ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 1, 2015

ISSN - 0974-2441

Research Article

SYNTHESIS AND ACETYLCHOLINESTERASE/BUTYRYLCHOLINESTERASE INHIBITION ACTIVITY OF ARECOLINE-, 4-THIAZOLIDINONE- AND PIPERIDINE- BASED CONJUGATES

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Received: 14 August 2014, Revised and Accepted: 13 September 2014

ABSTRACT

Objective: The aim of the study is to design, synthesize and identification of novel acetylcholinesterase/butyrylcholinesterase (AChE/BuChE) inhibitors.

Methods: The AChE and BuChE inhibition activity of a library of piperidine and 4-thiazolidinone substituted arecoline derivatives are described. The chemical structures of newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis.

Results: The cholinesterase inhibition assays indicated that few of the synthesized compounds exhibited considerable activity at micromolar range for AChE over BuChE. Compound 7c exhibited the most potent AChE inhibitory activity with an IC_{50} value of 6.62 µM for AChE and 13.78 µM for BuChE, which is comparable to the standard Neostigmine with an IC_{50} 2.05 µM for AChE and 3.64 µM for BuChE respectively.

Conclusion: Our results clearly demonstrate that arecoline-4-thiazolidinone derivatives open up a new avenue in the field of Alzheimer's disease.

Keywords: Arecoline analogs, Propylphosphonic anhydride (T3P®), Acetylcholinesterase/butyrylcholinesterase inhibitor, 4-thiazolidinones.

INTRODUCTION

Alzheimer's disease (AD) is one of the most predominant irreversible neurodegenerative diseases associated with age advancement that is characterized by progressive memory loss due to neuronal deterioration and cognitive dysfunction [1]. AD develops gradually and may remain masked for several years without a diagnosis. Dysfunction of the cholinergic system, aggregation of amyloid beta proteins, hyperphosphorylation of tau proteins are the major causes of AD [2]. In humans, the pivotal signaling molecule of cholinergic transmission is acetylcholine (ACh), which is one of the prime neurotransmitters with the dual role of excitatory and inhibitory neurotransmission [3]. It majorly operates at neuromuscular junction, central nervous system and synapses in the ganglia [4]. Levels of ACh at the cholinergic synapse are regulated by cholinesterases that include acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) [5]. AChE rapidly degrades the ACh into acetate and choline, thus terminates the nerve impulse transmission [6].

According to the cholinergic hypothesis, reduction of ACh is a responsible for the development of AD [7]. Inhibition of AChE could be a better strategy in improving the cognitive capacity of the AD patient [8]. The role of BuChE is not very clear but thought to be involved in the hydrolysis of ACh. In the normal conditions, AChE levels in the brain are higher than BuChE. On the contrary, AD Patients suffers from a substantial decline in the concentration of AChE and BuChE concentration remains same or may increase progressively. Furthermore, in the last few decades, cholinesterase inhibitors are the extensively used medications to prevent the advancement of AD [9]. Synthetic compounds like donepezil, tacrine, rivastigmine and galanthamine (Fig. 1), a natural alkaloid are the US-Food and Drug Administration approved cholinesterase inhibitors used to treat AD patients [10-12]. Therefore, targeting of cholinesterases is considered to be very effective in designing and formulating the therapeutic agent to break the progression of AD.

Arecoline is a natural alkaloid present in the arecanut, and it is known for its effective cognition skills enhancing effects in patients with AD and so arecoline derivatives have been considered as prime anti-AD compounds [13-15]. From past few years, our research group has been involved in designing and optimizing arecoline derivatives as cholinesterase inhibitors [16-17]. In continuation of our research work [18] in the field of medicinal chemistry for developing more potent and selective AChE/BuChE inhibitors, we have focused our attention to new class of arecoline analogues, by incorporating an extra carbon (-CH_2-) atom between 4-thiazolidinone ring and arecoline nucleus. Synthesis of homologated 4-thiazolidinone substituted arecoline derivatives has been achieved for the first time as shown in the Scheme 1.

METHODS

Reactions were monitored by thin layer chromatography (TLC) using precoated sheets of silica gel G/UV-254 of 0.25 mm thickness (Merck 60F254) using UV light for visualization. ^{1}H and^{13}C NMR spectra were recorded on an NMR spectrometer operating at 400 and 100 MHz, respectively, using the residual solvent peak as a reference relative to $SiMe_4$. Mass spectra were recorded using high resolution mass spectrometer (HRMS). Infrared spectra were recorded on Shimadzu FT-IR model 8300 spectrophotometer.

Chemicals used: All chemicals were obtained from Sigma-Aldrich, Fluka and Merck Chemicals.

Synthesis

General procedure for the synthesis of tert-butyl 4-(3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)-4-oxothiazolidin-2-yl)piperidine-1-carboxylate (5)

To an equimolar 3-picolyl amine (1), N-Boc piperdine 4-carbaldehyde (2) and thioglycolic acid (3) in ethyl acetate, T3P® (1.2 equiv) was added at 0°C and stirred the reaction mixture at room temperature for

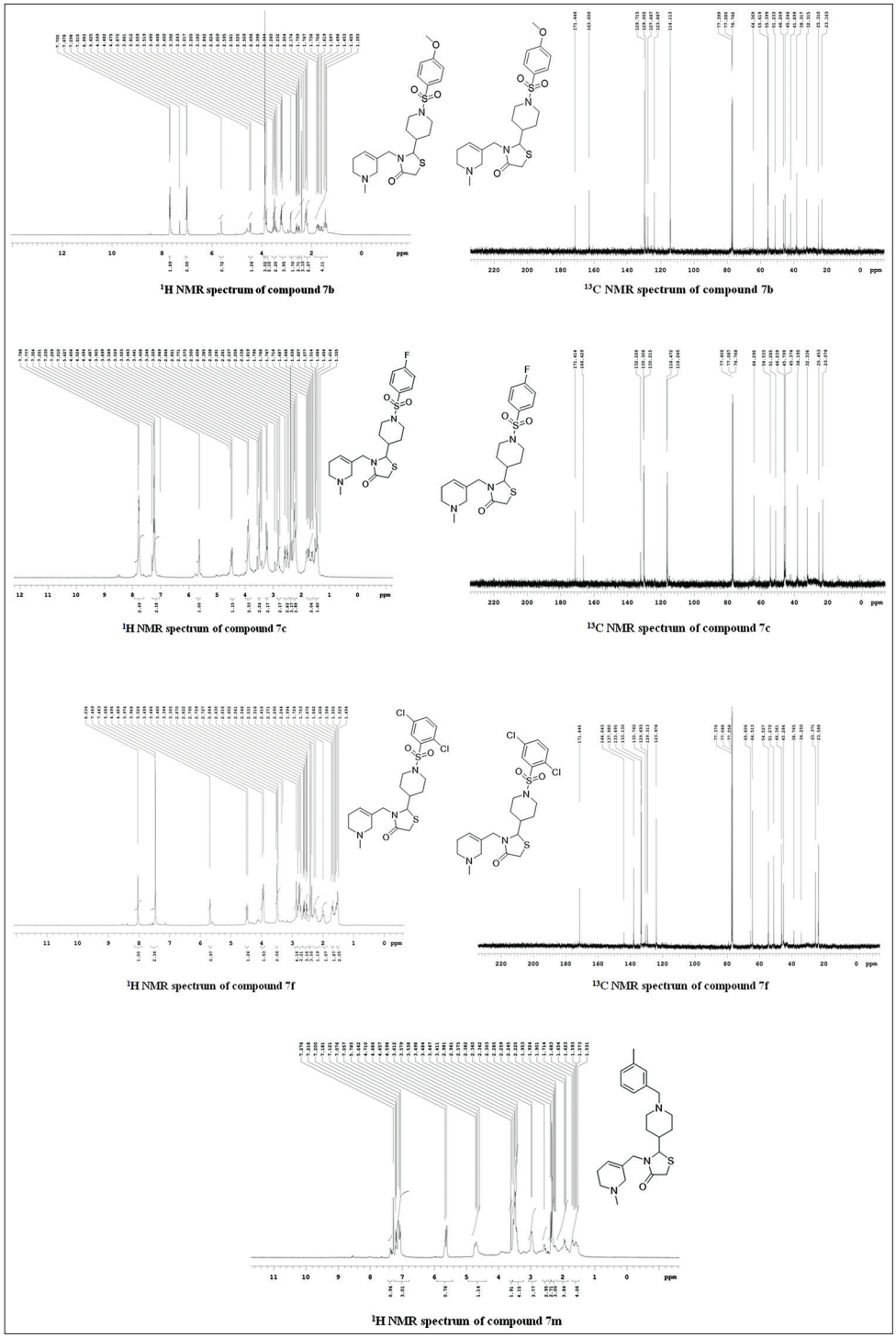
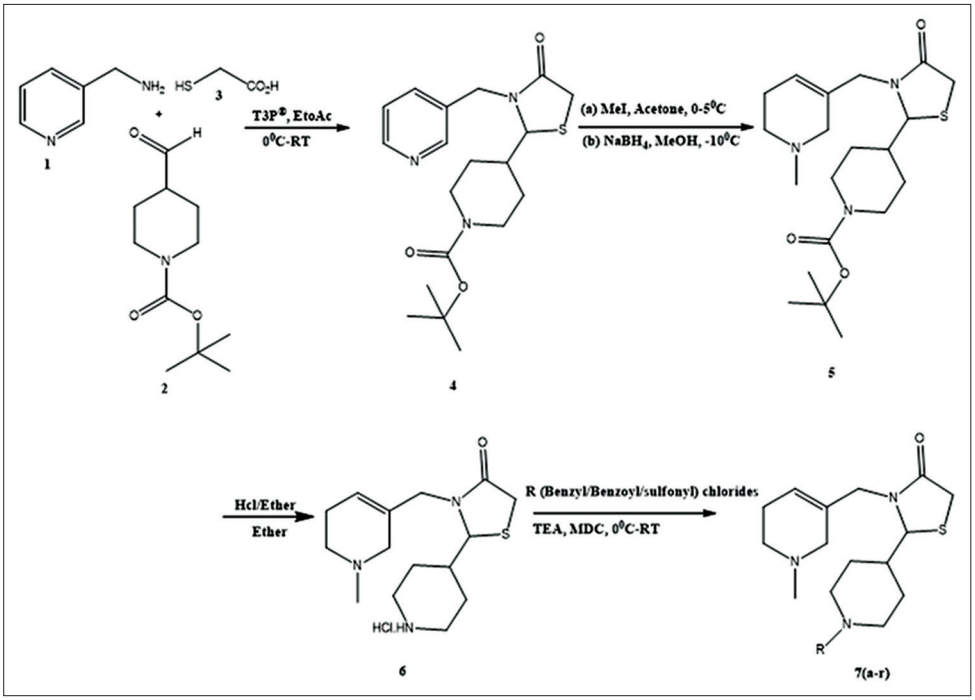


Fig. 1: Representational^{1}H NMR and^{13}C NMR

3 hrs [19]. The reaction was monitored by TLC. After the completion of the reaction, the mixture was diluted with water and neutralized by adding 10% $NaHCO_3$ solution. The product was extracted with ethyl acetate (twice) and the combined organic layers were washed with water followed by brine solution. The organic phase was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to afford the product (4) which was pure enough to do the next step. Compound (4) on stirring for 3 hrs with methyl iodide (2 equiv) in dry acetone at 0°C followed by reduction using sodium borohydride (1.2 equiv) in methanol at −10°C afforded compound (5).

General procedure for the synthesis of compounds 7(a-r)

The key intermediate (6) was synthesized by deprotection of compound (5) using HCl/ether in ether at 0°C. To a stirred solution of compound (6) in dichloromethane, triethyl amine (3 equiv) was added and cooled to 0-5°C, and then benzyl/benzoyl/benzene sulfonyl chloride (1.0 equiv) was added dropwise. The reaction mixture was stirred for 4-5 hrs at room temperature, the reaction was monitored by TLC. After completion of the reaction, the mixture was washed with water followed by brine solution. The organic layer was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to afford the title compounds 7(a-r) in good yields.



Scheme 1: Synthesis of arecoline 4-thiazolidinone derivatives. R: 7a=$C_6H_4SO_2$, 7b=4-OCH_3-C_6H_4-SO_2, 7c=4-F-C_6H_4-SO_2, 7d=4-NO_2-C_6H_4-SO_2, 7e=2,4,6-3CH_3-C_6H_2-SO_2, 7f=2,5-2Cl-C_6H_3-SO_2, 7g=3,4,5-3OCH_3-$C_6H_2SO_2$, 7h=3-CH_3-C_6H_4-CH_2, 7i=3-CF_3-C_6H_4-SO_2, 7j=3,4-2Cl-C_6H_3-CH_2, 7k=3-NO_2-C_6H_4-CH_2, 7l=3-F-C_6H_4-CO, 7m=2,6-2Cl-C_6H_3-CO, 7n=3-Br-C_6H_4-CO, 7o=4-Cl-C_6H_4-CO, 7p=2-OCH_3-C_6H_4-CO, 7q=3-NO_2-C_6H_4-CO, 7r=4-t-butyl-C_6H_4-CO

3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)-2-(1-(phenylsulfonyl)piperidin-4-yl)thiazolidin-4-one (7a)

Yellow gummy liquid Yield: 85% IR γmax (nujol) 3050, 1650, 1575, 1450, 1375, 1365, 1240, 1162/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 7.74-7.71 (m, 3H, ArH), 7.33-7.28 (m, 2H, ArH), 5.73 (m, 1H, arecoline-C-4H), 4.54-4.50 (d, *J*=4.2 Hz, 1H, thiazolidinone-CH), 3.85 (s, 2H, arecolinyl-CH_2), 3.76-3.74 (dd, *J* = 2.8 Hz, 2H, thiazolidinone-CH_2), 3.27-3.24 (m, 4H, piperidine-CH_2), 2.89 (s, 2H, arecoline-C-2H_2), 2.72-2.68 (m, 2H, arecoline-C-6H_2), 2.56-2.51 (m, 1H, piperidine-C-4H), 2.41 (s, 3H, N-CH_3), 2.25-2.20 (m, 2H, arecoline-C-5H_2), 1.78-1.68 (m, 2H, piperidine-CH_2), 1.65-1.52 (m, 2H, piperidine-CH_2).^{13}C NMR (100 MHz, $CDCl_3$); δ 174.6, 136.2, 135.7, 129.0, 125.6, 123.5, 121.1, 64.4, 52.8, 50.5, 46.0, 43.5, 42.6, 41.9, 38.2, 26.4, 23.0. HRMS (ESI) *m/z* Calcd for $C_{21}H_{29}N_3O_3S_2Na$ $[M+Na]^+$ 458.6033, found 458.6052.

2-(1-((4-methoxyphenyl)sulfonyl)piperidin-4-yl)-3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)thiazolidin-4-one (7b)

Pale yellow liquid Yield: 86% IR γmax (nujol) 3045, 1651, 1582, 1448, 1382, 1369, 1320, 1245, 1163/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 7.70-7.67 (d, *J* = 8.8 Hz, 2H, ArH), 7.01-6.99 (d, *J* = 8.0 Hz, 2H, ArH), 5.62 (m, 1H, arecoline-C-4 H), 4.48-4.47 (d, *J* = 3.8 Hz, 1H, thiazolidinone-CH), 3.87 (s, 3H, OCH_3), 3.81 (s, 2H, arecolinyl-CH_2), 3.519-3.512 (dd, *J* = 2.8 Hz, 2H, thiazolidinone-CH_2), 3.24-3.18 (m, 4H, piperidine-CH_2), 2.84 (s, 2H, arecoline-C-2H_2), 2.62-2.58 (m, 2H, arecoline-C-6H_2), 2.52-2.49 (m, 1H, piperidine-C-4H), 2.39 (s, 3H, N-CH_3), 2.34-1.79 (m, 2H, arecoline-C-5H_2), 1.77-1.39 (m, 4H, piperidine-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$); δ 171.4, 163.0, 129.7, 129.0, 127.6, 123.8, 114.1, 64.3, 55.6, 55.3, 51.2, 46.2, 45.2, 41.8, 38.3, 32.3, 25.3, 23.1. HRMS (ESI) *m/z* Calcd for $C_{22}H_{31}N_3O_4S_2Na$ $[M+Na]^+$ 465.6292, found 465.6301.

2-(1-((4-fluorophenyl)sulfonyl)piperidin-4-yl)-3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)thiazolidin-4-one (7c)

Pale brown liquid yield: 78% IR γmax (nujol) 3043, 1648, 1588, 1448, 1385, 1371, 1245, 1163, 1090/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 7.79-7.77 (d, *J* = 8.0 Hz, 2H, ArH), 7.30-720 (m, 2H, ArH), 5.62 (m, 1H, arecoline C-4H), 4.49-4.48 (d, *J* = 2.8 Hz, 1H, thiazolidinone-CH), 3.86 (s, 2H, arecolinyl-CH_2), 3.80-3.79 (dd, *J* = 2.4 Hz, 2H, thiazolidinone-CH_2), 3.24-3.20 (m, 2H, piperidine-CH_2), 2.94-2.77 (m, 2H, piperidine-CH_2), 2.57-2.45 (m, 3H, piperidine-C-4H and arecoline-C-2H_2), 2.38 (s, 3H, N-CH_3), 2.33-2.15 (m, 4H, arecoline-CH_2), 1.81-1.66 (m, 2H, piperidine-CH_2), 1.51-1.33 (m, 2H, piperidine-CH_2).^{13}C NMR (100 MHz, $CDCl_3$); δ 171.4, 166.4, 132.2, 130.3, 130.2, 116.4, 116.2, 64.2, 54.5, 51.2, 46.2, 45.7, 45.3, 38.1, 32.3, 25.4, 23.0. HRMS (ESI) *m/z* Calcd for $C_{21}H_{28}FN_3O_3S_2Na$ $[M+Na]^+$ 476.5937, found 476.5942.

3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)-2-(1-((4-nitrophenyl)sulfonyl) piperidin-4-yl)thiazolidin-4-one (7d)

Dark brown liquid yield: 76% IR γmax (nujol) 3040, 1648, 1587, 1535, 1449, 1385, 1370, 1360, 1242, 1165/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 8.12-8.10 (d, *J* = 8.0 Hz, 2H, ArH), 7.98-7.96 (d, *J* = 7.8 Hz, 2H, ArH), 5.64 (m, 1H, arecoline C-4H), 4.51-4.50 (d, *J* = 3.4 Hz, 1H, thiazolidinone-CH), 3.95 (s, 2H, arecolinyl-CH_2), 3.88-3.87 (dd, *J* = 2.4 Hz, 2H, thiazolidinone-CH_2), 3.12-2.91 (m, 4H, piperidine-CH_2), 2.82 (s, 2H, arecoline-C-2H_2), 2.52-2.49 (m, 2H, arecoline-C-6H_2), 2.45-2.36 (m, 1H, piperidine-C-4H), 2.31 (s, 3H, N-CH_3), 2.21-2.13 (m, 2H, arecoline-C-5H_2), 1.71-1.52 (m, 4H, piperidine CH_2). ^{13}C NMR (100 MHz, $CDCl_3$); δ 172.0, 148.2, 140.1, 138.2, 129.1, 124.1, 121.2, 62.5, 58.4, 53.9, 48.2, 45.9, 45.6, 36.2, 33.2, 25.5, 22.9. HRMS (ESI) *m/z* Calcd for $C_{21}H_{28}N_4O_5S_2Na$ $[M+Na]^+$ 503.6008, found 503.6016.

2-(1-(mesitylsulfonyl)piperidin-4-yl)-3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl) thiazolidin-4-one (7e)

Pale yellow gummy solid yield: 80% IR γmax (nujol) 3054, 2987, 1659, 1582, 1448, 1383, 1372, 1251, 1168/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 7.25 (s, 2H, ArH), 5.73-5.69 (m, 1H, arecoline-C-4H), 4.79-4.78 (d, *J* = 4.0 Hz, 1H, thiazolidinone-CH), 3.91 (s, 2H, arecolinyl-CH_2), 3.95-3.85 (dd, *J* = 2.8 Hz, 2H, thiazolidinone-CH_2), 3.12-2.82 (m, 4H, piperidine-CH_2), 2.80 (s, 2H, arecoline-C-2H_2), 2.68 (s, 6H, Ar-CH_3), 2.59-2.56 (m, 2H, arecoline-C-6H_2), 2.49-2.42

(m, 1H, piperidine-C-4H), 2.35 (s, 3H, Ar-CH_3), 2.32 (s, 3H, N-CH_3), 2.12-1.96 (m, 2H, arecoline-C-5H_2), 1.62-1.46 (m, 4H, piperidine CH_2). ^{13}C NMR (100 MHz, $CDCl_3$); δ 170.8, 148.2, 145.1, 139.2, 138.4, 132.8, 121.8, 63.1, 59.6, 55.2, 48.4, 47.2, 45.6, 37.4, 35.2, 24.6, 22.7, 22.6. HRMS (ESI) *m/z* Calcd for $C_{24}H_{35}N_3O_3S_2Na$ $[M+Na]^+$ 500.6830, found 500.6856.

2-(1-((2,5-dichlorophenyl)sulfonyl)piperidin-4-yl)-3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)thiazolidin-4-one (7f)

Pale brown gummy solid yield: 77% IR γmax (nujol) 3050, 1658, 1576, 1450, 1380, 1367, 1240, 1163, 730/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 8.03 (s, 1H, ArH), 7.46-7.44 (d, *J* = 8.0 Hz, 2H, ArH), 5.68 (s, 1H, arecoline-C-4H), 4.49-4.48 (d, *J* = 4.0 Hz, 1H, thiazolidinone-CH), 3.93 (s, 2H, arecolinyl-CH_2), 3.87-3.86 (dd, *J* = 2.4 Hz, 2H, thiazolidinone-CH_2), 2.87-2.79 (m, 4H, piperidine-CH_2), 2.75 (s, 2H, arecoline-C-2H_2), 2.64-2.51 (m, 3H, piperidine-C-4H and arecoline-C-6H_2), 2.41 (s, 3H, N-CH_3), 2.29-1.99 (m, 2H, arecoline-C-5H_2), 1.72-1.64 (m, 2H, piperidine-CH_2), 1.60-1.49 (m, 2H, piperidine-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$); δ 171.4, 144.0, 137.9, 133.4, 133.1, 130.7, 129.6, 129.3, 123.9, 65.6, 64.5, 54.5, 51.2, 46.3, 45.2, 38.7, 34.2, 25.3, 23.5. HRMS (ESI) *m/z* Calcd for $C_{21}H_{27}Cl_2N_3O_3S_2Na$ $[M+Na]^+$ 527.4934, found 527.4945.

3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)-2-(1-((3,4,5-trimethoxyphenyl) sulfonyl)piperidin-4-yl)thiazolidin-4-one (7g)

Pale yellow liquid yield: 90% IR γmax (nujol) 3050, 1657, 1576, 1448, 1381, 1369, 1240, 1210, 1165/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 7.29 (s, 2H, ArH), 5.69-5.68 (m, 1H, arecoline-C-4H), 4.72-4.71 (d, *J* = 4.2 Hz, 1H, thiazolidinone-CH), 3.92 (s, 2H, arecolinyl-CH_2), 3.89-3.85 (dd, *J* = 2.6 Hz, 2H, thiazolidinone-CH_2), 3.88 (s, 9H, OCH_3), 3.52-3.48 (m, 4H, piperidine-CH_2), 2.95 (s, 2H, arecoline-C-2H_2), 2.58-2.48 (m, 2H, arecoline-C-6H_2), 2.36-2.32 (m, 1H, piperidine-C-4H), 2.29 (s, 3H, N-CH_3), 2.14-2.09 (m, 2H, arecoline-C-5H_2), 1.56-1.42 (m, 4H, piperidine-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$); δ 172.5, 171.8, 153.2, 139.8, 136.9, 130.5, 119.9, 112.5, 61.7, 60.1, 57.2, 54.0, 47.5, 45.2, 36.5, 34.9, 25.9, 23.3. HRMS (ESI) *m/z* Calcd for $C_{24}H_{35}N_3O_6S_2Na$ $[M+Na]^+$ 548.6812, found 548.6819.

3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)-2-(1-(3-methylbenzyl)piperidin-4-yl)thiazolidin-4-one (7h)

Yellow liquid yield: 72% IR γmax (nujol) 3057, 1658, 1579, 1445, 1383, 1369, 1240, 1167/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 7.27 (s, 1H, ArH), 7.21-7.12 (m, 3H, ArH), 5.78-5.64 (m, 1H, arecoline-C-4H), 4.71-4.59 (d, *J* = 4.2 Hz, 1H, thiazolidinone-CH), 3.61 (s, 2H, arecolinyl-CH_2), 3.49-3.41 (m, 4H, thiazolidinone-CH_2 & benzyl-CH_2), 2.98-2.96 (m, 4H, piperidine-CH_2), 2.81 (s, 2H, arecoline-C-2H_2), 2.57-2.51 (m, 3H, piperidine-CH and arecoline C-6H_2), 2.38 (s, 3H, Ar-CH_3), 2.34 (s, 3H, N-CH_3), 1.95-1.90 (m, 2H, arecoline-C-5H_2), 1.71-1.53 (m, 4H, piperidine CH_2). ^{13}C NMR (100 MHz, $CDCl_3$); δ 171.1, 139.2, 138.4, 137.2, 130.4, 129.5, 128.3, 126.1, 121.5, 65.6, 62.2, 59.1, 54.3, 53.7, 48.1, 45.2, 38.1, 35.1, 26.2, 24.2, 21.5. HRMS (ESI) *m/z* Calcd for $C_{22}H_{31}N_3O_3S_2Na$ $[M+Na]^+$ 472.6298, found 472.6308.

3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)-2-(1-((3-(trifluoromethyl)phenyl) sulfonyl) piperidin-4-yl)thiazolidin-4-one (7i)

Dark brown liquid yield: 76% IR γmax (nujol) 3051, 1658, 1582, 1448, 1397, 1388, 1369, 1247, 1172/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 8.06 (s, 1H, ArH), 7.89-7.69 (m, 3H, ArH), 5.62-5.59 (m, 1H, arecoline C-4H), 4.64-4.63 (d, *J* = 3.8 Hz, 1H, thiazolidinone-CH), 3.81 (s, 2H, arecolinyl-CH_2), 3.79-3.76 (dd, *J* = 2.6 Hz, 2H, thiazolidinone-CH_2), 3.14-3.02 (m, 4H, piperidine-CH_2), 2.86 (s, 2H, arecoline-C-2H_2), 2.44-2.38 (m, 3H, arecoline-C-6H_2 and piperidine-CH), 2.29 (s, 3H, N-CH_3), 2.12-2.03 (m, 2H, arecoline-C-5H_2), 1.65-1.58 (m, 4H, piperidine-CH_2). ^{13}C NMR (100 MHz, CDCl3); δ 171.4, 141.6, 138.5, 131.5, 130.9, 129.6, 128.1, 125.4, 125.2, 125.1, 125.0, 123.8, 121.5, 62.5, 59.1, 55.2, 48.2, 47.5, 45.2, 37.2, 35.2, 26.2, 23.1. HRMS (ESI) *m/z* Calcd for $C_{22}H_{28}F_3N_3O_3S_2Na$ $[M+Na]^+$ 526.6012, found 526.6019.

2-(1-(3,4-dichlorobenzyl)piperidin-4-yl)-3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)thiazolidin-4-one (7j)

Brown gummy solid yield: 71% IR γmax (nujol) 3049, 1658, 1586, 1451, 1392, 1369, 1249, 1176, 786/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 7.74-7.72 (d, *J* = 8.2 Hz, 1H, ArH), 7.45 (s, 1H, ArH), 7.28-7.26 (d, *J* = 8.0 Hz, 1H, ArH), 5.66-5.62 (m, 1H, arecoline-C-4H), 4.68-4.67 (d, *J* = 3.6 Hz, 1H, thiazolidinone-CH), 3.89 (s, 2H, arecolinyl-CH_2), 3.78-3.74 (dd, *J* = 2.4 Hz, 2H, thiazolidinone-CH_2), 3.58 (s, 2H, benzyl-CH_2), 3.18-3.12 (m, 4H, piperidine-CH_2), 2.76 (s, 2H, arecoline-C-2H_2), 2.60-2.56 (m, 2H, arecoline-C-6H_2), 2.39-2.36 (m, 1H, piperidine-C-4H), 2.27 (s, 3H, N-CH_3), 2.19-2.16 (m, 2H, arecoline-C-5H_2), 1.61-1.49 (m, 4H, piperidine-CH_2). ^{13}C NMR (100 MHz, CDCl3); δ 170.8, 138.2, 134.8, 132.2, 129.9, 129.6, 129.2, 121.5, 64.9, 62.5, 59.3, 55.4, 54.2, 48.2, 44.8, 37.6, 35.2, 24.5, 23.9. HRMS (ESI) *m/z* Calcd for $C_{22}H_{29}Cl_2N_3OSNa$ $[M+Na]^+$ 477.4562, found 477.4569.

3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)-2-(1-(3-nitrobenzyl)piperidin-4-yl)thiazolidin-4-one (7k)

Pale yellow gummy solid yield: 70% IR γmax (nujol) 3037, 1652, 1593, 1541, 1452, 1389, 1378, 1363, 1245, 1165/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 8.12-8.01 (m, 2H, ArH), 7.56-7.51 (m, 2H, ArH), 5.66-5.62 (m, 1H, arecoline-C-4H), 4.56-4.55 (d, *J* = 4.2 Hz, 1H, thiazolidinone-CH), 3.89 (s, 2H, arecolinyl-CH_2), 3.92-3.81 (dd, *J* = 2.2 Hz, 2H, thiazolidinone-CH_2), 3.64 (s, 2H, benzyl-CH_2), 3.17-3.12 (m, 4H, piperidine-CH_2), 2.85 (s, 2H, arecoline-C-2H_2), 2.51-2.46 (m, 2H, arecoline-C-6H_2), 2.35-2.31 (m, 1H, piperidine-C-4H), 2.27 (s, 3H, N-CH_3), 2.22-2.18 (m, 2H, arecoline-C-5H_2), 1.95-1.86 (m, 2H, piperidine-CH_2), 1.56-1.42 (m, 2H, piperidine-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$); δ 171.6, 148.2, 136.9, 136.2, 135.2, 130.2, 125.6, 122.3, 121.6, 64.2, 62.5, 59.2, 55.2, 53.6, 47.8, 37.9, 35.2, 24.8, 23.2. HRMS (ESI) *m/z* Calcd for $C_{22}H_{30}N_4O_3SNa$ $[M+Na]^+$ 453.5636, found 453.5642.

2-(1-(3-fluorobenzoyl)piperidin-4-yl)-3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)thiazolidin-4-one (7l)

Brown gummy solid yield: 80% IR γmax (nujol) 3037, 1670, 1657, 1589, 1543, 1458, 1391, 1369, 1248, 1168/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 7.38-7.32 (m, 1H, ArH), 7.21-7.16 (m, 2H, ArH), 7.01-6.96 (m, 1H, ArH), 5.64-5.61 (m, 1H, arecoline-C-4H), 4.59-4.55 (d, *J* = 4.4 Hz, 1H, thiazolidinone-CH), 3.87 (s, 2H, arecolinyl-CH_2), 3.74-3.70 (dd, *J* = 2.4 Hz, 2H, thiazolidinone-CH_2), 3.15-3.09 (m, 4H, piperidine-CH_2), 2.80 (s, 2H, arecoline-C-2CH_2), 2.58-2.53 (m, 2H, arecoline-C-6H_2), 2.46-2.43 (m, 1H, piperidine-C-4H), 2.27 (s, 3H, N-CH_3), 2.22-2.17 (m, 2H, arecoline-C-5H_2), 1.58-1.52 (m, 2H, piperidine-CH_2), 1.39-1.35 (m, 2H, piperidine-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$); δ 171.6, 160.8, 138.3, 137.2, 125.9, 124.1, 122.8, 121.2, 65.6, 62.4, 59.3, 55.2, 53.6, 47.4, 42.9, 36.9, 33.8, 25.9, 24.1, 23.8. HRMS (ESI) *m/z* Calcd for $C_{22}H_{28}FN_3O_2SNa$ $[M+Na]^+$ 440.5400, found 440.5412.

2-(1-(2,6-dichlorobenzoyl)piperidin-4-yl)-3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)thiazolidin-4-one (7m)

Dark brown gummy solid yield: 82% IR γmax (nujol) 3051, 1680, 1661, 1591, 1453, 1397, 1372, 1251, 1176, 789/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 7.37-7.30 (m, 2H, ArH), 7.26-7.25 (m, 1H, ArH), 5.88-5.83 (m, 1H, arecoline-C-4H), 4.68-4.67 (d, *J* = 4.0 Hz, 1H, thiazolidinone-CH), 3.85 (s, 2H, arecolinyl-CH_2), 3.81-3.79 (dd, *J* = 2.4 Hz, 2H, thiazolidinone-CH_2), 3.20-3.16 (m, 4H, piperidine CH_2), 2.86 (s, 2H, arecoline-C-2H_2), 2.77-2.66 (m, 3H, piperidine-C-4H & arecoline-C-6H_2), 2.33 (s, 3H, N-CH_3), 2.25-2.05 (m, 2H, arecoline-C-5H_2), 1.68-1.26 (m, 4H, piperidine-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$); δ 172.1, 167.9, 136.3, 135.0, 131.6, 131.5, 127.7, 125.8, 65.4, 52.1, 50.1, 46.1, 45.7, 45.3, 38.8, 32.1, 24.1, 23.2. HRMS (ESI) *m/z* Calcd for $C_{22}H_{27}Cl_2FN_3O_2SNa$ $[M+Na]^+$ 491.4397, found 491.4405.

2-(1-(3-bromobenzoyl)piperidin-4-yl)-3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)thiazolidin-4-one (7n)

Pale yellow gummy solid yield: 84% IR γmax (nujol) 3053, 1684, 1665, 1596, 1453, 1397, 1372, 1254, 1176, 589/cm;^{1}H NMR

(400 MHz, $CDCl_3$); δ 8.01 (s, 1H, ArH), 7.89-7.76 (m, 3H, ArH), 5.65-5.63 (m, 1H, arecoline-C-4H), 4.59-4.58 (d, *J* = 3.8 Hz, 1H, thiazolidinone-CH), 3.96-3.91 (m, 4H, thiazolidinone-CH_2 and arecolinyl-CH_2), 3.36-3.21 (m, 4H, piperidine-CH_2), 2.86 (s, 2H, arecoline-C-2H_2), 2.46-2.41 (m, 3H, piperidine-C-4H and arecoline-C-6H_2), 2.28 (s, 3H, N-CH_3), 2.19-2.16 (m, 2H, arecoline-C-5H_2), 1.48-1.39 (m, 4H, piperidine-CH_2). ^{13}C NMR (100 MHz, CDCl3); δ 172.1, 171.6, 141.2, 138.5, 132.9, 129.9, 129.4, 126.4, 123.1, 121.5, 61.8, 59.6, 55.2, 48.5, 45.2, 44.7, 37.4, 34.3, 26.2, 23.1. HRMS (ESI) *m/z* Calcd for $C_{22}H_{28}BrN_3O_2SNa$ $[M+Na]^+$ 501.4456, found 501.4462.

2-(1-(4-chlorobenzoyl)piperidin-4-yl)-3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)thiazolidin-4-one (7o)

Pale yellow gummy solid yield: 83% IR γmax (nujol) 3047, 1680, 1661, 1596, 1450, 1397, 1370, 1254, 1176, 789/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 7.88-7.86 (d, *J* = 8.0 Hz, 2H, ArH), 7.74-7.72 (d, *J* = 8.2 Hz, 2H, ArH), 5.63-5.61 (m, 1H, arecoline-C-4H), 4.60-4.51 (d, *J* = 4.0 Hz, 1H, thiazolidinone-CH), 3.81 (s, 2H, arecolinyl-CH2), 3.79-3.76 (dd, *J* = 4.0 Hz, 2H, thiazolidinone-CH_2), 3.41-3.37 (m, 4H, piperidine-CH_2), 2.83 (s, 2H, arecoline-C-2H_2), 2.62-2.57 (m, 2H, arecoline-C-6H_2), 2.52-2.49 (m, 1H, piperidine-C-4H), 2.32 (s, 3H, N-CH_3), 2.16-2.08 (m, 2H, arecoline-C-5H_2), 1.47-1.41 (m, 4H, piperidine-CH_2). ^{13}C NMR (100 MHz, CDCl3); δ 170.8, 170.1, 141.2, 137.3, 134.1, 129.7, 128.9, 126.4, 121.2, 62.2, 59.2, 55.2, 48.2, 45.2, 37.2, 35.2, 26.2, 24.2. HRMS (ESI) *m/z* Calcd for $C_{22}H_{28}ClN_3O_2SNa$ $[M+Na]^+$ 456.9946, found 456.9952.

2-(1-(2-methoxybenzoyl)piperidin-4-yl)-3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)thiazolidin-4-one (7p)

Pale brown gummy solid yield: 88% IR γmax (nujol) 3042, 1682, 1659, 1596, 1448, 1397, 1372, 1254, 1220, 1178/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 7.90-7.84 (m, 2H, ArH), 7.25-7.21 (m, 2H, ArH), 5.63-5.60 (m, 1H, arecoline-C-4H), 4.64-4.63 (d, *J* = 4.2 Hz, 1H, thiazolidinone-CH), 3.96 (s, 2H, arecolinyl-CH_2), 3.91 (s, 3H, OCH_3), 3.52-3.51 (dd, *J* = 2.4 Hz, 2H, thiazolidinone-CH_2), 3.29-3.25 (m, 4H, piperidine-CH_2), 2.95 (s, 2H, arecoline-C-2H_2), 2.56-2.51(m, 2H, arecoline-C-6H_2), 2.48-2.46 (m, 1H, piperidine-C-4H), 2.35 (s, 3H, N-CH_3), 2.22-2.16 (m, 2H, arecoline-C-5H_2), 1.62-1.59 (m, 2H, piperidine-CH2), 1.36-1.33 (m, 2H, piperidine-CH_2). ^{13}C NMR (100 MHz, CDCl3); δ 172.6, 171.4, 158.2, 138.2, 131.2, 128.5, 127.8, 121.2, 120.6, 112.6, 62.4, 59.2, 56.2, 55.1, 48.2, 45.2, 44.8, 37.2, 35.1, 25.3, 23.2. HRMS (ESI) *m/z* Calcd for $C_{23}H_{31}N_3O_3SNa$ $[M+Na]^+$ 452.5755, found 452.5761.

3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)-2-(1-(3-nitrobenzoyl)piperidin-4-yl)thiazolidin-4-one (7q)

Pale brown liquid yield: 82% IR γmax (nujol) 3041, 1682, 1652, 1592, 1540, 1452, 1394, 1381, 1363, 1245, 1168/cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 8.51 (s, 1H, ArH), 8.38-8.32 (m, 2H, ArH), 7.76-7.72 (m, 1H, ArH), 5.64-5.61 (m, 1H, arecoline-C-4H), 4.62-4.61 (d, *J* = 3.8 Hz, 1H, thiazolidinone-CH), 3.88 (s, 2H, arecolinyl-CH_2), 3.69-3.64 (dd, *J* = 2.4 Hz, 2H, thiazolidinone-CH_2), 3.26-3.20 (m, 4H, piperidine-CH_2), 2.86 (s, 2H, arecoline-C-2H_2), 2.61-2.57 (m, 2H, aarecoline-C-6H_2), 2.42-2.38 (m, 1H, piperidine-C-4H), 2.32 (s, 3H, N-CH_3), 2.28-2.24 (m, 2H, arecoline-C-5H_2), 1.52-1.38 (m, 4H, piperidine-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$); δ 173.1, 171.6, 148.2, 138.2, 136.4, 132.9, 129.6, 125.2, 123.4, 121.5, 62.3, 59.2, 56.1, 48.1, 45.1, 44.9, 36.9, 35.2, 26.2, 23.8. HRMS (ESI) *m/z* Calcd for $C_{22}H_{28}N_4O_4SNa$ $[M+Na]^+$ 467.5471, found 467.5478.

2-(1-(4-tert-butylbenzoyl)piperidin-4-yl)-3-((1-methyl-1,2,5,6-tetrahydropyridin-3-yl)methyl)thiazolidin-4-one (7r)

Pale brown gummy solid yield: 86% IR γmax (nujol) 3045, 1688, 1662, 1597, 2234, 1451, 1399, 1372, 1330, 1254, 1220, 1178/ cm;^{1}H NMR (400 MHz, $CDCl_3$); δ 8.01-7.99 (d, *J* = 8.0 Hz, 2H, ArH), 7.46-7.44 (d, *J* = 7.8 Hz, 2H, ArH), 5.64-5.61 (m, 1H, arecoline-C-4H), 4.63-4.62 (d, *J* = 3.6 Hz, 1H, thiazolidinone-CH), 3.85 (s, 2H, arecolinyl-CH_2), 3.75-3.71 (dd, *J* = 2.6 Hz, 2H, thiazolidinone-CH_2), 3.38-3.32 (m, 4H, piperidine-CH_2), 2.85 (s, 2H, arecoline-C-2H_2), 2.62-2.59 (m, 2H, arecoline-C-6H_2), 2.49-2.45 (m, 1H, piperidine-C-4H), 2.34 (s, 3H, N-CH_3), 2.11-2.07 (m, 2H, arecoline-C-5H_2), 1.61-1.41 (m, 4H, piperidine-CH_2), 1.38 (s, 9H, t-butyl-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$); δ 172.9, 171.0, 148.1, 137.6, 136.1, 133.1, 129.1, 125.2, 122.9, 121.1, 62.2, 59.2, 55.1, 48.1, 44.9, 44.1, 37.2, 35.1, 26.2, 24.2. HRMS (ESI) *m/z* Calcd for $C_{26}H_{37}N_3O_2SNa$ $[M+Na]^+$ 478.6559, found 478.6565.

Pharmacology

In vitro inhibition studies on human AChE

The assay has been performed according to the guidelines provided in the AChE Assay Kit (DACE-100) purchased from Bio Assay systems #3191 Corporate Place, Hayward, CA 94545, USA. Briefly the assay medium (1 mL) consisted of phosphate buffer (pH 8.0), 50 µL of 0.01 M DTNB, 10 µL of enzyme, and 50 µL of 0.01 M substrate (ACh chloride solution). Briefly, the test compounds were added to the assay solution and pre incubated at 37°C with enzyme for 15 minutes followed by the addition of substrate. The activity was determined by measuring the increase of absorbance at 412 nm at 60 seconds intervals.

In vitro BuChE inhibition assay

The inhibitory activities of the prepared compounds against BuChE were performed by means of the method previously described by Ellman *et al*. [20] using neostigmine as the reference compound. This is based on the reaction of released thiocholine to give a colored product with a chromogenic reagent. The assay was performed in 0.1 M KH_2PO_4/ K_2HPO_4 buffers, pH 8.0, on a Shimadzu 2450 spectrophotometer. Enzyme solutions were prepared to give the concentration of 2.0 unit/ mL. The assay medium contained 0.01 M phosphate buffer (pH 8.0), DTNB, enzyme, and 0.01 M substrate BTC for BuChE. Test compounds were added to the assay medium and pre-incubated with the enzyme for 15 minutes at 37°C followed by the addition of substrate. The activity was determined by measuring the increase of absorbance at 412 nm with 1 minute interval at 37°C. The data was calculated according to the method of Ellman *et al*. [20].

RESULTS AND DISCUSSION

Chemistry

Previously we have reported √-ferrite catalyzed synthesis of arecoline thiazolidinone derivatives [21] and the synthesized compounds were subjected to *in vitro* muscarinic receptor binding studies as well as *in vivo* pharmacological evaluation of memory and learning in male wistar rats, few of them displayed significant activity in *in vitro* and *in vivo* experiments. In continuation of our research work towards the development of the new class of heterocyclic compounds for the treatment of AD, we herein report propylphosphonic anhydride (T3P®) catalyzed synthesis of homologated arecoline thiazolidinone derivatives. Mild reaction condition, ease of workup, easy purification, and good yields are the noteworthy features of this protocol.

Series of piperidine substituted arecoline 4-thiazolidinone derivatives were prepared by five synthetic steps. Cyclocondensation of 3-picolyl amine, thioglycolic acid with N-Boc piperdine 4-carbaldehyde was carried out in the presence of T3P® to afford 4-thiazolidinone at ambient temperature, then the pyridine ring of the compound 4 was reduced to an arecoline nucleus using earlier reported method [21] to get 5. The deprotection of compound 5 was achieved using HCl in ether to get 7. Finally using key intermediate 7 we have synthesized a good number of piperidine substituted arecoline 4-thiazolidinone derivatives 7(a-r) using substituted benzyl/benzoyl/benzene sulfonyl chlorides.

Pharmacology

Cholinesterase inhibitory activity

Research in the previous decades showed that cholinesterases are the leading and potential targets in treating AD. We investigated whether our newly synthesized compounds could inhibit AChE and BuChE. Effect of arecoline derivatives were evaluated against AChE using DACE-100 Quantichrom™ AChE assay kit based on an improved Ellman method, in which thiocholine produced by the action of AChE forms

a yellow color with 5,5'-dithiobis(2-nitrobenzoic acid). Half maximal inhibitory concentrations (IC_{50}) of the compounds 7a-r along with the IC_{50} of positive control neostigmine are summarized in Table 1. We identified compound 7c to be the most potent inhibitor of AChE with the IC_{50} of 6.62 μM. Additionally, we also investigated the effect of new molecules against BuChE and 7c exhibited as a very good inhibitor with the IC_{50} of 13.78 μM. Apart from our lead compound 7c, other members of the series displayed significant inhibitory activity against both esterases with variable efficacy. The IC_{50} values for AChE inhibition are ranged from 26.85 to 6.62 μM and for BuChE from 45.87 to 13.78 μM respectively. Interestingly, all synthesized compounds are significantly more active towards AChE than BuChE, Some of the derivatives proved to be comparatively selective for AChE than to BuChE (7c >7r > 7q > 7m.).

Structure–activity relationship (SAR)

SAR can be drawn from the *in vitro* assay for the synthesized derivatives 7(a-r). Among all the synthesized compounds, four derivatives 7c, 7m, 7r and 7q showed good AChE inhibition activity (Table 1), the potency of the molecules follows the order 7c > 7r > 7q > 7m. The remaining derivatives did not show noticeable AChE/BuChE inhibition. The compound 7c displayed most potent activity (IC_{50} 6.62 μM for AChE and 13.78 μM for BuChE) which is comparable with the standard neostigmine (IC_{50} 2.05 μM for AChE and 3.64 μM for BuChE). Compound 7c has a fluorine atom at para position of benzene sulfonyl group which is substituted to the amine group of the piperidine nucleus. Compounds with benzyl substitution (7h, 7j, 7k) had poor activity against both AChE and BuChE inhibition when compared to benzene sulfonyl and benzoyl derivatives. This suggests that, the presence of carbonyl or sulfonyl group between piperidine and aryl substitution played an important role for AChE/BuChE inhibition. Among the compounds with aryl sulfonyl/benzoyl group as substituents, different compounds exhibited different range of activity based on the atoms/groups attached to the phenyl ring.

CONCLUSION

In the field of drug discovery, enzymes are often serves as excellent targets in designing therapeutic agents for various pathological conditions. The present report focus on the tailoring of cholinesterase inhibitors is targeting the AD. Many researchers have proved that, cognitive behavior can be improved using cholinesterase inhibitors in AD patients. In this study, a new group of arecoline analogues, modified at the junction of Arecoline and 4-thiazolidinone by incorporating an extra carbon linkage have been prepared and all the synthesized compounds were evaluated for their inhibitory efficacy against AChE and BuChE. We identified 7c as a lead compound with the good inhibitory efficacy against both cholinesterases. This confirms that the fluorine atom on the benzene sulfonyl moiety may have a considerable influence on the cholinesterase activity. Thus, further structural modification of compound 7c can be carried out to develop effective cholinesterase inhibitors.

Table 1: Inhibitory activity of arecoline-thiazolidinone derivatives 7a-r and positive control against AChE and BuChE, determined by Ellman's method

Compounds	IC_{50} for AChE (μM)	IC_{50} for BuChE (μM)
7a	26.85466±1.94	45.87±1.0
7b	23.7816±1.09	40.08±1.06
7c	6.62±0.41	13.78±0.48
7d	20.98246±0.69	22.71±1.68
7e	27.81±1.03	36.17±0.64
7f	28.08±1.74	30.44±1.4
7g	24.21±2.8	20.40±2.19
7h	41.28063±1.75	53.36±0.77
7i	32.61538±2.35	ND
7j	34.7554±0.55	25.80±2.18
7k	30.36954±1.57	ND
7l	ND	ND
7m	19.90476±0.56	21.93±0.81
7n	26.6911±2.12	38.08±2.81
7o	25.81726±1	33.94±0.65
7p	21.9312±1.49	24.33±1.23
7q	16.28019±0.43	19.03±2.79
7r	11.57143±0.87	25.93±1.71
Neostigmine	2.051282±0.07	3.64±0.19

AChE: Acetylcholinesterase, BuChE: Butyrylcholinesterase

ACKNOWLEDGMENTS

The authors are grateful to the DST-IFCPAR, BRNS, Govt. of India for financial support to K.S.R. for the project vide NO. IFC/4303-1/2010-11 Dated 22-12-2010, Vide No. 2009/37/40/BRNS/2266 dated 23/11/2009. KSS and CDM thanks UGC and DST for RFSMS and INSPIRE fellowships.

REFERENCES

1. Jeong ML, Sandesh S, Jae WP. Noh IC, Woon DC, Jung HC. Ameliorative effect of novel vitamin formula with herbal extracts on scopolamine-induced alzheimer's disease. Asian J Pharm Clin Res 2013;6(2):175-9.
2. Jagdeep SD, Prasad DN, Avinash CT, Rajiv G. Role of traditional medicine in neuropsychopharmacology. Asian J Pharm Clin Res 2009;2(2):72-6.
3. Blokland A. Acetylcholine: A neurotransmitter for learning and memory? Brain Res Brain Res Rev 1995;21(3):285-300.
4. Sine SM. End-plate acetylcholine receptor: Structure, mechanism, pharmacology, and disease. Physiol Rev 2012;92(3):1189-234.
5. Greig NH, Utsuki T, Yu Q, Zhu X, Holloway HW, Perry T, *et al.* A new therapeutic target in Alzheimer's disease treatment: Attention to butyrylcholinesterase. Curr Med Res Opin 2001;17(3):159-65.
6. Colovic MB, Krstic DZ, Lazarevic-Pašti TD, Bondžic AM, Vasic VM. Acetylcholinesterase inhibitors: Pharmacology and toxicology. Curr Neuropharmacol 2013;11(3):315-35.
7. Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: A review of progress. J Neurol Neurosurg Psychiatry 1999;66(2):137-47.
8. Holden M, Kelly C. Use of cholinesterase inhibitors in dementia. Adv Psychiatr Treat 2002;8(2):89-96.
9. Terry AV Jr, Buccafusco JJ. The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: Recent challenges and their implications for novel drug development. J Pharmacol Exp Ther 2003;306(3):821-7.
10. Murray AP, Faraoni MB, Castro MJ, Alza NP, Cavallaro V. Natural AChE Inhibitors from Plants and their Contribution to Alzheimer's Disease Therapy. Curr Neuropharmacol 2013;11(4):388-413.
11. Tricco AC, Soobiah C, Berliner S, Ho JM, Ng CH, Ashoor HM, *et al.* Efficacy and safety of cognitive enhancers for patients with mild cognitive impairment: A systematic review and meta-analysis. Can Med Assoc J 2013;185(16):1393-401.
12. Adams RL, Craig PL, Parsons OA. Neuropsychology of dementia. Neurol Clin 1986;4(2):387-404.
13. Chang YF, Liu TY, Liu ST. Arecoline inhibits and destabilizes agrin-induced acetylcholine receptor cluster formation in C2C12 myotubes. Food Chem Toxicol 2013;60:391-6.
14. Bartolomeo AC, Morris H, Boast CA. Arecoline via miniosmotic pump improves AF64A-impaired radial maze performance in rats: A possible model of Alzheimer's disease. Neurobiol Learn Mem 1997;68(3):333-42.
15. Asthana S, Greig NH, Holloway HW, Raffaele KC, Berardi A, Schapiro MB, *et al.* Clinical pharmacokinetics of arecoline in subjects with Alzheimer's disease. Clin Pharmacol Ther 1996;60(3):276-82.
16. Sadashiva CT, Chandra JN, Kavitha CV, Thimmegowda A, Subhash MN, Rangappa KS. Synthesis and pharmacological evaluation of novel N-alkyl/aryl substituted thiazolidinone arecoline analogues as muscarinic receptor 1 agonist in Alzheimer's dementia models. Eur J Med Chem 2009;44(12):4848-54.
17. Malviya M, Kumar YC, Mythri RB, Venkateshappa C, Subhash MN, Rangappa KS. Muscarinic receptor 1 agonist activity of novel N-aryl carboxamide substituted 3-morpholino arecoline derivatives in Alzheimer's presenile dementia models. Bioorg Med Chem 2009;17(15):5526-34.
18. Sharath Kumar KS, Hanumappa A, Hegde M, Narasimhamurthy KH, Raghavan SC, Rangappa KS. Synthesis and antiproliferative effect of novel 4-thiazolidinone-, pyridine- and piperazine-based conjugates on human leukemic cells. Eur J Med Chem 2014;81:341-9.

19. Sharath Kumar KS, Swaroop TR, Harsha KB, Narasimhamurthy KH, Rangappa KS. $T_3P^{®}$-DMSO mediated one pot cascade protocol for the synthesis of 4-thiazolidinones from alcohols. Tetrahedron Lett 2012;53:5619-23.
20. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-95.
21. Chandra JN, Malviya M, Sadashiva CT, Subhash MN, Rangappa KS. Effect of novel arecoline thiazolidinones as muscarinic receptor 1 agonist in Alzheimer's dementia models. Neurochem Int 2008;52(3):376-83.