Medicinal chemistry

Ali et al. Med Chem 2012, 2:1
DOI: 10.4172/2161-0444.1000106

Research Article Open Access

Alzheimer Diseases: Substituted Spiro [2.3′] Oxindolespiro [3.2″]-5, 6-Dimethoxy-Indane-1″-One- Indolizine Analogue as Inhibitors of Acetylcholinesterase

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Abstract

Indanone derivatives share vital pharmacological properties, considered useful in alzheimer's disease (AD).The aim of this study was synthesis and evaluate indolizine analogues if possess acetyl cholinesterase (AChE)inhibitory activity. The easily accessible three-step synthesis of these compounds resulted to be significantly less difficult and expensive than that of donepezil. Several compounds possess anti-cholinesterase activity in the order of micro and sub-micromolar. Particularly, compound **3k** was the most potent inhibitors of the series. Compound **3k**, showed potent inhibitory activity against acetyl cholinesterase enzyme with IC_{50} 0.10 µmol/L. Indolizine analogues might be potential acetyl cholinesterase agents for AD.

Keywords: Alzheimer diseases; Acetyl cholinesterase; AchE inhibitor; 1,3-dipolar cycloaddition; Azomethineylide

Introduction

Senile dementia is undesired consequence of progressive aging. Alzheimer's disease is one form of senile dementia which occurs due to various neuropathological conditions such as senile plaques and neurofibrillary tangles. It is the most widely spread dementias that affects 50% of the population aged 85 years [1,2]. It is the seventh leading cause of death in the world affecting 5.3 million people. In AD, increasing numbers of nerve cells deteriorate and die along with loss in synapse through which information flows from and to the brain. This leads to cognitive impairment and dementia [3].

The neuropathology of AD is generally characterized by the presence of numerous plaques of amyloid β-peptide (Aβ) plaques, neurofibrillary tangles (NFT), and degeneration or atrophy of the basal forebrain cholinergic neurons. The loss of basal forebrain cholinergic cells results in an important reduction in acetylcholine (ACh), which plays an important role in the cognitive impairment associated with AD. Acetylcholine esterase plays an important role in accelerating Aβ plaque deposition. The levels of MAO-B are increased in temporal, parietal and frontal cortex of AD patients. The high levels of MAO-B increase hydroxyl radicals which in turn lead to the formation of Aβ plaques [4].

The increasing level of ACh has been regarded as one of the most promising approaches for the symptomatic treatment of AD. Acetyl choline esterase is an enzyme that inhibits the hydrolysis of acetylcholine there by it increases the level of acetyl choline [5]. Several drugs are under development targeting acetylcholine esterase (AChE). The commercially available AChE inhibitors include galanthamine, donepezil, rivastigmine and tacrine [6].

Based on the prevalence of disease and the availability of limited options of drugs for the treatment of AD, Continuation of our previous work [7] we planned to develop novel drugs for AD. In the present investigation potent heterocyclic compounds were developed targeting AChE.

Results and Discussion

Chemistry

Spiro [2.3′] oxindolespiro [3.2″]-5, 6-dimethoxy-indane-1″-one-indolizine analogue **3a-o** described in this study is shown in Table 1, and a reaction sequence for the preparation is outlined in Scheme 1. In the initial step, 5,6-dimethoxy-2-[(*E*)-1-phenylmethylidene]-1-indanone were synthesized by us earlier condensing 5,6-dimethoxy-1-indanone with appropriate aromatic aldehyde in dilute methanolic sodium hydroxide solution at room temperature, spiro Indolizidinene ring were synthesized via 1,3-dipolar cycloaddition of azomethine ylide generated by the decarboxylative condensation of appropriate isatin and pipecolinic acid with dipolarophiles (5,6-dimethoxy-2-[(*E*)-1-phenylmethylidene]-1-indanone) and to get titled compounds in 74-94% yield after recrystalization with pet ether: ethyl acetate (4:1). The purity of the compounds was checked by TLC and elemental analyses. Both analytical and spectral data (^{1}H-NMR, IR) of all the synthesized compounds were in full agreement with the proposed structures. The elemental analysis results were within ±0.4% of the theoretical values.

Anti-choline esterase inhibitory activity

Among the novel substituted Indolizine derivatives for treating AD, their anticholinesterase activities **(compounds 3a-3o)** was assayed according to Ellmann's method on AChE from electric eel using commercial donepezil HCl as the reference standard. The BuChE inhibitory on equine serum BuChE were also examined by the same

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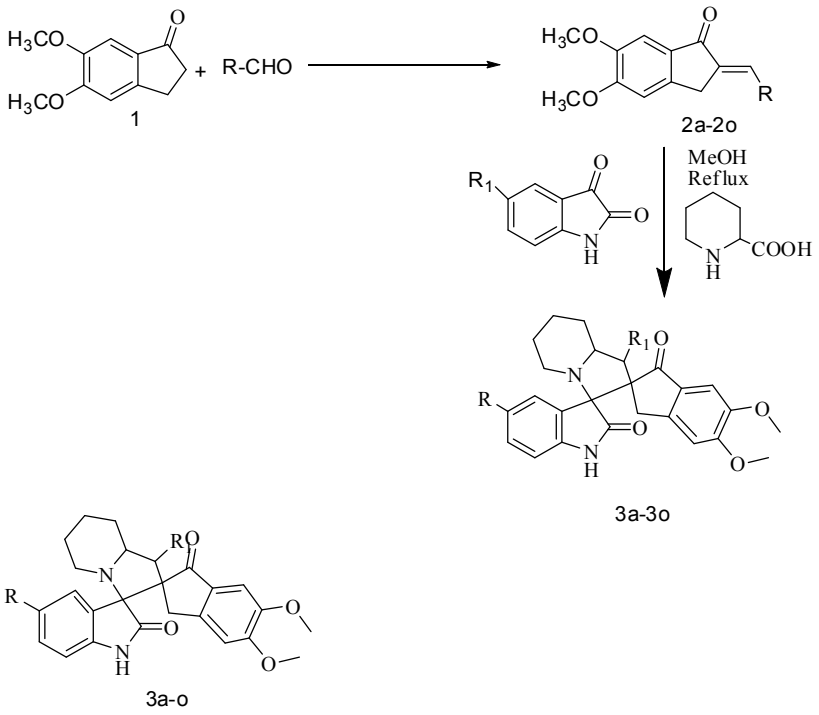
Received December 08, 2011; **Accepted** February 23, 2012; **Published** February 24, 2012

Citation: Ali MA, Ismail R, Choon, Kumar RS, Asad M, et al. (2012) Alzheimer Diseases: Substituted Spiro [2.3′] Oxindolespiro [3.2″]-5, 6-Dimethoxy-Indane-1″-One- Indolizine Analogue as Inhibitors of Acetylcholinesterase. Medchem 2:106. doi:10.4172/2161-0444.1000106

Comd	**R**	R_1	Rf Vlaue	Yield (%)	M.P(^{0}C)	AchE[a] IC_{50}(μM)±SEM	BuchE [b] IC_{50}(μM)±SEM	Selectivity for AChE[c]
3a	Pyridyl-	H	0.67	74	204	0.42±0.02	3.4±0.02	8.09
3b	4-flurophenyl-	H	0.64	65	183	1.12±0.02	2.12±0.02	1.89
3c	3,4-Dimethoxy phenyl--	H	0.69	72	194	3.2±0.02	1.2±0.02	0.37
3d	Phenyl-	H	0.62	80	161	1.12±0.1	3.10±0.1	2.76
3e	2-Chloro phenyl-	H	0.78	82	138	1.8±0.01	4.3±0.01	2.3
3f	Pyridyl-	Cl	0.73	85	113	0.37± 0.01	3.8± 0.01	10.20
3g	4-flurophenyl-	Cl	0.76	92	126	1.6±0.1	3.7±0.1	2.3
3h	3,4-Dimethoxy phenyl--	Cl	0.74	85	105	2.2±0.1	4.2±0.1	1.90
3i	Phenyl-	Cl	0.66	77	154	3.2±0.1	11.2±0.1	3.50
3j	2-Chloro phenyl-	Cl	0.70	82	162	2.6±0.1	6.6±0.1	2.50
3k	Pyridyl-	NO_2	0.78	90	126	0.10±0.1	4.10±0.1	36.41
3l	4-flurophenyl-	NO_2	0.82	56	178	8.6±0.1	1.6±0.1	0.18
3m	3,4-Dimethoxy phenyl-	NO_2	0.85	81	194	6.10±0.1	3.10±0.1	0.50
3n	Phenyl-	NO_2	0.59	76	172	13.2±0.1	2.2±0.1	0.16
3o	2-Chloro phenyl-	NO_2	0.83	72	182	7.6±0.1	1.6±0.1	0.20
Drug	Donepezil.HCl	-	-	-	-	0.12±0.01	3.6±0.1	30.0

Table 1: Physical constants and Inhibition of AchE and BuchE activities of the synthesized compounds.



Scheme 1: Protocol for synthesis.

method. Inhibition of AChE activities of the synthesized compounds is shown in Table 1. The data listed in Table 1 clearly show that most of the designed compounds exhibited good to moderate inhibitory activities toward the AChE and BuChE inhibition are summarized in Table 1. All indolizine derivatives were potent inhibitors of AChE, with IC_{50} values ranging from micromolar to sub-micromolar. A simple structure–activity relationship analysis showed that the AChE inhibitory potency closely related to the substitution of the indolizine and isatin ring. Compounds **3k**, **3f**, and **3a** with pyridine ring, were the best inhibitors in their series. Especially, compound **3k** showed the best AChE inhibitory activity of all the indolizine derivatives, with an IC_{50} value of 0.10μM. However, the same tendency was not shown for BuChE inhibition. Other compounds in this series showed a remarkably moderate inhibitory activity compared to the nitro group substitute with pyridine ring containing indolizine derivatives.

Nitro group with pyridine ring substituted derivatives showed most potent inhibitory activity of 4- to 5-fold more potent activity than **3a**-**3f** derivatives. The relative rigidity of the nitro with pyridine substituted scaffold could encumber penetration into the AChE ravine to reach the binding site.

From the IC_{50} values of the compounds, it appeared that the indolizine derivatives **3c**, **3i, 3o** showing a better activity toward BuChE than that of AChE. Compound **3i** the most potent for BuChE

inhibition, had an IC_{50} value of 1.2μM, about 2.6-fold higher than for AChE. Recent studies indicated that inhibition of brain BuChE may represent an important therapeutic target for AD. Among the newer derivatives, it is conceivable that derivatives showing AChE inhibitory activity can be further modified to exhibit better potency than the standard drugs. Further studies to acquire more information about Quantitative Structure-Activity Relationships (QSAR) are in progress in our laboratory. The indanone containing indolizine derivatives discovered in this study may provide valuable therapeutic intervention for the treatment of AD diseases.

Materials and Methods

The entire chemicals were supplied by E.Merck (Germany) and S.D fine chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in the solvent system toluene-ethyl formate-formic acid (5:4:1) and benzene-methanol (8:2), the spots were located under iodine vapors or UV light. IR spectrums were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr Pellets).^{1}H–NMR spectra were recorded or a Bruker AC 300 MHz spectrometer using TMS as internal standard in DMSO/ $CDCl_3$.

General procedure

General method for the preparation of 2-[(E)-1-(substituted aryl) methylidene]-5,6-dimethoxy-1-indanone (2a-2o)[6-7].

5,6-dimethoxy-1-indanone (1.92g, 0.01mol), appropriate aldehyde (1.02g,0.01 mol), were dissolved in ethanol and sodium hydroxide (30%, 5mL) with 10ml of Pet. Ether. The reaction mixture was stirred at room temperature for 4h. The resulting solution allowed standing overnight then poured into ice-cold water followed by neutralization with HCl. The solid separated was filtered off dried and purified from ethanol.

General procedure

Synthesis of Spiro [2.3′] oxindolespiro [3.2″]-5,6-dimethoxy-1″-indanone-4-(substituted)- Indolizine derivatives.

To a 5, 6-dimethoxy-2-[(*E*)-1-phenylmethylidene]-1-indanone of (0.001moles) methanol, appropriate isatin and pipecolinic acid in a molar ratio of 1:1:2 in methanol at reflux for 6–7.5 h furnished novel spiro indolizine derivatives. Excess of solvent was removed under reduced pressure and the reaction mixture was cooled poured on to crushed ice (20 gm). The product obtained was filtered, washed with water and recrystallized from methanol.

Spiro[2.3′]oxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-(pyridyl)-Indolizine (**3a**): IR(KBr): 1525, 1690,1724 and 3290 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.27, 1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72 (t, 1H, CH), 3.32 (s, 1H, CH), 3.84 (s, 6H, OCH_3),7.01-7.42 (m, 6H, aromatic), 7.42-8.4 (m, 4H, pyridine), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{30}H_{29}N_3O_4$: C, 72.71; H, 5.90; N, 8.48%; Found: 72.70; H, 5.88; N, 8.46%; EI-MS *m/z* 496 (M^{+1}).

Spiro[2.3′]oxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-(4-fluorophenyl)-Indolizine (**3b**): IR(KBr): 744,1525, 1690, 1724 and 3290 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.27, 1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72 (t, 1H, CH), 3.32 (s, 1H, CH), 3.84 (s, 6H, OCH_3), 7.01-7.42 (m, 10H, aromatic), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{31}H_{29}FN_2O_4$: C, 72.64; H, 5.70; N, 5.47%; Found: C, 72.62; H, 5.68; N, 5.45%; EI-MS *m/z* 513 (M^{+1}).

Spiro[2.3′]oxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-(3,4-dimethoxyphenyl)-Indolizine **(3c):**

IR(KBr): 1525, 1690, 1724 and 3290 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.27, 1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72 (t, 1H, CH), 3.32 (s, 1H, CH), 3.84 (s, 12H, OCH_3), 7.01-7.42 (m, 9H, aromatic), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{33}H_{34}N_2O_6$: C, 71.46; H, 6.18; N, 5.05%; Found: C,71.44; H, 6.16; N, 5.03%; EI-MS *m/z* 555(M^{+1}).

Spiro[2.3′]oxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-phenyl- Indolizine **(3d):** IR(KBr): 1525, 1690, 1724 and 3290 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 1.27,1.47-1.55, (m, 8H, CH_2 X5), 2.52-2.62(d, 2H, CH_2), 2.72 (t, 1H, CH), 3.32 (s, 1H, CH), 3.84 (s, 6H, OCH_3),7.01-7.42 (m, 11H, aromatic), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{31}H_{30}N_2O_4$: C, 75.28; H, 6.11; N, 5.66%; Found: C,75.26; H, 6.09; N, 5.64%; EI-MS *m/z* 495 (M^{+1}).

Spiro[2.3′]oxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-(2-chlorophenyl)-Indolizine **(3e):** IR(KBr): 744, 1525, 1690, 1724 and 3290 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.27,1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72 (t, 1H, CH), 3.32 (s, 1H, CH), 3.84 (s, 6H, OCH_3), 7.01-7.42 (m, 10H, aromatic), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{31}H_{29}ClN_2O_4$: C, 70.38; H, 5.53; N, 5.30%; Found: C,70.36; H, 5.51; N, 5.28%; EI-MS *m/z* 529 (M^{+1}).

Spiro[2.3′]oxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-(pyridyl)-Indolizine **(3f)** IR(KBr): 1525, 1690,1724 and 3290 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.27,1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72(t, 1H, CH), 3.32(s, 1H, CH), 3.84 (s, 6H, OCH_3),7.01-7.42 (m, 5H, aromatic), 7.42-8.4 (m, 4H, pyridine), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{30}H_{28}ClN_3O_4$: C, 67.98; H, 5.32; N,7.93%; Found: 67.66; H, 5.30; N,7.91%; EI-MS *m/z* 530 (M^{+1}).

Spiro[2.3′]-5-chlorooxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-(4-fluorophenyl)-Indolizine **(3g)** IR(KBr): 744,1525, 1690, 1724 and 3290 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.27, 1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72 (t, 1H, CH), 3.32 (s, 1H, CH), 3.84 (s, 6H, OCH_3), 7.01-7.42 (m, 9H, aromatic), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{31}H_{28}ClFN_2O_4$: C, 68.07; H, 5.16; N, 5.12%; Found: C, 68.05; H, 5.14; N, 5.10%; EI-MS *m/z* 547 (M^{+1}).

Spiro[2.3′]-5-chlorooxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-(3,4-dimethoxyphenyl)-Indolizine **(3h)** IR(KBr): 1525, 1690, 1724 and 3290 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.27, 1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72 (t, 1H, CH), 3.32 (s, 1H, CH), 3.84 (s, 12H, OCH_3), 7.01-7.42 (m, 8H, aromatic), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{33}H_{33}ClN_2O_6$: C, 67.28; H, 5.65; N, 4.76%; Found: C, 67.28; H, 5.65; N, 4.76%; EI-MS *m/z* 589 (M^{+1}).

Spiro[2.3′]-5-chlorooxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-phenyl-Indolizine **(3i)** IR(KBr): 1525, 1690, 1724 and 3290 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.27, 1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72 (t, 1H, CH), 3.32 (s, 1H, CH), 3.84 (s, 6H, OCH_3), 7.01-7.42 (m, 10H, aromatic), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{31}H_{29}ClN_2O_4$: C, 70.38; H, 5.53; N, 5.30%; Found: C, 70.36; H,5.51; N, 5.28%; EI-MS *m/z* 529 (M^{+1}).

Spiro[2.3′]-5-chlorooxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-(2-Chlorophenyl)-Indolizine **(3j)** IR(KBr): 1525, 1690, 1724 and 3290 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.27,1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72 (t, 1H, CH), 3.32 (s, 1H, CH), 3.84 (s, 6H, OCH_3),7.01-7.42 (m, 9H, aromatic), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{31}H_{28}Cl_2N_2O_4$: C, 66.08 H, 5.01; N, 4.97%; Found: C, 66.06 H, 4.98; N, 4.95%; EI-MS *m/z* 563 (M^{+1}).

Spiro[2.3′]-5-nitroooxindolespiro [3.2″]-5,6-dimethoxy-1″-indanone-4-(pyridyl)-Indolizine **(3k)** IR(KBr): 1525, 1690,1724 and

3290 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 1.27,1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72 (t, 1H, CH), 3.32 (s, 1H, CH), 3.84 (s, 6H, OCH_3),7.01-7.42 (m, 5H, aromatic), 7.42-8.4 (m, 4H, pyridine), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{30}H_{28}N_4O_6$: C, 66.66; H, 5.22; N,10.36%; Found: C, 66.66; H, 5.22; N, 10.36%; EI-MS *m/z* 541 (M^{+1}).

Spiro[2.3′]-5-nitroooxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-(4-fluorophenyl)-Indolizine **(3l)** IR(KBr): 1525, 1690, 1724 and 3290 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 1.27,1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72 (t, 1H, CH), 3.32 (s, 1H, CH), 3.84 (s, 6H, OCH_3),7.01-7.42 (m, 9H, aromatic), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{31}H_{28}FN_3O_6$: C, 66.78; H, 5.06; N, 7.54%; Found: C, 66.76; H, 5.04; N, 7.52%; EI-MS *m/z* 558 (M^{+1}).

Spiro[2.3′]-5-nitroooxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-(3,4-dimethoxyphenyl)-Indolizine **(3m)** IR(KBr): 1525, 1690, 1724 and 3290 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 1.27,1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72 (t, 1H, CH), 3.32 (s, 1H, CH), 3.84 (s, 12H, OCH_3), 7.01-7.42 (m, 8H, aromatic), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{33}H_{33}N_3O_8$: C, 66.10; H, 5.55; N, 7.01%; Found: C, 66.08; H, 5.53; N, 6.98%; EI-MS *m/z* 600 (M^{+1}).

Spiro[2.3′]-5-nitroooxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-(pyridyl)-Indolizine **(3n)** IR(KBr): 1525, 1690, 1724 and 3290 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 1.27,1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72 (t, 1H, CH); 3.32 (s, 1H, CH), 3.84 (s, 6H, OCH_3),7.01-7.42 (m, 10H, aromatic), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{31}H_{29}N_3O_6$: C, 69.00; H,5.42; N,7.79%; Found: C, 69.02; H,5.40; N,7.77%; EI-MS *m/z* 540 (M^{+1}).

Spiro[2.3′]-5-nitroooxindolespiro[3.2″]-5,6-dimethoxy-1″-indanone-4-(2-chlorophenyl)-Indolizine **(3o)** IR(KBr): 1525, 1690, 1724 and 3290 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): 1.27,1.47-1.55, (m, 8H, CH_2 X4), 2.52-2.62 (d, 2H, CH_2), 2.72 (t, 1H, CH), 3.32 (s, 1H, CH), 3.84 (s, 6H, OCH_3),7.01-7.42 (m, 9H, aromatic), 8.48 (s, 1H, NH); Anal. Calcd. for $C_{31}H_{28}ClN_3O_6$: C, 64.86 H, 4.92; N,7.32%; Found: C, 64.84 H, 4.90; N,7.30%; EI-MS *m/z* 574 (M^{+1}).

Acetylcholinesterase inhibitory activity

In vitro inhibition studies on AChE and BuChE

Acetylcholinesterase (AChE, from electric eel), butylcholinesterase (BuChE, from equine serum), 5,5′- dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, DTNB), acetylthiocholine chloride (ATC), butylthiocholine chloride (BTC), and hydrochloride were purchased from Sigma–Aldrich. Indolizine derivatives were dissolved in DMSO and diluted in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0) to provide a final concentration range. DMSO was diluted to a concentration in excess of 1 in 10,000, and no inhibitory action on either AChE or BuChE was detected in separate prior experiments.

In vitro AChE assay

All the assays were carried out under 0.1 M KH_2PO_4/K_2HPO_4 buffer, pH 8.0, using a Shimadzu UV-2450 Spectrophotometer. Enzyme solutions were prepared to give 2.0 units/mL in 2 mL aliquots. 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). Test compounds were added to the assay solution and pre incubated at 37 ^{0}C with the enzyme for 15 min followed by the addition of substrate. The activity was determined by measuring the increase in absorbance at 412 nm at 1 min intervals at 37°C. Calculations were performed according to the method of the equation in Ellman et al. [8]. Each concentration was assayed in triplicate. In vitro BuChE assay was similar with the method used for AChE.

Acknowledgements

The authors wish to express their thanks to Pharmacogenetic and Novel Therapeutic Research, Institute for Research in Molecular Medicine, Universiti of Sains Malysia, Pennag and this work were funded through Research Grant No.RUC (1001/PSK/8620012).

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