

5. Results and discussion

The work proposed in the aim and objectives is discussed in the following sections:

5.1. Chemical studies

5.1.1. Synthesis of vicinal diaryl pyrazole-based compounds (59-153)

5.1.2. Synthesis of benzofuran based compounds (156-227)

5.2. Biological studies

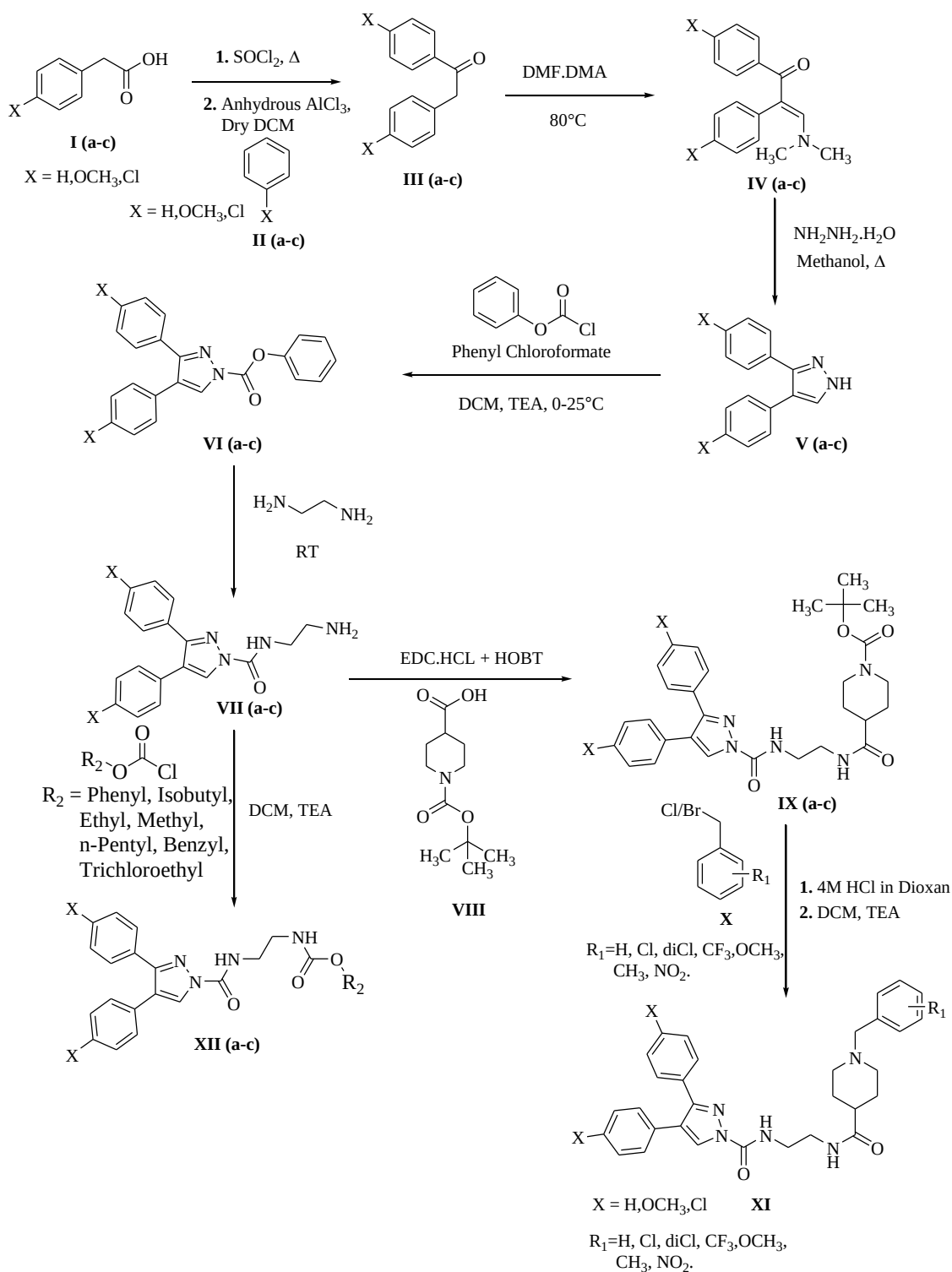
5.2.1. *In vitro* biological evaluation of vicinal diaryl pyrazole-based compounds (91-121, 130-153)

5.2.2. *In vitro* biological evaluation of benzofuran based compounds (156-227)

5.1. Chemical studies

The proposed compounds were synthesized according to the synthetic strategies illustrated in the general schemes. Using **general scheme-1**, two different series of vicinal diaryl substituted pyrazole viz. *N*-(2-(3,4-diphenyl-1*H*-pyrazole-1-carboxamido)ethyl)-1-benzylpiperidine-4-carboxamide derivatives [**XI (a-c)**] and 2-(3,4-diphenyl-1*H*-pyrazole-1-carboxamido)ethylcarbamate derivatives [**XII (a-c)**] were prepared.

Using thionyl chloride, the acid chlorides were initially made from substituted phenylacetic acids [**I (a-c)**]. 1,2-Diarylethanones [**III (a-c)**] were subsequently generated by Friedel-Crafts acylation employing substituted benzenes [**II (a-c)**] and the acid chlorides. Further, *N,N*-dimethylformamide dimethylacetal (DMF.DMA) was added and heated in solvent free condition to produce substituted (*E*)-3-(dimethylamino)-1,2-diarylprop-2-en-1-one [**IV (a-c)**] which was cyclized to substituted 3,4-diphenyl-1*H*-pyrazole [**V (a-c)**] using hydrazine hydrate. In the next step this compound was treated with phenyl chloroformate to produce substituted phenyl 3,4-diaryl-1*H*-pyrazole-1-carboxylate [**VI (a-c)**] which was then substituted with ethylene diamine to synthesize intermediate *N*-(2-aminoethyl)-3,4-diaryl-1*H*-pyrazole-1-carboxamide substituents [**VII (a-c)**]. This derivative was coupled with 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (**VIII**) via acid amine coupling in presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) and hydroxybenzotriazole (HOBt) under basic condition to prepare *tert*-butyl 4-(2-(3,4-diaryl-1*H*-pyrazole-1-carboxamido)ethylcarbamoyl)piperidine-1-carboxylate derivatives [**IX (a-c)**]. The protected amine was deprotected using 4*M* HCl in dioxan and was further reacted with different benzyl halides (**X**) to obtain final compound *N*-(2-(3,4-diaryl-1*H*-pyrazole-1-carboxamido)ethyl)-1-benzylpiperidine-4-carboxamide derivatives [**XI (a-c)**].



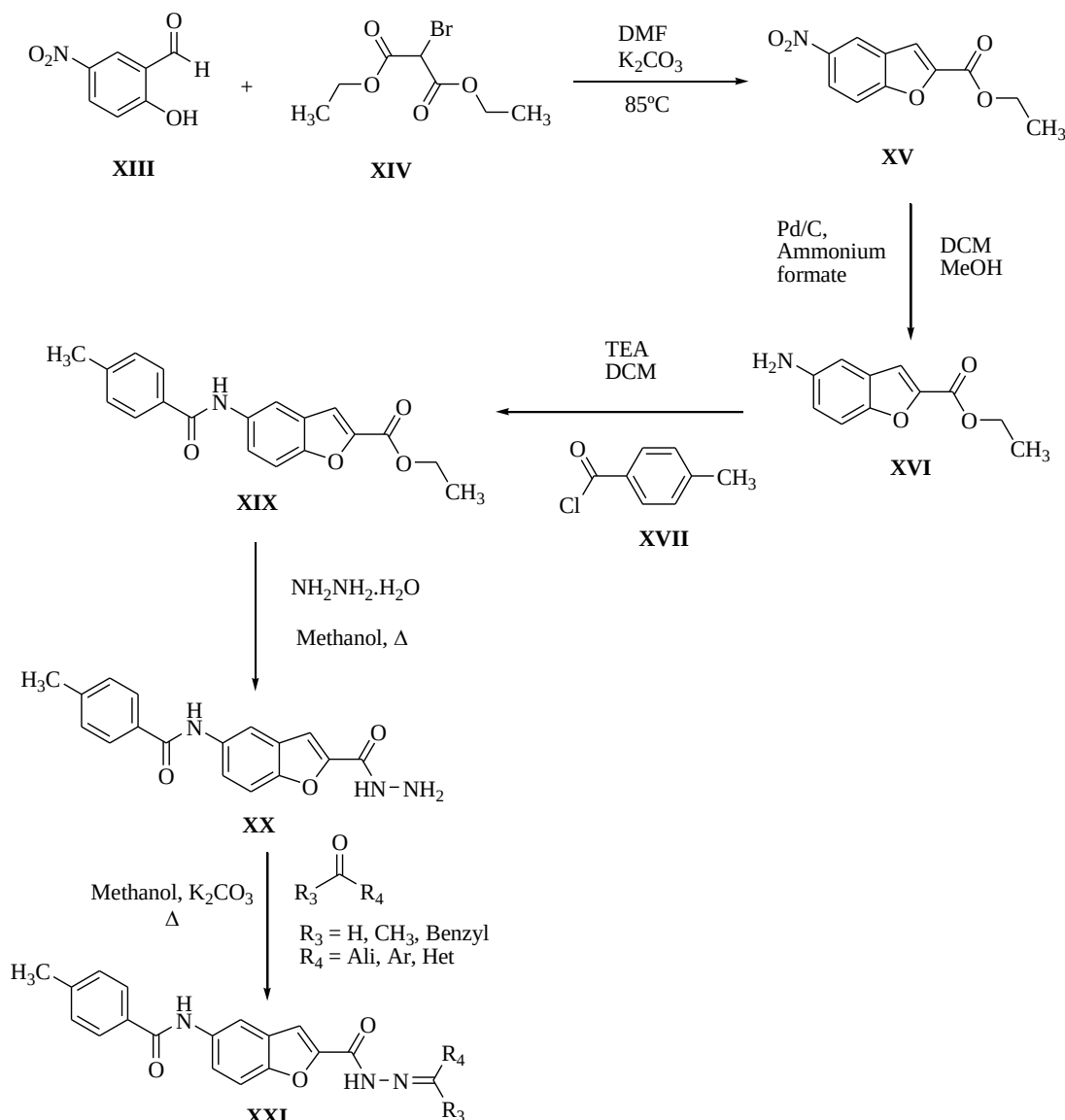
General Scheme-1

On the other hand, [**VII (a-c)**] reacted with various chloroformate derivatives to produce final compounds [**XII (a-c)**] containing carbamate derivatives.

To synthesize the envisaged compounds, **general scheme-2** was adopted, wherein 2-hydroxy-5-nitrobenzaldehyde (**XIII**) reacted with potassium carbonate. Subsequently, cyclization using diethyl 2-bromomalonate (**XIV**) produced ethyl 5-nitrobenzofuran-2-

carboxylate (**XV**). Further reduction was carried out using ammonium formate and Pd/C to obtain ethyl 5-aminobenzofuran-2-carboxylate (**XVI**).

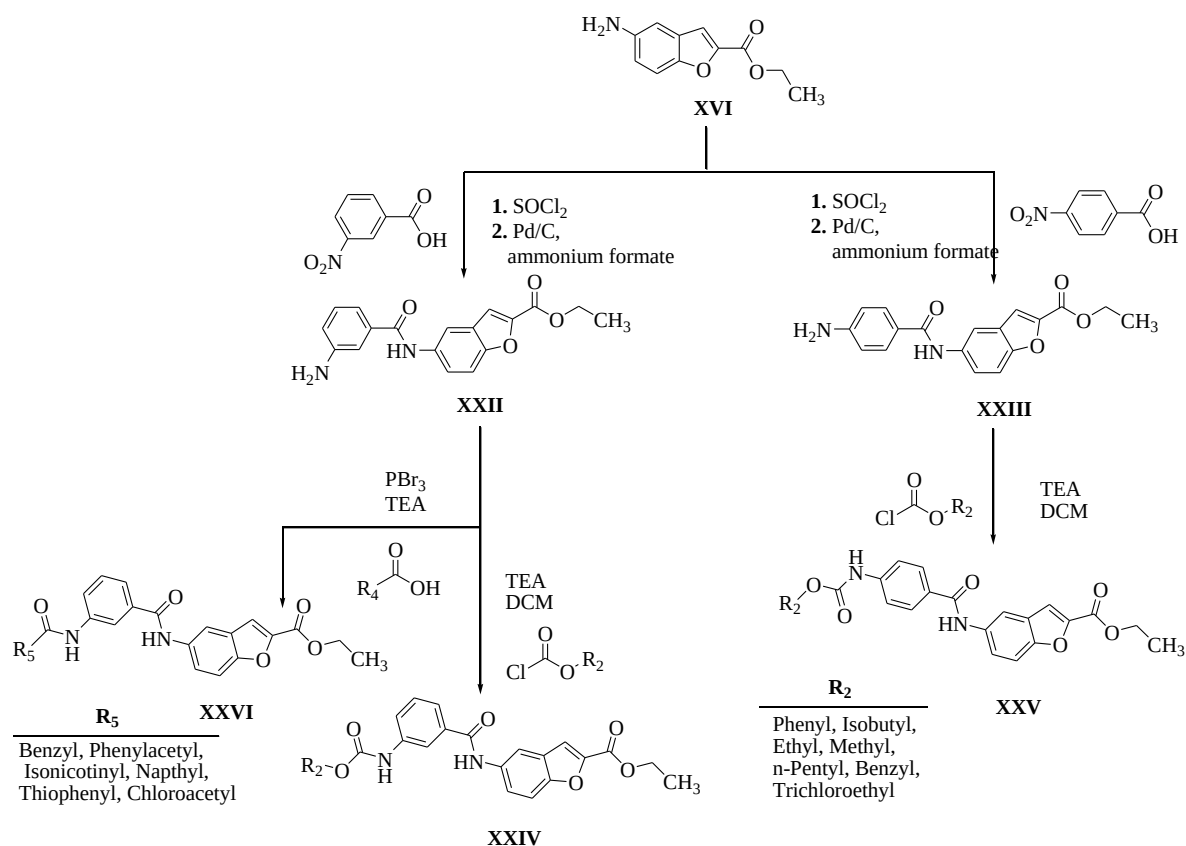
Ethyl 5-aminobenzofuran-2-carboxylate (**XVI**) and 4-methylbenzoyl chloride (**XVII**) produced ethyl 5-(4-methylbenzamido)benzofuran-2-carboxylate (**XVIII**) which when further reacted with hydrazine hydrate afforded 5-(4-methylbenzamido)benzofuran-2-carbohydrazide (**XIX**). The final step included generation of final Schiff base compounds (**XX**).



General Scheme 2

The acid chloride was prepared from 3-aminobenzoic acid using thionyl chloride followed by subsequent, nucleophilic substitution (SN_2) using ethyl 5-aminobenzofuran-2-carboxylate (**XVI**) and acid chloride to produce ethyl 5-(3-nitrobenzamido)benzofuran-2-carboxylate which was then reduced with Pd/C to produce intermediate 5-((3-

amino)benzamido)benzofuran-2-carboxylate (**XXII**). In the next step, substituted chloroformates were treated with Ethyl 5-((3-amino)benzamido)benzofuran-2-carboxylate (**XXII**) in the presence of triethyl amine in dichloromethane at room temperature by the nucleophilic substitution (SN_2) to obtained substituted Ethyl 5-((3-carbamate)benzamido)benzofuran-2-carboxylate (**XXIV**) on the other hand, various carboxylic acid derivatives were coupled with ethyl 5-((3-amino)benzamido)benzofuran-2-carboxylate (**XXII**) to produce ethyl 5-(3-substitutedbenzamido)benzofuran-2-carboxylate (**XXVI**).



General Scheme-3

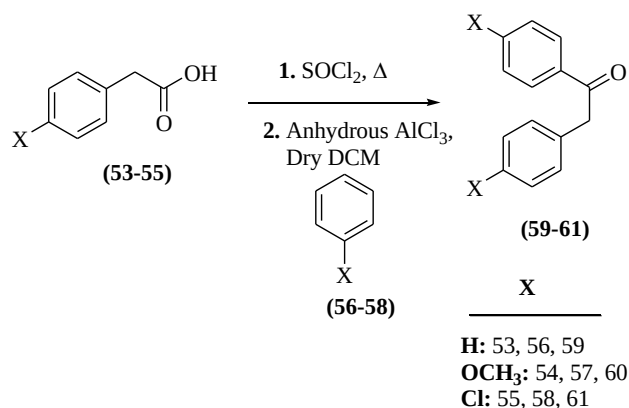
Another acid chloride was prepared from 4-aminobenzoic acid using thionyl chloride and ethyl 5-aminobenzofuran-2-carboxylate **XVI** to produced ethyl 5-(4-nitrobenzamido)benzofuran-2-carboxylate subsequently getting reduced in the presence of Pd/C to produce ethyl 5-((4-amino)benzamido)benzofuran-2-carboxylate **XXIII**. In the next step, substituted chloroformates were treated with ethyl 5-((4-amino)benzamido)benzofuran-2-carboxylate **XXIII** in the presence of triethyl amine and dichloromethane at room

temperature by the nucleophilic substitution ($\text{S}_{\text{N}}2$) to obtained substituted ethyl 5-((4-carbamate)benzamido)benzofuran-2-carboxylate **XXV**.

5.1.1. Synthesis of vicinal diaryl substituted pyrazoles

5.1.1.1. Synthesis of 1,2-diarylethanones (59-61)

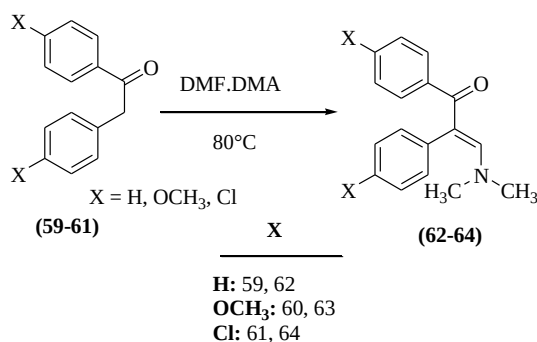
As shown in **Scheme 1**, substituted phenylacetic acids were treated with thionyl chloride to produce 4-substituted phenylacetyl chlorides. These chlorides then underwent Friedel-Crafts acylation with substituted benzenes (**56-58**) in the presence of anhydrous aluminum chloride, resulting in the formation of 1,2-diarylethanones (**59-61**). IR spectral data of compound (**59-61**) displayed the characteristic peaks for the $\text{C}=\text{O}$ carbonyl stretching at 1671, 1674, 1689 cm^{-1} along with $\text{C}-\text{O}$ stretching and bending peaks of methoxy at 1261 and 1023 cm^{-1} (**60**).



Scheme-1

5.1.1.2. Synthesis of (*E*)-3-(dimethylamino)-1,2-diarylprop-2-en-1-one (62-64)

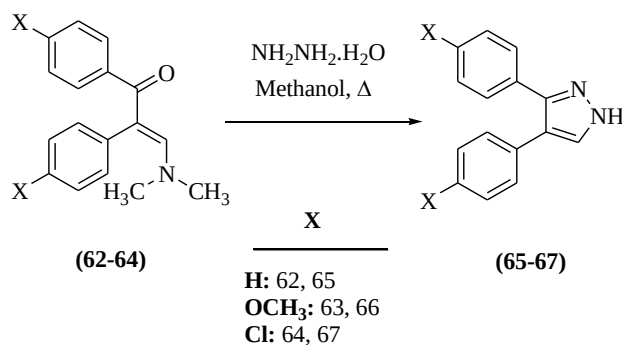
1,2-Diarylethanones (**59-61**) on reaction with dimethylacetamide dimethylacetal (DMF.DMA) at 80°C (**Scheme-2**) produced (*E*)-3-(dimethylamino)-1,2-diarylprop-2-en-1-one (**62-64**). IR spectral data of compounds (**62-64**) showed characteristic peaks for the $\text{C}=\text{O}$ carbonyl stretching at 1653, 1655 and 1662 cm^{-1} along with $\text{C}-\text{O}$ stretching and bending peaks of methoxy at 1262 and 1024 cm^{-1} for $-\text{OCH}_3$ containing derivative (**63**).



Scheme-2

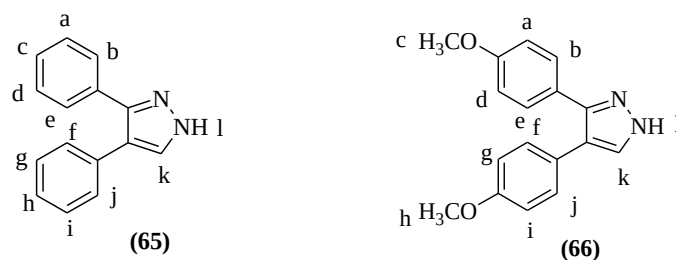
5.1.1.3. Synthesis of 3,4-diaryl-1*H*-pyrazole (65-67)

3,4-Diaryl-1*H*-pyrazole (**65-67**) was cyclized from (*E*)-3-(dimethylamino)-1,2-diarylprop-2-en-1-one (**62-64**) upon reaction with hydrazine hydrate in methanol.

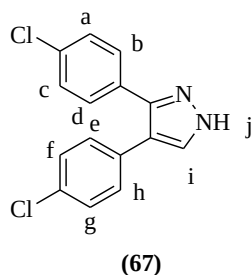


Scheme-3

Compound (**65**) in its PMR spectra produced aromatic protons at δ 7.65 (s, 1H_k), 7.47 (dd, J = 6.7, 3.0 Hz, 2H_{b,e}), 7.35 – 7.32 (m, 3H_{d,f,i}), 7.31 (d, J = 4.4 Hz, 3H_{a,g,i}), 7.26 (d, J = 6.8 Hz, 2H_{c,h}). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 220.9 [M]⁺.



Similarly, compound (**66**) in its PMR spectra exhibited aromatic protons at δ 7.61 (s, 1H_k), 7.42 – 7.34 (m, 2H_{b,e}), 7.25 – 7.21 (m, 2H_{f,i}), 6.90 – 6.82 (m, 4H_{a,d,g,i}) and aliphatic methoxy protons at 3.81 (s, 6H_{c,h}). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 281.2 [M+H]⁺.

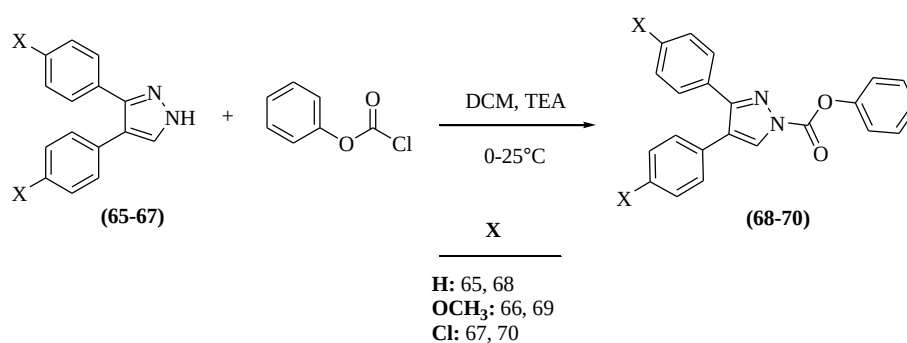


Compound (**67**) in its PMR spectra reported δ 7.69 (s, 1H_i), 7.41 (d, J = 8.2 Hz, 2H_{b,d}), 7.34 (dt, J = 7.4, 3.6 Hz, 4H_{a,c,e,h}), 7.24 (s, 2H_{f,g}). Mass spectra of the compound

displayed quasi-molecular ion peak at m/z 289 $[M+H]^+$ and $291[M+2H]^+$ representing presence of Cl atom in the structure.

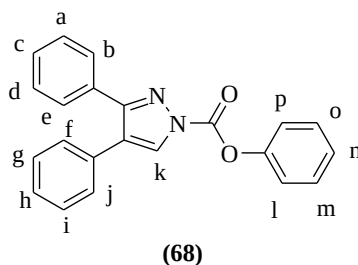
5.1.1.4. Synthesis of phenyl 3,4-diaryl-1*H*-pyrazole-1-carboxylate (68-70)

3,4-Diaryl-1*H*-pyrazole (65-67) was reacted with phenyl chloroformate via nucleophilic substitution reaction to produce (scheme-4) phenyl 3,4-diaryl-1*H*-pyrazole-1-carboxylate (68-70).



Scheme-4

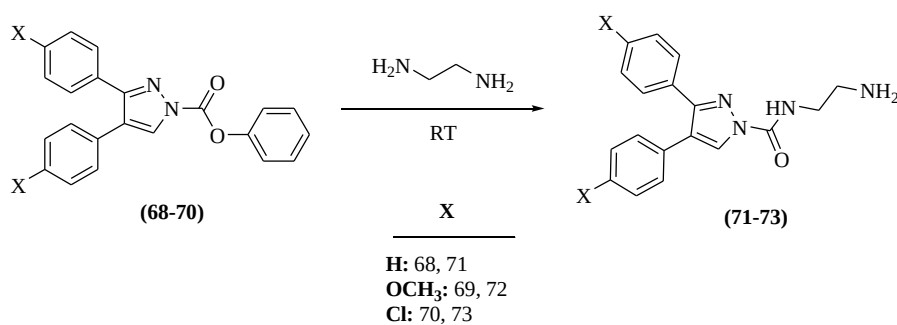
Compound (68) in its IR spectra showed C=O carbamate stretching at 1757 cm^{-1} . Its PMR spectra exhibited aromatic protons at δ 8.28 (s, 1H_k), 7.50 (dd, $J = 7.7, 1.9\text{ Hz}$, 2H_{b,f}), 7.39 (t, $J = 7.9\text{ Hz}$, 2H_{p,l}), 7.26 (d, 10H_{a,b,c,d,f,g,h,i,j,m,o}), 7.18 (s, 1H_n). Its mass spectra displayed quasi-molecular ion peak at m/z 341.0 $[M+H]^+$.



Other two compounds (69-70) in its IR spectra produced carbamate peaks at 1755 and 1752 cm^{-1} respectively along with an additional peak of C-O stretching at 1246 cm^{-1} confirming presence of methoxy group in (69). Mass spectra of compound (69) displayed quasi-molecular ion peak at m/z 401.2 $[M+H]^+$ while (70) produced quasi-molecular ion peak at m/z 408.7 $[M+H]^+$ along with $410.8 (M+2H)^+$ confirming presence of Cl atom.

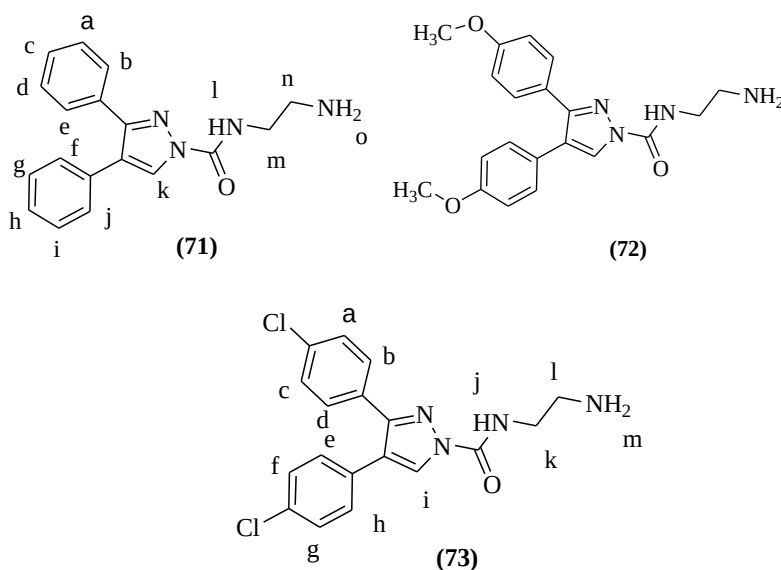
5.1.1.5. Synthesis of *N*-(2-aminoethyl)-3,4-diaryl-1*H*-pyrazole-1-carboxamide (71-73)

Phenyl 3,4-diaryl-1*H*-pyrazole-1-carboxylate (68-70) as per scheme-5 were treated with excess of ethylene diamine to produce desired derivatives *N*-(2-aminoethyl)-3,4-diaryl-1*H*-pyrazole-1-carboxamide (71-73).



Scheme-5

The structure of synthesized compound **(71)** was confirmed with IR where NH stretching peak was observed at 3376 and 3309 cm^{-1} along with C=O of urea at 1698 cm^{-1} . Its PMR spectra displayed pyrazole ring proton at δ 8.28 (s, 1H_k), triplet of NH urea proton at 7.57 (t, J = 6.1 Hz, 1H_l), aromatic protons at 7.47 (dd, J = 7.4, 2.2 Hz, 2H_{b,e}), 7.31 (d, J = 1.6 Hz, 2H_{f,j}), 7.30 – 7.29 (m, 1H_a), 7.28 (d, J = 1.5 Hz, 1H_d), 7.27 (d, J = 2.4 Hz, 2H_{g,i}), 7.25 (s, 1H_c), 7.22 (s, 1H_h) along with another NH amine protons at 4.79 (s, 2H_o) and aliphatic spacers at 3.50 – 3.47 (m, 2H_m), 2.94 (s, 2H_n) respectively. Its mass spectra displayed quasi-molecular ion peak at m/z 307 $[\text{M}+\text{H}]^+$.



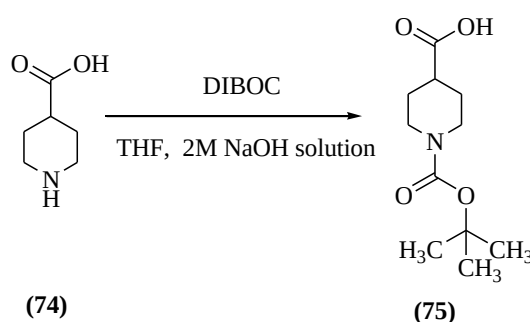
Compound **(72)** in its IR spectra reported C=O of urea at 1696 cm^{-1} and an additional peak of O-C stretching at 1236 cm^{-1} confirming presence of methoxy group in compound **(72)**. Its mass spectra displayed quasi-molecular ion peak at m/z 367.2 $[\text{M}+\text{H}]^+$

Compound **(73)** in its IR spectra reported NH stretching at 3313 and 3379 cm^{-1} along with C=O of urea at 1698 cm^{-1} . PMR spectra of the compound exhibited pyrazole ring proton at δ 8.37 (s, 1H_i) followed by urea NH proton as a triplet at 7.64 (t, J = 6.1 Hz, 1H_j) and

aromatic protons at 7.52 – 7.47 (m, 2H_{b,d}), 7.41 – 7.36 (m, 4H_{a,c,e,h}), 7.27 (s, 1H_f), 7.25 (d, *J* = 1.9 Hz, 1H_g) aliphatic protons at 3.62 – 3.57 (m, 2H_k), 3.06 (t, *J* = 5.9 Hz, 2H_l). Its mass spectra displayed quasi-molecular ion peak at *m/z* 374.8 (M)⁺ and 376.9 (M+2)⁺.

5.1.1.6. Synthesis of 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (75)

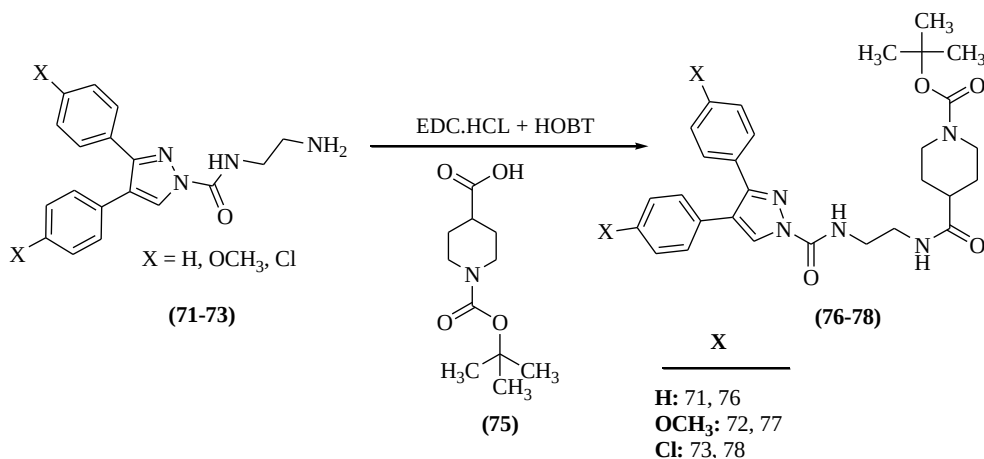
Secondary amine of piperidine-4-carboxylic acid (**74**) was protected using BOC anhydride in presence of 1M sodium hydroxide solution and THF to prepare 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid (**75**) (Scheme-6). In its IR data, the peaks for OH stretching at 3212 cm⁻¹, C=O carbonyl stretching of acid at 1735 cm⁻¹ and C=O carbonyl stretching of carbamate at 1661 cm⁻¹.



Scheme-6

5.1.1.7. Synthesis of *tert*-butyl 4-(2-(3,4-diaryl-1*H*-pyrazole-1-carboxamido)ethyl carbamoyl)piperidine-1-carboxylate (76-78)

N-(2-Aminoethyl)-3,4-diaryl-1*H*-pyrazole-1-carboxamide (**71-73**) was condensed with 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid via acid-amine coupling reaction potentiated by 3-(dimethylamino-propyl)-ethyl-carbodiimide hydrochloride (EDC.HCl) and hydroxybenzotriazole (HOBT) to obtain *tert*-butyl 4-(2-(3,4-diaryl-1*H*-pyrazole-1-carboxamido)ethylcarbamoyl)piperidine-1-carboxylate (**76-78**) as per scheme-7.



Scheme-7

The chemical structure is a complex molecule with 21 labeled atoms (a through v) for NMR assignment. The structure includes a biphenyl system, a pyrazole ring, a carbonyl group, a piperidine ring, and a tert-butyl ester group. The labels are as follows: a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s, t, u, v.

(76)

Chemical structures of compounds 1 and 2 are shown. Compound 1 is a pyrazole derivative with a 4-methoxyphenyl group (atoms a, b, c, d, e, f, g, h, i, j, k) and a 4-methoxyphenyl group (atoms l, m, n, o, p, q, r, s, t, u, v, w). Compound 2 is a pyrazole derivative with a 4-chlorophenyl group (atoms a, b, c, d, e, f, g, h, i, j, k) and a 4-chlorophenyl group (atoms l, m, n, o, p, q, r, s, t, u, v, w).

(77)

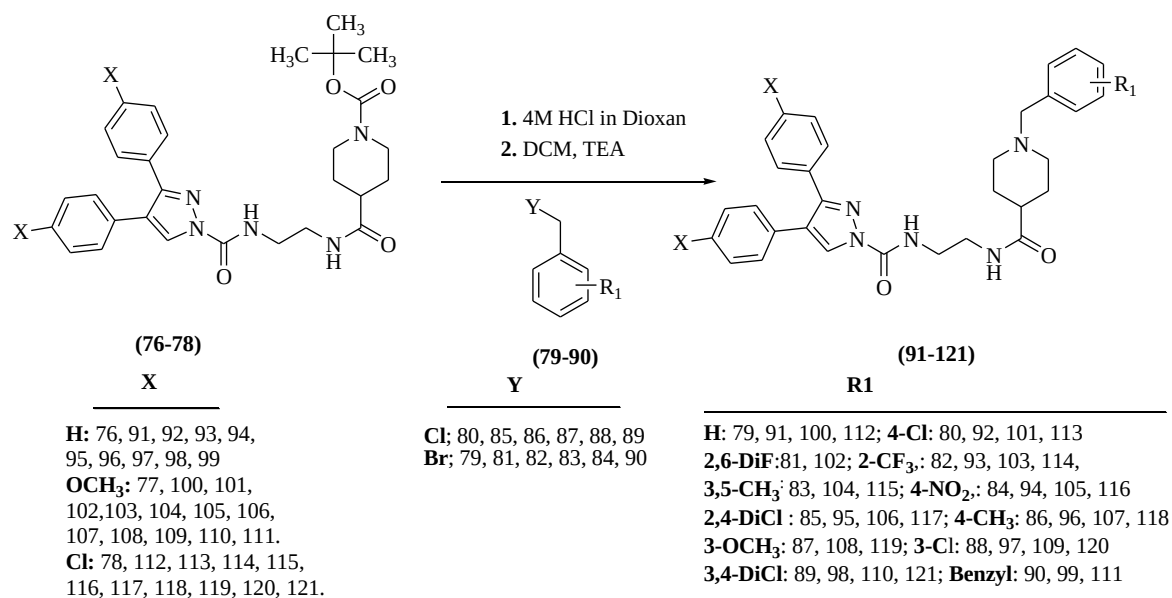
(78)

Compound (**78**) in its IR spectra reported NH stretching peak at 3377 and 3277 cm^{-1} along with C=O carbonyl stretching at 1730, 1693 and 1642 cm^{-1} . In its PMR spectra

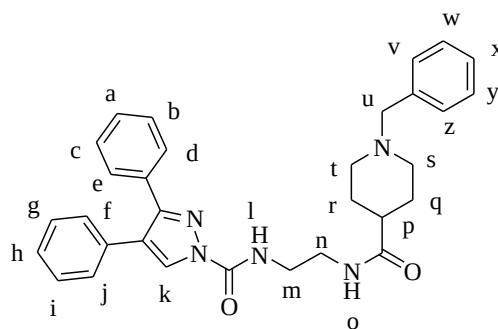
pyrazole proton at δ 8.27 (s, 1H_k) along with a triplet of NH of urea at 7.58 (s, 1H_i) followed by aromatic protons at 7.42 (d, J = 8.5 Hz, 2H_{c,d}), 7.35 – 7.29 (m, 4H_{a,b,e,h}), 7.22 – 7.16 (m, 2H_{f,g}). Another NH of amide at 6.20 (s, 1H_m) and aliphatic protons at 4.11 (s, 2H_{q1,r1}), 3.61 (t, J = 6.1 Hz, 2H_k), 3.54 (q, J = 5.6 Hz, 2H_i), 2.71 (d, J = 12.9 Hz, 2H_{q1,r1}), 2.23 (ddt, J = 11.5, 7.5, 3.8 Hz, 1H_n), 1.82 (s, 1H_{o1}), 1.60 (qd, J = 12.2, 4.3 Hz, 3H_{o1,p}), 1.44 (s, 9H_{s,t,u}). Its mass spectra depicted quasi-molecular ion peak at 586 (M+H)⁺ and 588.6 (M+2H)⁺ representing presence of Cl atom.

5.1.1.8. Synthesis of *N*-(2-(3,4-diaryl-1*H*-pyrazole-1-carboxamido)ethyl)-1-benzylpiperidine-4-carboxamides (91-121)

In the final step, *tert*-butyl 4-(2-(3,4-diaryl-1*H*-pyrazole-1-carboxamido)ethylcarbamoyl)piperidine-1-carboxylate (**76-78**) were deprotected as per **scheme-8** in presence of 4M dioxan in HCl and DCM to obtain *N*-(2-(3,4-diaryl-1*H*-pyrazole-1-carboxamido)ethyl)piperidine-4-carboxamide hydrochloride intermediates. These deprotected compounds were taken as it without any purification and further reacted with substituted benzyl halides (**79-90**) in presence of triethylamine and DCM to afford the targeted final compounds *N*-(2-(3,4-diaryl-1*H*-pyrazole-1-carboxamido)ethyl)-1-benzylpiperidine-4-carboxamides (**91-121**).

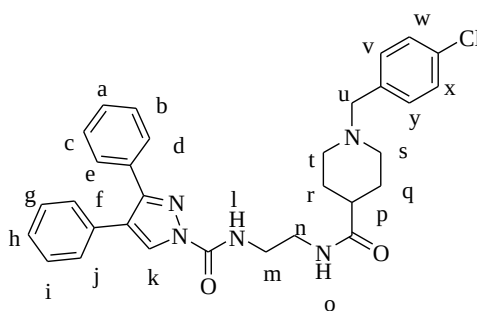


Scheme-8



(91)

IR spectral data of compound **(91)** showed characteristic NH stretching at 3318 cm^{-1} , and C=O carbonyl stretching at 1702 and 1638 cm^{-1} respectively. Its PMR showed one singlet peak at δ 8.28 (s, 1H_k) representing proton of heterocyclic pyrazole ring followed by NH proton of urea at 7.55 (t, 1H_l , $J = 6.0\text{ Hz}$) and two aromatic protons 7.51 (d, 1H_d , $J = 1.7\text{ Hz}$) and 7.49 (d, 1H_e , $J = 2.0\text{ Hz}$). Aromatic protons at 7.26 (m, $12\text{H}_{a,b,c,g,h,i,j,v,w,y,z}$) were obtained respectively followed by the most shielded aromatic proton 7.25 – 7.21 (t, 1H_x). Another NH proton was observed at 6.15 (s, 1H_o) followed by six aliphatic protons of spacer out of which four protons were observed as doublet of triplet at 3.61 (dt, 2H_m), 3.56 – 3.50 (dt, 2H_n) along with two singlet protons at 3.48 (s, 2H_u) respectively. The piperidine ring protons was observed at 2.90 (dt, $2\text{H}_{t,l,s_1}$), followed by 2.11 (m, 1H_p), 1.98 (t, $J = 11.2\text{ Hz}$, $2\text{H}_{t,l,s_1}$) and 1.83 – 1.72 (m, $4\text{H}_{p,q}$). In its ^{13}C -NMR NMR C=O peaks were observed at δ 175.90, 167.88 followed by aromatic C_{Ar} peaks at 152.13, 150.89, 132.54, 132.06, 131.87, 130.99, 129.60, 129.27, 128.90, 128.82, 128.77, 128.68, 128.57, 128.51, 128.33, 128.08, 127.56, 127.21, 124.05, C-N peaks at 68.27, 63.13, 53.01 40.26, 40.07 and C_{ali} peaks at 38.83, 30.46, 29.79, 29.02, 28.75. Mass spectra represented ion peaks at (m/z) 508.31 $(\text{M}+\text{H})^+$.



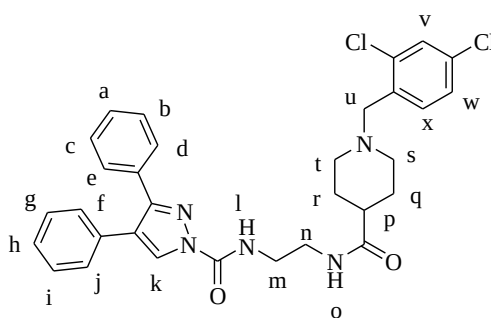
(92)

Compound **(92)** produced characteristic IR peak consisting of NH stretching at 3312 cm^{-1} along with C=O amide stretching at 1637 and 1697 cm^{-1} . While observing its PMR, heterocyclic pyrazole proton was obtained at δ 8.28 (s, 1H_k) along with one triplet NH proton of urea at 7.58 (t, 1H_l) followed by aromatic protons at 7.51 (d, $J = 1.7\text{ Hz}$, 1H_e) and 7.49 (d,

(93)

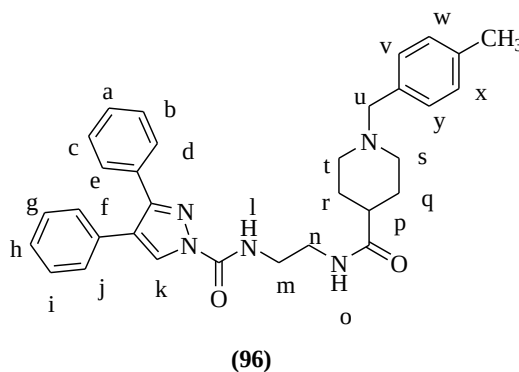
(94)

The IR spectral data of compound **(94)** showed NH stretching at 3312 cm^{-1} along with prominent C=O amide stretching at 1650 and 1696 cm^{-1} . The PMR spectrum of the compound exhibited singlet proton of pyrazole at δ 8.20 (s, $1H_k$) followed by two aromatic doublet protons at 8.07 (d, $2H_{w,x}$ $J = 8.6\text{ Hz}$). NH broad peak was observed at 7.52 (t, $1H_l$) then after, aromatic protons 7.48 – 7.39 (m, $4H_{d,e,v,y}$), 7.27 (d, $J = 6.4\text{ Hz}$, $1H_f$), 7.25 – 7.17 (m, $7H_{a,b,c,g,h,i,j}$) respectively. Another NH proton was present as a broad singlet at 6.21 (s, $1H_o$) followed aliphatic region which showed protons of aliphatic spacer at 3.57 – 3.43 (m, $6H_{m,n,u}$). Two protons of piperidine ring appeared at 2.81 (d, $2H_{s1, t1}$), 2.08 (s, $3H_{p,s1,t1}$), 1.74 (t, $J = 12.4\text{ Hz}$, $4H_{q,r}$) respectively. HRMS (ESI+) m/z was calculated as 553.2563 for $C_{31}H_{32}N_6O_4$ $[M+H]^+$; found, 553.2561.

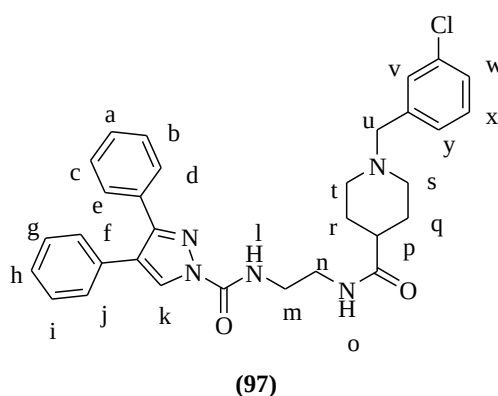


(95)

In the IR spectral data of compound **(95)** NH peak appeared at 3305 cm^{-1} along with amide C=O peaks at 1637 and 1699 cm^{-1} respectively. The PMR spectrum of the compound exhibited a singlet proton of pyrazole ring at δ 8.21 (s, $1H_k$) and a triplet NH proton at 7.49 (t, $1H$). Aromatic region showed protons at 7.46 – 7.41 (m, $2H_{d,e}$), 7.35 (d, $J = 8.3\text{ Hz}$, $1H_v$), 7.27 (q, $J = 2.2\text{ Hz}$, $3H_{w,f,j}$), 7.25 – 7.18 (m, $7H_{a,b,c,g,h,i,x}$). Second NH proton was present as broad singlet at 6.11 (s, $1H_o$) thereafter aliphatic region showed protons of spacers at 3.59 – 3.52 (m, $2H_m$) and 3.47 (d, $4H_{n,u}$) respectively. Piperidine protons were obtained at 2.86 – 2.76 (m, $2H_{s1, t1}$), 2.10 – 1.95 (m, $3H_{p, s1, t1}$), 1.76 – 1.67 (m, $4H_{q, r}$) respectively. ^{13}C -NMR represented C=O peaks at δ 175.78, 152.19 C_{Ar} peaks at 150.96, 134.77, 133.12, 132.05, 131.84, 131.48, 129.17, 128.86, 128.77, 128.72, 128.58, 128.53, 128.08, 127.61, 127.09, 124.12 C-N peaks at 58.74, 53.17, 40.25, 40.17 C_{ali} peaks at 29.80, 28.90 respectively. HRMS (ESI+) m/z was calculated as 576.1932 for $C_{31}H_{31}Cl_2N_5O_2$ $[M+H]^+$; found, 576.1901.



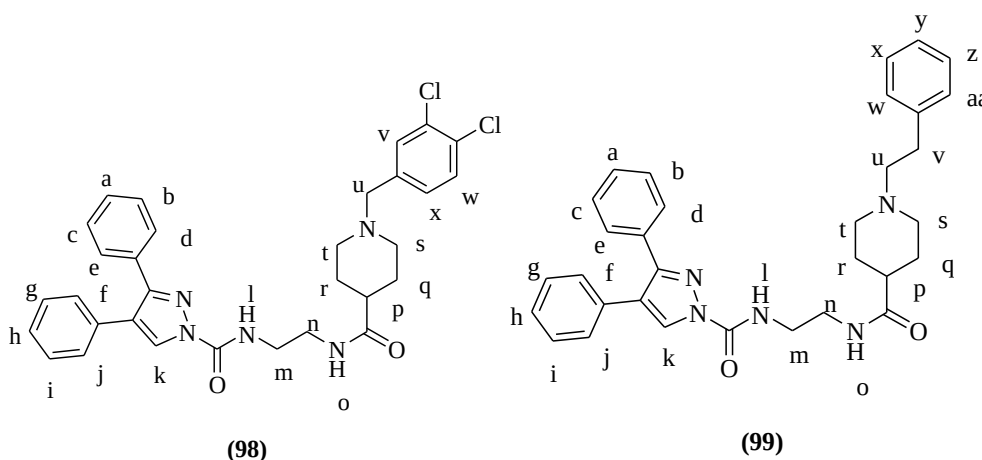
Compound **(96)** in spectral analysis of IR produced NH stretching peak at 3312 cm^{-1} and a couple of carbonyl $\text{C}=\text{O}$ amide groups at 1703 and 1637 cm^{-1} . Its PMR showed singlet proton of pyrazole at δ 8.27 (s, 1H_k) along with triplet proton of NH at 7.55 (t, $J = 6.1\text{ Hz}$, 1H_l) followed by aromatic protons at $7.51 - 7.48$ (m, $2\text{H}_{d,e}$), $7.34 - 7.30$ (m, $5\text{H}_{a,b,c,f,j}$), 7.28 (d, $J = 2.9\text{ Hz}$, $2\text{H}_{g,i}$), 7.25 (s, 1H_h), 7.18 (d, $J = 7.8\text{ Hz}$, $2\text{H}_{v,y}$), 7.10 (d, $J = 7.7\text{ Hz}$, $2\text{H}_{w,x}$) respectively. Broad singlet of NH was present at 6.17 (s, 1H_o) and in the aliphatic region, protons of spacers appeared at $3.63 - 3.58$ (m, 2H_m), 3.53 (m, 2H_n), 3.44 (s, 2H_u) respectively. Two protons of piperidine were observed at 2.90 (dt, $J = 11.5, 3.4\text{ Hz}$, $2\text{H}_{s,t}$), CH_3 protons were obtained at 2.32 (s, 3H), 2.11 (s, 1H_p), $2.00 - 1.92$ (m, $2\text{H}_{s,t}$) and 1.74 (qd, $J = 11.5, 3.7\text{ Hz}$, $4\text{H}_{q,r}$). ^{13}C -NMR depicted $\text{C}=\text{O}$ peaks at δ 175.92, 152.15 C_{Ar} peaks at 150.90, 136.80, 132.05, 131.87, 129.27, 129.01, 128.83, 128.78, 128.69, 128.58, 128.52, 128.29, 128.09, 127.56, 124.06 $\text{C}-\text{N}$ peaks at 62.89, 52.99, 40.23, 40.10 and C_{ali} peaks at 29.81, 28.79, 21.22. HRMS (ESI+) m/z was calculated as 522.2868 for $\text{C}_{32}\text{H}_{35}\text{N}_5\text{O}_2$ $[\text{M}+\text{H}]^+$; found, 522.2837. HPLC purity: 97.89 %.



In the IR spectral data of compound **(97)**, peak of NH amine stretching was observed at 3318 cm^{-1} along with $\text{C}=\text{O}$ carbonyl stretching of amide functional group at 1694 and 1655 cm^{-1} respectively. Its PMR spectrum represented most deshielded singlet pyrazole CH proton at δ 8.28 (s, 1H_k) along with NH proton of urea as broad singlet at 7.56 (s, 1H_l) and aromatic protons at $7.52 - 7.48$ (m, $2\text{H}_{d,e}$). A doublet proton was observed at 7.35 (d, $J =$

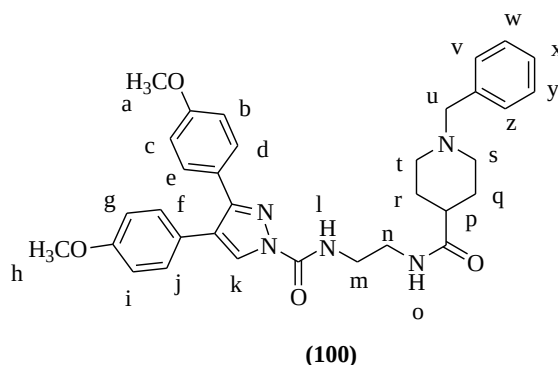
2.7 Hz, 1H_a) followed by other aromatic protons at 7.32 (dd, $J = 5.3, 2.0$ Hz, 5H_{b,c,f,h,j,i}), 7.28 (d, $J = 2.9$ Hz, 2H_{g,i}), 7.26 (m, 2H_{v,w}), 7.22 (d, $J = 4.5$ Hz, 2H_{x,y}). Another NH proton was present as broad singlet at (s, 1H_o) followed aliphatic spacers at 3.65 – 3.57 (m, 2H_m), 3.54 (q, $J = 5.6$ Hz, 2H_n), 3.45 (s, 2H_u). Finally piperidine ring was observed at 2.89 (bs, 2H_{s1,t1}), 2.12 (bs, 1H_p), 2.00 (bs, 2H_{s1,t1}), 1.82 (bs, 4H_{q,r}). HRMS (ESI⁺) m/z was calculated as 542.2322 for C₃₁H₃₂ClN₅O₂ [M+H]⁺; found, 542.2306.

IR spectral data of compound **(98)** represented NH stretching peak at 3305 cm⁻¹ and C=O carbonyl stretching of amide functional group at 1706 and 1639 cm⁻¹. The PMR spectra exhibited singlet proton of pyrazole at δ 8.28 (s, 1H_k) along with triplet proton of NH at 7.55 (t, 1H_i) followed by aromatic protons at 7.52 – 7.48 (dd, 2H_{d,e}), 7.41 (d, $J = 2.0$ Hz, 1H_a), 7.37 – 7.27 (m, 9H_{b,c,f,g,h,i,j,v,w}), 7.14 (d, $J = 8.2$ Hz, 1H_x). Another NH proton was observed as broad singlet at 6.16 (s, 1H_o) which was followed by aliphatic protons consisting of spacers 3.66 – 3.59 (m, 2H_m), 3.54 (q, $J = 5.6$ Hz, 2H_n) and 3.41 (s, 2H_u). Piperidine ring showed protons at 2.85 (d, $J = 11.1$ Hz, 2H_{s1,t1}), 2.10 (d, $J = 11.3$ Hz, 1H_p), 1.99 (t, $J = 11.1$ Hz, 2H_{s1,t1}), 1.87 – 1.72 (m, 4H_{q,r}). Its mass spectra depicted quasi-molecular ion peak at (m/z) 576.28 (M+H)⁺ along with isotopic of Cl atom at 578.27 (M+2H)⁺.

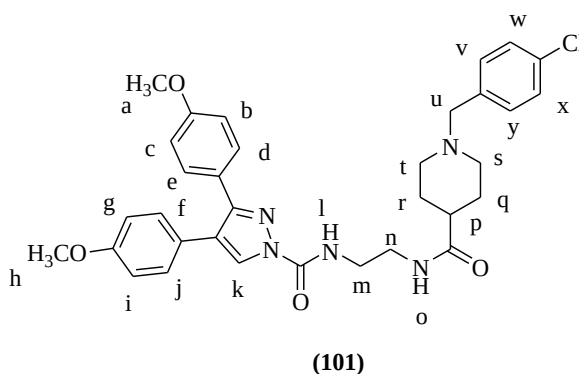


Compound **(99)** showed IR spectrum containing NH stretching at 3307 cm⁻¹ and C=O carbonyl stretching of amide at 1705 and 1644 cm⁻¹. The PMR spectra exhibited singlet proton of pyrazole ring at δ 8.29 (s, 1H_k) followed by a triplet of NH proton at 7.62 (t, 1H_i) along with aromatic protons 7.51 (dd, $J = 7.4, 2.3$ Hz, 2H_{d,e}), 7.32 (dq, $J = 5.3, 2.6$ Hz, 7H_{a,b,c,f,g,i,j}), 7.29 – 7.26 (m, 3H_{h,w,aa}), 7.19 (dd, $J = 15.7, 7.3$ Hz, 3H_{x,y,z}). Second NH proton was present as broad singlet at 6.45 (s, 1H_o) which was followed by spacers from the aliphatic region at, 3.62 (t, $J = 5.9$ Hz, 2H_m), 3.55 (t, $J = 5.7$ Hz, 2H_n) and second spacer at 2.86 (dd, $J = 10.3, 5.9$ Hz, 2H_u), 2.66 (dd, $J = 10.4, 6.0$ Hz, 2H_v). Piperidine protons were

observed at 3.14 – 3.09 (m, 2H_{s1,t1}), 2.30 (bs, 3H_{p,s1,t1}), 2.01 (bs, 2H_q), 1.89 (m, $J = 11.6$ Hz, 2H_r). HRMS (ESI+) m/z was calculated as 522.2868 for C₃₂H₃₅N₅O₂ [M+H]⁺; found, 522.2867.

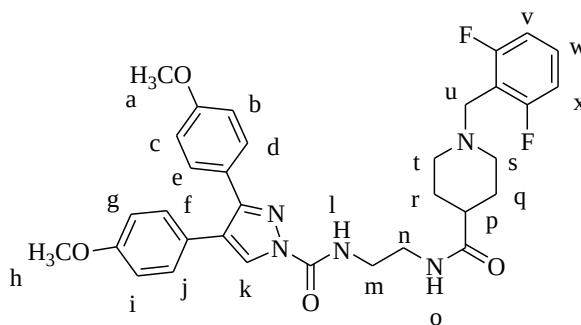


IR spectral data of compound **(100)** reported NH stretching at 3321 cm⁻¹ along with couple of amide C=O carbonyl stretching at 1728 and 1633 cm⁻¹. Additional C-O stretching of ether group was observed at 1241 cm⁻¹. Its PMR spectra exhibited singlet proton of pyrazole ring at δ 8.19 (s, 1H_k) along with a triplet of NH proton of urea at 7.55 (t, 1H_l) followed by protons of aromatic ring at 7.44 (d, $J = 2.1$ Hz, 1H_d), 7.43 (d, $J = 2.1$ Hz, 1H_e), 7.29 (d, $J = 4.3$ Hz, 4H_{f,j,v,z}), 7.26 – 7.17 (m, 3H_{w,x,y}), 6.87 (d, $J = 2.9$ Hz, 2H_{b,c}), 6.85 (d, $J = 3.1$ Hz, 2H_{g,i}). Second amide NH proton was observed as a triplet at 6.25 (t, $J = 5.2$ Hz, 1H_o) followed by six protons of methoxy group as was observed at 3.81 (d, $J = 4.2$ Hz, 6H_{a,h}). Aliphatic spacers were reported at 3.61 – 3.56 (m, 2H_m), 3.52 (dd, $J = 6.8, 4.8$ Hz, 2H_n) and 3.47 (s, 2H_u) respectively. Most shielded piperidine ring was seen at 2.90 (dt, 2H_{s1,t1}), 2.10 (ddt, 1H_p), 2.02 – 1.94 (m, 2H_{s1,t1}), 1.85 – 1.72 (m, 4H_{q,r}). Mass spectra represented quasi-molecular ion peak at (m/z) 568.4 (M+H)⁺.



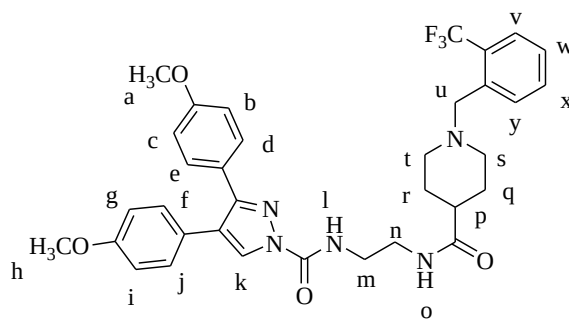
IR spectral data of compound **(101)** presented NH stretching at 3326 cm⁻¹ along with amide C=O carbonyl stretching at 1737 and 1642 cm⁻¹. Additional C-O stretching of ether group was observed at 1244 cm⁻¹. Its PMR spectra exhibited singlet proton of pyrazole ring at δ 8.19 (s, 1H_k) along with a triplet of NH proton of urea at 7.51 (t, 1H_l) followed by protons

of aromatic ring at 7.45 (d, $J = 2.1$ Hz, $1H_d$), 7.43 (d, $J = 2.1$ Hz, $1H_e$), 7.24 (d, $J = 4.3$ Hz, $4H_{f,j,w,x}$), 7.21 – 7.17 (m, $2H_{v,y}$), 6.87 (d, $J = 3.6$ Hz, $2H_{b,c}$), 6.85 (d, $J = 3.8$ Hz, $2H_{g,i}$). Another amide NH proton was observed as a triplet at 6.17 (t, $1H_o$) followed by six protons of methoxy group as was observed at 3.82 (d, $J = 3.2$ Hz, $6H_{a,h}$). Aliphatic spacers were reported at 3.64 – 3.57 (m, $2H_m$), 3.53 (td, $J = 6.5, 4.4$ Hz, $2H_n$) and 3.43 (s, $2H_u$) respectively. Most shielded piperidine ring was seen at 2.90-2.83 (m, $2H_{s1,t1}$), 2.07 (bs, $1H_p$), 2.02 – 1.93 (m, $2H_{s1,t1}$), 1.83 – 1.72 (m, $4H_{q,r}$). Its ^{13}C -NMR represented C=O peaks at δ 175.79, 151.9, 151.08, along with C_{AR-OCH_3} peaks at 160.06 and 159.13 followed by C_{Ar} peaks at 137.10, 132.71, 130.33, 129.96, 129.79, 128.41, 127.62, 124.57, 124.31, 123.50, 114.14, 113.92 along with C-O peak at 55.35 and C-N peaks at 62.40, 53.10, 43.19 and C_{ali} peaks at 40.18, 28.88 respectively. Its mass spectra depicted quasi-molecular ion peak at (m/z) 602.5 (M+H) $^+$, along with isotopic of Cl atom at 604 (M+2H) $^+$.



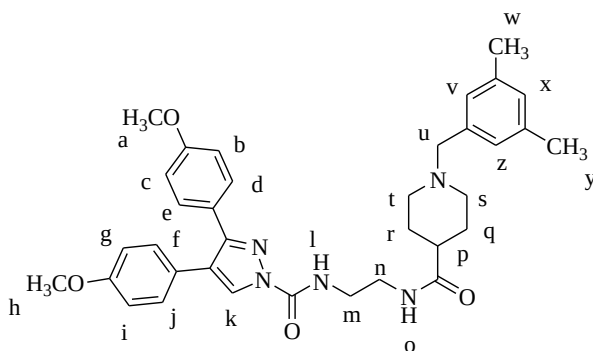
(102)

In the IR spectrum of **(102)**, NH stretching appeared at 3323 cm^{-1} together with two C=O amide stretching at 1643 and 1736 cm^{-1} . Its PMR spectra exhibited singlet proton of pyrazole ring at δ 8.19 (s, $1H_k$) along with a triplet of NH proton of urea at 7.50 (t, $1H_l$) followed by protons of aromatic ring at 7.45 (d, $J = 2.1$ Hz, $1H_d$), 7.43 (d, $J = 2.1$ Hz, $1H_e$), 7.25-7.18 (m, $3H_{f,j,w}$), 6.98-6.84 (m, $6H_{b,c,g,i,v,x}$). Another amide NH proton was observed as a triplet at 6.16 (t, $1H_o$) followed by six protons of methoxy group as was observed at 3.83 (d, $6H_{a,h}$). Aliphatic spacers were reported at 3.68 (t, $J = 1.6$ Hz $2H_m$), 3.59 (td, $J = 6.7, 4.4$ Hz, $2H_n$) and 3.5 (td, $J = 5.8, 2.8$ Hz, $2H_u$) respectively. Most shielded piperidine ring was seen at 2.96 (d, $J = 11.5$ Hz, $2H_{s1,t1}$), 2.12 – 1.99 (m, $3H_{p,s1,t1}$), 1.86 – 1.70 (m, $4H_{q,r}$). Its mass spectra depicted quasi-molecular ion peak at 604.5 (M+H) $^+$.



(103)

Compound **(103)** in spectral analysis of IR showed NH stretching at 3374 cm^{-1} followed by C=O amide stretching at 1633 and 1727 cm^{-1} along with ether stretching at 1239 cm^{-1} . The PMR spectra revealed singlet peak of pyrazole at $\delta\ 8.20$ (s, $1H_k$) along with aromatic protons 7.80 (d, $J = 7.8\text{ Hz}$, $1H_v$) and 7.60 (d, $J = 7.8\text{ Hz}$, $1H_y$). NH proton of urea was merged with one aromatic proton between $7.56 - 7.47$ (m, $2H_{l,x}$) followed by other aromatic protons at $7.47 - 7.42$ (m, $2H_{d,e}$), 7.30 (t, $J = 7.6\text{ Hz}$, $1H_w$), $7.22 - 7.17$ (m, $2H_{f,j}$) and $6.89 - 6.83$ (m, $4H_{b,c,g,i}$) respectively. Another NH proton of amide was observed at 6.20 (t, $J = 5.1\text{ Hz}$, $1H_o$) which was then followed by aliphatic region starting with six methoxy proton at 3.82 (d, $J = 4.0\text{ Hz}$, $6H$) along with spacers at 3.61 (d, $J = 6.2\text{ Hz}$, $4H_{m,u}$) and $3.56 - 3.51$ (m, $2H_n$). Protons of piperidine ring were observed at 2.87 (dt, $J = 11.8, 3.6\text{ Hz}$, $2H_{s,l,t}$), 2.08 (dtd, $J = 22.6, 10.8, 4.1\text{ Hz}$, $3H_{p,s,l,t}$), $1.85 - 1.74$ (m, $4H_{q,r}$). Its ^{13}C -NMR showed C=O peaks at $\delta\ 175.90, 151.94, 151.10$, along with $\text{C}_{\text{Ar}}\text{-OCH}_3$ peaks at $160.07, 159.12$ and C_{Ar} peaks at $138.30, 131.88, 130.19, 129.98, 129.81, 128.61, 128.31, 127.64, 126.69, 125.96, 125.69, 125.63, 124.58, 124.32, 123.52, 123.24, 114.13, 113.94$ followed by C-O peak at 55.36 , C-N peaks at $58.35, 53.40, 43.30, 40.25$ and C_{ali} peaks at $40.18, 29.10$. Mass spectra produced quasi-molecular ion peak at $(m/z)\ 636.6\ (\text{M}+\text{H})^+$.

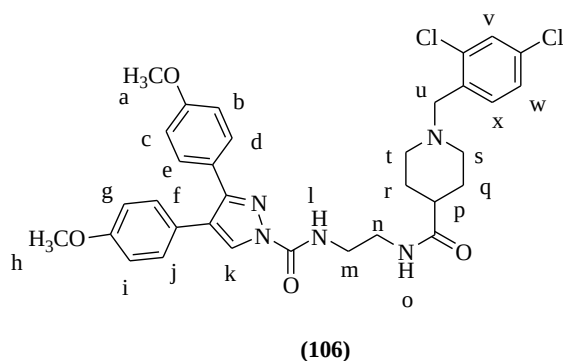
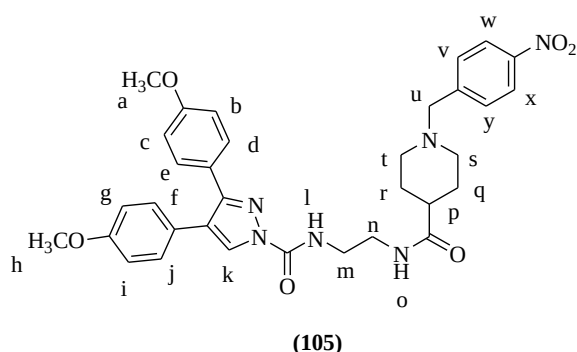


(104)

Compound **(104)**, in spectral analysis of IR showed NH stretching at 3319 and 3261 cm^{-1} and amide C=O carbonyl stretching at 1703 and 1639 cm^{-1} along with C-O ether

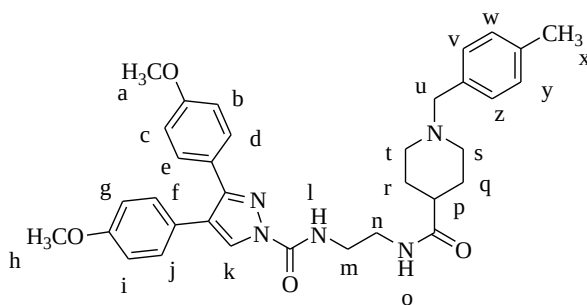
stretching at 1244 cm^{-1} . Its PMR spectrum exhibited singlet proton of pyrazole ring at δ 8.20 (s, $1H_k$) and a triplet proton of NH at 7.52 (t, $J = 6.0\text{ Hz}$, $1H_l$) followed by aromatic protons at 7.45 (d, $J = 2.1\text{ Hz}$, $1H_d$), 7.43 (d, $J = 2.0\text{ Hz}$, $1H_e$), 7.20 (d, $J = 2.1\text{ Hz}$, $1H_f$), 7.19 (d, $J = 2.1\text{ Hz}$, $1H_j$), 6.91 (bs, $2H_{b,c}$), 6.87 (m, $J = 2.4\text{ Hz}$, $3H_{v,x,z}$), 6.85 (d, $J = 2.5\text{ Hz}$, $2H_{g,i}$) respectively. Another amide NH proton was present at 6.18 (s, $1H_o$) followed by six deshielded aliphatic protons of methoxy group at 3.82 (d, $J = 3.3\text{ Hz}$, $6H_{a,h}$) and spacers at 3.60 (td, $2H_m$), 3.55 – 3.49 (m, $2H_n$) and 3.40 (s, $2H_u$). Another six protons of methyl group was observed at 2.29 (s, $6H_{w,y}$), and protons of piperidine ring 2.91 (dt, $J = 11.6$, $2H_{s1,t1}$), 2.15 – 2.07 (m, $1H_p$), 1.96 (t, $J = 11.3\text{ Hz}$, $2H_{s1,t1}$), 1.82 – 1.74 (m, $4H_{q,r}$). Mass spectra produced quasi-molecular ion peak at $(m/z) 596.44 (M+H)^+$.

IR spectral data of compound **(105)** showed NH stretching at 3312 cm^{-1} along with C=O carbonyl stretching of amide at 1701 and 1641 cm^{-1} and a characteristic ether peak of methoxy C-O stretching at 1243 cm^{-1} . Its PMR spectra revealed pyrazole ring singlet proton at δ 8.19 (s, $1H_k$) along with aromatic protons adjacent to nitro group at 8.16 (d, $J = 1.8\text{ Hz}$, $1H_w$) and 8.14 (d, $J = 1.9\text{ Hz}$, $1H_x$) followed by NH proton of urea group at 7.54 (t, $J = 6.1\text{ Hz}$, $1H_l$) after which other aromatic protons were observed 7.48 (d, $J = 8.2\text{ Hz}$, $2H_{d,e}$), 7.46 – 7.42 (m, $2H_{f,j}$), 7.19 (d, $J = 2.0\text{ Hz}$, $1H_v$), 7.18 (d, $J = 2.2\text{ Hz}$, $1H_y$), 6.87 (d, $J = 1.8\text{ Hz}$, $1H_b$), 6.87 – 6.85 (m, $2H_{f,i}$), 6.84 (d, $J = 2.2\text{ Hz}$, $1H_c$) respectively. Another NH proton was found at 6.24 (s, $1H_o$) which was then trailed by deshielded methoxy protons at 3.82 (d, $6H_{a,h}$), spacers were reported at 3.61 (td, $2H_m$), 3.57 – 3.50 (m, $4H_{n,u}$). Piperidine protons were present at 2.90 – 2.82 (m, $2H_{s1,t1}$), 2.13 (m, $1H_p$), 2.05 (s, $2H_{s1,t1}$) and 1.87 – 1.74 (m, $4H_{q,r}$) respectively. Mass spectra produced quasi-molecular ion peak at $(m/z) 613.42 (M+H)^+$. HRMS (ESI+) m/z was calculated as 613.2774 for $C_{33}H_{36}N_6O_6 [M+H]^+$; found, 613.2761.



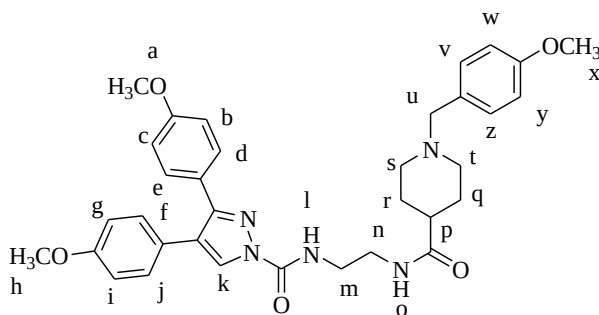
Compound **(106)** produced IR spectrum having NH stretching at 3347 cm^{-1} along with C=O carbonyl stretching of amide at 1738 and 1648 cm^{-1} and an ether C-O stretching at 1241 cm^{-1} . The PMR spectrum depicted a singlet proton of the pyrazole CH at δ 8.20 (s, $1H_k$)

and triplet proton of NH at 7.52 (t, 1H_i) followed by aromatic protons at 7.46 – 7.40 (m, 3H_{d,e,v}), 7.34 (d, $J = 2.2$ Hz, 1H_w), 7.19 (dt, $J = 6.4, 1.9$ Hz, 3H_{f,j,x}), 6.87 (d, $J = 2.7$ Hz, 2H_{b,c}) and 6.85 (d, $J = 2.7$ Hz, 2H_{g,i}). Another NH proton of amide was observed at 6.20 (t, 1H_o) after which aliphatic protons were reported. Six methoxy protons at 3.82 (d, $J = 3.0$ Hz, 6H_{a,h}) were seen followed by spacers at 3.63 – 3.59 (m, 2H_m), 3.53 (d, $J = 4.6$ Hz, 4H_{n,u}), 2.88 (dt, $J = 11.7, 3.6$ Hz, 2H), 2.10 (qd, $J = 11.4, 5.0$ Hz, 3H), 1.85 – 1.74 (m, 4H). HRMS (ESI⁺) m/z was calculated as 636.2144 for C₃₃H₃₅Cl₂N₅O₄ [M+H]⁺; found, 636.2126.



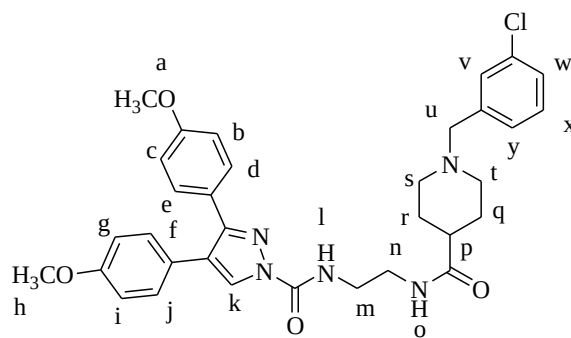
(107)

IR spectral data of compound **(107)** showed NH stretching at 3314 and 3274 cm⁻¹ together with amide C=O carbonyl stretching at 1724 and 1642 cm⁻¹ and with a characteristic ether C-O stretching at 1243 cm⁻¹. The PMR spectra exhibited pyrazole proton at δ 8.20 (s, 1H) and a triplet proton of NH at 7.54 (t, $J = 6.1$ Hz, 1H). It was followed by aromatic protons at 7.45 (d, $J = 2.1$ Hz, 1H_d), 7.43 (d, $J = 2.1$ Hz, 1H_e), 7.21 – 7.16 (m, 4H_{b,c,f,j}), 7.10 (d, $J = 7.8$ Hz, 2H_{g,i}), 6.87 (d, $J = 2.4$ Hz, 2H_{w,y}), 6.85 (t, $J = 2.3$ Hz, 2H_{v,z}) respectively. Another NH proton was seen at 6.26 (t, $J = 5.0$ Hz, 1H_o) followed by aliphatic protons of methoxy at 3.82 (d, $J = 2.7$ Hz, 6H_{a,h}) spacers at 3.62 – 3.57 (m, 2H_m), 3.54 – 3.49 (m, 2H_n) and 3.44 (s, 2H_u). Methyl protons were observed at 2.33 (s, 3H_x) along with piperidine protons at 2.93 – 2.88 (m, 2H_{s1,t1}), 2.11 (ddt, 1H_p), 2.02 – 1.94 (m, 2H_{s1,t1}), 1.83– 1.71 (m, 4H_{q,r}). HRMS (ESI⁺) m/z was calculated as 582.3080 for C₃₄H₃₉N₅O₄ [M+H]⁺; found, 582.3072.



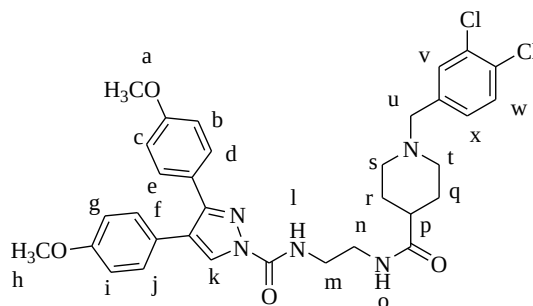
(108)

IR spectral data of compound **(108)** showed NH stretching at 3326 cm^{-1} . Its PMR recorded pyrazole singlet peak at δ 8.20 (s, 1H_k) NH urea peak at 7.56 (t, $J = 6.1\text{ Hz}$, 1H_l) and aromatic protons at 7.47 – 7.41 (m, $2\text{H}_{d,e}$), (b,c,f,g,i,j,w,y,v,z) 7.24 – 7.17 (m, 3H), 6.91 (s, 1H), 6.87 (ddd, $J = 9.8, 6.0, 2.3\text{ Hz}$, 5H) 6.80 (dd, $J = 8.3, 2.5\text{ Hz}$, 1H), followed by another NH peak at 6.33 (s, 1H). Aliphatic protons were observed at 3.82 (d, $J = 4.5\text{ Hz}$, $6\text{H}_{a,h}$), 3.79 (s, 3H_x), 3.60 (td, $J = 6.8, 4.4\text{ Hz}$, 2H_m), 3.55 – 3.48 (m, $4\text{H}_{n,u}$) along with piperidine protons 2.97 – 2.92 (m, $2\text{H}_{s1,t1}$), 2.11 (d, $J = 31.8\text{ Hz}$, $3\text{H}_{p,s1,t1}$), 1.88 – 1.74 (m, $4\text{H}_{q,r}$). HRMS (ESI+) m/z was calculated as 598.3029 for $\text{C}_{34}\text{H}_{39}\text{N}_5\text{O}_5$ $[\text{M}+\text{H}]^+$; found, 598.3016.



(109)

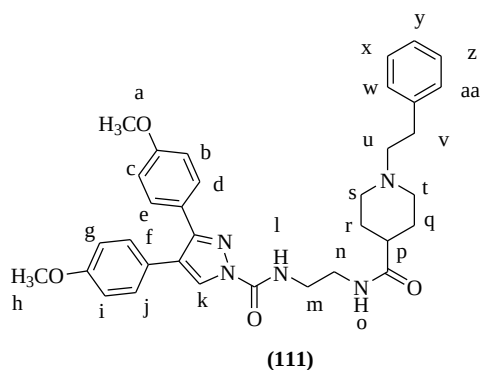
IR spectral data of compound **(109)** showed NH stretching at 3327 cm^{-1} along with C=O carbonyl stretching at 1704 cm^{-1} . Its PMR recorded pyrazole singlet peak at δ 8.20 (s, 1H_k) NH urea peak at 7.53 (t, $J = 6.1\text{ Hz}$, 1H_l) and aromatic protons at 7.46 – 7.41 (m, $2\text{H}_{d,e}$), (b,c,f,g,i,jv,w,x,y,) 7.31 (d, $J = 1.8\text{ Hz}$, 1H), 7.23 – 7.17 (m, 5H), 6.88 – 6.84 (m, 4H) followed by another NH peak at 6.22 (s, 1H_o). Aliphatic protons were observed at 3.82 (d, $J = 3.1\text{ Hz}$, $6\text{H}_{a,h}$), 3.60 (td, $J = 6.8, 4.5\text{ Hz}$, 2H_m), 3.55 – 3.50 (m, 2H_n), 3.44 (s, 2H_u) along with protons of piperidine ring 2.89 – 2.86 (m, $2\text{H}_{s1,t1}$), 1.98 (d, $J = 12.4\text{ Hz}$, $3\text{H}_{p,s1,t1}$), 1.84 – 1.71 (m, $4\text{H}_{q,r}$). HRMS (ESI+) m/z was calculated as 602.2533 for $\text{C}_{33}\text{H}_{36}\text{ClN}_5\text{O}_4$ $[\text{M}+\text{H}]^+$; found, 602.2533.



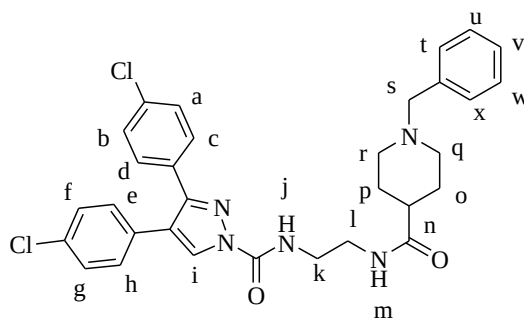
(110)

IR spectral data of compound **(110)** showed NH stretching at 3336 cm^{-1} along with C=O carbonyl stretching at 1635 and 1733 cm^{-1} . Its PMR recorded pyrazole singlet peak at δ

8.19 (s, 1H_k) NH urea peak at 7.53 (t, $J = 6.1$ Hz, 1H_l) and aromatic protons at 7.46 – 7.42 (m, 2H_{d,e}), (b,c,f,g,i,jv,w,x) 7.40 (d, $J = 2.0$ Hz, 1H), 7.35 (d, $J = 8.2$ Hz, 1H), 7.21 – 7.17 (m, 2H), 7.16 – 7.12 (m, 1H), 6.88 – 6.84 (m, 4H) followed by another NH peak at 6.22 (s, 1H_o). Aliphatic protons were observed at 3.82 (d, $J = 3.4$ Hz, 6H_{a,h}), 3.60 (td, $J = 6.8, 4.4$ Hz, 2H_m), 3.56 – 3.50 (m, 2H_n), 3.40 (s, 2H_u) along with protons of piperidine ring 2.85 (dt, $J = 11.9, 3.5$ Hz, 2H_{s1,t1}), 2.16 – 2.06 (m, 1H_p), 2.03 – 1.94 (m, 2H_{s1,t1}), 1.81 – 1.68 (m, 4H_{q,r}). HRMS (ESI⁺) m/z was calculated as 636.2144 for C₃₃H₃₅Cl₂N₅O₄ [M+H]⁺; found, 636.2095.

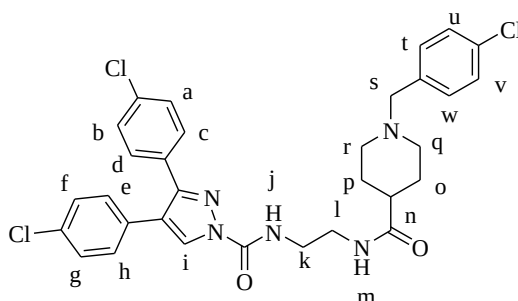


IR spectral data of compound **(111)** showed NH stretching at 3292 cm⁻¹ along with C=O carbonyl stretching at 1705 cm⁻¹. Its PMR recorded pyrazole singlet peak at δ 8.20 (s, 1H_k) NH urea peak at 7.55 (t, $J = 6.1$ Hz, 1H_l) and aromatic protons at 7.47 – 7.42 (m, 2H_{d,e}), (b,c,f,g,i,jv,w,x,y,z,aa) 7.30 – 7.27 (m, 1H), 7.25 (s, 1H), 7.22 – 7.16 (m, 5H), 6.88 – 6.84 (m, 4H) followed by another NH peak at 6.27 (s, 1H_o). Aliphatic protons were observed at 3.82 (d, $J = 2.7$ Hz, 6H_{a,h}), 3.64 – 3.58 (m, 2H_m), 3.53 (q, $J = 5.3$ Hz, 2H_n), 3.03 (dt, $J = 11.9, 3.6$ Hz, 2H_u), 2.83 – 2.77 (m, 2H_v), 2.58 (dd, $J = 10.3, 6.1$ Hz, 2H_{s1,t1}), 2.14 (d, $J = 12.0$ Hz, 3H_{p,s1,t1}), 1.90 (d, $J = 12.7$ Hz, 2H_{q1}), 1.80 (td, $J = 12.4, 3.7$ Hz, 2H_r). ¹³C-NMR of the compound represented C=O peaks at δ 175.63, 152.01, 151.16 along with C_{AR}-OCH₃ peaks at 160.10, 159.15 followed by aromatic peaks C_{Ar} at 130.01, 129.85, 128.83, 128.53, 127.68, 126.20, 124.59, 124.35, 123.58, 114.17, 113.98 C-O peak at 53.18 C-N peaks at 55.40, 40.34, 40.21 and C_{ali} peaks at 33.66, 28.80. The molecular of quasi-molecular ion peak of the compound was observed at 582.41 (M+H)⁺. HPLC purity: 96.59%.



(112)

Compound **(112)** produced IR spectrum containing NH spectrum at 3363 cm^{-1} along with C=O peak of urea and amide at 1729 and 1640 cm^{-1} respectively. PMR spectrum of the compound exhibited pyrazole singlet proton at δ 8.23 (s, 1H_i) and a triplet proton of urea NH at 7.58 (t, $J = 5.9\text{ Hz}$, 1H_j) followed by 7.40 (d, $J = 1.9\text{ Hz}$, 1H_c), 7.38 (d, $J = 2.1\text{ Hz}$, 1H_d), 7.30 – 7.23 (m, 9H_{a,b,e,h,t,u,v,w,x}), 7.15 (d, $J = 2.1\text{ Hz}$, 1H_f), 7.13 (d, $J = 1.9\text{ Hz}$, 1H_g). Another NH proton of amide was obtained at 6.18 (t, $J = 5.4\text{ Hz}$, 1H_m) after which aliphatic protons of spacer were observed at 3.57 (td, $J = 6.7, 4.4\text{ Hz}$, 2H_k), 3.54 – 3.47 (m, 2H_l), 3.45 (s, 2H_s), 2.93 – 2.83 (m, 2H_{q1,r1}), 2.08 (tt, $J = 11.3, 4.2\text{ Hz}$, 2H_{q1,r1}), 1.95 (td, $J = 11.5, 2.8\text{ Hz}$, 1H_n), 1.83 – 1.65 (m, 4H_{o,p}). HRMS (ESI⁺) m/z was calculated as 576.1932 for C₃₁H₃₁Cl₂N₅O₂ [M+H]⁺; found, 576.1895.



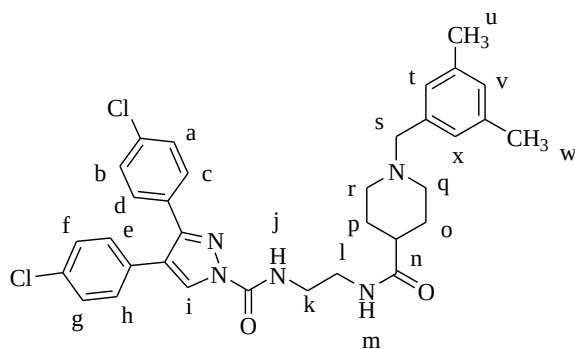
(113)

IR spectral data of compound **(113)** showed C=O carbonyl stretching of amide and urea at 1644 and 1729 cm^{-1} . It PMR spectrum exhibited singlet pyrazole proton at δ 8.26 (s, 1H_i) and NH proton of urea at 7.55 (t, $J = 6.4\text{ Hz}$, 1H_j) followed by aromatic protons at 7.46 – 7.39 (m, 2H_{c,d}), 7.35 – 7.26 (m, 8H_{e,f,g,h,t,u,v,w}), 7.20 – 7.17 (m, 2H_{a,b}) respectively. Another amide NH proton was seen at 6.09 (s, 1H_m) followed by aliphatic protons 3.61 (t, $J = 6.1\text{ Hz}$, 2H_k), 3.54 (q, $J = 5.6\text{ Hz}$, 2H_l), 3.45 (s, 2H_u). Piperidine protons were observed at 2.88 (bs, 2H_{q1,r1}), 2.11 (bs, 1H_n), 1.99 (bs, 2H_{q1,r1}), 1.80 (bs, 4H_{o,p}). ¹³C-NMR spectra represented C=O peaks at δ 175.68, 150.68, 150.42 C_{Ar} peaks at 138.15, 134.95, 133.72, 130.30, 130.14,

[illegible]

(114)

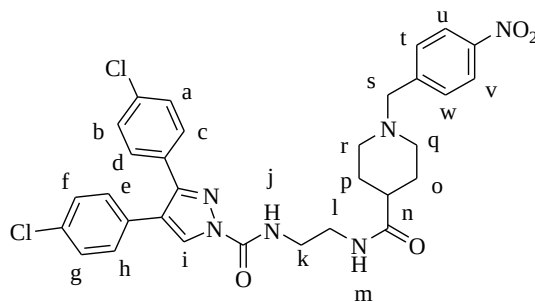
IR spectral data of compound (**114**) showed NH stretching at 3329 cm^{-1} together with C=O carbonyl stretching of amide and urea at $1638, 1734\text{ cm}^{-1}$. Its PMR spectrum exhibited singlet pyrazole proton at $\delta\ 8.26$ (s, 1H), an aromatic proton at 7.79 (d, $J = 7.8$ Hz, 1H), 7.60 (d, $J = 7.3$ Hz, 2H_{c,d}) along with NH of urea at 7.49 (t, $J = 7.6$ Hz, 1H), aromatic protons $7.45 - 7.40$ (m, 2H_{e,h}), 7.31 (d, $J = 8.3$ Hz, 5H_{a,b,u,v,w}), $7.20 - 7.15$ (m, 2H_{f,g}) respectively. Another amide NH proton was observed as broad singlet at 6.17 (s, 1H) followed by aliphatic region $3.66 - 3.59$ (m, 4H_{k,s}), 3.55 (dd, 2H_i), $2.92 - 2.83$ (m, 2H_{q1,r1}), $2.20 - 2.02$ (m, 3H_{n, q1,r1}), $1.86 - 1.75$ (m, 4H_{o,p}). HRMS (ESI+) m/z was calculated as 644.1806 for $\text{C}_{32}\text{H}_{30}\text{Cl}_2\text{F}_3\text{N}_5\text{O}_2$ $[\text{M}+\text{H}]^+$, found, 644.1810 .



(115)

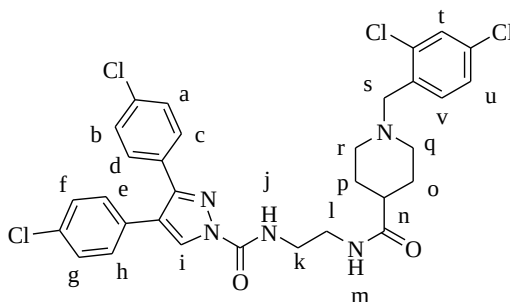
IR spectral data of compound (**115**) showed NH stretching at 3348 cm^{-1} together with C=O carbonyl stretching of amide and urea at $1639, 1731\text{ cm}^{-1}$. It PMR spectrum exhibited singlet pyrazole proton at δ 8.33 (s, 1H_i), NH proton of urea at 7.68 (t, $J = 5.9\text{ Hz}$, 1H) followed by aromatic protons at 7.50 (d, $J = 8.5\text{ Hz}$, 2H_{c,d}), 7.40 – 7.36 (m, 4H_{a,b,e,h}), 7.34 (s, 1H_v), 7.27 (d, $J = 2.1$, 1H_x), 7.25 (d, $J = 2.1\text{ Hz}$, 1H_t), 7.05 (bs, 1H_f), 7.00 (bs, 1H_g). Aliphatic protons were found at 3.71 – 3.66 (m, 2H_k), 3.61 (dd, $J = 7.5, 4.1\text{ Hz}$, 4H_{l,s}), 3.20 (t, $J = 7.3$

Hz, $2H_{q1,r1}$), 3.09 (bs, $2H_{q1,r1}$), 2.41 (bs, $1H_n$), 2.37 (s, $6H_{u,w}$), 1.95 (bs, $4H_{o,p}$). ^{13}C -NMR displayed C=O peaks at δ 175.68, 150.68, 150.42 followed by C_{Ar} peaks at 138.15, 134.95, 133.72, 130.30, 130.14, 130.02, 129.81, 129.03, 128.84, 128.31, 127.66, 122.68 C-N peaks at 62.53, 52.36, 40.50, 39.68 and C_{ali} 46.20, 29.77, 27.82, 21.31 respectively. HRMS (ESI+) m/z was calculated as 604.2245 for $\text{C}_{33}\text{H}_{35}\text{Cl}_2\text{N}_5\text{O}_2$ $[\text{M}+\text{H}]^+$, found, 604.2240.



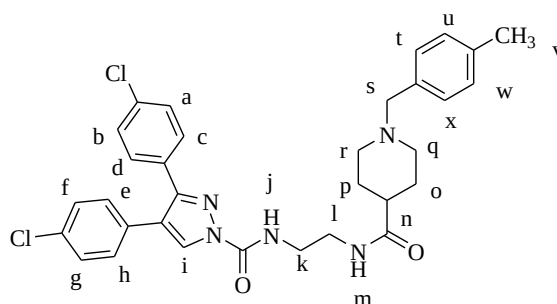
(116)

IR spectral data of compound (116) showed NH stretching at 3321 cm^{-1} together with C=O carbonyl stretching of amide and urea at $1638, 1733\text{ cm}^{-1}$. Its PMR spectrum exhibited singlet pyrazole proton at δ 8.26 (s, $1H$) deshielded aromatic protons at 8.16 (d, $J = 1.8\text{ Hz}$, $1H_t$), 8.15 (d, $J = 2.0\text{ Hz}$, $1H_w$) NH proton of urea at 7.58 (t, $J = 6.0\text{ Hz}$, $1H_j$) and other aromatic protons at 7.48 (d, $J = 8.3\text{ Hz}$, $2H_{c,d}$), 7.43 (d, $J = 1.9\text{ Hz}$, $1H_u$), 7.42 (d, $J = 2.1\text{ Hz}$, $1H_v$), 7.32 (d, $J = 2.0\text{ Hz}$, $2H_{e,h}$), 7.30 (d, $J = 2.0\text{ Hz}$, $2H_{a,b}$), 7.19 (d, $J = 2.0\text{ Hz}$, $1H_f$), 7.17 (d, $J = 2.0\text{ Hz}$, $1H_g$). Another NH proton of amide was present at 6.14 (s, $1H_m$) and aliphatic protons were seen at 3.62 (m, $2H_k$), 3.56 (m, $4H_l$), 2.86 (dd, $J = 9.7, 5.7\text{ Hz}$, $2H_{q1,r1}$), 2.12 (m, $1H_n$), 2.07 – 2.01 (m, $2H_{q1,r1}$), 1.83 – 1.77 (m, $4H_{o,p}$). ^{13}C -NMR displayed C=O peaks at δ 175.79, 150.82, 150.64 followed by C_{Ar} peaks at 147.21, 146.46, 135.04, 133.82, 130.21, 130.05, 129.99, 129.77, 129.53, 129.49, 129.09, 129.00, 128.88, 128.27, 123.62, 122.82 C-N peaks at 62.26, 53.18, 40.46, 40.02, and C_{ali} peaks at 42.92, 38.80, 29.79, 28.79, 23.82, 23.08 respectively. HRMS (ESI+) m/z was calculated as 621.1783 for $\text{C}_{31}\text{H}_{30}\text{Cl}_2\text{N}_6\text{O}_4$ $[\text{M}+\text{H}]^+$, found, 621.1734.



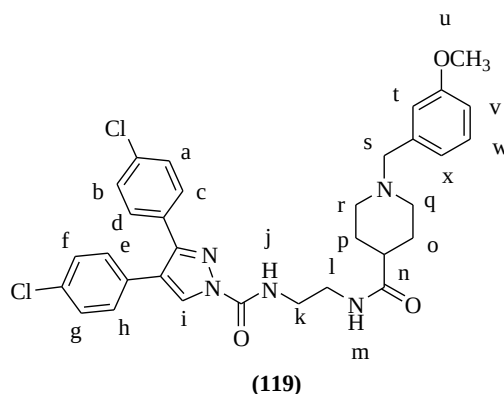
(117)

IR spectral data of compound **(117)** showed NH stretching at 3264 and 3348 cm^{-1} together with C=O carbonyl stretching of amide and urea at 1640 and 1731 cm^{-1} . It PMR spectrum exhibited singlet pyrazole proton at δ 8.26 (s, 1H_i) NH proton of urea at 7.57 (t, J = 6.0 Hz, 1H_j) followed by aromatic protons at 7.43 (d, J = 2.1 Hz, 1H_t), 7.42 (d, J = 2.2 Hz, 2H_{c,d}), 7.34 (d, J = 2.1 Hz, 1H_u), 7.33 (d, J = 1.6 Hz, 2H_{e,h}), 7.31 (d, J = 1.5 Hz, 2H_{a,b}), 7.21 – 7.17 (m, 3H_{f,g,v}). Another NH proton was obtained at 6.11 (s, 1H_m) followed by aliphatic protons at 3.61 (td, J = 6.8, 4.5 Hz, 2H_k), 3.57 – 3.52 (m, 4H_{l,s}), piperidine protons at 2.91 – 2.86 (m, 2H_{q1,r1}), 2.09 (m, 3H_{n, q1,r1}), 1.83 – 1.73 (m, 4H_{o,p}). Quasi-molecular ion peak in mass spectra was observed at 644.30 (M+H)⁺ along with isotopic peak of Cl at 646.22 (M+2H)⁺.

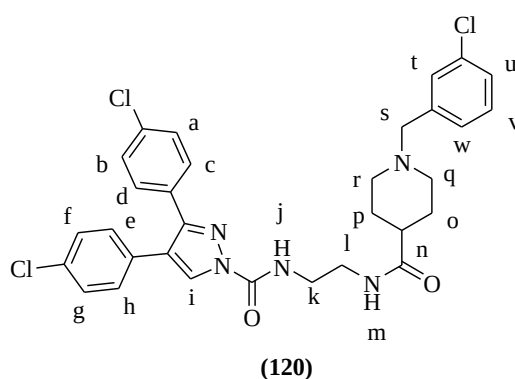


(118)

IR spectral data of compound **(118)** showed NH stretching at 3267 and 3349 cm^{-1} together with C=O carbonyl stretching of amide and urea at 1641 and 1731 cm^{-1} . It PMR spectrum exhibited singlet pyrazole proton at δ 8.26 (s, 1H_i), NH proton of urea at 7.56 (t, J = 6.0 Hz, 1H_j) followed by aromatic protons at 7.43 (d, J = 2.0 Hz, 1H_c), 7.41 (d, J = 2.2 Hz, 1H_d), 7.32 (s, 2H_{e,h}), 7.30 (d, J = 2.0 Hz, 2H_{a,b}), 7.19 (d, J = 1.9 Hz, 2H_{f,g}), 7.17 (s, 2H_{u,w}), 7.11 (d, J = 7.8 Hz, 2H_{t,x}). Another NH proton was obtained at 6.11 (s, 1H), followed by aliphatic protons at 3.63 – 3.59 (m, 2H_k), 3.54 (dd, J = 5.8, 3.7 Hz, 2H_l), 3.46 (s, 2H_s), 2.91 (d, 2H_{q1,r1}), 2.33 (s, 3H_v), 2.11 (bs, 1H_n), 2.00 (d, J = 10.5 Hz, 2H_{q1,r1}), 1.76 (td, J = 12.0, 3.5 Hz, 4H_{o,p}) respectively. Quasi-molecular ion peak in mass spectra was observed at 590.32 (M+H)⁺ along with isotopic peak of Cl at 592.27 (M+2H)⁺.

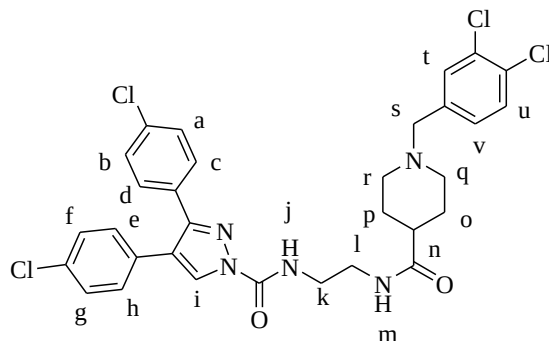


IR spectral data of compound **(119)** showed NH stretching at 3330 cm^{-1} together with C=O carbonyl stretching of amide and urea at 1639 and 1704 cm^{-1} . Additional O-C ether stretching was seen at 1255 cm^{-1} . Its PMR spectrum exhibited singlet pyrazole proton at δ 8.26 (s, 1H_i), NH proton of urea at 7.57 (t, $J = 6.0$ Hz, 1H_j) followed by aromatic protons at 7.44 – 7.40 (m, 2H_{c,d}), 7.31 (dd, $J = 8.3, 1.4$ Hz, 4H_{a,b,e,h}), 7.23 – 7.16 (m, 3H_{t,v,x}), 6.87 (d, $J = 7.5$ Hz, 2H_{f,g}), 6.81 – 6.77 (m, 1H_w). Another NH proton was obtained at 6.11 (s, 1H) followed by methoxy protons at 3.79 (s, 3H) and other aliphatic protons at 3.61 (td, $J = 6.8, 4.4$ Hz, 2H_k), 3.54 (dd, $J = 7.8, 4.0$ Hz, 2H_l), 3.46 (s, 2H_s) piperidine protons at 2.91 (dt, $J = 11.8, 3.5$ Hz, 2H_{q1,r1}), 2.11 (dt, $J = 11.3, 6.7$ Hz, 1H_n), 2.00 (d, $J = 10.0$ Hz, 2H_{q1,r1}), 1.76 (td, $J = 11.8, 3.5$ Hz, 4H_{o,p}). ^{13}C -NMR produced C=O peaks at δ 176.01, 159.74 along with C_{Ar} peaks at 150.84, 150.60, 139.87, 135.04, 133.79, 130.27, 130.14, 130.05, 129.81, 129.28, 129.08, 128.90, 128.32, 122.82, 121.50, 114.62, 112.66 C-O peak at 55.30 C-N peaks at 63.11, 53.10, 40.49, 39.95 C_{ali} peaks at 43.22, 29.81, 28.86 respectively. Quasi-molecular ion peak in mass spectra was observed at 606.35 (M+H)⁺ along with isotopic peak of Cl at 608.28 (M+2H)⁺.



IR spectral data of compound **(120)** showed NH stretching at 3363 cm^{-1} together with C=O carbonyl stretching of amide and urea at 1725 and 1633 cm^{-1} . Its PMR spectrum showed singlet proton peak of pyrazole at δ 8.26 (s, 1H_i) NH of urea at 7.57 (t, $J = 6.0$ Hz, 1H_j) followed by aromatic protons at 7.44 – 7.41 (m, 2H_{c,d}), 7.33 – 7.30 (m, 5H_{e,h,t,u,w}), 7.23 (d, $J = 4.6$ Hz, 2H_{a,b}), 7.20 – 7.16 (m, 3H_{f,g,v}). Another NH proton of amide was found at 6.15

(s, 1H_m) and aliphatic protons were found at 3.65 – 3.58 (m, 2H_k), 3.55 (t, $J = 5.7$ Hz, 2H_l), 3.48 (d, $J = 6.2$ Hz, 2H_s), 2.95 – 2.86 (m, 2H_{q1,s1}), 2.14 (s, 1H_n), 2.01 (s, 2H_{q1,s1}), 1.72 (s, 4H_{o,p}). Quasi-molecular ion peak in mass spectra was observed at 610.30 (M+H)⁺ along with isotopic peak of Cl at 612.26 (M+2H)⁺.



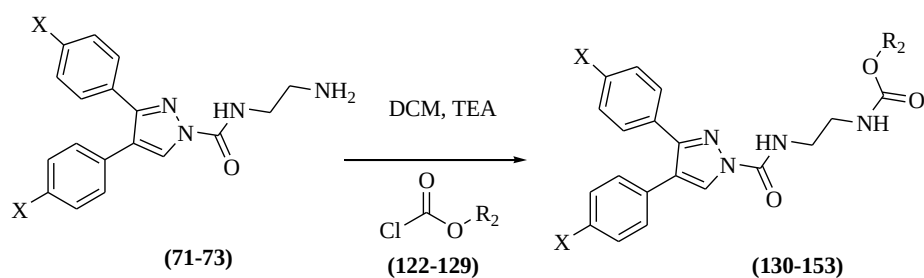
(121)

IR spectral data of compound **(121)** showed NH stretching at 3336 cm⁻¹ together with C=O carbonyl stretching of amide and urea at 1642 and 1729 cm⁻¹. Its PMR spectrum showed singlet proton peak of pyrazole at δ 8.26 (s, 1H_i) NH of urea at 7.56 (t, $J = 6.0$ Hz, 1H_j) followed by aromatic protons at 7.45 – 7.40 (m, 2H_{c,d}), 7.36 (d, $J = 8.1$ Hz, 1H_u), 7.32 (dd, $J = 8.3, 1.6$ Hz, 5H_{e,h,a,b,t}), 7.20 – 7.16 (m, 2H_{f,g}). Another amide proton NH at 6.10 (s, 1H_m) followed by aliphatic protons at 3.62 (q, $J = 4.0$ Hz, 2H_k), 3.55 (t, $J = 5.9$ Hz, 2H_l), 3.42 (s, 2H_s) piperidine protons at 2.86 (d, $J = 10.8$ Hz, 2H_{q1,r1}), 2.12 (s, 1H_n), 1.99 (s, 2H_{q1,r1}), 1.78 (d, $J = 15.5$ Hz, 4H_{o,p}). ¹³C-NMR showed C=O at δ 175.68, 150.60, 150.39 followed by C_{AR} peaks at 134.84, 133.61, 132.22, 130.91, 130.64, 130.14, 130.09, 129.94, 129.84, 129.62, 128.90, 128.70, 128.19, 128.11, 122.60 C-N peaks at 61.60, 52.78, 45.82, 40.33, 39.73 and C_{ali} peaks at 28.53; HRMS (ESI⁺) m/z was calculated as 644.1153 for C₃₁H₂₉Cl₄N₅O₂ [M+H]⁺, found, 644.1112, [M+2H]⁺ 646.1112. HPLC purity: 96.66%.

5.1.1.9. Synthesis of substituted 2-(3,4-diaryl-1H-pyrazole-1-carboxamido)

ethylcarbamates (130-153)

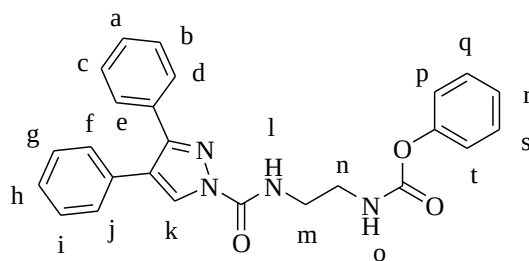
In the final step according to **scheme 9**, *N*-(2-aminoethyl)-3,4-diaryl-1H-pyrazole-1-carboxamide (**71-73**) was reacted with various chloroformates (**122-129**) in presence of TEA to obtained substituted 2-(3,4-diaryl-1H-pyrazole-1-carboxamido)ethylcarbamates (**130-153**).



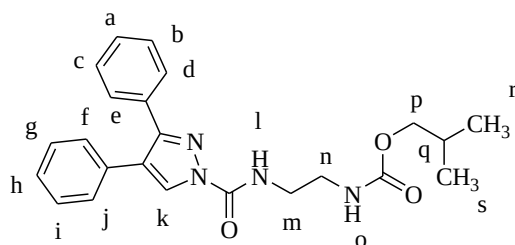
X	R ₂
H: 71, 130, 131, 132, 133, 134, 135, 136, 137, 138.	Phenyl: 122, 130, 139, 147
OCH₃: 72, 139, 140, 141, 142, 143, 144, 145, 146.	Isobutyl: 123, 131, 140, 148
Cl: 73, 147, 148, 149, 150, 151, 152, 153, 154.	Ethyl: 124, 132, 141, 149
	Methyl: 125, 134, 142, 150
	n-Pentyl: 126, 135, 143, 151
	2,2,2-Trichloroethyl: 127, 136, 144, 152
	Benzyl: 128, 137, 145, 153
	(9H)-Fluorenyl methyl: 129, 138, 146, 154

Scheme 9

Compound **(130)** showed NH stretching at 3348 cm^{-1} and C=O carbonyl stretching at 1717 cm^{-1} . Its PMR spectra exhibited a singlet δ 8.32 (s, 1H_k) triplet of NH of urea at 7.60 (t, $J = 6.1\text{ Hz}$, 1H_l) followed by aromatic protons at 7.53 – 7.49 (m, 2H_{d,e}), 7.37 – 7.27 (m, 10H_{a,b,c,f,g,h,i,j,q,t}), 7.21 – 7.15 (m, 1H_q), 7.14 – 7.08 (m, 2H_{p,u}) along with other NH at 5.52 (d, $J = 6.2\text{ Hz}$, 1H_o) and aliphatic spacers at 3.68 (td, $J = 6.6, 4.8\text{ Hz}$, 2H_m), 3.57 (td, $J = 6.6, 4.8\text{ Hz}$, 2H_o). Its mass spectra produced quasi-molecular ion peak at (m/z) 427.27 (M+H)⁺.



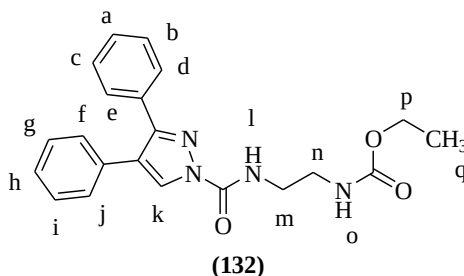
(130)



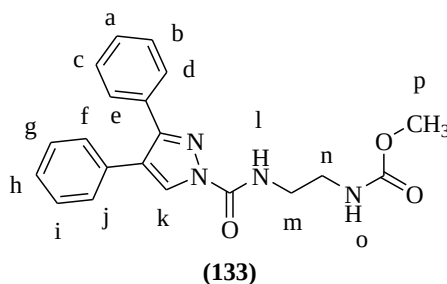
(131)

Compound **(131)** showed NH stretching at 3334 cm^{-1} and C=O carbonyl stretching at 1714 and 1694 cm^{-1} . Its PMR spectra exhibited a singlet δ 8.30 (s, 1H_k) NH of urea and an

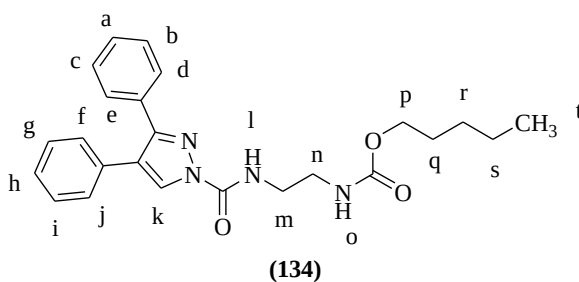
aromatic proton merged at 7.57 – 7.51 (m, 2H_{l,d}) along with other aromatic protons at 7.50 (d, $J = 2.3$ Hz, 1H_e), 7.36 – 7.27 (m, 8H_{a,b,c,f,g,h,i,j}). Another NH of amide at 5.06 (s, 1H_o) and aliphatic protons at 3.85 (d, $J = 6.7$ Hz, 2H_p), 3.61 (q, $J = 6.0$ Hz, 2H_m), 3.48 (dd, $J = 8.5, 3.9$ Hz, 2H_n), 1.89 (m, $J = 6.7$ Hz, 1H_q), 0.90 (d, $J = 6.7$ Hz, 6H_{r,s}). Its mass spectra produced quasi-molecular ion peak at (m/z) 407.28 (M+H)⁺.



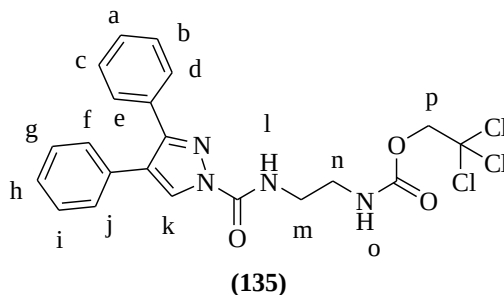
IR spectrum of **(132)** NH stretching at 3345cm⁻¹ along with C=O carbonyl stretching at 1726 and 1693 cm⁻¹. PMR spectra reported pyrazole proton at δ 8.30 (s, 1H_k) NH of urea at 7.56 (s, 1H_l) aromatic protons at 7.53 – 7.48 (m, 2H_{d,e}), 7.36 – 7.27 (m, 8H_{a,b,c,f,g,h,i,j}). Another NH proton at 5.11 (d, $J = 7.9$ Hz, 1H_o) aliphatic protons at 4.12 (q, $J = 7.1$ Hz, 2H_p), 3.60 (q, $J = 5.9$ Hz, 2H_m), 3.46 (q, $J = 6.0$ Hz, 2H_n) and 1.23 (t, $J = 7.1$ Hz, 3H_q) respectively. Its mass spectra produced quasi-molecular ion peak at (m/z) 379.23 (M+H)⁺.



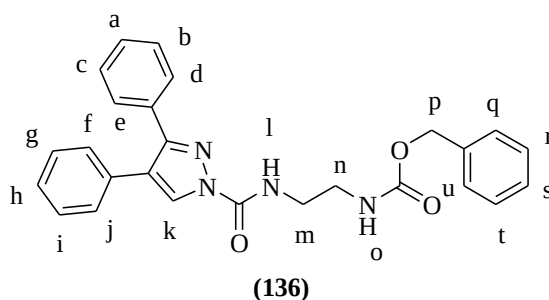
IR spectrum of **(133)** NH stretching at 3341cm⁻¹ along with C=O carbonyl stretching at 1712 and 1700 cm⁻¹. PMR spectra reported pyrazole proton at δ 8.25 (s, 1H) NH of urea at 7.51 (s, 1H) aromatic protons at 7.48 – 7.44 (m, 2H_{d,e}), 7.34 – 7.27 (m, 4H_{b,c,f,j}), 7.26 – 7.19 (m, 4H_{a,g,h,i}). NH of amide at 5.12 (s, 1H_o) aliphatic protons at 3.63 (s, 3H_p), 3.55 (q, $J = 6.0$ Hz, 2H_m), 3.41 (q, $J = 5.8$ Hz, 2H_n). HRMS (ESI⁺) m/z was calculated as 365.1613 for C₂₀H₂₀N₄O₃ [M+H]⁺, found, 365.1595.



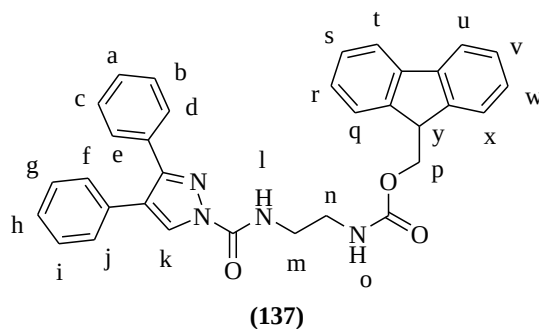
IR spectrum of **(134)** NH stretching at 3309 cm^{-1} along with C=O carbonyl stretching at 1712 and 1680 cm^{-1} . PMR spectra reported pyrazole proton at $\delta\ 8.30$ (s, 1H_k) NH of urea at $7.59 - 7.53$ (m, 1H_l) aromatic protons at 7.51 (dd, $J = 7.1, 2.2\text{ Hz}$, $2\text{H}_{d,e}$), $7.36 - 7.28$ (m, $8\text{H}_{a,b,c,f,g,h,i,j}$). Another NH of amide at 5.08 (s, 1H_i) followed by aliphatic protons at 4.06 (t, $J = 6.8\text{ Hz}$, 2H_p), 3.60 (q, $J = 6.0\text{ Hz}$, 2H_m), $3.51 - 3.41$ (m, 2H_n), $1.65 - 1.59$ (m, 2H_q), 1.30 (p, $J = 3.8\text{ Hz}$, $4\text{H}_{r,s}$), $0.90 - 0.86$ (m, 3H_t) respectively. HRMS (ESI⁺) m/z was calculated as 421.2239 for $\text{C}_{24}\text{H}_{28}\text{N}_4\text{O}_3$ $[\text{M}+\text{H}]^+$, found, 421.2261 .



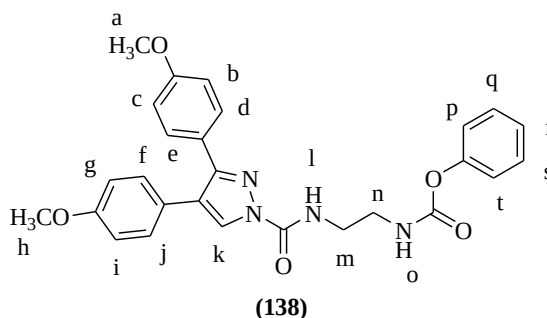
IR spectrum of **(135)** NH stretching at 3369 and 3292 cm^{-1} along with C=O carbonyl stretching at 1728 and 1705 cm^{-1} . PMR spectra reported pyrazole proton at $\delta\ 8.30$ (s, 1H_k) NH proton at 7.55 (d, $J = 5.9\text{ Hz}$, 1H_l) aromatic protons at 7.50 (dd, $J = 7.6, 2.0\text{ Hz}$, $2\text{H}_{d,e}$), $7.37 - 7.28$ (m, $8\text{H}_{a,b,c,f,g,h,i,j}$). Other NH proton of amide at 5.47 (d, $J = 6.3\text{ Hz}$, 1H_o) followed by aliphatic protons at 4.74 (s, 2H_p), 3.65 (td, $J = 6.6, 4.8\text{ Hz}$, 2H_m), 3.53 (td, $J = 6.6, 4.8\text{ Hz}$, 2H_n). ^{13}C -NMR spectra showed C=O peaks at $\delta\ 155.09, 152.22$, C_{AR} peaks at $150.55, 132.07, 131.84, 128.88, 128.79, 128.71, 128.62, 128.55, 128.17, 127.59, 124.08$, C-O peaks at 95.61 , C-Cl peak at 74.69 and C-N peaks at $41.35, 40.35$. HRMS (ESI⁺) m/z : calculated, 481.0600 for $\text{C}_{21}\text{H}_{19}\text{Cl}_3\text{N}_4\text{O}_3$ $[\text{M}+\text{H}]^+$, found, 481.0586 .



IR spectrum of **(136)** NH stretching at 3344 cm^{-1} along with C=O carbonyl stretching at 1707 and 1691 cm^{-1} . PMR spectra reported pyrazole proton at δ 8.24 (s, 1H_k) NH proton at 7.53 (t, 1H_l) aromatic protons at 7.49 – 7.43 (m, 2H_{d,e}), 7.31 – 7.26 (m, 8H_{a,b,c,f,g,h,i,j}), 7.26 – 7.20 (m, 5H_{q,r,s,t,u}). Other NH proton of amide at 5.22 (t, $J = 4.3\text{ Hz}$, 1H_o) followed by aliphatic protons at 5.06 (s, 2H_p), 3.55 (q, $J = 5.9\text{ Hz}$, 2H_m), 3.42 (td, $J = 6.5, 3.7\text{ Hz}$, 2H_n). HRMS (ESI+) m/z was calculated as 441.1926 for C₂₆H₂₄N₄O₃ [M+H]⁺, found, 441.1926.

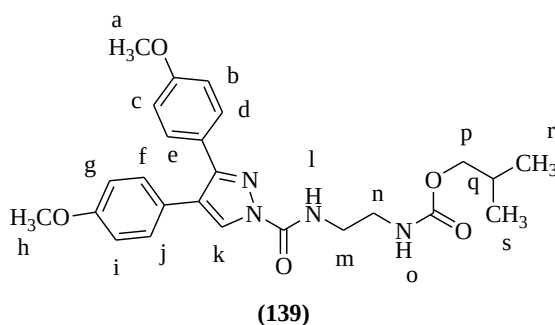


IR spectrum of **(137)** NH stretching at 3313 cm^{-1} along with C=O carbonyl stretching at 1699 and 1722 cm^{-1} . PMR spectra reported pyrazole proton at δ 8.28 (s, 1H_k) 7.73 (d, $J = 7.6\text{ Hz}$, 2H_{t,u}), 7.57 (d, $J = 7.5\text{ Hz}$, 2H_{q,x}) NH proton of urea at 7.54 (s, 1H_l) other aromatic protons 7.51 – 7.47 (m, 2H_{d,e}), 7.38 – 7.29 (m, 10H_{b,c,f,g,i,j,r,s,v,w}), 7.26 – 7.21 (m, 2H_{a,h}). Another NH proton at 5.21 (s, 1H_o) aliphatic protons at 4.41 (d, $J = 6.9\text{ Hz}$, 2H_p), 4.19 (t, $J = 6.9\text{ Hz}$, 1H_y), 3.64 – 3.58 (m, 2H_m), 3.49 (q, $J = 4.5\text{ Hz}$, 2H_n). ¹³C-NMR displayed C=O peaks at δ 156.88, 152.09 followed C_{AR} peaks at 150.50, 143.96, 141.40, 132.10, 131.86, 128.80, 128.75, 128.66, 128.60, 128.50, 128.13, 127.77, 127.50, 127.12, 125.16, 123.93, 120.06 along with C-O peak at 66.93, C_y peak at 47.30 and C-N peaks at 41.01, 40.61. HRMS (ESI+) m/z was calculated as 529.2239 for C₃₃H₂₈N₄O₃ [M+H]⁺, found, 529.2228.

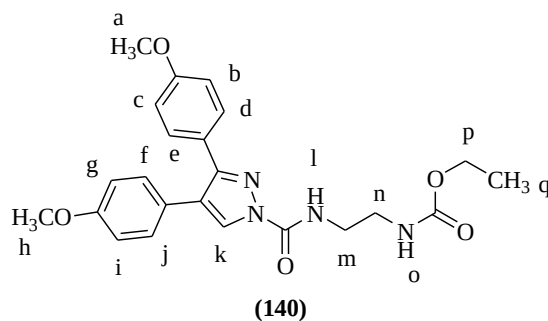


Compound **(138)** showed NH stretching at 3363 and 3313 cm^{-1} and C=O carbonyl stretching at 1710 cm^{-1} . Its PMR spectra exhibited a singlet δ 8.23 (s, 1H_k) triplet of NH of urea at 7.57 (t, $J = 6.2\text{ Hz}$, 1H_l) followed by aromatic protons at 7.47 – 7.40 (m, 2H_{d,e}), 7.32 (t, $J = 7.9\text{ Hz}$, 2H_{q,s}), 7.23 – 7.15 (m, 3H_{p,r,t}), 7.11 (dd, $J = 7.8, 1.6\text{ Hz}$, 2H_{f,j}), 6.89 – 6.84 (m,

4H_{b,c,g,i}) along with other NH at 5.54 (t, $J = 5.8$ Hz, 1H_o) and aliphatic spacers at 3.82 (d, $J = 1.4$ Hz, 6H_{a,h}), 3.70 – 3.64 (m, 2H_m), 3.55 (q, $J = 5.7$ Hz, 2H_n). ¹³C-NMR of the compound displayed peaks at δ C_{AR-OCH₃} 160.03, 159.06 C=O peaks 155.18, 151.89, 151.03 followed by other C_{AR} peaks at 150.74, 129.96, 129.84, 129.37, 127.73, 125.43, 124.61, 124.33, 123.43, 121.69, 114.11, 113.93, C-O peaks at 55.37, 55.34, C-N peaks at 41.33, 40.39. Mass spectra produced parent quasi-molecular ion peak (m/z) 487.27 (M+H)⁺.

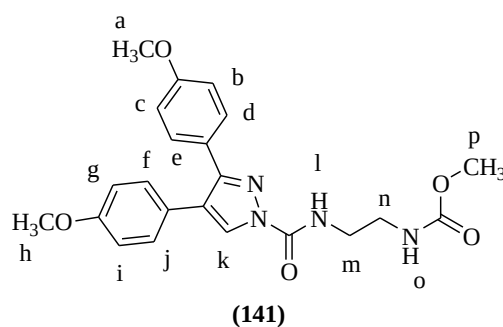


Compound **(139)** in its PMR spectrum showed NH stretching at 3374, 3423cm⁻¹ along with C=O carbonyl stretching at 1611 and 1706cm⁻¹. Its PMR spectrum exhibited pyrazole singlet peak at δ 8.21 (s, 1H_k) along with NH proton of urea at 7.50 (s, 1H_i) and other aromatic protons at 7.45 (d, $J = 8.7$ Hz, 2H_{d,e}), 7.22 – 7.18 (m, 2H_{f,j}), 6.88 – 6.85 (m, 4H_{b,c,g,i}) NH of carbamate at 5.08 (s, 1H_o) and other aliphatic protons at 3.85 (d, $J = 6.7$ Hz, 2H_p), 3.82 (s, 6H_{a,h}), 3.60 (q, $J = 6.0$ Hz, 2H_m), 3.49 – 3.43 (m, 2H_n), 1.89 (dt, $J = 13.4, 6.7$ Hz, 1H_q), 0.90 (d, $J = 6.7$ Hz, 6H_{r,s}). ¹³C-NMR represented C_{AR-OCH₃} peaks at δ 160.02, 159.06 followed C=O peaks at 157.24, 151.78 and other C_{AR} peaks at 150.57, 129.97, 129.82, 127.69, 124.67, 124.41, 123.34, 114.11, 113.91 along with C-O peaks at 71.33, 55.37, 55.34 and C-N peaks at 41.01, 40.61 and finally C_{ali} peaks at 28.07, 19.14. HRMS (ESI⁺) m/z was calculated as 467.2294 for C₂₅H₃₀N₄O₅ [M+H]⁺, found, 467.2305.

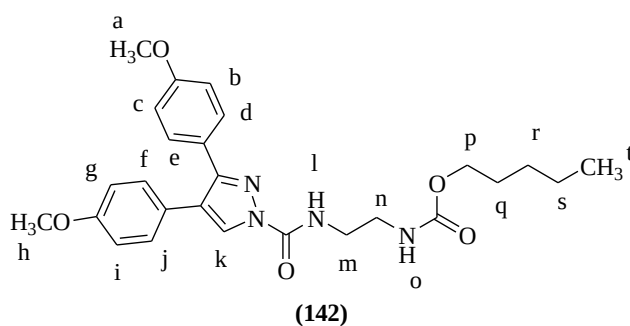


Compound **(140)** in its PMR spectrum showed NH stretching at 3370 cm⁻¹ along with C=O carbonyl stretching at 1613 and 1705 cm⁻¹. Its PMR spectrum exhibited pyrazole singlet peak at δ 8.21 (s, 1H_k) along with NH proton of urea at 7.52 (s, 1H_i) and other aromatic

protons at 7.45 (d, $J = 8.4$ Hz, $2H_{d,e}$), 7.21 – 7.18 (m, $2H_{f,j}$), 6.87 (t, $2H_{b,c}$), 6.86 (t, $J = 2.0$ Hz, $2H_{g,i}$) NH of carbamate at 5.11 (s, $1H_o$) and other aliphatic protons at 4.15 – 4.10 (m, $2H_p$), 3.82 (s, $6H_{a,h}$), 3.59 (q, $J = 6.0$ Hz, $2H_m$), 3.44 (dd, $2H_n$), 1.24 (d, $J = 7.1$ Hz, $3H_q$). ^{13}C -NMR of the compound showed C_{AR-OCH_3} peaks at δ 160.04, 159.09 along with $C=O$ peaks at 157.07, 151.84 and other C_{AR} peaks at 150.60, 129.99, 129.84, 127.71, 124.68, 124.42, 123.38, 114.13, 113.94, 113.85 followed by C-O peak at 61.15, 60.54, 55.39, C-N 41.06, 40.62, C_{ali} peak as rotamers at 14.71 and 14.32. HRMS (ESI+) m/z was calculated as 439.1981 for $C_{23}H_{26}N_4O_5$ $[M+H]^+$, found, 439.1976. HPLC purity: 95.62 %.

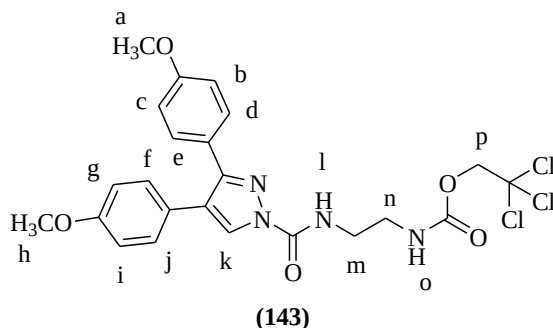


Compound **(141)** in its PMR spectrum showed NH stretching at 3346 cm^{-1} along with $C=O$ carbonyl stretching at 1613 and 1705 cm^{-1} . Its PMR spectrum exhibited pyrazole singlet peak at δ 8.21 (s, $1H_k$) along with NH proton of urea at 7.51 (d, $J = 6.9$ Hz, $1H_l$) and other aromatic protons at 7.47 – 7.43 (m, $2H_{d,e}$), 7.22 – 7.18 (m, $2H_{f,j}$), 6.88 (t, $2H_{b,c}$), 6.86 (t, $J = 2.0$ Hz, $2H_{g,i}$) NH of carbamate at 5.15 (s, $1H_o$) and other aliphatic protons at 3.82 (s, $6H_{a,h}$), 3.68 (s, $3H_p$), 3.59 (q, $J = 6.1$ Hz, $2H_m$), 3.45 (d, $J = 6.0$ Hz, $2H_n$). HRMS (ESI+) m/z was calculated as 425.1824 for $C_{22}H_{24}N_4O_5$ $[M+H]^+$, found, 425.1823.

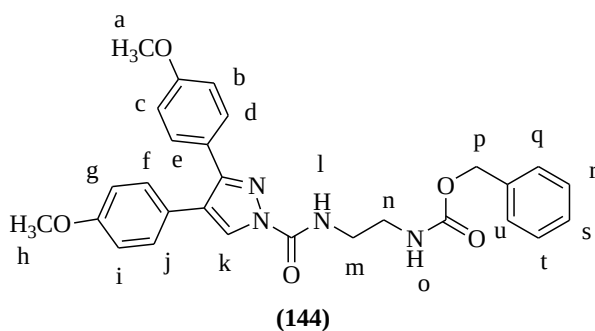


Compound **(142)** in its PMR spectrum showed NH stretching at 3361 cm^{-1} along with $C=O$ carbonyl stretching at 1685 and 1728 cm^{-1} . Its PMR spectrum exhibited pyrazole singlet peak at δ 8.21 (s, $1H_k$) along with NH proton of urea at 7.51 (s, $1H_l$) and other aromatic protons at 7.47 – 7.43 (m, $2H_{d,e}$), 7.22 – 7.18 (m, $2H_{f,j}$), 6.88 (t, $2H_{b,c}$), 6.86 (t, $J = 6.0$ Hz, $2H_{g,i}$) NH of carbamate at 5.08 (s, $1H_o$) and other aliphatic protons at 4.06 (t, $J = 6.7$ Hz,

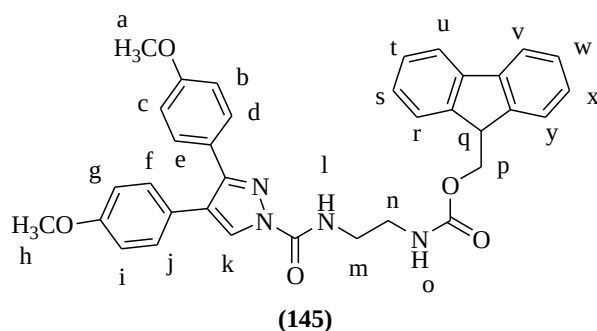
2H_p), 3.82 (s, 6H_{a,h}), 3.58 (t, $J = 6.0$ Hz, 2H_m), 3.45 (d, $J = 6.3$ Hz, 2H_n), 1.59 (d, $J = 6.4$ Hz, 2H_q), 1.31 (h, $J = 3.2$ Hz, 4H_{r,s}), 0.89 (q, $J = 4.0$ Hz, 3H_t). Mass spectra of the compound depicted quasi-molecular ion peak at (m/z) 481.30 (M+H)⁺.



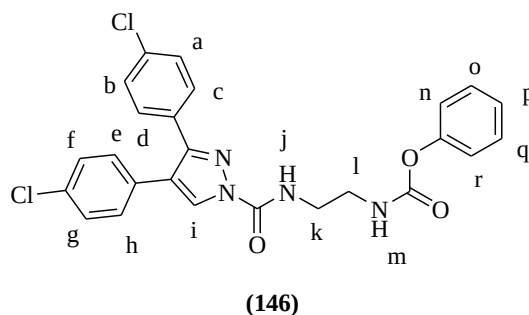
Compound **(143)** in its PMR spectrum showed NH stretching at 3285 and 3368 cm⁻¹ along with C=O carbonyl stretching at 1702 and 1726 cm⁻¹. Its PMR spectrum exhibited pyrazole singlet peak at δ 8.21 (s, 1H_k) along with NH proton of urea at 7.49 (t, $J = 6.2$ Hz, 1H) and other aromatic protons at 7.46 – 7.43 (m, 2H_{d,e}), 7.22 – 7.18 (m, 2H_{f,j}), 6.89 - 6.85 (m, 4H_{b,c,g,i}), NH of carbamate at 5.46 (s, 1H_o) and other aliphatic protons at 4.73 (s, 2H_p), 3.82 (s, 6H_{a,h}), 3.65 (dd, $J = 6.6, 4.9$ Hz, 2H_m), 3.55 – 3.51 (m, 2H_n). HRMS (ESI⁺) m/z was calculated as 541.0812 for C₂₃H₂₃Cl₃N₄O₅ [M+H]⁺, found, 541.0800.



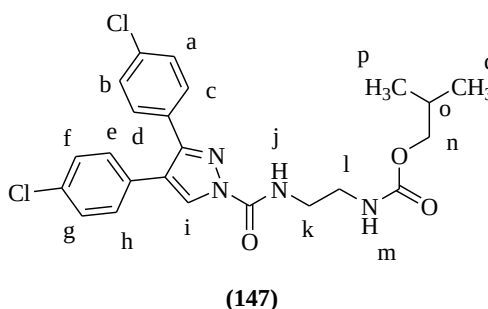
Compound **(144)** in its PMR spectrum showed NH stretching at 3372 cm⁻¹ along with C=O carbonyl stretching at 1726 and 1686 cm⁻¹. Its PMR spectrum exhibited pyrazole singlet peak at δ 8.20 (s, 1H_k) along with NH proton of urea at 7.53 (d, $J = 5.4$ Hz, 1H_l) followed by aromatic protons at 7.44 (d, $J = 8.5$ Hz, 2H_{d,e}), 7.35 – 7.30 (m, 5H_{f,j,r,t,s}), 7.21 – 7.18 (m, 2H_{q,u}), 6.87 (d, $J = 2.1$ Hz, 2H_{b,c}), 6.85 (d, $J = 2.1$ Hz, 2H_{f,i}). NH amide present at 5.22 (s, 1H_o) and aliphatic protons at 5.11 (s, 2H_p), 3.82 (d, $J = 1.9$ Hz, 6H_{a,h}), 3.60 (d, $J = 6.0$ Hz, 2H_m), 3.50 – 3.46 (m, 2H_n).



Compound **(145)** in its PMR spectrum showed NH stretching at 3330 cm^{-1} along with broad C=O carbonyl stretching at 1713 cm^{-1} . Its PMR spectra represented singlet pyrazole proton at $\delta\ 8.19$ (s, 1H_k) and aromatic protons at 7.73 (d, $J = 7.6\text{ Hz}$, $2\text{H}_{u,v}$), 7.56 (d, $J = 7.5\text{ Hz}$, $2\text{H}_{r,y}$) NH proton of carbamate at 7.51 (s, 1H_i) and other aromatic protons $7.43 - 7.40$ (m, $2\text{H}_{d,e}$), 7.37 (t, $J = 7.5\text{ Hz}$, $2\text{H}_{s,t}$), 7.29 (d, $J = 7.5\text{ Hz}$, $2\text{H}_{w,x}$), $7.18 - 7.14$ (m, $2\text{H}_{f,j}$), $6.86 - 6.82$ (m, $4\text{H}_{b,c,g,i}$). Another NH of amide at 5.22 (s, 1H_o) and aliphatic at 4.40 (d, $J = 7.0\text{ Hz}$, 2H_p), 4.19 (t, $J = 6.9\text{ Hz}$, 1H_q), 3.81 (d, $J = 5.4\text{ Hz}$, $6\text{H}_{a,h}$), $3.64 - 3.56$ (m, 2H_m), $3.52 - 3.44$ (m, 2H_n) respectively. Mass spectra produced quasi-molecular ion peak at $(m/z)\ 589.39$ $(\text{M}+\text{H})^+$.

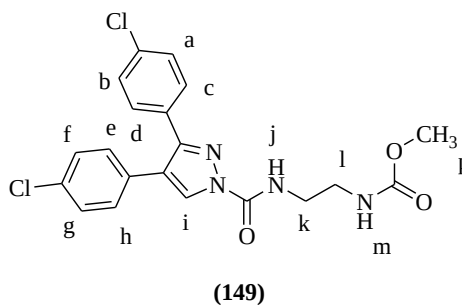
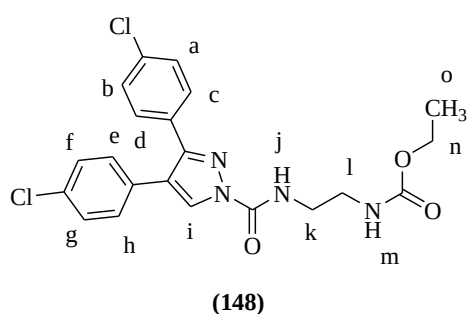


IR spectral data of compound **(146)** reported NH peak at $3296, 3349\text{ cm}^{-1}$ together with C=O of amide and urea at 1598 and 1717 cm^{-1} . A singlet peak of pyrazole was obtained at $\delta\ 8.29$ (s, 1H_i) along with a triplet of urea NH at 7.60 (t, $J = 6.1\text{ Hz}$, 1H_j) and other aromatic protons at $7.45 - 7.39$ (m, $2\text{H}_{c,d}$), $7.33 - 7.29$ (m, $6\text{H}_{a,b,d,e,h,o,q}$), $7.20 - 7.17$ (m, $3\text{H}_{n,p,r}$), 7.10 (dd, $J = 7.7, 1.7\text{ Hz}$, $2\text{H}_{f,g}$). Another NH amide 5.50 (s, 1H_m) aliphatic protons at 3.68 (q, $J = 5.8\text{ Hz}$, 2H_k), 3.56 (q, $J = 5.8\text{ Hz}$, 2H_l). HRMS (ESI⁺) m/z was calculated as 495.0990 for $\text{C}_{25}\text{H}_{20}\text{Cl}_2\text{N}_4\text{O}_3$ $[\text{M}+\text{H}]^+$, found, 495.0956 .



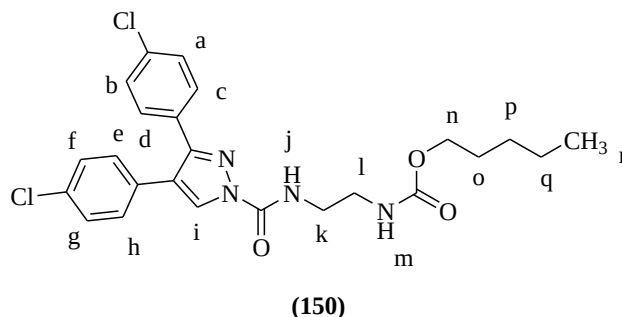
IR spectral data of compound **(147)** reported NH peak at 3377 and 3334 cm^{-1} together with C=O of amide 1726 and urea at 1680 cm^{-1} . A singlet peak of pyrazole was obtained at δ 8.28 (s, 1H_i) along with a triplet of urea NH at 7.56 (t, J = 6.2 Hz, 1H_j) and other aromatic protons at 7.43 (d, J = 8.2 Hz, 2H_{c,d}), 7.34 – 7.29 (m, 4H_{a,b,d,e,h}), 7.20 – 7.17 (m, 3H_{f,g,r}). Another NH amide 5.09 (d, J = 6.5 Hz, 1H_m) aliphatic protons at 3.85 (d, J = 6.7 Hz, 2H_n), 3.61 (q, J = 5.9 Hz, 2H_k), 3.51 – 3.42 (m, 2H_l), 1.89 (dq, J = 13.4, 6.2 Hz, 1H_o), 0.90 (d, J = 6.7 Hz, 6H_{p,q}). HRMS (ESI⁺) m/z was calculated as 475.1303 for C₂₃H₂₄Cl₂N₄O₃ [M+H]⁺, found, 475.1291.

Compound **(148)** produced IR spectrum showing NH at 3349 and 3370 cm^{-1} amide peak C=O at 1686 and 1732 cm^{-1} . PMR spectra exhibited pyrazole at δ 8.28 (s, 1H_i) and NH of urea at 7.57 (s, 1H_j) and aromatic protons at 7.43 (d, J = 8.1 Hz, 2H_{c,d}), 7.34 – 7.29 (m, 4H_{a,b,e,h}), 7.20 – 7.17 (m, 2H_{f,g}). Another NH of amide at 5.08 (s, 1H_m) and aliphatic protons at 4.12 (q, J = 7.1 Hz, 2H_n), 3.60 (q, J = 5.9 Hz, 2H_k), 3.46 (q, J = 6.2 Hz, 2H_l), 1.23 (t, J = 7.1 Hz, 3H_o). HRMS (ESI⁺) m/z was calculated as 447.0990 for C₂₁H₂₀Cl₂N₄O₃ [M+H]⁺, found, 447.0957.

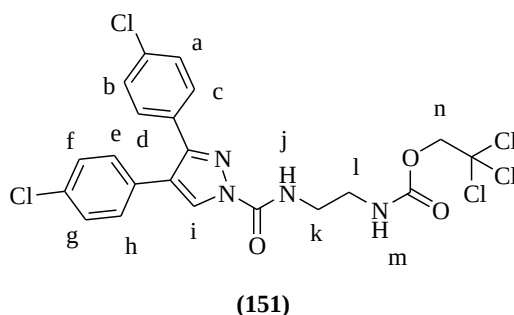


Compound **(149)** produced IR spectrum showing NH at 3328 cm^{-1} along with C=O at 1695 and 1736 cm^{-1} . PMR spectra exhibited pyrazole at δ 8.28 (s, 1H_i) and NH of urea at 7.53 (d, J = 7.2 Hz, 1H_j) and aromatic protons at 7.43 (d, J = 8.4 Hz, 2H_{c,d}), 7.34 – 7.30 (m, 4H_{a,b,e,h}), 7.19 (d, J = 2.1 Hz, 1H_i), 7.18 (d, J = 1.9 Hz, 1H_j). Another NH of amide at 5.10 (s, 1H_m) and aliphatic protons at 3.68 (s, 3H_p), 3.60 (q, J = 6.0 Hz, 2H_k), 3.51 – 3.42 (m, 2H_l).

^{13}C -NMR predicted C=O peaks at δ 157.39, 150.63, 150.04 along with C_{AR} peaks at 134.89, 133.62, 130.15, 130.00, 129.88, 129.67, 128.94, 128.82, 128.76, 128.22, 122.58, and C-O peak at 52.29 while C-N peak at 40.92, 40.63. Mass spectra depicted quasi-molecular ion peak at (m/z) 433.19 $(\text{M}+\text{H})^+$ along with isotope peak of diCl at 435.16 $(\text{M}+2\text{H})^+$.

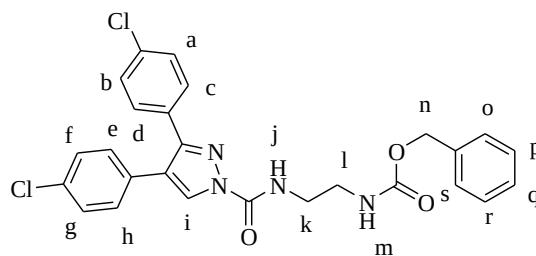


Compound **(150)** produced IR spectrum showing NH at 3325 and 3346 cm^{-1} amide peak C=O at 1675 and 1737 cm^{-1} . PMR spectra exhibited pyrazole at δ 8.28 (s, 1H_i) and NH of urea at 7.56 (d, J = 7.2 Hz, 1H_j) and aromatic protons at 7.43 (d, J = 8.5 Hz, 2H_{c,d}), 7.33 – 7.30 (m, 4H_{a,b,e,h}), 7.20-7.17 (m, 2H_{i,j}). Another NH of amide at 5.05 (s, 1H_m) and aliphatic protons at 4.05 (t, J = 6.8 Hz, 2H_n), 3.60 (q, J = 5.9 Hz, 2H_k), 3.47 (t, J = 6.0 Hz, 2H_l), 1.60 (d, J = 6.6 Hz, 2H_o), 1.30 (q, J = 3.4 Hz, 4H_{p,q}), 0.90 – 0.86 (m, 3H_r). ^{13}C -NMR showed C=O peak δ 157.16, 150.57, 150.01 followed by C_{AR} peaks at 134.86, 133.59, 130.16, 130.01, 129.87, 129.66, 128.92, 128.74, 128.20, 122.54, and C-O peak at 65.30 C-N peaks 40.73 and C_{ali} peaks at 28.61, 27.92, 22.29, 13.94. HRMS (ESI+) m/z was calculated as 489.1459 for $\text{C}_{24}\text{H}_{26}\text{Cl}_2\text{N}_4\text{O}_3$ $[\text{M}+\text{H}]^+$, found, 489.1420.



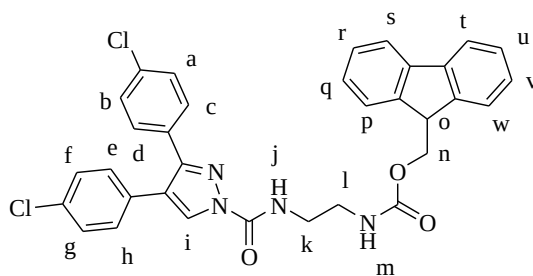
Compound **(151)** produced IR spectrum showing NH at 3290 and 3370 cm^{-1} amide peak C=O at 1705 and 1726 cm^{-1} . PMR spectra exhibited pyrazole at δ 8.21 (s, 1H_i) and NH of urea at 7.49 (t, J = 6.1 Hz, 1H_j) and aromatic protons at 7.47 – 7.42 (m, 2H_{c,d}), 7.21 (d, J = 2.1 Hz, 1H_e), 7.19 (d, J = 2.1 Hz, 1H_h), 6.88 (d, J = 2.1 Hz, 2H_{a,b}), 6.86 (d, J = 2.1 Hz, 2H_{f,g}). Another NH of amide at 5.46 (s, 1H_m) and aliphatic protons at 4.73 (s, 2H_n), 3.64 (td, J = 6.7,

4.8 Hz, 2H_k), 3.56 – 3.50 (m, 2H_l). HRMS (ESI⁺) m/z was calculated as 548.9821 for C₂₁H₁₇Cl₅N₄O₃ [M+H]⁺, found, 548.9754, [M+2H]⁺, 550.9754.



(152)

Compound **(152)** produced IR spectrum showing NH at 3311 and 3407 cm⁻¹ amide peak C=O at 1685 and 1720cm⁻¹. PMR spectra exhibited pyrazole at δ 8.26 (s, 1H_i) and NH of urea at 7.55 (d, *J* = 6.1 Hz, 1H_j) and aromatic protons at 7.42 (d, *J* = 8.2 Hz, 2H_{c,d}), 7.35 – 7.29 (m, 9H_{a,b,e,h,o,p,q,r,s}), 7.20 – 7.16 (m, 2H_{f,g}). Another NH proton was observed at 5.18 (s, 1H_m), 5.11 (s, 2H_n), 3.61 (q, *J* = 5.8 Hz, 2H_k), 3.49 (dd, *J* = 7.1, 4.7 Hz, 2H_l). ¹³C-NMR exhibited C=O peaks of amide at δ 156.94, 150.75, 150.18 followed by C_{AR} peaks at 136.40, 135.02, 133.75, 130.31, 130.15, 130.02, 129.82, 129.07, 128.95, 128.88, 128.64, 128.37, 128.30, 128.22, 122.70 along with C-O peak at 67.05 and C-N peaks at 41.02, 40.78. Mass spectra showed quasi-molecular ion peak at (m/z) 509.15 (M+H)⁺ along isotope peak of diCl 511.99 (M+2H)⁺. HPLC purity 95.61 %.



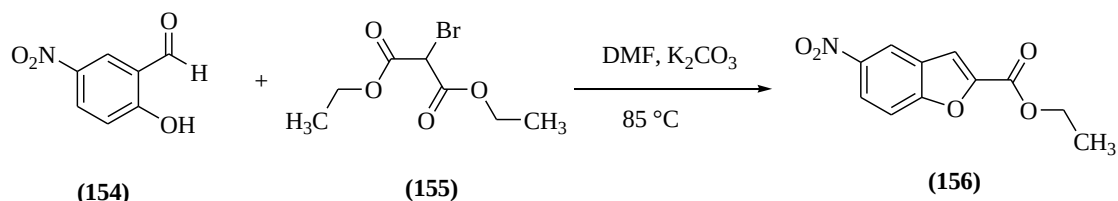
(153)

Compound **(153)** produced IR spectrum showing NH at 3335 cm⁻¹ amide peak C=O at 1679, 1747 cm⁻¹. PMR spectra exhibited pyrazole at δ 8.22 (s, 1H_i), aromatic protons as 7.72 (d, *J* = 7.6 Hz, 2H_{p,w}) NH of urea at 7.56 (d, *J* = 5.5 Hz, 1H_j) other aromatic protons at 7.53 (d, *J* = 7.5 Hz, 2H_{c,d}), 7.39 – 7.32 (m, 4H_{e,h,s,u}), 7.29 – 7.24 (m, 6H_{a,b,q,r,u,v}), 7.10 (d, *J* = 8.2 Hz, 2H_{e,g}). Another NH of amide at 5.21 (t, *J* = 5.9 Hz, 1H_m), 4.38 (d, *J* = 7.0 Hz, 2H_n), 4.15 (t, *J* = 7.1 Hz, 1H_o), 3.61 (q, *J* = 5.8 Hz, 2H_k), 3.50 (q, *J* = 5.9 Hz, 2H_l) respectively. HRMS (ESI⁺) m/z was calculated as 597.1459 for C₃₃H₂₆Cl₂N₄O₃ [M+H]⁺, found, 597.1416.

5.1.2. Synthesis of benzofuran derivatives (156-227)

5.1.2.1. Synthesis of ethyl 5-nitrobenzofuran-2-carboxylate (156)

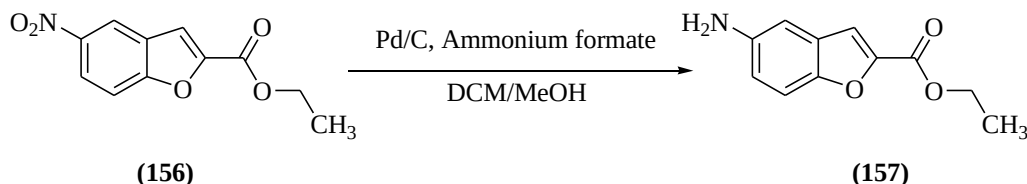
Ethyl 5-nitrobenzofuran-2-carboxylate (**156**) was synthesized using **scheme-10** by cyclization reaction wherein 2-hydroxy-5-nitrobenzaldehyde (**154**) reacted with diethyl 2-bromomalonate (**155**) in presence of potassium carbonate, to produce white solid ethyl 5-nitrobenzofuran-2-carboxylate (**156**). The synthesized compound was confirmed with m.p. 175-177 °C (Lit.¹ 176 -178 °C).



Scheme-10

5.1.2.2. Synthesis of ethyl 5-aminobenzofuran-2-carboxylate (157)

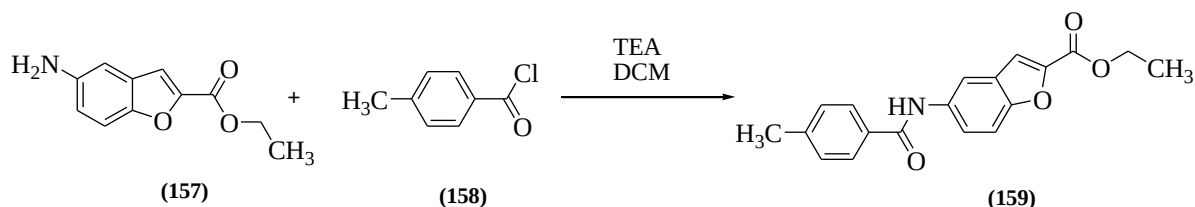
Ethyl 5-nitrobenzofuran-2-carboxylate (**156**) by reduction reaction with ammonium formate and Pd/C (10%) in presence of methanol using **scheme-11** produced ethyl 5-aminobenzofuran-2-carboxylate. (**157**)



Scheme-11

5.1.2.3. Synthesis of ethyl 5-(4-methylbenzamido)benzofuran-2-carboxylate (159)

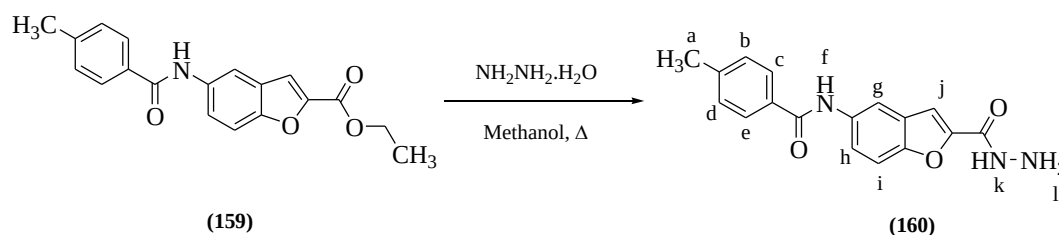
Scheme-12 represents synthesis of compound (**159**) wherein, 4-methyl benzoyl chloride (**158**) was added via nucleophilic substitution reaction with ethyl 5-aminobenzofuran-2-carboxylate (**157**).



Scheme-12

5.1.2.4. Synthesis of 5-(4-methylbenzamido)benzofuran-2-carbohydrazide (160)

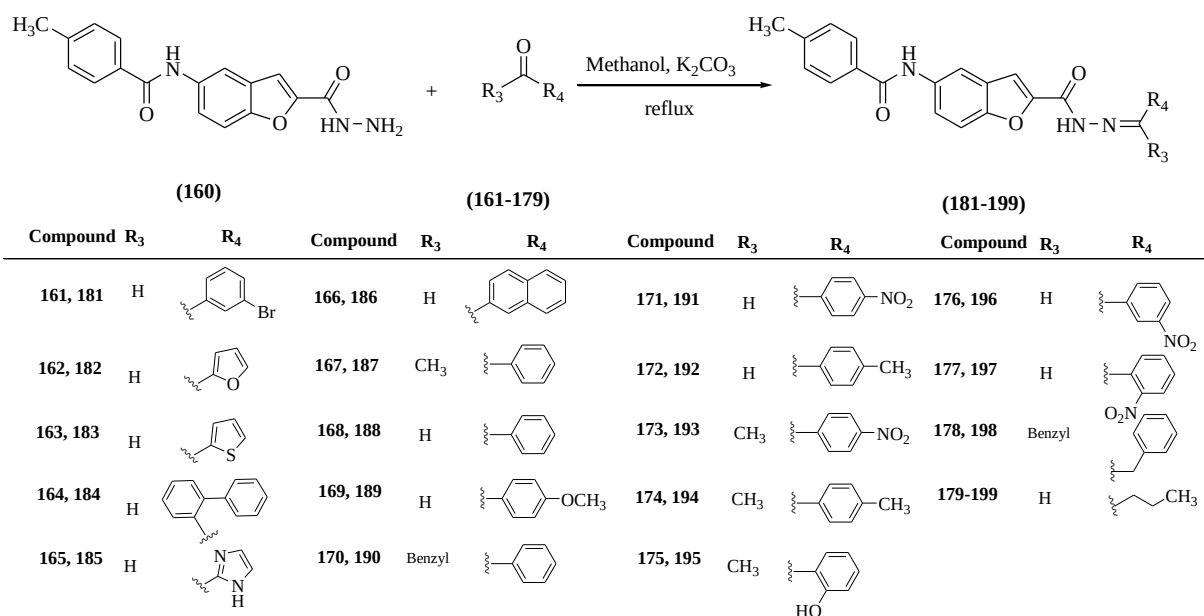
Ethyl 5-(4-methylbenzamido)benzofuran-2-carboxylate (**159**) underwent reaction with hydrazine hydrate to produce 5-(4-methylbenzamido)benzofuran-2-carbohydrazide (**160**) as shown in Scheme-13.



Scheme-13

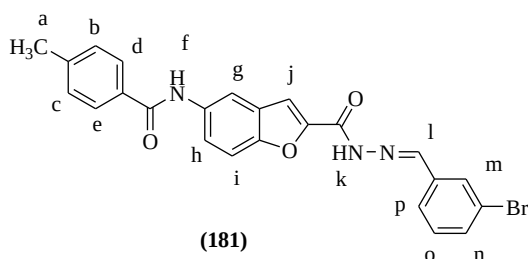
Compound (**160**) was characterized by IR spectra in which NH stretching was observed at 3324 cm^{-1} followed by C=O carbonyl stretching at 1651 cm^{-1} . PMR of the compound showed NH peak at δ 10.26 (s, 1H_f) and 10.01 (s, 1H_k) respectively. Aromatic protons at 8.25 (d, $J = 2.1\text{ Hz}$, 1H_j), 7.94 – 7.86 (m, 2H_{c,e}), 7.72 (dd, $J = 9.0, 2.2\text{ Hz}$, 1H_h), 7.61 (d, $J = 8.9\text{ Hz}$, 1H_i), 7.52 (d, $J = 0.9\text{ Hz}$, 1H_g), 7.35 (d, $J = 8.0\text{ Hz}$, 2H_{b,d}). Another NH₂ proton at 4.65 – 4.52 (m, 2H_i) and aliphatic CH₃ protons at 2.39 (s, 3H_a). The mass spectra exhibited m/z 310.3 (M+H)⁺ peak.

5.1.2.5. Synthesis of 5-(4-methylbenzamido)-N'-(substituted)benzofuran-2-carbohydrazide derivatives (**181-199**)



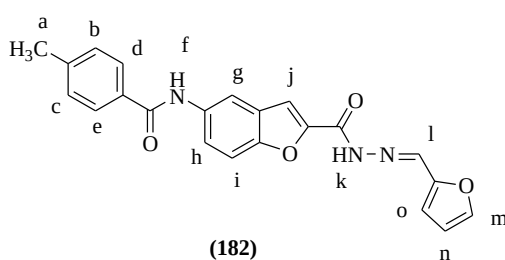
Scheme-14

In the final step of synthesis, various substituted aldehydes and ketones were refluxed in presence of K₂CO₃ to produce Schiff base derivatives of 5-(4-methylbenzamido)-N'-(substituted)benzofuran-2-carbohydrazide derivatives (**181-199**) as per scheme-14.

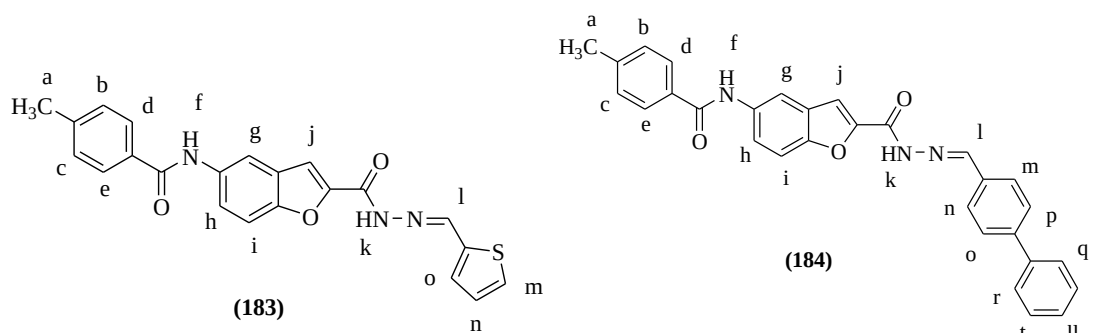


Compound **(181)** in its IR spectra reported NH stretching peaks at 3321 and 3399 cm^{-1} along with C=O of amide and carbonylhydrazide at 1678 and 1657 cm^{-1} . Its PMR spectra exhibited NH protons at δ 12.34 (s, 1H_k) and 10.32 (s, 1H_f), benzylidene proton at 8.50 (s, 1H_l), followed by aromatic protons (b,c,d,e,g,h,i,j,m,n,o,p) at 8.34 (s, 1H), 7.94 – 7.90 (m, 3H), 7.80 (d, J = 8.6 Hz, 2H), 7.75 (d, J = 7.8 Hz, 1H), 7.68 (dd, J = 17.3, 7.9 Hz, 2H), 7.44 (d, J = 7.9 Hz, 1H), 7.36 (d, J = 7.9 Hz, 2H) and aliphatic methyl protons at 2.40 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 476.4 $[\text{M}+\text{H}]^+$ along with 478 $[\text{M}+2\text{H}]^+$ which represent presence of bromine.

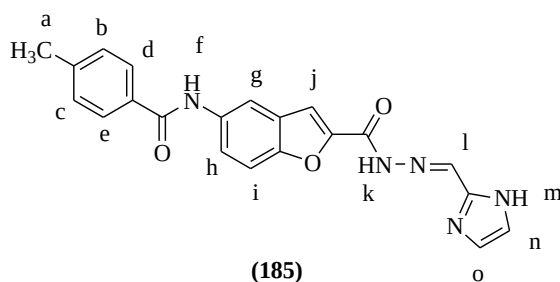
Compound **(182)** in its IR spectra reported NH stretching peaks at 3247 cm^{-1} along with C=O of amide and carbonylhydrazide at 1656 and 1644 cm^{-1} respectively. Its PMR spectra exhibited NH protons at δ 12.16 (s, 1H_k) and 10.31 (s, 1H_f), benzylidene proton at 8.41 (s, 1H_l), followed by aromatic protons (b,c,d,e,g,h,i,j,m,n,o) at 8.32 (d, J = 2.1 Hz, 1H), 7.90 (dd, J = 8.8, 7.0 Hz, 3H), 7.82 – 7.67 (m, 3H), 7.35 (d, J = 8.0 Hz, 2H), 6.97 (d, J = 3.4 Hz, 1H), 6.66 (dd, J = 3.5, 1.8 Hz, 1H), and aliphatic methyl protons at 2.40 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 388.3 $[\text{M}+\text{H}]^+$.



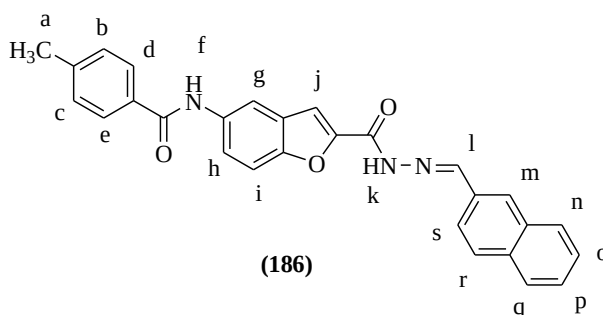
Compound **(183)** in its IR spectra reported NH stretching peaks at 3264 cm^{-1} along with C=O of amide and carbonylhydrazide at 1756 and 1645 cm^{-1} . Its PMR spectra exhibited NH protons at δ 12.18 (s, 1H_k) and 10.31 (s, 1H_f), benzylidene proton at 8.72 (s, 1H_l), followed by aromatic protons (b,c,d,e,g,h,i,j,m,n,o) at 8.31 (s, 1H), 7.95 – 7.88 (m, 2H), 7.78 (dd, J = 9.1, 2.2 Hz, 1H), 7.70 (dd, J = 12.1, 8.0 Hz, 3H), 7.49 (d, J = 3.6 Hz, 1H), 7.35 (d, J = 7.9 Hz, 2H), 7.16 (dd, J = 5.0, 3.6 Hz, 1H) and aliphatic methyl protons at 2.40 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 404.4 $[\text{M}+\text{H}]^+$.



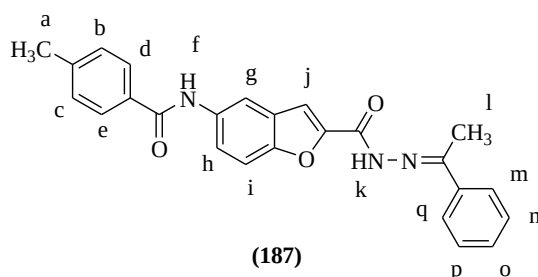
Compound **(184)** in its IR spectra reported NH stretching peaks at 3316 and 3256 cm^{-1} along with C=O of amide and carbohydrazone at 1665 and 1642 cm^{-1} . Its PMR spectra exhibited NH protons at δ 12.26 (s, 1H_k) and 10.33 (s, 1H_f), benzylidene proton at 8.56 (s, 1H_l), followed by aromatic protons (b,c,d,e,g,h,i,j,m,n,o,p,q,r,s,t,u) at 8.31 (s, 1H), 7.92 (d, $J = 7.9$ Hz, 2H), 7.83 (d, $J = 8.1$ Hz, 2H), 7.79 – 7.72 (m, 6H), 7.68 (d, $J = 9.0$ Hz, 1H), 7.50 (t, $J = 7.6$ Hz, 2H), 7.41 (d, $J = 7.4$ Hz, 1H), 7.35 (d, $J = 7.9$ Hz, 2H) and aliphatic methyl protons at 2.40 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 474.4 $[\text{M}+\text{H}]^+$.



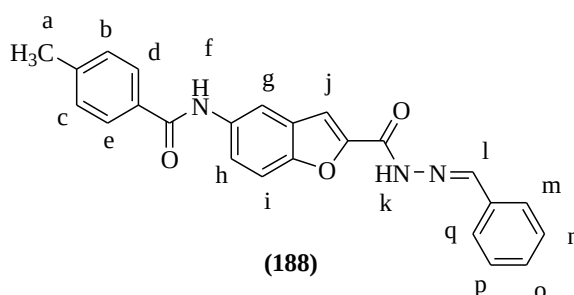
Compound **(185)** in its IR spectra reported NH stretching peaks at 3309 and 3236 cm^{-1} along with C=O of amide and carbohydrazone at 1663 and 1640 cm^{-1} . Its PMR spectra exhibited NH protons at δ 12.87 (s, 1H_m), 12.30 (s, 1H_k) and 10.31 (s, 1H_f) benzylidene proton at 8.33 (s, 1H) and aromatic protons (b,c,d,e,g,h,i,j,n,o) at 7.95 – 7.87 (m, 2H), 7.83 – 7.67 (m, 3H), 7.35 (d, $J = 7.9$ Hz, 2H), 7.11 (s, 2H) and aliphatic methyl protons at 2.39 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 388.2 $[\text{M}+\text{H}]^+$.



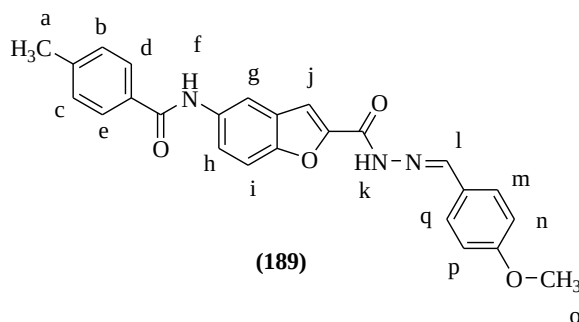
Compound **(186)** in its IR spectra reported NH stretching peaks at 3329 and 3267 cm^{-1} along with C=O of amide and carbohydrazide at 1664 and 1648 cm^{-1} . Its PMR spectra exhibited NH protons at δ 12.30 (s, 1H_k) and 10.32 (s, 1H_f) benzylidene proton at 8.70 (s, 1H) followed aromatic protons (b,c,d,e,g,h,i,j,l,m,n,o,p,q,r,s) at 8.34 (s, 1H), 8.18 (s, 1H), 8.04 (dt, $J = 7.0$, 3.5 Hz, 1H), 8.00 (s, 2H), 7.97 (dd, $J = 6.1$, 3.5 Hz, 1H), 7.92 (d, $J = 7.8$ Hz, 2H), 7.79 (s, 2H), 7.72 (d, $J = 9.0$ Hz, 1H), 7.59 (dt, $J = 6.3$, 3.5 Hz, 2H), 7.36 (d, $J = 7.8$ Hz, 2H), and aliphatic methyl protons at 2.40 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 448.3 $[\text{M}+\text{H}]^+$.



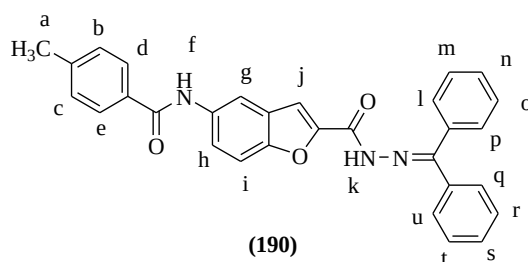
Compound **(187)** in its IR spectra reported NH stretching peaks at 3333 cm^{-1} along with C=O of amide and carbohydrazide at 1676 and 1644 cm^{-1} . Its PMR spectra exhibited NH protons at δ 10.95 (s, 1H_k) and 10.32 (s, 1H_f) followed aromatic protons (b,c,d,e,g,h,i,j,m,n,o,p,q) at 8.35 – 8.30 (m, 1H), 7.90 (dd, $J = 11.3$, 5.7 Hz, 4H), 7.82 – 7.77 (m, 2H), 7.71 (d, $J = 9.0$ Hz, 1H), 7.47 (dd, $J = 5.5$, 2.1 Hz, 3H), 7.36 (d, $J = 7.8$ Hz, 2H) and aliphatic methyl protons at 2.41 (d, $J = 8.3$ Hz, 6H_{a,l}). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 412.2 $[\text{M}+\text{H}]^+$.



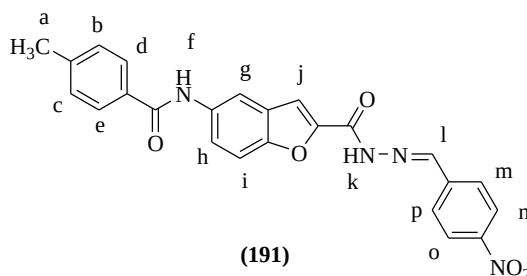
Compound **(188)** in its IR spectra reported NH stretching peaks at 3243, 3288 cm^{-1} along with C=O of amide and carbohydrazide at 1660 and 1637 cm^{-1} . Its PMR spectra exhibited NH protons at δ 12.32 (s, 1H_k) and 10.42 (s, 1H_f) benzylidene proton at 8.65 (s, 1H_l) followed aromatic protons (b,c,d,e,g,h,i,j,m,n,o,p,q) at 8.44 (s, 1H), 7.94 (d, $J = 62.3$ Hz, 7H), 7.52 (d, 5H) and aliphatic methyl protons at 2.61 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 398.2 $[\text{M}+\text{H}]^+$.



Compound **(189)** in its IR spectra reported NH stretching peaks at 3290, 3351 cm^{-1} along with C=O of amide and carbonyl at 1664 and 1650 cm^{-1} . Its PMR spectra exhibited NH protons at δ 12.05 (s, 1H_k) and 10.31 (s, 1H_f) benzylidene proton at 8.47 (s, 1H_l) followed aromatic protons (b,c,d,e,g,h,i,j,m,n,p,q) at 8.32 (d, J = 2.1 Hz, 1H), 7.91 (d, J = 7.9 Hz, 2H), 7.78 (dd, J = 8.9, 2.1 Hz, 1H), 7.74 (s, 2H), 7.69 (d, J = 8.2 Hz, 2H), 7.35 (d, J = 7.9 Hz, 2H), 7.04 (d, J = 8.5 Hz, 2H), and aliphatic methyl protons at 3.82 (s, 3H_o), 2.40 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 428.2 $[\text{M}+\text{H}]^+$.

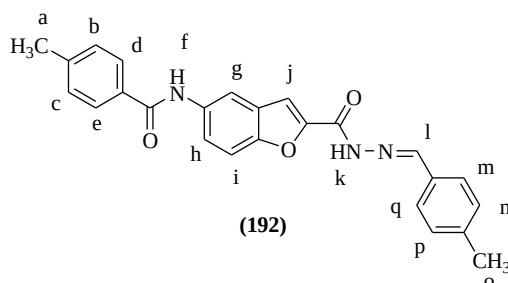


Compound **(190)** in its IR spectra reported NH stretching peaks at 3340 cm^{-1} along with C=O at 1667 cm^{-1} . Its PMR spectra exhibited NH protons at δ 10.29 (s, 1H_f), 9.98 (s, 1H_k) followed aromatic protons (b,c,d,e,g,h,i,j,l,m,n,o,p,q,r,s,t,u) at 8.27 (d, J = 17.1 Hz, 1H), 7.90 (t, J = 8.1 Hz, 2H), 7.71 (dd, J = 21.3, 4.7 Hz, 5H), 7.60 – 7.51 (m, 3H), 7.47 (s, 5H), 7.34 (d, J = 7.7 Hz, 2H) and aliphatic methyl protons at 2.40 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 474.2 $[\text{M}+\text{H}]^+$.

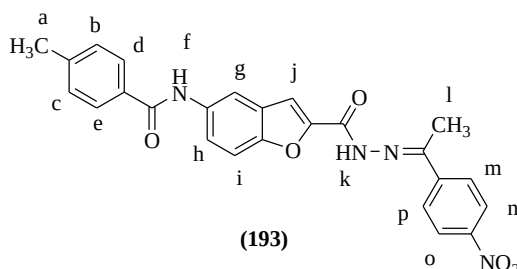


Compound **(191)** in its IR spectra reported C=O at 1659 cm^{-1} . Its PMR spectra exhibited NH protons at δ 12.48 (s, 1H_k), 10.32 (s, 1H_f) benzylidene proton at 8.63 (s, 1H_l)

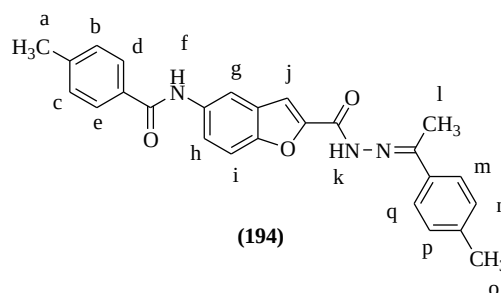
followed aromatic protons (b,c,d,e,g,h,i,j,m,n,o,p) at 8.32 (d, $J = 8.8$ Hz, 3H), 8.01 (d, $J = 8.4$ Hz, 2H), 7.91 (d, $J = 7.8$ Hz, 2H), 7.80 (d, $J = 11.3$ Hz, 2H), 7.71 (d, $J = 9.1$ Hz, 1H), 7.35 (d, $J = 7.7$ Hz, 2H), and aliphatic methyl protons at 2.40 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 443.1 $[M+H]^+$.



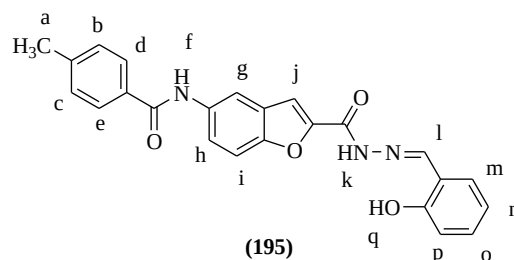
Compound **(192)** in its IR spectra reported C=O at 1659 cm^{-1} . Its PMR spectra exhibited NH protons at δ 12.13 (s, 1H_k), 10.32 (s, 1H_f) benzylidene proton at 8.50 (s, 1H_i) followed aromatic protons (b,c,d,e,g,h,i,j,m,n,p,q) at 8.33 (s, 1H), 7.91 (d, $J = 7.9$ Hz, 2H), 7.81 – 7.74 (m, 2H), 7.69 (d, $J = 8.9$ Hz, 1H), 7.64 (d, $J = 7.8$ Hz, 2H), 7.36 (d, $J = 7.8$ Hz, 2H), 7.29 (d, $J = 7.8$ Hz, 2H) and aliphatic methyl protons at 2.38 (d, $J = 15.6$ Hz, 6H_{a,o}). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 412.2 $[M+H]^+$.



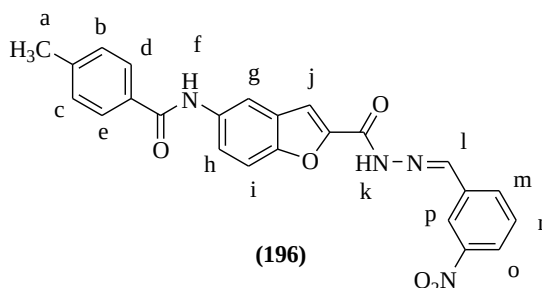
Compound **(193)** in its IR spectra reported NH stretching peaks at 3362 cm^{-1} along with C=O at 1662 cm^{-1} . Its PMR spectra exhibited NH protons at δ 11.13 (s, 1H_k), 10.32 (s, 1H_f) followed aromatic protons (b,c,d,e,g,h,i,j,m,n,o,p) at 8.37 – 8.28 (m, 3H), 8.12 (d, $J = 8.5$ Hz, 2H), 7.95 – 7.86 (m, 3H), 7.79 (dd, $J = 9.0, 2.2$ Hz, 1H), 7.72 (d, $J = 8.9$ Hz, 1H), 7.35 (d, $J = 7.9$ Hz, 2H) and aliphatic methyl protons at 2.48 (s, 3H_i), 2.40 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 457.2 $[M+H]^+$.



Compound **(194)** in its IR spectra reported NH stretching peaks at 3333 cm^{-1} along with C=O carbonyl stretching of amide and carbohydrazide at 1674 and 1644 cm^{-1} . Its PMR spectra exhibited NH protons at δ 10.88 (s, 1H_k), 10.30 (s, 1H_f) followed aromatic protons (b,c,d,e,g,h,i,j,m,n,p,q) at 8.32 (s, 1H), 7.90 (s, 2H), 7.74 (d, $J = 21.7\text{ Hz}$, 5H), 7.37 – 7.28 (m, 4H) and aliphatic methyl protons at 2.40 (s, 9H_{a,l,o}). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 426.2 $[M+H]^+$.

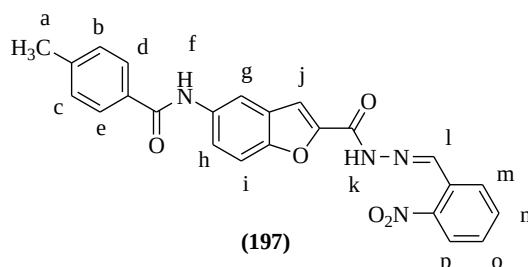


Compound **(195)** in its IR spectra reported C=O at 1601 and 1637 cm^{-1} . Its PMR spectra exhibited NH protons at δ 13.10 (s, 1H_k), 10.25 (s, 1H_f) benzylidene proton at 8.53 (s, 1H_l) followed aromatic protons (b,c,d,e,g,h,i,j,m,n,o,p) at 8.21 (s, 1H), 7.91 (d, $J = 7.8\text{ Hz}$, 2H), 7.69 (d, $J = 8.9\text{ Hz}$, 1H), 7.61 (d, $J = 8.7\text{ Hz}$, 1H), 7.45 (s, 1H), 7.37 (dd, $J = 15.0, 7.6\text{ Hz}$, 3H), 7.19 (t, $J = 8.2\text{ Hz}$, 1H), 6.86 – 6.78 (m, 2H) and aliphatic methyl protons at 2.39 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 414.2 $[M+H]^+$.

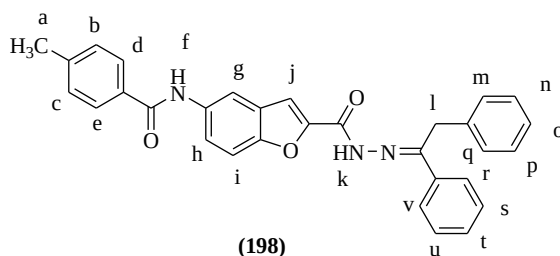


Compound **(196)** in its IR spectra showed NH stretching peaks at 3288 cm^{-1} along with C=O at 1604 and 1657 cm^{-1} . Its PMR spectra exhibited NH protons at δ 12.46 (s, 1H_k), 10.32 (s, 1H_f) benzylidene proton at 8.64 (s, 1H_l) followed aromatic protons (b,c,d,e,g,h,i,j,m,n,o,p)

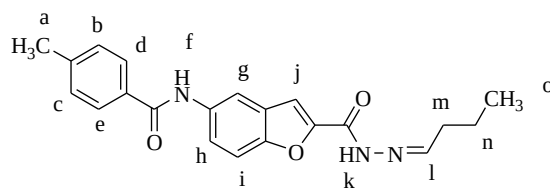
at 8.55 (t, $J = 2.0$ Hz, 1H), 8.34 (s, 1H), 8.27 (dd, $J = 8.3, 2.4$ Hz, 1H), 8.17 (d, $J = 7.7$ Hz, 1H), 7.91 (d, $J = 7.9$ Hz, 2H), 7.83 – 7.72 (m, 3H), 7.70 (d, $J = 9.0$ Hz, 1H), 7.35 (d, $J = 7.9$ Hz, 2H) and aliphatic methyl protons at 2.39 (s, 3H_a). Mass spectra of the compound produced quasi-molecular ion peak at (m/z) 443.4 ($M+H$)⁺.



Compound **(197)** in its IR spectra showed NH stretching peaks at 3243, 3300 cm^{-1} along with C=O at 1638, 1660 cm^{-1} . Its PMR spectra exhibited NH protons at δ 12.55 (s, 1H_k), 10.32 (s, 1H_f) benzylidene proton at 8.95 (s, 1H_l) followed aromatic protons (b,c,d,e,g,h,i,j,m,n,o,p) at 8.37 – 8.32 (m, 1H), 8.18 – 8.07 (m, 2H), 7.94 – 7.77 (m, 5H), 7.75 – 7.66 (m, 2H), 7.36 (d, $J = 7.9$ Hz, 2H) and aliphatic methyl protons at 2.40 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 443.4 [$M+H$]⁺.



Compound **(198)** in its IR spectra showed NH stretching peaks at 3349, 3368 cm^{-1} along with C=O at 1647 cm^{-1} . Its PMR spectra exhibited NH protons at δ 11.26 (s, 1H_k), 10.30 (s, 1H_f) followed aromatic protons (b,c,d,e,g,h,i,j,m,n,o,p,q,r,s,t,u,v) at 8. 8.26 (s, 1H), 7.94 – 7.83 (m, 4H), 7.78 – 7.72 (m, 1H), 7.70 – 7.60 (m, 2H), 7.37 (dd, $J = 15.2, 7.7$ Hz, 5H), 7.29 (q, $J = 6.2$ Hz, 4H), 7.20 – 7.15 (m, 1H) and aliphatic methyl protons at 4.45 (s, 2H_l), 2.40 (s, 3H_a). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 488.2 [$M+H$]⁺.



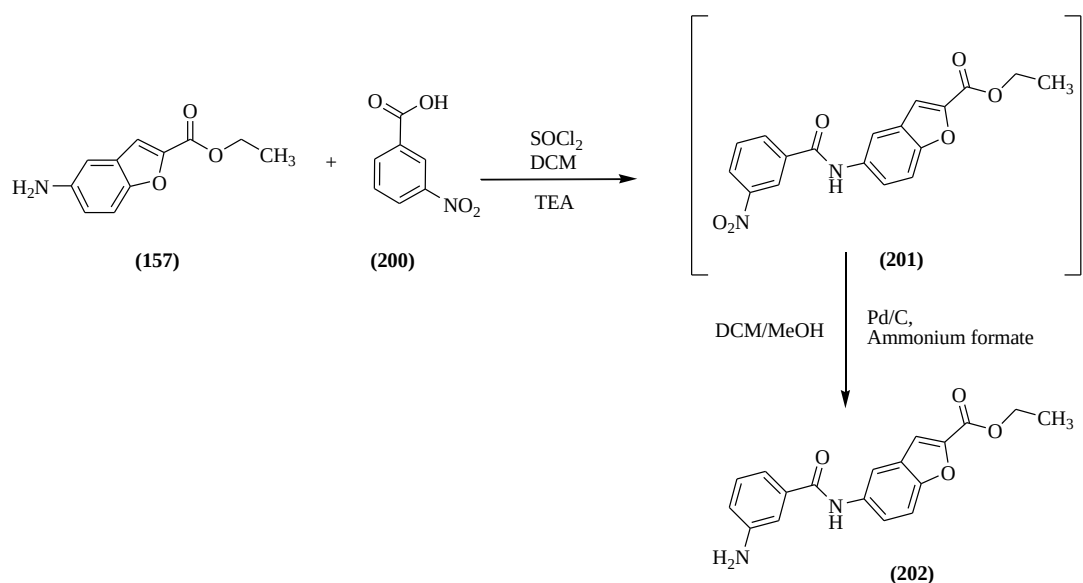
(199)

Compound **(199)** in its IR spectra showed NH stretching peaks at 3301 cm^{-1} along with C=O at 1659 cm^{-1} . Its PMR spectra exhibited NH protons at δ 11.76 (s, 1H_k), 10.30 (s, 1H_f) benzylidene proton at 8.30 (s, 1H_i) followed aromatic protons (b,c,d,e,g,h,i,j) at 7.91 (d, $J = 7.7\text{ Hz}$, 2H), 7.80 (dd, $J = 24.0, 7.3\text{ Hz}$, 2H), 7.66 (d, $J = 10.0\text{ Hz}$, 2H), 7.35 (d, $J = 7.8\text{ Hz}$, 2H) and aliphatic methyl protons at 2.40 (s, 3H_a), 2.26 (q, $J = 6.9\text{ Hz}$, 2H_m), 1.54 (p, $J = 7.4\text{ Hz}$, 2H_n), 0.94 (t, $J = 7.3\text{ Hz}$, 3H_o). Mass spectra of the compound displayed quasi-molecular ion peak at m/z 364.2 $[M+H]^+$.

5.1.2.6. Synthesis of ethyl 5-((3-amino)benzamido)benzofuran-2-carboxylate (**202**)

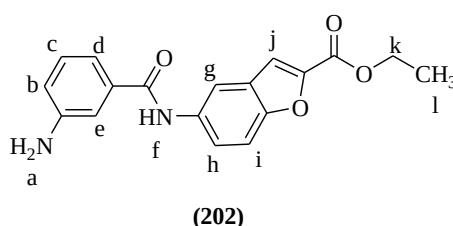
Ethyl 5-aminobenzofuran-2-carboxylate (**157**) as shown in **scheme-15** was reacted with 3-nitrobenzoic acid (**200**) after producing acid chloride to obtain intermediate ethyl 5-((3-nitro)benzamido)benzofuran-2-carboxylate (**201**) which was further reduced to ethyl 5-((3-amino)benzamido)benzofuran-2-carboxylate (**202**).

The compound was characterized by IR spectra in which NH stretching was reported at 3408 and 3330 cm^{-1} along with C=O carbonyl stretching peaks at 1710 and 1639 cm^{-1} . In its mass spectrum, quasi-molecular ion peak was observed at m/z 325.08 $[M+H]^+$.



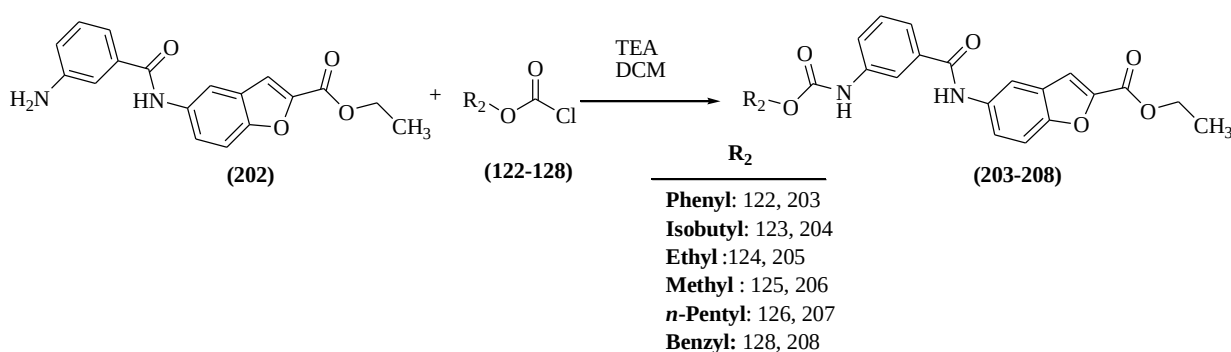
Scheme-15

Its PMR spectrum exhibited signals for secondary amine at δ 10.19 (s, 1H_f) and for primary amine at δ 5.29 (s, 2H_a). Aromatic protons observed at δ 7.07-7.05 (t, 2H_{d, c}) and δ 6.72-6.71 (d, 1H_b, $J = 5.4$ Hz). While singlet observed at δ 7.12 (s, 1H_e). Aromatic protons of benzofuran ring observed at δ 8.28 (s, 1H_g) also, aromatic protons of benzofuran ring observed at δ 7.74-7.72 (d, 2H_{h, i}) and aromatic proton observed at δ 7.66 (s, 1H_j) as a singlet of benzofuran ring. Among aliphatic protons, protons of ethyl group displayed at δ 4.32-4.31 (d, 2H_k, $J = 7.1$ Hz) and singlet at δ 1.29 (s, 3H_l).



5.1.2.7. Synthesis of ethyl 5-((3-substituted carbamate)benzamido)benzofuran-2-carboxylate (203-208)

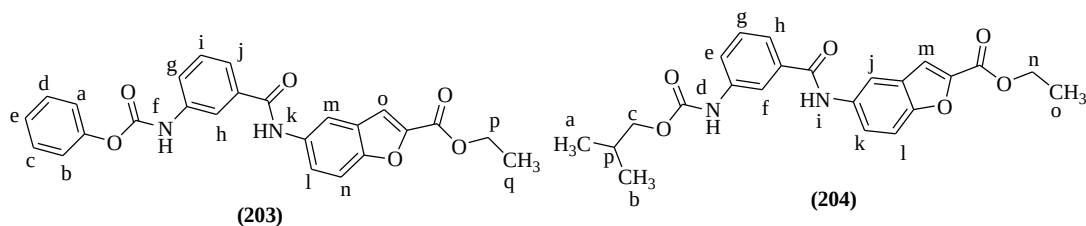
Various substituted chloroformate derivatives (122-128) were treated with ethyl 5-((3-amino)benzamido)benzofuran-2-carboxylate (202) as per **scheme-16** in the presence of triethyl amine and dichloromethane at room temperature by the nucleophilic substitution (S_N2) to obtained ethyl 5-((3-substituted carbamate)benzamido)benzofuran-2-carboxylated derivatives (203-208).



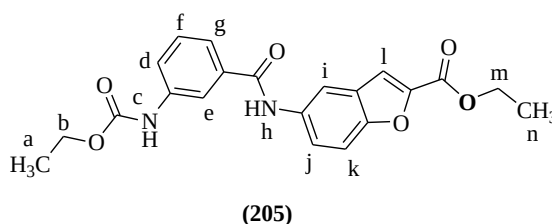
Scheme-16

Compound (203) in spectral analysis of IR showed characteristic peaks for NH stretching at 3321 cm⁻¹, 3142 cm⁻¹ for aromatic C-H stretching, characteristic peaks of C=O carbonyl stretching at 1746 cm⁻¹ for carbamate, C=O carbonyl stretching at 1706 cm⁻¹ for ester and C=O carbonyl stretching at 1647 cm⁻¹ for amide along with peak at 1189 cm⁻¹ for aromatic C-O stretching. Its PMR spectrum exhibited signals for secondary amines at δ 10.45

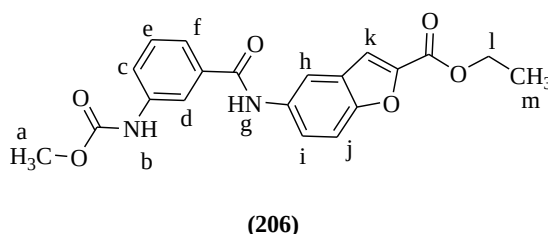
(s, 1H_k) and 10.43 (s, 1H_f). Aromatic protons at δ 7.80-7.78 (d, 2H_{g, j}) exhibited doublet and triplet at δ 7.52-7.48 (t, 1H_i). While singlet observed at δ 8.33 (s, 1H_h). Aromatic protons of benzofuran ring observed at δ 8.09 (s, 1H_m) also, aromatic protons of benzofuran ring observed at δ 7.73 (bs, 1H_{l, o}) and doublet of aromatic proton observed at δ 7.68-7.66 (d, 1H_n) of benzofuran ring. While triplet observed at δ 7.46-7.43 (t, 2H_{c, d}) and multiplet at δ 7.29-7.24 (m, 3H_{a, b, e}) of the phenyl ring of carbamate moiety. Among aliphatic protons, protons of ethyl group displayed multiplets at δ 4.37-4.36 (m, 2H_p) and triplet at δ 1.36-1.33 (t, 3H_q). The mass spectrum of compound (**203**) displayed the peak at m/z 445.25 [M+H]⁺.



IR spectral data of compound (**204**) showed the characteristic peaks for NH stretching at 3330 cm⁻¹, characteristic peaks of C=O carbonyl stretching at 1730 cm⁻¹ for carbamate, peak of C=O carbonyl stretching at 1696 cm⁻¹ for ester and C=O carbonyl stretching at 1641 cm⁻¹ for amide along with peak at 1218 cm⁻¹ for aromatic C-O stretching. Its PMR spectrum exhibited signals for secondary amines at δ 10.39 (s, 1H_i) and 9.84 (s, 1H_d). Aromatic protons at δ 8.34-8.33 (d, 1H_e, J = 2.2 Hz) exhibited *ortho* coupling with protons observed at δ 7.47-7.43 (t, 1H_g, J = 7.9 Hz) and δ 7.78-7.77 (d, 1H_h, J = 2.3 Hz). While singlet observed at δ 8.04 (s, 1H_j). Aromatic protons of benzofuran ring observed at δ 7.81 (s, 1H_j, J = 2.2 Hz) also, an aromatic proton of benzofuran ring observed at δ 7.73-7.72 (d, 1H_k, J = 9 Hz) exhibited *ortho* coupling with *meta* coupling proton observed at δ 7.60-7.58 (d, 1H_l, J = 7.9 Hz) and aromatic proton observed at δ 7.68 (s, 1H_m) as a singlet of benzofuran ring. Among aliphatic protons, protons of ethyl group displayed multiplets at δ 4.40-4.35 (m, 2H_n, J = 7.1 Hz) and triplet at δ 1.37-1.33 (t, 3H_o, J = 7.1 Hz) and protons of isobutyl chain displayed doublet at δ 3.91-3.90 (d, 2H_c, J = 6.7 Hz). Multiplets observed at δ 2.45-2.41 (m, 1H_p, J = 8.1 Hz) and doublets of methyl groups of isobutyl chain observed at δ 0.96-0.94 (d, 6H_{a, b}, J = 6.7 Hz). In its mass spectrum [M+H]⁺ quasi-molecular ion peak was observed at 425.16 m/z .

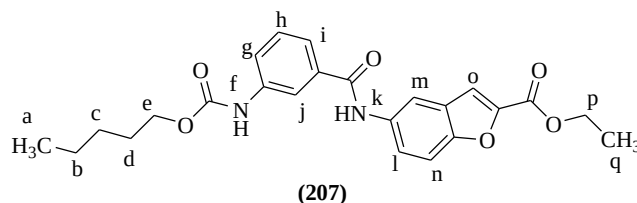


Compound (**205**) in spectral analysis of IR showed characteristic peaks for NH stretching at 3324 cm^{-1} , 3086 cm^{-1} for aromatic C-H stretching, characteristic peaks of C=O carbonyl stretching at 1731 cm^{-1} for carbamate, C=O carbonyl stretching at 1690 cm^{-1} for ester and C=O carbonyl stretching at 1658 cm^{-1} for amide along with peak at 1230 cm^{-1} for aromatic C-O stretching. Its PMR spectrum exhibited signals for secondary amines at δ 10.39 (s, 1H_h) and 9.83 (s, 1H_c). Aromatic protons at δ 8.33-8.32 (d, 1H_d , $J = 2.2\text{ Hz}$), singlet observed at δ 8.03 (s, 1H_e). An aromatic proton of benzofuran ring observed at δ 7.81 (s, 1H_i) also, aromatic proton of benzofuran ring observed at δ 7.78-7.77 (d, 1H_j , $J = 9\text{ Hz}$), 7.76-7.72 (d, 1H_k , $J = 8\text{ Hz}$), 7.67 (s, 1H_l) and 7.46-7.42 (t, $J = 7.9\text{ Hz}$, 1H_f). Among aliphatic protons, protons of ethyl group displayed multiplets at δ 4.40-4.34 (m, 2H_m , $J = 7.1\text{ Hz}$) and triplet at δ 1.37-1.33 (t, 3H_n , $J = 7.1\text{ Hz}$) and protons of ethyl group of carbamate moiety displayed multiplets at δ 4.18-4.13 (m, 2H_b , $J = 7.1\text{ Hz}$) and triplet at δ 1.28-1.24 (t, 3H_a , $J = 7.1\text{ Hz}$). The mass spectrum of compound displayed the peak at m/z 397.13 $[\text{M}+\text{H}]^+$.

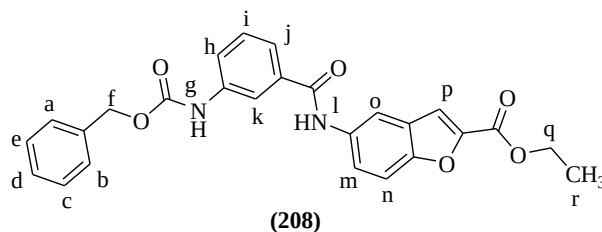


IR spectral data of compound (**206**) showed the characteristic peaks for NH stretching at 3363 cm^{-1} , 3141 cm^{-1} for aromatic C-H stretching, characteristic peaks of C=O carbonyl stretching at 1705 cm^{-1} for ester and C=O carbonyl stretching at 1652 cm^{-1} for amide along with peak at 1244 cm^{-1} for aromatic C-O stretching. Its PMR spectrum exhibited signals for secondary amines at δ 10.39 (s, 1H_g) and 9.86 (s, 1H_b). Aromatic protons at δ 8.33-8.32 (d, 1H_c, J = 2.3 Hz) exhibited *ortho* coupling with protons observed at δ 7.47-7.43 (t, 1H_e, J = 7.9 Hz) and δ 7.78-7.77 (d, 1H_f, J = 2.2 Hz). While singlet observed at δ 8.01 (s, 1H_d). Aromatic protons of benzofuran ring observed at δ 7.81 (s, 1H_h) also, aromatic protons of benzofuran ring observed at δ 7.73-7.71 (d, 1H_i, J = 9 Hz) exhibited *ortho* coupling with *meta* coupling proton observed at δ 7.61-7.59 (d, 1H_j, J = 8 Hz) and aromatic proton observed at δ

7.68 (s, 1H_k) as a singlet of benzofuran ring. Among aliphatic protons, protons of ethyl group displayed multiplets at δ 4.40-4.35 (m, 2H_l, J = 7.1 Hz) and triplet at δ 1.37-1.33 (t, 3H_m, J = 7.1 Hz) and protons of methyl group of carbamate moiety displayed singlet at δ 3.70 (s, 3H_a). In its mass spectrum $[M+H]^+$ quasi-molecular ion peak was observed at 383.20 m/z.



Compound (207) in spectral analysis of IR showed characteristic peaks for NH stretching at 3345 cm⁻¹, 2954 cm⁻¹ for aromatic C-H stretching, characteristic peaks of C=O carbonyl stretching at 1731 cm⁻¹ for carbamate, C=O carbonyl stretching at 1706 cm⁻¹ for ester and C=O carbonyl stretching at 1646 cm⁻¹ for amide along with peak at 1223 cm⁻¹ for aromatic C-O stretching. Its PMR spectrum exhibited signals for secondary amines at δ 10.38 (s, 1H_k) and 9.82 (s, 1H_f). Aromatic protons at δ 8.33-8.32 (d, 1H_g, J = 2.2 Hz) exhibited *ortho* coupling with protons observed at δ 7.46-7.42 (t, 1H_h, J = 7.9 Hz) and δ 7.78-7.77 (d, 1H_i, J = 2.2 Hz). While singlet observed at δ 8.03 (s, 1H_j). Aromatic protons of benzofuran ring observed at δ 7.81 (s, 1H_m) also, an aromatic proton of benzofuran ring observed at δ 7.73-7.70 (d, 1H_l, J = 9.1 Hz) exhibited *ortho* coupling with *meta* coupling proton observed at δ 7.60-7.58 (d, 1H_n, J = 7.9 Hz) and aromatic proton observed at δ 7.67 (s, 1H_o) as a singlet of benzofuran ring. Among aliphatic protons, protons of ethyl group displayed multiplets at δ 4.40-4.34 (m, 2H_p, J = 7.1 Hz) and protons of pentyl chain displayed doublet at δ 4.12-4.08 (t, 2H_e, J = 6.6 Hz) also, multiplet observed at δ 1.66-1.60 (m, 2H_d, J = 6.9 Hz) and triplet observed at δ 0.91-0.88 (t, 3H_a). While multiplet observed at δ 1.36-1.33 (m, 7H_{q, b, c}). The mass spectrum of compound displayed the peak at m/z 439.27 $[M+H]^+$.



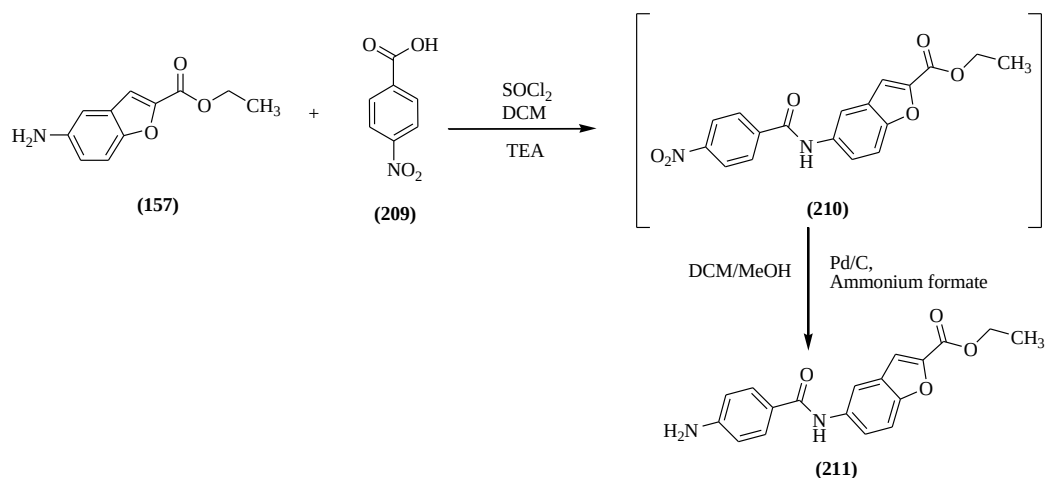
IR spectral data of compound (208) showed the characteristic peaks for NH stretching at 3319 cm⁻¹, 3105 cm⁻¹ for aromatic C-H stretching, characteristic peaks of C=O carbonyl stretching at 1730 cm⁻¹ for Carbamate, peak of C=O carbonyl stretching at 1696 cm⁻¹ for ester

and C=O carbonyl stretching at 1643 cm^{-1} for amide along with peak at 1220 cm^{-1} for aromatic C-O stretching. Its PMR spectrum exhibited signals for secondary amines at δ 10.39 (s, $1H_l$) and 9.99 (s, $1H_g$). Aromatic protons at δ 7.80-7.78 (d, $1H_h$) exhibited *ortho* coupling with protons observed at δ 7.77-7.76 (d, $1H_j$). While singlet observed at δ 8.32 (s, $1H_k$). Aromatic protons of benzofuran ring observed at δ 8.04 (s, $1H_o$) also, an aromatic proton of benzofuran ring observed at δ 7.73-7.70 (d, $1H_m$). Doublet of aromatic proton observed at δ 7.68-7.66 (d, $1H_n$) and singlet at δ 7.60 (s, $1H_p$) of benzofuran ring. While multiplets of aromatic protons observed at δ 7.47-7.33 (m, $6H_{a,b,c,d,e,i}$). Among aliphatic protons, protons of ethyl group displayed multiplets at δ 4.39-4.34 (m, $2H_q$) and triplet at δ 1.36-1.33 (t, $3H_r$) also, aliphatic protons of carbamate moiety observed at δ 5.18 (s, $2H_f$). In its mass spectrum $[M+H]^+$ quasi-molecular ion peak was observed at 459.21 m/z.

5.1.2.8. Synthesis of ethyl 5-((4-amino)benzamido)benzofuran-2-carboxylate (211)

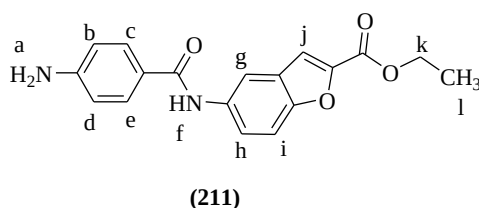
As per **scheme-17** ethyl 5-aminobenzofuran-2-carboxylate (**157**) was reacted with 4-nitrobenzoic acid (**209**) after producing acid chloride to obtain intermediate ethyl 5-((4-nitro)benzamido)benzofuran-2-carboxylate (**210**) which was further reduced to ethyl 5-((4-amino)benzamido)benzofuran-2-carboxylate (**211**).

IR spectral data of compound (**211**) showed the characteristic peaks for NH_2 stretching at 3394 cm^{-1} , for NH stretching peak at 3318 cm^{-1} , 3130 cm^{-1} for aromatic C-H stretching, characteristic peaks of C=O carbonyl stretching at 1706 cm^{-1} for ester and C=O carbonyl stretching at 1658 cm^{-1} for amide along with peak at 1222 cm^{-1} for aromatic C-O stretching. In its mass spectrum $[M+H]^+$ quasi-molecular ion peak was observed at 325.11 m/z.



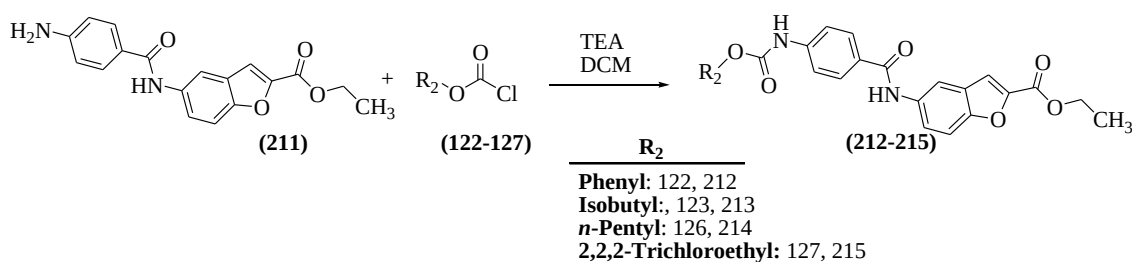
Scheme-17

PMR spectrum of **(211)** exhibited signals for secondary amines at δ 9.92 (s, 1H_f) and for primary amine at δ 5.57 (s, 2H_a). Aromatic protons at δ 7.79-7.78 (d, 1H_c, J = 2.2 Hz) exhibited *ortho* coupling with protons observed at δ 6.62-6.60 (d, 2H_{b, d}, J = 8.7 Hz). While aromatic proton observed at δ 7.78-7.76 (d, 2H_{e, h}). Aromatic proton of benzofuran ring observed at δ 8.30 (s, 1H_g) also, aromatic protons of benzofuran ring observed at δ 7.68-7.66 (d, 1H_i, J = 9.1 Hz) and δ 7.73 (s, 1H_j, J = 8.9 Hz). Among aliphatic protons, protons of ethyl group displayed multiplets at δ 4.39-4.34 (m, 2H_k, J = 7.1 Hz) and triplet observed at δ 1.35-1.33 (t, 3H_l). Mass spectra produced quasi-molecular ion peak at (m/z) 325.11 [M+H]⁺.



5.2.1.9. Synthesis of ethyl 5-((4-substituted carbamate)benzamido)benzofuran-2-carboxylate (**212-215**)

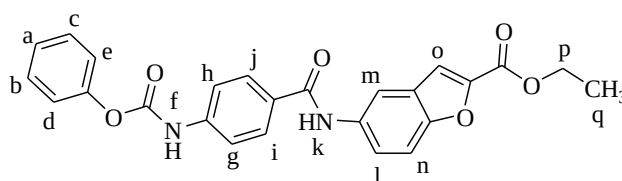
Various substituted chloroformate derivatives (**122-127**) were treated with ethyl 5-((4-amino)benzamido)benzofuran-2-carboxylate (**211**) as per **scheme-18** in the presence of triethyl amine and dichloromethane at room temperature by the nucleophilic substitution (S_N2) to obtained ethyl 5-((3-substituted carbamate)benzamido)benzofuran-2-carboxylated derivatives (**212-215**).



Scheme-18

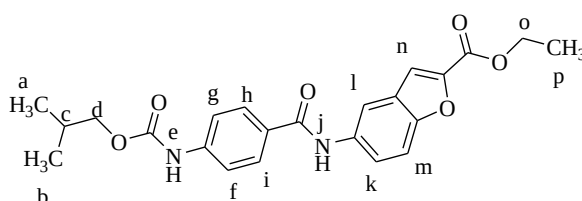
Compound **(212)** in spectral analysis of IR showed characteristic peaks for NH stretching at 3377 cm⁻¹, 3146 cm⁻¹ for aromatic C-H stretching, characteristic peaks of C=O carbonyl stretching at 1756 cm⁻¹ for carbamate, C=O carbonyl stretching at 1698 cm⁻¹ for ester and C=O carbonyl stretching at 1655 cm⁻¹ for amide along with peak at 1199 cm⁻¹ for aromatic C-O stretching. PMR spectrum exhibited signals for secondary amines at δ 10.58 (s, 1H_k) and 10.29 (s, 1H_f). Aromatic protons at δ 8.36-8.32 (d, 2H_{i, j}, J = 15.1 Hz) exhibited *ortho* coupling with protons observed at δ 7.80-7.78 (d, 3H_{g, h, m}, J = 7.3 Hz). Similarly, an

ortho coupling was observed between aromatic protons showing doublets at δ 8.00-7.98 (d, $1H_l$, $J = 8.8$ Hz). A singlet appeared at δ 7.73 (s, $1H_o$) for aromatic proton of benzofuran ring. Aromatic proton at δ 7.68-7.66 (d, $1H_n$, $J = 8.9$ Hz) exhibited *meta* coupling of benzofuran ring. Aromatic proton at δ 7.48-7.44 (t, $2H_{b, c}$, $J = 7.9$ Hz) exhibited *meta* coupling with proton observed at δ 7.31-7.26 (t, $3H_{a, d, e}$, $J = 10.0$ Hz). Among aliphatic protons, protons of ethyl groups displayed multiplets at δ 4.40-4.34 (m, $2H_p$) and triplet at δ 1.37-1.33 (t, $3H_q$). The mass spectrum displayed the quasi-molecular ion peak at m/z 445.18 $[M+H]^+$.

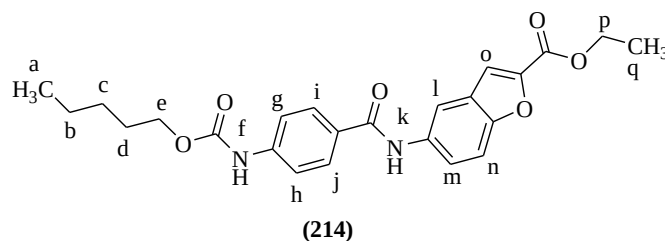


(212)

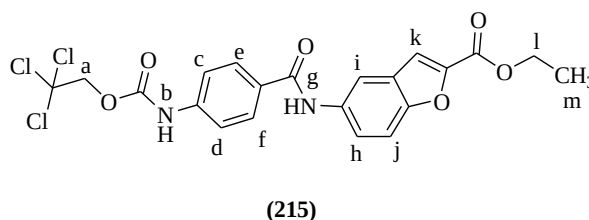
IR spectral data of compound (213) showed the characteristic peaks for NH stretching at 3326 cm^{-1} , 3111 cm^{-1} for aromatic C-H stretching, characteristic peaks of C=O carbonyl stretching at 1714 cm^{-1} for carbamate, peak of C=O carbonyl stretching at 1696 cm^{-1} for ester and C=O carbonyl stretching at 1642 cm^{-1} for amide along with peak at 1223 cm^{-1} for aromatic C-O stretching. Its PMR spectrum exhibited signals for secondary amines at δ 10.23 (s, $1H_j$) and 9.96 (s, $1H_e$). Aromatic protons at δ 8.32-8.31 (d, $2H_{i, h}$, $J = 2.2$ Hz) exhibited *ortho* coupling with protons observed at δ 7.95-7.93 (d, $2H_{g, f}$, $J = 8.9$ Hz). Similarly, an *ortho* coupling was observed at δ 7.80 (s, $1H_l$). Aromatic protons of benzofuran ring observed at δ 7.78-7.77 (d, $1H_k$, $J = 2.2$ Hz) exhibited *ortho* coupling with *meta* coupling protons observed at δ 7.63-7.60 (d, $1H_m$, $J = 8.8$ Hz). Aromatic proton observed at δ 7.72 (s, $1H_n$) as a singlet of benzofuran ring. Among aliphatic protons, protons of ethyl group displayed multiplets at δ 4.40-4.34 (m, $2H_o$, $J = 7.0$ Hz) and triplet at δ 1.36-1.33 (t, $3H_p$, $J = 7.1$ Hz) and protons of isobutyl group displayed doublet at δ 3.92-3.90 (d, $2H_d$, $J = 6.7$ Hz). Multiplets observed at δ 3.55-3.49 (m, $1H_c$) and doublets of methyl groups of isobutyl chain observed at δ 0.96-0.94 (d, $6H_{a, b}$, $J = 6.7$ Hz). In its mass spectrum $[M+H]^+$ quasi-molecular ion peak was observed at 425.16 m/z .



(213)



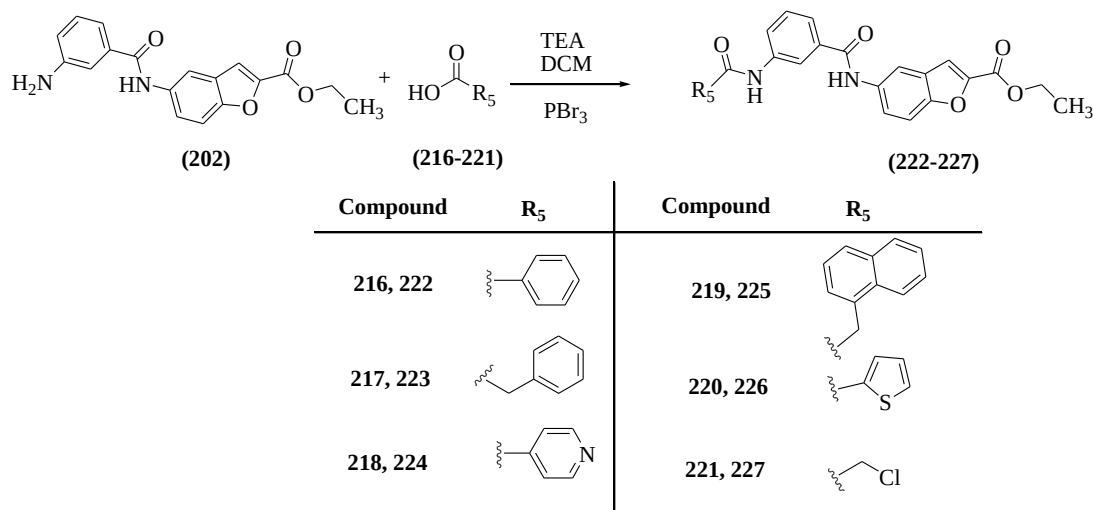
Compound **(214)** in spectral analysis of IR showed characteristic peaks for NH stretching at 3346 cm^{-1} , 3108 cm^{-1} for aromatic C-H stretching, characteristic peaks of C=O carbonyl stretching at 1732 cm^{-1} for carbamate, C=O carbonyl stretching at 1701 cm^{-1} for ester and C=O carbonyl stretching at 1647 cm^{-1} for amide along with peak at 1222 cm^{-1} for aromatic C-O stretching. Its PMR spectrum exhibited signals for secondary amines at δ 10.20 (s, $1H_k$) and 9.92 (s, $1H_n$). Aromatic protons at δ 7.90-7.88 (d, $2H_{i,j}$, $J = 8.8\text{ Hz}$) exhibited *ortho* coupling with protons observed at δ 7.75-7.73 (d, $2H_{g,h}$, $J = 9.9\text{ Hz}$). Aromatic proton of benzofuran ring observed at δ 8.27 (s, $1H_l$). Aromatic protons of benzofuran ring observed at δ 7.67-7.65 (d, $1H_m$, $J = 9.2\text{ Hz}$) exhibited *ortho* coupling with protons observed at δ 7.58-7.56 (d, $2H_{n,o}$, $J = 8.9\text{ Hz}$). Among aliphatic protons, protons of ethyl group displayed multiplets at δ 4.34-4.30 (m, $2H_p$, $J = 7.1\text{ Hz}$) and protons of pentyl chain displayed doublet at δ 4.07-4.05 (t, $2H_e$, $J = 6.6\text{ Hz}$) also, doublet observed at δ 1.59-1.60 (d, $2H_d$) and singlet observed at δ 0.85 (s, $3H_a$). While singlet observed at δ 1.30 (s, $7H_{q,b,c}$). The mass spectrum of compound displayed the quasi-molecular ion peak at m/z 439.24 $[M+H]^+$.



IR spectral data of compound **(215)** showed the characteristic peaks for NH stretching at 3344 cm^{-1} , 2984 cm^{-1} for aromatic C-H stretching, characteristic peaks of C=O carbonyl stretching at 1712 cm^{-1} for carbamate and C=O carbonyl stretching at 1640 cm^{-1} for amide along with peak at 1230 cm^{-1} for aromatic C-O stretching. Its PMR spectrum exhibited signals for secondary amines at δ 10.49 (s, $1H_g$) and 10.28 (s, $1H_b$). Aromatic protons at δ 7.99-7.97 (d, $2H_{e,f}$, $J = 8.9\text{ Hz}$) exhibited *ortho* coupling with protons observed at δ 7.80-7.78 (d, $2H_{c,d}$). Aromatic proton of benzofuran ring observed at δ 8.32 (s, $1H_i$) also, aromatic protons of benzofuran ring observed at δ 7.68-7.66 (d, $2H_{h,j}$, $J = 8.2\text{ Hz}$) and δ 7.73 (s, $1H_k$, $J = 8.9\text{ Hz}$). Among aliphatic protons, protons of ethyl group displayed multiplets at δ 4.39-4.35 (m, $2H_l$) and protons observed at δ 1.36-1.34 (t, $3H_m$). While singlet observed at δ 4.99

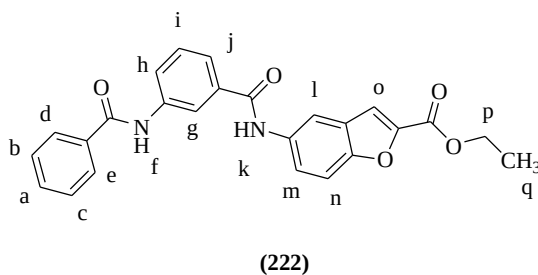
(s, 2H_a). In its mass spectrum [M+H]⁺ quasi-molecular ion peak at 499.14 m/z along with isotope peak of trichloro at 501.34 [M+2H]⁺.

5.2.1.10. Synthesis of ethyl 5-((3-substitutedamido)benzamido)benzofuran-2-carboxylate (222-227)



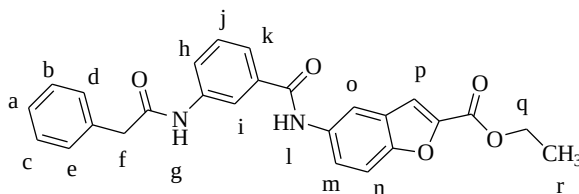
Scheme-19

Various substituted carboxylic derivatives (216-221) were converted to acid bromide using phosphorous tribromide and were coupled with ethyl 5-((3-amino)benzamido)benzofuran-2-carboxylate (222-227) as per **scheme-19** in the presence of triethyl amine and dichloromethane at room temperature by the nucleophilic substitution (S_N2) to obtained ethyl 5-((3-substituted amido)benzamido)benzofuran-2-carboxylated derivatives (222-227).



Compound (222) in its IR spectra reported NH stretching peaks at 3348 and 3297 cm⁻¹ along with amide stretching of C=O at 1728 and 1643 cm⁻¹. Its PMR exhibited signals for secondary amines at δ 10.46 (d, J = 12.3 Hz, 2H_{f,k}) followed aromatic protons at (a,b,c,d,e,g,h,i,j,l,m,n,o) 8.35 (d, J = 2.2 Hz, 2H), 8.06 – 7.99 (m, 3H), 7.80 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 9.6 Hz, 2H), 7.62 – 7.51 (m, 4H). Aliphatic protons were present at 4.37 (q, J = 7.1

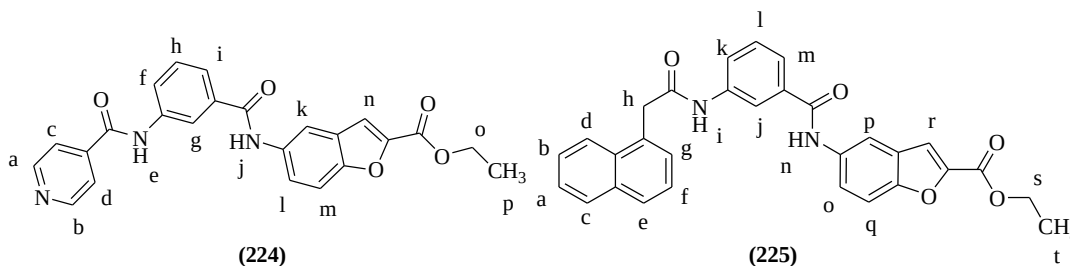
Hz, 2H_p). 1.35 (t, $J = 7.1$ Hz, 3H_q). Its mass spectra produced quasi-molecular ion peak at m/z 429.3 [M+H]⁺.



(223)

Compound (223) in its IR spectra reported NH stretching peaks at 3390 and 3263 cm⁻¹ along with amide stretching of C=O at 1711, 1680 and 1644 cm⁻¹. Its PMR exhibited signals for secondary amines at δ 10.40 (d, $J = 5.7$ Hz, 2H_{g,l}) followed by aromatic protons (a,b,c,d,e,h,i,j,k,m,n,o,p) at 8.32 (d, $J = 2.1$ Hz, 1H), 8.12 (s, 1H), 7.88 – 7.62 (m, 5H), 7.47 (t, $J = 7.9$ Hz, 1H), 7.39 – 7.23 (m, 5H). Aliphatic protons at 4.37 (q, $J = 7.1$ Hz, 2H_q), 3.67 (s, 2H_f), 1.34 (t, $J = 7.1$ Hz, 3H_r). Its mass spectra produced quasi-molecular ion peak at m/z 443.25 [M+H]⁺.

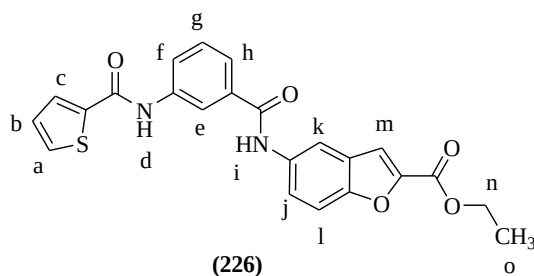
Compound (224) in its IR spectra reported NH stretching peaks at 3395 and 3265 cm⁻¹ along with amide stretching of C=O at 1715, 1688 and 1654 cm⁻¹. Its PMR exhibited signals for secondary amines at δ 10.40 (d, $J = 5.7$ Hz, 2H_{e,j}) followed by aromatic protons (a,b,c,d,f,g,h,i,k,m,n) at 8.32 (d, $J = 2.1$ Hz, 1H), 8.12 (s, 1H), 7.88 – 7.62 (m, 5H), 7.47 (t, $J = 7.9$ Hz, 1H), 7.39 – 7.23 (m, 5H). Aliphatic protons at 4.37 (q, $J = 7.1$ Hz, 2H_o), 1.34 (t, $J = 7.1$ Hz, 3H_p). Its mass spectra produced quasi-molecular ion peak at m/z 430.20 [M+H]⁺.



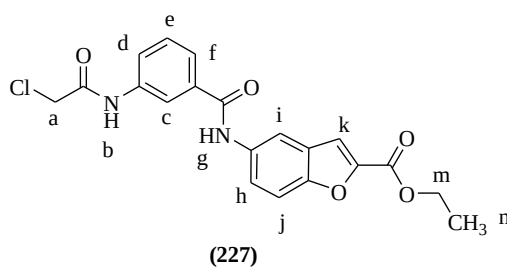
(224)

(225)

Compound (225) in its IR spectra reported amide stretching of C=O at 1677 and 1606 cm⁻¹. Its PMR exhibited signals for secondary amines at δ 10.56 (s, 1H_n), 10.41 (s, 1H_i), followed by aromatic protons (a,b,c,d,e,f,g,h,i,j,k,l,m,o,p,q,r) at 8.32 (d, $J = 2.1$ Hz, 1H), 8.16 (d, $J = 8.3$ Hz, 2H), 7.97 – 7.93 (m, 2H), 7.86 (dd, $J = 8.4, 3.9$ Hz, 2H), 7.79 (d, $J = 5.8$ Hz, 1H), 7.76 (d, $J = 2.2$ Hz, 1H), 7.53 (td, $J = 5.8, 3.8$ Hz, 4H), 7.48 – 7.44 (m, 2H). Aliphatic protons at 4.37 (t, $J = 7.1$ Hz, 2H_s), 4.19 (s, 2H_h), 1.34 (t, $J = 7.1$ Hz, 3H_t). Its mass spectra produced quasi-molecular ion peak at m/z 493.25 [M+H]⁺.



Compound **(226)** in its IR spectra reported NH stretching peak at 3308 cm^{-1} along with amide stretching of C=O at 1711 , 1651 and 1630 cm^{-1} . Its PMR exhibited signals for secondary amines at δ 10.45 (d, $J = 3.1\text{ Hz}$, $2\text{H}_{\text{d,i}}$), followed by aromatic protons (a,b,c,e,f,g,h,j,k,l,m) at 8.34 (d, $J = 2.1\text{ Hz}$, 1H), 8.27 (t, $J = 1.9\text{ Hz}$, 1H), 8.08 (dd, $J = 3.8$, 1.2 Hz , 1H), 8.01 (dd, $J = 8.4$, 2.1 Hz , 1H), 7.89 (dd, $J = 5.0$, 1.1 Hz , 1H), 7.83 – 7.78 (m, 2H), 7.75 – 7.71 (m, 2H), 7.53 (t, $J = 7.9\text{ Hz}$, 1H), 7.25 (dd, $J = 5.0$, 3.7 Hz , 1H). Aliphatic protons at 4.37 (q, $J = 7.1\text{ Hz}$, 2H_{n}), 1.35 (t, $J = 7.1\text{ Hz}$, 3H_{o}). Its mass spectra produced quasi-molecular ion peak at m/z 435.19 $[\text{M}+\text{H}]^+$.



Compound **(227)** in its IR spectra reported NH stretching peaks at 3252 cm^{-1} along with amide stretching of C=O at 1712 , 1667 and 1647 cm^{-1} . Its PMR spectra reported NH peaks at δ 10.52 (s, 1H_{g}), 10.44 (s, 1H_{b}) followed by aromatic protons at c,d,e,f,h,i,j,k 8.33 (d, $J = 2.1\text{ Hz}$, 1H), 8.12 (t, $J = 2.0\text{ Hz}$, 1H), 7.86 – 7.77 (m, 3H), 7.74 – 7.69 (m, 2H), 7.51 (t, $J = 7.9\text{ Hz}$, 1H) and aliphatic protons at 4.38 (t, $J = 7.1\text{ Hz}$, 2H_{m}), 4.29 (s, 2H_{a}), 1.34 (t, $J = 7.1\text{ Hz}$, 3H_{n}). Its mass spectra produced quasi-molecular ion peak at m/z 401.13 $(\text{M}+\text{H})^+$ and 403.17 $(\text{M}+2\text{H})^+$ representing Cl atom.

5.3. Biological activity

5.3.1. Biological studies of vicinal diaryl pyrazole-based compounds (91-121, 130-153)

5.3.1.9. *In vitro* cholinesterase (AChE and BuChE) and monoamine oxidase (MAO-B) inhibitory studies

The synthesized series of compounds (**91-121** and **130-153**) were evaluated for cholinesterase inhibition (AChE and BuChE) along with monoamine oxidase (MAO-B) inhibitory activity. The cholinesterase inhibition was performed using protocol described by Ellman² while MAO-B inhibition was carried out using Amplex® Red Monoamine Oxidase Assay Kit (Invitrogen, USA)³. All the compounds were primarily screened at a fixed concentration of 10 μ M to obtain the %inhibition. Physostigmine (eserine) was used as standard drug for cholinesterase inhibition whereas pargyline was used as standard drug in MAO-B inhibition assay (**Table 5.1**).

Table 5.1: Percentage inhibition of AChE, BuChE and MAO-B enzyme of vicinal diaryl pyrazole containing benzyl piperidine derivatives (**91-121**).

Compound	Code	% Inhibition $\pm$ SEM at 10 μ M		
		<i>h</i> AChE	<i>h</i> BuChE	MAO-B
91	K26	16 $\pm$ 0.223	20.6 $\pm$ 8.966	96.76 $\pm$ 3.245
92	K27	-11 $\pm$ 2.112	6 $\pm$ 5.746	98.27 $\pm$ 1.145
93	K28	-8 $\pm$ 0.824	3 $\pm$ 2.487	100 $\pm$ 0
94	K30	-3 $\pm$ 2.683	-2 $\pm$ 5.291	83.62 $\pm$ 0.97
95	K31	19 $\pm$ 1.595	4 $\pm$ 4.123	95.12 $\pm$ 2.985
96	K32	3 $\pm$ 2.616	6 $\pm$ 3.616	100 $\pm$ 0
97	K34	2 $\pm$ 0.884	9 $\pm$ 7.201	100 $\pm$ 0
98	K35	9 $\pm$ 2.214	1 $\pm$ 6.460	96.57 $\pm$ 1.53
99	K36	10 $\pm$ 1.708	1 $\pm$ 1.936	91.81 $\pm$ 6.095
100	K37	5 $\pm$ 1.353	19.1 $\pm$ 4.295	100 $\pm$ 0
101	K38	7 $\pm$ 2.020	9 $\pm$ 5.746	98.77 $\pm$ 1.23
102	K39	13 $\pm$ 2.752	1 $\pm$ 2.487	95.26 $\pm$ 1.475
103	K40	6 $\pm$ 0.916	-2 $\pm$ 2.378	96.02 $\pm$ 0.365
104	K41	4 $\pm$ 1.585	7 $\pm$ 5.291	89.85 $\pm$ 9.495
105	K42	13 $\pm$ 1.451	5 $\pm$ 4.123	86.77 $\pm$ 6.37

106	K43	18 ± 2.415	1 ± 3.616	81.83 ± 0.345
107	K44	16 ± 1.557	2 ± 7.201	86.62 ± 0.955
108	K45	15 ± 2.709	1 ± 2.121	88.35 ± 2.435
109	K46	16 ± 3.477	2 ± 6.460	89.89 ± 1.77
110	K47	18 ± 2.415	1 ± 11.207	89.59 ± 0.84
111	K48	17 ± 5.517	2 ± 4.253	94.83 ± 3.215
112	K49	18 ± 1.222	22.5 ± 3.776	92.51 ± 3.475
113	K50	16 ± 4.585	8 ± 2.754	82.26 ± 3.275
114	K51	8 ± 2.709	1 ± 3.828	83.63 ± 5.11
115	K52	7 ± 2.273	3 ± 4.068	79.86 ± 0.98
116	K53	16 ± 2.981	3 ± 3.618	82.94 ± 0.845
117	K54	8 ± 5.517	2 ± 6.217	88.75 ± 4.895
118	K55	7 ± 3.384	3 ± 6.071	75.33 ± 5.6
119	K56	2 ± 2.983	2 ± 1.221	83.2 ± 0.525
120	K57	7 ± 1.544	3 ± 1.921	84.38 ± 7.335
121	K58	8 ± 1.802	2 ± 2.212	100 ± 0
Physostigmine		99.7 ± 0.137	96 ± 0.531	-
Pargyline		-	-	100 ± 0

*Data is mean ± standard error of mean (in percentage) of sexuplicate experiments for AChE and BuChE assay and duplicate for MAO-B inhibitory assay.

As observed in **Table 5.2**, compound **(144/K16)** and compound **(152/K24)** showed potent inhibition of 89.5 % and 94.7% of AChE respectively. Both the compounds were analogous of each other bearing a -OCH₃ and -Cl group on the para position of 3,4-diaryl substitution of pyrazole which is further linked with benzyl carbamate moiety. Surprisingly compound **(132/K4)** and **(140/K12)** which are also both analogous bearing a -H and -OCH₃ group on the para position of 3,4-diaryl substitution of pyrazole linked with ethyl carbamate moiety were proved be activators of AChE enzyme showing -23% and -24% inhibition at 10μM respectively. The negative inhibition of the mentioned molecule indeed opens a new scope of research to further modify the compounds and investigate as cholinesterase reactivators which can be modified as a therapeutic agent in case of organophosphorus poisoning.^{4,5} However, other compounds from the series produced moderate to low AChE enzyme inhibition while all the synthesized compounds displayed negligible BuChE inhibitory activity at 10 μM.

Table 5.2: Percentage inhibition of AChE, BuChE and MAO-B enzyme of vicinal diaryl pyrazole containing carbamate derivatives (**130-153**).

Compound	Compound Code	% Inhibition $\pm$ SEM at 10 μ M		
		<i>h</i> AChE	<i>h</i> BuChE	MAO-B
130	K1	1.3 $\pm$ 1.774	2.5 $\pm$ 3.797	100 $\pm$ 0
131	K3	-16.3 $\pm$ 0.763	1.0 $\pm$ 8.240	94.94 $\pm$ 1.76
132	K4	-23.1 $\pm$ 1.586	4.8 $\pm$ 5.439	84.22 $\pm$ 2.065
133	K5	21.6 $\pm$ 0.449	1.6 $\pm$ 5.132	82.84 $\pm$ 0.105
134	K6	12.3 $\pm$ 1.268	7.0 $\pm$ 3.143	89.58 $\pm$ 1.76
135	K7	15.1 $\pm$ 0.430	0.7 $\pm$ 5.136	95.13 $\pm$ 3.16
136	K8	-10.9 $\pm$ 2.46	1.6 $\pm$ 2.169	91.99 $\pm$ 1.52
137	K9	1.7 $\pm$ 0.242	-2.9 $\pm$ 4.676	81.21 $\pm$ 0.475
138	K10	7.2 $\pm$ 0.763	3.4 $\pm$ 2.988	100 $\pm$ 0
139	K11	-11.1 $\pm$ 1.143	2.2 $\pm$ 1.740	98.75 $\pm$ 1.255
140	K12	-24.5 $\pm$ 2.616	3.8 $\pm$ 1.073	100 $\pm$ 0
141	K13	-19.7 $\pm$ 1.419	4.5 $\pm$ 5.816	100 $\pm$ 0
142	K14	34.2 $\pm$ 0.901	9.0 $\pm$ 2.439	99.19 $\pm$ 0.815
143	K15	-1.7 $\pm$ 0.806	4.7 $\pm$ 1.268	95.33 $\pm$ 0.095
144	K16	89.5 $\pm$ 0.620	6.4 $\pm$ 2.302	93.78 $\pm$ 0
145	K17	35.6 $\pm$ 0.440	1.4 $\pm$ 3.550	87.57 $\pm$ 1.62
146	K18	38.7 $\pm$ 1.476	7.1 $\pm$ 5.384	93.15 $\pm$ 0.025
147	K19	26.4 $\pm$ 1.767	6.0 $\pm$ 1.150	98.51 $\pm$ 1.49
148	K20	-13.2 $\pm$ 1.582	6.7 $\pm$ 2.309	82.9 $\pm$ 1.885
149	K21	-2.3 $\pm$ 1.116	9.7 $\pm$ 2.144	92.67 $\pm$ 7.195
150	K22	1.6 $\pm$ 0.882	12.3 $\pm$ 2.999	99.15 $\pm$ 1.07
151	K23	5.0 $\pm$ 1.823	9.7 $\pm$ 5.484	95.59 $\pm$ 1.245
152	K24	94.7 $\pm$ 0.285	14.1 $\pm$ 6.968	99.11 $\pm$ 0.89
153	K25	69.0 $\pm$ 0.262	7.1 $\pm$ 3.671	14.12 $\pm$ 3.815
Physostigmine		99.7 $\pm$ 0.137	96 $\pm$ 0.531	NA
Pargyline		NA	NA	100 $\pm$ 0

*Data is mean $\pm$ standard error of mean (in percentage) of sextuplicate experiments for AChE and BuChE assay and duplicate for MAO-B inhibitory assay.

The initial screening of the Amplex red assay revealed that nearly every compound was a strong inhibitor of the MAO-B enzyme at 10 μ M. **Fig 5.1.** shows almost all the compounds showed >75% inhibition, 21 compounds exhibited 80-90% inhibition, 8 compound showed 90-95% inhibition, 16 compounds exhibited 95-100% inhibition while (**K1, K10, K12, K13, K28, K32, K34, K37**) inhibited 100% of MAO-B enzyme. The data proves that the designed vicinal diaryl pyrazole compounds are potent inhibitors of MAO-B enzyme and as hypothesized using molecular docking studies discussed in **chapter-4**.

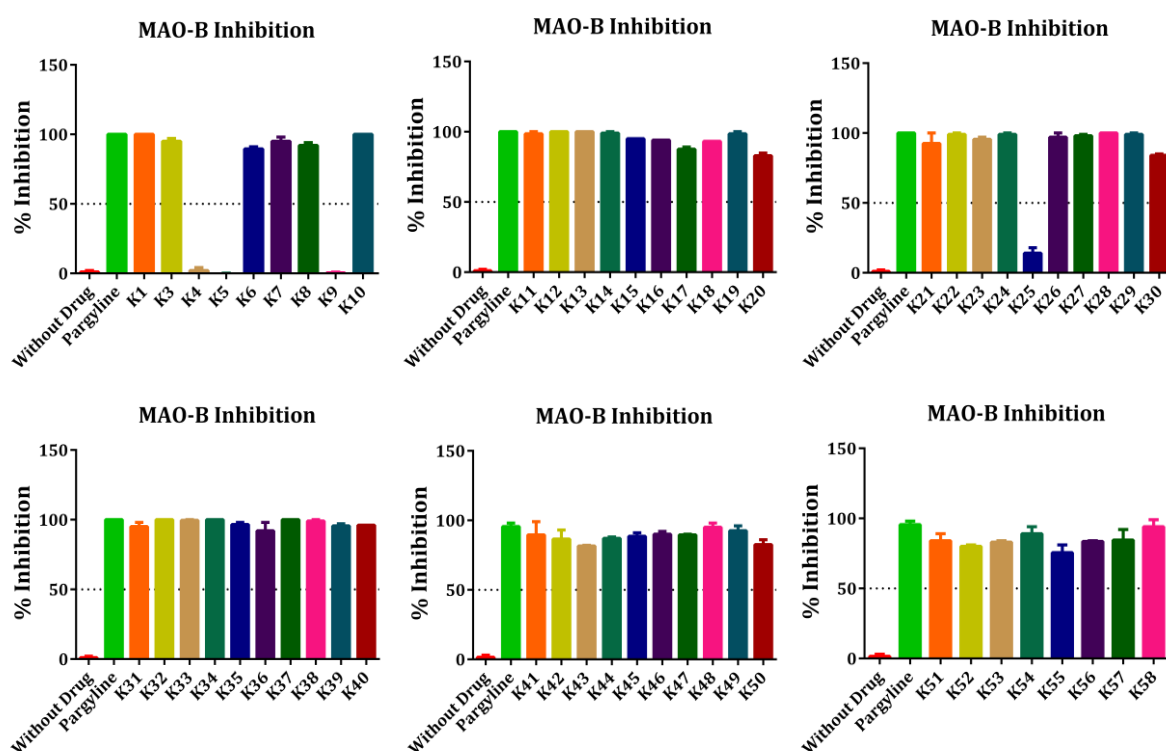
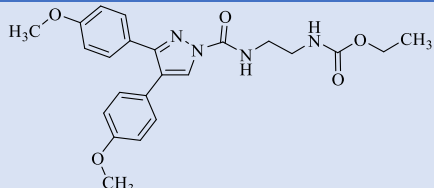
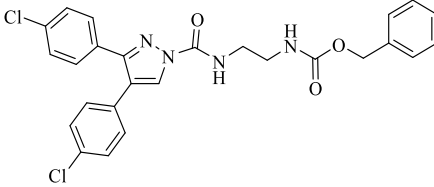
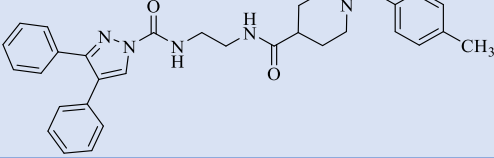
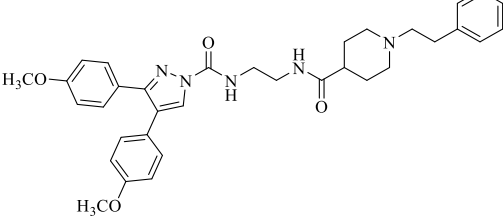
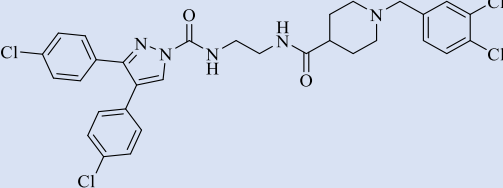


Fig 5.1: The graphs represent MAO-B activity of compounds K1-58 in *STHdh*^{Q7/Q7} cells at 10 μ M. Bar graph contains % inhibition of MAO-B enzyme on y axis vs compounds K1-58 on x axis. The inhibition of the compounds was compared to negative control (without drug) and pargyline was used as positive control. The data points represent the mean values from duplicate experiments, and error bars indicate standard error of mean.

These findings led to the selection of the five most effective compounds (**K12, K24, K32, K48, and K58**) for IC₅₀ analysis. GraphPad Prism, version 6, was used to determine the IC₅₀ values by utilizing the curves of log (inhibitor) vs. normalized response with a varied slope. For IC₅₀ evaluation, starting chemical concentrations between 0 and 10 μ M were utilized. (**K24**) contains a vicinal diaryl pyrazole along with a urea spacer attached with a benzyl carbamate moiety was considered because of it showed potent activity for both AChE and MAO-B whereas (**K12**) had compounds (**K32, K48 and K58**) were considered individually for three different analogues (-H, -OCH₃ and -Cl) of diaryl substitution of

pyrazole. All the compounds exhibited inhibitory concentration in nanomolar wherein IC₅₀ of (**K24**) was found to be 92.86 ± 7.66 nM followed by (**K58**, **K32**, **K48** and **K12**) having IC₅₀ of 101.4 ± 10.62 nM, 109.3 ± 20.78 nM, 146.8 ± 22.15 nM and 448.5 ± 18.25 nM respectively (**Table 5.3** and **Fig 5.2**).

Table 5.3: IC₅₀ values compounds **K12**, **K24**, **K32**, **K48** and **K58** against MAO-B inhibition.

Compound	Compound code	Structure	MAO-B IC ₅₀ (nM) $\pm$ SEM
140	K12		448.5 ± 18.25
152	K24		92.86 ± 7.66
96	K32		109.3 ± 20.78
111	K48		146.8 ± 22.15
121	K58		101.4 ± 10.62

*Data is mean $\pm$ standard error of mean (in percentage) of duplicate experiments.

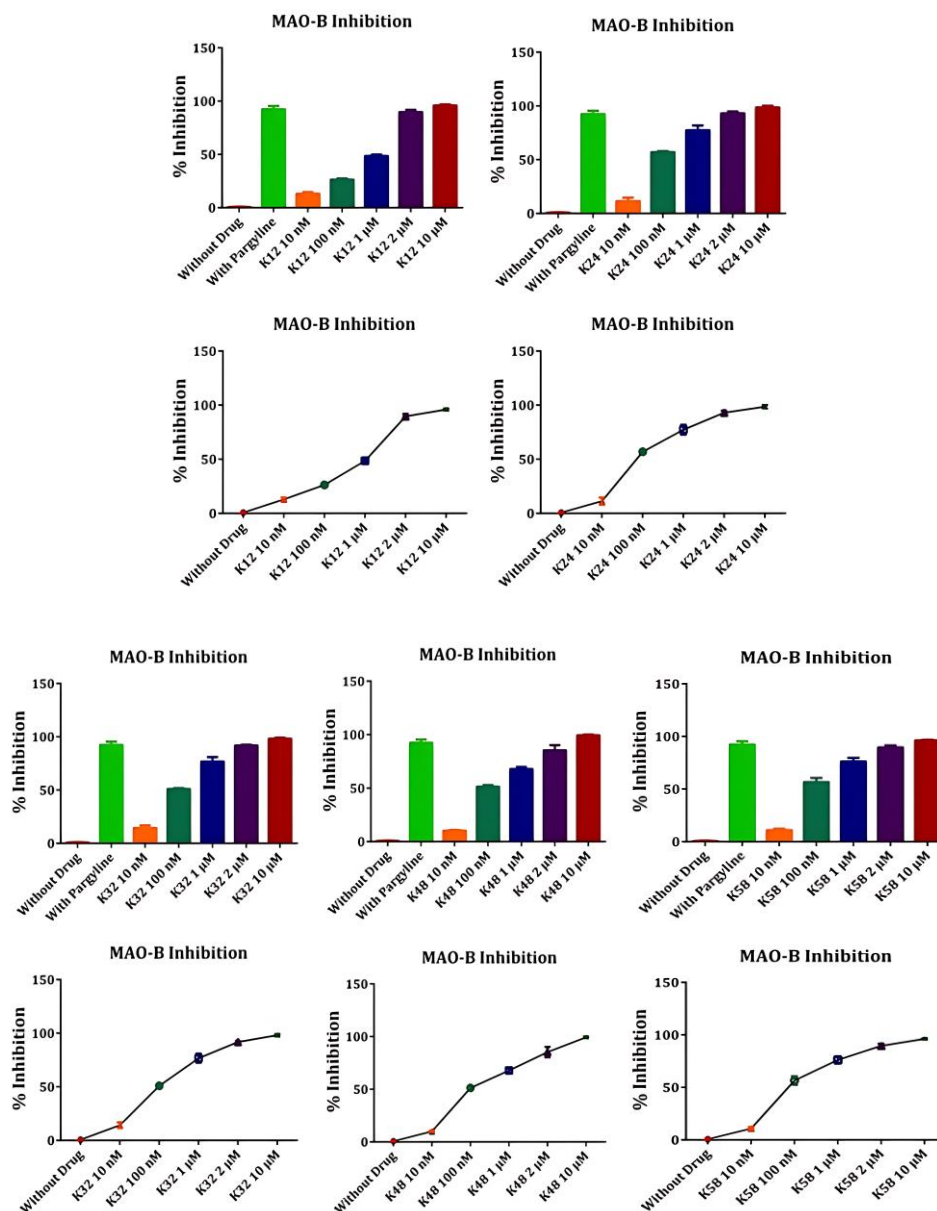


Fig 5.2: Bar graph represents %MAO-B inhibition of *STHdh*^{Q7/Q7} cells at 10 nM, 100 nM, 1 μM, 2 μM and 10 μM concentration for compounds K12, K24, K32, K48 and K58 compared to negative control (without drug) and pargyline as positive control. Line graph represents %MAO-B inhibition of *STHdh*^{Q7/Q7} cells at 10 nM, 100 nM, 1 μM, 2 μM and 10 μM concentration for compounds **K12, K24, K32, K48** and **K58** for calibration curvatures of log (inhibitor) vs normalized response with a variable slope.

5.3.1.10. Cytotoxic effect of compounds on cell line by MTT assay

In this study, the cytotoxic effects of selected compounds (**K12, K24, K32, K48** and **K58**) i.e (were investigated at varying concentrations on *STHdh*^{Q7/Q7} cells. The cells were exposed to range of concentrations and assessed their viability using the MTT assay. Aim of MTT assay was to elucidate dose-dependent responses on viability and their significance.

Table 5.4: Percentage cell viability of *STHdh*^{Q7/Q7} cells at different concentrations of **K12**, **K24**, **K32**, **K48** and **K58**

Compound	Compound code	10 nM	100 nM	1 μ M	2 μ M	10 μ M
140	K12	88.86 $\pm$ 5.8	86.74 $\pm$ 5.6	77.96 $\pm$ 6	73.86 $\pm$ 5	66.97 $\pm$ 5
152	K24	84.47 $\pm$ 3.9	81.21 $\pm$ 6	73.45 $\pm$ 5	73.47 $\pm$ 4.7	67.10 $\pm$ 5.4
96	K32	98.16 $\pm$ 2.8	94.16 $\pm$ 2.8	94.47 $\pm$ 4.1	91.79 $\pm$ 6	91.32 $\pm$ 4.4
111	K48	96.20 $\pm$ 5.7	94.17 $\pm$ 5	89.06 $\pm$ 3.6	82.01 $\pm$ 4.8	72.05 $\pm$ 3.7
121	K58	94.45 $\pm$ 1.6	88.13 $\pm$ 2.6	85.17 $\pm$ 3.1	86.18 $\pm$ 5.4	83.67 $\pm$ 4.7

*Data is mean $\pm$ standard error of mean (in percentage)

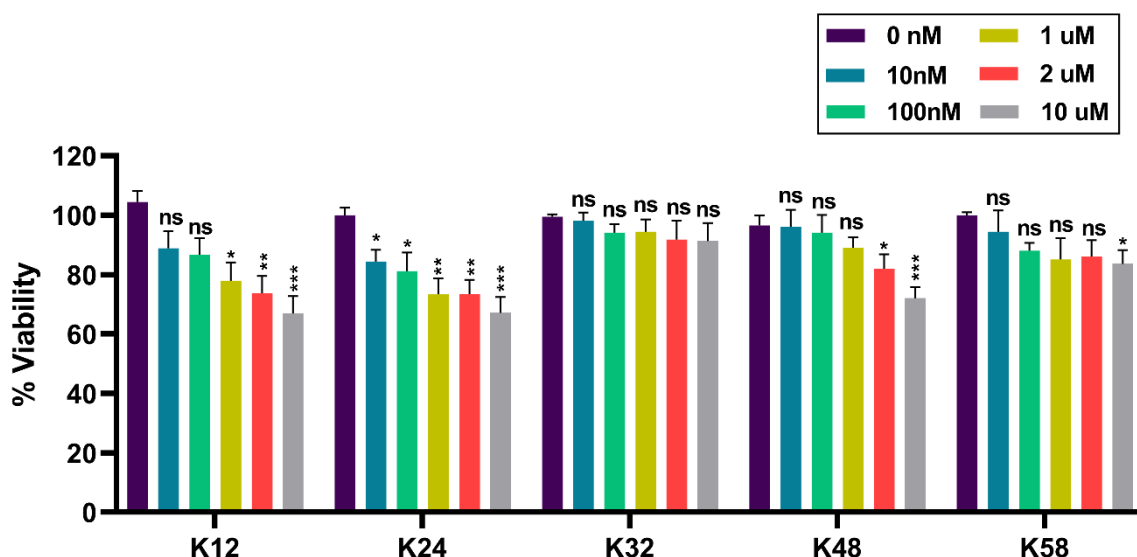


Fig 5.3: % Cell viability from MTT assay - The graph represents the percentage cell viability of *STHdh*^{Q7/Q7} cells following treatment with compounds **K12**, **K24**, **K32**, **K48** and **K58** at the final concentrations of 10 nM, 100 nM, 1 μ M, 2 μ M, and 10 μ M. The data points represent the mean values from triplicate experiments, and error bars indicate standard error of mean. Cell viability percentages are normalized relative to untreated control cells (0 nM). Significance levels are indicated as follows: n.s. for p-value > 0.05 , *p-value < 0.05 , **p-value < 0.01 , ***p-value < 0.001 . (n=3)

The presented data (**Fig. 5.3**) represents the results of a cell viability assay conducted on *STHdh*^{Q7/Q7} cells treated with compounds (**K12**, **K24**, **K32**, **K48** and **K58**) at range of concentrations (10 nM, 100 nM, 1 μ M, 2 μ M, and 10 μ M). Compared to the control group (0 nM), which exhibited 100% cell viability, treatment with all the concentrations of (**K32** and **K58**) compounds showed non-significant effect on cell viability, with values ranging from

approximately 83% to 98% of the control. This suggests a non-cytotoxic effect at these compounds.

However, (**K12**, **K24** and **K48**) compounds showed nearly non-significant effect on viability at lower concentration (10 nM and 100 nM), with a noticeable decline in cell viability observed in a dose-dependent manner as the concentration of the compounds escalated (1 μ M, 2 μ M, and 10 μ M), indicating a concentration-related cytotoxic effect. This pattern highlights the non-cytotoxic nature of these compounds at lower concentrations, with only marginal effects seen at higher concentrations of the compounds.

5.3.1.11. Metal chelation activity

All the compounds having >90% MAO-B inhibition was considered for evaluating their metal chelation capabilities. In initial investigation, metal-chelating assay using ferrozine and ferrous chloride was performed at a low concentration at 10 μ M. 8-Hydroxyquinoline a well-known metal chelator^{6,7} and EDTA were used as standards (**Table 5.5**). Majority of compounds showed better ability to chelate Fe^{2+} at low concentration of 10 μ M than standard 8-hydroxyquinoline. Compound **97 (K34)** showed the most significant metal chelating ability showing 28.94% chelation was almost close to EDTA.

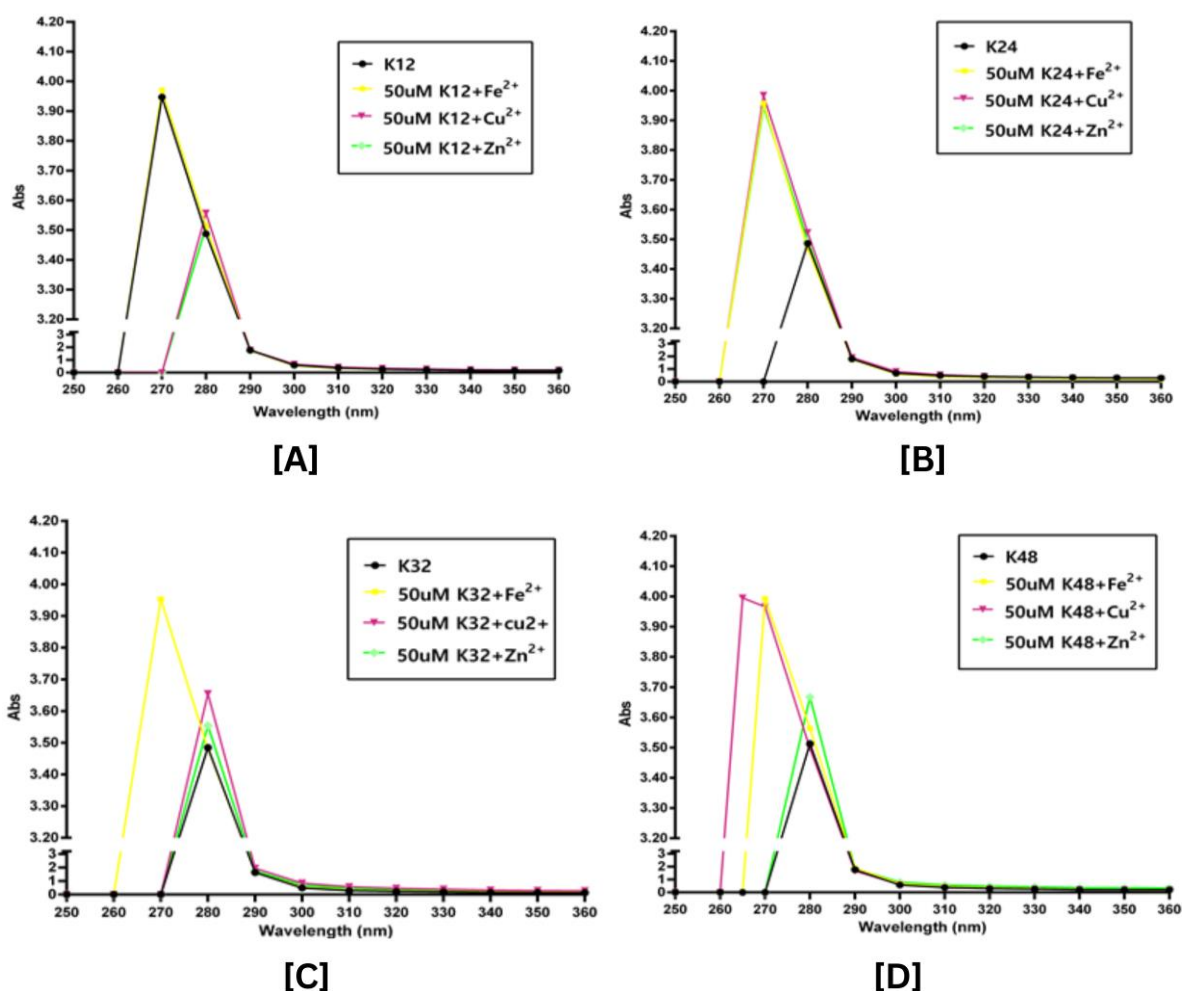
Table 5.5: Metal chelation activity on ferrozine and ferrous chloride biometals at 10 μ M concentration.

Compound	Code	% MCA	$\pm$ SEM	Compound	Code	% MCA	$\pm$ SEM
	Blank	0	0	92	K27	14.33	2.23
	8-HQ	3.09	0.88	93	K28	6.00	0.68
130	K01	6.00	0.10	95	K31	5.71	2.32
131	K03	7.84	2.13	96	K32	14.52	0.10
135	K07	0.10	2.13	97	K34	28.94	2.13
136	K08	-1.45	0.00	98	K35	6.39	1.84
138	K10	-0.68	1.74	99	K36	12.39	1.26
139	K11	0.10	2.71	100	K37	10.78	0.67
140	K12	0.87	0.19	101	K38	20.33	0.29
141	K13	-2.52	0.68	102	K39	2.90	2.61
142	K14	-4.84	2.23	103	K40	5.42	1.65
143	K15	1.83	1.54	104	K41	19.85	0.58
144	K16	-1.44	0.58	105	K42	11.91	3.10

147	K19	-2.03	0.58	108	K45	18.30	4.26
149	K21	-3.19	3.29	109	K46	8.37	0.58
150	K22	-2.42	3.49	111	K48	16.94	3.87
151	K23	-2.42	0.39	112	K49	10.84	0.29
152	K24	-3.56	0.58	117	K54	-3.10	0.48
91	K26	18.59	0.87	120	K57	8.81	0.97
				121	K58	-0.97	1.07

*Data is mean $\pm$ standard error of mean (in percentage)

Furthermore, a UV-vis spectroscopy assay was performed to evaluate the ability of compounds (**K12**, **K24**, **K32**, **K48** and **K58**) to chelate biometals, such as Cu^{2+} , Fe^{2+} , and Zn^{2+} . The compounds were evaluated at a higher concentration of 50 μM alone and with different metal solutions. The experimental results revealed distinct changes in the absorption peaks or overall absorption upon interaction of the compounds with the respective metal solutions, indicating the formation of new metal-compound complexes **Fig 5.4**.



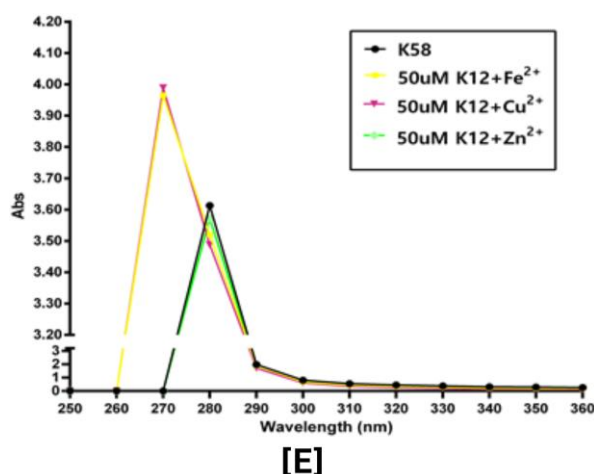


Fig 5.4. UV spectra of the compounds (50 μ M) alone and in the presence of CuSO_4 (50 μ M), FeCl_2 (50 μ M), or ZnCl_2 (50 μ M) in buffer (20 mM HEPES, 150 mM NaCl, pH 7.4): (a) **K12 (140)** (b) **K24 (152)**, (c) **K32 (96)**, (d) **K48 (111)**, and (e) **K58 (121)**.

Compound (**K12**) showed absorption peak at 260 nm and on addition of CuSO_4 and ZnCl_2 shifted the absorption peak to 280 nm indicating its ability to chelate Cu^{2+} and Zn^{2+} ions. However, no effect was observed in the shifting of the peak on addition of FeCl_2 [Fig. 5.4. (a)]. In contrast, compound (**K24**) displayed absorption peak at 280 nm which shifted to 260 nm in all three solutions, suggesting its ability to chelate all the three tested metals [(Fig. 5.4. (b))]. Compound (**K32**) showed a shift in the absorption peak upon interaction with FeCl_2 , but showed only a change in the maximum absorption at 280 nm upon interaction with Cu^{2+} and Zn^{2+} [Fig. 5.4. (c)]. Both **K48** and **K58** showed a shift in the absorption peak from 280 to 270 or 265 nm upon interaction with Cu^{2+} and Fe^{2+} , but no effect was observed in the presence of Zn^{2+} [Fig. 5.4. (d and e)], indicating their ability to chelate Cu^{2+} and Fe^{2+} and not Zn^{2+} , illustrating their chelation potential. These findings underscore the diverse abilities of compounds to chelate various metal ions, thus showing valuable chelation properties.

5.3.1.12. Antioxidant activity

The compounds were checked for its antioxidant property using DPPH assay. The preliminary testing was performed at a low concentration of 10 μ M. Compound **102 (K39)** was found to possess best antioxidant property with % radical scavenging of 5.78% compared to 5.97% of 8-hydroxyquinoline at 10 μ M (Table 5.6).

Table 5.6: Antioxidant radical scavenging activity using DPPH assay.

Compound	Code	% RSA	$\pm$ SEM	Compound	Code	% RSA	$\pm$ SEM
	Blank	0	0	92	K27	-2.697	1.734
	8-Hq	5.973	2.312	93	K28	-4.432	0.000

130	K01	-5.973	0.771	95	K31	0.193	0.385
131	K03	-1.349	1.927	96	K32	-6.936	0.578
135	K07	-4.817	1.541	97	K34	-0.771	0.963
136	K08	-6.166	0.193	98	K35	-2.312	1.349
138	K10	-4.046	2.312	99	K36	-3.083	1.349
139	K11	-7.322	0.963	100	K37	0.771	0.963
140	K12	-3.468	2.890	101	K38	-5.202	0.000
141	K13	0.578	1.156	102	K39	5.780	2.890
142	K14	-6.551	0.193	103	K40	0.963	1.927
143	K15	-0.771	0.963	104	K41	-2.759	3.103
144	K16	0.578	1.156	105	K42	-0.104	0.276
147	K19	-4.432	1.541	108	K45	-6.897	1.724
149	K21	-1.927	1.349	109	K46	-8.793	4.310
150	K22	0.963	0.385	111	K48	-5.517	0.690
151	K23	0.000	0.578	112	K49	-2.759	1.724
152	K24	-6.936	0.578	117	K54	-1.207	0.172
91	K26	-1.927	0.963	120	K57	-1.724	0.690
				121	K58	0.172	0.172

*Data is mean $\pm$ standard error of mean (in percentage)

Total five compounds (**K12**, **K24**, **K32**, **K48** and **K58**) were selected to check the radical scavenging activity by using DPPH assay. The compounds were tested for range of concentration (10 μ M to 500 μ M of final concentration). The compounds showed varying degree of radical scavenging activity (**Table 5.7**) out of which compound (**K12**) was found to possess best activity followed by compounds (**K48** and **K32**).

Table 5.7: Percentage radical scavenging activity at final concentration range from 10 to 500 μ M.

Compound	Code	10 μ M	50 μ M	100 μ M	200 μ M	500 μ M
140	K12	5.58 $\pm$ 0.70	6.5 $\pm$ 1.2	4.1 $\pm$ 1.3	3.0 $\pm$ 0.0	2.7 $\pm$ 1.0
152	K24	2.6 $\pm$ 1.2	1.3 $\pm$ 0.81	3.3 $\pm$ 0.93	2.6 $\pm$ 0.16	1.6 $\pm$ 0.00
96	K32	1.9 $\pm$ 0.47	3.3 $\pm$ 0.36	3.8 $\pm$ 0.43	4.0 $\pm$ 1.6	5.3 $\pm$ 0.82
111	K48	4.1 $\pm$ 0.35	4.4 $\pm$ 0.70	4.7 $\pm$ 0.27	4.1 $\pm$ 0.12	3.6 $\pm$ 0.12
121	K58	1.8 $\pm$ 0.66	2.3 $\pm$ 0.48	2.6 $\pm$ 0.47	3.1 $\pm$ 0.12	3.7 $\pm$ 0.70

*Data is mean $\pm$ standard error of mean (in percentage) of duplicate

5.3.2. Biological studies of benzofuran based compounds (181-199, 203-208, 212-215 and 222-227)

5.3.2.1. *In vitro* cholinesterase (AChE and BuChE) and monoamine oxidase (MAO-B) inhibitory studies

Compounds containing benzofuran fused carbamate derivatives (203-208, 212-215) and benzofuran fused amide derivatives (222-227) were evaluated for AChE and BuChE inhibitory activity using Ellman assay at 10 μ M primary concentration and results are shown in **Table 5.8**. Compound (223) having 75% inhibition was found out to be the most potent AChE inhibitor whereas compound (222) was found to be the best inhibitor of BuChE enzyme.

Table 5.8: Percentage inhibition of AChE, BuChE and MAO-B enzyme (181-199), (203-208, 212-215).

Compound	Code	% Inhibition $\pm$ SEM at 10 μ M		
		MAO-B	hAChE	hBuChE
181	K96	-19	-	-
182	K97	-46	-	-
183	K98	-118	-	-
184	K99	-28	-	-
185	K100	-41	-	-
186	K101	-26	-	-
187	K102	-71	-	-
188	K103	-63	-	-
189	K104	-46	-	-
190	K105	17	-	-
191	K106	-39	-	-
192	K107	-30	-	-
193	K108	-20	-	-
194	K109	-48	-	-
195	K110	-24	-	-
196	K111	-33	-	-
197	K112	-6	-	-

198	K116	-141	-	-
199	K117	-162	-	-
203	K86	-38	26 ± 1.980	15 ± 2.264
204	K87	-34	21 ± 2.034	9 ± 2.913
205	K88	-46	27 ± 2.151	-1 ± 1.886
206	K89	-73	10 ± 4.767	0 ± 1.714
207	K90	-74	29 ± 2.626	1 ± 2.781
208	K91	-50	18 ± 4.085	3 ± 3.549
212	K92	-57	16 ± 2.780	2 ± 2.446
213	K93	-41	22 ± 2.266	0 ± 0.916
214	K94	-106	13 ± 3.648	-1 ± 4.653
215	K95	81	9 ± 3.400	-7 ± 2.599
222	K73	69	24 ± 1.859	20 ± 2.542
223	K75	79	75 ± 0.590	1 ± 2.414
224	K76	72	17 ± 2.558	1 ± 3.094
225	K79	-96	29 ± 1.751	1 ± 2.664
226	K80	5	9 ± 2.917	1 ± 1.831
227	K81	-282	7 ± 2.546	-3 ± 1.027
Physostigmine		-	99.7 ± 0.137	96 ± 0.531
Pargyline		100 ± 0	-	-

**Data is mean ± standard error of mean (in percentage) of sextuplicate experiments for AChE and BuChE assay and duplicate for MAO-B inhibitory assay

The synthesized compounds of benzofuran containing carbohydrazide derivatives (**181-199**) were evaluated for MAO-B inhibitory activity using Amplex® Red Monoamine Oxidase Assay Kit (Invitrogen, USA). Primary screening was carried at 10 µM concentration and % inhibition is reported in **Table 5.8**. Pargyline was taken as standard drug in MAO-B inhibition assay. Compound (**190**) having 17% inhibition was found to be the most potent MAO-B inhibitor from the entire series of compounds. Surprisingly, other compounds of the series exhibited negative enzyme inhibition, amongst them compounds (**183, 198 and 199**) showed more than -100% inhibition.

Similarly, at 10 µM concentration, another series of benzofuran derivatives containing carbamate (**203-208, 212-215**) and amide (**222-227**) functional groups were also evaluated for MAO-B inhibition. Two compounds (**215 and 223**) were found to be the most potent

having 81% and 79% inhibition respectively. In contrary, compounds (**214**, **225** and **227**) turned out to be having negative MAO-B inhibition with activity of -106%, -96% and -282% respectively.

The negative inhibition of MAO-B enzyme opens up a new area of research altogether where these types of compounds can truly be considered further in the development into potential therapeutic and diagnostic agents like PET tracers to estimate the progress of various neurological disorders in patients.^{8–10}

MAO-B inhibitory activity was carried out using Amplex® Red Monoamine Oxidase Assay Kit (Invitrogen, USA). Pargyline was used a standard drug to check the response of the assay which produced 100% inhibition. This assures that the results obtained from the assay conducted cannot be considered as false positive and therefore the negative inhibition produced by the evaluated series of benzofuran compounds has to be further investigated in depth to confirm the actual reason of negative inhibition.

According to the preliminary investigation done on the basis of *in silico* and *in vitro* analysis, we hypothesize that the compounds could be binding to some allosteric site which might be present on MAO-B enzyme protein. However, this hypothesis could be justified by carrying out kinetic studies of the compounds which could give us more idea regarding binding of the ligand with protein as competitive or non-competitive. Furthermore, according to the discussion of MAO-B active binding site done in **chapter-3**, there are two important components in the active site; the entrance cavity and the substrate. The substrate cavity is further divided into an aromatic cage which contains FAD and a bridge that links the entrance cavity to substrate cavity. Tyr326 and Ile199 are reported as key amino acids for the translation of the bridge while Tyr398 and Tyr435 are reported as key amino acids of the aromatic cage^{11–14}. In general, for MAO-B functioning to occur, the ligand has to pass through the entrance cavity, then bind at the bridge which opens the cavity to aromatic cage via translation and then finally to aromatic cage in order bind with the FAD which is responsible for the metabolism of catecholamines (**Fig. 5.5**). In correlation with the results of the docking studies, it can be hypothesized that the benzofuran containing ligand has the ability to enter from the entrance cavity and produce a significant interaction at the bridge which indeed opens the gate to the aromatic cage. However, it fails to bind in the aromatic cage thereby leaving the gate open for the catecholamines to bind with FAD and enhancing the rate of metabolism which apparently is responsible for negative inhibition results.

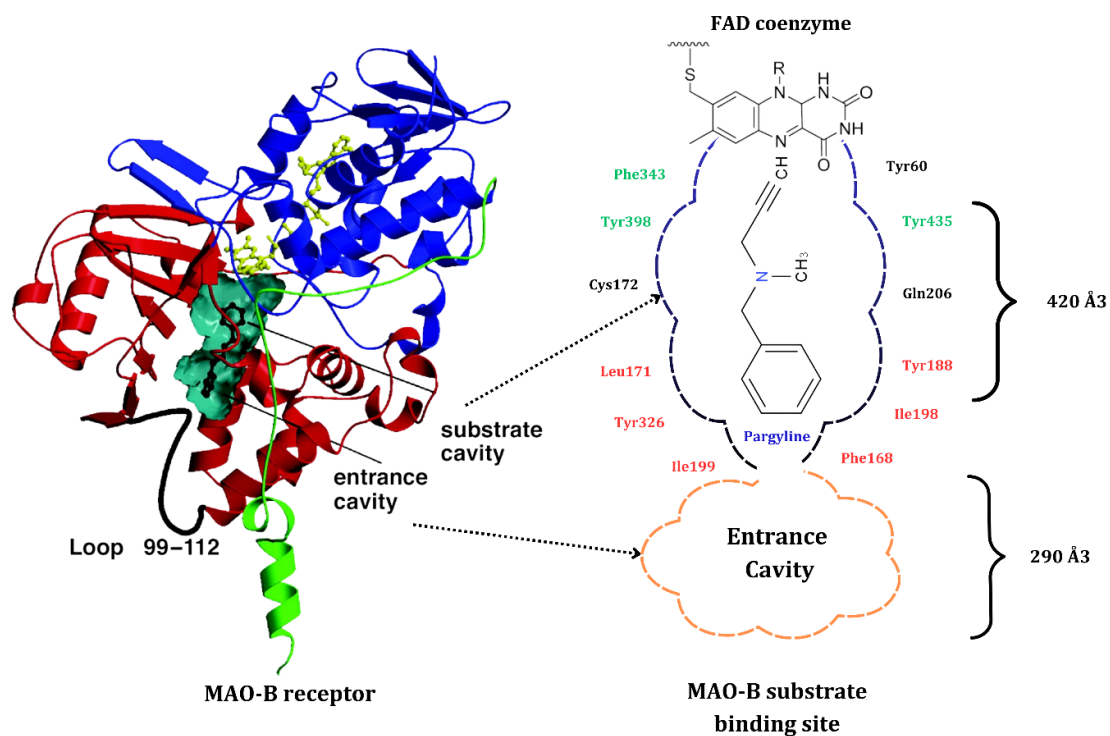


Fig. 5.5: Schematic diagram of active binding site of MAO-B.

Still a considerable amount of study needs to be conducted in order to confirm the hypothesis which can further taken as potential research for the development of these type of compounds.

5.3.2.2. Antioxidant activity

Using the DPPH test, the radical scavenging activity of the compounds was examined at a concentration of 10 μ M. All of the compounds exhibited a relatively modest percentage of radical scavenging action, as indicated by the antioxidant activity data (**Table 5.10**). The low percentage scavenging activity shown for the compounds raises the possibility that, at the studied doses, i.e 10 μ M, the compounds are ineffective antioxidants.

Table. 5.10: % Radical Scavenging Activity (RSA) at 10 μ M concentrations.

Compound	Code	% RSA	$\pm$ SEM
	Blank	0	0
	Hq 10uM	5.973	2.312
	VitC 10uM	31.407	4.239
225	K79	-6.207	2.069
227	K81	-3.621	0.172
214	K94	-4.138	0.690
215	K95	-4.138	0.690

183	K98	-2.931	0.517
198	K116	7.414	1.552
199	K117	-1.207	1.207

*Data is mean $\pm$ standard error of mean (in percentage) of duplicate for DPPH assay.

5.3.2.3. Metal chelation activity

Metal chelation activity was performed using Ferrozine and Ferrous Chloride was performed. **Table. 5.11** showed that all the six selected compounds were tested at 10 μ M concentration. Results have shown that most of the compounds have metal chelation activity compared to the standard drug 8-hydroxyquinoline. On the other hand, all the compounds have less metal chelation activity compared to EDTA. The highest metal chelation was shown by the compound **198 (K116)** with 16.06 ± 1.8 % MCA.

Table 5.11: Metal chelation activity using ferrozine and ferrous chloride of compounds at 10 μ M concentrations.

Compound	Code	% MCA	$\pm$ SEM
	EDTA 10uM	34.65	1.059
	8-HQ 10uM	3.09	0.88
225	K79	-1.74	0.68
227	K81	-1.94	1.65
214	K94	1.94	1.26
215	K95	-1.74	0.48
183	K98	0.48	1.74
198	K116	16.07	1.84
199	K117	5.58	1.44

*Data is mean $\pm$ standard error of mean (in percentage) of duplicate for metal chelation assay.

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