Journal of Enzyme Inhibition and Medicinal Chemistry, February 2005; 20(1): 41–47

Synthesis and inhibitory potential towards acetylcholinesterase, butyrylcholinesterase and lipoxygenase of some variably substituted chalcones

AURANGZEB HASAN[1], KHALID M. KHAN[2], MOHAMMED SHER[1], GHULAM M. MAHARVI[2], SARFRAZ A. NAWAZ[2], M.I. CHOUDHARY[2], ATTA-UR-RAHMAN[2], & CLAUDIU T. SUPURAN[3]

[1]*Department of Chemistry, Quaid-i-Azam University, Islamabad-45320, Pakistan,* [2]*HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Karachi-75270, Pakistan, and* [3]*University of Florence, Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Via della Lastruccia 3, Rm. 188, Polo Scientifico, 50019 Sesto Fiorentino (Firenze), Italy*

(Received 21 June 2004; in final form 6 August 2004)

Abstract

A series of variably substituted chalcones were synthesized by condensation of substituted acetophenones with mono-, di- or trisubstituded benzaldehydes. It was observed that some of these compounds have the potential to inhibit acetylcholinesterase, whereas others show activity against butyrylcholinesterase, depending on the substitution pattern at the two aromatic rings of these chalcones. Similarly, lipoxygenase was inhibited by two of these compounds. It has been observed that inhibition of the three enzymes was concentration dependent with the IC_{50} values ranging from 28.2–134.5 μM against acetylcholinesterase, 16.0–23.1 μM against butyrylcholinesterase and 57.6–71.7 μM against lipoxygenase, respectively.

Keywords: *Chalcones, acetylcholinesterase, butyrylcholinesterase, lipoxygenase*

Introduction

Chalcones, or 1,3-diaryl-2-propen-1-ones, are natural/synthetic compounds belonging to the flavonoid family. Chalcones possess a broad spectrum of biological activities, including antibacterial, anthelmintic, amoebicidal, antiulcer, antiviral, insecticidal, antiprotozoal, anticancer, cytotoxic, and immunosuppressive activities [1,2]. The present study was designed to determine the inhibitory potential of a series of variably substituted chalcones against acetylcholinesterase, butyrylcholinesterase and lipoxygenase enzymes.

Acetylcholinesterase (AChE) is a key component of cholinergic brain synapses and neuromuscular junctions. The major biological role of the enzyme is the termination of impulse transmission by rapid hydrolysis of the cationic neurotransmitter acetylcholine.[3] According to the cholinergic hypothesis, the memory impairment in the patients with senile dementia of *Alzheimer's* type results from a deficiency in cholinergic function in the brain [4]. Hence the most promising therapeutic strategy for activating central cholinergic functions has been the use of cholinomimetic agents. Thus enzyme acetylcholinesterase (AChE) has long been an attractive target for the rational drug design and discovery of inhibitors for the treatment of *Alzheimer's* disease (AD). Many acetylcholinesterase inhibitors like tacrine, galanthamine and eserine etc. are used for the symptomatic treatment of *Alzheimer's* disease. Recently it has also been found that butyrylcholinesterase (BChE) inhibition may also be an effective tool for the treatment of AD and related dementias [5]. Cholinesterase

Correspondence: C. T. Supuran, University of Florence, Dipartimento di Chimica, Laboratorio di Chimica Bioinorganica, Via della Lastruccia 3, Rm. 188, Polo Scientifico, 50019 Sesto Fiorentino (Firenze), Italy. Tel.: 39 055 4573005. Fax: 39 055 4573385. E-mail: claudiu.supuran@unifi.it

ISSN 1475-6366 print/ISSN 1475-6374 online © 2005 Taylor & Francis Ltd
DOI: 10.1080/14756360400015231

inhibitors may act as potential leads in the discovery of clinically useful inhibitors for nervous system disorders and in *Alzheimer's* disease.

Lipoxygenases (LOXs) are involved in arachidonic acid metabolism through lipoxygenase pathways, generating various biologically active lipids that play important roles in thrombosis and tumor progression. Angiogenesis, the formation of new capillary vessels from pre-existing ones, underlines a number of physiological processes and participates in the development of several pathological conditions such as arthritis, cancer.[6] Lipoxygenases are therefore potential target for the rational drug design and discovery of inhibitors for the treatment of variety of disorders such as bronchial asthma, inflammation, cancer and autoimmune diseases. Thus a search for new lipoxygenase inhibitors appears to be a promising approach to develop new drug candidates.

In continuation of our drug discovery program [7–14], we synthesized a variety of substituted chalcones and randomly screened them for their inhibitory effects against acetylcholinesterase, butyrylcholinesterase and lipoxygenase. The structures of synthesized compounds were determined using spectroscopic and microanalytic techniques like, NMR, mass spectroscopy, MS, IR and purity was determined by elemental analysis.

Materials and methods

General

All reactions were carried out in dried glass apparatus. Thin layer chromatography was performed on pre-coated DC-Alufolien Kieselgel 60 F 254 (E Mereck, Darmstardt, Germany). ^{1}H NMR spectra were recorded on a Bruker AH-400 spectrometer (Rheinstetten-Forchhein, Germany), operating at 400 MHz for ^{1}H nuclei. Proton chemical shifts are recorded in δ (parts per million) and coupling constants in Hertz. Infrared spectra were recorded on a Bio-Red FTIR (USA) spectrometer. The EIMS were performed on a Finnigan MAT 311 A (Bremen, Germany) spectrometer.

Chalcones **1–5** were synthesized following the Claisen–Schmidt condensation reaction, in which 80 ml of 4.0 M solution of sodium hydroxide was dissolved in 60 ml of ethanol. The solution was cooled in an ice bath to 0°C and 1.86 ml (0.016 mol) of freshly distilled acetophenone (**16**) was added. The mixture was stirred for 0.5 h in an ice bath keeping the temperature at 0–5°C. Then 1.91 ml (0.016 mol) of 2-hydroxybenzaldehyde for (**1**), 2.19 g (0.016 mol) of 3-hydroxybenzaldehyde for (**2**) 2.19 g (0.016 mol) of 4-hydroxybenzaldehyde for (**3**) 1.94 ml (0.016 mol) of 4-methoxybenzaldehyde for (**4**) and 2.66 g (0.016 mol) of 3,4-dimethoxybenzaldehyde for (**5**), respectively, were added and mixture was stirred vigorously for 2–3 h. The reaction mixture was kept overnight at 0–5°C. The thick oil obtained was diluted with chilled water and aqueous hydrochloric acid was added to neutralize it. The products were obtained as yellow precipitates. The precipitates were filtered and recrystallized from aqueous ethanol to afford the respective chalcone (**1–5**).

*3-(2-Hydroxyphenyl)-1-Phenyl-2-Propene-1-one (**1**)*. Yield: 81%. Mp = 130°C; Anal calc. for $C_{15}H_{12}O_2$ (224.26) [Found: C, 80.31; H, 5.38. Requires: C, 80.34; H, 5.398]; IR (KBr): 1596, 1635, 3205 cm^{-1}; ^{1}HNMR (400 MHz, CD_3OD): δ 7.63 (*dd*, $\mathcal{J}$ = 1.3, 7.22 Hz, 1H), 7.51 (*m*, 1H), 7.23 (*ddd*, $\mathcal{J}$ = 1.4, 8.2, 7.19 Hz, 1H), 6.87 (*dd*, $\mathcal{J}$ = 8.3, 1.4 Hz, 1H), 7.77 (*d*, $\mathcal{J}$ = 15.7 Hz, 1H), 8.09 (*d*, $\mathcal{J}$ = 15.8 Hz, 1H), 8.0–8.02 (*m*, 2H), 7.55–7.57 (*m*, 2H), 7.58–7.60 (*m*, 1H), 9.30 (*s*, 1H, exchangeable by D_2O); *m/z*: 224 (M^+, 29), 223 (8), 207 (43), 196 (19), 147 (54), 119 (33), 105 (73), 77 (100), 51 (68%).

*3-(3-Hydroxyphenyl)-1-Phenyl-2-Propene-1-one (**2**)*. Yield = 68%. Mp = 151°C; Anal calc. for $C_{15}H_{12}O_2$ (224.26) [Found: C, 80.36; H 5.38. Requires: C, 80.34; H, 5.398]; IR (KBr): 1591, 1648, 3195 cm^{-1}. ^{1}HNMR (400 MHz, CD_3OD): δ 6.80 (*dd*, $\mathcal{J}$ = 1.40, 1.48 Hz, 1H), 6.85 (*m*, 1H), 6.78 (*m*, 1H), 7.01 (*dd*, $\mathcal{J}$ = 8.01, 7.95 Hz, 1H), 7.61 (*d*, $\mathcal{J}$ = 14.83, 1H), 7.75 (*d*, $\mathcal{J}$ = 14.92 Hz, 1H), 7.98–8.0 (*m*, 2H), 7.63–7.67 (*m*, 3H), 9.23 (*s*, 1H, exchangeable by D_2O). *m/z*: 224 (M^+, 21), 223 (10), 207 (48), 196 (12), 194 (32), 147 (100), 119 (31), 107 (13), 105 (91), 77 (28), 51 (48%).

*3-(4-Hydroxyphenyl)-1-Phenyl-2-Propene-1-one (**3**)*. Yield = 75%; Mp = 101°C; Anal calc. for $C_{15}H_{12}O_2$ (224.26) [Found: C, 80.36; H, 5.40. Requires: C, 80.34; H, 5.398]; IR (KBr): 1595, 1663, 3305 cm^{-1}. ^{1}HNMR (400 MHz, CD_3OD): δ 7.49–7.53 (*m*, 2H), 6.83 (*dd*, $\mathcal{J}$ = 1.9, 6.97 Hz, 2H), 7.54 (*d*, $\mathcal{J}$ = 14.22, 1H), 7.73 (*d*, $\mathcal{J}$ = 15.44, 1H), 8.0–8.02 (*m*, 2H), 7.56–7.61 (*m*, 2H), 7.73–7.76 (*m*, 1H), 9.13 (*s*, 1H, exchangeable by D_2O). *m/z*: 224 (M^+, 25), 223 (27), 207 (31), 196 (23), 190 (21), 147 (8), 121 (100),119 (12), 105 (28), 77 (17), 51 (19%).

*3-(4-Methoxyphenyl)-1-Phenyl-2-Propene-1-one (**4**)*. Yield = 81%. Mp = 65°C; Anal calc. for $C_{16}H_{14}O_2$ (238.29) [Found: C, 80.63; H, 5.94. Requires: C, 80.65; H, 5.928]; IR (KBr): 1635, 1596 cm^{-1}. ^{1}H-NMR (400 MHz, CD_3OD): δ 6.99 (*dd*, $\mathcal{J}$ = 1.9, 7.02 Hz, 2H), 6.97 (*dd*, $\mathcal{J}$ = 1.9, 7.03 Hz, 2H), 7.59 (*d*, $\mathcal{J}$ = 15.47 Hz, 1H), 7.75 (*d*,$\mathcal{J}$ = 15.49 Hz, 1H), 8.05 (*ddd*, $\mathcal{J}$ = 1.9, 1.4, 7.3 Hz, 2H), 7.50–7.54 (*m*, 2H), 7.68 (*m*, 1H), 3.84 (*s*, 3H). *m/z*: 238 (100, M^+), 223 (27), 210 (16), 208 (42), 161 (49), 131(15), 121 (9), 105 (61), 77 (62), 51 (24%).

*3-(3,4-Dimethoxyphenyl)-1-Phenyl-2-Propene-1-one (**5**)*. Yield = 75%; Mp = 61°C; Anal calc. for $C_{17}H_{16}O_3$ (268.31) [Found: C, 76.08; H, 5.09. Requires: C, 76.10; H, 6.018]; IR (KBr): 1594, 1694 cm^{-1}. ^{1}HNMR (400 MHz, CD_3OD): δ 7.36 (*d*, $\mathcal{J}$ = 1.8, 1H), 6.98 (*d*, $\mathcal{J}$ = 9.01, 1H), 7.29 (*dd*, $\mathcal{J}$ = 9.3, 1.7 Hz, 1H), 7.61 (*d*, $\mathcal{J}$ = 15.58 Hz, 1H), 7.73 (*d*, $\mathcal{J}$ = 15.53 Hz, 1H), 7.81 (*ddd*, $\mathcal{J}$ = 1.4, 1.6, 7.8, 2H), 7.81 (*ddd*, $\mathcal{J}$ = 7.7, 7.3, 1.5 Hz, 2H), 7.57–7.59 (*m*, 1H), 3.86 (*s*, 3H), 3.89 (*s*, 3H). *m/z*: 268 (M^+, 87), 267 (54), 253 (55), 240 (15), 238 (35), 191 (70), 163 (20), 151 (18), 105 (32), 77 (100), 51 (98%).

Chalcones **6–9** were synthesized by following a general procedure in which 50 g (0.53 mol) of phenol (**18**) were added to 100 ml of 10% NaOH solution. The mixture was stirred for 15 min so that the phenol dissolved. The mixture was cooled in an ice bath and 65 ml (0.53 mol) of acetyl chloride was added dropwise. The mixture was shaken vigorously for 10 min and the emulsion formed was extracted with carbon tetrachloride (3 × 50 mL). The organic layer was washed carefully with dilute Na_2CO_3 solution until effervescence ceased. Distillation of carbon tetrachloride afforded 54 g (76%) of phenyl acetate (**19**). To 56 g (0.42 mol) of anhydrous aluminum chloride was added 40 ml of dried CS_2 dropwise. After 10 min, 46 ml (0.37 mol) of phenyl acetate **(19)** was added and the mixture kept overnight at room temperature. The solidified oil was diluted with toluene and $MgSO_4$ was added to dry the solution. Distillation of toluene at atmospheric pressure afforded an oil, which was distilled under reduced pressure (15–20 mm of Hg) until the *p*-isomer begins to collect in the condenser. Finally 16 ml (41%) of 2-hydroxy acetophenone was collected at 105–106° at 20 mm Hg. Using the Claisen–Schmidt condensation reaction, 2-hydroxy-acetophenone was then reacted with 1.52 ml (0.015 mol) of substituted-benzaldehyde [for (**6**), 1.82 ml (0.015 mol) of 3-methoxy benzaldehyde, for (**7**), 1.82 ml (0.015 mol) of 4-methoxy benzaldehyde and for (**8**) and 2.50 g (0.015 mol) of 3,4-dimethoxybenzaldehyde for (**9**) respectively]. The mixture was stirred vigorously for 2–3 h and then was kept at 0–5°C overnight. The thick oil obtained was diluted with cold water and aqueous hydrochloric acid was added to neutralize it. The products were obtained as yellow precipitates which were filtered and recrystallized from aqueous ethanol to afford the respective chalcones.

*3-Phenyl-1-(2′-Hydroxyphenyl)-2-Propene-1-one (**6**)*. Yield = 80%; Mp = 88°C; Anal calc. for $C_{15}H_{12}O_2$ (224.26) [Found: C, 80.37; H, 5.38. Requires: C, 80.34; H, 5.398]; IR (KBr): 1567, 1640, 3295 cm^{-1}. ^{1}HNMR (400 MHz, CD_3OD): δ 7.21–7.30 (*m*, 5H), 7.85 (*d*, $\mathcal{J}$ = 13.9 Hz, 1H), 8.04 (*d*, $\mathcal{J}$ = 14.2 Hz, 1H), 7.64 (*dd*, $\mathcal{J}$ = 8.1, 1.7 Hz, 1.H), 6.92 (*dd*, $\mathcal{J}$ = 8.3, 1.5 Hz, 1H), 7.38–7.39 (*m*, 1H), 6.99–7.01 (*m*, 1H), 9.6 (*s*, 1H, exchangeable with D_2O); *m/z*: 224 (M^+, 42), 223 (34), 207 (18), 147 (71), 131 (100), 103 (34), 120 (23), 104 (89), 93 (34), 92 (13), 77 (98%),

*3-(3-Methoxyphenyl)-1-(2′-Hydroxyphenyl)-2-Propene-1-one (**7**)*. Yield = 77%. Mp = 72°C; Anal calc. for $C_{16}H_{14}O_3$ (242.28) [Found: C, 80.34; H, 5.39. Requires: C, 75.57; H, 5.558]; IR (KBr): 1590, 1635, 3340 cm^{-1}. ^{1}HNMR (400 MHz, CD_3OD): δ 7.20 (*dd*, $\mathcal{J}$ = 1.4, 1.6 Hz, 1-H), 7.16–7.18 (*m*, 1H), 7.16–7.18 (*m*, 1H), 7.13–7.15 (*m*, 1H), 7.27 (*dd*, $\mathcal{J}$ = 7.81, 7.85 Hz, 1H), 7.56 (*d*, $\mathcal{J}$ = 15.4, 1H), 7.91 (*d*, $\mathcal{J}$ = 15.7 Hz, 1H), 7.95 (*dd*, $\mathcal{J}$ = 1.6, 7.9 Hz, 1H), 7.33 (*dd*, $\mathcal{J}$ = 1.6, 8.1, 1H), 7.51–7.53 (*m*, 1H), 7.20–7.21 (*m*, 1H), 9.23 (*s*, 1H, exchangeable by D_2O), 3.86 (*s*, 3H). *m/z*: 254 (M^+, 67), 253 (32), 239 (21), 237 (13), 161 (43), 147 (76), 134 (87), 121 (39), 120 (22), 107 (39), 93 (100%).

*3-(4-Methoxyphenyl)-1-(2′-Hydroxyphenyl)-2-Propene-1-one (**8**)*. Yield = 80%; Mp = 110°C; Anal calc. for $C_{16}H_{14}O_3$ (254.29) [Found: C, 75.54; H, 5.54. Requires: C, 75.57; H, 5.558]; IR (KBr): 1587, 1637, 3315 cm^{-1}. ^{1}HNMR (400 MHz, CD_3OD): δ 6.90 (*dd*, $\mathcal{J}$ = 1.9, 7.3 Hz, 2H), 7.01 (*dd*, $\mathcal{J}$ = 1.3, 7.1 Hz, 2H), 7.65 (*d*, $\mathcal{J}$ = 15.21, 1H), 7.90 (*d*, $\mathcal{J}$ = 15.29 Hz, 1H), 8.05 (*dd*, $\mathcal{J}$ = 1.37, 7.6 Hz, 1H), 7.33 (dd, $\mathcal{J}$ = 1.34, 8.1 Hz, 1H), 7.47 (*ddd*, $\mathcal{J}$ = 8.4, 7.8, 1.4 Hz, 1H), 7.24 (*ddd*, $\mathcal{J}$ = 7.6, 7.9, 1.3 Hz, 1H), 9.12 (*s*, 1H, exchangeable by D_2O), 3.87 (*s*, 3H). *m/z*: 254 (M^+, 97), 253 (2.3), 237 (35), 161 (61), 147 (86), 134 (100), 121 (76), 107 (35), 93 (21%).

*3-(3,4-Dimethoxyphenyl)-1-(2′-Hydroxyphenyl)-2-Propene-1-one (**9**)*. Yield = 73%; Mp = 167°C; Anal calc. for $C_{17}H_{16}O_4$ (284.31) [Found: C, 71.84; H, 5.66. Requires: C, 71.82; H, 5.678]; IR (KBr): 1648, 1603 cm^{-1}. ^{1}HNMR (400 MHz, CD_3OD): δ 7.02 (*d*, $\mathcal{J}$ = 1.96 Hz, 1H), 6.96 (*d*, $\mathcal{J}$ = 7.60 Hz, 1H), 7.42 (*dd*, $\mathcal{J}$ = 2.5, 8.34 Hz, 1H), 7.68 (*d*, $\mathcal{J}$ = 15.40 Hz, 1H), 7.87 (*d*, $\mathcal{J}$ = 15.36, 1H), 7.34 (*dd*, $\mathcal{J}$ = 1.75, 8.3 Hz, 1H), 7.50 (*ddd*, $\mathcal{J}$ = 8.4, 7.9, 1.3 Hz, 1H), 7.22 (*ddd*, $\mathcal{J}$ = 8.3, 8.1, 1.5 Hz, 1H), 8.17 (*dd*, $\mathcal{J}$ = 1.18, 7.90 Hz, 1H), 3.88 (*s*, 3H), 3.92 (*s*, 3H), 9.51 (*s*, 1H, exchangeable by D_2O): *m/z*: 284 (M^+, 69), 283 (29), 269 (9), 267 (7.8), 191 (8), 164 (100), 147 (29), 137 (1.2), 121 (46), 93 (18%).

Chalcones **10–12** were synthesized from 70 g (0.54 mol) of *p*-chlorophenol (**18a**), to which was added 200 ml of 10% NaOH solution with stirring. The reaction mixture was kept in an ice bath to maintain the temperature at 0–5°C with constant stirring and 66 ml (0.84 mol) of acetyl chloride was added dropwise. The temperature was kept low for 1 h. The oil formed was extracted with dichloromethane (2 × 30 ml), to afford 39 g (82%) of *p*-chlorophenyl acetate (**19a**). 45 g (0.33 mol) of anhydrous aluminum chloride was introduced into a dry 2-necked round bottom flask with a drying tube and gas trapping

apparatus and 30 g (0.175 mol) of dried *p*-chlorophenylacetate (**19a**) was added. The mixture was heated on an oil bath at 120°C for 75 min. The reaction mixture was allowed to cool at room temperature and 100 g of crushed ice was added followed by 200 ml of chilled water. Then 100 ml of toluene was added and the solution stirred for 1 h. After extraction the toluene was distilled off to leave a residual oil. Vacuum distillation (20 mm/Hg, 25°C) of the oil afforded 20 g of 5-chloro-2-hydroxyacetophenone. Similarly for the synthesis of chalcones **10–12**, the Claisen–Schmidt reaction was used and 2 g (0.012 mol) of freshly distilled 5-chloro-2-hydroxyacetophenone was reacted with 1.22 ml (0.012 mol) of benzaldehyde for (**10**), 1.68 g (0.012 mol) of 4-chlorobenzaldehyde for (**11**) and 1.44 g (0.012 mol) of 3-methylbenzaldehyde for (**12**), respectively. The mixture was stirred vigorously for 2–3 h and was kept at 0–5°C overnight. The thick oil obtained was diluted with chilled water and aqueous hydrochloric acid was added to neutralize it. The products were obtained as yellow precipitates. The precipitate was filtered and recrystallized from aqueous ethanol to afford the respective chalcone.

*3-Phenyl-1-(2′-Hydroxy-5′-Chlorophenyl)-2-Propene-1-one (**10**)*. Yield = 58%; Mp = 98°C; Anal calc. for $C_{15}H_{11}O_2Cl$ (258.71) [Found: C, 69.67; H, 4.28; Cl 13.71. Requires: C, 69.64; H, 4.29; Cl, 13.708]; IR (KBr): 1642, 1587 cm^{-1}. ^{1}HNMR (400 MHz, CD_3OD): δ 7.36–7.53 (*m*, 5H), 7.84 (*d*, $\mathcal{J}$ = 15.42 Hz, 1H), 7.93(*d*, $\mathcal{J}$ = 15.42 Hz), 6.97 (*d*, $\mathcal{J}$ = 8.90Hz, 1H), 7.79 (*dd*, $\mathcal{J}$ = 6.94, 1.08 Hz, 1H), 8.12(*d*, $\mathcal{J}$ = 1.20, 1H), 9.42 (*s*, 1H, exchangeable by D_2O); *m/z*: 260 ($[M+2]^+$, Cl^{37}, 24), 258 (M^+, 73), 257 (52), 241 (17), 181 (57), 154 (100), 131 (15), 126 (21), 103 (42.03), 77 (41), 51 (26%).

*3-(4-Chlorophenyl)-1-(2′-Hydroxy-5′-Chlorophenyl)-2-Propene-1-one (**11**)*. Yield = 75%; Mp = 93°C; Anal calc. for $C_{15}H_{10}O_2Cl_2$ (293.15) [Found: C, 61.49; H, 3.43; Cl, 24.208. Requires: C, 61.46; H, 3.44; Cl, 24.198]; IR (KBr): 1587, 1642, 3317 cm^{-1}; ^{1}HNMR (400 MHz, CD_3OD): δ 7.28 (*dd*, $\mathcal{J}$ = 7.8, 1.7 Hz, 2H), 7.22 (*dd*, $\mathcal{J}$ = 1.65, 8.12 Hz, 2H), 7.56 (*d*, $\mathcal{J}$ = 14.35 Hz, 1H), 7.9 (*d*, $\mathcal{J}$ = 7.9, 1.45 Hz, 1H), 7.60 (*d*, $\mathcal{J}$ = 1.45 Hz, 1H), 8.93(*s*, 1H, exchangeable by D_2O). *m/z*: 299 ($[M+4]^+$, Cl^{37} Cl^{37}, 21), 297 ($[M+2]^+$, Cl^{37} Cl^{35}, 4), 295 (M^+, 33), 293 (M^+, 40), 291 (12), 275 (23), 181 (91), 165 (81), 154 (100), 138 (47), 127 (43), 111 (17%).

*3-(3-Methylphenyl)-1-(2′-Hydroxy-5′-Chlorophenyl)-2-Propene-1-one (**12**)*. Yield = 75%; Mp = 112°C; Anal calc. for $C_{16}H_{13}O_2Cl$ (272.73) [Found: C, 70.49; H, 4.81; Cl, 13.02. Requires: C, 70.46; H, 4.80; Cl, 13.008]; IR (KBr): 688, 1575, 1650, 3312 cm^{-1}. ^{1}HNMR (400 MHz, CD_3OD). δ 7.15 (*dd*, $\mathcal{J}$ = 1.4, 1.3 Hz, 1H), 7.12 (*dd*, $\mathcal{J}$ = 8.2, 1.3 Hz, 1H), 7.05 (*dd*, $\mathcal{J}$ = 7.4, 8.1 Hz, 1H), 6.93–26.94(*m*, 1H), 6.93 (*d*, $\mathcal{J}$ = 13.98 Hz, 1H), 7.5(*d*, $\mathcal{J}$ = 14.0 Hz, 1H), 6.89 (*d*, $\mathcal{J}$ = 7.4 Hz, 1H), 7.31 (*dd*, $\mathcal{J}$ = 7.56, 1.39 Hz, 1H), 7.53 (*d*, $\mathcal{J}$ = 1.33 Hz, 1H), 9.81 (*s*, 1H, exchangeable by D_2O), 2.35 (*s*, 3H); *m/z*: 274 ($[M+2]^+$, Cl^{37}, 24), 272 (M^+, 72), 271 (8), 255 (63), 181 (67), 155 (100), 145 (43), 127 (32), 118 (43), 91 (71%),

Enzyme inhibition assays

In vitro *cholinesterase inhibition assay*. Electric-eel acetylcholinesterase (EC 3.1.1.7), horse-serum butyrylcholinesterse (E.C 3.1.1.8), acetylthiocholine iodide, butyrylthiocholine chloride, 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB) and galanthamine were purchased from Sigma (St. Louis, MO, USA). Buffer and other chemicals were of analytical grade. The 'test compounds' inhibiting activities on acetylcholinesterase and butyrylcholinesterase were measured by slightly modifying the spectrophotometric method developed by Ellman *et al.*[26] Acetylthiocholine iodide and butyrylthiocholine chloride were used as substrates to assay acetylcholinesterase and butyrylcholinesterase, respectively. The 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB) was used for the measurement of cholinesterase activity. 140 μL of 100 mM sodium phosphate buffer (pH 8.0), 10 μL of (0.25mM) DTNB, 20 μL of test-compound solution and 20 μL of acetylcholinesterase or butyrylcholinesterase solution were mixed and incubated for 15 min at 25°C. The reaction was then initiated by the addition of 10μL acetylthiocholine (0.4 mM) or butyrylthiocholine (0.2 mM), respectively. The hydrolysis of acetylthiocholine and butyrylthiocholine were monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine and butyrylthiocholine, respectively, at a wavelength of 412 nm. Test-compounds and the control were dissolved in EtOH. All the inhibition studies were performed in 96-well microtiter-plates using *SpectraMax 340* (Molecular Devices, CA, USA).

In vitro *lipoxygenase inhibition assay*. Lipoxygenase inhibiting activity was conveniently measured by slightly modifying the spectrometric method developed by Tappel.[27] Lipoxygenase (1.13.11.12) type I-B and linoleic acid were purchased from sigma (St. Loius, MO, USA). 160 μL of 100 mM sodium phosphate buffer (pH 8.0), 10 μL of test-compound solution and 20 μL of Lipoxygenase solution were mixed and incubated for 5 min at 25°C. The reaction was then initiated by the addition of (0.2 mM) 10 μL linoleic acid (substrate) solution, with the formation of (9*Z*,11*E*)-(13*S*)-13-hydroperoxyoctadeca-9,11-dienoate, and the change of absorbance was

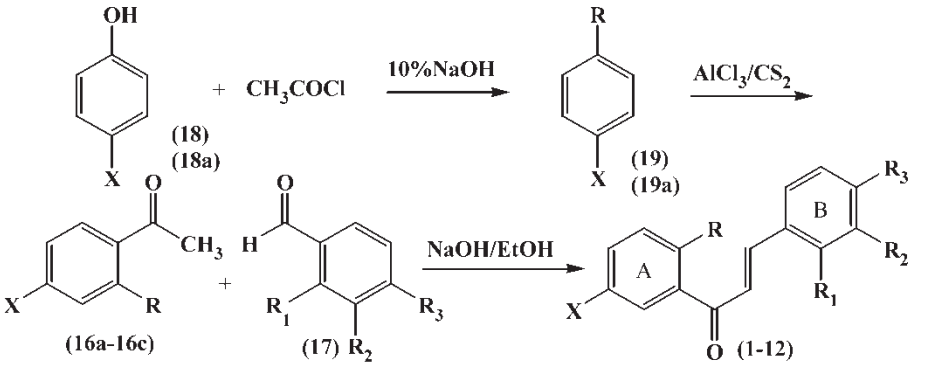
followed for 10 min at a wavelength of 234 nm. Test-compounds and the control were dissolved in MeOH. All the reactions were performed in triplicate. The IC_{50} values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA).

Estimation of IC_{50} values. The concentrations of test compounds that inhibited the hydrolysis of substrates by 50% (IC_{50}) were determined by monitoring the effect of various concentrations of these compounds in the assays on the inhibition values. The IC_{50} values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA).

Results and discussion

Chemistry

A variety of methods [[15–23]] are available for the synthesis of chalcones. The chalcones used in the present study were synthesized by the Claisen–Schmidt condensation reaction,[[24]] which involves reaction of



Comp No	X	R	R1	R2	R3	Yield %
16a/1	H/H	H/H	–/OH	–/H	–/H	81
16a/2	H/H	H/H	–/H	–/OH	–/H	68
16a/3	H/H	H/H	–/H	–/H	–/OH	75
16a/4	H/H	H/H	–/H	–/H	–/OCH_3	81
16a/5	H/H	H/H	–/H	–/OCH_3	–/OCH_3	75
16b/6	H/H	OH/OH	–/H	–/H	–/H	80
16b/7	H/H	OH/OH	–/H	–/OCH_3	–/H	77
16b/8	H/H	OH/OH	–/H	–/H	–/OCH_3	80
16b/9	H/H	OH/OH	–/H	–/OCH_3	–/OCH_3	73
16c/10	Cl/Cl	OH/OH	–/H	–/H	–/H	58
16c/11	Cl/Cl	OH/OH	–/H	–/H	–/Cl	75
16c/12	Cl/Cl	OH/OH	–/H	–/CH_3	–/H	75
13	H	H	H	H	H	81
18*	H	–	–	–	–	–
18a*	Cl	–	–	–	–	–
19	H	$OCOCH_3$	–	–	–	81
19a	Cl	$OCOCH_3$	–	–	–	79

* starting material

Scheme 1. Synthesis of chalcones **1–12** by Claisen–Schmidt condensation.

equimolar amounts of a substituted acetophenone with a substituted benzaldehyde in the presence of aqueous/alcoholic alkaline hydroxide (Scheme 1) [25]. The synthetic reaction gave good yields (58 to 81%) for all the chalcones **1–12** reported here.

The chalcones of type **1–12** were synthesized with ring B (substituents incorporating hydroxyl) substitution at the 2′-position of ring A and 2′-hydroxy 5′-chloro ring A substitution. Synthesis of chalcones **1–12** commenced by the conversion of commercially available or appropriately synthesized substituted-benzaldehydes **17** followed by their condensation with freshly distilled acetophenone **16**. As a result five compounds (**1–5**) having ring B substituents were obtained.

For the synthesis of chalcone **6–9,** phenol **18** was converted to the phenyl acetate **19** which on Fries rearrangement was converted to *o*-hydroxyacetophenone (Scheme 1). This was in turn condensed with variably substituted benzaldehydes **17**, leading to the four chalcones **6–9**. The synthesis of chalcones **10–12** was also achieved by following the above-mentioned procedure. However, in this case 4-chlorophenol (**18a**) was converted to 4-chlorophenyl acetate (**19a**) and then transformed to the chalcones **10–12**.

Biology

The synthesized chalcones were screened for their inhibition potential against acetylcholinesterase, butyrylcholinesterase and lipoxygenase. The results are presented in Table I.

From these results it is evident that the hydroxyl (-OH) group present in ring A has a vital role in the inhibition of acetylcholinsterase. Compounds **6–10** and **12** have a -OH group in ring A and showed IC_{50} values in the range 28.2 − 138.5 μM. In comparison, compounds **1–5**, which do not have a -OH group in ring A, are completely inactive. Compound **4** closely resembles compound **8** but only lacks a -OH group in ring A, and is totally inactive whereas compound **8** showed weak activity. Similarly compound **5** which resembles the most active compound **9**, showed no activity at all because it also lacks a -OH group in ring A. Although compounds **1**, **2** and **3** have a hydroxyl group in the molecule they are still inactive, because they all have a -OH group in ring B and not in ring A. Compound **10** exhibited lesser activity than compound **9**. This may be due to the presence of a chloro substituent in ring A. Compound **11** is totally inactive, probably due to the presence of two chloro substituents, one in each ring. In addition, the methyl group may be another substituent that further deactivates the chalcone molecule against acetylcholinesterase activity, as observed in compound **12**. For acetylcholinesterase inhibitory activity it may be concluded that a hydroxyl group *ortho* to the side chain is very much responsible for AChE inhibitory activity. Substituents like methoxy, chloro or methyl may increase or decrease the AChE activity of chalcones in the present set of compounds.

Interestingly when the present series of compounds were screened for their butyrylcholinesterase inhibition activity, it was found that all those compounds, which exhibited good activities against AChE, were inactive against butyrylcholinesterase. The activity difference was once again rationalized on the basis of their structures. Compound **1** was found to be a potent compound; it has a hydroxyl group on ring B *ortho* to the side chain, which indicates that hydroxyl group at the same position may be responsible for butyryl cholinesterase inhibitory activity. It is also obvious from the activity of compounds **2** and **3**, which exhibited lower activity than compound **1**, although both have hydroxyl group on ring B but *meta* or *para* to the side chain. Another substituent effect was also found in the series due to the presence of a methoxy group on ring B, which may also be responsible for butyrylcholinestrease activity but to lesser extent as compared to a hydroxyl group *ortho* to the side chain. These effects can be seen in compounds **4** and **5**. For example, compound **4** which has a methoxy group *para* to the side chain was less inhibitory as compared to compound **5**, which contains two methoxy group *meta* and *para* to the side chain. A very weak butyryl cholinesterase inhibitory activity was shown by compound **13**, which is an unsubstituted chalcone, supporting our hypothesis that a suitable substituent like a -OH group on ring B *ortho* to the side chain is responsible for enhanced butyrylcholinesterase inhibitory activity in this series of derivatives. In view of these results we can state that a hydroxyl group *ortho* to the side chain and a methoxy in the *meta* and *para*

Table I. *In vitro* inhibition data for acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and lipoxygenase (LOX) with compounds **1–15**.

	$IC_{50}(\mu M)$ ± SEM*		
Compounds	*AChE*	*BChE*	*LOX*
1	00	16.0 ± 0.3	00
2	00	46.2 ± .1.2	00
3	00	40.0 ± 1.4	00
4	00	39.0 ± 5.0	00
5	00	23.1 ± 2.9	00
6	59.2 ± 5.0	00	00
7	66.0 ± 0.8	00	00
8	92.9 ± 5.4	00	00
9	28.2 ± 0.5	00	00
10	39.2 ± 0.53	00	57.6 ± 2.6
11	00	00	71.7 ± 0.5
12	134.5 ± 0.45	00	00
13 (Unsub. Chalcone)	00	109. 0 ± 1.0	00
14 (Galanthamine**)	0.5 ± .01	8.5 ± 0.5	00
15 (Baicalein***)	00	00	22.5 ± 1.5

*Standard means error of five assays, **Standard inhibitor of acetylcholinesterase and butyrylchlinesterase, ***Standard inhibitor of lipoxygenase.

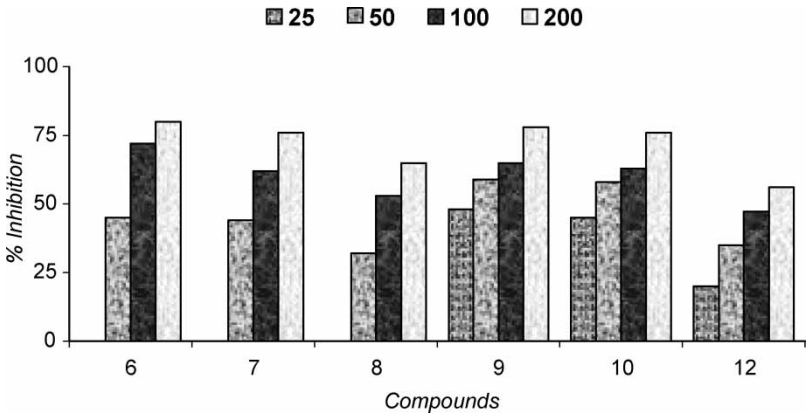


Figure 1. Inhibition [%] of acetylcholinesterase enzyme at 25, 50, 100 and 200 μM for compounds **6**, **7**, **8**, **9**, **10** and **12**.

positions are inducing the butyrylcholinesterase inhibitory activity in this small series of derivatives.

The compounds **1–13** were also screened for their effects on lipoxygenase inhibition and it was observed that all the compounds except **10** and **11** were completely inactive. Lipoxygenase inhibition with compounds **10** and **11** may also be rationalized on the basis of their structural features. Both the compounds have chlorine atoms in their structures, the decreasing activity of compound **11** being probably due to the presence of an additional chlorine atom on ring B. The complete loss of activity of compound **12** may be due to the presence of a methyl group on ring B. It may be concluded from the results for lipoxygenase inhibitory activity that a chlorine atom *para* to a hydroxyl group on ring A may be responsible for inhibitory activity, whereas chlorine and methyl substituents on ring B dramatically decrease activity.

In conclusion the group of chalcones under study provides a firm base to explore these types of compounds for their effects against acetylcholinesterase, butyrylcholinesterase and lipoxygenase and to search for potent and selective inhibitors for these enzymes. Inhibition [%] of acetylcholinesterase enzyme at various concentrations (μM) of compounds **6**, **7**, **8**, **9**, **10** and **12** and a graphical presentation of the comparative IC_{50} values of the compounds **1**, **2**, **3**, **4**, **5** and **13** with **14** (standard inhibitor of acetylcholinesterase and butyrylcholinsterase) and inhibition [%] of lipoxygenase at various concentrations of compounds **10** and **11** as compared to baicalein **15** (standard inhibitor of lipoxygenase), are presented in Figure 1.

References

[1] Dhar DN. The Chemistry of Chalcones and Related Compounds. New York: Wiley-Interscience; 1981.
[2] Dimmock JR, Elias DW, Beazely MA, Kandepu NM, Curr Med Chem 1999;6:1125.
[3] Tougu V, Curr. Med. Chem. 2001;1:155.
[4] Perry EK, Br. Med. Bull. 1986;42:63.
[5] Yu SQ, Holloway HW, Utsuki T, Brossi A, Greig NH, J Med Chem 1999;42:1855.
[6] Nie D, Honn KV. Cell Mol Life Sci 2002;59:799.
[7] Khan KM, Maharvi GM, Abbaskhan A, Hayat S, Khan MTH, Makhmoor T, Choudhary MI, Shaheen F, Helv Chim Acta 2003;86:457.
[8] Atta-ur-Rahman, Choudhary MI, Thomsen WJ. Bioassay Techniques for Drug Development. The Netherlands: Harwood Academic Publishers; 2001. p 16.
[9] Khan KM, Saify ZS, Zeeshan, Khan A, Ahmed M, Saeed M, Abdel-Jalil RJ, Grubler G, Voelter W, Z. Naturforsch 1999;54b:1210.
[10] Khan KM, Saify ZS, Zeeshan, Khan A, Ahmed M, Saeed M, Schick M, Kohlbau HJ, Voelter W, Arzneim-Forsch/Drug Res 2000;50:915.
[11] Saify ZS, Khan KM, Haider SM, Zeeshan, Shah STA, Saeed M, Shekhani MS, Voelter W, Z. Naturforsch 1999;54b:1327.
[12] Zaidi JH, Naeem F, Iqbal R, Choudhary MI, Khan KM, Shah STA, Hayat S, Voelter W, Z. Naturforsch 2001;56b:689.
[13] Khan KM, Iqbal S, Lodhi MA, Maharvi GM, Perveen S, Choudhary MI, Rahman AU, Chohan ZH, Supuran CT, J Enz Inhib Med Chem 2004; in press.
[14] Khan KM, Rahat S, Choudhary MI, Atta-ur-Rahman, Ghani U, Perveen S, Khatoon S, Dar A, Malik A, Helv Chim Acta 2002;85:559.
[15] Dershowitz S, Proshauer S, J Org Chem 1961;26:3595.
[16] Dershowitz, S. (1963) *U.S. Patent* 3-104, p. 257–259.
[17] Kozlov NS, Pinegina LY, Selezneva EA, Zh Obshch Khim 1962;32:436.
[18] Wadsworth Jr., WS, Emmons WQD, J Am Chem Soc 1961;83:1733.
[19] Obara H, Takahashi H, Hirano H, Bull Chem Soc Jp 1969;42:560.
[20] Tsukervanik IP, Galustyan GG, Zh Obshch Khim 1961;31:528.
[21] Mehra HS, Mathur KBL, J Indian Chem Soc 1955;32:465.
[22] Koehetkov NK, Belyaev VF, Zh Obshch Khim 1960;30:1495.
[23] Belyacy VF, Zh Obshch Khim 1964;34:861.
[24] Geissman TA, Clinton RO, J Chem Soc 1946;68:697.
[25] Corrêa R, Pereira MAS, Buffon D, Santos L, Cechinel Filho V, Santos ARS, Nunes RJ, Arch Pharm 2001;334:332.
[26] Ellman GL, Courtney KD, Andres V, Featherstone RM, Biochem Pharmacol 1961;7:88.
[27] Tappel AL. Methods in Enzymology., 5 New York: Academic Press; 1962. p 539.