibition activity which has not been reported yet. 2'-Hydroxy-4'-O-prenyl-2,6-dichlorochalcone **7** displayed a very weak activity against the acetylcholinesterase with a higher detection limit 0.1 mM than the standard control (galanthamine). Moreover, 4'-hydroxy-2,6-dichlorochalcone **1**, 2'-hydroxy-4-nitrochalcone **4**, 2'-hydroxy-4-(dimethyl)aminochalcone **5** and 2'-hydroxy-4-methoxychalcone **6** showed moderate AchE inhibitory activity with higher percentage inhibition of 54.24, 46.14 and 49.32 % respectively in the microplate assay.

Acknowledgement

The authors extends their appreciation to the Kano State Government for the scholarship and to the Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia for providing all the necessary equipments required for the research and funding the work through the research Vote No. RJ130000. 7826.4F142.

References

- [1] Kutaiba, I. A., Mohamed, A. M. 2012. Chemistry, Biochemistry and Antioxidant Activity. *Journal of Pharmacy Research*. 5(8): 4013–4020.
- [2] Srinivasa, M. R., Kotes, J., Narukulla, R., Duddeck., H. 2004. Synthesis and Spectroscopic Characterization of Some Chromanochalcones and Their Dihydro Derivatives. *Archive of Organic Chemistry*. 14: 96–102.
- [3] Reddy, C. S., Nagaraj, A. 2008. Synthesis and Biological Study of Novel Bis-chalcones, Bis-thiazines and Bis-pyrimidines. *Journal of the Iranian Chemical Society. Soc. 5*: 262–267.
- [4] Hamdi, N., Fischmeister, C., Puerta, M. C., Valerga, P. 2010. A Rapid Access to New Coumarinylchalcone and Substituted Chromeno [4,3-c]pyrazol-4(1H)-ones and Their Antibacterial and DPPH Radical Scavenging Activities. *Medicinal Chemistry Research*. 19: 1–16.
- [5] Kamal, A., Prabhakar, S., Ramaiah, M. J., Reddy, P. V., Reddy, C. R., Mallareddy, A., Shankaraiah, Lakshmi, Narayan, Reddy, T. L. N., Pushpavalli, S. N. C. V. L., Pal-Bhadra, M. 2011. Synthesis and Anticancer Activity of Chalcone-pyrrolbenzodiazepine Conjugates Linked Via 1,2,3-Triazole Ring Side-armed with Alkane Spacers. *European Journal of Medicinal Chemistry*. 46: 3820–3831.
- [6] Niti, G. G., Rajput, P. R., Banewar, V. W., Raut, A. R. 2012. Synthesis and Antimicrobial Activity of Some Chalcones and Flavones Having 2-Hydroxy Acetophenone Moiety. *International Journal of Pharma and Bio Science*. 3(3): 389–395.
- [7] Prashar, H., Chawla, A., Sharma, A. K., Kharb, R. 2012. Chalcone as a Versatile Moiety for Diverse Pharmacological Activities. *International Journal of Pharmaceutical Sciences and Research. Sci. Res.* 3(7): 1913–1927.
- [8] Bano, S., Javed, K., Ahmad, S., Rathish, I. G., Singh, S., Chaitanya, M., Arunasree, K. M., Alam, M. S. 2013. Synthesis of Some Novel Chalcones, Flavanones and Flavones and Evaluation of Their Anti-inflammatory Activity. *European Journal of Medicinal Chemistry*. 65: 51–59.
- [9] Dimmock, J. R., Kandepu, N. M., Hetherington, M., Quail, J. W., Pugazhenthii, U., Sudom, A. M., Chamankah, M., Rose, P., Pass, E., Allen, T. M., Halleran, S., Szydowski, J., Mutus, B., Tannous, M., Manavathur, E. K., Myers, T. G., De Clercq, E., Balzarini, J. 1998. Cytotoxic Activities of Mannich Bases of Chalcones and Related Compounds. *Journal of Medicinal Chemistry*. 41: 1014–1026.
- [10] Bakthir, H., Ali, N. A. A., Arnold, N., Teichert, A., Wessjohann, L. 2011. Anticholinesterase Activity of Endemic Plant Extracts from Soqotra. *African Journal of Traditional Complement and Alternative Medicine*. 8(3): 296–299.
- [11] Sugamoto, K., Matsusita, Y., Matsui, K., Kurogi, C., Matsui, T. 2011. Synthesis and Antibacterial Activity of Chalcones Bearing Prenyl or Geranyl Groups from *Angelica keiskei*. *Tetrahedron Letters*. 67: 5346–5359.
- [12] Huang, W., Chien, P., Yang, C., Lee, A., 2003. Novel synthesis of flavonoids of *Scutellaria baicalensis*. *Chemical and Pharmaceutical Bulletin*. 51: 339–340.
- [13] Sharma, A., Anand, N., Sharma, R., Chaturvedi, U., Khanna, A. K., Bhatia, G., Tripathi, R. P. 2012. Synthesis of 5-aryl-6-cinnamoyl-7-methyl-Flavanones as Novel Antioxidants and Antihyperlipidemics. *Journal of Enzyme Inhibition and Medicinal Chemistry*. 27(2): 211–22.
- [14] Kamboj, R. C., Arora, R., Sharma, G., Kumar, D., Sharma, C., Joshi, R., Aneja, K. R. 2010. Eco-friendly Synthesis and Antimicrobial Activity of Chalcones. *Der PharmaChemica*. 2(3): 157–170.
- [15] Singh, H. P., Pandeya, S. N., Chauhan, C. S., Chandra, Shekhar, Sharma, C. S. 2011. Synthesis and Pharmacological Screening of Some Novel Chalconyl Derivatives of Substituted Phenyl Semicarbazide. *Medicinal Chemistry Research*. 20: 74–80.
- [16] Ahmed, N., Ali, H., van Lier, J. E. 2005. Silica Gel Supported InBr₃ and InCl₃: New Catalysts for the Facile and Rapid Oxidation of 2-

- Hydroxychalcones and Flavanones to Their Corresponding Flavones Under Solvent Free Conditions. *Tetrahedron Letters*. 46: 253–256.
- [17] Ohkatsu, Y., Satoh, T. 2008. Antioxidant and Photo-antioxidant Activities of Chalcone Derivatives. *Journal of the Japan Petroleum Institute*. 51(5): 298–308.
- [18] Bombardelli, E., Valenti, P. 2002. Preparation of Chalcones having Antiproliferative Activity. US 6423740 B1 20020723. 1–10.
- [19] Kumar, D., Patel, G., Kumar, A., Roy, Ram, K. 2009. Ionic liquid catalyzed expeditious synthesis of 2-aryl-2,3-dihydroquinolin-4(1H)-ones and 2-aryl-2,3-dihydro-4H-chromen-4-ones Under Microwave Irradiation. *Journal of Heterocyclic Chemistry*. 46(4): 791–795.
- [20] Ellman, G. L., Courtney, D., Andles, V., Featherstone, R. M. 1961. *Biochem. Pharm.* 7: 88–95.
- [21] Rhee, I. K., van Rijn, R. M., Verpoorte, R. 2003. Qualitative Determination of False-positive Effects in the Acetylcholinesterase Assay Using Thin Layer Chromatography. *Phytochemical Analysis*. 14: 127–131.
- [22] Yoon, Y. K., Mohamed Ashraf Ali, A. A., Wei, A. C., Tan Soo Choon, T. S., Khaw, K. Y., Murugaiyah, V., Osman, H., Masand, V. H. 2013. Synthesis, Characterization, and Molecular Docking Analysis of Novel Benzimidazole Derivatives as Cholinesterase inhibitors. *Bioorganic Chemistry*. 49: 33–39.