Synthesis and *in vitro* cholinesterase inhibitory potential of dihydropyridine derivatives

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Abstract: Twelve derivatives of dihydropyridine derivatives (6-17) were synthesized and evaluated for in-vitro cholinesterases (AChE, BChE) inhibitory activity. All compounds showed potent activity with IC_{50} values between 0.21±0.003 to 147.14±0.12µM for AChE and among them five compounds showed potent activity with IC_{50} values 17.16±0.02 to 231.6±0.12µM for BChE when compared with standard Eserine (IC_{50} = 0.85±0.0001 µM (AChE) & 0.04±0.0001µM (BChE). The most potent compound 11 can be considered as potential lead compound showed an inhibition of 95.35±0.11 and IC_{50}= 0.21±0.003 while compound 7 showed an inhibition of 83.45±0.13 and IC_{50}= 17.16±0.02. It is concluded from structural activity relationship that the presence of nitro group at C-2 and C-4 position of dihydropyridine ring increase the acetyl cholinesterase and butyrylcholinesterase activities of these compounds while presence of -Br and -Cl also enhances the activities.

Keywords: Dihydropyridines, hantzsch reaction, MCR, alzheimer, acetylcholinesterase, butyryl-cholinesterase.

INTRODUCTION

Alzheimer's disease is a complex disease with multiple, intertwining pathological factors (Savelief *et al*., 2013), including; progressive cerebral failure and memory loss (Scarpini *et al*., 2003). Acetylcholines play significant roles in the pathophysiology of Alzheimer's disease (Savelief *et al*., 2013) and can be degraded by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) (Girard *et al*., 2007, Azzouz *et al*., 2018). Primarily AChE is a regulatory enzyme for cholinergic synapses, while BChE known as co-regulator (Schwarz *et al*., 2014). Alzheimer's disease can be controlled by inhibition of AChE and BChE (Manral *et al*., 2015), however BChE has limited quantity and is localized in the peripheral tissues. Thus, discovery of new selective AChE inhibitors with lesser peripheral inhibition is therapeutically suitable for the treatment of Alzheimer's disease (Sang *et al*. 2015, Maryamabadi *et al*. 2016).

In recent years, dihydropyridines synthesis gain significant attention because of their remarkable biological and pharmaceutical activities. 1,4-dihrydropyrimidines as analogues of NADH coenzyme and other related derivatives are extensively used as calcium channel blockers for the treatment of many diseases like cardiovascular disorder including hypertension, angina and cardiac arrhythmias. A new family of tacrine dihydropyridine hybrids, named as tacripyrine was found as a potent and selective inhibitor AChE. Some derivatives of dihydropyridines are available commercially for the treatment of hypertension such as nifedipine (1), amlodipine (2), felodipine (3) and nicardipine (4) (Nasr-Esfahani *et al*., 2014)

Likewise it was reported that this class of compounds shown remarkable biological properties like anti-inflammatory, anti-ulcer, anti-convulsant, anti-tubercular and anti-microbial (Joshi *et al*., 2014, Sharma *et al*., 2018). A recent study on dihydropyridines showed that the compound (5) and its derivatives were identified as a new class of anti-oxidants and it was predicted that these compounds could serve as a lead molecule in the development of new antioxidants with therapeutic potential (Anwar *et al*., 2014).

Moreover a detail review on chemotherapeutic activities of 1, 4-dihydropyridines is given by Saghi Sepehri *et al* in 2015 (Sepehri *et al*., 2015). In view of these significant properties, we have undertaken synthesis and biological screening of novel 1,4-dihydropyridines. Recently we reported synthesis of some arylfuryl-1,4-dihydropyridines (Zafar *et al*., 2017) and now we like to present the synthesis and acetyl- and butyrylcholin esterase properties of novel 4-arylfuran-2-yl-1,4-dihydropyridines (6-17).

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MATERIALS AND METHODS

All chemicals were purchased from Sigma-Aldrich (USA) and Merck (Germany) and of analytical grade and were as used as without any further purification. Pre-coated silica gel, GF-254 (Merck, Germany) was used for thin layer chromatography. Visualization of TLC was performed under ultraviolet light at 254 and 366 nm. Melting points were taken on a Gallen Kamp melting point apparatus and were uncorrected. Elemental analyses were performed on Perkin Elmer (2400 series II) CHN elemental analyzer. FTIR spectra were recorded on a Bruker Tensor 27. The ^{1}H-NMR and ^{13}C-NMR spectra were taken on Bruker DPX instrument at 300 and 400 MHz. All chemical shifts are reported in δ downfield from TMS. Coupling constants (J) are reported in Hz. Multiplicity in ^{1}H-NMR is reported as singlet(s), doublet (d), triplet (t), quartet (q) and multiplet (m).

General procedure for the synthesis of compounds (6-17)

A mixture of 5-arylfuran-2-carbaldehyde (1mmol), ethyl acetoacetate (2mmol), and ammonium acetate (1mmol) was refluxed in ethanol (25mL) for three hours (reaction monitored through TLC). After cooling, the reaction mixture was poured down into ice-cold water. Precipitates were filtered, washed with water and cold ethanol, dried and recrystallized from ethanol to give the desired products (Scheme 1).

Diethyl-2,6-dimethyl-4-(5'-(4′′-nitrophenyl)furan-2′-yl)-1,4-dihydropyridine-3, 5-dicarbox-ylate (6)

Yield: 95%, M. P. = 145-150°C, IR (υ-cm^{-1}): 3834.9 (N-H stretching), 3107.0 (Ar-H), 2984.0 (C-H stretching of CH_3), 1714.3 (C=O ester), 1512.3, 1330.4 (NO_2); Mass: m/z (%): 440.2 (M) 96.7%, 411.2 (M-2CH_3) 100%, 395.2 (M-NO_2) 30.1%, 383.1 (M-2, C_2H_5) 23.3%, 367.2 (M-$COOC_2H_5$) 100%, 337.2 (M-$COOC_2H_5$-2CH_3) 26.7%, 321.1 (M- $COOC_2H_5$-C_2H_5O) 30.4%, 293.1 (M-2$COOC_2H_5$) 11.7%, 252.1 (M-$C_{10}H_6No_3$) 14.9%, 196.1 (M-$C_{10}H_6NO_3$-2C_2H_5) 13.8%, 179.1 (M- $C_{10}H_6NO_3$-2C_2H_5-CH_3) 10.7%, 150.1 (M-$C_{10}H_6NO_3$-C_2H_5O-CH_3) 15.4%, 120.1 (M-$C_{17}H_{20}NO_5$) 13.0%, 55.1 (C_4H_5) 6.5%, 43.0 (C_2H_4O) 9.9%. ^{1}H-NMR ($CDCl_3$) δ: 2.379(s, 6H, 2CH_3), 1.319(t, 6H, 2CH_3CH_2, *J*=10.68Hz), 4.234(q, 4H, 2CH_3CH_2, *J*=12.63Hz), 7.74(H-6", 2"), 8.25(H-3", 5"); ^{13}C-NMR ($CDCl_3$) δ:14.08 (CH_3), 61.70 (CH_3-CH_2), 167.20 (C=O), 107.70 (C=C), 149.98 (C-NH), 14.39 (CH_3), 33.83 (CH-C), 150.21 (CH=C-O), 100.37 (CH-CH), 107.70 (CH-CH), 154.79 (CH-aromatic ring), 136.91, 130.83, 123.2, 124.80, 125.59, 149.81 (aromatic carbons). CHN Microanalysis: calculated: C, 62.72; H, 5.49; N, 6.36; Found: C, 62.65; H, 5.42; N, 6.30.

Diethyl-2,6-dimethyl-4-(5'-(2''-chloro-4′′-nitrophenyl)furan-2′-yl)1,4-dihydropyridine-3-carboxylate (7)

Yield: 59%, M.P. =220°C, IR (υ-cm^{-1}): 3311.9 (N-H stretching), 3087.5 (Ar-H), 2983.5 (C-H stretching of CH_3), 1652.7 (C=O ester), 1487.3, 1332.7 (NO_2). Mass: m/z (%): 476.0 (M+2)14.4%, 473.9 (M) 49.6%, 444.9 (M-2CH_3) 54.4%, 401.0 (M-$COOC_2H_5$) 100%, 371.0 (M-$COOC_2H_5$-2CH_3) 6.55%, 328.0 (M-2$COOC_2H_5$) 6.9%, 252.1 (M-$C_{10}H_5ClNO_3$) 14.4%, 223.0 (M-$C_{13}H_{18}NO_4$) 5.3%, 183.9 (M-$C_{10}H_5ClNO_3$-$COOC_2H_5$) 10.2%, 150.0 (M-$C_{10}H_5ClNO_3$-$COOC_2H_5$-2CH_3) 7.3%. ^{1}H-NMR ($CDCl_3$) δ: 2.374(s, 6H, 2CH_3), 1.302(t, 6H, 2$\underline{CH}_3CH_2$, *J*=10.62Hz), 4.184(q, 4H, 2$CH_3\underline{CH_2}$, *J*=10.41Hz), 5.303(s, 1H, CH), 7.28(d, 4H, H-6",*J*=6.00Hz), 8.275(s, 1H, H-3") ; ^{13}C-NMR ($CDCl_3$) δ: 14.40 ($\underline{C}H_3CH_2$), 60.02 ($CH_3\underline{C}H_2$), 167.13 ($\underline{C}$=O), 100.33 , 145.42 (C$\underline{C}$NH), 19.62 ($\underline{C}H_3CNH$), 33.81 ($\underline{C}$H-furan), 145.42, 100.33, 107.92, 146.61 (furan ring), 145.27, 129.19, 116.01, 135.42, 126.24, 134.90 (phenyl ring). CHN Microanalysis: calculated: C, 58.17; H, 4.88; Cl, 7.47; N, 5.90, Found: C, 58.14; H, 4.85; N, 5.88.

Diethyl-2,6-dimethyl-4-(5'-(2′′-nitrophenyl)furan-2'-yl)-1,4-dihydropyridine-3-carboxylate: (8)

Yield: 50%, M.P. = 120-122°C, IR (υ-cm^{-1}): 3732.1(N-H stretching), 3347.7 (Ar-H), 2984.9 (C-H stretching of CH_3), 1648.2 (C=O ester), 1478.8, 1336.2 (NO_2). Mass: m/z (%): 440.2 (M) 27.4%, 423.1 (M-CH_3) 22.3%, 395.1 (M-NO_2) 15.8%, 367.1 (M-$COOC_2H_5$) 100%, 337.1 (M-$COOC_2H_5$-2CH_3) 5.3%, 293.1 (M-2$COOC_2H_5$) 5.0%, 252.1 (M-$C_{10}H_6No_3$)18.5%, 224.1 (M-$C_{10}H_6No_3$-2CH_3) 6.3%, 150.0 (M-$C_{10}H_6NO_3$-2CH_3-$COOC_2H_5$) 7.8%, 77.0 (M-$C_{17}H_{20}NO_5$-NO_2) 3.4%. ^{1}H-NMR ($CDCl_3$) δ: 2.361(s, 6H, 2CH_3), 1.288(t, 6H, 2CH_3CH_2, *J*=10.62Hz), 4.181(q, 4H, 2CH_3CH_2, *J*=10.04Hz), 5.138(s, 1H, CH), 6.120, 6.127 (2H of furan ring, H-3′, H-4′), 6.593(d, 1H, H-6′′, *J*=3.57Hz), 7.958 and 7.82(H-2′′, and H-3 ′′respectively). ^{13}C-NMR ($CDCl_3$) δ: 14.36 (CH_3CH_2), 59.82 (CH_3CH_2), 167.21 (C=O), 99.82 (2C), 146.129 (2C), 19.567 (2CH_3), 33.378 (CH), 99.699 (CH-furan), 146.494 (C-furan), 106.453 (CH-furan), 147.037 (C-furan), 127.561 (C-aromatic), 127.228 (CH-aromatic), 127.561 (C-NO_2-aromatic). CHN Microanalysis: calculated: C, 62.72; H, 5.49; N, 6.36; Found: C, 62.70; H, 5.50; N, 6.34.

Diethyl-2,6-dimethyl-4-(5'-(5''-chloro-2′′-nitrophenyl)furan-2'-yl)-1,4-dihydropyridine-3-carboxylate (9)

Yield: 59%, M.P. =135°C, IR (υ-cm^{-1}): 3745.5 (N-H stretching), 3353.1 (Ar-H), 2985.4 (C-H stretching of CH_3), 1651.4 (C=O ester), 1476.3, 1363.8 (NO_2). Mass: m/z (%): 476.1 (M+2) 16.9%, 474.1 (M) 57.5%, 457.1 (M-CH_3) 48.0%, 444.1 (M-2CH_3) 8.2%, 429.1 (M-NO_2) 26.6%, 401.1 (M-$COOC_2H_5$) 100%, 373.1 (M-$COOC_2H_5$-2CH_3) 11.2%, 355.1 (M-$COOC_2H5$-NO_2) 26.1%, 327.1 (M-2$COOC_2H_5$) 9.7%, 283.1 (M-2$COOC_2H_5$-NO_2) 16.2%, 252.1 (M-$C_{10}H_5ClNO_3$) 48.9%, 224.1 (M-$C_{13}H_{18}NO_4$) 12.0%, 150.1 (M-$C_{10}H_5ClNO_3$-$COOC_2H_5$-2CH_3) 19.0%. ^{1}H-NMR ($CDCl_3$) δ: 2.355(s, 6H, 2CH_3),

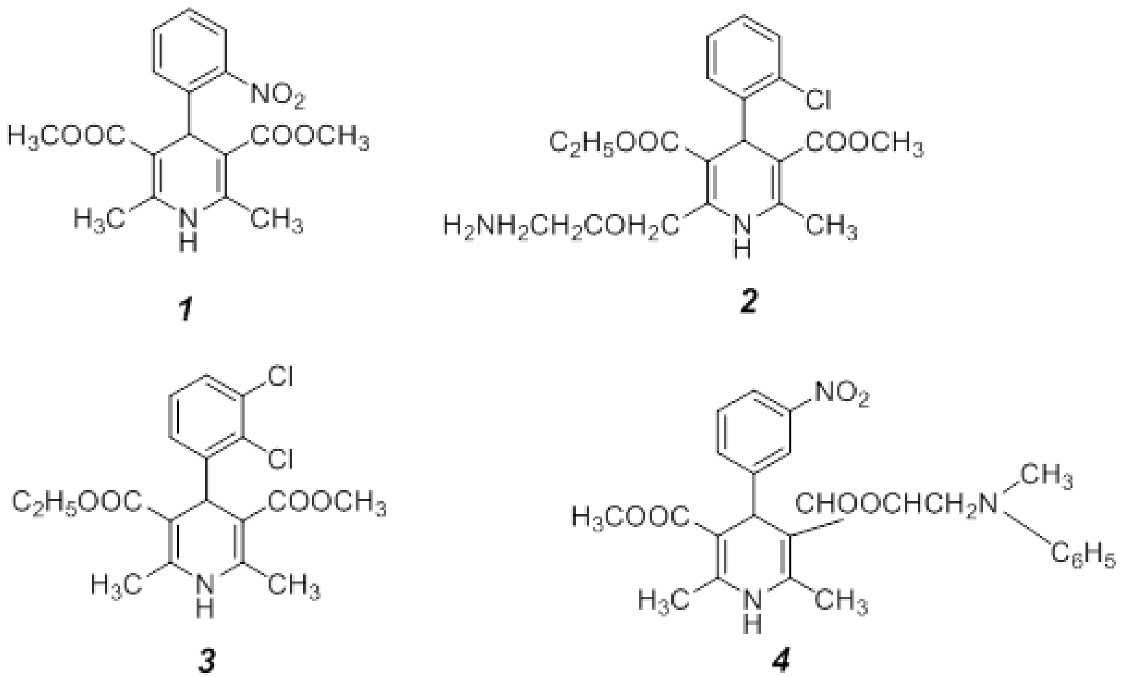


Fig. 1: Some commercially available dihydropyridines for treatment of Hypertensive

1.267(t, 6H, 2$\underline{CH}_3$CH$_2$, *J*=10.68Hz), 4.179(q, 4H, 2CH$_3\underline{CH_2}$, *J*=9.66Hz), 5.140(s,1H,CH), 6.127(d, 1H, H-3″, *J*=2.45Hz), 6.576(s, 1H, H-4″), 7.502(s, 1H, H-6″) ; ^{13}C-NMR (CDCl$_3$) δ: 14.34 ($\underline{C}$H$_3$CH$_2$), 59.83 (CH$_3\underline{C}$H$_2$), 167.216 (2C=O), 99.672(2C), 146.958 (2C), 19.611 (2CH$_3$), 33.499 (2CH), 99.672 (CH-furan), 145.481 (C-furan), 106.675 (CH-furan), 132.567 (C-aromatic), 131.537 (CH-aromatic), 128.529 (CH-aromatic), 123.779 (CH-aromatic), 146.107 (C-NO$_2$-aromatic). Micro analysis: Calculated: C, 58.17; H, 4.88; Cl, 7.47; N, 5.90; Found: C, 58.17; H, 4.88; Cl, 7.47; N, 5.90.

Diethyl 4-(3′-nitrophenyl)-2,6-diphenyl-1,4-dihydropyridine-3,5- dicarboxylate (10)

Yield: 64 %, M.P. = 158-160°C, Mass: m/z (%): 499.1 (4.2) (M+1), 498.1, (13.3) (M), 469.0 (17.1) (M-CH$_3$), 425.1 (47.4) (M-COOC$_2$H$_5$), 397.1 (9.2) (M-COOC$_2$H$_5$-C$_2$H$_5$), 376.1 (100) (M-C$_6$H$_4$NO$_2$), 348.1(17.3) (M-COOC$_2$H$_5$-C$_6$H$_5$), 320.1 (15.3) (M-C$_6$H$_4$NO$_2$-C$_2$H$_5$), 302.0 (8.2) (M-C$_6$H$_4$NO$_2$-C$_6$H$_5$),274.0 (5.9) (M-2COOC$_2$H$_5$-C$_6$H$_5$), 230.1 (8.4) (M-2COOC$_2$H$_5$-C$_6$H$_5$-NO$_2$), 203.1 (7.0) (M-2COOC$_2$H$_5$-2C$_6$H$_5$), 77.0 (8.0) (C$_6$H$_5$). ^{1}H-NMR (CDCl$_3$) δ: 0.911(s, 6H, 2CH$_3$CH$_2$, *J*=10.80Hz), 3.909(q, 4H, 2CH$_3$CH$_2$, *J*=7.82Hz), 5.333(s, 1H, CH), 7.366(t, 2H, H-3', 3″,*J*=1.02Hz), 7.386(t, 4H, H-3′, 5', 3″ , 5″, *J*=5.28Hz), 7.397(d, 4H, H-2′, 6', 6" , 2", *J*=3.15Hz), 7.412(t, 1H, H-5''' *J*=2.25Hz (Nitro phenyl part)), 7.420(s, 1H, H-2''', (Nitro phenyl part)), 8.427(s, 1H, H-4''', (Nitro phenyl part)). ^{13}C-NMR (CDCl$_3$) δ: 13.669 (2CH$_3$), 60.093 (2CH$_2$), 166.492 (2C=O), 103.342 (2C), 40.081 (CH), 146.498 (2C), 134.370 (2C, aromatic), 128.512 (4CH, aromatic), 129.132 (4CH, aromatic), 128.114 (2CH, aromatic), 148.553 (C, aromatic), 136.136 (CH, aromatic), 129.576 (CH, aromatic), 121.734 (CH, aromatic), 149.356 (C-N, aromatic), 122.760 (CH, aromatic). CHN Microanalysis: Calculated: C, 62.72; H, 5.49; N, 6.36; Found: C, 62.70; H, 5.46; N, 6.37.

Diethyl 4-(5′- (4″-bromophenyl)furan-2′-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate (11)

Yield: 28 %, M.P. = 134°C, Mass: m/z (%): 599.9 (M+2), 598 (M+1), 582.1 (10.08) (M-CH$_3$), 506.0 (5.16) (M-CH$_3$-C$_6$H$_5$), 431.0 (4.92) (M-CH$_3$-2C$_6$H$_5$), 396.1 (6.63) (M-Br-C$_2$H$_5$O-C$_6$H$_5$), 352.1 (11.74) (M-CH$_3$-C$_6$H$_5$-C$_6$H$_4$Br), 322.1 (6.81) (M-CH$_3$-C$_6$H$_5$-C$_6$H$_4$Br-C$_2$H$_5$), 215.1 (13.56) (M-C$_{10}$H$_6$BrO-CH$_3$-C$_3$H$_5$O$_2$- C$_6$H$_5$), 185.0 (24.64) (M-C$_{10}$H$_6$BrO-CH$_3$-C$_3$H$_5$O$_2$-C$_6$H$_5$-CH$_3$O), 139.1 (8.46) (M-CH$_3$-C$_{10}$H$_6$BrO-2C$_6$H$_5$- C$_3$H$_5$O$_2$), 105.1 (100) (M-C$_{10}$H$_6$BrO-2C$_6$H$_5$- C$_3$H$_5$O$_2$-C$_2$H$_5$O), 79.9 (7.46) (Br) , 77.1 (46.43) (C$_6$H$_5$), ^{1}H-NMR (CDCl$_3$) δ: 1.147 (t, 6H, 2CH$_3$CH$_2$COO, *J*=14.1Hz), 4.184 (q, 4H, 2CH$_3$CH$_2$COO, *J*=13.8Hz), 3.313 (s, 1H, C-4), 7.495 (d, 2H, H-2 ′,6, *J*=8.4Hz), 7.556 (d, 2H, H-3 ′, 5, *J*=7.2Hz), The phenyl groups of two amide linkages present at 3- and 5-position show following values: 7.495(d, 4H, H-1, H-6, of both phenyl groups, *J*=8.4Hz), 7.164 (t, 4H, H-3, 5, of both phenyl groups, *J*=3.3Hz), 7.119 (t, 2H, H-4, of both phenyl groups, *J*=5.1Hz), ^{13}C-NMR (CDCl$_3$) δ: 14.101 (2CH$_3$), 61.509 (2CH$_2$), 30.951 (CH), 165.016 (2C=O), 148.779 (2C), 136.408 (2CH-aromatic), 128.735 (4CH-aromatic), 126.920 (4CH-aroamtic), 156.462 (2C), 108.198 (CH-furan), 129.231 (3CH-aromatic), 133.715 (2CH-aromatic), 122.570 (CBr). CHN Microanalysis: Calculated: C, 58.24; H, 5.10; N, 2.95; Found: C, 58.20; H, 5.12; N, 2.92.

Table 1: Acetylcholinesterase and Butyrylcholinesterase activity of 6-9

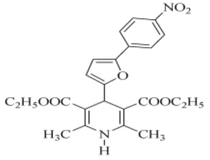
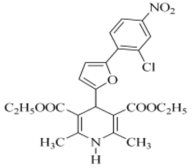
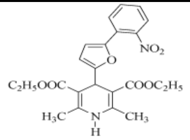
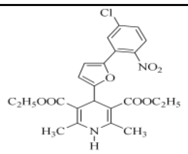
Compounds	Structure	Inhibition (%) at 0.5 mM for AChE	IC_{50} (µM)	Inhibition (%) at 0.5 mM for BChE	IC_{50} (µM)
6		75.89±0.23	62.81±0.12	29.44±0.27	-
7		87.15±0.17	15.91±0.05	83.45±0.13	17.16±0.02
8		81.45±0.16	22.62±0.07	77.39±0.11	29.93±0.06
9		85.09±0.12	11.93±0.05	76.59±0.15	46.27±0.08
Standard	Eserine	82.82±1.09	0.85±0.0001	91.29±1.17	0.04±0.0001

Table 2: Acetylcholinesterase and Butyrylcholinesterase activity of 10-13

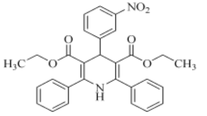
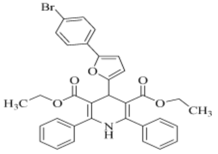
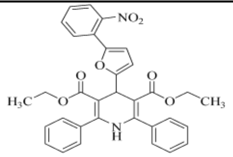
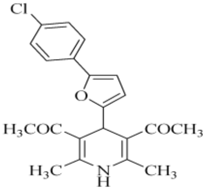
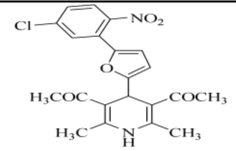
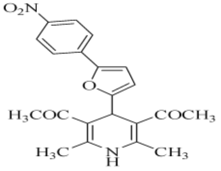
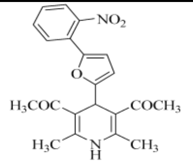
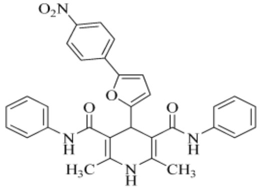
Compounds	Structure	Inhibition (%) at 0.5 mM for AChE	IC_{50} (µM)	Inhibition (%) at 0.5 mM for BChE	IC_{50} (µM)
10		75.29±0.23	47.52±0.18	30.37±0.18	-
11		95.35±0.11	0.21±0.003	75.35±0.25	74.18±0.17
12		81.25±0.15	29.87±0.09	19.32±0.29	-
13		72.56±0.24	76.14±0.16	48.07±0.14	-
Standard	Eserine	82.82±1.09	0.85±0.0001	91.29±1.17	0.04±0.0001

Table 3: Acetylcholinesterase and Butyrylcholinesterase activity of 14-17

Compounds	Structure	Inhibition (%) at 0.5 mM for AChE	IC_{50} (µM)	Inhibition (%) at 0.5 mM for BChE	IC_{50} (µM)
14		77.87±0.16	43.26±0.02	47.91±0.13	-
15		62.77±0.36	132.93±0.25	27.63±0.11	-
16		77.34±0.11	33.27±0.04	56.25±0.21	231.6±0.12
17		63.84±0.37	147.14±0.12	34.21±0.19	-
Standard	Eserine	82.82±1.09	0.85±0.0001	91.29±1.17	0.04±0.0001

Diethyl 4-(5′-(2″-nitrophenyl)furan-2′-yl)-2,6-diphenyl-1,4-dihydropyridine-3,5-dicarboxylate (12)

Yield: 60 %, M.P. = 102°C, Mass: m/z (%): 564.2 (2.2) (M), 532.2 (4.92) (M-C_2H_5), 519.2 (1.2) (M-NO_2), 491.2 (11.37) (M-$COOC_2H_5$), 445.2 (1.2) (M-$COOC_2H_5$-NO_2), 429.2 (3.1) (M-C_6H_5-NO_2-CH_3), 391.1 (1.1) (M-C_6H_5-NO_2-CH_3-CH_2O), 360.2 (8.27) (M-$C_{10}H_6NO_3$-CH_3), 346.1 (5.41) (M-$C_{10}H_6NO_3$-2CH_3), 302.1 (4.27) (M-$C_{10}H_6NO_3$- $C_3H_5O_2$), 258.1 (14.13) (M-$C_{10}H_6NO_3$-$C_3H_5O_2$-C_2H_5O),229.1 (13.15) (M-$C_{10}H_6NO_3$-$C_3H_5O_2$-C_6H_5), 201.1 (5.46) (M-$C_{10}H_6NO_3$- $C_3H_5O_2$- C_6H_5-C_2H_5), 188.1 (5.46) (M- $C_{23}H_{22}NO_4$), 106.0 (7.49) (M-$C_{10}H_6NO_3$-2C_6H_5- $C_3H_5O_2$), 105.0 (100)(M- $C_{10}H_6NO_3$-2C_6H_5- $C_3H_5O_2$- C_2H_5O), 77.0 (74.26) (C_6H_5), ^{13}C-NMR($CDCl_3$) δ: 14.094 (2CH_3), 61.658 (2CH_2), 112.760 (2C), 127.753 (4CH),147.153 (2C), 133.827 (3CH-aromatic), 136.290 (3CH), 150.923 (C), 128.948 (10CH), 164.791(2C=O). CHN Microanalysis: Calculated: C, 70.20; H, 5.00; N, 4.96; Found: C, 70.22; H, 5.00; N, 4.95.

3,5-diacetyl-4-(5′-(4″-chlorophenyl) furan-2'-yl)-2,6-dimethyl-1,4-dihydropyridine (13)

Yield: 56 %, M. P. = 140-142°C, Mass: m/z (%): 371.0 (1.2) (M+2), 369.0 (11.1) (M^+), 326.0 (26.8) (M-$COCH_3$), 288.0 (100) (M-C_5H_6O), 273.0 (58.3) (M-C_5H_6O-CH_3), 253.0 (17.2) (M-C_5H_6O-Cl), 231.0 (93.4) (M-C_5H_6O-$COCH_3$-CH_3), 192.1 (7.3) (M-$C_{10}H_6ClO$), 177.0 (9.6) (M-$C_{11}H_{14}NO_2$), 149.0 (22.2) (M-$C_{10}H_6ClO$-$COCH_3$), 139.0 (30.7) (M-$C_{10}H_6ClO$-$COCH_3$-CH_3), 111.0 (10.1) (M-$C_{15}H_{16}NO_3$),97.1 (6.5) (M-$C_{10}H_6ClO$-2$COCH_3$-CH_3), 83.1 (6.8) (M-$C_{10}H_6ClO$-2$COCH_3$-2CH_3), 57.1 (10.8)(M-$C_{10}H_6ClO$-C_5H_7O-$COCH_3$-CH_3), 43.0 (44) (M-$C_{19}H_{17}ClNO_2$).^{13}C-NMR ($CDCl_3$) δ: 20.39(2CH_3), 26.12(2CH_3CO), 29.56(CH), 148.36(2CH_3-CNH), 108.60(2C-CO-CH_3), 157.10(2C-furan, C-2', C-5'), 106.11(C-3′), 107.41(C-4′), 128.81 (3C-aromatic, -1″, 2″, 6″), 129.31(2C-aromatic, C-3″, 5″), 134.89(C-Cl, aromatic, C-4″). CHN Microanalysis: Calculated: C, 68.20; H, 5.45; N, 3.79; Found: C, 68.18; H, 5.44; N, 3.76.

3,5-diacetyle-4-(5'-(5''-chloro-2''-nitrophenyl)furan-2'-yl)-2, 6-dimethyl-1,4-dihydro-pyridine (14)

Yield = 43%, M.P = 152°C, Mass: m/z (%): 416.0 (M+2), 414.0 (6.7) (M^+), 397.0 (67.9) (M-CH_3), 371.0 (70.5) (M-$COCH_3$), 356.9 (6.2) (M-$COCH_3$-CH_3), 339.1 (3.6) (M-$COCH_3$-Cl), 324.0 (7.0) (M-$COCH_3$-NO_2), 224.0 (9.0) (M-$C_6H_3ClNO_3$-CH_3), 228.0 (54.1) (M-$C_6H_3ClNO_3$-2CH_3), 218.1 (17.4) (M-$C_6H_3ClNO_3$-$COCH_3$), 204.1 (17.4) (M-$C_6H_3ClNO_3$-$COCH_3$-CH_3), 192.1 (33.6) (M-$C_{10}H_5ClNO_3$), 176.1 (13.6) (M-$C_{10}H_5NO_3$-CH_3), 165.0 (10.5) (M-$C_{10}H_5NO_3$-2CH_3), 149.1 (12.8) (M-$C_{10}H_5NO_3$-$COCH_3$), 137.0 (13.5) (M-$C_{10}H_5NO_3$-$COCH_3$-CH_3), 106.1

(6.8) (M-$C_{10}H_5NO_3$-$2COCH_3$), ^{13}C-NMR: (75MHz) (DMSO) δ: 20.551 ($2CH_3$), 26.141 ($2CH_3$), 196.720 (2C=O), 113.238 (2C), 149.772 (2C), 29.885 (CH), 149.772 (C-Furan), 107.182 (CH-Furan), 109.713 (CH-Furan), 145.143 (C-Furan), 132.749 (C-aromatic), 218.210 (CH-aromatic), 139.427 (C-Cl), 130.423 (CH), 123.872 (CH), 145.143 (C-N). CHN Microanalysis: Calculated: C, 60.80; H, 4.62;; N, 6.75; Found: C, 60.81; H, 4.62; N, 6.73.

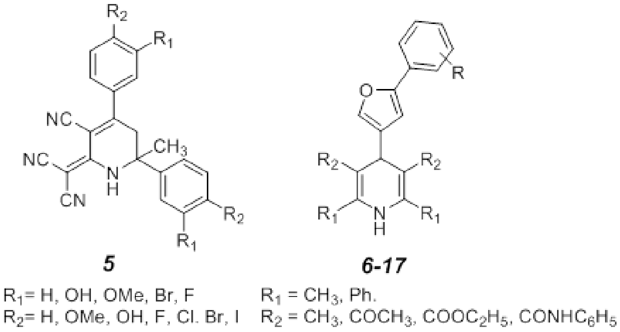
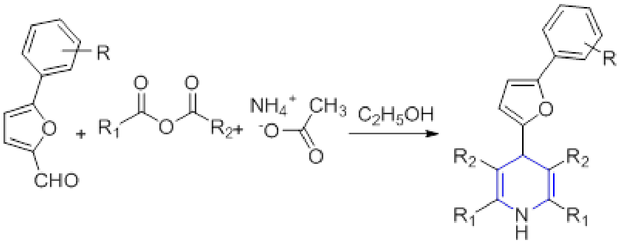


Fig. 2: Antioxidant and acetyl- and butyrylcholin esterase active dihydropyridines



Schemes 1: Synthesis of compounds (6-17)

3, 5-diacetyl-4-(5'-(4''-nitrophenyl)furan-2'-yl)-2, 6-dimethyl-1,4-dihydropyridine (15)

Yield: 39%, M.P. = 222°C, Mass: m/z (%): 380.2 (17.6) (M), 350.2 (24.81) (M-$2CH_3$), 337.1 (52.91) (M-$COCH_3$), 333.2 (M-NO_2), 307.2 (41.06) (M-$COCH_3$-$2CH_3$), 291.1 (23.75) (M-$COCH_3$-NO_2), 263.1 (M-$COCH_3$-NO_2-$2CH_3$), 230.1 (51.90) (M-$C_6H_4NO_2$-$2CH_3$), 216.1 (M-$C_6H_4NO_4$-$COCH_3$), 192.1 (21.91) (M-$C_{10}H_6NO_3$), 176.1 (40.82) (M-$C_{10}H_6NO_3$-CH_3), 149.0 (20.36) (M-$C_{10}H_6NO_3$-$COCH_3$), 120.1 (44.90) (M-$C_{10}H_6NO_3$-$COCH_3$-$2CH_3$), 106.1 (26.44) (M-$C_{10}H_6NO_3$-$2COCH_3$), 83.1 (28.75) (M-$C_{10}H_6NO_3$-$2COCH_3$-$2CH_3$), 77 (33.60) (C_6H_5).

3,5-diacetyl-4-(5'-(2''-nitrophenyl)furan-2'-yl)-2,6-dimethyl-1,4-dihydropyridine (16)

Yield = 62%, M.P = 155-158°C, Mass: m/z (%): 381.2 (M+1), 380.2 (7.86) (M), 364.2 (M-CH_3), 337.2 (100) (M-$COCH_3$), 321.2 (8.59) (M-$COCH_3$-CH_3), 290.1 (7.62) (M-$COCH_3$-NO_2), 276.1 (M-$COCH_3$-NO_2-CH_3), 262.1 (7.18) (M-$COCH_3$-NO_2-$2CH_3$), 228.1 (79.11) (M-$C_6H_4NO_2$-$2CH_3$), 192.1 (39.50) (M-$C_{10}H_6NO_3$), 176.1 (12.39) (M-$C_{10}H_6NO_3$-CH_3), 149.1 (14.48) (M-$C_{10}NO_3$-$COCH_3$), 105.0 (7.51) (M-$C_{10}NO_3$-$2COCH_3$), 77.0 (6.49) (M-$C_{15}H_{16}NO_3$-NO_2), 43.0 (21.71) (M-$C_{19}H_{17}N_2O_4$) ^{13}C-NMR: (75MHz) (DMSO) δ: 20.529 ($2CH_3$), 147.168 (2C), 110.146 (2C), 197.003 (2C=O), 29.927 ($2CH_3$), 147.301 (2C), 107.037 (2CH), 131.621 (2C), 127.913 (2CH), 131.621 (CH), 123.666 (CH), 145.457 (C-N). CHN Microanalysis: Calculated: C, 66.31; H, 5.30; N, 7.36; Found: C, 66.30; H, 5.30; N, 7.32.

2,6-dimethyl-4-(5'-(4''-nitrophenyl)furan-2'-yl)-N3, N5-diphenyl-1,4-dihydropyridine-4-3, 5-dicarboxamide (17)

M.P. =210°C, Yield =55%, ^{1}H-NMR: (300MHZ) (DMSO) δ: 2.107 (s, 6H, $2CH_3$), 5.339 (s, 1H, H-4, CH), 8.013 (s, 2H, 2NH-amide), 9.566 (s, 1H, NH-dihydropridine), 5.339 (s, 1H, H-3'), 6.266 (d, 1H, H-4', *J*=3.3Hz), 7.782 (d, 2H, H-2'', 6'', *J*= 6.2Hz), 8.132 (d, 2H, H-3'', 5'', *J*=5.43Hz), 7.631 (d, 4H, H-2, 6 (3-amide), H-2, 6 (5-amide), *J*=6.2Hz), 7.232 (t, 4H, H-3, 5 (3-amide), H-3, 5 (5-amide), *J*=14.40Hz), 7.057 (t, 2H, H-4(3-amide), H-4(5-amide), *J*=3.0Hz). ^{13}CNMR: (75MHz) (DMSO) δ: 17.473 (2C, $2CH_3$), 26.237 (CH, C-4), 150.476 (2C, C-2, 6), 102.356 (3C, C-3, 5), 165.282 (2C=O, amide), 150.476 (C-2'), 107.775 (C-4'), 153.925 (C-5'), 146.474, 121.505, 128.526, 134.442 (C-6'', 1''), The phenyl group of both the amide groups show peaks at following positions: 134.376 (C-1), 122.809 (2C, C-2, C-6), 129.034 (C-3, 5) 124.284 (C-4). CHN Microanalysis: Calculated: C, 69.65; H, 4.90; N, 10.48; Found: C, 69.65; H, 4.90; N, 10.45.

Biochemical assays

Acetylcholinesterase and butyrylcholinesterase activity

Acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8) consist of an enzymes family, which includes serine hydrolases. The diverse specificities for the substrates and inhibitors for these enzymes are due to the dissimilarity in amino acid remains of the active sites of AChE and BChE. Actually the system of enzyme is responsible for the termination of acetylcholine at cholinergic synapses (Elumalai *et al.*, 2015). These are key components of cholinergic brain synapses and neuromuscular junctions. The central function of AChE and BChE is to catalyze the hydrolysis of the neurotransmitter acetylcholine and termination of the nerve impulse in cholinergic synapses (Mohsen 2012). It has been found that BChE is present in appreciably higher quantity in Alzheimer's plaques than in the normal age linked dementia of brains. H-1 and H-2 receptor antagonists possess AChE inhibitory activities (Cygler *et al.*, 1993).

Cholinesterase inhibitors raise the quantity of acetylcholine available for neuronal and neuromuscular transmission through their ability to reversibly or irreversibly. Hence, the search for new cholinesterase inhibitors is important strategy to introduce new drug candidates for the treatment of Alzheimer’s disease and other related diseases (Gauthier 2001).

Acetylcholinesterase assay

The activity of AChE inhibition was carried out according to the process 15 with slight alteration. Total volume of the reaction mixture was 100µL. It contained 60 µL Na_2HPO_4 buffer with concentration of 50mM and pH 7.7 (Siddiqui *et al*. 2013). Ten µL test compound (0.5mM well^{-1}) was added, followed by the addition of 10µL (0.005 unit well^{-1}) enzyme. The contents were mixed and pre-incubated for 10 min at 37°C. The reaction was initiated by the addition of 10µL of 0.5mM well^{-1} substrate (acetylthiocholine iodide), followed by the addition of 10µL DTNB (0.5mM well^{-1}). After 15 min of incubation at 37°C, absorbance was measured at 405 nm using 96-well plate reader Synergy HT, Biotek, USA. All experiments were carried out with their respective controls in triplicate. Eserine (0.5mM well^{-1}) was used as a positive control.

Butyrylcholinesterase assay

The BChE inhibition activity was performed according to the method (Zahra *et al*., 2013) with slight modifications. Total volume of the reaction mixture was 100 µL containing 60 µL, Na_2HPO_4 buffer, 50 mM and pH 7.7. Ten µL test compound 0.5mM well^{-1} was added followed by the addition of 10µL (0.5 unit well^{-1}) BChE (Sigma Inc.). The contents were mixed and pre-read at 405 nm and then pre-incubated for 10min at 37°C. The reaction was initiated by the addition of 10µL of 0.5mM well^{-1} substrate (butyrylthiocholine chloride). Followed by the addition of 10µL DTNB, 0.5mM well^{-1}. After 15 min of incubation at 37°C, absorbance was measured at 405 nm using 96-well plate reader Synergy HT, Biotek, USA. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM well^{-1}) was used as positive control.

STATISTICAL ANALYSIS

IC_{50} values were calculated using EZ–Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA). The percent inhibition was calculated by the help of following equation.

$$\text{Percentage Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

RESULTS

In the present work, various dihydropyridines (6-17) were prepared via MCR (multicomponent reaction) method and characterized by different spectroscopic techniques like NMR, FT-IR, EI-MS and CHN. These synthesized compounds were evaluated for *in-vitro* cholinesterases (AChE, BChE) inhibitory activity. The results are given below in the form of tables 1-3.

DISCUSSION

A library of dihydropyridines (6-17) was synthesized by reacting ethyl acetoacetate with different aryl furan aldehydes in the presence of ammonium acetate in ethanol under reflux for 3h. The mixture was cooled at room temperature and poured on to cold water for precipitation. The pure compounds were collected after filtration.

General structure is shown in Scheme 1 and confirmed from spectral techniques, showing NH absorption peak approximately at 3700-3800 cm^{-1} and C=O at around 1600-1700 cm^{-1} in FTIR. Similarly, NH at 8-9 ppm was confirmed by proton NMR and C=O at 163-168 ppm was confirmed by ^{13}C-NMR. Structure of these compounds were also confirmed from molecular ion peak and fragmentation pattern in EIMS. Percentage of each element (C, H, and N) was confirmed from micro-elemental analysis (CHN).

Acetylcholinesterase and Butyrylcholinesterase activity of Dihydropyridines

All synthetic dihydropyridines (6-17) were evaluated for in vitro acetylcholinesterase and butyrylcholinesterase inhibitory activity. All compounds showed potent activity with IC_{50} values between 0.21±0.003 to 147.14±0.12 µM for AChE and among them five compounds showed potent activity with IC_{50} values 17.16±0.02 to 231.6±0.12 µM for BChE when compared with standard Eserine (IC_{50} = 0.85±0.0001 µM (AChE) & 0.04±0.0001 µM (BChE). The most potent compound (11) can be considered as potential lead compound showed an inhibition of 95.35±0.11 and IC_{50}= 0.21±0.003 lower than the standard (Eserine). While compounds (7), (12) and (13) showed comparable acetylcholinsterase activity having MIC_{50} values 23.45±0.06, 29.87±0.09 and 15.91±0.05 respectively. Structure-activity relationships (SAR) studies of this series showed that the presence of nitro group at C-2 and C-4 position enhances the acetylcholinesterase and butyrylcholinesterase activities, while presence of –Br at 4-position and -Cl at position 2, 4 & 5 also have positive effects. The data is given in tables 1-3.

CONCLUSION

The compounds (6-17) were synthesized and tested via available spectroscopic techniques. The synthesized compounds were tested for Acetylcholinesterase and Butyrylcholinesterase Activity and majority of compounds were found very active. Thus, it is concluded that these compounds (7, 11, 12 & 13) can be used important pharmacological drugs after testing toxicity.

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