Chapter 4  Spiroimidazol-4-ones....

4.0 Imidazol-4-ones

Imidazolidinones constitute a class of heterocyclic compounds with wide ranging biological activities. The 2-imidazolidinone and 4-imidazolidinone scaffolds can be found in compounds with CCR3 and 5-HT2c receptor antagonists activity,\(^1\) angiogenic,\(^2\) and antibacterial\(^3\) properties, and in phosphodiesterase inhibitors\(^4\). The imidazolones serves as useful synthetic and chiral auxiliaries\(^5\).

The rapid synthesis of diverse libraries of small organic molecules for biochemical studies and drug discovery is an active research field\(^6,7\). Among many classes of small molecule libraries, 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one \(^8\) is a very attractive template for combinatorial synthesis, due to its high biological profile.

Irbesartan (Figure-22) (SR47436, BMS-186295), chemically designated as 2-butyl-3-[[29-(1H-tetrazole-5-yl)[1,19-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4,4]non-1-en-4-one, is a potent, long-acting angiotensin II (AII)\(^1\) receptor antagonist, with high selectivity for the AT\(^1\) subtype. Irbesartan and other AII receptor antagonists have the potential to offer advantages in safety and tolerability over earlier classes of drugs for the treatment of hypertension, diabetic nephropathy, and heart failure\(^9,10,11\). A clinical study in hypertensive subjects has demonstrated that irbesartan effectively lowers blood pressure with once-daily administration\(^12\).

From literature it is known that, spiro compounds represent an important class of naturally occurring substances characterized by their highly pronounced biological properties. The asymmetric characteristic of the molecule due to the chiral spiro carbon is one of the important criteria of the biological activities. Recently, the effects of azaindolizinone derivative ZSET1446, spiroimidazo[1,2-a]pyridine-3,2-indon]-2(3\(H\))-one, were assessed in rats with learning deficits induced by A\(\beta\)\(_{1-40}\) or scopolamine suggesting that ZSET1446 (Figure-23) may be a potential candidate for development as a therapeutic agent to treat cognitive impairment associated with conditions such as Alzheimer’s disease\(^13\). Keeping this in mind, we have synthesized, 2-butyl-1,3-diazaspiro[4,4]non-1-en-4-one \(^8\) by following the reported procedure\(^14\).
The association of bioactive nucleus with other pharmacological agents is hoped to improve efficacy of treatment by combining effects from the different pharmacological mechanisms of action. In this perspective, a number of 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one derivatives 83(a-j) have been synthesized by interaction of 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one 81 with different aralkyl halides having substituted phenyl ring, biphenyl ring with heterocyclic moiety and fused rings. Structure elucidation of the new compounds has been carried out with the help of elemental analysis and spectral data. All the synthesized compounds have been screened for their ability to inhibit acetylcholinesterase. Some derivatives (83a, 83b, 83j) in this class showed good inhibition against AChE as compared to neostigmine as standard.

Figure-22

Figure-23

The synthesis of the compounds 83(a-j) was done by the substitution of different bioactive aralkyl halides with 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one 81 in presence of powdered potassium carbonate in N,N-dimethylformamide (DMF). The compounds were also obtained by microwave irradiation method. The yields under conventional and microwave irradiation method were in the range of 60-65% and 80-90% respectively.

In the present work, the synthesis of spiromimidazol-4-one analogs involves the following steps:

(i) Synthesis of 1-amino-1-cyano-cyclopentane hydrochloride
(ii) Synthesis of 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one
(iii) N-alkylation of 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one
4.1 Synthesis of spiroimidazol-4-one derivatives 83(a-j)

The synthetic scheme for the preparation of spiroimidazol-4-one analogs 83(a-j) is as shown in Scheme 34.

4.1.1 Synthesis of 1-amino-1-cyano-cyclopentane hydrochloride (80)

A mixture of cyclopentanone (20g, 0.238mole) and ammonium formate (15g, 0.238mole) in 125 ml water were stirred together to give a clear solution. To that solution, potassium cyanide (15.50g, 0.238mole) were spooned in over 5 minutes with a little heat evolution; an oily phase began to separate. The reaction mixture was stirred at ambient temperature for about 24 hours. The upper layer was separated and it was diluted to 75 ml with toluene and then dried over anhydrous sodium sulfate. The toluene mixture was saturated with dry hydrogen chloride gas, giving separation of solid (a precipitate). The solid was filtered, washed with toluene and hexane, and dried at 50°C under vacuum. Yield 68%; mp: 156-158°C.
IR $\nu_{\text{max}}$ (KBr): 3340, 2985, 2224, 1480 cm$^{-1}$.

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.5-1.95 (m, 8H, cyclopentyl), 2.2-2.4 (s, 2H, -NH$_2$).


4.1.2 Synthesis of 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one monohydrochloride (81)

To the solution of 1-amino-1-cyanocyclopentane (15g, 0.1361mole) in 150 ml of toluene triethylamine (20.6g, 0.204mole; 1.5 eq) was added and to this solution valeroyl chloride (19.69g, 0.1632mole; 1.2 eq) was added dropwise, under cooling, at 20°C. After 10-15 minutes the temperature was raised to 80°C and the mixture was stirred at that temperature for about 2 hours. The reaction mixture was then cooled to room temperature and it was extracted consecutively with 50 ml of water, 40 ml of 2% hydrochloric acid solution and 40 ml of water. The toluene solution was evaporated under vacuo. To the residue a solution of potassium hydroxide (3.82g, 0.068mole; 0.5eq) in 50 ml of methanol, were added at first and then under cooling a 30 ml of 30% hydrogenperoxide solution. After the addition of hydrogen peroxide the temperature was maintained at 50°C for about 30 minutes. The solution was cooled to room temperature and added potassium hydroxide (15.28g, 0.272mole; 2eq) solution. The mixture was heated under reflux condition for about 2 hours. The reaction was then freezed by the addition of 30 g of ammonium chloride and methanol was distilled out. The reaction mixture was then extracted with ethyl acetate (50x3ml). The combined organic phase was evaporated in vacuo, the residue was dissolved in 5-fold amount of acetone, the resulting solution was filtered, its pH was adjusted to 1-2 by the addition of conc. hydrochloric acid solution. The suspension thus obtained was stirred for 1hour, cooled to 0°C. The crystals were filtered off, washed with cold acetone and dried to obtain the product 81. Yield 55%; mp: 68°C.

IR $\nu_{\text{max}}$ (KBr): 2995, 1480, 1455, 1365 cm$^{-1}$.

$^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 1.5-1.95 (m, 8H, cyclopentyl), 7.8-8.2 (s, 1H, -NH), 0.88-0.92 (t, 3H, -CH$_3$), 1.2-1.4 ((m, 6H, -CH$_2$-CH$_2$-CH$_2$),

Anal. calcd. for C$_{11}$H$_{18}$N$_2$O: C: 68.01, H: 9.34, N: 14.42. Found C: 68.03, H: 9.35, N: 14.45%.
4.1.3 General procedures for the synthesis of 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one derivatives 83(a-j).

[a] Conventional method:
A mixture of 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one 81 (1 eq), alkyl halides 82(a-j) (1.2 eq) and powdered potassium carbonate (5 eq) in N,N-dimethylformamide (10 ml) was stirred at room temperature for about 6-8 hours. The reaction was monitored by thin layer chromatography. After the completion of the reaction, deionized water was added to the reaction mixture and extracted with ethyl acetate (3 x 10 ml). The combined organic layer was dried over anhydrous Na$_2$SO$_4$. The crude products 83(a-j) obtained on evaporation of the solvent under reduced pressure were purified by column chromatography using hexane and ethyl acetate as eluents.

[b] Microwave irradiation method:
A 25 ml conical flask charged with 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one 81, (1 g, 3.29 mmole), different aralkyl halides 82(a-j) (1.2 eq), and DMF (10 ml), was irradiated in the microwave oven at 20% power level (60 W) for 90-150 s. After completion of the reaction (tlc), 10 equivalent of water was added to the cooled (rt) contents of the flask. Using the above workup procedure isolated the pure products. The reaction condition and physical data of the synthesized compounds are depicted in the Table 14.

4.1.3.1 Synthesis of 4’-(2-butyl-4-oxo-1,3-diaza-spiro[4.4]non-1-en-3-ylmethyl)-biphenyl-2-carbonitrile (83a)
The product 83a was obtained from 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one 81 (1 g, 5.147 mmol), 4’-bromomethyl-biphenyl-2-carbonitrile 82a (1.681 g, 6.176 mmol) and potassium carbonate (3.557 g, 25.74 mmol).

IR $\nu_{\text{max}}$ (KBr): 3095, 2930, 1735, 1574, 1480 cm$^{-1}$.

$^1$H NMR (CDCl$_3$ 400 MHz) $\delta$: 1.51-1.81 (m, 8H, -cyclopentane), 4.34-4.36 (s, 2H, -CH$_2$-), 1.2-1.4 (m, 6H, -CH$_2$-CH$_2$-CH$_2$-), 0.88-0.92 (t, 3H, -CH$_3$), 7.15-8.00 (m, 8H, Ar-H).

Anal. calcd. for C$_{25}$H$_{27}$N$_3$O: C: 77.89, H: 7.06, N: 10.90. Found C: 77.91, H: 7.04, N: 10.89%.
4.1.3.2 Synthesis of 3-(2-bromo-4,5-dimethoxybenzyl)-2-butyl-1,3-diazaspiro[4,4]non-1-en-4-one (83b)

The product 83b was obtained from 2-butyl-1,3-diazaspiro[4,4]non-1-en-4-one 81 (1g, 5.147mmol), 1-Bromo-2-bromomethyl-4,5-dimethoxy-benzene 82b (1.914g, 6.174mmol) and potassium carbonate (3.557g, 25.74mmol).

IR $\nu_{max}$ (KBr): 3105, 2940, 1739, 1572, 1482, 1455, 1367 cm$^{-1}$.

$^1$H NMR (CDCl$_3$ 400 MHz) $\delta$: 1.51-1.81 (m, 8H, -cyclopentane), 4.34-4.36 (s, 2H, -CH$_2$-), 1.2-1.4 (m, 6H, -CH$_2$-CH$_2$-CH$_2$-), 0.88-0.92 (t, 3H, -CH$_3$), 3.81 (s, 6H-OCH$_3$), 6.46 (s,1H, Ar-H), 6.75 (s, 1H, Ar-H).

Anal. calcd. for C$_{20}$H$_{27}$BrN$_2$O$_3$: C: 56.74, H: 6.43, N: 6.62. Found C: 56.73, H: 6.43, N: 6.64%.

4.1.3.3 Synthesis of 2-butyl-3-(4-nitrobenzyl)-1,3-diazaspiro[4,4]non-1-en-4-one (83c)

The product 83c was obtained from 2-butyl-1,3-diazaspiro[4,4]non-1-en-4-one 81 (1g, 5.147mmol), 1-Bromomethyl-4-nitro-benzene 82c (1.334g, 6.174mmol) and potassium carbonate (3.557g, 25.74mmol).

IR $\nu_{max}$ (KBr): 3100, 2935, 1729, 1565, 1480, 1445, 1355 cm$^{-1}$.

$^1$H NMR (CDCl$_3$ 400 MHz) $\delta$: 1.51-1.81 (m, 8H, -cyclopentane), 4.34-4.36 (s, 2H, -CH$_2$-), 1.2-1.4 (m, 6H, -CH$_2$-CH$_2$-CH$_2$-), 0.88-0.92 (t, 3H, -CH$_3$), 7.15-7.18 (d, 4H, Ar-H).


4.1.3.4 Synthesis of 2-[2-(2-butyl-4-oxo-1,3-diazaspiro[4,4]non-1-en-3-yl)ethyl]isoindole-1,3-dione (83d)

The product 83d was obtained from 2-butyl-1,3-diazaspiro[4,4]non-1-en-4-one 81 (1g, 5.147mmol), 2-(2-chloro-ethyl)-isoindole-1,3-dione 82d (1.208g, 6.174mmol) and potassium carbonate (3.557g, 25.74mmol).

IR $\nu_{max}$ (KBr): 3099, 2932, 1734, 1578, 1495, 1465, 1365 cm$^{-1}$.

$^1$H NMR (CDCl$_3$ 400 MHz) $\delta$: 1.51-1.81 (m, 8H, -cyclopentane), 3.65-3.85 (t, 4H, -CH$_2$-CH$_2$-), 1.2-1.4 (m, 6H, -CH$_2$-CH$_2$-CH$_2$-), 0.88-0.92 (t, 3H, -CH$_3$), 7.72-8.35 (m, 4H, Ar-H).
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Anal. calcd. for C_{21}H_{25}N_{3}O_{3}: C: 68.64, H: 6.86, N: 11.44. Found C: 68.63, H: 6.87, N: 11.45%.

4.1.3.5 Synthesis of 2-buty1-3-(6-methylbenzo[1,3]dioxole-5-ylmethyl)-1,3-diaza
spiro[4,4]non-1-en-4-one (83e)

The product 83e was obtained from 2-buty1-1,3-diaza-spiro[4,4]non-1-en-4-one 81 (1g, 5.147mmol), 5-Chloromethyl-6-methyl-benzo[1,3]dioxole 82e (1.415g, 6.177mmol) and potassium carbonate (3.557g, 25.74mmol).

IR ν_{max} (KBr): 3095, 2930, 1735, 1574, 1480, 1465, 1369 cm^{-1}.

_{1}^{1}H NMR (CDCl_{3} 400 MHz) δ: 1.51-1.81 (m, 8H, -cyclopentane), 4.35-4.41 (s, 2H, -CH_{2}-), 1.2-1.4 (m, 6H, -CH_{2}-CH_{2}-CH_{2}-), 0.88-0.92 (t, 3H, -CH_{3}), 6.46 (s, 4H, Ar-H), 5.95 (s, 2H, 5.8 (s, 2H, -CH_{2}-)

Anal. calcd. for C_{20}H_{26}N_{2}O_{3}: C: 70.15, H: 7.65, N: 8.18. Found C: 70.13, H: 7.65, N: 8.19%.

4.1.3.6 Synthesis of 2-buty1-3-[2-(4-chlorophenyl)-2-oxoethyl]-1,3-diaza
spiro[4,4]non-1-en-4-one (83f)

The product 83f was obtained from 2-buty1-1,3-diaza-spiro[4,4]non-1-en-4-one 81 (1g, 5.147mmol), 2-Bromo-1-(4-chloro-phenyl)-ethanone 82f (1.442g, 6.175mmol) and potassium carbonate (3.557g, 25.74mmol).

IR ν_{max} (KBr): 3115, 2940, 1743, 1572, 1488, 1455, 1345 cm^{-1}.

_{1}^{1}H NMR (CDCl_{3} 400 MHz) δ: 1.51-1.81 (m, 8H, -cyclopentane), 4.35-4.41 (s, 2H, -CH_{2}-), 1.2-1.4 (m, 6H, -CH_{2}-CH_{2}-CH_{2}-), 0.88-0.92 (t, 3H, -CH_{3}), 7.65-7.82 (d, 4H, Ar-H).

Anal. calcd. for C_{19}H_{23}ClN_{2}O_{2}: C: 65.79, H: 6.68, N: 8.08. Found C: 65.78, H: 6.69, N: 8.10%.

4.1.3.7 Synthesis of 2-buty1-3-[2'-(1-trityl-tetrazol-5-yl)biphenyl-4-ylmethyl]-1,3-diaza
spiro[4,4]non-1-en-4-one (83g)

The product 83g was obtained from 2-buty1-1,3-diaza-spiro[4,4]non-1-en-4-one 81 (1g, 5.147mmol), 5-(4'-bromomethyl-biphenyl-2-yl)-1-trityl-1H-tetrazole 82g (3.443g, 6.175mmol) and potassium carbonate (3.557g, 25.74mmol).

IR ν_{max} (KBr): 3114, 2945, 1740, 1570, 1486, 1451, 1340 cm^{-1}.
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\(^1\)H NMR (CDCl\(_3\) 400 MHz) \(\delta\): 1.51-1.81 (m, 8H, -cyclopentane), 4.34-4.36 (s, 2H, -CH\(_2\)-), 1.2-1.4 (m, 6H, -CH\(_2\)-CH\(_2\)-CH\(_2\)-), 0.88-0.92 (t, 3H, -CH\(_3\)), 6.79-8.05 (m, 24H, Ar-H).

4.1.3.8 Synthesis of 4′-(2-butyl-4-oxo-1,3-diazaspiro[4,4]non-1-en-3-ylmethyl)-biphenyl-2-carboxylic acid methyl ester (83h)

The product 83h was obtained from 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one 81 (1g, 5.147mmol), 4′-Bromomethyl-biphenyl-2-carboxylic acid methyl ester 82h (1.885g, 6.176mmol) and potassium carbonate (3.557g, 25.74mmol).
IR \(\nu_{max}\) (KBr): 3105, 2937, 1748, 1576, 1467, 1465, 1354cm\(^{-1}\).

\(^1\)H NMR (CDCl\(_3\) 400 MHz) \(\delta\): 1.51-1.81 (m, 8H, -cyclopentane), 4.34-4.36 (s, 2H, -CH\(_2\)-), 1.2-1.4 (m, 6H, -CH\(_2\)-CH\(_2\)-CH\(_2\)-), 0.88-0.92 (t, 3H, -CH\(_3\)), 3.92-3.93 (s, 3H, -OCH\(_3\)), 7.11-8.00 (m, 8H, Ar-H).

4.1.3.9 Synthesis of 4′-(2-butyl-4-oxo-1,3-diazaspiro[4,4]non-1-en-3-ylmethyl)-biphenyl-2-carboxylic acid tert-butyl ester (83i)

The product 83i was obtained from 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one 81 (1g, 5.147mmol), 4′-Bromomethyl-biphenyl-2-carboxylic acid tert-butyl ester 82i (2.144g, 6.174mmol) and potassium carbonate (3.557g, 25.74mmol).
IR \(\nu_{max}\) (KBr): 3017, 2945, 1758, 1578, 1493cm\(^{-1}\).

\(^1\)H NMR (CDCl\(_3\) 400 MHz) \(\delta\): 1.51-1.81 (m, 8H, -cyclopentane), 4.34-4.36 (s, 2H, -CH\(_2\)-), 1.2-1.4 (m, 6H, -CH\(_2\)-CH\(_2\)-CH\(_2\)-), 0.88-0.92 (t, 3H, -CH\(_3\)), 0.97-1.00 (s, 9H, -C(CH\(_3\))\(_3\)), 7.12-7.89 (m, 8H, Ar-H).
Anal. calcd. for C\(_{29}\)H\(_{36}\)N\(_2\)O\(_3\): C: 75.62, H: 7.88, N: 6.08. Found C: 75.61, H: 7.87, N: 6.09%.
**Chapter 4**

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### 4.1.3.10 Synthesis of 3-(4-bromobenzyl)-2-butyl-1,3-diazaspiro[4,4]non-1-en-4-one (83j)

The product 83j was obtained from 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one 81 (1g, 5.147mmol), 1-bromo-4-bromomethyl-benzene 82j (1.544g, 6.178mmol) and potassium carbonate (3.557g, 25.74mmol).

IR ν\textsubscript{max} (KBr): 3098, 2941, 1740, 1579, 1478, 1462, 1345cm\textsuperscript{-1}.

\(^1\)H NMR (CDCl\textsubscript{3} 400 MHz) δ: 1.51-1.81 (m, 8H, -cyclopentane), 4.34-4.36 (s, 2H, -CH\textsubscript{2}-), 1.2-1.4 (m, 6H, -CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{2} -), 0.88-0.92 (t, 3H, -CH\textsubscript{3}), 7.10-7.45 (d, 4H, Ar-H).

Anal. calcd. for C\textsubscript{18}H\textsubscript{23}BrN\textsubscript{2}O: C: 59.51, H: 6.38, N: 7.71. Found C: 59.51, H: 6.39, N: 7.70%.

**Table 14: Reaction condition and physical data of spiroimidazol-4-one derivatives 83(a-j).**

<table>
<thead>
<tr>
<th>Spiroimidazol-4-ones</th>
<th>Reaction time</th>
<th>R\textsubscript{f} Value</th>
<th>Yield (%)</th>
<th>mp°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional (hrs)</td>
<td>MW irr (min)</td>
<td>Conventional</td>
<td>MW irr</td>
</tr>
<tr>
<td>83a</td>
<td>6</td>
<td>75</td>
<td>0.75</td>
<td>65</td>
</tr>
<tr>
<td>83b</td>
<td>5</td>
<td>65</td>
<td>0.62</td>
<td>60</td>
</tr>
<tr>
<td>83c</td>
<td>6</td>
<td>75</td>
<td>0.56</td>
<td>65</td>
</tr>
<tr>
<td>83d</td>
<td>6</td>
<td>65</td>
<td>0.62</td>
<td>62</td>
</tr>
<tr>
<td>83e</td>
<td>6</td>
<td>65</td>
<td>0.68</td>
<td>65</td>
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<td>0.59</td>
<td>60</td>
</tr>
<tr>
<td>83g</td>
<td>5</td>
<td>70</td>
<td>0.65</td>
<td>63</td>
</tr>
<tr>
<td>83h</td>
<td>5</td>
<td>65</td>
<td>0.58</td>
<td>63</td>
</tr>
<tr>
<td>83i</td>
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<td>65</td>
<td>0.49</td>
<td>65</td>
</tr>
<tr>
<td>83j</td>
<td>5</td>
<td>70</td>
<td>0.69</td>
<td>65</td>
</tr>
</tbody>
</table>
4.2 Pharmacology: Acetylcholinesterase inhibitory activity of spiroimidazol-4-ones (a-j)

[a] Materials and methods

The Materials and methods used for the inhibition of acetylcholinesterase are same as given in Chapter-2 (Section 2.6).

[b] Results

The results obtained from the inhibitory activities of spiroimidazol-4-one against different sources of AChE are depicted in the Table 15.

Table 15: Comparative inhibitory activities of spiroimidazol-4-one derivatives (a-j) against AChE from different sources

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rat brain Homogenate</th>
<th>Human serum</th>
<th>Electric Eel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC$_{50}$ (nM)</td>
<td>IC$_{50}$ (nM)</td>
<td>IC$_{50}$ (nM)</td>
</tr>
<tr>
<td>83a</td>
<td>90.00</td>
<td>82.50</td>
<td>90.00</td>
</tr>
<tr>
<td>83b</td>
<td>77.50</td>
<td>82.5</td>
<td>80.00</td>
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<tr>
<td>83c</td>
<td>133.6</td>
<td>135.4</td>
<td>149.2</td>
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<tr>
<td>83d</td>
<td>233.8</td>
<td>230.8</td>
<td>250.1</td>
</tr>
<tr>
<td>83e</td>
<td>216.2</td>
<td>213.5</td>
<td>212.4</td>
</tr>
<tr>
<td>83f</td>
<td>280.4</td>
<td>286.4</td>
<td>279.6</td>
</tr>
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<td>83g</td>
<td>356.5</td>
<td>383.5</td>
<td>373.2</td>
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<tr>
<td>83h</td>
<td>243.2</td>
<td>240.6</td>
<td>235.7</td>
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<td>268.8</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Neostigmine</td>
<td>35.00</td>
<td>45.00</td>
<td>47.50</td>
</tr>
</tbody>
</table>
Inhibition of Rat brain homogenate AChE by spiroimidazol-4-one derivatives

![Figure 24]

Inhibition of human serum AChE by spiroimidazol-4-one derivatives

![Figure 25]
4.3 Results and discussion

4.3.1 Chemistry

The compounds 83(a-j) were synthesized in two different methods viz., conventional and microwave irradiation. In comparison to conventional method, microwave heating offers more advantages such as reduced reaction time (50-60s), low cost, simplicity in processing, reduced pollution and high yield. In the present work, all the synthesized compounds were characterized by the spectral analysis (IR, \(^1\)H NMR) and elemental analysis.

The IR spectra of all the synthesized compounds 80, 81 and 83 (a-j) were recorded on KBr pellets in the range of 4000-400cm\(^{-1}\) and spectral data were given. The presence of a sharp absorption band in the region of 2220-2218cm\(^{-1}\) for CN (nitrile) and bending vibrations bands of primary amines in the region of 3500-3400cm\(^{-1}\) for primary amino group confirms the formation of the compound 80. The absence of CN peak, NH\(_2\) peak and the existence of C=O group peak at 1742cm\(^{-1}\) and CH\(_2\) bending absorption at 1455 cm\(^{-1}\) confirms the formation of compound 81. The presence of aromatic C=C stretching at 1575 and 1475cm\(^{-1}\) confirms the formation of the compound 83 (a-j).
4.3.2 $^1$H NMR spectral data

The $^1$H NMR spectra of the synthesized molecules 80, 81, and 83(a-j) were recorded and data are presented. The existence multiplet peaks in the range of $\delta$: 1.5-1.95 for cyclopentyl ring confirms the formation of compound 80. The presence of multiplet peaks in the range of $\delta$: 0.8-1.4 for open chain aliphatic group confirms the formation of the compound 81. The presence of singlet at 4.34-4.38 and multiplet in the range of $\delta$: 6.5-8.0 confirms the formation of products 83(a-j).

4.3.3 Acetylcholinesterase inhibition studies of spiroimidazol-4-one derivatives

The inhibitory studies of the newly synthesized compounds against AChE using different sources such as rat brain homogenate AChE, human serum AChE and electric eel AChE are as shown in Figures 24, 25 and 26 respectively. Activities of the synthesized compounds were compared with the inhibitory activity shown by the known standard inhibitor Neostigmine. The effects of different or versatile aromatic substituents are explored for their inhibitory activity against AChE. It can be deduced from the results that, the electron withdrawing atoms or the substituents (R) with less bulky groups shows greater activity. The order of potency is 83j > 83b > 83a.

The remaining substituents (83d, 83e, 83g, 83h and 83i) show weak inhibitory activity, probably because of the bulky hydrophobic ring and the absence of electron withdrawing atoms or groups. Among the molecules screened for the AChE inhibitory activity, the compound 83j (IC$_{50}$=70, 70 and 67.5nM) having bromo group at the 4$^{th}$ position shows highest AChE inhibitory activity. The substituent 83b and 83a having bromo atom on the 2$^{nd}$ position and cyano group on the biphenyl ring respectively are also effective in blocking the AChE enzyme activity (IC$_{50}$=77.5, 82.5, 80.00; 90.00, 82.5, 90.00nM respectively) (Table 15). But the compound 83g, 83h, 83i having biphenyl ring with bulky substituent did not show any inhibitory activity. Smaller the substituent, the more favorable activity at the biphenyl methyl site of the molecule (83a). The compound 83c and 83f shows moderate activity. This is probably due to the presence of electron withdrawing nitro group (83c) and chlorine atom at 4$^{th}$ position of the substituent and also because of the ester spacer at the 1$^{st}$ position of the substituent R. Compound 83d and 83e having fused rings did not show good inhibitory activity.
From the SAR studies, it is observed that the spiromidazol-one ring containing single or non-fused aromatic rings (83j, 83b and 83a) showed a better activity than the fused aromatic rings (83d, 83e). From the AChE inhibitory results, it may be concluded that, for an effective binding and blocking of the AChE activity, molecule needs to bind with the peripheral site and the active site of the enzyme and it may be possible that the spiromidazolone-4-one binds at the active site gorge and the different substituents bind to peripheral site separated by the spacer such as methylene or acyl or ester groups. Furthermore, the bulky n-butyl chain present on second position of the spiromidazol-4-one enhances the hydrophobicity may helps in binding to the active site of the enzyme. Therefore, it can be summarized that substitution of other smaller rings with smaller electron withdrawing groups on spiromidazol-4-one basic nucleus needs to be studied for better AChE inhibitory activity. Compound 83j is the most potent of all the compounds tested. This leads to the suggestion that compounds like 83j or further modified can lead to the development of a potent AChE inhibitor. The structures of the potent AChE inhibitor are shown in Figure 27.

![Figure 27: Structures of potent AChE inhibitor](image)

### 4.4 Conclusion

In summary, we have synthesized novel 2-butyl-1,3-diaza-spiro[4,4]non-1-en-4-one derivatives 83(a-j) under both conventional and microwave irradiation technique (solution phase). It is thus concluded that under microwave heating, the products 83(a-j) were conveniently and efficiently prepared in good yields, typically in the range of 80-85%. From AChE inhibitory activity data, it reveals that, the compounds 83j, 83b and 83a are active site-directed irreversible inhibitors of AChE activity. These results could aid in the design of new AChE inhibitors and give rise to new inhibitor design and synthesis, resulting in greater selectivity as well as an increase in new inhibitor potency.
References


