4. RESULTS AND DISCUSSION

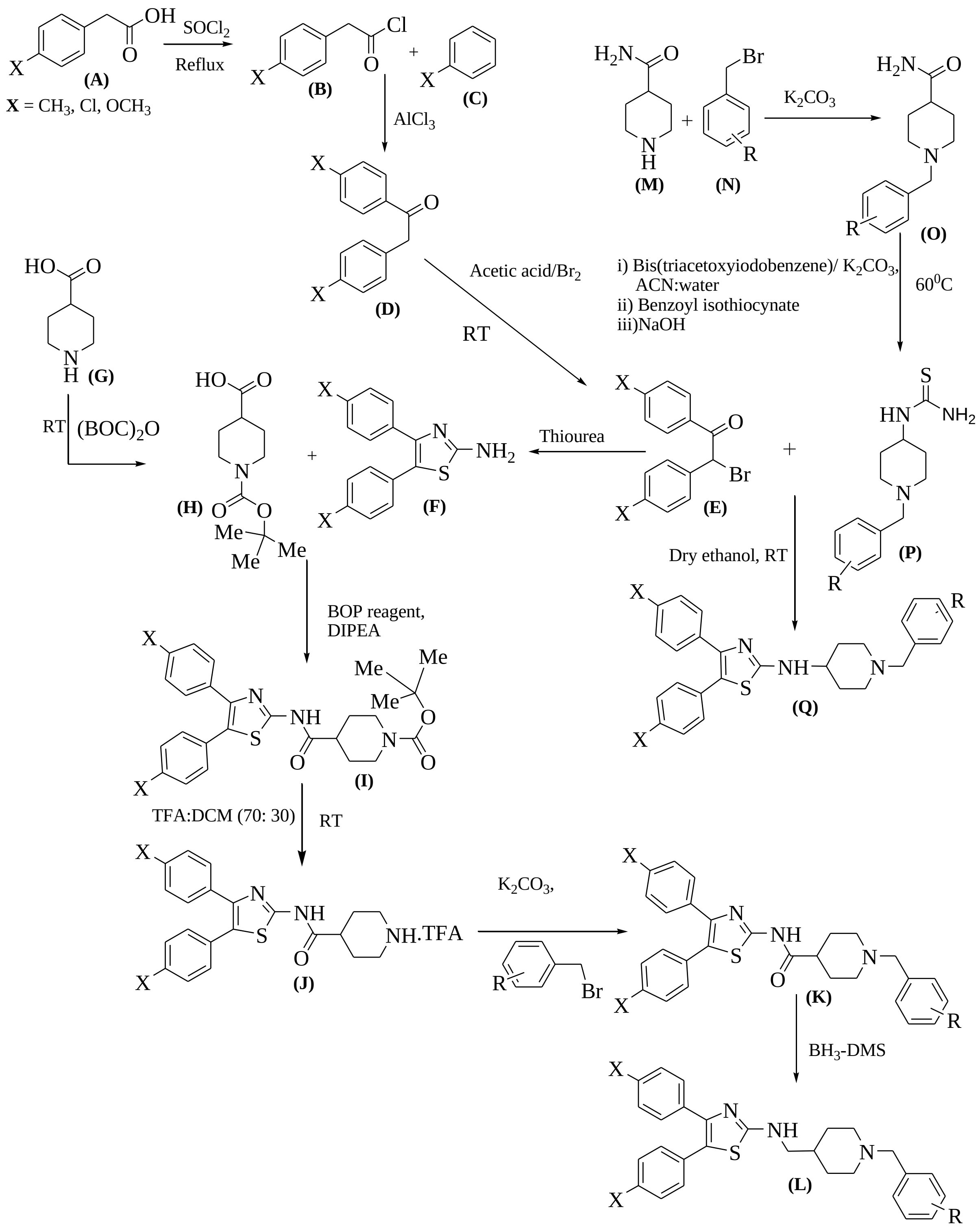
The work carried out towards achieving the proposed plan has been discussed under the following two main headings:

1. Chemical studies and
2. Biological studies

4.1 Chemical Studies

To synthesize the envisaged compounds, general scheme-1 was adopted, wherein 4-(substituted phenyl)acetic acids (**A**) were reacted with thionyl chloride to obtain 4-(substituted phenyl)acetyl chlorides **(B)**. The acetyl chloride derivatives **(B)** were reacted with 4-subsitityed benzenes **(C)** to get 1,2-di(4-substituted phenyl)ethanones **(D).** Bromination of 1,2-di(4-substituted phenyl)ethanones was carried out to obtain 2-bromo-1,2-di (4-substituted phenyl)ethanones **(E).** 2-Bromo-1,2-di(4-substituted phenyl)ethanones were cyclized to obtain 4,5-bis(substituted phenyl)thiazol-2-ylamines **(F).** Piperidine-4-carboxylic acid **(G)** was protected under the basic condition to obtain 1-(*t*.butoxycarbonyl)piperidine-4-carboxylic acid **(H)**. The coupling of 4,5-bis(substituted phenyl)thiazol-2-ylamines **(F)** and 1-(*t*.butoxycarbonyl)piperidine-4-carboxylic acid **(H)** offered *t*.butyl 4-[4,5-bis(substituted phenyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylates **(I)**. Deprotection of *t*.butyl 4-[4,5-bis(substituted phenyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylates **(I)** under acidic conditions offered *N*-[4,5-bis(substituted phenyl)thiazol-2-yl]piperidine-4-carboxamides **(J)**, which were further reacted with different substituted benzyl bromides in presence of potassium carbonate to obtain 1-(substituted benzyl)-*N*-[4,5-bis(4-substituted phenyl)thiazolyl]piperidine-4-caroxamides **(K)**, which were further reduced using borane dimethyl sulfide to get 1-(substituted benzyl)piperidin-4-yl-4,5-bis(substituted phenyl)thiazol-2-ylamines **(L)**.

4-Piperidine carboxamide **(M)** was reacted with substituted benzyl bromides **(N)** to obtain 1-(substituted benzyl)piperidine-4-carboxamides **(O)**, which were further treated with bis(trifluoroacetoxy)iodobenzene and benzoyl isothiocynate to obtain 1-[substituted benzyl)piperidin-4-yl]thioureas **(P)**. Reaction of 1-[substituted benzyl)piperidin-4-yl] thioureas **(P)** with 2-bromo-1,2-di(4-substituted phenyl)ethanones **(E)** yielded 1-(substituted benzyl)-*N*-[4,5-bis(4-substituted phenyl)thiazol-2-yl]piperidin-4-ylamines **(Q)**.



General Scheme 1

In order to execute scheme-1, 4-(substituted phenyl)acetic acids, piperidine-4-carboxylic acid and 4-subsitituted benzoic acids available commercially were used as such. The work related to the synthesis of 4-(substituted phenyl)acetyl chlorides **(B)**, 1,2-di(4-substituted phenyl)ethanones **(D)**, 2-bromo-1,2-di-(4-substituted phenyl)ethanones **(E)** and 4,5-bis(substituted phenyl)thiazol-2-ylamines **(F)** and also the intermediates **(I, J** and **P)** and the targeted products **(K, L** and **Q)** has been described under the following major heads:

4.1.1 Synthesis of substituted 1,2-di(4-substituted phenyl)ethanones **(4-6)**

4.1.2 Synthesis of 2-bromo-1,2-di(4-substituted phenyl)ethanones **(7-9)**

4.1.3 Synthesis of 4,5-bis(substituted phenyl)thiazol-2-ylamines **(10-12)**

4.1.4 Synthesis of 1-(*t*.butoxycarbonyl)piperidine-4-carboxylic acid **(13)**

4.1.5 Synthesis of *t*.butyl 4-[4,5-bis(substituted phenyl)thiazol-2-ylcarbamoyl] piperidine-1-carboxylates **(14-16)**

4.1.6 Synthesis of *N*-[4,5-bis(substituted phenyl)thiazol-2-yl]piperidine-4-carboxamides **(17- 19)**

4.1.7 Synthesis of 1-(substituted benzyl)-*N*-[4,5-bis(4-substituted phenyl)thiazolyl] piperidine-4-caroxamides **(20-45)**

4.1.8 Synthesis of *N*-[1-(substituted benzyl)piperidin-4-yl]-4,5-bis(substituted phenyl)thiazol-2-ylamines **(46-56)**

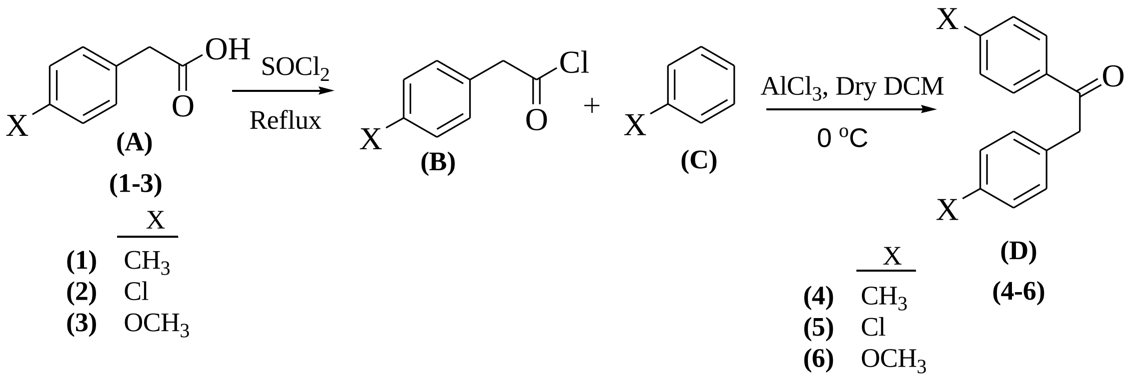
4.1.9 Synthesis of 1-(substituted benzyl)piperidine-4-carboxamides **(57-62)**

4.1.10 Synthesis of 1-[substituted benzyl)piperidin-4-yl]thioureas **(63-68)**

4.1.11 Synthesis of 1-(substituted benzyl)-*N*-[4,5-bis(4-substituted phenyl)thiazol-2-yl]piperidin-4-ylamines **(70-79)**

4.1.1 Synthesis of substituted 1,2-di(4-substituted phenyl)ethanones (4-6)

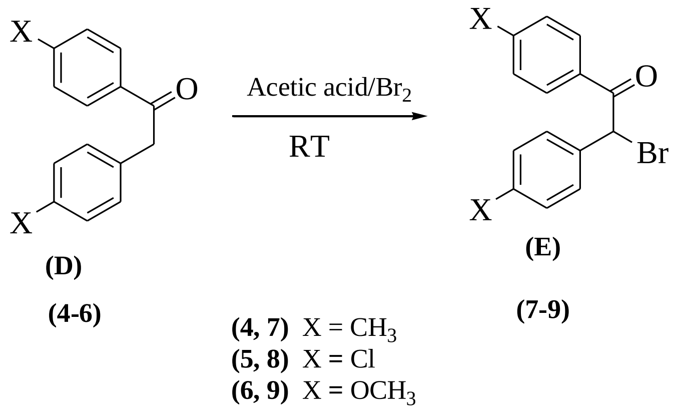
Following Scheme 2, 4-(substituted phenyl)acetic acids **(1-3)** were reacted with thionyl chloride to get 4-(substituted phenyl)acetyl chlorides **(B),** which were further reacted with 4-subsititued benzenes **(C)** to obtain 1,2-di(4-substituted phenyl)ethanones (**4-6**) by using Friedel-Craft acylation in the presence of anhydrous aluminum chloride at 0^oC. In the IR spectrum of compound **(4)**, the C=O stretching of carbonyl was seen at 1688 cm^{-1}. Its mass spectrum showed molecular ion peak at 224.61 $(M)^+$. In the IR spectrum of compound **(5)**, the C=O stretching of carbonyl was seen 1689 cm^{-1}. Its mass spectrum showed molecular ion peak at 265.14 $(M)^+$. In the IR spectrum of compound **(6)**, the C=O stretching of carbonyl was seen at 1689 cm^{-1}. Its mass spectrum showed molecular ion peak at 256.38 $(M)^+$.



Scheme-2

4.1.2 Synthesis of 2-bromo-1,2-di(4-substituted phenyl)ethanones (7-9)

1,2-Di(4-substituted phenyl)ethanones (**4-6**) were subjected for free radical bromination using bromine and acetic acid to obtain 2-bromo-1,2-di(4-substituted phenyl)ethanone **(7-9)** (Scheme-3). In the IR spectrum of compound **(7)**, the C=O stretching of carbonyl was seen at 1676 cm^{-1}. Its mass spectrum showed molecular ion peak at 303.29 $(M)^+$. In the IR spectrum of compound **(8),** the C=O stretching of carbonyl was seen at 1680 cm^{-1}. Its mass spectrum showed molecular ion peak at 344.77 $(M)^+$. In the IR spectrum of compound **(9),** the C=O stretching of carbonyl was seen at 1658 cm^{-1}. Its mass spectrum showed molecular ion peak at 335.31 $(M)^+$.

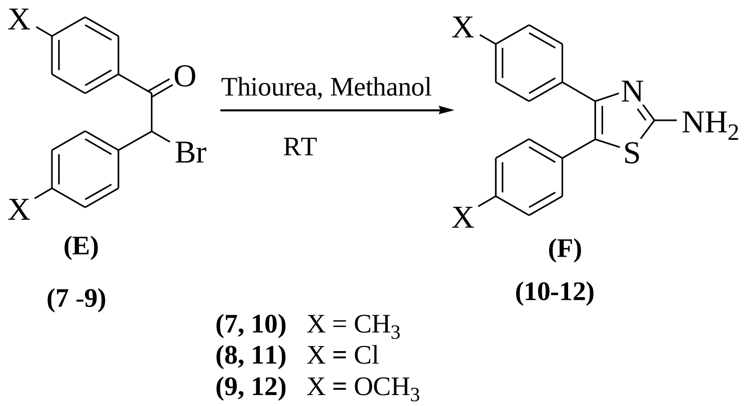


Scheme-3

4.1.3 Synthesis of 4,5-bis(substituted phenyl)thiazol-2-ylamine (10-12)

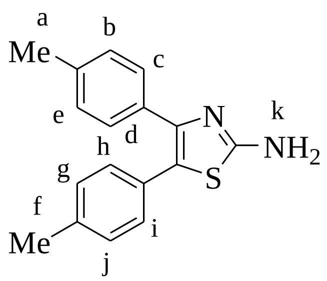
Scheme-4 was followed for the synthesis of the desired intermediates **(10-12)**. 2-Bromo-1,2-di(4-substituted phenyl)ethanones (**7-9**) were reacted with thiourea using methanol as a solvent to get the compounds 4,5-bis(substituted phenyl)thiazol-2-ylamines

(10-12). IR spectrum of compound **(10)** showed N-H stretching vibrations as two strong peaks at 3436 and 3250 cm^{-1} and N-H deformation as moderate peak at 1615 cm^{-1}. Its PMR



Scheme-4

spectrum showed aromatic protons at δ 7.34-7.32 (d, $2H_{c,d}$) as doublet which coupled with proton ($CH_{b,e}$) observed at δ 7.05-7.03 as a doublet with a coupling constant of 8.0 Hz. Other signals were observed at δ 7.16-7.14 (d, $2H_{h,i}$) as doublet which coupled with proton ($CH_{g,j}$) observed at δ 7.05-7.03 as a doublet with coupling constant of 8.0 Hz for the other aromatic



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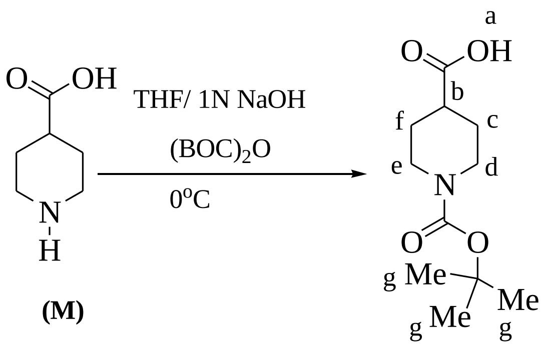
protons. A broad singlet was observed at δ 5.49 (bs, *N*-H_k) due to the protons attached to amine nitrogen. The aliphatic protons were observed at δ 2.31 as a singlet for six protons due to the methyl groups on diaryl moiety. Its mass spectrum showed molecular ion peak at 280.19 $(M)^+$. In the IR spectrum, compound **(11)** showed N-H stretching vibrations as two strong peaks at 3444 and 3274 cm^{-1} and N-H deformation as moderate peak at 1630 cm^{-1}. Its mass spectrum showed molecular peak at 321.32 $(M)^+$. In the IR spectrum, compound **(12)** showed N-H stretching vibrations as two strong peaks at 3390 and 3292 cm^{-1} and N-H deformation as a moderate peak at 1638 cm^{-1}. Its mass spectrum showed molecular ion peak at 312.24 $(M)^+$.

4.1.4 Synthesis of 1-(*t*.butoxycarbonyl)piperidine-4-carboxylic acid (13)

In order to protect the secondary amine, piperidine-4-carboxylic **(M)** was reacted with di-*t*.butyl dicarbonate using 1N/NaOH and THF at $0^{o}C$ to get 1-(*t*.butoxycarbonyl)piperidine-4-carboxylic acid **(13)** as per Scheme-5. In the IR spectrum of compound **(13),** the C=O stretching of carbamate was observed at 1736 cm^{-1}. The peak at 1660 cm^{-1} was due to the C=O stretching of the acid. Its PMR spectrum showed a broad singlet at δ 10.37 (bs, $1H_a$) due to proton attached to carboxylic group. Aliphatic protons were observed at δ 4.01 (bs 2H), 2.89-2.83 (t, 2H), 2.51-2.45 (m, 1H), 1.92-1.89 (m, 2H) and 1.69-1.59 (m, 2H) The other signal was observed at 1.46 (s, $9H_g$) for nine protons due to *t*.butyl group. Its mass spectrum showed molecular peak at 229.05 $(M)^+$.

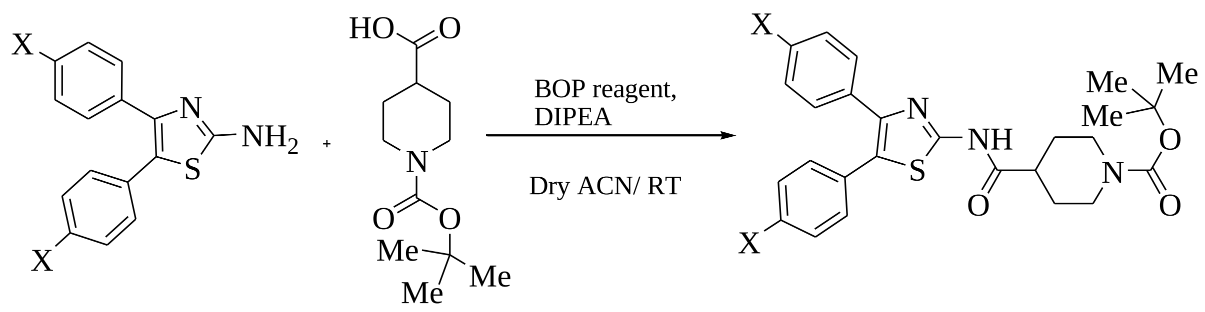


Scheme-5

4.1.5 Synthesis of *t*.butyl 4-[4,5-bis(substituted phenyl)thiazol-2-ylcarbamoyl] piperidine-1-carboxylates (14-16)

In the scheme 6, 4,5-bis(substituted phenyl)thiazol-2-yl-amines **(10-12)** were treated with 1-(*t*.butyloxycarbonyl)piperidine-4-carboxylic acid **(13)** in presence of *N*,*N*-diisopropylethylamine and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) as the coupling reagent under basic conditions to get *t*.butyl 4-[4,5-bis(substituted phenyl)thiazol-2-ylcarbamoyl]piperidine-1-carboxylates **(14-16)**.

In the IR spectrum of compound **(14)**, the C=O stretching of the amide was observed at 1688 cm^{-1}. Its mass spectrum showed molecular ion peak at 491.07 $(M)^+$. In the IR spectrum compound **(15)**, the C=O stretching of the amide and carbamate were observed at 1687 cm^{-1}. Its mass spectrum showed molecular ion peak at 532.00 $(M)^+$ as the base peak. In the IR spectrum of compound **(16)**, the C=O stretching of the amide and the carbamate were



(10-12) **(13)** **(14-16)**

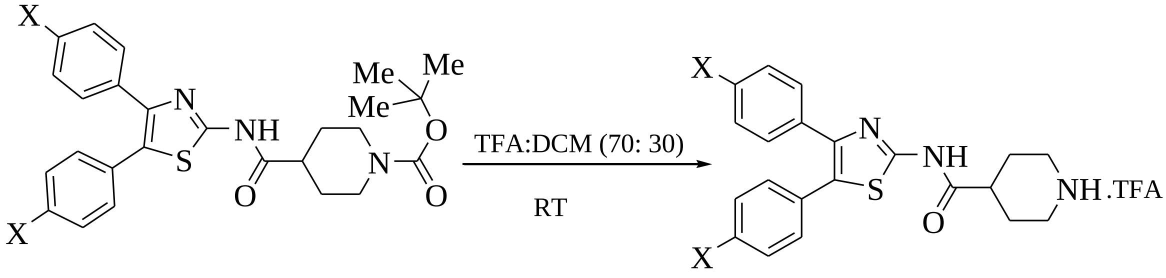
(10, 14) X = CH_3
(11, 15) X = Cl
(12, 16) X = OCH_3

Scheme-6

observed at 1691 cm^{-1}. Its mass spectrum showed molecular ion peak at 523.14 $(M)^+$ as the base peak.

4.1.6 Synthesis of *N*-[4,5-bis(substituted phenyl)thiazol-2-yl]piperidine-4-carboxamides (17-19)

In the Scheme-7, *t*.butyl 4-[4,5-bis(substituted phenyl) thiazol-2-ylcarbamoyl] piperidine-1-carboxylates **(14-16)** were reacted with trifluroacetic acid and DCM to get *N*-[4,5-bis(substituted phenyl)thiazol-2-yl]piperidine-4-carboxamides **(17-19)**. In the IR spectrum of compound **(17)**, the C=O stretching of the amide was observed at 1674 cm^{-1}. Its mass spectrum showed molecular ion peak at 391.33 $(M)^+$. In the IR spectrum of compound **(18)**, the C=O stretching of the amide was observed at 1677 cm^{-1}. Its mass spectrum showed molecular ion peak at 431.70 $(M)^+$. In the IR spectrum of compound **(19)**, the C=O stretching of amide was observed at 1675 cm^{-1}. Its mass spectrum showed molecular ion peak at 423.17 $(M)^+$.



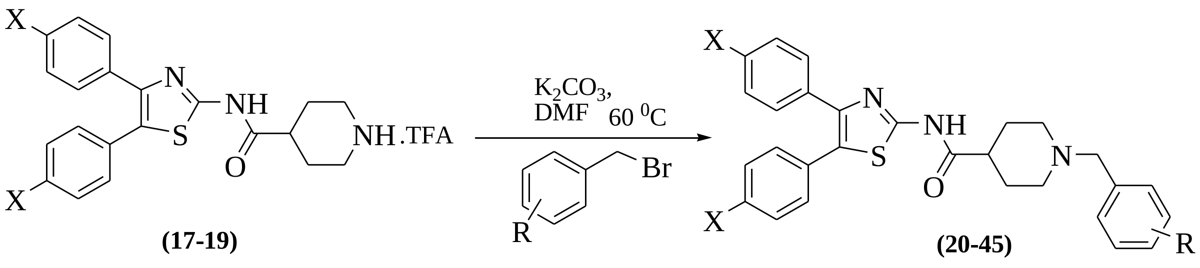
(14-16) **(17-19)**

(14, 17) X = CH_3
(15, 18) X = Cl
(16, 19) X = OCH_3

Scheme-7

4.1.7 Synthesis of 1-(substituted benzyl)-*N*-[4,5-bis(4-substituted phenyl)thiazol-2-yl] piperidine-4-carboxamides (20-45)

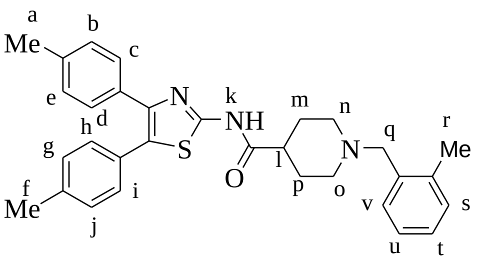
For *N*-substituted derivatives, *N*-[4,5-bis(substituted phenyl)thiazol-2-yl]piperidine-4-carboxamides **(17-19)** were reacted with substituted benzyl bromides using potassium carbonate and DMF to afford the desired compounds **(20-45)** (Scheme-8).



(17, 20-29) X = CH_3
(18, 30-37) X = Cl
(19, 38-45) X = OCH_3

Scheme-8

In the IR spectrum of compound **(20)**, C=O stretching of the amide was observed at 1683 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 11.05 (s, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons ($CH_{c,d}$) were observed at δ 7.40-7.38 as doublet which coupled with protons ($CH_{b,e}$) observed at δ 7.26-7.24 as a doublet with coupling constant of 8.1 Hz whereas the other aromatic protons were observed at δ 7.18-7.07 (m, $8H_{h,i,g,j,s,t,u,v}$) for eight protons. Among the aliphatic protons signals were obtained at δ 3.33 (s, $2H_q$) for two protons, δ 2.72-2.70 (d, $2H_{n,o}$) for two protons with coupling constant of 9.3 Hz. A singlet at δ 2.36 (s, $2H_r$) for three protons due to the methyl group on aromatic moiety, a singlet at δ 2.30 (s, $6H_{a,f}$) for six protons to the methyl groups attached on diaryl



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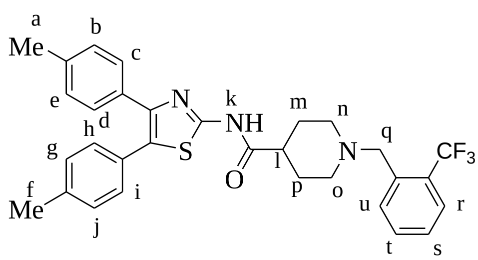
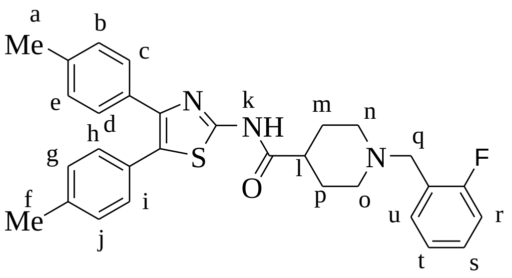


(21)

moiety. Other signals were observed at δ 1.68-1.65 (m, 5H) and δ 1.49-1.46 (d, $2H_{m,p}$) for two protons with coupling constant of 9.3 Hz. Its ^{13}C-NMR spectrum showed peak at δ 173.37 due to C=O carbon of the amide. Aromatic carbons appeared at δ 156.54, 143.60, 137.69, 137.66, 137.54, 131.95, 130.26, 129.72, 129.49, 129.35, 129.20, 129.13, 128.80, 127.01, 126.60 and 125.45, whereas the aliphatic carbons appeared at δ 60.94, 52.62, 29.72, 28.51, 21.26 and 19.23. Its mass spectrum showed molecular ion peak at 495.85 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 5.95 min retention time.

In the IR spectrum of compound **(21),** the C=O stretching of the amide was observed at 1685 cm^{-1}. Its PMR spectrum offered a broad singlet at δ 11.74 (s, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons were observed at δ 7.74-7.72 (d, 1H, *J* = 8.0 Hz), 7.59-7.57 (d, 1H, *J* = 8.0 Hz), 7.49-7.42 (m, 3H), 7.30-7.25 (m, 3H), 7.15-7.13 (d, 2H, *J* = 8.0 Hz) and 7.11-7.09 (d, 2H, *J* = 8.0 Hz). Among the aliphatic protons a singlet was observed at δ 3.49 (s, $2H_q$) for two protons and δ 2.64-2.62 (d, $2H_{n,o}$) for two protons with coupling constant of 8.6 Hz. Signals were obtained at δ 2.37 (s, $3H_a$) and δ 2.28 (s, $3H_f$) as singlets for six protons due to the methyl groups on the diaryl moiety. Other signals appeared at δ 1.67-1.58 (m, 4H), δ 1.54-1.51 (m, 1H) and δ 1.38-1.36 (d, $2H_{m,p}$) for two protons with coupling constant of 8.6 Hz. Its ^{13}C-NMR spectrum showed peak at δ 173.84 due to C=O carbon of the amide. Aromatic carbons appeared at δ 157.52, 143.44, 138.09, 137.85, 137.77, 131.86, 131.77, 130.13, 129.54, 129.35, 129.04, 128.93, 128.22, 126.61, 126.54, 125.55 and 125.50, whereas the aliphatic carbons appeared at δ 58.13, 52.55, 41.99, 28.45 and 21.22. Its mass spectrum showed molecular ion peak at 549.80 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 7.01 min retention time.

In the IR spectrum of compound **(22),** the C=O stretching of the amide was observed at 1681 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 11.68 (s, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons at ($CH_{c,d}$) were observed at δ 7.39-7.37 as doublet which coupled with protons ($CH_{b,e}$) observed at δ 7.15-7.13 as a doublet with a coupling constant of 8.0 Hz. Protons ($CH_{h,i}$) were observed at δ 7.26-7.24 as doublet which coupled with protons ($CH_{g,j}$) observed at δ 7.07-7.05 as a doublet with a coupling constant of 8.1 Hz. Other aromatic protons were observed at δ 7.21-7.19 (m, $1H_t$) for one proton, δ 6.98-6.95 (m, $2H_{\boldsymbol{u,s}}$) for two protons and δ 6.93-6.88 (m, 1H). Among the aliphatic protons, a singlet was observed at δ 3.35 (s, $2H_q$) for two protons and δ 2.66-2.64 (d, $2H_{n,o}$) for two protons with coupling constant of 11.5 Hz. Signals were obtained at δ 2.37 (s, $3H_a$) and δ 2.29 (s, $3H_f$) as singlets for six protons due to the methyl groups attached to the diaryl moiety. Other signals were observed at δ 1.65-1.49 (m, 5H) and δ 1.39-1.36 (d, $2H_{m,p}$) for two protons with coupling constant of 11.5 Hz). Its ^{13}C-NMR spectrum showed peak at δ 173.72 due to C=O carbon of the amide. Aromatic carbons appeared at δ 164.12, 161.67, 157.50, 143.37, 141.02, 140.95, 137.86, 131.86, 129.60, 129.33, 129.28, 129.05, 128.90, 126.50, 124.43, 115.76 and 113.74, whereas the aliphatic carbons appeared at δ 62.43, 52.22, 41.90, 28.27 and 21.25. Its mass spectrum showed molecular ion peak at 499.47 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 5.93 min retention time.



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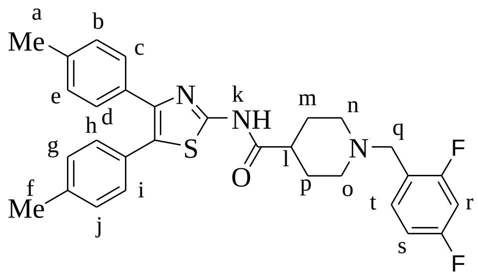

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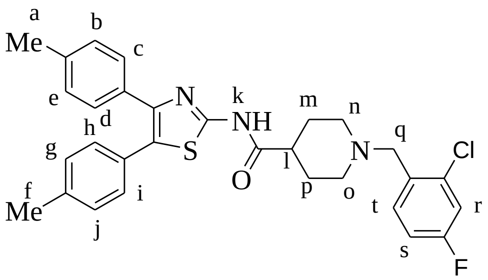
In the IR spectrum of compound **(23)**, the C=O stretching of the amide was observed at 1686 cm^{-1}. Its PMR spectrum offered peak for a broad singlet at δ 11.48 (s, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons at ($CH_{c,d}$) were observed at δ 7.40-7.38 as doublet which coupled with protons ($CH_{b,e}$) observed at δ 7.17-7.13 as a doublet with a coupling constant of 8.1 Hz. Protons ($CH_{h,i}$) were observed at δ 7.26-7.24 as doublet which coupled with protons ($CH_{g,j}$) observed at δ 7.09-7.07 as doublet with coupling constant of 8.0 Hz. Other aromatic peaks were observed at δ 6.81-6.77 (m, 2H) and δ 6.67-6.62 (m, 1H). Among the aliphatic protons signals were obtained at δ 3.33 (s, $2H_q$) for two protons, δ 2.66-2.63 (d, $2H_{n,o}$) for two protons with coupling constant 9.8 Hz. Signals were obtained at δ 2.39 (s, $3H_a$) and δ 2.30 (s, $3H_f$) as singlets for six protons due to the methyl groups attached to the diaryl moiety. Other signals were observed at δ 1.70-1.54 (m, 5H) and δ 1.43-1.37 (d, $2H_{m,p}$) for two protons with coupling constant of 9.8 Hz. Its ^{13}C-NMR spectrum showed peak at δ 173.69 due to C=O carbon of the amide. Aromatic carbons appeared at δ 164.20, 161.74, 157.59, 143.35, 142.92, 142.78, 137.80, 131.87, 130.80, 129.32, 129.02, 128.54, 126.54, 111.16 and 102.30 whereas the aliphatic carbons appeared at δ 62.13, 52.26, 41.79, 29.37 and 21.28. Its mass spectrum showed molecular ion peak at 517.10 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 6.21 min retention time.

In the IR spectrum of compound **(24)**, the C=O stretching of the amide was observed at 1679 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 11.16 (s, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons were observed at 7.40-7.38 (d, 3H, J = 8.0 Hz), 7.26-7.24 (m, 2H), 7.15-7.13 (d, 2H, J = 8.0 Hz), 7.09-7.07 (m, 3H) and 6.94-6.90

(m, 1H). Among the aliphatic protons a singlet was observed at δ 3.45 (s, $2H_q$) for two protons and δ 2.72-2.70 (d, $2H_{n,o}$) for two protons with coupling constant of 9.8 Hz. Signals were observed at δ 2.37 (s, $3H_a$) and δ 2.30 (s, $3H_f$) as singlets for six protons due to the methyl groups attached to the diaryl moiety. Other signals were observed at δ 1.75-1.67 (m, 7H). Its ^{13}C-NMR spectrum showed peak at δ 173.52 due to C=O carbon of the amide. Aromatic carbons appeared at δ 157.15, 143.47, 137.77, 134.59, 134.48, 131.89, 131.59, 131.50, 129.52, 129.33, 129.05, 128.87, 126.58, 116.66, 116.41, 113.94 and 113.73 whereas the aliphatic carbons appeared at δ 58.48, 52.34, 42.07, 28.37 and 21.25. Its mass spectrum showed molecular ion peak at 533.04 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.68 min retention time.



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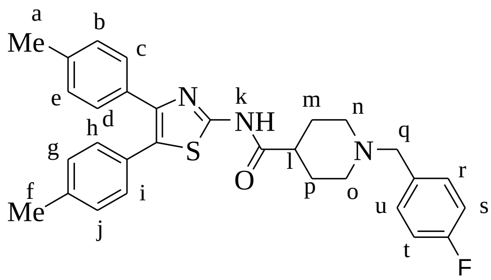
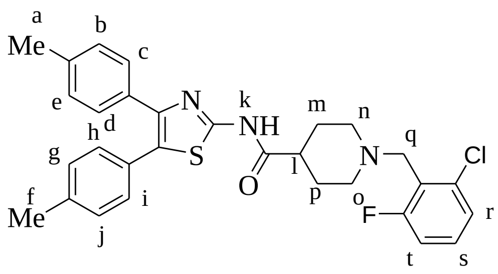


(25)

In the IR spectrum of compound **(25)**, the C=O stretching of amide was observed at 1675 cm^{-1}.

In the IR spectrum of compound **(26)**, the C=O stretching of the amide was observed at 1680 cm^{-1}. Its PMR spectrum offered a broad singlet at δ 11.35 (s, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons at ($CH_{c,d}$) were observed at δ 7.35-7.33 as doublet which coupled with protons ($CH_{b,c}$) observed at δ 7.15-7.13 as a doublet with coupling constant of 8.0 Hz. Protons ($CH_{h,i}$) were observed at δ 7.26-7.24 as doublet which coupled with protons ($CH_{g,j}$) observed at δ 7.15-7.13 as a doublet with a coupling constant of 8.0 Hz. Other peaks for the aromatic protons were observed at δ 7.04-7.02 (d, $2H_{r,t}$) for two protons with a coupling constant of 8.0 Hz and δ 6.95-6.91 (m, $1H_s$) for one proton. Among the aliphatic protons signals were observed at δ 3.60 (s, $2H_q$) for two protons and δ 2.77-2.74 (d, $2H_{n,o}$) for two protons with a coupling constant of 11.1 Hz. Other signals were observed at δ 2.37 (s, $3H_a$ and δ 2.29 (s, $3H_f$) as singlets for six protons due to the methyl groups attached to the diaryl moiety. Signals were also observed at δ 1.77-1.52 (m, 3H), δ 1.65-1.62 (m, 2H) and δ 1.42-1.39 (d, $2H_{m,p}$) for two protons with coupling constant of 11.1 Hz. Its ^{13}C-NMR spectrum showed peak at δ 173.66 due to C=O carbon of the amide. Aromatic carbons appeared at δ 163.31, 160.84, 157.39, 143.39, 137.75, 137.66, 136.73, 136.67, 131.86, 129.49, 129.07, 128.86, 126.45, 125.40, 123.91, 114.01 and 113.77 whereas the aliphatic carbons appeared at δ 52.34, 51.87, 41.90, 28.27 and 21.25. Its mass spectrum showed molecular ion peak at 533.24 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.69 min retention time.



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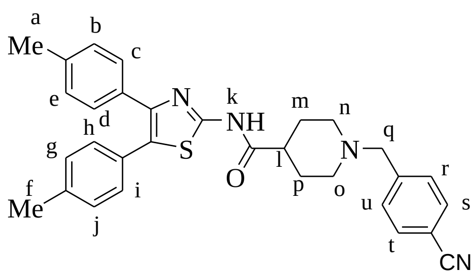
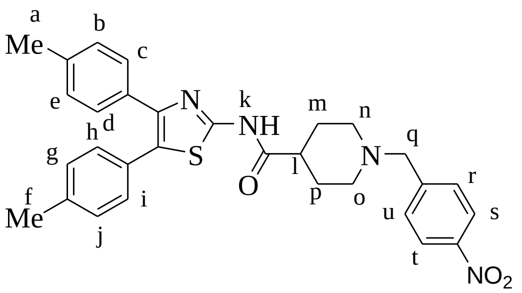


(27)

In the IR spectrum of compound **(27)**, C=O stretching of the amide was observed at 1659 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 11.46 (bs, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons ($CH_{s,t}$) were observed at δ 7.58-7.56 as doublet which coupled with protons ($CH_{u,r}$) observed at δ 7.26-7.24 as doublet with a coupling constant of 8.0 Hz. Protons ($CH_{c,d}$) were observed at δ 7.39-7.37 as doublet which coupled with protons ($CH_{b,e}$) observed at δ 7.15-7.13 as a doublet with a coupling constant of 8.0 Hz. Other peaks for aromatic protons ($CH_{h,i}$) were observed at δ 7.38-7.36 as doublet which coupled with protons ($CH_{g,j}$) observed at δ 7.08-7.06 as a doublet with a coupling constant of 8.0 Hz. Among the aliphatic protons, a singlet was observed at δ 3.52 (s, $2H_q$) for two protons and δ 2.66-2.64 (d, $2H_{n,o}$) for two protons with a coupling constant of 8.6 Hz. Signals were obtained at δ 2.38 (s, $3H_a$) and δ 2.29 (s, $3H_f$) as singlets for six protons due to the methyl groups attached to the diaryl moiety. Other signals were observed at δ 1.66-1.65 (m, 5H) and δ 1.50 (bs, 2H). Its ^{13}C-NMR spectrum showed peak at δ 173.24 due to C=O carbon of the amide. Aromatic carbons appeared at δ 156.83, 143.48, 137.82, 137.77, 132.11, 131.86, 129.54, 129.35, 129.30, 129.23, 129.00, 128.82, 118.90, 116.25 and 111.01 whereas the aliphatic carbons appeared at δ 62.44, 52.46, 42.03, 29.72, 28.30 and 21.29. Its mass spectrum showed molecular ion peak at 506.92 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 5.97 min retention time.

In the IR spectrum of compound **(28)**, C=O stretching of the amide was observed at 1680 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 11.75 (bs, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons ($CH_{s,t}$) were observed at δ 8.14-8.12 as doublet which coupled with protons ($CH_{u,r}$) observed at δ 7.26-7.24 as doublet with a coupling constant of 8.6 Hz. Protons ($CH_{c,d}$) were observed at δ 7.43-7.38 as doublet which coupled with protons ($CH_{b,e}$) observed at δ 7.16-7.14 as doublet with a coupling constant of 8.0 Hz. Other peaks for the aromatic protons ($CH_{h,i}$) were observed at δ 7.43-7.38 as doublet which coupled with protons ($CH_{g,j}$) observed at δ 7.08-7.06 as doublet with a coupling constant of 8.0 Hz. Among the aliphatic protons signals were observed at δ 3.47 (s, $2H_q$) for two protons and δ 2.64-2.62 (d, $2H_{n,o}$) for two protons with coupling constant of 6.4 Hz. Signals were obtained at δ 2.38 (s, $3H_a$) and δ 2.29 (s, $3H_f$) as singlets for six protons due to the methyl groups attached to the diaryl moiety. Other signals were at δ 1.66-1.59 (m, 4H), δ 1.54-1.52 (d, 1H) and δ 1.27-1.24 (d, 2H). Its ^{13}C-NMR spectrum showed peak at δ 173.44 due to C=O carbon of the amide. Aromatic carbons appeared at δ 157.22, 147.13, 146.39, 143.39, 137.86, 137.82, 131.84, 129.56, 129.32, 129.29, 128.97, 128.87, 126.61, 125.25 and 123.51, whereas the aliphatic carbons appeared at δ 62.14, 52.42, 41.80, 28.28 and 21.29. Its mass spectrum showed molecular ion peak at 527.2 $(M+1)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 6.06 min retention time.



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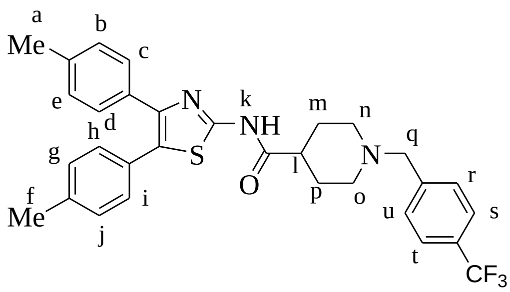
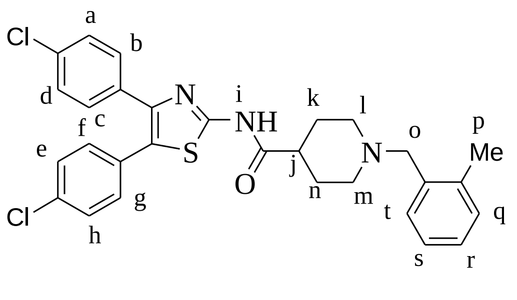


(29)

In the IR spectrum of compound **(29)**, C=O stretching of the amide was observed at 1687 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 11.48 (s, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons ($CH_{c,d}$) were observed at δ 7.38-7.36 as doublet which coupled with protons ($CH_{b,e}$) observed at δ 7.15-7.10 as doublet with a coupling constant of 8.0 Hz. Protons ($CH_{h,i}$) were observed at δ 7.26-7.24 as doublet which coupled with protons ($CH_{g,j}$) observed at δ 7.15-7.10 as doublet with a coupling constant of 8.0 Hz. Other signals for the aromatic protons ($CH_{s,t}$) were observed at δ 7.26-7.24 as doublet which coupled with protons ($CH_{r,u}$) observed at δ 7.05-7.03 as a doublet with a coupling constant of 8.0 Hz. Among the aliphatic protons signals were observed at δ 3.37 (s, $2H_q$) for two protons and δ 2.66-2.64 (d, $2H_{n,o}$) for two protons with a coupling constant of 9.7 Hz. Signals were observed at δ 2.37 (s, $3H_a$) and δ 2.28 (s, $3H_b$) as singlets for six protons due to the methyl groups attached to the diaryl moiety. Other signals were observed at δ 1.55-1.26 (m, 5H) and δ 1.41-1.39 (d, $2H_{m,p}$) for two protons with coupling constant of 9.7 Hz. Its ^{13}C-NMR spectrum showed peak at δ 173.68 due to C=O carbon of the amide. Aromatic carbons appeared at δ 157.47, 148.23, 143.36, 137.79, 136.92, 131.85, 130.18, 129.53, 129.31, 129.26, 129.04, 128.90, 126.50, 120.68 and 119.21, whereas the aliphatic carbons appeared at δ 62.10, 52.13, 41.89, 28.27 and 21.23. Its mass spectrum showed molecular ion peak at 549.52 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 7.01 min retention time.

In the IR spectrum of compound **(30)**, C=O stretching of the amide was observed at 1688 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 10.13 (s, $1H_i$) due to the proton attached to the amidic nitrogen. The aromatic protons ($CH_{b,c}$) were observed at δ 7.41-7.38 as doublet which coupled with protons ($CH_{a,d}$) observed at δ 7.28-7.26 as doublet with a coupling constant of 8.5 Hz. Protons ($CH_{f,g}$) were observed at δ 7.32-7.30 as doublet which coupled with protons ($CH_{e,h}$) observed at δ 7.28-7.26 as doublet with a coupling constant of 8.5 Hz. Other signals for the aromatic protons (CH_q) were observed at δ 7.21-7.19 as doublet with coupling constant of 6.7 Hz and δ 7.16-7.12 (m, $3H_{r,s,t}$) for three protons. Among the aliphatic protons, a singlet was observed at δ 3.39 (s, $2H_o$) for two protons and δ 2.84-2.81 (d, $2H_{l,m}$) for two protons with a coupling constant of 11.2 Hz. Other signals were obtained at δ 2.33 (s, $3H_p$) as a singlet for three protons due to the methyl group on the aromatic ring. Other signals were observed at δ 1.99-1.93 (m, 1H), δ 1.85-1.77 (m, 2H) and δ 1.75-1.65 (m, 4H). Its ^{13}C-NMR spectrum showed peak at δ 173.08 due to C=O carbon of the amide. Aromatic carbons appeared at δ 156.48, 143.29, 137.57, 136.36, 134.15, 134.03, 132.92, 130.72, 130.32, 130.24, 130.12, 129.75, 129.22, 128.81, 127.09, 126.23 and 125.48 whereas the aliphatic carbons appeared at δ 60.97, 52.71, 42.95, 28.54 and 19.23. Its mass spectrum showed molecular ion peak at 535.93 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 6.04 min retention time.



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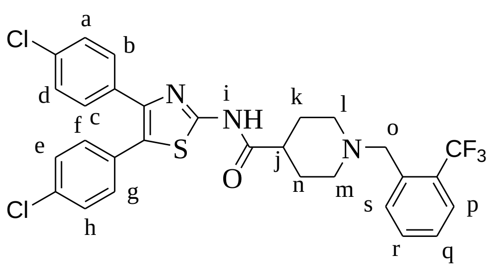
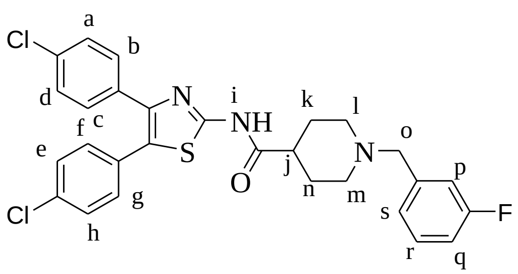


(31)

In the IR spectrum of compound **(31)**, the C=O stretching of the amide was observed at 1690 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 10.62 (s, $1H_i$) due to the proton attached to the amidic nitrogen. The aromatic protons were observed at 7.75-7.73 (d, 1H, *J* = 7.8 Hz), 7.61-7.59 (d, 1H, *J* = 7.8 Hz), 7.51-7.43 (m, 1H), 7.41-7.40 (m, 2H) and 7.34-7.24 (m, 7H). Among the aliphatic protons, a singlet was observed at δ 3.56 (s, $2H_o$) for two protons and δ 2.79-2.76 (d, $2H_{l,m}$) for two protons with a coupling constant of 10.6 Hz. Other signals were observed at δ 1.89-1.71 (m, 5H) and δ 1.62-1.58 (d, 2H). Its mass spectrum showed molecular ion peak at 589.33 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 5.30 min retention time.

In the IR spectrum of compound **(32)**, the C=O stretching of the amide was observed at 1681 cm^{-1}. Its PMR spectrum offered a broad singlet at δ 12.25 (s, $1H_i$) due to the proton attached to the amidic nitrogen. The aromatic protons ($CH_{b,c}$) were observed at δ 7.42-7.39 as doublet which coupled with protons ($CH_{a,d}$) observed at δ 7.35-7.33 as doublet with a coupling constant of 8.5 Hz. Protons ($CH_{f,g}$) were observed at δ 7.29-7.27 as doublet which coupled with protons ($CH_{e,h}$) observed at δ 7.27-7.26 as a doublet with a coupling constant of 6.5 Hz. Other signals for the aromatic protons were at δ 7.38 (s, $1H_p$) for one proton, δ 7.18-7.13 (m, $2H_{r,s}$) for two protons and δ 7.03-6.99 (m, 1H) . Among the aliphatic protons, a singlet was observed at δ 3.78 (s, $2H_o$) for two protons and δ 3.02-3.00 (d, $2H_{l,m}$) for two protons with a coupling constant of 10.8 Hz. Other signals were observed at δ 2.64 (s, 1H), δ 2.00 (bs, 2H), and δ 1.92-1.80 (m, 4H). Its ^{13}C-NMR spectrum showed peak at δ 173.34 due to C=O carbon of the amide. Aromatic carbons appeared at δ 163.40, 160.97, 156.21, 142.99, 133.22, 132.84, 132.55, 130.69, 129.97, 129.84, 128.88, 128.22, 124.98, 124.48, 115.77, 114.20 and 114.00 whereas the aliphatic carbons appeared at δ 60.93, 51.87, 29.01 and 27.40. Its mass spectrum showed molecular ion peak at 539.50 $(M)^+$.



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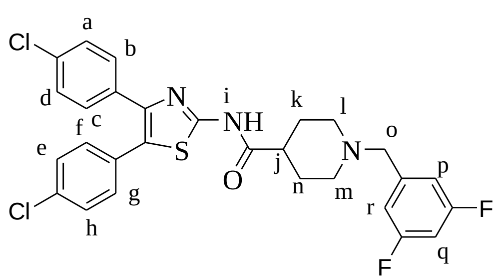
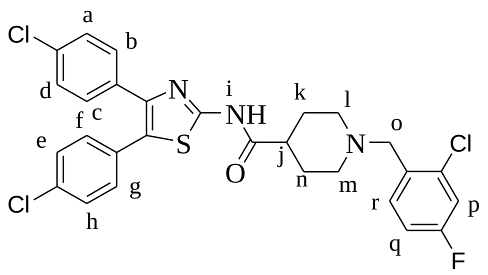


(33)

In the IR spectrum of compound **(33)**, the C=O stretching of amide was observed at 1688 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 10.35 (s, $1H_i$) due to the proton attached to the amidic nitrogen. The aromatic protons ($CH_{b,c}$) were observed at δ 7.37-7.35 as doublet which coupled with protons ($CH_{a,d}$) observed at δ 7.26-7.24 as doublet with a coupling constant of 8.5 Hz. Protons ($CH_{f,g}$) were observed at δ 7.33-7.31 as doublet which coupled with protons ($CH_{e,h}$) observed at δ 7.26-7.24 as doublet with a coupling constant of 8.5 Hz. Other signals for the aromatic protons were observed at δ 7.19-7.17 (m, 2H) and δ 6.99-6.94 (m, 1H). Among the aliphatic protons, a singlet was obtained at δ 3.66 (s, $2H_o$) for two protons and δ 2.91-2.88 (d, $2H_{l,m}$) for two protons with a coupling constant of 11.4 Hz. Other signals were obtained at δ 2.04-1.94 (m, 2H) and 1.76-1.62 (m, 5H). Its ^{13}C-NMR spectrum showed peak at δ 173.41 due to C=O carbon of the amide. Aromatic carbons appeared at δ 163.31, 160.84, 157.61, 142.97, 136.75, 134.21, 132.76, 130.71, 130.17, 129.25, 129.11, 128.92, 126.13, 125.46, 114.05 and 113.82 whereas the aliphatic carbons appeared at δ 52.36, 42.38, 29.71 and 28.31.

In the IR spectrum of compound **(34)**, the C=O stretching of amide was observed at 1686 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 10.94 (s, $1H_i$) due to the proton attached to the amidic nitrogen. The aromatic protons ($CH_{b,c}$) were observed at δ 7.42-7.40 as doublet which coupled with protons ($CH_{a,d}$) observed at δ 7.29-7.25 as doublet with a coupling constant of 8.5 Hz. Protons ($CH_{f,g}$) were observed at δ 7.34-7.31 as doublet which coupled with protons ($CH_{e,h}$) observed at δ 7.29-7.25 as doublet with a coupling constant of 8.5 Hz. Other signals for the aromatic protons were observed at δ 7.38 (s, $1H_p$) for one proton and δ 7.09-7.06 as doublet for one proton (CH_q) which coupled with protons (CH_r) observed at δ 6.96-6.91 as doublet with a coupling constant of 8.5 Hz. Among the aliphatic protons, a singlet was observed at δ 3.47 (s, $2H_o$) for two protons and δ 2.79-2.77 (d, $2H_{l,m}$) for two protons with coupling constant of 8.8 Hz. Other signals were observed at δ 1.89-1.70 (m, 4H), δ 1.55-1.53 (m, 2H) and δ 1.30-1.25 (m, 1H). Its ^{13}C-NMR spectrum showed peak at δ 173.26 due to C=O carbon of the amide. Aromatic carbons appeared at δ 164.32, 160.84, 157.28, 143.10, 134.26, 134.20, 132.83, 130.69, 130.18, 130.11, 129.27, 128.92, 126.25, 116.74, 116.50, 114.50 and 113.79 whereas the aliphatic carbons appeared at δ 58.52, 52.46, 42.44 and 28.40. Its mass spectrum showed molecular ion peak at 573.54 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 6.38 min retention time.



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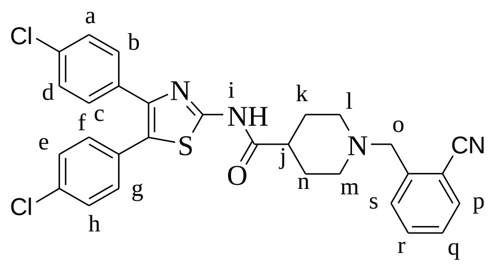
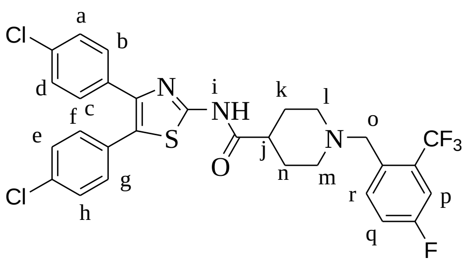


(35)

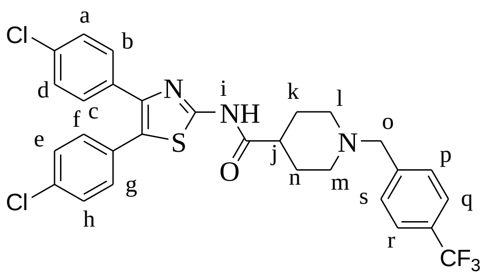
In the IR spectrum of compound **(35)**, the C=O stretching of the amide was observed at 1685 cm^{-1}. The peak at 2231 cm^{-1} was appeared due to the nitril group. Its PMR spectrum showed a broad singlet at δ 10.89 (s, $1H_i$) due to the proton attached to the amidic nitrogen. The aromatic proton (CH_p) was observed at δ 7.63-7.61 as doublet with a coupling constant of 7.6 Hz. Protons ($CH_{b,c}$) were observed at δ 7.54-7.52 as doublet which coupled with protons ($CH_{a,d}$) observed at δ 7.29-7.27 as doublet with a coupling constant of 8.0 Hz. Protons ($CH_{f,g}$) were observed at δ 7.42-7.40 as doublet which coupled with protons ($CH_{e,h}$) observed at δ 7.29-7.27 as doublet with a coupling constant of 8.0 Hz whereas the other signals for the aromatic protons were at δ 7.34-7.32 (m, $3H_{q,r,s}$) for three protons. Among the aliphatic protons signals were obtained at δ 3.61 (s, $2H_o$) for two protons and δ 2.79-2.76 (d, $2H_{l,m}$) for two protons with a coupling constant of 10.6 Hz. Other signals were observed at δ 1.85-1.68 (m, 5H) and δ 1.57-1.55 (d, 2H). Its ^{13}C-NMR spectrum showed peak at δ 172.99 due to C=O carbon of the amide. Aromatic carbons appeared at δ 156.79, 143.21, 134.21, 134.09, 132.85, 132.67, 130.72, 130.15, 129.87, 129.25, 128.86, 127.60, 126.25, 177.80 and 112.84 whereas the aliphatic carbons appeared at δ 60.46, 52.54, 42.51, 29.71 and 28.41. Its mass spectrum showed molecular ion peak at 546.82 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 5.72 min retention time.

In the IR spectrum of compound **(36)**, the C=O stretching of amide was observed at 1665 cm-1.

In the IR spectrum of compound **(37)**, the C=O stretching of amide was observed at 1688 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 9.92 (bs, $1H_i$) due to the proton attached to the amidic nitrogen. The aromatic protons ($CH_{b,c}$) were observed at δ 7.31-7.29 as doublet which coupled with protons ($CH_{a,d}$) observed at 7.09-7.07 as doublet with a coupling constant of 8.5 Hz and δ 7.25-7.23 (d, $4H_{f,j,e,h}$) for four protons with coupling constant of 8.0 Hz. Other signals for the aromatic protons were observed at δ 7.20-7.16 (m, 4H). Among the aliphatic protons, a singlet was observed at δ 3.39 (s, $2H_o$) for two protons and δ 2.79-2.76 (d, $2H_{l,m}$) for two protons with a coupling constant of 11.2 Hz. Other signals were observed at



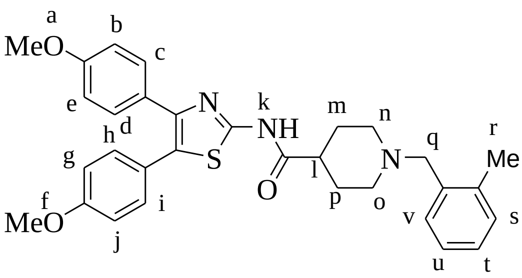
(36)



(37)

δ 2.02-1.97 (m, 1H), δ 1.85-1.78 (m, 6H). Its ^{13}C-NMR spectrum showed peak at δ 173.14 due to C=O carbon of the amide. Aromatic carbons appeared at δ 157.02, 148.28, 143.13, 36.87, 134.23, 132.81, 130.68, 130.19, 130.16, 130.13, 129.25, 128.87, 126.21, 121.76, 120.78 and 119.21 whereas the aliphatic carbons appeared at δ 62.15, 52.43, 42.52 and 31.94. Its mass spectrum showed molecular ion peak at 607.1 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.62 min retention time.

In the IR spectrum of compound **(38)**, the C=O stretching of amide was observed at 1665 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 11.11 (bs, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons ($CH_{c,d}$) were observed at δ 7.44-7.42 as doublet with a coupling constant of 8.5 Hz. Other signals for the aromatic protons were observed at δ 7.58 (s, 1H), δ 7.19-7.13 (m, 6H) and δ 6.87-6.82 (m, 3H). Among the aliphatic protons signals were obtained at δ 3.92 (s, $3H_a$) and δ 3.78 (s, $3H_f$) as singlets for six protons due to the methyl groups on the diaryl moiety. Other signals were obtained at δ 3.92 (s, $2H_q$) for two protons and δ 2.73-2.71 (d, $2H_{n,o}$) for two protons with a coupling constant of 5.5 Hz. Signals were observed at δ 2.31 (s, $3H_r$) for three protons due to the methyl group attached to the aromatic ring. Other peaks for the aliphatic protons were observed at δ 1.68-163 (m, 5H) and 1.49 (bs, 2H). Its ^{13}C-NMR spectrum showed peak at δ 173.69 due to C=O carbon of the amide. Aromatic carbons appeared at δ 159.29, 159.25, 156.90, 142.92, 137.53, 136.48, 130.74, 130.25, 130.16, 129.72, 127.28, 126.99, 125.55, 125.42, 124.34, 114.29 and 113.95 whereas the aliphatic carbons appeared at δ 60.93, 55.32, 52.58, 42.44, 28.50 and 19.23. Its mass spectrum showed molecular ion peak at 527.82 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 5.59 min retention time.



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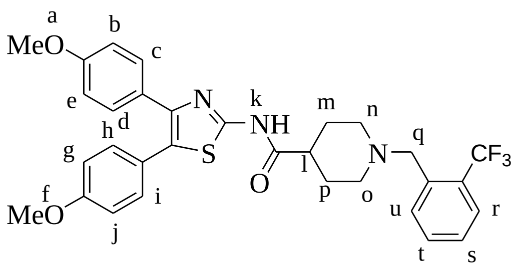
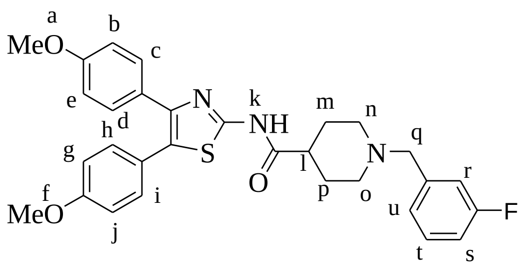


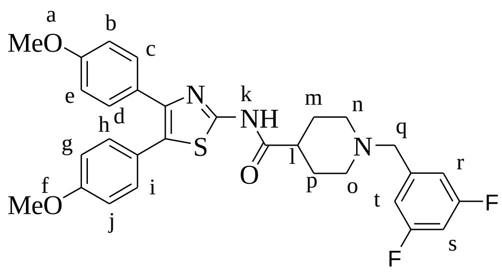
(39)

In the IR spectrum of compound **(39)**, the C=O stretching of the amide was observed at 1685 cm^{-1}. Its PMR spectrum offered a broad singlet at δ 11.32 (bs, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons were observed at δ 7.76-7.75 (m, 1H), 7.60-7.56 (m, 2H), 7.51-7.43 (m, 3H), 7.32-7.24 (m, 3H), 6.90-6.81 (m, 3H). Among the aliphatic protons signals were observed at δ 3.92 (s, $3H_a$) and δ 3.78 (s, $3H_f$) as singlets for six protons due to the methyl groups attached to the diaryl moiety. Other signals were observed at δ 3.59 (s, $2H_q$) for two protons, 2.70 (bs, 2H), 1.87-1.84 (m, 4H), 1.49-1.42 (m, 2H) and 1.25-0.97 (m, 1H). Its ^{13}C-NMR spectrum showed peak at δ 173.59 due to C=O carbon of the amide. Aromatic carbons appeared at δ 159.50, 159.30, 157.18, 155.59, 143.66, 134.12, 131.83, 130.74, 130.15, 129.73, 128.24, 126.83, 125.80, 123.89, 114.32, 113.99, 112.03 and 111.92 whereas the aliphatic carbons appeared at δ 56.29, 55.29, 52.65, 42.23 and 28.44. Its mass spectrum showed molecular ion peak at 581.46 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.39 min retention time.

In the IR spectrum of compound **(40)**, the C=O stretching of the amide was observed at 1677 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 11.46 (bs, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons ($CH_{c,d}$) were observed at δ 7.42-7.40 as doublet which coupled with protons ($CH_{b,e}$) observed at δ 6.89-6.87 as doublet with a coupling constant of 8.7 Hz. Protons ($CH_{h,i}$) were observed at δ 7.29-7.26 as doublet which coupled with protons ($CH_{g,j}$) observed at δ 6.82-6.80 as doublet with a coupling constant of 8.7 Hz. Other signals for the aromatic protons were observed at δ 7.02-6.99 (m, 2H) and 6.97-6.91 (m, 1H). Among the aliphatic protons signals were observed at δ 3.84 (s, $3H_a$) and δ 3.77 (s, $3H_f$) for six protons due to the methyl groups attached to the diaryl moiety. Other signals were observed at δ 3.39 (s, $2H_q$) for two protons, 2.71 (bs, 2H), 1.67 (bs, 5H) and 1.47 (bs, 2H). Its ^{13}C-NMR spectrum showed peak at δ 173.50 due to C=O carbon of the amide. Aromatic carbons appeared at δ 164.11, 161.67, 159.33, 157.01, 142.84, 130.72, 130.14, 129.64, 129.56, 127.21, 125.58, 124.50, 124.26, 115.83, 115.62, 114.31 and 113.95 whereas the aliphatic carbons appeared at δ 62.37, 55.31, 52.26, 42.02 and 28.25. Its mass spectrum showed molecular ion peak at 532.3 $(M+1)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 5.59 min retention time.



(40)

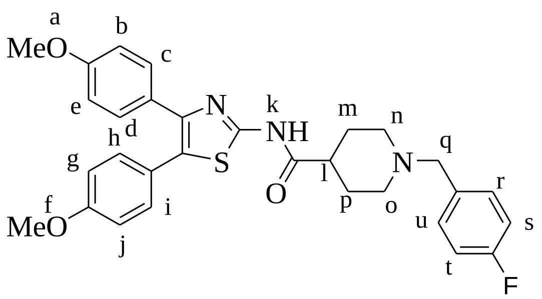


(41)

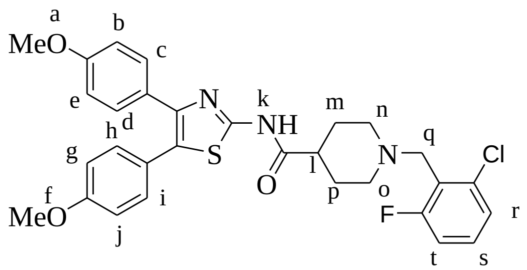
In the IR spectrum of compound **(41)**, the C=O stretching of amide was observed at 1680 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 11.45 (bs, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons ($CH_{c,d}$) were observed at δ 7.45-7.43 as doublet coupled with protons ($CH_{b,e}$) observed at δ 6.89-6.87 as a doublet with a coupling constant of 8.7 Hz. Protons ($CH_{h,i}$) were observed at δ 7.29-7.27 as doublet coupled with protons ($CH_{g,j}$) observed at δ 6.82-6.80 as doublet with a coupling constant of 8.7 Hz. Other signals for the aromatic protons were observed at δ 6.79-6.77 (m, 2H) and δ 6.68-6.63 (m, 1H). Among the aliphatic protons signals were observed at δ 3.84 (s, $3H_a$) and δ 3.78 (s, $3H_f$) for six protons due to the methyl groups attached to the diaryl moiety. Signals were observed at δ 3.34 (s, $2H_q$) for two proton and δ 2.68-2.65 (d, $2H_{n,o}$) for two protons with a coupling constant of 8.2 Hz. Other signals for the aliphatic protons were observed at δ 1.65-1.60 (bs, 5H) and 1.45-1.43 (d, 2H). Its ^{13}C-NMR spectrum showed peak at δ 173.55 due to C=O carbon of the amide. Aromatic carbons appeared at δ 164.27, 161.80, 161.08, 159.36, 157.16, 142.81, 130.72, 130.15, 127.23, 125.61, 124.23, 114.33, 113.98, 111.38, 111.20, 102.58 and 102.07 whereas the aliphatic carbons appeared at δ 62.13, 55.31, 52.33, 41.95 and 28.29. Its mass spectrum showed molecular ion peak at 549.73 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.57 min retention time.

In the IR spectrum of compound **(42)**, the C=O stretching of amide was observed at 1688 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 10.95 (bs, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons ($CH_{c,d}$) were observed at δ 7.34-7.32 as doublet which coupled with protons ($CH_{b,e}$) observed at δ 6.81-6.79 as doublet with a coupling constant of 8.5 Hz. Protons ($CH_{h,i}$) were observed at δ 7.21-7.19 as doublet which coupled with protons ($CH_{g,j}$) observed at δ 6.73-6.71 as a doublet with a coupling constant of

8.5 Hz. Other signals for the aromatic protons were observed at δ 7.16-7.12 (m, 2H) and δ 6.91-6.87 (m, 2H). Among the aliphatic protons signals were appeared at δ 3.71 (s, $6H_{a,f}$) as singlets for six protons due to the methyl groups attached to the diaryl moiety. Other signals were observed at δ 3.32 (s, $2H_q$) for two protons, δ 2.66 (bs, 2H), 1.69-1.61 (m, 4H) and 1.48 (bs, 3H). Its ^{13}C-NMR spectrum showed peak at δ 173.59 due to C=O carbon of the amide. Aromatic carbons appeared at δ 163.28, 160.85, 159.32, 157.04, 142.92, 133.76, 130.78, 130.56, 127.29, 125.65, 124.34, 115.14, 114.93, 114.13 and 112.12 whereas the aliphatic carbons appeared at δ 62.22, 55.38, 52.26, 42.22 and 29.78. Its mass spectrum showed molecular ion peak at 531.23 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 5.56 min retention time.



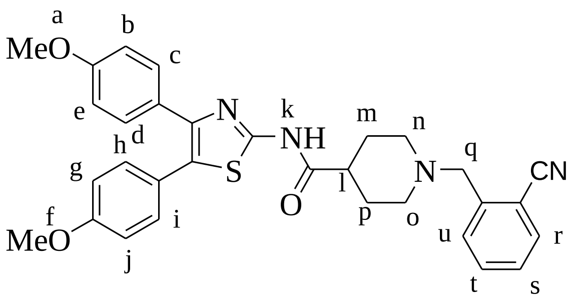
(42)



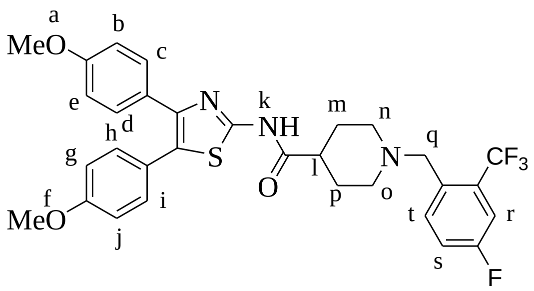
(43)

In the IR spectrum of compound **(43)**, the C=O stretching of amide was observed at 1683 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 11.08 (bs, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons were observed at δ 7.35-7.33 (d, 2H), δ 7.29-7.16 (m, 2H), δ 6.84-6.74 (m, 6H) and δ 6.64-6.57 (m, 1H). Among the aliphatic protons, a singlet was observed at δ 3.71 (s, $6H_{a,f}$) for six protons due to the methyl groups attached to the diaryl moiety, another signal was obtained at δ 3.85 (s, $2H_q$) for two protons. Other signals were observed at δ 3.31 (bs, 2H), 2.65 (bs, 3H) and 1.18 (bs, 4H). Its mass spectrum showed molecular ion peak at 564.75 $(M)^+$.

In the IR spectrum of compound **(44)**, the C=O stretching of the amide was observed at 1684 cm^{-1}. The peak observed at 2219 cm^{-1} was due to the nitril group. Its PMR spectrum showed a broad singlet at δ 11.34 (bs, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons were observed at δ 7.61-7.59 (d, $1H_r$) for one proton as doublet with coupling constant of 7.6 Hz, proton ($CH_{c,d}$) at δ 7.51-7.49 as doublet which coupled with protons ($CH_{b,e}$) observed at δ 6.89-6.87 as doublet with a coupling constant of 8.6 Hz. Protons ($CH_{h,i}$) were observed at δ 7.45-7.42 as doublet which coupled with protons ($CH_{g,j}$) observed at δ 6.83-6.81 as a doublet with a coupling constant of 8.6 Hz. Other signals for the aromatic protons appeared at δ 7.34-7.26 (m, $3H_{s,t,u}$) for three protons. Among the aliphatic protons signals were observed at δ 3.84 (s, $3H_a$) and δ 3.78 (s, $3H_f$) for six protons due to the methyl groups attached to the diaryl moiety. A singlet was obtained at δ 3.59 (s, $2H_q$) for two protons. Other signals were observed at δ 2.73-2.71(d, $2H_{n,o}$) as doublet for two protons with coupling constant of 9.7 Hz, δ 1.78-1.70 (m, 5H) and 1.50 (bs, 2H). Its ^{13}C-NMR spectrum showed peak at δ 173.31 due to C=O carbon of the amide. Aromatic carbons appeared at δ 159.31, 159.26, 156.72, 142.92, 142.52, 132.73, 132.65, 130.73, 130.13, 129.82, 127.48, 127.20, 125.64, 124.21, 117.78, 114.31, 113.96 and 112.70 whereas the aliphatic carbons appeared at δ 60.39, 55.29, 52.44, 42.13, 29.70 and 28.38. Its mass spectrum showed molecular ion peak at 538.44 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 6.13 min retention time.



(44)

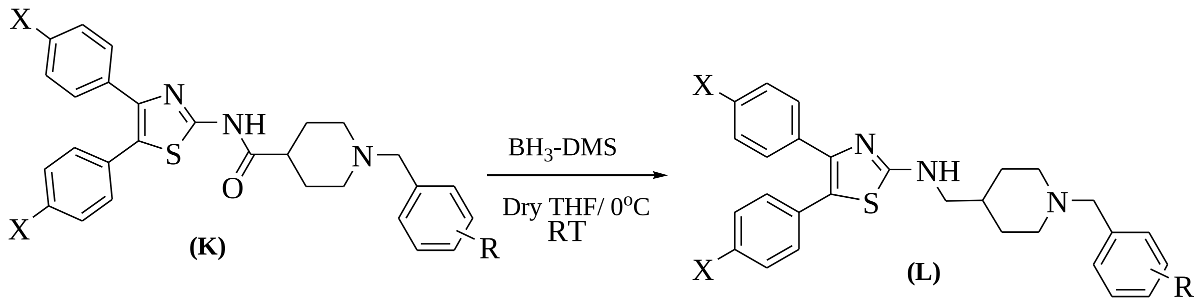


(45)

In the IR spectrum of compound **(45)**, C=O stretching of the amide was observed at 1682 cm^{-1}. Its PMR spectrum showed a broad singlet at δ 11.60 (s, $1H_k$) due to the proton attached to the amidic nitrogen. The aromatic protons were observed at δ 7.74-7.71 (m, 1H), proton ($CH_{c,d}$) at δ 7.47-7.45 as doublet which coupled with protons ($CH_{b,e}$) observed at δ 6.89-6.87 as doublet with a coupling constant of 8.7 Hz. Protons ($CH_{h,i}$) were observed at δ 7.20-7.18 as doublet which coupled with protons ($CH_{g,j}$) observed at δ 6.84-6.82 as doublet with a coupling constant of 8.3 Hz. Another aromatic protons were observed at δ 7.30-7.24 (m, $2H_{t,u,}$) for two protons. Among the aliphatic protons signals were observed at δ 3.84 (s, $3H_a$) and δ 3.77 (s, $3H_f$) for six protons due to the methyl groups attached to the diaryl moiety. A singlet was obtained at δ 3.46 (s, $2H_q$) for two protons. Other signals were observed at δ 2.65 (bs, 2H), 1.67-1.57 (m, 5H) and 1.49 (bs, 2H). Its ^{13}C-NMR spectrum showed peak at δ 173.56 due to C=O carbon of the amide. Aromatic carbons appeared at δ 162.15, 159.49, 157.25, 156.99, 155.60, 142.91, 134.12, 133.70, 132.33, 130.73, 129.72, 127.24, 125.66, 124.23, 118.81, 114.22, 113.99 and 112.04 whereas the aliphatic carbons appeared at δ 57.53, 55.29, 52.56, 42.13 and 28.47. Its mass spectrum showed molecular ion peak at 599.29 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 6.83 min retention time.

4.1.8 Synthesis of *N*-[1-(substituted benzyl)piperidin-4-yl]methyl-4,5-bis(substituted phenyl)thiazol-2-ylamines (46-56)

N-[(1-Substituted benzyl)piperidin-4-yl]methyl-4,5-diarylthiazol-2-ylamines **(46-56)** were prepared by reduction of 1-subsutited benzyl-*N*-(4,5-disubstituted phenylthiazol-2-yl)piperidine-4-carboxamides **(20, 21, 23, 25, 30, 33, 34, 36, 38, 39** and **43)** in presence of borane-dimethyl sulphide complex in THF at 0 °C (Scheme-8).



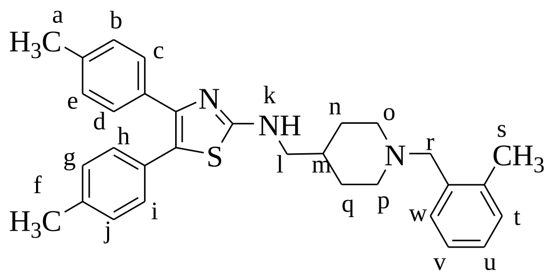
(20, 21, 23, 25, 30, 33, 34, 36, 38, 39, 43) **(46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56)**

(20, 21, 23, 25,	**46, 47, 48, 49)**	X = CH_3
(30, 33, 34, 36,	**50, 51, 52, 53)**	X = Cl
(38, 39, 43,	**54, 55, 56)**	X = OCH_3

Scheme-8

IR spectrum of compound **(46)** showed N-H stretching vibration as a strong peak at 3186 cm^{-1} and N-H deformation as a moderate peak at 1557 cm^{-1}. Its PMR spectrum showed aromatic protons ($CH_{c,d}$) at δ 7.36-7.34 as doublet which coupled with protons ($CH_{b,e}$) observed at δ 7.06-7.04 as doublet a with coupling constant of 8.0 Hz. Protons ($CH_{h,i}$) were observed at δ 7.27-7.25 as a doublet which coupled with protons ($CH_{g,j}$) observed at δ 7.06-7.04 as doublet with a coupling constant of 8.0 Hz. Other signals for aromatic protons were observed at δ 7.16-7.11 (m, $4H_{t,u,v,w}$) for four protons. A singlet was observed at δ 5.70 (s, $1H_k$) due to the proton attached to the amine nitrogen. Other signals were observed at δ 3.44 (s, $2H_r$) for two protons. A broad singlet was appeared at δ 3.09 (s, $2H_l$) for two protons. Other signals were observed at δ 2.90-2.87 (d, $2H_{o.p}$) for two protons with a coupling constant of 11.8 Hz and a singlet for six protons appeared at δ 2.34 (s, $6H_{a,f}$) due to the methyl groups attached to the diaryl moiety. Signals were obtained at δ 2.31 (s, $3H_s$) for three protons due to the methyl group attached to the aromatic ring, δ 2.00-1.94 (m, 3H), 1.71-1.62 (d, $2H_{n,q}$) for two protons with a coupling constant of 11.8 Hz and δ 1.32-1.25 (t, 2H). Its ^{13}C-

NMR spectrum showed aromatic carbons appeared at δ 167.81, 145.73, 137.51, 137.14, 136.71, 132.85, 130.29, 130.17, 129.84, 129.27, 129.16, 128.91, 128.78, 127.12, 125.55 and 119.69 whereas the aliphatic carbons appeared at δ 60.99, 53.49, 36.15, 30.16, 21.34 and 19.34. Its mass spectrum showed molecular ion peak at 481.61 $(M)^+$.



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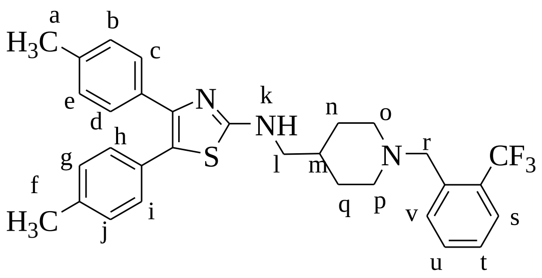
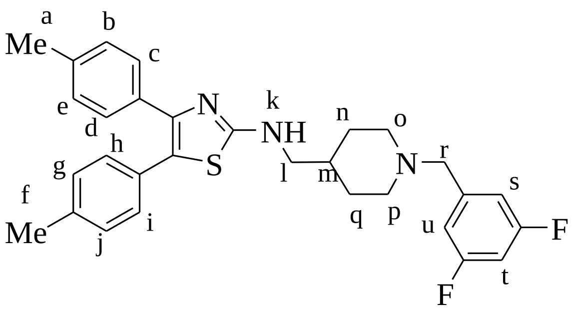


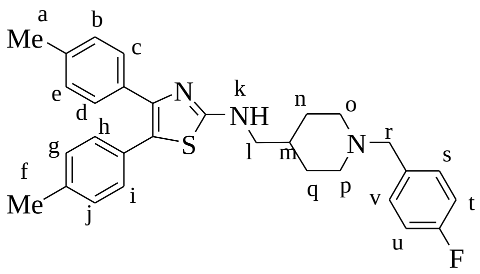
(47)

IR spectrum of compound **(47)** showed N-H stretching vibration as a strong peak at 3200 cm^{-1} and N-H deformation as a moderate peak at 1584 cm^{-1}. Its PMR spectrum offered peaks for aromatic protons at δ 7.81-7.80 (d, 1H, *J* =7.7 Hz), 7.62-7.60 (d, 2H, *J* =7.7 Hz), 7.53-7.49 (m, 1H), 7.36-7.29 (m, 3H), 7.17-7.15 (d, 2H, *J* = 8.1 Hz) and 7.06-7.04 (d, 4H, *J* = 8.1 Hz). A singlet was observed at δ 5.73 (s, $1H_k$) due to the proton attached to the amine nitrogen. It also gave a singlet at δ 3.64 (s, $2H_r$) for two protons and δ 3.17-3.15 (d, $2H_l$) for two protons with a coupling constant of 6.3 Hz. Other signals were observed at δ 2.89-2.86 (d, $2H_{o,p}$) for two protons with coupling constant of 11.4 Hz, a singlet at δ 2.32 (s, $6H_{a,f}$) for six protons due to the methyl groups attached to the diaryl moiety, δ 2.09-2.03 (m, 2H) and δ 1.74-1.67 (m, 5H). Its mass spectrum showed molecular ion peak at 536.10 $(M)^+$.

IR spectrum of compound **(48)** showed N-H stretching vibration as a strong peak at 3198 cm^{-1} and N-H deformation as a moderate peak at 1583 cm^{-1}. Its PMR spectrum showed aromatic protons ($CH_{c,d}$) at δ 7.29-7.27 as doublet which coupled with protons ($CH_{b,e}$) observed at δ 6.99-6.95 as doublet with a coupling constant of 7.6 Hz. Protons ($CH_{h,i}$) were observed at δ 7.08-7.06 as doublet which coupled with protons ($CH_{g,j}$) observed at δ 6.99-6.95 as doublet with a coupling constant of 7.6 Hz. Other aromatic protons observed at δ 6.77-6.76 (m, 2H) and δ 6.60-6.56 (m, 1H). Signal was observed at δ 6.20 (s, $1H_k$) due to the proton attached to the amine nitrogen. A singlet was observed at δ 3.33 (s, $2H_r$) for two protons. A broad singlet was appeared at δ 2.95 (s, $2H_l$) for two protons. Other signals were observed at δ 2.73-2.70 (d, $2H_{o,p}$) for two protons with a coupling constant of 12.0 Hz. A singlet was observed at δ 2.33 (s, $6H_{a,f}$) for six protons due to the methyl groups attached to the diaryl moiety, δ 1.89-1.79 (m, 2H), δ 1.59-1.56 (d, $2H_{n,q}$) for two protons with a coupling constant of 12.0 Hz and δ 1.18-1.10 (m, 3H). ^{13}C-NMR spectrum showed aromatic carbons appeared at δ 167.97, 164.29, 161.83, 145.69, 143.35, 137.11, 136.63, 132.86, 130.10, 129.21, 128.89, 119.40, 111.39, 111.21, 102.49 and 101.98 whereas the aliphatic carbons appeared at δ 62.36, 53.32, 52.16, 35.85, 30.01 and 21.29. Its mass spectrum showed molecular ion peak at 503.84 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.61 min retention time.



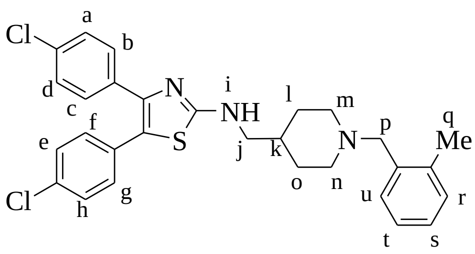
(48)



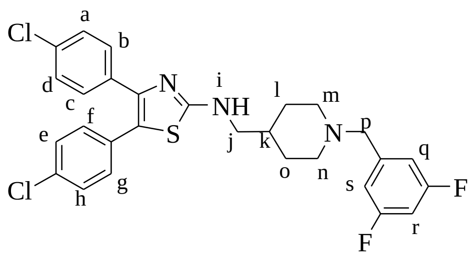
(49)

IR spectrum of compound **(49)** showed N-H stretching vibration as a strong peak at 3196 cm^{-1} and N-H deformation as a moderate peak at 1582 cm^{-1}. Its PMR spectrum showed aromatic protons ($CH_{c,d}$) at δ 7.36-7.34 as a doublet which coupled with protons ($CH_{b,e}$) observed at δ 7.06-7.04 as doublet with a coupling constant of 8.1 Hz. Protons ($CH_{h,i}$) were observed at δ 7.15-7.14 as doublet which coupled with protons ($CH_{g,j}$) observed at δ 7.06-7.04 as doublet with a coupling constant of 8.1 Hz. Other aromatic protons were observed at δ 7.26-7.24 (m, 2H) and δ 7.01-6.96 (m, 2H). Among the aliphatic protons, signal was observed at δ 5.58 (s, $1H_k$) due to the proton attached to the amine nitrogen. A singlet was observed at δ 3.45 (s, $2H_r$) for two protons and δ 3.13-3.10 (m, 2H). Other signals were observed at δ 2.88-2.86 (d, $2H_{o,p}$) for two protons with a coupling constant of 12.0 Hz. It also gave a singlet at δ 2.35 (s, $6H_{a,f}$) for six protons due to the methyl groups attached to the diaryl moiety. Few peaks were observed at δ 1.91-1.90 (m, 3H), 1.64-1.62 (d, 2H, J = 12.0 Hz) and 1.36-1.25 (m, 2H). Its ^{13}C-NMR spectrum showed aromatic carbons appeared at δ 166.02, 144.65, 136.19, 135.97, 132.76, 130.35, 129.96, 129.01, 128.67, 128.36, 128.31, 117.73, 114.73 and 114.52 whereas the aliphatic carbons appeared at δ 61.45, 52.76, 40.20, 39.37, 38.95, 29.61 and 20.78. Its mass spectrum showed molecular ion peak at 486.01 $(M)^+$.

IR spectrum of compound **(50)** showed N-H stretching vibration as a strong peak at 3200 cm^{-1} and N-H deformation as a moderate peak at 1580 cm^{-1}. Its PMR spectrum offered peaks for aromatic protons at δ 7.39-7.36 (m, 2H), δ 7.26-7.21 (m, 5H) and δ 7.18-7.13 (m, 5H). A singlet was observed at δ 5.53 (s, $1H_i$) due to the proton attached to the amine nitrogen. Signals were observed at δ 3.44 (s, $2H_p$) for two protons and δ 3.15-3.12 (m, $2H_j$). Other signals were observed at δ 2.91-2.89 (d, $2H_{m,n}$) for two protons with a coupling constant of 11.3 Hz. A singlet was observed at δ 2.35 (s, $3H_q$) for three protons due to the methyl group attached to the aromatic ring, δ 2.01-1.96 (t, 2H), δ 1.70-1.64 (m, 2H) and δ 1.35-1.25 (m, 3H). Its ^{13}C-NMR spectrum showed aromatic carbons appeared at 167.90, 145.26, 137.44, 133.57, 133.50, 133.05, 131.09, 130.44, 130.24, 130.21, 129.79, 128.87, 128.48, 127.02, 125.50 and 119.16 whereas the aliphatic carbons appeared at δ 60.82, 53.35, 51.97, 36.08, 30.02 and 19.27. Its mass spectrum showed molecular ion peak at 521.86 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 6.04 min retention time.



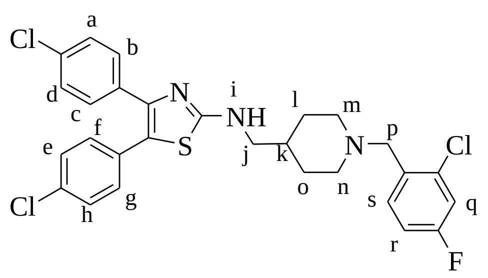
(50)



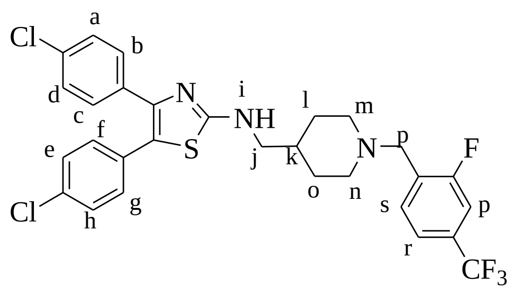
(51)

In the IR spectrum of compound **(51)** showed N-H stretching vibration as strong peak at 3195 cm^{-1} and N-H deformation as a moderate peak at 1579 cm^{-1}. Its PMR spectrum showed aromatic protons at δ 7.38-7.35 (m, 2H), δ 7.26-7.14 (m, 8H) and δ 7.00-6.95 (m, 1H). Signals were appeared at δ 5.53 (s, $1H_i$) due to the proton attached to the amine nitrogen. It also gave a singlet at δ 3.70 (s, $2H_p$) for two protons. Signal was observed at δ 3.14-3.11 (t, $2H_j$). Other signals were observed at δ 2.99-2.96 (d, $2H_{m,n}$) for two protons with a coupling constant of 11.5 Hz, δ 2.71-2.13 (m, 2H), δ 1.74-1.71 (d, 2H) and δ 1.37-1.27 (m, 3H). Its mass spectrum showed molecular ion peak at 544.32 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.47 min retention time.

IR spectrum of compound **(52)** showed N-H stretching vibration as a strong peak at 3199 cm^{-1} and N-H deformation as a moderate peak at 1579 cm^{-1}. Its PMR spectrum showed aromatic protons at δ 7.93-7.90 (m, 1H), 7.43-7.39 (m, 2H), 7.28-7.23 (m, 4H), 7.22-7.19 (m, 2H), 7.16-7.15 (m, 1H), 6.96-6.93 (m, 1H). A singlet was observed at δ 5.62 (s, $1H_i$) due to the proton attached to the amine nitrogen. Signal was appeared at δ 3.57 (s, $2H_p$) for two protons and δ 3.16-3.14 (m, 2H). Other signals were observed at δ 2.93-2.90 (d, $2H_{m,n}$) for two protons with a coupling constant of 11.2 Hz, δ 2.17-2.08 (m, 2H), δ 1.76-1.69 (d, $2H_{l,o}$) for two protons with a coupling constant of 12.8 Hz, δ 1.66-1.64 (m, 1H) and δ 1.37-1.36 (m, 2H). Its mass spectrum showed molecular ion peak at 561.03 $(M+1)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.66 min retention time.



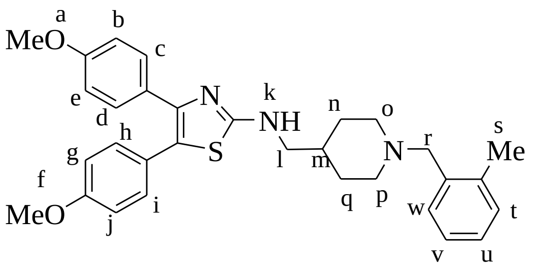
(52)



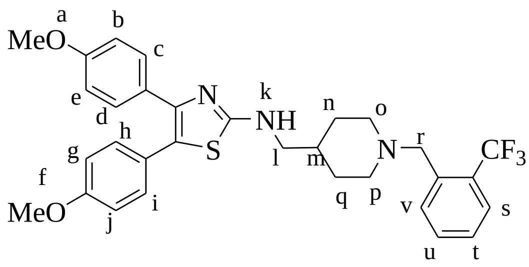
(53)

IR spectrum of compound **(53)** showed N-H stretching vibration as a strong peak at 3195 cm^{-1} and N-H deformation as a moderate peak at 1579 cm^{-1}. Its PMR spectrum showed aromatic protons at δ 7.79-7.76 (m, 1H), 7.39-7.36 (m, 2H), 7.33-7.30 (d, 1H), 7.26-7.20 (m, 5H) and δ 7.18-7.15 (m, 1H). A singlet was observed at δ 5.61 (s, $1H_i$) due to the proton attached to the amine nitrogen. Other signals were observed at δ 3.58 (s, $2H_p$) for two protons and δ 3.17-3.14 (t, $2H_j$). Signals were observed at δ 2.85-2.82 (d, $2H_{m,n}$) for two protons with a coupling constant of 11.5 Hz, δ 2.17-2.08 (t, 2H), δ 1.75-1.72 (d, 2H) and δ 1.43-1.27 (m, 3H). Its ^{13}C-NMR spectrum showed aromatic carbons appeared at 168.14, 162.13, 145.30, 134.03, 133.66, 133.56, 133.06, 131.09, 130.59, 130.26, 129.82, 128.96, 128.53, 124.42, 122.21, 119.07, 118.71 and 113.22 whereas the aliphatic carbons appeared at δ 57.75, 53.38, 52.09, 35.95 and 29.71. Its mass spectrum showed molecular ion peak at 594.12 $(M+1)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.93 min retention time.

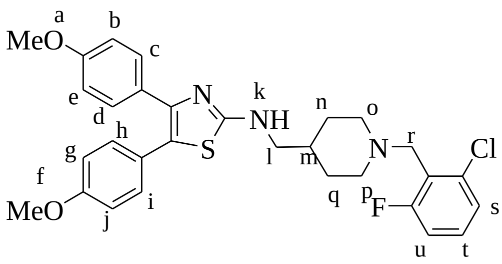
IR spectrum of compound **(54)** showed N-H stretching vibration as a strong peak at 3200 cm^{-1} and N-H deformation as a moderate peak at 1579 cm^{-1}. Its PMR spectrum offered peaks for aromatic protons at δ 7.40-7.38 (m, 2H), δ 7.20-7.19 (m, 2H), δ 7.18-7.17 (m, 4H) and δ 6.81-6.75 (m, 4H). A singlet was observed at δ 5.49 (s, $1H_k$) due to proton attached to the amine nitrogen. Singles were observed at δ 3.79 (s, $3H_a$) and δ 3.79 (s, $3H_f$) for six protons due to the methoxy groups attached to the diaryl moiety. It also gave a singlet at δ 3.44 (s, $2H_r$) for two protons. Other signals were observed at δ 3.12 (bs, 2H), 2.77-2.75 (d, $2H_{o,p}$) for two protons with a coupling constant of 11.4 Hz. It gave a singlet at δ 2.35 (s, $3H_s$) for three protons due to methyl group attached to the aromatic moiety, δ 2.04-1.98 (m, 2H), δ 1.71-1.67 (m, 2H) and δ 1.36-1.22 (m, 3H). Its ^{13}C-NMR spectrum showed aromatic carbons appeared at 167.49, 158.82, 144.93, 137.40, 136.67, 133.82, 130.49, 130.18, 129.71, 129.48, 128.65, 128.18, 126.89, 125.44, 118.67, 113.95 and 111.74 whereas the aliphatic carbons appeared at δ 60.92, 55.22, 53.43, 52.09, 36.09, 30.13 and 19.25. Its mass spectrum showed molecular ion peak at 513.62 $(M+1)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.51 min retention time.



(54)



(55)



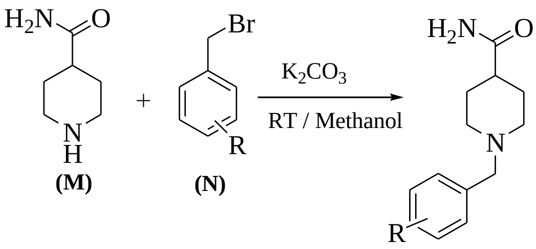
(56)

IR spectrum of compound **(55)** showed N-H stretching vibration as a strong peak at 3207 cm^{-1} and N-H deformation as a moderate peak at 1583 cm^{-1}. Its PMR spectrum showed aromatic protons at δ 7.81-7.79 (d, 1H, J = 7.8 Hz), 7.62-7.60 (d, 1H, J = 7.7 Hz), 7.53-7.49 (m, 2H), 7.41-7.37 (m, 2H), 7.33-7.26 (m, 1H), 7.15-7.13 (m, 1H) and 6.82-6.76 (m, $4H_{s,t,u,v}$) for four protons. A singlet was observed at δ 5.70 (s, $1H_k$) due to proton attached to the amine nitrogen. Signals were observed at δ 3.89 (s, $3H_a$) and δ 3.79 (s, $3H_f$) for six protons due to the methoxy groups attached to the diaryl moiety. A singlet was observed at δ 3.64 (s, $2H_r$) for two protons. Other signals were observed at δ 3.15-3.13 (m, 2H), 2.88-2.85 (d, 2H), δ 2.07-2.02 (m, 2H), δ 1.75-1.67 (m, 3H) and δ 1.42-1.25 (m, 2H). Its ^{13}C-NMR spectrum showed aromatic carbons appeared at δ 167.64, 159.09, 154.85, 145.82, 133.90, 131.74, 130.20, 129.61, 128.61, 128.31, 127.75, 126.96, 125.66, 116.95, 114.01, 113.66 and 111.83 whereas the aliphatic carbons appeared at δ 67.92, 58.30, 56.25, 55.25, 35.97 and 30.14. Its mass spectrum showed molecular ion peak at 568.4 $(M+1)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.58 min retention time.

IR spectrum of compound **(56)** showed N-H stretching vibration as a strong peak at 3206 cm^{-1} and N-H deformation as a moderate peak at 1596 cm^{-1}. Its PMR spectrum showed aromatic protons at δ 7.49-7.48 (d, 1H), 7.39-7.37 (m, 2H), 7.13-7.12 (m, 1H), 6.88-6.87 (d, 2H, J = 6.2 Hz), 6.81-6.76 (m, 4H) and 6.70-6.65 (m, 1H). A singlet was appeared at δ 5.68 (s, $1H_k$) due to proton attached to the amine nitrogen. Signals were observed at δ 3.88 (s, $3H_a$) and δ 3.79 (s, $3H_f$) for six protons due to the methoxy groups attached to the diaryl moiety. Signal was observed at δ 3.47 (s, $2H_r$) as a singlet for two protons. Other signals were observed at δ 3.16-3.14 (d, $2H_l$) for two protons with a coupling constant of 5.9 Hz, δ 2.90-2.84 (d, $2H_{o,p}$) for two protons with a coupling constant of 12.2 Hz, δ 2.03-1.98 (m, 2H), δ 1.77-1.74 (d, $2H_{n,q}$) for two protons with a coupling constant of 12.2 Hz, δ 2.03-1.98 (m, 2H) and δ 1.64-1.61 (m, 3H). Its mass spectrum showed molecular ion peak at 551.29 $(M)^+$.

4.1.9 Synthesis of 1-(substituted benzyl)piperidine-4-carboxamides (57-62)

4-Piperidinecarboxamide **(M)** was treated with substituted benzyl bromides **(N)** using potassium carbonate in methanol to get 1-(substituted)piperidine-4-carboxamide derivatives **(57-62)** (Scheme-9)**.**



(M) **(N)** **(O)**

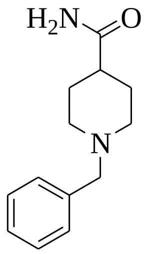
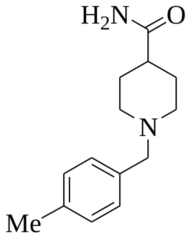
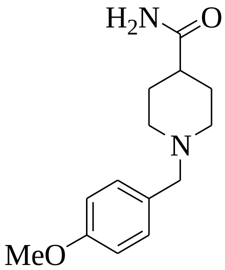
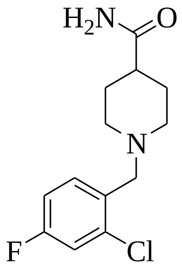
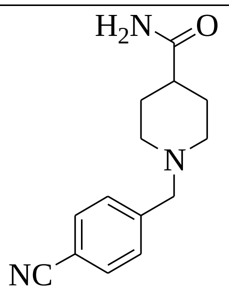
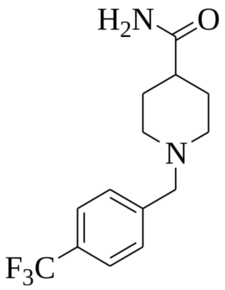
(57-62)

Compd	**R**
(57)	H
(58)	4-Me
(59)	4-OMe
(60)	2-Cl,4-F
(61)	4-CN
(62)	4-CF_3

Scheme-9

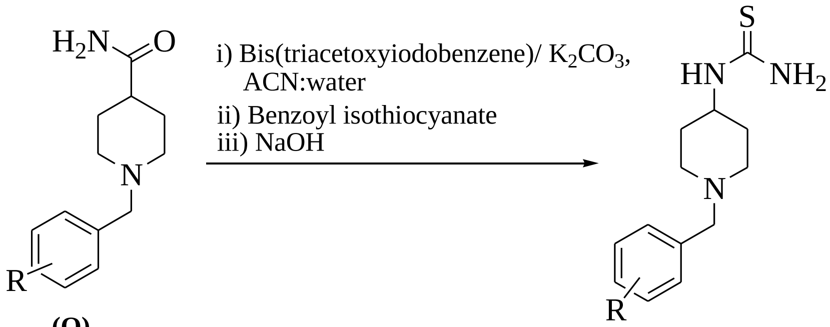
Spectral data of the 1-(substituted benzyl)piperidine-4-carboxamide derivatives **(57-62)** are shown in table 1

Table 1: Spectral data of the 1-(substituted benzyl)piperidine-4-carboxamide derivatives **(57-62)**

Comp.	Comp structure	IR peaks (cm^{-1})	MS peaks (m/z)
57		3348 (N-H stretching) 1635 (C=O stretching of amide)	-
58		3342 (N-H stretching) 1630 (C=O stretching of amide)	232.01(M)$^+$
59		3343 (N-H stretching) 1636 (C=O stretching of amide), 1245 (C-O stretching)	248.92 (M)$^{+\cdot}$
60		3381 (N-H stretching) 1654 (C=O stretching of amide)	270.19 (M)$^+$
61		3445 (N-H stretching), 1673 (C=O stretching of amide), 2224 (CN stretching)	243.94 (M)$^+$
62		3334 (N-H stretching), 1636 (C=O stretching of amide)	286.46 M)$^+$

4.1.10 Synthesis of 1-[substituted benzyl)piperidine-4-yl]thiourea derivatives (63-68)

Acidic Hofmann rearrangement of 1-(substituted benzyl)piperidine-4-carboxamides **(57-62)** using bis(triacetoxyiodobenzene) in aqueous solution of ACN at 60 ^{0}C gave yellow colored oil of amines as intermediates. The intermediate amines without characterization were dissolved as such in DCM and reacted with benzoyl isothiocynate over night, followed by basic hydrolysis yielding the desired thioureas as **(63-68)** (Scheme 10).



(O) **(P)**

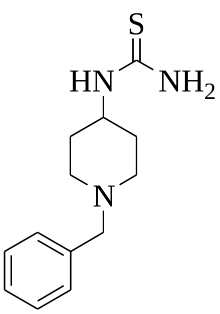
(57-62) **(63-68)**

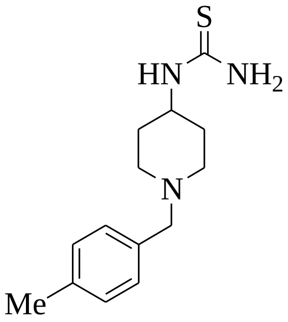
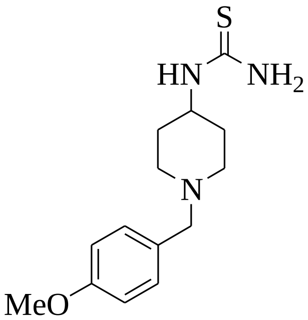
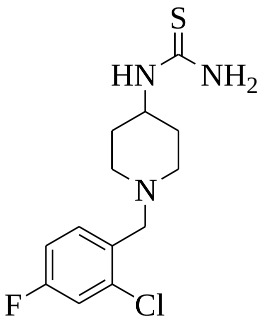
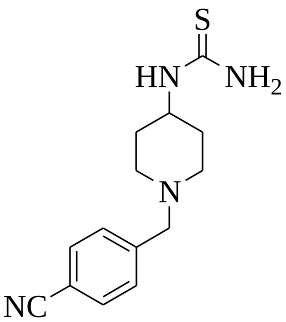
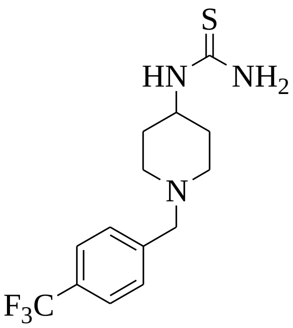
Comp	R
(57, 63)	H
(58, 64)	4-Me
(59, 65)	4-OMe
(60, 66)	2-Cl-4-F
(61, 67)	4-CN
(62, 68)	4-CF_3

Scheme-10

Spectral data of the 1-[substituted benzyl)piperidin-4-yl]thiourea derivatives **(63-68)** are shown in table 2

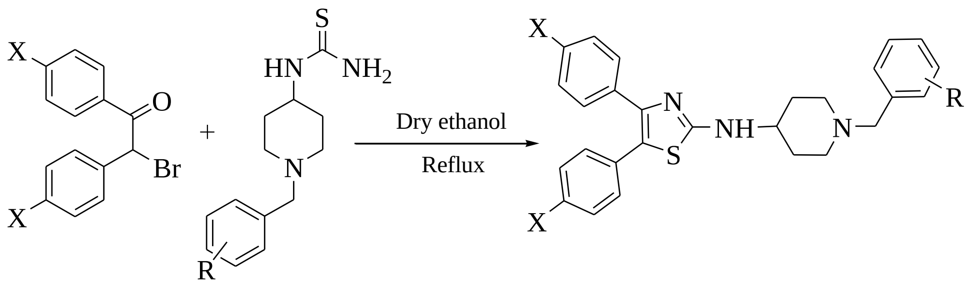
Table 2: Spectral data of the 1-[substituted benzyl)piperidin-4-yl]thiourea derivatives **(63-68)** synthesized in Scheme- 10

Comp.	Comp. structure	IR peaks (cm-1)	MS peaks (m/z)
63		3298 (N-H stretching), 1630 (N-H deformation), 1437 (C=S stretching)	-

64		3298 (N-H stretching) 1630 (N-H deformation), 1437 (C=S stretching)	-
65		3394 (N-H stretching) 1630 (N-H deformation), 1437 (C=S stretching)	-
66		3405 (N-H stretching) 1627 (N-H deformation), 1491 (C=S stretching)	301.17 $(M)^+$
67		3405 (N-H stretching), 1630 (N-H deformation), 2230 (CN stretching), 1565 (C=S stretching)	274.90 $(M)^+$
68		3394 (N-H stretching), 1630 (N-H deformation), 1437 (C=S stretching)	-

4.1.11 Synthesis of 1-(substituted benzyl)-*N*-[4,5-bis(4-substituted phenyl)thiazol-2-yl] piperidin-4-ylamines (70-79)

1-[Substituted benzyl)piperidin-4-yl]thiourea derivatives **(63-68)** were treated with 2-bromo-1,2-diarylethanones **(69a-69c)** in dry ethanol to obtain the desired 1-(substituted benzyl)-*N*-[4,5-bis(4-substituted phenyl)thiazol-2-yl]piperidin-4-ylamines **(70-79)** as per Scheme 11.



(E)
(69a-69c)

Compd	**X**
(69a)	Me
(69b)	Cl
(69c)	OMe

(P)
(63-68)

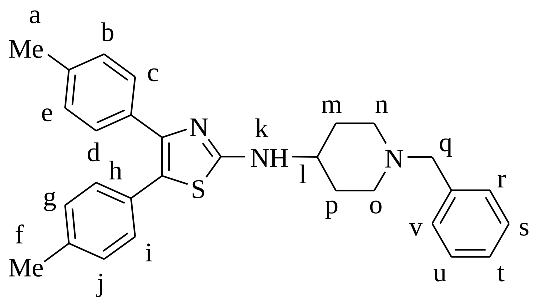
Compd	**R**
(63)	H
(64)	4-Me
(65)	4-OMe
(66)	2-Cl-4-F
(67)	4-CN
(68)	4-CF_3

(Q)
(70-79)

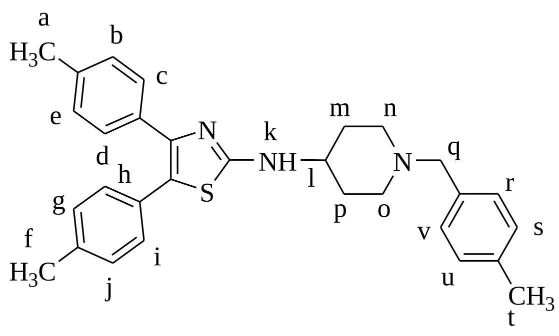
X			**R**
Me	**Cl**	**OMe**	
(70)	**(72)**	–	H
(71)	**(73)**	**(77)**	4-Me
–	**(74)**	**(78)**	4-OMe
–	–	**(79)**	4-CF_3
–	**(75)**	–	2-Cl, 4-F
–	**(76)**	–	4-CN

Scheme-11

IR spectrum of compound **(70)** showed N-H stretching vibration as a strong peak at 3396 cm^{-1} and N-H deformation as a moderate peak at 1534 cm^{-1}. Its PMR spectrum offered peaks for aromatic protons at δ 7.29-7.27 (d, $2H_{c,d}$) for two protons and δ 7.23-7.21 (d, $2H_{h,i}$) for two protons. Other aromatic protons appeared at δ 7.19-7.15 (m, 2H), δ 7.09-7.07 (m, 3H) and δ 6.97-6.95 (m, 4H). A doublet was observed at δ 5.21-5.19 (d, $1H_k$) due to the proton attached to the amine with coupling constant of 6.7 Hz. Other signals were at δ 3.42 (s, $2H_q$) for two protons, δ 3.30 (bs, $1H_l$) for one proton and δ 2.74-2.71 (d, 2H). Other signals were observed at δ 2.22 (s, $3H_a$) and δ 2.21 (s, $3H_f$) for six protons due to methyl groups attached to the diaryl moiety, δ 2.11-2.06 (t, 2H), δ 2.02-1.99 (d, 2H) and δ 1.54-1.49 (m, 2H). Its ^{13}C-NMR spectrum showed signals for aromatic carbons at δ 165.91, 145.61, 138.19, 137.08, 136.76, 132.71, 130.10, 129.25, 129.18, 128.84, 128.80, 128.28, 127.13 and 119.93 whereas the aliphatic carbons appeared at δ 63.68, 52.96, 51.97, 32.20, 21.30 and 21.23. Its mass spectrum showed molecular ion peak at 453.05 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.57 min retention time.



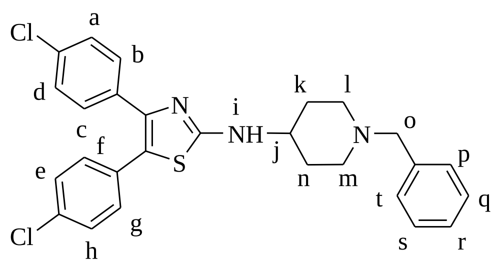
(70)



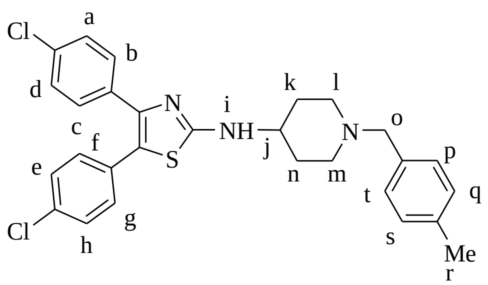
(71)

IR spectrum of compound **(71)** offered N-H stretching vibration as a strong peak at 3201 cm^{-1} and N-H deformation as a moderate peak at 1553 cm^{-1}. Its PMR spectrum showed aromatic protons ($CH_{c,d}$) at δ 7.37-7.35 as doublet which coupled with protons ($CH_{b,e}$) observed at δ 7.17-7.15 as doublet with a coupling constant of 8.0 Hz. Protons ($CH_{h,i}$) were observed at δ 7.19-7.17 as doublet which coupled with protons ($CH_{g,j}$) observed at δ 7.13-7.11 as a doublet with a coupling constant of 8.0 Hz. Other signals for the aromatic protons were at δ 7.05-7.03 (d, $4H_{r,s,u,v}$) for four protons with coupling constant of 8.0 Hz. A doublet was observed at δ 5.17-5.15 (d, $1H_k$) due to the proton attached to the amine with coupling constant of 7.5 Hz. Other signals were observed at δ 3.47 (s, $2H_q$) for two protons, δ 3.38 (bs, $1H_l$) for one proton and δ 2.81-2.78 (d, 2H). It gave a singlet at δ 2.33 (s, $3H_t$) for three protons due to methyl group attached to the aromatic moiety. Signals were observed at δ 2.31 (s, $6H_{a,f}$) for six protons due to methyl groups attached to the diaryl moiety, δ 2.18-2.13 (m, 2H), δ 2.10-2.07 (d, 2H) and δ 1.62-1.54 (m, 2H). Its ^{13}C-NMR spectrum showed signals for aromatic carbons at δ 165.84, 145.63, 137.06, 136.75, 135.01, 132.71, 130.10, 129.19, 128.95, 128.82 and 119.96 whereas the aliphatic carbons appeared at δ 62.79, 52.96, 51.88, 32.23, 29.73 and 14.15. Its mass spectrum showed molecular ion peak at 467.17 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 5.97 min retention time.

IR spectrum of compound **(72)** showed N-H stretching vibration as a strong peak at 3394 cm^{-1} and N-H deformation as a moderate peak at 1561 cm^{-1}. Its PMR spectrum showed aromatic protons at δ 7.32-7.31 (m, 3H), δ 7.25-7.23 (m, 2H), 7.17-7.14 (m, 6H) and δ 7.09-7.07 (m, 2H). A doublet was observed at δ 5.18-5.16 (d, $1H_i$) due to the proton attached to the amine with coupling constant of 7.2 Hz. It also gave a singlet at δ 3.46 (s, $2H_o$) for two protons, δ 3.32 (bs, $1H_j$) for one proton and δ 2.78-2.75 (d, 2H). Other signals were observed at δ 2.15-2.09 (t, 2H) and δ 2.05-2.01 (d, 2H) and δ 1.58-1.49 (m, 2H). Its ^{13}C-NMR spectrum showed aromatic carbons at δ 166.30, 145.22, 138.00, 133.57, 133.51, 133.12, 131.11, 130.51, 130.23, 129.19, 128.92, 128.49, 128.30, 127.19 and 119.30 whereas the aliphatic carbons appeared at δ 63.04, 51.90 and 32.12. Its mass spectrum showed molecular ion peak at 494.62 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.53 min retention time.



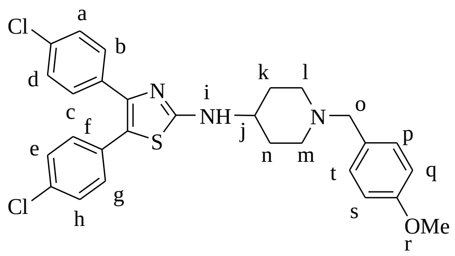
(72)

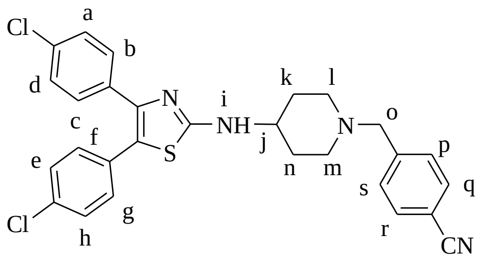


(73)

IR spectrum of compound **(73)** showed N-H stretching vibration as a strong peak at 3182 cm^{-1} and N-H deformation as a moderate peak at 1551 cm^{-1}. Its PMR spectrum showed aromatic protons at δ 7.38-7.36 (d, 2H), δ 7.25-7.23 (m, 5H), δ 7.22-7.20 (m, 2H) and δ 7.14-7.12 (m, 3H). A doublet was observed at δ 5.22-5.20 (d, $1H_i$) due to the proton attached to the amine with coupling constant of 7.9 Hz. It also gave signals at δ 3.49 (s, $2H_o$) for two protons, δ 3.39 (bs, $1H_j$) for one proton and δ 2.83-2.80 (d, 2H). A singlet at δ 2.34 (s, $3H_r$) was observed for three protons due to methyl group attached to the aromatic moiety. Other signals were at δ 2.19-2.14 (m, 2H) and δ 2.11-2.07 (d, 2H) and δ 1.63-1.55 (m, 2H). Its ^{13}C-NMR spectrum showed signals for aromatic carbons at δ 166.29, 145.25, 136.79, 134.88, 133.60, 133.50, 133.12, 131.14, 130.51, 130.22, 129.17, 128.96, 128.91, 128.47 and 119.30 whereas the aliphatic carbons appeared at δ 62.75, 53.05, 32.14, 29.72 and 21.13. Its mass spectrum showed molecular ion peak at 507.33 $M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.56 min retention time.

IR spectrum of compound **(74)** showed N-H stretching vibration as strong peak at 3399 cm^{-1} and N-H deformation as a moderate peak at 1565 cm^{-1}. Its PMR spectrum showed aromatic protons at δ 7.49-7.47 (d, $2H_{b,c}$) for two protons with a coupling constant of 7.8 Hz, δ 7.25-7.21 (m, 6H), δ 7.15-7.14 (d, 2H) and δ 6.87-6.85 (d, 2H). A singlet was observed at δ 5.27 (bs, $1H_i$) due to the proton attached to the amine. It also gave a signal at δ 3.80 (s, $3H_r$) for three protons due to methoxy group attached to the aromatic moiety. Signals were observed at δ 3.49 (s, $2H_o$) for two protons and at δ 3.40 (bs, $1H_j$) for one proton. Other signals were at δ 2.85-2.83 (d, 2H), δ 2.21-2.16 (d, 2H), δ 2.12-2.09 (d, 2H) and δ 1.66-1.58 (m, 2H). Its mass spectrum showed molecular ion peak at 524.54 (M)$^+$.

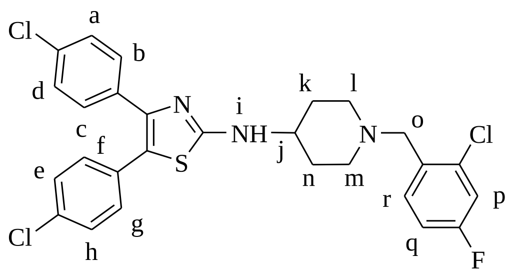




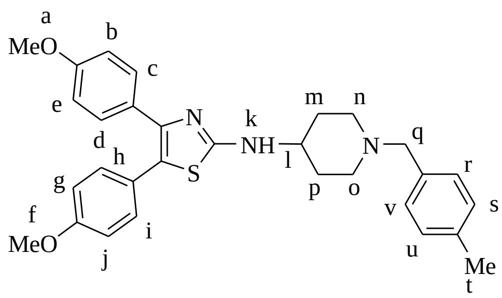
(74) **(75)**

IR spectrum of compound **(75)** showed N-H stretching vibration as a strong peak at 3394 cm^{-1} and N-H deformation as a moderate peak at1598 cm^{-1}. The peak observed at 2223 cm^{-1} was due to the nitril group. Its PMR spectrum offered peaks for aromatic protons ($CH_{r,q}$) at δ 7.62-7.60 as doublet which coupled with protons ($CH_{p,s}$) observed at δ 7.17-7.15 as doublet with a coupling constant of 8.3 Hz. Protons ($CH_{b,c}$) were observed at δ 7.46-7.44 as doublet which coupled with protons ($CH_{a,d}$) observed at δ 7.26-7.24 as doublet with a coupling constant of 8.1 Hz. Other signals for the aromatic protons ($CH_{f,g}$) were observed at δ 7.38-7.36 as doublet which coupled with protons ($CH_{e,h}$) observed at δ 7.23-7.21 as doublet with a coupling constant of 8.3 Hz. A singlet was observed at δ 5.36 (bs, $1H_i$) due to the proton attached to the amine. Signals were observed at δ 3.57 (s, $2H_o$) for two protons and δ 3.43 (bs, $1H_j$) for one proton. Other signals were observed at δ 2.82-2.79 (d, 2H), δ 2.26-2.24 (m, 2H), δ 2.11-2.08 (m, 2H) and δ 1.67-1.59 (m, 2H). Its mass spectrum showed molecular ion peak at 519.84 (M)$^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.68 min as retention time.

In the IR spectrum of compound **(76)** showed N-H stretching vibration as strong peak at 3210 cm^{-1} and N-H deformation as a moderate peak at 1601 cm^{-1}. Its PMR spectrum showed aromatic protons at δ 7.50-7.44 (m, 1H), δ 7.37-7.35 (m, 2H), δ 7.25-7.21 (m, 4H), δ 7.18-7.16 (m, 2H), δ 7.12-7.09 (d, 1H) and δ 6.99-6.94 (m, 1H). A doublet was observed at δ 5.31-5.30 (d, $1H_i$) due to proton attached to the amine with coupling constant of 6.7 Hz. It also gave signals at δ 3.59 (s, $1H_o$) for two protons and δ 3.44 (bs, $1H_j$) for one proton. Other signals were observed at δ 2.86-2.83 (d, $2H_{l,m}$) for two protons with a coupling constant of 11.7 Hz, δ 2.31-2.26 (d, 2H), δ 2.12-2.10 (d, 2H, J = 10.5 Hz) and δ 1.65-1.56 (m, 2H). ^{13}C-NMR spectrum showed signals for aromatic carbons at 166.23, 162.68, 160.21, 145.16, 134.78, 134.68, 133.50, 131.11, 130.46, 129.49, 128.89, 128.45, 119.30, 116.80 and 113.98 whereas the aliphatic carbons appeared at δ 58.48, 51.88, 31.56 and 29.68.



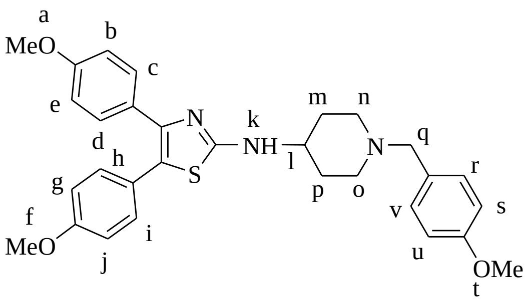
(76)



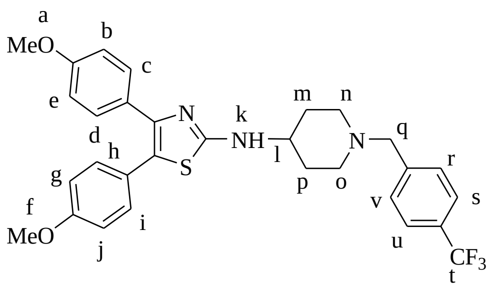
(77)

In the IR spectrum of compound **(77)** showed N-H stretching vibration as strong peak at 3391 cm^{-1} and N-H deformation as a moderate peak at 1550 cm^{-1}. Its PMR spectrum offered signals for aromatic protons at δ 7.54-7.52 (d, $1H_c$) for one proton with a coupling constant of 8.4 Hz, δ 7.48-7.47 (d, $2H_{d,h}$) for two protons, δ 7.21-7.19 (d, $2H_{i,b}$) for two protons, δ 7.14-7.12 (d, $3H_{e,g,j}$) for three protons and δ 6.79-6.75 (m, $4H_{r,s,u,v}$) for four protons. Among the aliphatic protons, it gave a broad singlet at δ 5.24 (bs, $1H_k$) due to proton attached to amine, δ 3.85 (s, $3H_a$) and a signal at δ 3.73 (s, $3H_f$) as a singlet for six protons due to methoxy groups attached to the diaryl moiety. A singlet at δ 3.52 (s, $2H_q$) was observed for two protons. Other signals were observed at δ 3.39 (bs, $1H_l$) for one proton, δ 2.87-2.84 (d, 2H). Other signals were observed at δ 2.30 (s, $3H_t$) as a singlet for three protons due to methyl group attached to the aromatic moiety, δ 2.28-2.12 (m, 2H), δ 2.10-2.07 (d, 2H) and δ 1.67-1.60 (m, 2H). Its mass spectrum showed molecular ion peak at 499.58 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 5.92 min as retention time.

IR spectrum of compound **(78)** showed N-H stretching vibration as a strong peak at 3153 cm^{-1} and N-H deformation as a moderate peak at 1570 cm^{-1}. Its PMR spectrum showed aromatic protons at δ 7.49-7.48 (d, 1H), δ 7.40-7.36 (m, 2H), δ 7.26-7.22 (m, 3H), δ 7.15-7.12 (d, 1H), δ 6.87-6.85 (d, 2H) and δ 6.79-6.75 (m, 3H). A broad singlet was observed at δ 5.27 (bs, $1H_k$) due to proton attached to the amine. Signal was observed at δ 3.88 (s, $3H_t$) for three protons due to methoxy group attached to the aromatic moiety. It also gave a singlet at δ 3.80 (s, $6H_{a,f}$) for six protons due to methoxy groups attached to the diaryl moiety. Other signals were at δ 3.49 (s, $2H_q$) a singlet for two protons, δ 3.39 (bs, $1H_l$) for one proton, δ 2.85-2.83 (d, 2H), δ 2.28-2.16 (m, 2H), δ 2.13-2.10 (m, 2H) and δ 1.66-1.61 (m, 2H). Its ^{13}C-NMR spectrum showed signals for aromatic carbons at 165.81, 159.15, 154.89, 145.64, 133.92, 132.49, 130.82, 130.60, 129.61, 128.99, 127.60, 126.83, 117.19, 114.40, 113.80 and 111.82 whereas aliphatic carbons appeared at δ 62.02, 56.25, 55.29, 52.28, 51.49 and 31.94. Its mass spectrum showed molecular ion peak at 515.01 $(M)^+$. The compound also showed high LC-MS/MS purity (> 98 %) with 2.59 min retention time.



(78)



(79)

IR spectrum of compound **(79)** showed N-H stretching vibration as a strong peak at 3384 cm^{-1} and N-H deformation as a moderate peak at 1550 cm^{-1}. Its PMR spectrum showed aromatic protons at δ 7.49-7.48 (d, 1H), δ 7.40-7.36 (m, 2H), δ 7.29-7.22 (m, 2H), δ 7.15-7.12 (m, 1H), δ 7.03-6.97 (m, 3H) and δ 6.81-6.75 (m, 3H). A broad singlet was observed at δ 5.27 (bs, $1H_k$) due to proton attached to the amine. Signals were observed at δ 3.86 (s, $3H_a$) and δ 3.79 (s, $3H_f$) for six protons due to methoxy groups attached to the diaryl moiety. Other signal gave a singlet at δ 3.48 (s, $2H_q$) for two protons, δ 3.39 (bs, $1H_l$) for one proton, δ 2.82-2.79 (d, 2H) for two protons, δ 2.20-2.17 (d, 2H) for two protons, δ 2.14-2.10 (t, 2H) and δ 1.64-1.55 (m, 2H). Its ^{13}C-NMR spectrum showed signals for aromatic carbons at 165.94, 160.85, 159.06, 154.88, 145.69, 133.92, 132.49, 130.69, 129.20, 113.63 and 111.83 whereas aliphatic carbons appeared at δ 62.51, 56.25, 52.91, 51.83, 32.65 and 29.72. Its mass spectrum showed molecular ion peak at 553.40 $(M)^+$.

4.2 Biological work

4.2.1 *In vitro* AChE and BuChE inhibition assays: The synthesized compounds **(20-56** and **70-79)** were evaluated for their potential to treat AD. AChE and BuChE inhibition activities were evaluated by the method described by Ellman [94] wherein tacrine and donepezil were used as reference standards **(Table 3)**. Compounds **(47-49, 52** and **56)** having benzyl piperidine and 4,5-disubstituted thiazolyl-2-amine linked to the aminomethylene spacer exhibited higher AChE inhibitory activity [IC_{50} values (nM): 398.0± 3.9, 296.0± 4.0, 374.4±3.9, 519.1± 8.7 and 357± 3.5 respectively] than the rest of the derivatives.

Compounds **(20-22, 24** and **29)** having 4,5-bis(*p*-tolyl)thiazol-2-ylamine and substituted benzyl piperidines linked through the carboxamide group showed poor AChE inhibitory activity [IC_{50} values (nM): >10000]. Compounds **(77-79)** having 4,5-bis(4-methoxypheny)thiazol-2yl-amine linked to the substituted benzylpiperidines without a carbon spacer also showed good inhibitory activities [IC_{50} values (nM): 826.1± 30.1, 767.2± 30.8, 745.5± 29.4 respectively].

It has been reported that BuChE levels remain constant or increase in advanced AD while AChE concentration in certain brain regions decreases. [11-13] These reports concluded that a balanced inhibition of both AChE and BuChE could be beneficial for the treatment of patients with cognitive deficit observed in AD. Thus, it was decided to check the targeted compounds for BuChE inhibitory activities also. Majority of the compounds exhibited good BuChE inhibitory activity. Compounds **(20, 30, 32, 40** and **43)** showed better BuChE inhibitory activity [IC_{50} values (nM): 1497.3± 21.5, 1035.4± 21.8, 715.1± 14.1, 1538.2± 29.3 and 1572.4±36.1 respectively].

The two compounds **(48, 52)** which showed the best AChE inhibitory activities [IC_{50} values (nM): 296.0± 4.0 and 519.1± 8.7 respectively] also showed satisfactory BuChE inhibitory activities [IC_{50} values (nM): 1841.2± 29.7 and 740± 13.2 nm respectively].

Table 3. AChE and BuChE inhibitory activities (IC_{50} values), inhibition of AChE induced $A\beta_{1-42}$ aggregation and selectivity ratio of the compounds (20-56 and 70-79)

Compound	**AChE**	**BuChE**	**Selectivity ratio**[b]	**$A\beta_{1-42}$ aggregation**[c]
	(IC_{50} nM) ± SEM[a]	**(IC_{50} nM) ± SEM**[a]		**% Inhibition**
20	>10000	1497.3±21.5	7.0	nd[d]
21	7573.2±25.4	1881.4±30.6	4.0	nd[d]
22	>10000	1967.4±24.1	5.1	nd[d]
23	2500.2±30.3	3513.4±27.6	0.7	nd[d]
24	>10000	1604.2±25.9	7.8	nd[d]
26	2880.4±22.3	2772.4±25.6	1.0	nd[d]
27	1281.4±24.7	5321.4±38.4	0.2	nd[d]
28	2322.4±15.1	>10000	0.1	nd[d]
29	>10000	2778.2±25.4	10.3	nd[d]
30	1101.4±15.3	1035.4±21.8	1.1	nd[d]

31	2773.4±24.6	3359.6±34.1	0.8	nd[d]
32	650.5±8.1	715.1±14.1	0.9	18.82±2.23
33	636.5±7.7	3034±37.5	0.2	12.02±2.35
34	3696.4±16.4	>10000	0.3	nd[d]
35	1771.2±18.3	1593.2±32.7	1.1	nd[d]
37	1380.2±28.5	2176.3±33.7	0.6	nd[d]
38	1336.2±27.5	1851.2±26.4	0.7	nd[d]
39	677.2±14.2	1918.2±18.4	0.4	14.46±2.15
40	570.5±14.3	1538.2±29.3	0.4	21.17±1.21
41	2448.2±13.6	>10000	0.1	nd[d]
42	1155.0±27.7	1733.2±26.4	0.7	nd[d]
43	414.4±15.7	1572.4±36.1	0.3	16.56±1.75
44	2797.6±11.5	3579.4±13.7	0.8	nd[d]
45	1234.3±24.6	1780.4±31.5	0.7	nd[d]
46	501.8±13.9	2511.2±12.6	0.2	16.17±1.96
47	398.5±3.9	2198.7±28.9	0.2	26.35±2.46
48	**296.0±4.0**	**1841.2±29.7**	0.2	**27.65±2.91**
49	374.4±3.9	1952.4±31.7	0.2	25.79±3.53
50	869.7±11.2	1429.3±17.4	0.6	23.26±2.51
51	632.2±3.7	818.0±8.3	0.8	19.39±2.65
52	**519.1±8.7**	**740.5±13.2**	0.7	15.00±2.13
53	1243.5±38.8	3297.6±15.0	0.4	nd[d]
54	586.8±8.4	2210.1±29.3	0.3	25.64±2.77
55	694.6±4.3	8013.2±17.1	0.1	20.60±2.35
56	357.7±3.5	2150.4±13.4	0.2	**27.20±3.15**
70	1278.2±25.5	3589.4±11.8	0.4	nd[d]
71	1089.3±27.7	6030.5±35.9	0.2	nd[d]
72	1375.4±24.9	3297.3±32.4	0.4	nd[d]
73	3186.4±10.9	>10000	0.3	nd[d]

74	365.8±5.8	1969.3±17.1	0.2	nd[d]
75	1022.4±30.4	1720.5±31.7	0.6	nd[d]
76	365.8±5.8	1969.3±17.1	0.2	**27.47±2.85**
77	826.1±30.1	2248.6±29.5	0.4	19.24±2.38
78	767.2±30.8	2529.3±29.8	0.3	11.42±1.43
79	745.5±29.4	1675.4±25.5	0.4	18.93±2.85
Tacrine	51.89±7.1	10.6±1.8	4.9	30.05±3.12
Donepezil	11.64±3.2	1255.4±7.1	0.01	37.26±2.69

[a]Results are the mean ± SEM of at least three determinations. [b]Selectivity ratio = (IC_{50} of AChE)/(IC_{50} of BuChE). [c]Inhibition of AChE induced Aβ1-42 aggregation produced by the test compounds at 10 μM. [d]Not determined

4.2.2 Inhibition of AChE induced $A\beta_{1-42}$ aggregation: Evidences indicated that AChE localized with Aβ in senile plaques promoted the assembly of Aβ into fibrils and accelerated Aβ peptide deposition. AChE binds with Aβ directly through its peripheral binding site to promote aggregatory effect. [95, 96] Blockade of the peripheral site might attenuate the AChE induced Aβ peptide aggregation.[97] On the basis of *in vitro* ChE inhibition studies, compounds with potent AChE inhibitory activity from the series were selected for the thioflavin T (ThT) assay [98, 99] **(Table 3)** to evaluate their ability to inhibit AChE induced $A\beta_{1-42}$ aggregation. The results showed that three compounds **(48, 56** and **76)** were found to be the most potent inhibitors of AChE induced $A\beta_{1-42}$ aggregation with ~27% inhibition at 10 μM concentration.

4.2.3 *In vitro* blood-brain barrier permeation assay: Successful crossing of a drug through the blood−brain barrier (BBB) is of great importance for treatment of CNS disorders. To explore whether the synthesized hybrid diarylthiazol-benzylpiperidine derivatives have the ability to penetrate into the brain, a parallel artificial membrane permeation assay of the blood-brain barrier (PAMPA-BBB) was performed as described by Di *et al*.[100-102] This model has the advantage of predicting passive diffusion of any molecule through BBB with a high success rate.

Table 4. Permeability (P_e, 10^{-6} cm s^{-1}) results from the PAMPA-BBB assay for some compounds with their predictive penetration in CNS

Compd	P_e (10^{-6} cm s^{-1})[a]	Prediction[b]	Compd	P_e (10^{-6} cm s^{-1})[a]	Prediction[b]
32	15.9±1.2	CNS+	51	12.5±1.3	CNS+
33	8.8±0.4	CNS+	52	13.2±1.5	CNS+
39	12.3±0.8	CNS+	54	12.3±0.8	CNS+
40	12.5±0.8	CNS+	55	8.4±0.8	CNS+
43	8.2±0.4	CNS+	56	12.2±0.6	CNS+
46	10.6±1.1	CNS+	76	8.4±0.8	CNS+
47	12.8±0.8	CNS+	77	9.6±1.1	CNS+
48	14.5±1.5	CNS+	78	10.3±0.6	CNS+
49	10.1±0.6	CNS+	79	8.6±0.2	CNS+
50	8.8±0.4	CNS+			

[a]Data expressed as mean ± SEM of three independent experiments. [b]CNS+ indicates good passive CNS permeation.

The *in vitro* permeability (P_e) of the selected hybrid diarylthiazol-benzylpiperidine derivatives **(Table 4)** and nine commercially available drugs **(Table 5)** through a lipid extract of porcine brain lipid in PBS/ethanol (70:30) was determined. Validation of the assay was performed by comparing the experimental permeability [P_e(exp)] of some commercial drugs with the reported values of permeation [P_e(ref)] that gave a linear relationship *i.e.* P_e(exp.) = 1.171P_e(ref.) + 1.489 (R^2= 0.983) **(Figure 6)**. From this equation and taking the limits established by Di *et al*,[44] for BBB permeation into consideration, it was concluded that compounds with permeability values (P_e) greater than 5.9 × 10^{-6} cms^{-1} **(Table 6)** were capable of crossing the BBB. All the tested compounds demonstrated permeability values above this limit. Therefore the measured permeability values (P_e) pointed that the compounds described here should easily cross the BBB by passive diffusion.

Table 5. Permeability (P_e 10^{-6} cm s^{-1}) of nine commercial drugs in the PAMPA-BBB assay used in the experimental validation.

Commercial drugs	P_e (10^{-6} cm s^{-1})	
	Reference value[a]	Experimental value[b]
Diazepam	16	21.2±1.8
Verapamil	13	15.1±1.2
Progesterone	9.3	12.2±1.1

Clonidine	6.2	9.4±0.4
Corticosterone	5.1	8.2±0.5
Lemofloxacin	1.1	2.3±0.2
Ofoxacin	0.8	1.5±0.3
Atenolol	0.8	3.2±0.4
Dopamine	0.2	1.8±0.2

[a]Taken from reference.[67] [b]Data are expressed as mean ± SEM of three independent experiments.

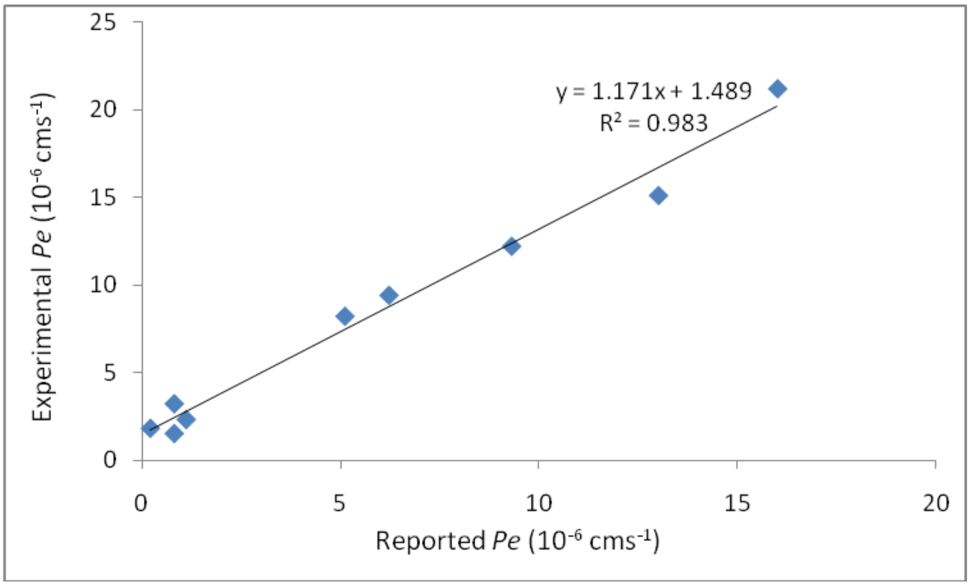


Figure. 6: Linear correlation between experimental and reported permeabilities of nine commercial drugs using PAMPA-BBB assay. P_e(exp.) = 1.171P_e(bibl.) + 1.489 (R^2 = 0.983)

Table 6. Ranges of permeability (P_e, 10^{-6} cm s^{-1}) of PAMPA-BBB assay.

Compounds with differing BBB Permeation	PBS/ethanol (70:30)
Compounds of high BBB permeation (CNS+)	$P_e > 6.2$
Compounds of uncertain BBB permeation (CNS+/-)	$6.2 > P_e > 3.8$
Compounds of low BBB permeation (CNS-)	$P_e > 3.8$

4.2.4 Cell viability and neuroprotective studies: To check therapeutic suitability of the selected hybrid diarylthiazole-benzylpiperidine derivatives, their effect on cell viability and neuroprotective ability against oxidative stress were evaluated using the human neuroblastoma SH-SY5Y cell line. For assessment of cytotoxicity of the test compounds, cells were exposed to relatively high concentrations of the compounds

Table 7. Cell viability, neuroprotection and free radical scavenging activity of some compounds in the human neuroblastoma SH-SY5Y cell line and DPPH assay

Compound	Cell viability (%)[a]		Neuroprotection (%)[b]		RP of DPPH (%)[c]	
	40 μM	80 μM	10 μM	20 μM	10 μM	20 μM
32	93.6±2.5	91.1±2.9	29.2±2.4	50.8±3.4	40.6±3.1	61.7±2.8
33	95.6±2.4	92.8±3.1	31.5±3.7	53.4±2.9	43.5±2.4	64.6±2.8
39	92.4±3.7	93.1±2.1	21.9±3.2	38.7±2.7	43.0±2.9	63.6±2.9
40	94.0±2.7	90.3±3.9	33.3±2.3	39.9±3.0	40.5±3.3	62.6±3.1
43	92.9±2.2	91.6±4.3	32.9±2.6	55.7±2.9	46.0±2.7	63.6±2.4
46	91.4±3.1	90.1±3.1	26.9±2.9	52.7±2.8	49.0±2.8	58.9±2.7
47	93.5±2.8	91.7±3.6	29.3±2.8	48.8±3.3	48.9±2.1	59.4±3.1
48	**95.2±2.6**	**91.3±2.9**	**39.6±2.3**	**59.5±2.4**	**54.6±2.7**	**70.5±2.6**
49	91.8±2.0	89.7±3.2	28.8±1.4	42.5±2.6	51.2±2.7	56.5±3.3
50	95.4±1.9	90.9±2.4	23.6±2.8	49.7±3.1	45.4±2.9	63.4±2.4
51	92.4±3.7	91.7±2.8	29.8±2.2	42.9±3.2	44.8±2.2	60.9±2.2
53	92.7±2.6	88.9±3.6	31.5±2.7	53.7±2.6	46.3±3.2	57.4±2.3
54	91.6±3.3	87.2±3.9	34.2±2.1	41.4±2.2	42.5±3.1	63.4±2.7
55	94.2±3.6	90.1±2.5	33.2±3.1	48.4±2.2	51.8±2.8	66.9±2.6
56	94.2±2.2	87.1±3.6	28.8±2.4	41.5±2.9	48.3±2.5	57.1±2.8
74	94.2±3.2	92.5±1.9	32.0±3.3	53.2±2.7	42.2±2.4	63.2±3.2
77	95.4±2.7	89.5±2.7	29.9±2.1	47.4±2.3	47.2±3.1	63.4±2.3
78	92.1±3.6	89.5±3.2	25.5±2.8	40.1±3.4	49.3±2.2	65.5±2.9
79	94.5±2.6	90.4±2.4	30.4±2.4	42.0±2.2	50.0±2.9	66.2±2.4
Tacrine	89.4±2.3	90.0±3.4	44.7±3.3	57.9±2.1	54.9±2.1	68.8±3.7
Donepezil	96.9±1.4	92.3±2.4	48.2±2.4	62.0±2.5	59.9±2.6	75.5±2.9
Ascorbic acid	nd	nd	nd	nd	56.4±2.7	67.7±2.7

[a]Percentage cell viability of SH-SY5Y cells exposed at relatively high concentrations (40 µM and 80 µM) of test compounds. [b]Percentage neuroprotection of SH-SY5Y cells at relatively lower concentrations (10 µM and 20µM) of the test compounds against H_2O_2 (100 µM) insult. Results are the mean ± SEM of at least three independent experiments. [c]RP of DPPH **(%) =** reduction percentage of DPPH. Results are the mean ± SEM of at least three independent experiments. nd = not determined.

(40 µM and 80 µM) for 24 hr followed by determination of the cell viability using 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) assay. In the given experimental conditions all of the selected hybrids showed negligible cell death even at such high concentrations **(Table 7)**.

In another set of experiments, neuroprotective potential of the selected hybrid diarylthiazole-benzylpiperidine derivatives was assessed. Oxidative stress-like condition in SH-SY5Y cells was induced using H_2O_2 as a toxic insult. [101, 103] In this assay,[101] H_2O_2 (100 µM) addition to the growth media induced significant cell death which was evidenced by reduced cell viability (52-56%) as compared to the control cells. To assess the neuroprotective potential, the cells were exposed to the test compounds (5 µM, 10 µM and 20 µM) 2 hr prior to the H_2O_2 insult for 24 hr. At 5 µM concentration, the compounds did not show promising protective effects (data not shown).

However, as shown in **(Table. 7)**, the selected derivatives exhibited significant neuroprotective effect at 10µM and 20µM concentrations. Amongst all of the hybrid compounds, compound **(48)** showed the highest neuroprotection against H_2O_2 insult. The results of these experiments revealed that compounds **(32, 33, 39, 40, 43, 46-51, 53-56, 74** and **77-79)** can protect neuronal cells against oxidative stress-associated cell death.

4.2.5 Antioxidant activity: Diphenyl-1-picrylhydrazyl (DPPH) is one of the few stable and commercially available organic nitrogen radicals. DPPH assay is commonly used for preliminary screening of compounds capable of scavenging the activated oxygen species.[104] The selected hybrid diarylthiazole-benzylpiperidine derivatives were evaluated for their anti-oxidant activity using DPPH assay. All the tested compounds exhibited significant free radical scavenging activity. At 10 µM concentration, the compounds showed free radical scavenging activity in the range of 40-55% and compound **(48)** showed the highest activity of 55%. Moreover, at 20 µM concentration, the test compounds exhibited free radical scavenging activity ranging from 56-70% with compound **(48)** showing 70% activity which was more than that of ascorbic acid.

4.2.6 ROS estimation in primary hippocampal neuronal culture: Hippocampal region of brain is considered as a memory origin centre having ACh as the major neurotransmitter. Therefore, degradation of hippocampal neurons causes memory impairment and ultimately neurodegeneration.[105, 106] Elevated level of ROS is a sign of increased oxidative stress in various pathological conditions.[107] $A\beta_{1-42}$ toxicity is known to induce oxidative stress in AD pathogenesis.[108] Here, anti-oxidant potential of the most potent test compound **(48)** was evaluated by estimating intracellular ROS level against Aβ_{1-42} toxicity of primary rat hippocampal neuronal cells using 2′,7′-dichlorofluorescine diacetate (DCFH-DA) assay.[67, 109] DCFH-DA crosses cell membrane and is readily hydrolyzed by intracellular esterases to nonfluorescent DCFH. In the presence of ROS, DCFH is rapidly oxidized to the highly fluorescent 2′,7′-dichlorofluorescine (DCF). As shown in **Figure 7A,** Aβ_{1-42} insult significantly elevated ROS level as compared to the control cells (p<0.001). However, the test compound **(48)** (10 µM and 20 µM) (**Figure 7A**, p<0.001) dose dependently attenuated ROS generation against Aβ_{1-42} toxicity like the standard drug donepezil (**Figure 7A**, p<0.001). Thus, the results revealed the potential of compound **(48)** to act as an efficient agent for the treatment of AD with ROS scavenging ability.

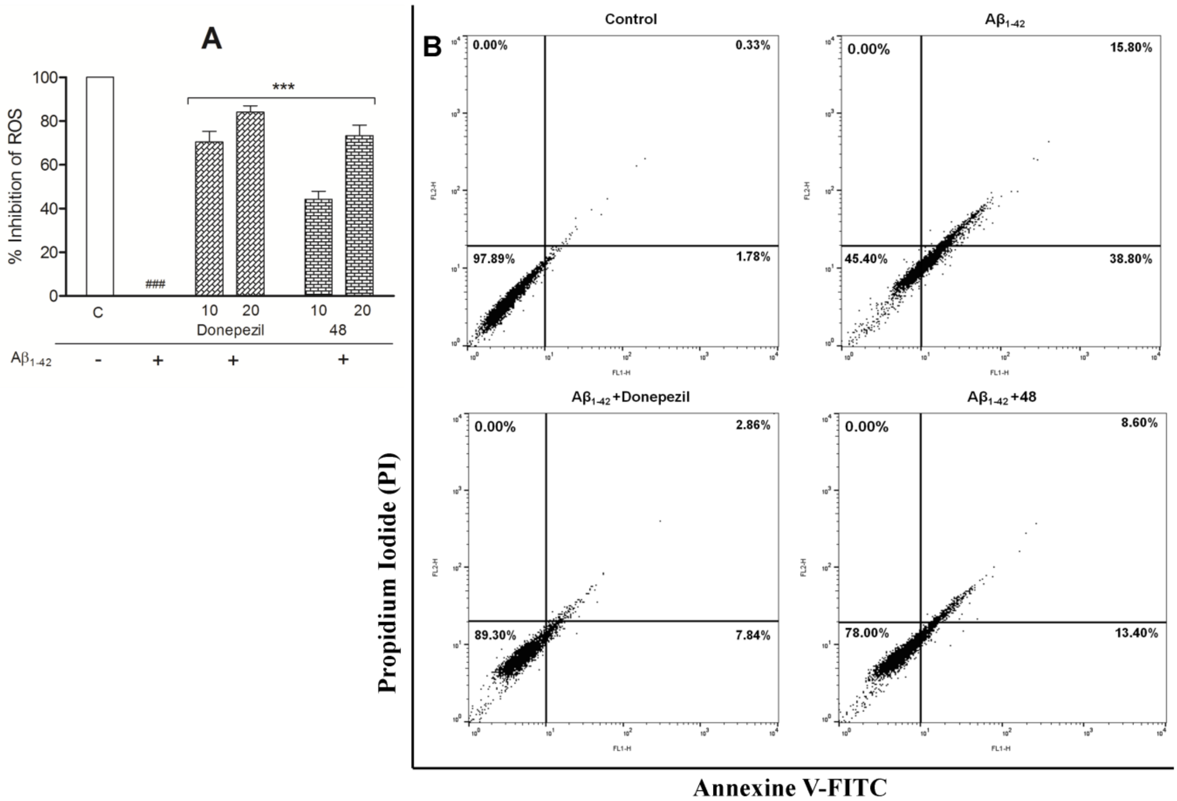


Figure 7: ROS scavenging and anti-apoptotic potential of compound **(48)** *in vitro* against Aβ_{1-42} (10 µM) insult in primary rat hippocampal neuronal culture. (A) Percentage inhibition of ROS generation assessed by DCFH-DA assay. (B) Flow cytometric assessment of apoptosis using Annexine V-FITC and PI staining. Cells in the lower left quadrant are viable (Annexine V-FITC-/PI-). Cells in the lower right quadrant are early apoptotic (Annexine V-FITC+/PI-) and those in the upper right quadrant are late apoptotic or nacrotic (Annexine V-FITC+/PI+). Data are expressed as mean ± SEM. $^{\#\#\#}p$<0.001 *vs.* control cells. *** p<0.00 *vs.* Aβ_{1-42}-treated control cells. C=control cells.

4.2.7 Flow cytometry: Apoptosis is a key event which plays an essential role in the development of various neurodegenerative diseases.[107, 110] It has also been reported that Aβ_{1-42}, a pathological hallmark of AD, causes apoptosis which ultimately leads to neurodegeneration.[111-113] The apoptosis was assessed using flow cytometry by staining the primary rat hippocampal neurons with annexine V-FITC and propidium iodide (PI). Annexin V enters the cells in both the early and late stages of apoptosis stages while PI stains the cells in the late apoptosis or necrosis stage. Aβ_{1-42} (10 µM) toxicity significantly increased percentage of early apoptotic cells (38.80%) as compared to the control cells (1.78%). Compound **(48)** (20 µM) (13.40%) significantly abrogated percentage of early apoptotic cells induced by Aβ_{1-42} insult in a manner similar to donepezil (7.84%). The results demonstrated anti-apoptotic effect of compound **(48)** against Aβ_{1-42} toxicity which further supported its anti-AD potential.

4.2.8 Behavioural model. To determine *in vivo* anti-cholinesterase activity of the most potent compound **(48)** in the series, scopolamine model was adopted.[114-116] Scopolamine induced amnesia in rodent model is an accepted standard protocol in behavioural pharmacology for evaluation of ChE inhibitors as anti-AD drug candidates. Scopolamine distinctly blocks muscarinic cholinergic receptors that lead to impairment of cognitive functions. To explore the anti-amnesic effect of compound **(48)**, Morris Water Maze (MWM) test was performed. During last five days of the treatment period, escape latency time (ELT) and number of platform area crossings were recorded for different experimental groups. The escape latencies were significantly prolonged (**Figure 8A**, p<0.001) while the number of platform area crossings (**Figure 8B**, p<0.001) were significantly reduced by scopolamine treatment (1.4 mg kg^{-1}, i.p.). Donepezil (at 5 mg kg^{-1}, p.o.) significantly reduced ELT (**Figure 8A**, p<0.001) and increased platform area crossings (**Figure 8B**, p<0.01) as compared to the scopolamine-treated control group. At an equivalent dose corresponding to donepezil, compound **(48)** significantly shortened ELT (**Figure 8A**, p<0.001) and increased the number of platform area crossings (**Figure 8B**, p<0.05) as compared to the scopolamine-treated control group. The results revealed anti-amnesic effect of compound **(48)** against scopolamine induced AD-like phenotype.

Next, the effect of compound **(48)** on AChE and BuChE activities was evaluated in the mice brain using Ellman's method. [94, 115] As described in our previous report, [93] scopolamine treatment significantly elevated AChE (**Figure 8C**, p<0.001) and BuChE (**Figure 8D**, p<0.001) levels as compared to the vehicle-treated control group. However, the elevated AChE (**Figure 8C**, $p<0.001$) and BuChE (**Figure 8D**, $p<0.001$) levels were significantly attenuated by compound **(48)** at an equivalent dose corresponding to donepezil.

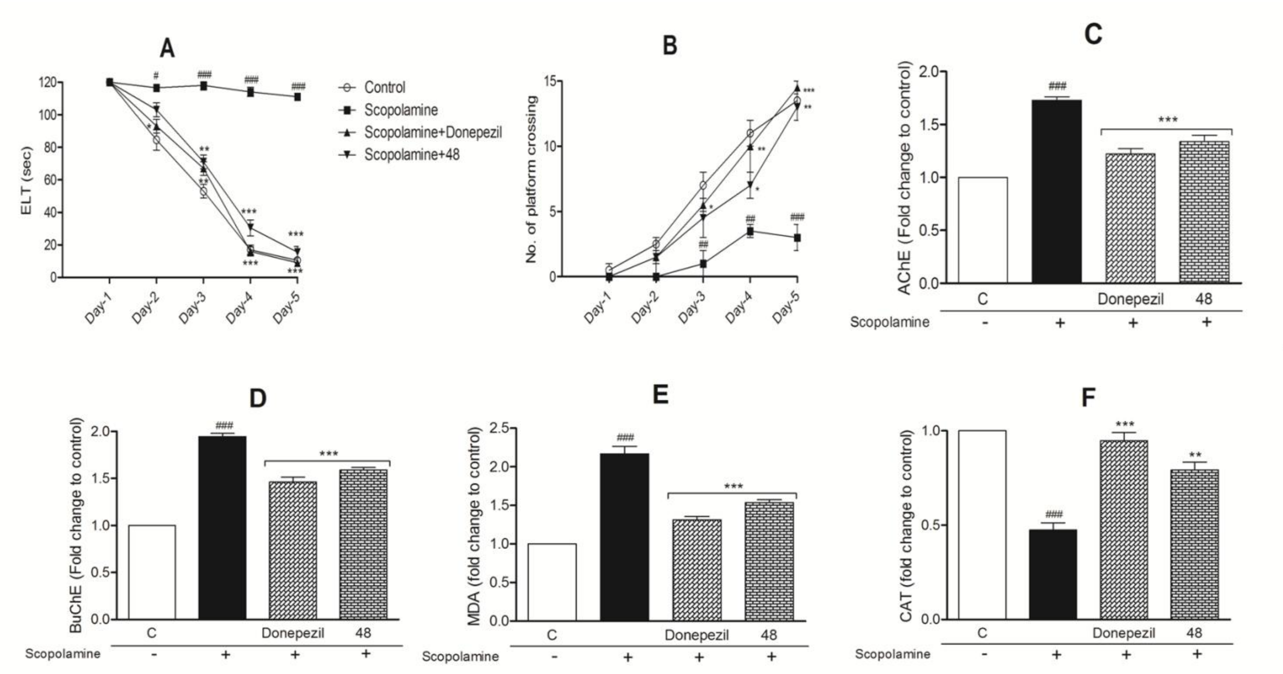


Figure 8. Compound **(48)** improved spatial learning and memory in mice treated with scopolamine through anti-cholinesterase and antioxidant activities. In MWM test, scopolamine (1.4 mg kg^{-1}, i.p.) increased (A) ELT while (B) reduced number of platform area crossings. This alteration was significantly reversed by **(48)** similar to donepezil at 5 mg kg^{-1}. (C) AChE and (D) BuChE levels elevated by scopolamine treatment were significantly attenuated by **(48).** Oxidative stress parameters are represented as (E) MDA and (F) CAT levels. Altered MDA and CAT levels after scopolamine treatment were significantly reversed by **(48)**. Data are expressed as mean ± SEM (n=6). $^{###}p<0.001$, $^{##}p<0.01$, $^{#}p<0.05$ *vs.* vehicle-treated control group.*** $p<0.001$, ** $p<0.01$, * $p<0.05$ *vs.* scopolamine-treated control group. C=vehicle-treated control group.

In order to further elucidate the mechanism of anti-amnesic activity of compound **(48)**, malondialdehyde (MDA - a by product of lipid peroxidation) and catalase (CAT - which catalyzes H_2O_2 decomposition) levels were determined following MWM test. Scopolamine treatment significantly elevated MDA level (**Figure 8E**, $p<0.001$) while reduced the CAT level (**Figure 8F**, $p<0.001$) compared to the vehicle-treated control group. However, the treatment of amnesic mice with compound **(48)** significantly attenuated MDA level (**Figure 8E**, $p<0.001$) while elevated the CAT level (**Figure 8F**, $p<0.01$). Thus, the results revealed *in vivo* ChE inhibition and antioxidant activities of compound **(48)** similar to donepezil, which further substantiated anti-AD potential of the most promising test compound **(48)**.

4.2.9 Y maze test: To develop AD like phenotype, animals were subjected to intracerebroventricular (ICV) injection of Aβ_{1-42} in the hippocampal region of brain. This is a promising model to evaluate the neuroprotective potential of any drug candidate in AD like condition.[93, 117] The working memory impairment was assessed using Y maze test. "Spontaneous alteration" behaviour displays the working memory. Aβ_{1-42}-treated control animals exhibited significantly reduced percentage of "spontaneous alterations" throughout the experimental session as compared to the vehicle-treated control animals (**Figure 9**, p<0.001). Animals treated with compound **(48)** displayed increased percentage of "spontaneous alterations" (**Figure 9**, p<0.01). However, the mean number of arm entries remained unchanged across all the experimental groups which suggested that the general locomotor activity was not hampered by Aβ_{1-42} toxicity. Thus, compound **(48)** improved hippocmpal dependant working memory worsened by Aβ_{1-42}.

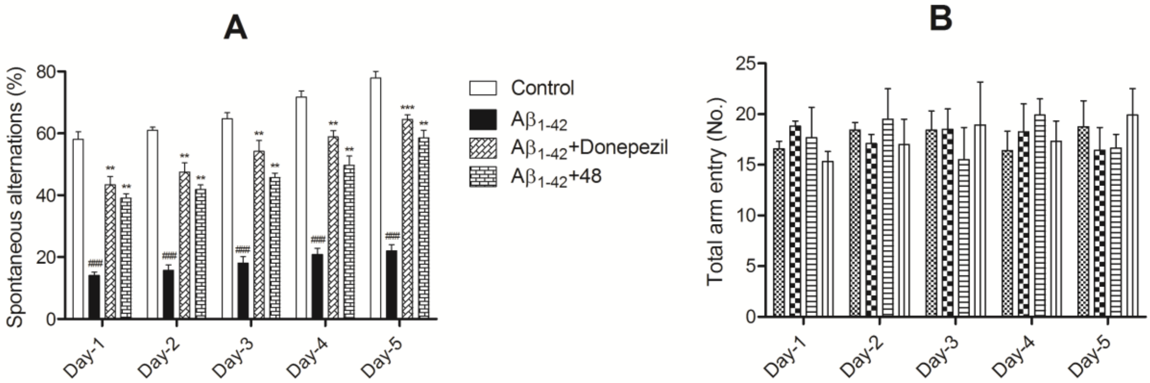


Figure 9. Compound **(48)** improved immediate working memory in rats which received ICV injection of Aβ_{1-42} in Y maze test. (A) Percentage spontaneous alterations and (B) total arm entries were recorder by a blind observer. Data are expressed as mean±SEM (n=6). $^{###}p$<0.001, $^{##}$ p<0.01, $^{#}$ p<0.05 *vs.* vehicle-treated control group.*** p<0.001, ** p<0.01, * p<0.05 *vs.* scopolamine-treated control group. C=vehicle-treated control group.

4.2.10 Western blot analysis: *In vivo* neuroprotective potential of compound **(48)** was assessed by determining the levels of pathological hallmarks of AD in the hippocampal region of rat brain which underwent ICV injection of Aβ_{1-42}. Elevated levels of Aβ_{1-42} and p-tau are key indicators of AD development. [118] At the end of the ICV experimental model, animals from different groups were scarified to estimate the levels of Aβ_{1-42} and p-Tau. A significant rise in the levels of Aβ_{1-42} (**Figure 10B**, p<0.001) and p-Tau (**Figure 10C**, p<0.01) was observed in the Aβ_{1-42}-treated control group as compared to the vehicle-treated control group. Supporting the results of *in vitro* Aβ_{1-42} aggregation inhibition (ThT) assay, treatment of the animals with compound **(48)** significantly attenuated the levels of Aβ_{1-42} (**Figure 10B**, *p*<0.01) and *p*-Tau (**Figure 10C**, *p*<0.05) similar to donepezil. The results confirmed anti-Aβ_{1-42} aggregatory and furthermore anti-AD potential of compound **(48)**. Further, to assess the *in vivo* anti-apoptotic potential of compound **(48)**, levels of cleaved-caspase-3 and cleaved-PARP were determined in the hippocampal region of brains of the animals which received Aβ_{1-42} insult. It has been well documented that cleavage/activation of caspase-3 is a key event of apoptosis.[110] It has been demonstrated that caspase-3 is partially or totally resp-

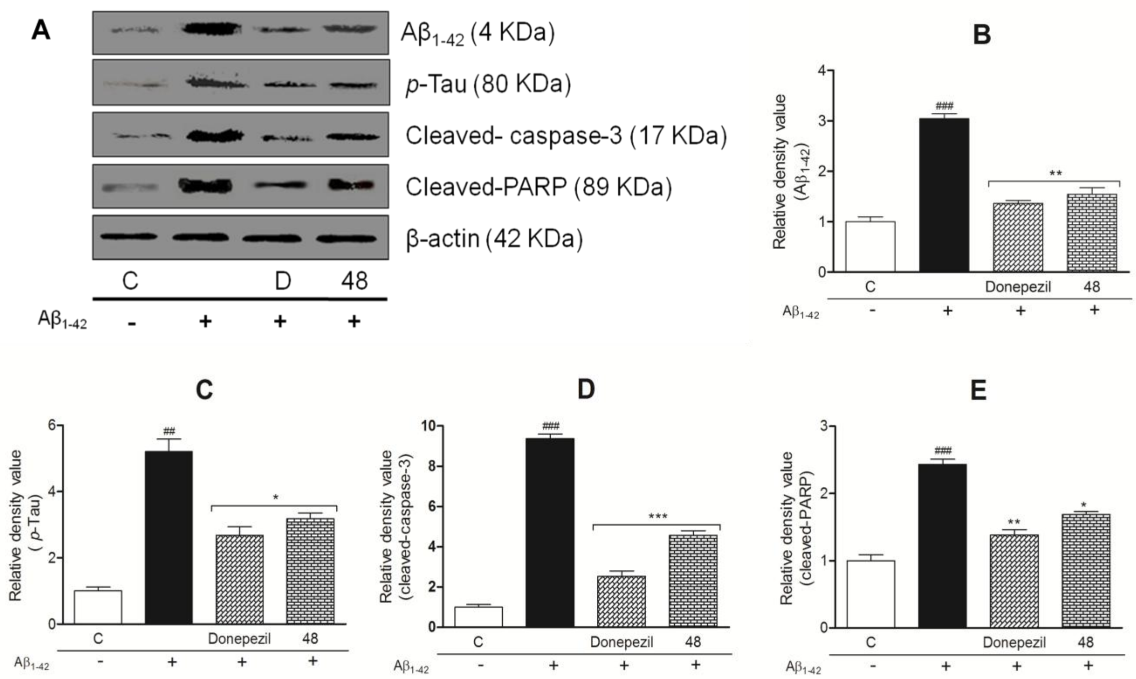


Figure 10. *In vivo* neuroprotective and anti-apoptotic potential of compound **(48)**. (A) The expression of Aβ_{1-42}, *p*-Tau, cleaved-caspase-3 and cleaved-PARP was assessed by Western blot analysis in the hippocampal region of rat brain which was given ICV injection of Aβ_{1-42}. Densitometric analysis revealed attenuation of (B) Aβ_{1-42}, (C) p-Tau, (D) cleaved-caspase-3 and (E) cleaved-PARP levels by compound **(48)** elevated by Aβ_{1-42} toxicity. Data are expressed as mean±SEM (n=6). $^{###}p<0.001$, $^{##}p<0.01$, $^{\#}p<0.05$ *vs.* vehicle-treated control group.*** *p*<0.001, ** *p*<0.01, * *p*<0.05 *vs.* Aβ_{1-42}-treated control group. C=vehicle-treated control group

onsible for the proteolytic cleavage of poly (ADP-ribose) polymerase-1 (PARP), a nuclear-DNA binding protein [119, 120] which is involved in numerous cellular events including DNA repair.[121] PARP overactivation is associated with different neurodegenerative conditions including AD.[122] It has been reported earlier that Aβ peptide induced PARP activity in the hippocampal region of adult rat brain. [123, 124] Cleavage of PARP is followed by the caspase-3 activation which subsequently leads to apoptosis and ultimately neuronal cell death. Thus, cleaved-caspase-3 and cleaved-PARP are the two significant key markers of apoptosis. [125] In this study, the levels of cleaved-caspase-3 (**Figure 10D**, $p<0.001$) and cleaved-PARP (**Figure 10E**, $p<0.001$) were significantly elevated in the hippocampal region of $A\beta_{1-42}$-treated control animal brain as compared to the brain of the vehicle-treated control group. Supporting the *in vitro* results, compound **(48)** demonstrated significant anti-apoptotic potential by lowering the levels of cleaved-caspase-3 (**Figure 10D**, $p<0.001$) and cleaved-PARP (**Figure 10E**, $p<0.05$) similar to donepezil, which were raised by $A\beta_{1-42}$ toxicity. In conclusion, compound **(48)** has exhibited a multi-target directed action which highlighted its potential as a possible therapeutic alternate for the treatment of AD.

4.2.11 Acute toxicity study: For the development of a new drug, acute toxicity profile of a compound is considered to be an important parameter. The most promising anti-AD candidate arising out of the current study is compound **(48)**. To investigate its safety profile, acute toxicity of compound **(48)** was determined in male Swiss Albino mice at doses of 0, 677, 1333 and 2000 mg kg^{-1} (n = 5 per group) by oral administration. Mice were regularly observed for 14 days after treatment. After the observation period of 14 days, all the animals remained alive and appeared healthy in terms of fur sleekness, water and food consumption, and body weight. On the 15th day, all the animals were sacrificed for macroscopic examination to evaluate possible damage to the heart, liver and kidneys. The results showed that mice treated with compound **(48)** did not produce any acute toxicity or mortality immediately or during the post-treatment period. Therefore, compound **(48)** was found to be non-toxic and well tolerated at doses of up to 2000 mg kg^{-1}.