5 Experimental work

5.1 Chemistry

The chemicals utilized in the synthesis of intermediates and target compounds were procured from various commercial suppliers, including Sigma Aldrich (USA), SD Fine Chemicals (India), Merck (Germany), TCI (Japan), CombiBlock (Switzerland), Spectrochem (India), Alfa Aesar (USA), and Avra Synthesis (India). The reagents and solvents underwent standard methods of drying and distillation before their utilization. The progress of chemical reactions was monitored by using thin-layer chromatography (TLC) on silica gel 60 F254 plates from Merck, Germany. These plates were then examined under a UV lamp emitting light at a wavelength of 254 nm. The NMR spectra, specifically ^{1}H and ^{13}C, were acquired using a 500 MHz Bruker NMR spectrometer. The deuterated solvents, $CDCl_3$ and DMSO-d_6, and internal standard tetramethylsilane (TMS) were used. The chemical shifts (d) were denoted in units of parts per million (ppm), and the coupling constants were presented in Hertz (Hz). The proton coupling patterns were recognized as singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). TheDepartment of Chemistry (BHU) provides the facility of QTOF-Chem-BHU X500R machines to obtain HRMS (high-resolution mass spectroscopy) data via electrospray ionization (HRMS/ESI). The reported compounds' purity was over 95%, characterized by ^{1}H NMR, ^{13}C NMR, and HRMS spectra, as stated in the Supporting Information (1. spectral data section). The nomenclature of compounds was determined using the software ChemDraw Professional 15.0 (Perkin Elmer).

5.1.1 General procedure for the synthesis of intermediate 2a-2c

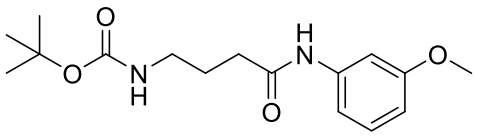
Into a stirring solution of alanine (0.8 g, 9.09 mmol) in DCM (30 ml), we added (Boc)2O (2.37 g, 10.90 mmol), and NaOH (0.29 g, 7.27 mmol) at 0°C. The reaction mixture was stirred at

room temperature for 12 h and reaction progress was monitored with TLC. Once the reaction was completed, then, we added DCM and water for workup. The aqueous layer underwent two washes with DCM (2 × 10 ml). The organic layers were combined, and anhydrous sodium sulfate (Na_2SO_4) was added to remove residual water from the organic phase. After this step, the organic solution was filtered, and the solvent was removed under reduced pressure utilizing a rotatory evaporator. The crudes (**2a-2c**) obtained were further used in the next step without purification.

5.1.2 Synthesis of intermediate 3a-3c

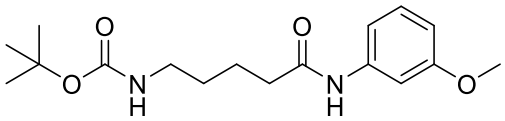
The intermediates **2a-2c** were synthesized by dissolving Boc-protected amino acids (200 mg) in THF. Subsequently, 200 mg of EDCI·HCl and 200 mg of HOBt were added to activate the carboxylic acid of the Boc-protected amino acids. The reaction mixture was stirred for 15 minutes, after which triethylamine (TEA) and meta-anisidine were added. The reaction was allowed to stir for 12-14 hr, and the progress of the reaction was monitored with the help of TLC. The THF solvent was removed using a rota-evaporator, and then water and ethyl acetate were used for workup. The ethyl acetate phase underwent two water washes, and the organic layer was separated and combined together. The ethyl acetate was removed under reduced pressure using a rota-evaporator. Compounds **3a-3c** were isolated via column chromatography using an eluent mixture of 20:80 ethyl acetate and hexane.

Tert-butyl (4-((3-methoxyphenyl)amino)-4-oxobutyl)carbamate (3a)



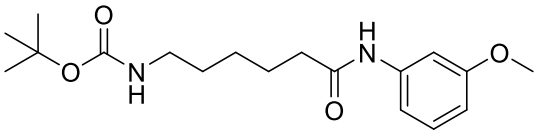
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (s, 1H), 7.40 (s, 1H), 7.22 (t, *J* = 8.1 Hz, 1H), 7.11 (d, *J* = 7.3 Hz, 1H), 6.66 (dd, *J* = 8.1, 2.1 Hz, 1H), 3.82 (s, 3H), 3.26-3.25 (m, 2H), 2.39 (t, *J* = 6.5 Hz, 2H), 1.89 (qu, J = 6.5 Hz, 2H), 1.48 (s, 9H).

5.1.2.1 Tert-butyl (5-((3-methoxyphenyl)amino)-5-oxopentyl)carbamate (3b)



^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.35 (s, 1H), 7.21 (t, *J* = 8.1 Hz, 1H), 7.01 (d, *J* = 7.6 Hz, 1H), 6.67 (dd, *J* = 8.2, 1.9 Hz, 1H), 3.82 (s, 3H), 3.19 (dd, *J* = 12.3, 5.3 Hz, 2H), 2.41 (t, *J* = 7.5 Hz, 2H), 1.78 (qu, *J* = 7.0, 7.3 Hz, 2H), 1.58 (qu, *J* = 7.0 Hz, 2H), 1.46 (s, 9H).

5.1.2.2 Tert-butyl (6-((3-methoxyphenyl)amino)-6-oxohexyl)carbamate (3c)



^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (s, 1H), 7.30 (s, 1H), 7.22 (t, *J* = 8.1 Hz, 1H), 6.99 (d, *J* = 8.2 Hz, 1H), 6.68 (dd, *J* = 8.2, 1.8 Hz, 1H), 3.83 (s, 3H), 3.15 (q, *J* = 6.5 Hz, 2H), 2.38 (t, *J* = 7.5 Hz, 2H), 1.78 (qu, *J* = 7.5 Hz, 2H), 1.54 (q, *J* = 7.1 Hz, 2H), 1.46 (s, 9H) 1.42 (m, , 2H).

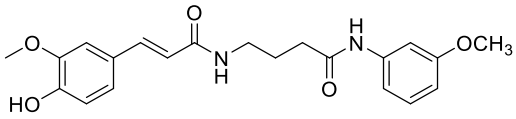
5.1.3 Synthesis of intermediate 4a-4c

The intermediate **3a-3c** were dissolved in DCM, followed by adding trifluoroacetic acid (TFA) and allowing the reaction to stir for 2h, and the reactions were monitored with the help of TLC. The solvent was evaporated to obtain the crude product. The crude product was washed twice with dried diethyl ether to get intermediates **4a-4c**, which was used further without purification.

5.1.4 General procedure for the synthesis of target compounds 5a-5c

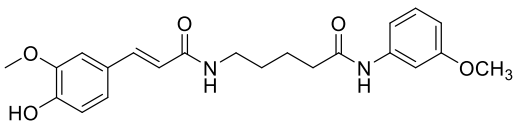
The target compounds **5a-5c** were synthesized by dissolving ferulic acid in 15 ml dried THF and adding EDC.HCl and HOBt and the reaction mixture was allowed to stir for 15 minutes, followed by the addition of triethylamine (TEA) and **4a-4c** (amines). The reaction mixture was stirred for 10-12 h at rt under a nitrogen atmosphere. Upon completion of the reaction, the workup was carried out using ethyl acetate and water. The solvent was evaporated, and crude was purified using column chromatography using ethyl acetate and hexane (80:20) ratio to yield target compounds **5a-5c**.

5.1.4.1 (E)-4-(3-(4-Hydroxy-3-methoxyphenyl)acrylamido)-N-(3-methoxyphenyl)butanamide (5a).



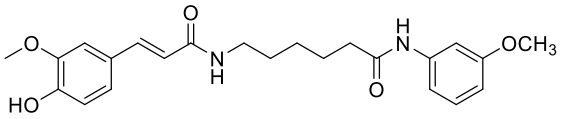
White solid powder, 1H NMR (500 MHz, DMSO-d_6) δ 9.90 (s, 1H), 9.41 (s, 1H), 8.01 (t, J = 5.3 Hz, 1H), 7.34-7.31 (m, 2H), 7.20-7.17 (m, 1H), 7.12 (bs, 2H), 6.99 (d, J = 8.1 Hz, 1H), 6.80 (d, J = 8.0 Hz, 1H), 6.61 (d, J = 8.0 Hz, 1H), 6.44 (d, J = 15.7 Hz, 1H), 3.81 (s, 3H), 3.72 (s, 3H), 3.24 (q, J = 6.5, 2H), 2.34 (t, J = 7.5 Hz, 2H), 1.77 (qu, J = 7.5 Hz, 2H).^{13}C NMR (126 MHz, DMSO-d_6,) δ 171.37, 165.90, 159.96, 148.71, 148.29, 140.95, 139.37, 129.89, 126.90, 121.93, 119.49, 116.13, 111.82, 111.24, 108.87, 105.34, 56.00, 55.40, 38.73, 34.40, 25.73. ESI-HRMS for $C_{21}H_{24}N_2O_5$. (M+H)+ calcd. 385.1763, found 385.1761.

5.1.4.2 (E)-5-(3-(4-Hydroxy-3-methoxyphenyl)acrylamido)-N-(3-methoxyphenyl)pentanamide (5b)



White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.86 (bs, 1H), 9.41 (bs, 1H), 7.97 (t, *J* = 5.7 Hz, 1H), 7.32 – 7.29 (m, 2H), 7.18 (t, *J* = 8.1 Hz, 1H), 7.12 – 7.11 (m, 2H), 6.98 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.79 (d, *J* = 8.1 Hz, 1H), 6.63 – 6.58 (m, 1H), 6.44 (d, *J* = 15.7 Hz, 1H), 3.81 (s, 3H), 3.72 (s, 3H), 3.20-3.17 (m, 2H), 2.32 (t, *J* = 7.4 Hz, 2H), 1.61 (qu, *J* = 7 Hz, 2H), 1.48 (qu, *J* = 7.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.65, 165.76, 159.95, 148.67, 148.28, 140.97, 139.26, 129.89, 126.92, 121.94, 119.56, 116.11, 111.78, 111.18, 108.86, 105.29, 55.98, 55.40, 38.83, 36.58, 29.34, 23.11. ESI-HRMS for $C_{22}H_{26}N_2O_5$. (M+H)+ calcd. 399.1920, found 399.1923.

5.1.4.3 (E)-6-(3-(4-Hydroxy-3-methoxyphenyl)acrylamido)-N-(3-methoxyphenyl)hexanamide (5c)



White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.85 (bs, 1H), 9.42 (bs, 1H), 7.96 (t, *J* = 5.6 Hz, 1H), 7.32 – 7.29 (m, 2H), 7.18 (t, *J* = 8.1 Hz, 1H), 7.12-7.11 (m, 2H), 6.98 (dd, *J* = 8.2, 1.5 Hz, 1H), 6.79 (d, *J* = 8.1 Hz, 1H), 6.60 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.44 (d, *J* = 15.7 Hz, 1H), 3.81 (s, 3H), 3.71 (s, 3H), 3.17 (q, *J* = 6.5 Hz, 2H), 2.30 (t, *J* = 7.4 Hz, 2H), 1.60 (quat, J= 7.5 2H), 1.47 (quat, *J* = 7.5, 2H), 1.32 (quat, *J* = 8.00 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.74, 165.75, 159.94, 148.65, 148.28, 140.97, 139.24, 129.88, 126.93, 121.94, 119.56, 116.10, 111.81, 111.18, 108.86, 105.31, 60.23, 55.99, 55.39, 39.01, 36.87, 29.51, 26.65, 25.30. ESI-HRMS for $C_{23}H_{28}N_2O_5$ (M+H)+ calcd. 413.2076, found 413.2076.

5.1.5 General procedure for the preparation of intermediate 7a-7b and 10a-10o

The different aromatic amines were dissolved in anhydrous DCM in the round bottom flask (RBF). Subsequently, K_2CO_3 (2.0 mmol) was introduced, followed by the dropwise addition

of chloroacetyl chloride (1.0 mmol) at 0°C. The reaction mixture was allowed to come to RT and stirred for 2h. The progression of the reaction was monitored using TLC, and upon completion of the reaction, a workup was carried out using DCM and water. The water layer was washed twice with DCM (2×10 ml). Anhydrous Na_2SO_4 was added to the combined organic layer. Afterward, the organic layer was filtered and evaporated using a rotatory evaporator under reduced pressure to yield the desired compounds **7a-7b** and **10a**-10o in good yield and further used without purification.

5.1.6 General procedure for the preparation of intermediate 8a-8b and 11a-11o

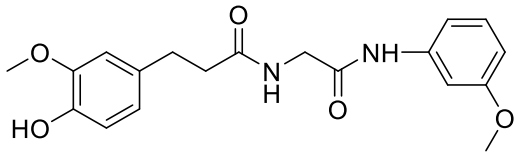
The **8a-8b** and **11a-11o** were synthesized using the intermediate **7a-7b** and **10a-10o**. The intermediates (7a-7b or 10a-10o, 1 mMol) were dissolved in 1,4-dioxane, followed by the addition of excess ammonia solution. The reaction was monitored via TLC; upon completion, the solvent was concentrated under reduced pressure, and the crude product was washed with dried diethyl ether. The crude was dried under reduced pressure and utilized in the next step without purification.

5.1.7 General procedure for the preparation of targets 9a-9f and 12a-12o

The various substituted phenyl propanoic acids for (**9a-9f**) or ferulic acid for (**12a-12o**) were dissolved in dried THF, followed by the addition of EDC-HCl and HOBt to generate active esters of respective acids. The reaction mixture was stirred for 15 minutes at RT; after that, amines (**8a-8b or 11a-11o**) were added, followed by the addition of triethylamine (TEA), and the reaction mixture was allowed to stir for 10-12 hr. TLC monitored the reaction, and the workup procedure was carried out using DCM and water upon completion of the reaction. The aqueous layer was treated twice with 20 ml DCM. The organic layers were combined and treated with Na_2SO_4 to remove water residues. The organic layer was filtered and concentrated

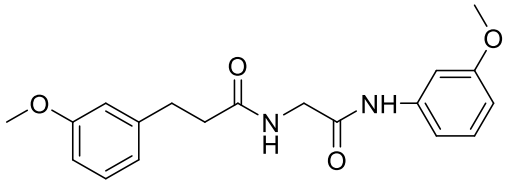
under reduced pressure using a rota-evaporator. The desired compounds were purified through column chromatography, using a mixture of ethyl acetate and hexane (80:20) as an eluting solvent.

5.1.7.1 3-(4-Hydroxy-3-methoxyphenyl)-N-(2-((3-methoxyphenyl)amino)-2-oxoethyl)propenamide (9a)



Yellowish white solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.92 (bs, 1H), 8.65 (s, 1H), 8.15 (t, *J* = 5.5 Hz, 1H), 7.29 (bs, 1H), 7.20 (t, *J* = 8.1 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 6.77 (s, 1H), 6.66-6.58 (m, 3H), 3.86 (d, *J* = 5.5 Hz, 2H), 3.73 (d, *J* = 7 Hz, 6H), 2.77 (t, *J* = 7.5 Hz, 2H), 2.42 t, *J* = 7.5 Hz, 2H).^{13}C NMR (126 MHz, DMSO-d_6,) δ 172.65, 168.41, 160.04, 147.90, 145.12, 140.57, 132.66, 130.06, 120.76, 115.79, 112.95, 111.92, 109.15, 105.49, 56.02, 55.48, 43.21, 37.74, 31.16. ESI-HRMS for $C_{19}H_{22}N_2NaO_5$ (M+Na)+ calcd. 381.1426, found 381.1410.

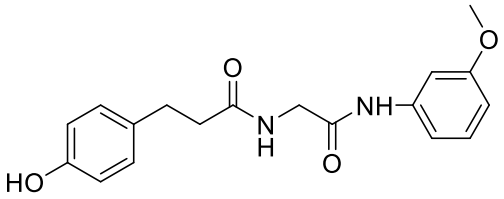
5.1.7.2 3-(3-Methoxyphenyl)-N-(2-((3-methoxyphenyl)amino)-2-oxoethyl)propenamide (9b)



White solid powder, ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.99 (s, 1H), 7.22 – 7.15 (m, 2H), 7.05 (d, *J* = 7.5 Hz, 1H), 6.87 (bs, 1H), 6.76 (m, 4H), 6.68 (d, *J* = 7.8 Hz, 1H), 4.08 (s, 2H), 3.77 (d, J = 13.5 Hz, 6H), 2.98 (t, *J* = 7. Hz, 2H), 2.62 (t, *J* = 7.5 Hz, 2H).^{13}C NMR (126 MHz, $CDCl_3$)

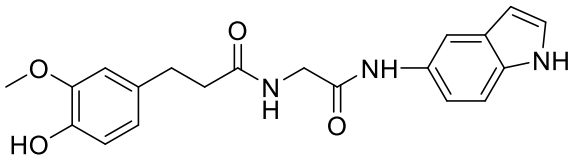
δ 173.41, 167.26, 160.10, 159.77, 141.97, 138.89, 129.62, 120.58, 114.15, 112.23, 111.58, 110.20, 105.80, 55.19, 44.61, 37.78, 31.59. ESI-HRMS for C19H22N2NaO4 (M+Na)+ calcd. 365.1477, found 365.1483.

5.1.7.3 3-(4-Hydroxyphenyl)-N-(2-((3-methoxyphenyl)amino)-2-oxoethyl)propenamide (9c)



White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.94 (s, 1H), 9.13 (s, 1H), 8.18 (d, *J* = 5.3 Hz, 1H), 7.31 (s, 1H), 7.21 (t, *J* = 8.1 Hz, 1H), 7.16 – 7.10 (m, 1H), 7.05 – 6.99 (m, 2H), 6.71 – 6.66 (m, 2H), 6.64 (d, *J* = 8.1 Hz, 1H), 3.88 (d, *J* = 5.8 Hz, 2H), 3.73 (s, 3H), 2.73-2.71 (m,, 2H), 2.43-2.40 (m, 2H). 13C NMR (126 MHz, DMSO-d_6) δ172.60, 172.56, 168.39, 160.00, 155.91, 140.54, 131.87, 130.01, 129.51, 115.53, 111.91, 109.11, 105.49, 55.42, 43.21, 37.72, 30.69. ESI-HRMS for $C_{18}H_{20}N_2NaO_4$ (M+Na)+ calcd. 351.1321, found 351.1320.

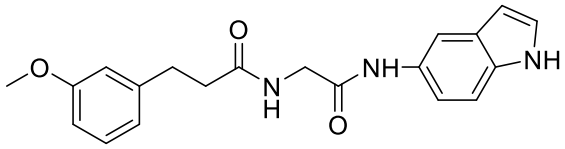
5.1.7.4 N-(2-((1H-Indol-5-yl)amino)-2-oxoethyl)-3-(4-hydroxy-3-methoxyphenyl)propenamide (9d)



Yellowish sticky mass. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.04 (bs, 1H), 9.76 (bs, 1H), 8.72 (s, 1H), 8.22 (t, J = 5.5 Hz, 1H), 7.89 (s, 1H), 7.37-7.35 (m, 2H), 7.24 (d, J = 8.5 Hz, 1H), 6.84 (s, 1H), 6.72-6.71 (m, 1H), 6.65 (d, J = 7.9 Hz, 1H), 6.42 (bs, 1H), 3.94 (d, J = 5.7 Hz, 2H),

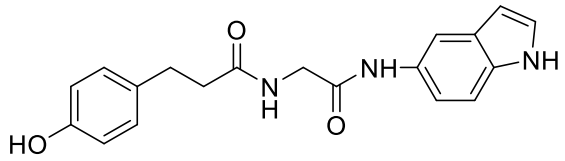
3.80 (s, 3H), 2.79 (t, *J* = 7 Hz, 2H), 2.49 (t, *J* = 6.5 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.53, 167.63, 147.84, 145.05, 133.16, 132.63, 131.29, 127.90, 126.35, 120.69, 115.73, 115.25, 112.86, 111.62, 111.15, 101.53, 55.94, 43.13, 37.74, 31.15. ESI-HRMS for $C_{20}H_{21}N_3O_4$ (M+H)+ calcd. 368.1610, found 368.1610.

5.1.7.5 N-(2-((1H-Indol-5-yl)amino)-2-oxoethyl)-3-(3-methoxyphenyl)propenamide (9e)



White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6,) δ 11.00 (bs, 1H), 9.71 (s, 1H), 8.20 (t, *J* = 5.5 Hz, 1H), 7.84 (bs, 1H), 7.32-7.30 (m, 2H), 7.20-7.17 (m, 2H), 6.81-6.80 (m, 2H), 6.75 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.37 (bs, 1H), 3.89 (d, *J* = 5.5 Hz, 2H), 3.73 (s, 3H), 2.82 (t, *J* = 7.5 Hz,, 2H), 2.51-2.49 (m, 2H).^{13}C NMR (126 MHz, DMSO-d_6) δ 172.32, 167.61, 159.75, 143.46, 133.19, 131.28, 129.74, 127.91, 126.35, 120.92, 115.30, 114.28, 111.87, 111.62, 111.21, 101.53, 55.35, 43.17, 37.17, 31.52. ESI-HRMS for $C_{20}H_{21}N_3NaO_3$ (M+Na)+ calcd. 374.1481, found 374.1479.

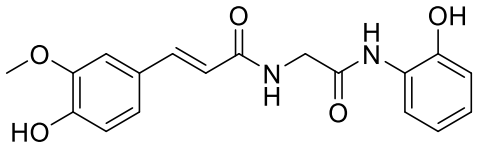
5.1.7.6 N-(2-((1H-Indol-5-yl)amino)-2-oxoethyl)-3-(4-hydroxyphenyl)propenamide (9f)



White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6,) δ 11.01 (s, 1H), 9.72 (s, 1H), 9.17 (s, 1H), 8.19 (t, *J* = 5.7 Hz, 1H), 7.85 (bs, 1H), 7.33 -7.31 (m, 2H), 7.20 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.02 (d, *J* = 8.3 Hz, 2H), 6.67 (d, *J* = 8.3 Hz, 2H), 6.38 (bs, 1H), 3.88 (d, *J* = 5.7 Hz, 2H), 2.74

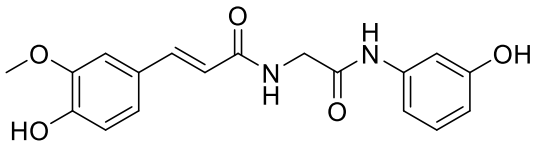
– 2.71 (m, 2H), 2.44 – 2.41 (m, 2H).^{13}C NMR (126 MHz, DMSO-d_6) δ 172.50, 167.65, 155.89, 133.16, 131.88, 131.28, 129.52, 127.90, 126.36, 115.69, 115.65, 115.39, 111.63, 111.16, 101.53, 43.13, 37.76, 30.70. ESI-HRMS for $C_{19}H_{19}N_3NaO_3$ (M+Na)+ calcd. 360.1324, found 360.1318.

5.1.7.7 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-((2-hydroxyphenyl)amino)-2-oxoethyl)acrylamide (12a)



^{1}H NMR (600 MHz, DMSO-d_6) δ 9.85 (bs, 1H), 9.47 (bs, 1H), 9.14 (s, 1H), 8.40 (t, *J* = 5.6 Hz, 1H), 7.88 (d, *J* = 7.9 Hz, 1H), 7.39 (d, *J* = 15.7 Hz, 1H), 7.17-7.16 (m, 1H), 7.03 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.92 (t, *J* = 7.2 Hz, 1H), 6.85 (d, *J* = 7.6 Hz, 1H), 6.81 – 6.75 (m, 2H), 6.59 (d, *J* = 15.7 Hz, 1H), 4.04 (d, *J* = 5.6 Hz, 2H), 3.81 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.44, 166.52, 148.91, 148.31, 147.66, 140.32, 126.72, 126.53, 124.82, 122.18, 121.68, 119.49, 118.70, 116.13, 115.69, 111.39, 56.02, 43.69. ESI-HRMS for $C_{18}H_{18}N_2NaO_5$ (M+Na)+ calcd. 365.1113, found 365.1105.

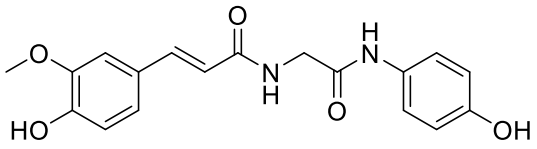
5.1.7.8 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-((3-hydroxyphenyl)amino)-2-oxoethyl)acrylamide (12b)



White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.89 (s, 1H), 9.44 (bs, 1H), 9.38(bs, 1H) 8.24 (t, *J* = 5.7 Hz, 1H), 7.37 (d, *J* = 15.7 Hz, 1H), 7.17 (d, *J* = 6.9 Hz, 2H), 7.08 (t, *J* =

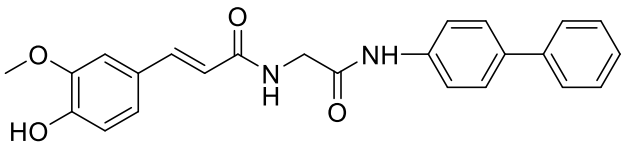
8.1 Hz, 1H), 7.03 (d, *J* = 8.1 Hz, 1H), 6.97 (d, *J* = 8.0 Hz, 1H), 6.81 (d, *J* = 8.1 Hz, 1H), 6.61 (d, *J* = 15.7 Hz, 1H), 6.46 (dd, *J* = 8.0, 1.3 Hz, 1H), 4.00 (d, *J* = 5.7 Hz, 2H), 3.82 (s, 3H).^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 168.15,166.29, 162.78, 158.09, 148.84, 148.31, 140.40, 139.92, 129.86, 126.85, 122.06, 119.11, 116.15, 111.41, 110.86, 110.37, 106.77, 56.02, 43.40. ESI-HRMS for $C_{18}H_{18}N_2NaO_5$ (M+Na)+ calcd. 365.1113, found 365.1105.

5.1.7.9 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-((4-hydroxyphenyl)amino)-2-oxoethyl)acrylamide (12c)



White solid powder, ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 9.77 (s, 1H), 9.46 (bs, 1H), 9.21 (bs, 1H), 8.23 (t, *J* = 5.7 Hz, 1H), 7.36 (m, 3H), 7.16-7.15 (m, 1H), 7.02 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.80 (d, *J* = 8.1 Hz, 1H), 6.70 (d, *J* = 8.8 Hz, 2H), 6.60 (d, *J* = 15.7 Hz, 1H), 3.97 (d, *J* = 5.7 Hz, 2H), 3.82 (bs, 3H).^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 167.56, 166.26, 153.79, 148.30, 139.86, 131.00, 126.82, 122.06, 121.40, 119.13, 116.13, 115.55, 111.34, 56.00, 43.20.

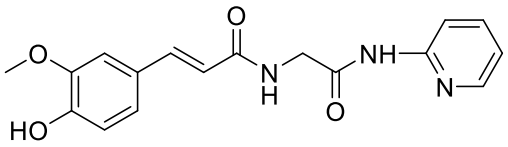
5.1.7.10 (E)-N-(2-([1,1'-Biphenyl]-4-ylamino)-2-oxoethyl)-3-(4-hydroxy-3-methoxyphenyl)acrylamide (12d)



White solid powder, ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 10.17 (s, 1H), 9.47 (s, 1H), 8.31-8.30 (m, 1H), 7.71 (d, *J* = 8.6 Hz, 2H), 7.67 – 7.61 (m, 4H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.38 (d, *J* = 15.6 Hz, 1H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.17 (s, 1H), 7.04 (d, *J* = 8.2 Hz, 1H), 6.82 (d, *J* = 8.1

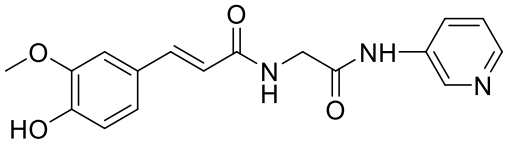
Hz, 1H), 6.63 (d, *J* = 15.7 Hz, 1H), 4.06 (d, *J* = 5.5 Hz, 2H), 3.82 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.41, 166.37, 148.88, 148.31, 140.07, 138.85, 135.37, 129.37, 127.47, 126.75, 122.11, 119.97, 119.02, 116.15, 111.36, 56.01, 43.40. ESI-HRMS for $C_{24}H_{22}N_2NaO_4$ (M+Na)+ calcd. 425.1477, found 425.1464.

5.1.7.11 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-oxo-2-(pyridin-2-ylamino)ethyl)acrylamide (12e)



White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.48 (bs, 1H), 9.43 (bs, 1H), 8.3-8.31 (m, 1H), 8.25 (t, *J* = 5.5 Hz, 1H), 8.06 (d, *J* = 8.2 Hz, 1H), 7.79 (t, *J* = 7.8 Hz, 1H), 7.37 (d, *J* = 15.7 Hz, 1H), 7.17 (bs, 1H), 7.15 – 7.07 (m, 1H), 7.03 (d, *J* = 8.2 Hz, 1H), 6.81 (d, *J* = 8.1 Hz, 1H), 6.60 (d, *J* = 15.7 Hz, 1H), 4.09 (d, *J* = 5.5 Hz, 2H), 3.82 (s, 3H).^{13}C NMR (126 MHz, DMSO-d_6) δ 169.19, 166.38, 152.25, 148.88, 148.48, 148.32, 140.06,138.69, 126.82, 122.11, 119.88, 118.98, 116.15, 113.84, 111.45, 56.05, 43.43. ESI-HRMS for $C_{17}H_{17}N_3O_4$ (M+H)+ calcd. 328.1297, found 328.1286.

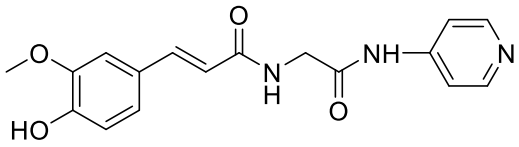
5.1.7.12 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-oxo-2-(pyridin-3-ylamino)ethyl)acrylamide (12f)



White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.26 (s, 1H), 9.45 (s, 1H), 8.76 (t, *J* = 2.4 Hz, 1H), 8.31 (t, *J* = 5.8 Hz, 1H), 8.27 (dd, *J* = 4.7, 1.4 Hz, 1H), 8.05-8.03 (m, 1H), 7.37

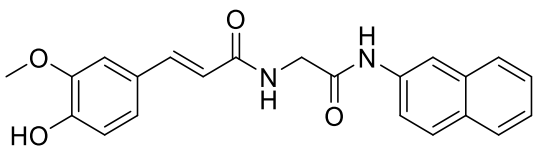
(m, 2H), 7.17-7.16 (m, 1H), 7.03 (dd, *J* = 8.2, 1.9 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 6.60 (d, *J* = 15.7 Hz, 1H), 4.05 (d, *J* = 5.8 Hz, 2H), 3.82 (s, 3H).^{13}C NMR (126 MHz, DMSO-d_6) δ 169.01, 166.36, 148.87, 148.31, 144.72, 141.28,140.03, 136.03, 126.81, 126.58, 124.14, 122.08, 119.00, 116.16, 111.42, 56.03, 43.31. ESI-HRMS for $C_{17}H_{17}N_3O_4$ (M+H)+ calcd. 328.1297, found 328.1287.

5.1.7.13 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-oxo-2-(pyridin-4-ylamino)ethyl)acrylamide (12g)



^{1}H NMR (600 MHz, DMSO-d_6) δ 10.44 (s, 1H), 9.47 (s, 1H), 8.44 (s, 1H), 8.33 (t, *J* = 5.8 Hz, 1H), 7.58 (bs, 2H), 7.37 (d, *J* = 15.7 Hz, 1H), 7.17-7.16 (m, 1H), 7.03 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.81 (d, *J* = 8.1 Hz, 1H), 6.60 (d, *J* = 15.7 Hz, 1H), 4.05 (d, *J* = 5.8 Hz, 2H), 3.82 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.65, 166.40, 150.90, 148.88, 148.30 , 145.90, 140.11, 126.76 , 122.10, 118.86, 116.14, 111.38 , 56.01, 43.53. ESI-HRMS for $C_{17}H_{17}N_3O_4$ (M+H)+ calcd. 328.1297, found 328.1297.

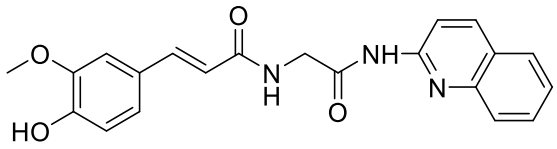
5.1.7.14 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-(naphthalen-2-ylamino)-2-oxoethyl)acrylamide (12h)



^{1}H NMR (600 MHz, DMSO-d_6) δ 10.03 (bs, 1H), 9.46 (bs, 1H), 8.37-8.36 (m, 1H), 8.10 (d, *J* = 7.4 Hz, 1H), 7.94 (m, 1H), 7.78 (d, *J* = 8.1 Hz, 1H), 7.69-7.68 (m, 1H), 7.57 – 7.54 (m, 2H),

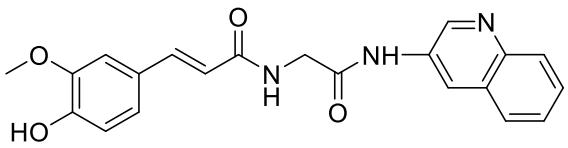
7.50 (t, *J* = 6.5 Hz, 1H), 7.41 (d, *J* = 13.5 Hz, 1H), 7.17 (bs, 1H), 7.04 (d, *J* = 7.0 Hz, 1H), 6.81 (d, *J* = 6.5 Hz, 1H), 6.63 (d, *J* = 13.0 Hz, 1H), 4.20 (d, *J* = 3.6 Hz, 2H), 3.81 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.21, 166.50, 148.84 148.31, 140.00, 134.18, 133.82, 128.58, 126.84, 126.54, 126.33, 126.06, 125.87, 123.27, 122.11, 119.11, 116.14, 111.36, 56.01, 43.37. ESI-HRMS for $C_{22}H21N_2NaO_4$ (M+Na)+ calcd. 399.1321, found 399.1309.

5.1.7.15 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-oxo-2-(quinolin-2-ylamino)ethyl)acrylamide (12i)



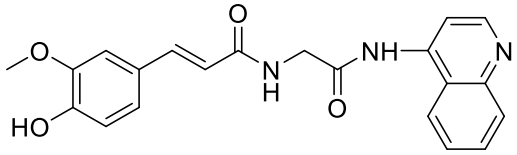
Slightly yellowish white solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.45 (s, 1H), 9.50 (s, 1H), 8.83 (bs, 1H), 8.42-8.36 (m, 2H), 7.93 (d, *J* = 8.5 Hz, 1H), 7.73 (d, *J* = 7.5 Hz, 1H), 7.49 – 7.29 (m, 2H), 7.17 (s, 1H), 7.04 (d, *J* = 7.5 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 6.62 (d, *J* = 15.7 Hz, 1H), 4.11 (d, *J* = 4.8 Hz, 2H), 3.82 (s, 3H).^{13}C NMR (126 MHz, DMSO-d_6) δ 168.95, 166.49, 151.41, 148.83, 148.31, 140.14, 136.10, 129.07, 126.78, 124.87, 122.17, 120.66, 118.92, 116.43, 116.13, 111.32,56.01, 43.50. ESI-HRMS for $C_{21}H_{19}N_3O_4$ (M+H)+ calcd. 378.1454, found 378.1449.

5.1.7.16 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-oxo-2-(quinolin-3-ylamino)ethyl)acrylamide (12j)



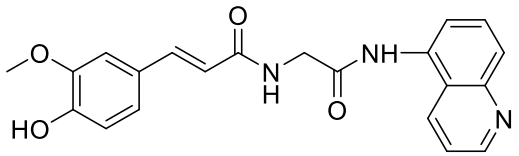
Brownish-yellow solid powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.40 (s, 1H), 9.45 (s, 1H), 9.20 (bs, 1H) 8.80 (dd, *J* = 4.2, 1.6 Hz, 1H), 8.36-8.29 (m, 2H), 7.99 (d, *J* = 9.1 Hz, 1H), 7.84 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.51-7.48 (m, 1H), 7.37 (d, *J* = 15.7 Hz, 1H), 7.18-7.17 (m, 1H), 7.03 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.81 (d, *J* = 8.1 Hz, 1H), 6.61 (d, *J* = 15.8 Hz, 1H), 4.09 (d, *J* = 5.8 Hz, 2H), 3.82 (s, 3H). 13C NMR (126 MHz, DMSO-d_6) δ 168.81, 166.84, 149.43, 149.01, 148.35, 140.58, 138.42, 137.09, 134.50, 128.29, 127.48, 126.74,122.68, 122.45, 122.24, 118.56, 116.63, 116.19, 111.54, 56.08, 44.44. ESI-HRMS for $C_{21}H_{19}N_3O_4$ (M+H)+ calcd. 378.1454, found 378.1450.

5.1.7.17 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-oxo-2-(quinolin-4-ylamino)ethyl)acrylamide (12k)



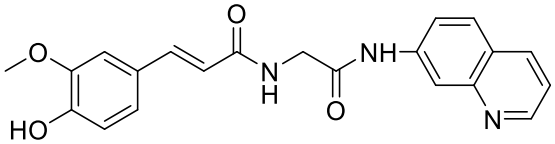
White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.31 (s, 1H), 9.41 (s, 1H), 8.76 (d, *J* = 4.0 Hz, 1H), 8.34 (s, 2H), 8.05 (d, *J* = 4.3 Hz, 1H), 7.97 (d, *J* = 8.1 Hz, 1H), 7.74 (t, *J* = 7.0 Hz, 1H), 7.61 (t, *J* = 6.8 Hz, 1H), 7.35 (d, *J* = 15.8 Hz, 1H), 7.13 (s, 1H), 6.99 (d, *J* = 7.5 Hz, 1H), 6.76 (d, *J* = 8.1 Hz, 1H), 6.58 (d, *J* = 15.4 Hz, 1H), 4.23 (d, *J* = 5.0 Hz, 2H), 3.77 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.86, 166.42, 149.50, 148.89, 148.33, 145.17, 140.02, 137.30, 135.93, 130.04, 128.79, 126.84, 123.76, 122.25, 122.09, 119.05, 116.18, 115.45, 111.46, 56.05, 43.54. ESI-HRMS for $C_{21}H_{19}N_3O_4$ (M+H)+ calcd. 378.1454, found 378.1445.

5.1.7.18 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-oxo-2-(quinolin-5-ylamino)ethyl)acrylamide (12l)



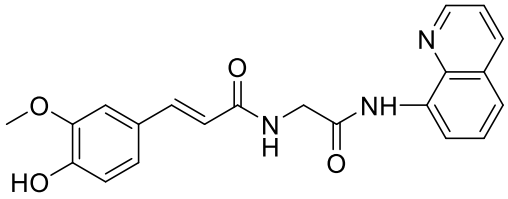
Yellowish white solid powder. 1H NMR (500 MHz, DMSO-d_6): δ 11.00 (s, 1H), 9.82 (s, 1H), 9.47 (s, 1H), 8.25 (t, *J* = 5.5 Hz, 1H), 7.85 (d, *J*= 5.5 Hz, 1H), 7.37 (d, J=15.7 Hz, 1H) 7.32-7.29 (m, 2H), 7.21 (t, J = 5 Hz, 2H), 7.02 (d, J = 10 Hz, 1H), 6.80 (d, *J* = 10 Hz, 1H), 6.62 (d, *J* = 15 Hz, 1H), 6.37 (s, 1H) 4.01 (d, J =5.5 Hz, 2H), 3.81 (s, 3H), 13C NMR (126 MHz, DMSO-d_6): δ 165.78, 162.39, 147.77, 147.07, 143.18, 131.68, 131.64, 129.19, 127.55, 123.97, 123.94, 123.85, 123.73, 121.95 115.43, 115.25, 115.08 114.11, 110.07, 67.93, 56.00. ESI-HRMS for $C_{21}H_{19}N_3O_4$ (M+H)+ calcd. 378.1454, found 378.1445.

5.1.7.19 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-oxo-2-(quinolin-7-amino)ethyl)acrylamide (12m).



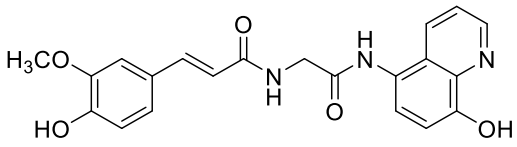
White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.43 (bs, 1H), 9.48 (bs, 1H), 8.84 (s, 1H), 8.44 (s, 1H), 8.39 – 8.22 (m, 2H), 7.94 (d, *J* = 8.0 Hz, 1H), 7.74 (d, *J* = 6.0 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.18 (s, 1H), 7.05-7.03 (m, 1H), 6.82-6.81 (m, 1H), 6.64 (d, *J* = 15.6 Hz, 1H), 4.12 (s, 2H), 3.83 (s, 3H).^{13}C NMR (126 MHz, DMSO-d_6) δ 166.38, 151.40, 148.92, 148.31, 140.03, 136.02, 129.04, 125.47, 124.85, 122.10, 120.64, 119.02, 116.47,116.15, 111.38, 56.01, 43.54. ESI-HRMS for $C_{21}H_{19}N_3O_4$ (M+H)+ calcd. 378.1454, found 378.1444.

5.1.7.20 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-oxo-2-(quinolin-8-ylamino)ethyl)acrylamide (12n)



Yellow solid powder 1H NMR (500 MHz, DMSO-d_6) δ 10.39 (s, 1H), 9.47 (s, 1H), 8.87 (dd, J = 4.1, 1.4 Hz, 1H), 8.69 – 8.59 (m, 2H), 8.41 (dd, J = 8.3, 1.3 Hz, 1H), 7.68 (d, J = 7.6 Hz, 1H), 7.63-7.58 (m, 2H), 7.44 (d, J = 15.7 Hz, 1H), 7.21 (bs, 1H), 7.08 (dd, J = 8.1, 1.4 Hz, 1H), 6.83 (d, J = 8.1 Hz, 1H), 6.66 (d, J = 15.8 Hz, 1H), 4.18 (d, J = 5.8 Hz, 2H), 3.84 (s, 3H). 13C NMR (126 MHz, DMSO-d_6) δ 168.81, 166.84, 149.44, 149.02, 148.36, 140.58, 138.43, 137.09, 134.51, 128.30, 127.48, 126.74, 122.68, 122.45, 122.24, 118.57, 116.63, 116.20, 111.56, 56.09, 44.44. ESI-HRMS for $C_{21}H_{19}N_3O_4$ (M+H)+ calcd. 378.1454, found 378.1442.

5.1.7.21 (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-((8-hydroxyquinolin-5-yl)amino)-2 oxoethyl)acrylamide (12o)



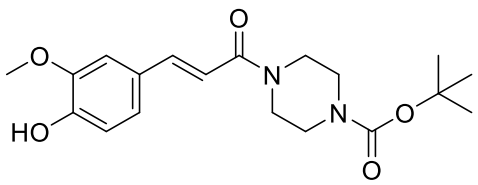
Yellow solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.89 (s, 1H), 9.83 (s, 1H), 9.47 (s, 1H), 8.87 (s, 1H), 8.36 (d, *J* = 7.2 Hz, 2H), 7.61 (dd, *J* = 8.3, 3.9 Hz, 1H), 7.40 (m, 2H), 7.17 (s, 1H), 7.04 (d, *J* = 8.1 Hz, 2H), 6.81 (d, *J* = 8.1 Hz, 1H), 6.62 (d, *J* = 15.7 Hz, 1H), 4.13 (d, *J* = 5.6 Hz, 2H), 3.82 (s, 3H). 13C NMR (126 MHz, DMSO-d_6) δ 168.87 , 166.41, 149.51, 148.89, 148.31, 145.15, 140.02, 137.30, 135.94, 130.04, 128.78, 126.80, 123.74, 122.27,

122.08, 118.99, 116.15, 115.41, 111.40, 56.01, 43.52. ESI-HRMS for $C_{21}H_{19}N_3O_5$. (M+H)+ calcd. 394.1403, found 394.1388.

5.1.8 General procedure for the synthesis of 14 and 15

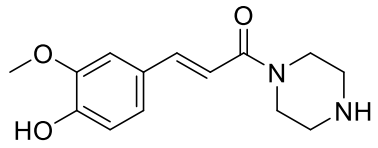
The commercially available ferulic acid (13), 2 gm, 10.30 mmol, was dissolved in THF, followed by EDCI.HCl (2.5 gm, 0.396 13.39 mmol), HOBt (2.06 gm, 13.39 mmol), and the mixture stirred for 10−15 min, at rt. Then Tert-Butyl piperazine-1-carboxylate was added to the stirring mixture, followed by the addition of diisopropylethylamine (DIPEA, 2.3 mL, 13.39 mmol), and the reaction was stirred for 12 hours. The reaction progress was monitored with TLC; once the reaction was completed, the workup of the reaction was carried out in water and ethyl-acetate. The ethyl-acetate layer was separated and evaporated under reduced pressure to get the crude product and purify this crude by column chromatography using ethyl acetate and hexane (0.4:0.6) to get intermediate **14**. Intermediate **14** was dissolved in DCM, then TFA was added, and the reaction was stirred for 2 hr. Once the reaction was completed, the work was performed using DCM and water. The DCM layer was separated and evaporated under reduced pressure to get the crude product and the compound was purified by using column chromatography to get intermediate **15** using a methanol and ethyl acetate (2:98) ratio.

5.1.8.1 Tert-butyl (E)-4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazine-1-carboxylate (14)



White solid crystal. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.44 (s, 1H), 7.42 (d, J = 15.5 Hz, 1H), 7.31 (s, 1H), 7.09 (d, J = 7.5, 1H), 7.04 (d, J = 15.5 Hz, 1H), 6.77(d, J = 8.0 Hz, 1H), 3.82 (s, 3H), 3.68 (bs, 2H), 3.54 (bs, 2H), 3.35 (bs, 4H), 1.41 (s, 9H).

5.1.8.2 (E)-3-(4-Hydroxy-3-methoxyphenyl)-1-(piperazin-1-yl)prop-2-en-1-one (15)



Yellowish oily mass. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.42 (d, J = 48.7 Hz, 1H), 7.46 (d, J = 15.2 Hz, 1H), 7.34 (s, 1H), 7.11 (t, J = 11.6 Hz, 2H), 6.79 (d, J = 8.1 Hz, 1H), 3.84 (s, 3H), 3.76 (s, 2H), 3.64 (s, 2H), 3.38 (s, 4H).

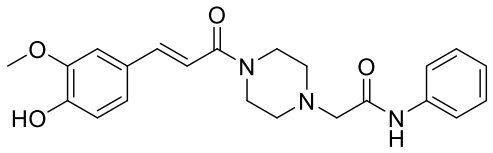
5.1.9 General procedure for the synthesis of intermediate 17a-17m and 20a-20f.

The various aromatic amines (**16a-16m**) or (**20a-20f**) were dissolved in dry DCM in RBF, followed by the addition of K_2CO_3 (2.0 mmol). The reaction mixture was cooled to 0 °C for 10 minutes. Once the reaction mixture reached 0 °C, chloroacetyl chloride (1.0 mmol) was slowly added. Then, the reaction mixture was allowed to reach room temperature, and the reaction was stirred for two hours. After 2 hr, the reaction was monitored with the help of TLC. Once the reaction was completed, the workup was done in DCM and water. The water layer was washed twice with DCM (2×10 ml). Then, both organic layers were combined, and anhydrous sodium sulfate (Na_2SO_4) was added to remove water from the organic layer. After that, the organic layer was filtered and concentrated under reduced pressure using a rotary evaporator. This process yielded the desired compounds **17a-17m** and **20a-20f** in good yield, which were used in the next step without further purification.

5.1.10 General procedure for synthesis target compounds 18a-18m and 21a-21f

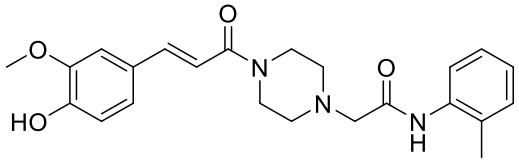
The intermediates **17a-17m** or **20a-20f** were dissolved in ethanol in RBF, then added K_2CO_3 and intermediate **FAPIP (15)** and refluxed the reaction for 6 hr. The reaction was monitored with the help of TLC. Evaporate the solvent under reduced pressure using a rota-evaporator. Then water and ethyl-acetate were added to the reaction mixture to separate the organic layer, and the solvent was to get crude product and purified with the help of column chromatography using methanol and ethyl-acetate (0.3:7) to get target compounds **18a-18m** or **21a-21f**.

5.1.10.1 (E)-2-(4-(3-(4-Hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-phenylacetamide (18a)



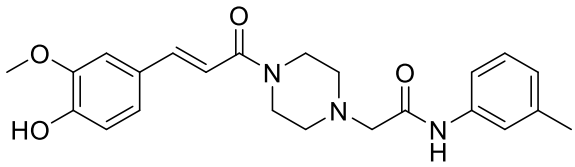
White solid powder, 1H NMR (500 MHz, DMSO-d_6) δ 9.77 (s, 1H), 9.43 (s, 1H), 7.64 (d, J = 7.6 Hz, 2H), 7.42 (d, J = 15.2 Hz, 1H), 7.32-7.29 (m, 3H), 7.10-7.07 (m, 2H), 7.06 (d, J = 6.2 Hz, 1H), 6.77 (d, J = 8.1 Hz, 1H), 3.83 (s, 3H), 3.78 (s, 2H), 3.64 (s, 2H), 3.19 (s, 2H), 2.57 (s, 4H). 13C NMR (126 MHz, DMSO-d_6) δ 168.58, 165.30, 148.91, 148.30, 142.77, 139.06, 129.13, 127.16, 123.90, 123.02, 119.98, 115.88, 114.91, 111.62, 61.88, 56.25. ESI-HRMS for $C_{22}H_{25}N_3O_4$. (M+H)+ calcd. 396.1923, found 396.1911.

5.1.10.2 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-(o-tolyl) acetamide (18b)



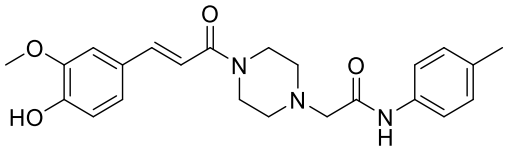
White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.54 (s, 1H), 9.52 (s, 1H), 7.82 (d, *J* = 8.0 Hz, 1H), 7.53 (d, *J* = 15.0 Hz, 1H), 7.44 (d, *J* = 1.5 Hz, 1H), 7.34 – 7.27 (m, 2H), 7.21 – 7.15 (m, 2H), 6.88 (d, *J* = 8.0 Hz, 1H), 3.93 (s, 2H), 3.90 (s, 2H), 3.76 (s, 2H), 3.45 (s, 2H), 2.70 (d, *J* = 10.0 Hz, 4H), 2.35 (s, 2H).^{13}C NMR (126 MHz, DMSO-d_6) δ 168.29, 165.39, 148.95, 148.31, 142.87, 136.51, 130.74, 130.07, 127.15, 126.67, 125.08, 123.23,123.08, 115.88, 114.87, 111.61, 61.66, 56.26, 18.04. ESI-HRMS for $C_{23}H_{27}N_3O_4$. (M+H)+ calcd. 410.2080, found 410.2066.

5.1.10.3 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-(o-tolyl) acetamide (18c)



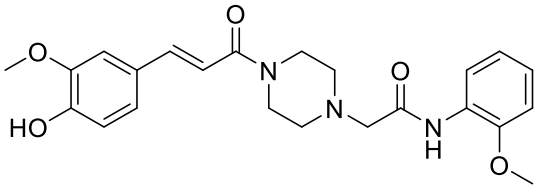
White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.70 (s, 1H), 9.46 (s, 1H), 7.47-7.40 (m, 2H), 7.34 (d, *J* = 7.2 Hz, 1H), 7.19 (t, *J* = 7.8 Hz, 1H), 7.12-7.05 (m, 3H), 6.89 (d, *J* = 7.4 Hz, 1H), 6.80 – 6.77 (m, 1H), 3.83 (s, 3H), 3.77 (s, 2H), 3.64 (s, 2H), 3.18 (s, 2H), 2.57 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.52, 165.53, 165.31, 149.01, 148.31, 142.79, 138.96, 138.34, 128.99, 127.14, 127.09, 124.60, 123.04, 120.49, 117.13, 115.91, 114.89, 111.66, 111.59, 61.87, 56.25, 21.62. ESI-HRMS for $C_{23}H_{27}N_3O_4$. (M+H)+ calcd. 410.2080, found 410.2067.

5.1.10.4 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-(p-tolyl)acetamide (18d)



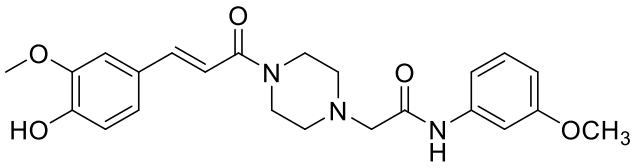
Yellowish white solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.68 (s, 1H), 9.44 (s, 1H), 7.52 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 15.2 Hz, 1H), 7.33 (s, 1H), 7.12 – 7.10 (m, 2H), 7.109 – 7.05 (m, 2H), 6.78 (d, J = 8.1 Hz, 1H), 3.83 (s, 3H), 3.78 (s, 2H), 3.64 (s, 2H), 3.17 (s, 2H), 2.56 (s, 4H), 2.275(s, 1H).^{13}C NMR ($CDCl_3$, 126 MHz): δ 168.34, 165.31, 148.92, 148.30, 142.78, 136.54, 132.82, 129.50, 127.16, 123.02, 120.01, 115.88, 114.91, 111.62,61.85, 56.52, 56.26 20.92. ESI-HRMS for $C_{23}H_{27}N_3O_4$. (M+H)+ calcd. 410.2080, found 410.2069.

5.1.10.5 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-(2-methoxyphenyl) acetamide (18e)



Greyish solid powder^{1}H NMR (500 MHz, D DMSO-d_6) δ 9.72 (s, 1H), 9.43 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 7.44 (d, *J* = 15.0 Hz, 1H), 7.34-7.33 (m, 1H), 7.12-7.10 (m, 2H), 7.07-7.06 (m, 2H), 6.95 – 6.92 (m, 1H), 6.78 (d, *J* = 8.5 Hz, 1H), 3.90 (s, 3H), 3.83-3.81 (m, 5H), 3.66 (s, 2H), 3.20 (s, 2H), 2.57 (s, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.14, 165.39, 148.96, 148.71, 148.31, 142.94, 127.49, 127.15, 124.27, 123.06, 121.02, 119.41, 115.90, 114.84, 111.68, 111.38, 61.63, 56.48, 56.28. ESI-HRMS for $C_{23}H_{27}N_3O_5$. (M+H)+ calcd. 426.2029, found 410.2020.

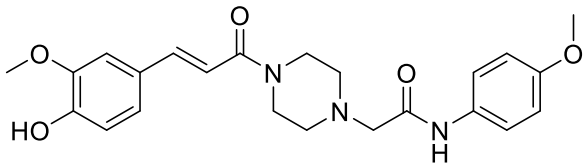
5.1.10.6 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-(3-methoxyphenyl) acetamide (18f)



White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.75 (s, 1H), 9.43 (s, 1H), 7.42 (d, J = 15.5 Hz, 1H), 7.35 – 7.33 (m, 2H), 7.21-7.20 (m, 2H), 7.11 – 7.05 (m, 2H), 6.78 (d, J = 8.0 Hz, 1H), 6.66-6.64 (m, 1H), 3.83 (s, 3H), 3.78 (s, 2H), 3.74 (s, 3H), 3.64 (s, 2H), 3.19 (s, 2H), 2.57 (s, 4H).^{13}C NMR (126 MHz, DMSO-d_6) δ 168.65, 165.31, 159.97, 148.92, 148.30, 142.77, 140.25, 129.93, 127.17, 123.02, 115.89, 114.91, 112.20, 111.64, 109.36, 105.71, 61.90, 56.26, 55.48. ESI-HRMS for $C_{23}H_{27}N_3O_5$. (M+H)+ calcd. 426.2029, found 410.2021.

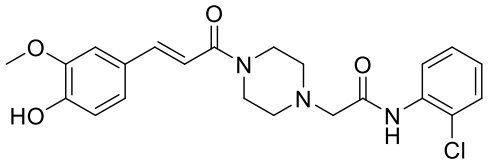
5.1.10.7 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-(4-methoxyphenyl)

acetamide (18g)



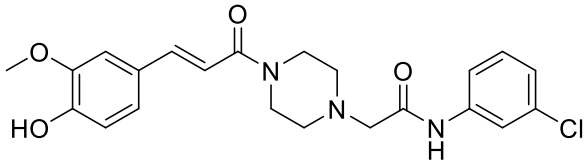
White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.64 (s, 1H),9.47(s,1H),7.54 (d, J = 9.0 Hz, 2H), 7.42 (d, J = 15.0 Hz, 1H), 7.33 (d, J = 1.5 Hz, 1H), 7.11 – 7.05 (m, 2H), 6.89 (d, J = 9.0 Hz, 2H), 6.78 (d, J = 8.0 Hz, 1H), 3.83 (s,3H), 3.78 (s, 2H), 3.73 (s, 3H), 3.64 (s, 2H), 3.16 (s, 2H), 2.56 (s, 4H).^{13}C NMR (126 MHz, DMSO-d_6) δ 168.08, 165.31, 155.84, 148.32, 142.78, 132.20, 123.03, 121.63, 115.89, 114.89, 114.24, 111.62, 61.85, 60.23, 56.26, 55.64. . ESI-HRMS for $C_{23}H_{27}N_3O_5$. (M+H)+ calcd. 426.2029, found 410.2018.

5.1.10.8 (E)-N-(2-chlorophenyl)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl) acetamide (18h)



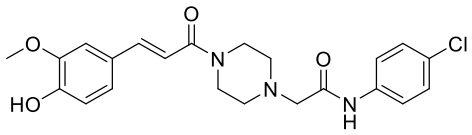
White solid powder, 1H NMR (500 MHz, DMSO-d_6) δ 9.92 (s, 1H), 9.43 (s, 1H), 7.69 (d, J = 8.9 Hz, 2H), 7.42 (d, J = 15.2 Hz, 1H), 7.37 (d, J = 8.9 Hz, 2H), 7.33 (s, 1H), 7.10 – 7.04 (m, 2H), 6.78 (d, J = 8.1 Hz, 1H), 3.83 (s, 3H), 3.77 (s, 2H), 3.64 (s, 2H), 3.20 (s, 2H), 2.57 (s, 4H). 13C NMR (DMSO-d_6, 126 MHz,): δ 168.82, 165.30, 148.91, 148.30, 142.77, 138.05, 129.02, 127.45, 127.16, 123.02, 121.57, 115.88, 114.90, 111.62, 61.84, 56.26. ESI-HRMS for $C_{22}H_{24}ClN_3O_4$. (M+H)+ calcd. 430.1534, found 430.1521.

5.1.10.9 (E)-N-(3-chlorophenyl)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl) acetamide (18i)



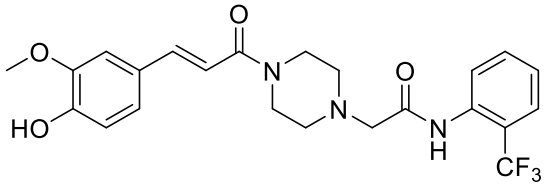
White solid powder, ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.93 (s, 1H), 9.45 (s, 1H), 8.23 (d, J = 8.2 Hz, 1H), 7.54 (d, J = 7.9 Hz, 1H), 7.44 (d, J = 15.2 Hz, 1H), 7.38-7.33 (m, 2H), 7.16 (t, J = 7.7 Hz, 1H), 7.10 (m, 2H), 6.78 (d, J = 8.1 Hz, 1H), 3.84 (s, 5H), 3.67 (s, 2H), 3.25 (s, 2H), 2.63 (s, 4H). ^{13}C NMR (DMSO-d_6, 126 MHz): δ168.74, 165.46, 148.96, 148.31, 142.93, 134.86, 129.76, 128.35, 127.14, 125.62, 123.68, 123.08, 122.16, 115.89, 114.83, 111.64, 61.49, 56.27. ESI-HRMS for $C_{22}H_{24}ClN_3O_4$. (M+H)+ calcd. 430.1534, found 430.1517.

5.1.10.10 (E)-N-(4-chlorophenyl)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl) acetamide (18j)



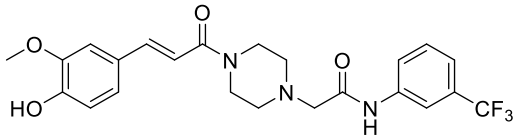
White solid powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.92 (s, 1H), 9.44 (s, 1H), 7.69 (d, J = 8.7 Hz, 2H), 7.42 (d, J = 15.2 Hz, 1H), 7.37 (d, J = 8.9 Hz, 2H), 7.33 (s, 1H), 7.10– 7.05 (m, 2H), 6.78 (d, J = 8.1 Hz, 1H), 3.83 (s, 3H), 3.77 (s, 2H), 3.63 (s, 2H), 3.20 (s, 2H), 2.56 (s, 4H). 13C NMR ($CDCl_3$, 126 MHz): δ 168.83, 165.31, 148.92, 148.30, 142.78, 138.05, 129.02, 127.46, 127.16, 123.02, 121.58, 115.89, 114.90, 111.62, 61.84, 56.26. ESI-HRMS for $C_{22}H_{24}ClN_3O_4$. (M+H)+ calcd. 430.1534, found 430.1514.

5.1.10.11 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-(2-(trifluoromethyl) phenyl) acetamide (18k)



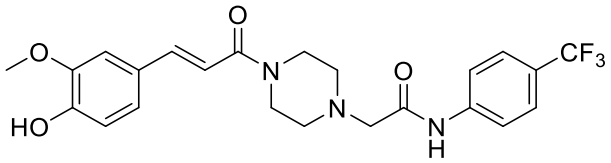
White solid powder ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.16 (s, 1H), 9.50 (s, 1H), 7.87 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 8.7 Hz, 2H), 7.42 (d, J = 15.2 Hz, 1H), 7.31 (d, J = 1.3 Hz, 1H), 7.10 – 7.05 (m, 2H), 6.78 (d, J = 8.5 Hz, 1H), 3.82 (s, 3H), 3.77 (s, 2H), 3.64 (s, 2H), 3.24 (s, 2H), 2.56 (d, J = 16.2 Hz, 4H).^{13}C NMR (126 MHz, DMSO-d_6) δ 169.42, 165.39, 148.90, 148.30, 142.85, 142.59, 127.14, 126.46, 123.02, 119.90, 115.88, 114.85, 111.55, 61.79, 56.23. ESI-HRMS for $C_{23}H_{24}F_3N_3O_4$. (M+H)+ calcd. 464.1797, found 464.1793.

5.1.10.12 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-(3-(trifluoromethyl) phenyl) acetamide (18l)



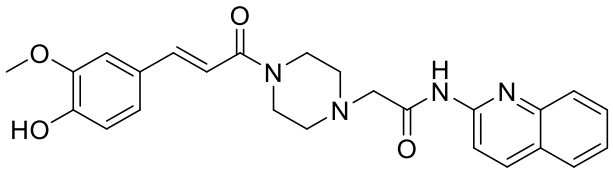
White solid powder 1H NMR (500 MHz, DMSO-d_6) δ 10.14 (s, 1H), 9.42 (s, 1H), 7.88 (d, J = 8 Hz, 2H), 7.68 (d, J = 8.5 Hz, 2H), 7.44 – 7.40 (m, 1H), 7.32 (s, 1H), 7.09 -7.04 (m, 2H), 6.77 (dd, J = 8.0, 2 Hz, 1H), 3.82 (s, 3H), 3.77 (s, 2H), 3.64 (s, 2H), 3.24 (s, 2H), 2.57-2.50 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.55, 149.25, 148.33, 143.71, 139.30, 130.71, 126.93, 123.59, 123.15, 115.98, 114.16, 111.95, 56.32. ESI-HRMS for $C_{23}H_{24}F_3N_3O_4$. (M+H)+ calcd. 464.1797, found 464.1798.

5.1.10.13 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-(4-(trifluoromethyl) phenyl) acetamide (18m)



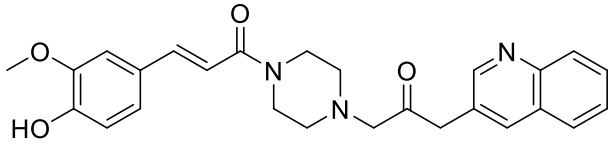
White solid powder ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.15 (s, 1H), 9.43 (s, 1H), 7.89 (d, *J* = 8.0 Hz, 2H), 7.69 (d, *J* = 8.0 Hz, 2H), 7.49 – 7.38 (m, 1H), 7.33 (s, 1H), 7.10-7.05 (m, 2H), 6.78 (dd, *J* = 8.0, 2.0 Hz, 1H), 3.83 (s, 3H), 3.78 (s, 2H), 3.65 (s, 2H), 3.25 (s, 2H), 2.57 (d, *J* = 14.5 Hz, 4H).^{13}C NMR (126 MHz, DMSO-d_6) δ 169.36, 165.32, 148.94, 148.31, 142.79,142.68, 127.16, 126.44, 125.92, 123.77, 123.01, 119.85, 115.89, 114.90, 111.64, 61.85, 56.25. ESI-HRMS for $C_{23}H_{24}F_3N_3O_4$. (M+H)+ calcd. 464.1797, found 464.1789.

5.1.10.14 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-(quinolin-2-yl) acetamide (21a)



Yellowish solid powder ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.39 (s, 1H), 8.39 (d, J = 9.0 Hz, 1H), 8.33 (d, J = 9.0 Hz, 1H), 7.93 (d, J = 7.5 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.73 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.5 Hz, 1H), 7.42 (d, J = 15.2 Hz, 1H), 7.33 (s, 1H), 7.10-7.06 (m, 2H), 6.80 (d, J = 8.1 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 2H), 3.64 (s, 2H), 2.82 (s, 2H), 2.62 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.17, 165.37, 148.95, 148.32, 142.83, 134.15, 128.77, 127.18, 126.59, 126.09, 125.64, 123.07, 122.44, 121.27, 115.90, 115.43, 114.92, 111.65, 61.77, 56.28. ESI-HRMS for $C_{25}H_{26}N_4O_4$. (M+H)+ calcd. 447.2032, found 447.2029.

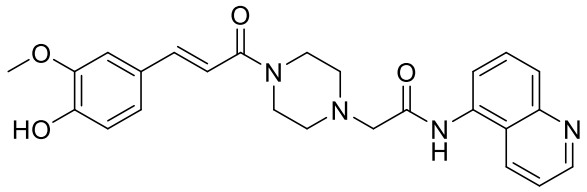
5.1.10.15 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl) piperazin-1-yl)-N-(quinolin-3-yl)acetamide (21b)



Yellowish solid powder ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.12 (s, 1H), 9.41 (s, 1H), 8.79 (dd, J = 4.5, 1.5 Hz 1H), 8.41 (d, J = 2.2 Hz, 1H), 8.30 (d, J = 8.2 Hz, 1H), 7.98 (d, J = 9.1 Hz, 1H), 7.90 (dd, J = 9.1, 2.3 Hz, 1H), 7.50-7.47 (m, 1H), 7.43 (d, J = 15.2 Hz, 1H), 7.33 (d, J = 1.8 Hz, 1H), 7.11 – 7.06 (m, 2H), 6.78 (d, J = 8.1 Hz, 1H), 3.83 (s, 3H), 3.80 (s, 2H), 3.67 (s, 2H), 3.28 (s, 2H), 2.62 (s, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.14, 165.33, 149.57, 148.93, 148.31, 145.26, 142.77, 136.99, 135.94, 129.89, 128.72, 127.18, 124.15, 123.01,

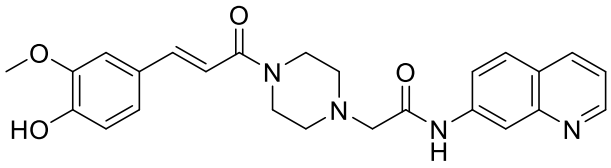
122.25, 115.87, 114.94, 111.68, 61.89, 56.28, 55.38, 49.07. ESI-HRMS for $C_{25}H_{26}N_4O_4$. (M+H)+ calcd. 447.2032, found 447.2022.

5.1.10.16 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl) piperazin-1-yl)-N-(quinolin-5-yl)acetamide (21c)



Redish solid powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.16 (s, 1H), 9.46 (s, 1H), 8.94 (dd, *J* = 4.1, 1.6 Hz, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 8.3 Hz, 1H), 7.83 (d, *J* = 7.1 Hz, 1H), 7.78 – 7.75 (m, 1H), 7.61 (dd, *J* = 8.6, 4.1 Hz, 1H), 7.44 (d, *J* = 15.2 Hz, 1H), 7.35 (d, *J* = 1.8 Hz, 1H), 7.12-7.08 (m, 2H), 6.79 (d, *J* = 8.1 Hz, 1H), 3.88 (s, 2H), 3.84 (s, 3H), 3.71 (s, 2H), 3.39 (s, 2H), 2.70 -2.68(m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.35, 151.03, 148.94, 148.56, 148.31, 142.91, 133.95, 131.71, 127.14, 123.30, 123.12, 121.69, 115.95, 114.87, 111.57, 56.35. ESI-HRMS for $C_{25}H_{26}N_4O_4$. (M+H)+ calcd. 447.2032, found 447.2027.

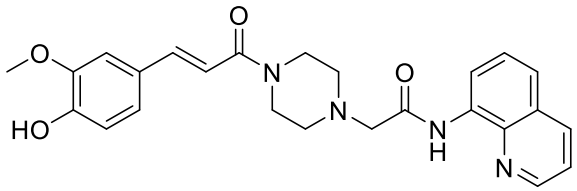
5.1.10.17 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-(quinolin-7-yl)acetamide (21d)



White solid powder 1H NMR (500 MHz, $CDCl_3$) δ 9.41 (s, 1H), 8.91 (s, 1H), 8.22 – 8.16 (m, 1H), 8.11 (d, J = 8.8 Hz, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.66 (d, J = 15.3 Hz, 1H), 7.40 (dd, J = 8.1, 4.3 Hz, 3H), 7.13 (d, J = 8.1 Hz, 1H), 7.03 (s, 1H), 6.94 (d, J = 8.2 Hz, 1H), 6.75 (d, J =

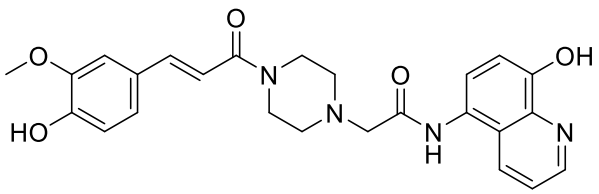
15.3 Hz, 1H), 3.96 (s, 3H), 3.87 (s, 3H), 3.29 (s, 2H), 2.74 (s, 3H). 13C NMR (126 MHz, $CDCl_3$) δ 166.38, 151.40, 148.92, 148.31, 140.03, 136.02, 129.04, 125.47, 124.85, 122.10, 120.64, 119.02, 116.47, 116.15, 111.38, 56.01, 43.54. ESI-HRMS for $C_{25}H_{26}N_4O_4$. (M+H)+ calcd. 447.2032, found 447.2028.

5.1.10.18 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-(quinolin-8-yl) acetamide (21e)



White solid powder ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.40 (s, 1H), 9.45 (s, 1H), 8.99 (dd, *J* = 4.1, 1.6 Hz, 1H), 8.66 (dd, *J* = 7.6, 1.0 Hz, 1H), 8.43 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.69 (d, *J* = 8.3 Hz, 1H), 7.66-7.63 (m 1H), 7.61 (t, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 15.2 Hz, 1H), 7.35 (s, 1H), 7.15 – 7.09 (m, 2H), 6.78 (d, *J* = 8.1 Hz, 1H), 3.90 (s, 2H), 3.84 (s, 2H), 3.82 (s, 3H), 3.77 (s, 2H), 2.67-2.64 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.86, 165.44, 149.84, 148.96, 148.32, 142.92, 138.55, 137.05, 134.38, 128.32, 127.50, 127.16, 123.07, 122.73, 122.30, 115.94, 114.89, 111.68, 61.91, 56.28. ESI-HRMS for $C_{25}H_{26}N_4O_4$. (M+H)+ calcd. 447.2032, found 447.2015.

5.1.10.19 (E)-2-(4-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)piperazin-1-yl)-N-(8-hydroxyquinolin-5-yl) acetamide (12f)



Yellowish solid powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.75 (s, 1H), 9.40 (s, 1H), 8.83 (d, *J* = 3.5 Hz, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 7.57-7.56 (dd, *J* = 8.0, 4.0 Hz, 1H), 7.45-7.37 (m, 2H), 7.29 (s, 1H), 7.04 (dd, *J* = 14.5, 5.0 Hz, 3H), 6.74 (d, *J* = 8.5 Hz, 1H), 3.79 (s, 4H), 3.65 (s, 3H), 3.27 (s, 2H), 3.25 (s, 2H), 2.60 (s, 4H). 13C NMR (126 MHz, DMSO-d_6) δ 169.32, 166.17, 147.84, 146.01, 139.89, 135.15, 128.84, 125.14, 123.46, 121.29, 120.98, 118.77, 117.99, 116.22, 114.29, 113.36, 112.19, 42.74, 25.45. ESI-HRMS for $C_{25}H_{26}N_4O_5$. (M+H)+ calcd. 463.1981, found 463.1979.

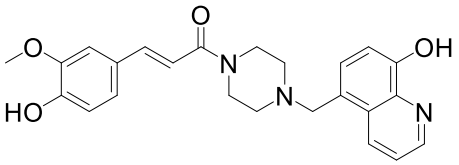
5.1.11 General procedure for the synthesis of intermediate 23

In a stirring solution of 8-hydroxyquinoline (22) formaldehyde (4ml, 37%) and 32% hydrochloric acid (4 ml), hydrogen chloride gas was purged for 6 hours at room temperature. Following filtration, the resulting product was subjected to crystallization with ethanol, forming compound 23 with a yield of 80%.

5.1.12 General procedure for the synthesis of target compound 24a

The intermediate 23 was treated with intermediate **15** following the procedure mentioned in section 5.1.10. yield a target compound **24a**.

5.1.12.1 (E)-3-(4-hydroxy-3-methoxyphenyl)-1-(4-((8-hydroxyquinolin-5yl)methyl)piperazin-1-yl)prop-2-en-1-one (24a)



Yellowish solid powder. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.76 (s, 1H), 9.47 (s, 1H), 8.86 (dd, J = 4.1, 1.5 Hz, 1H), 8.68 (dd, J = 8.6, 1.5 Hz, 1H), 7.60 (dd, J = 8.6, 4.1 Hz, 1H), 7.41 (d, J = 15.2 Hz, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.32 (d, J = 1.8 Hz, 1H), 7.09 – 7.06 (m, 2H), 7.03 –

7.05 (m, 2H), 6.77 (d, J = 8.1 Hz, 1H), 3.82 (s, 3H), 3.81 (s, 2H), 3.66 (s, 2H), 3.51 (s, 2H), 2.44-2.40 (d, J = 21.5 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.18, 153.41, 148.90, 148.29, 142.72, 139.32, 134.27, 129.52, 128.35, 127.16, 124.20, 123.00, 121.97, 115.88, 114.91, 111.59, 110.41, 59.79, 56.24, 0.58. ESI-HRMS for $C_{24}H_{25}N_3O_4$. (M+H)+ calcd. 420.1923, found 420.1906.

5.2 Biological Evaluation

5.2.1 In Vitro cholinesterase enzyme inhibition Assays.

Acetylcholinesterase (AChE) from human erythrocytes, as well as butyrylcholinesterase (BChE) from equine serum, Acetylthiocholine iodide (ATCI), butyrylthiocholine iodide (BTCI), and 5,5′-dithiobis(2-nitrobenzoic acid) DTNB-reagent were purchased from Sigma Aldrich. Donepezil, a positive control, was also obtained from Sigma Aldrich. 50 mM Tris-HCl buffer (8 pH) was used in this experiment. The ChE inhibitory activity of test compounds on acetylcholinesterase (AChE) from human erythrocytes and butyrylcholinesterase (BChE) from equine serum was determined using the method reported by Ellman et al. [181]. All the stock concentrations (2.5mM) of test compounds were prepared following the earlier procedure using molecular biology grade DMSO (0.8%) with ≥ 99.9% purity. Further dilution was performed to the desired concentration using Tris HCl buffer (8 pH). The assay was conducted following the procedure reported in a previous publication [147] and briefly incubated 10 µl of test compound with different concentrations (20, 10, 1, 0.1, and 0.01 µm final concentration) with 50 mL of AChE (0.022 U/mL) or BChE((0.06 U/mL) in 96 well plates. We added 10 µl of Tris HCl buffer in place of the test compound for control. After 30 minutes of incubation, we added 30 µl ATCI (1.5 mM) or BTCI (15 mM). After 30 minutes, we added 160 µl DTNB (1.5 mM), and immediately, absorption was measured using a BioTek

spectrophotometer. The IC_{50} indicates the test compound concentration required to inhibit enzyme activity by 50%. Each test compound was evaluated at five different concentrations. These experiments were performed in triplicate, and IC_{50} values for the compounds were computed using GraphPad Prism software version 8.0.1 (244).

5.2.2 Enzyme kinetic assay of lead compound 12o and 24a

Enzyme kinetic studies were conducted to explore the binding kinetics between ligands and the enzymes (AChE and BChE). An enzyme stock solution (*h*AChE or *eq*BChE) with a concentration of 500 U/mL was diluted to a final concentration of 0.022 U/mL (*h*AChE) or 0.066 U/mL (*eq*BChE) before use. A stock solution of the ligand (2.5 mM) was prepared in molecular biology-grade DMSO (99.9% purity) and further diluted with Tris-HCl buffer (pH = 8.0) to achieve the desired final concentration. In brief, a 50 µL portion of the enzyme solution (*h*AChE or eqBChE) was preincubated with compounds **12o** and **24a** at 37 °C for 30 minutes. Subsequently, substrate (ATCI at 1, 5, and 10 µM or BTCI at concentrations of 7.5, 15, and 30 µM) was added. Finally, 160 µL of DTNB-Ellman's reagent (0.15 mM for AChE or 1.5 mM for BChE) was introduced, and the absorbance was recorded at 415 nm utilizing a SynergyTM HT, Bio-Tek Instruments, Inc. Each assay was carried out in triplicate.

5.2.3 Molecular docking studies of 12o, EJMC-4e, 24a and DPZ

Molecular docking experiments were performed using Autodock Vina to examine the interactions between molecules and the active sites of AChE and BChE. The lead compounds **12o**, **24a**, and DPZ were docked into protein structures 4EY7 and 4BDS, respectively. The protein structures 4EY7 and 4BDS were retrieved from the Protein Data Bank. Before conducting the docking process, energy minimization of **12o, 24a,** and **DPZ** was carried out using the MOPAC energy minimization tool within the ChemDraw Ultra 8.0 software.

Subsequently, the protein and ligand files were converted into pdbqt format using Autodock Tools 1.5.6. Various parameters were adjusted in Autodock Vina for the molecular docking study. For 4EY7, the center was set to 70.086, -13.139, and -44.694 for the X, Y, and Z axes, respectively, with grid box dimensions 26 Å for all axes and an exhaustiveness of 8. For 4BDS, the center was set to 133.333, 115.917, and 41.389 for the X, Y, and Z-axes, respectively, with grid box dimensions 26 Å for all axes and an exhaustiveness of 8. Finally, Discovery Studio was used to visualize various docking poses for the ligands.

5.2.4 Molecular dynamics:

All-atom classical molecular dynamics simulations investigate the interaction between ligands and specific target proteins. Specifically, the most promising hits, **12o** and **24a**, were selected and subjected to MD simulation using GROMACS 2020 software and the CHARMM36 force field [186]. Docking was performed for **12o** and **24a** against 4EY7 and 4BDS, resulting in protein-ligand complexes. These complexes were subsequently solvated and ionized with Na+ and Cl- ions. Energy minimization and equilibration runs were performed using the NVT and NPT ensemble. During the equilibration phase, position restraints were applied to the complexes to prevent any distortion within the system during production runs. Temperature coupling was maintained using V-rescale, and pressure coupling was achieved with Parinello-Rahman, both set to sustain a temperature of 300 K and pressure of 1 bar, with coupling constants of 0.1 ps for temperature and 2 ps for pressure. The Particle Mesh Ewald (PME) method was employed to compute long-range electrostatic interactions and Van der Waals interactions with a short-range Van der Waals cut-off set at 1.2 nm. The LINCS algorithm was utilized to maintain bond constraints, and a time step of 0.002 ps was applied during the simulation. Subsequently, a production simulation run of 100 ns was conducted.

5.2.5 DPPH Assay

The DPPH assay was conducted to investigate the antioxidant activity of compounds. In this assay, the antioxidant molecule reduces DPPH, causing a decrease in absorbance at 517 nm. The test was conducted in methanol, employing the test compounds at five distinct concentrations (200, 160, 80, 40, and 20 μM final concentration). The outcomes were determined by measuring the absorbance of the DPPH (100 mM final concentration) solution (75 μl) after the addition of the test compounds (75 μl) in four wells. The reduction percentage of DPPH was then calculated based on the absorbance of the control and test solutions. The following equation was used to determine the % radical scavenging.

% Radical Scavenging = $[(A_0\text{-Ac})/Ao] \times 100$

A0 = absorbance of the blank sample without compound

Ac = absorbance of the sample with a tested compound

The assay was conducted in triplicate to ensure accuracy.

5.2.6 PAS binding assay

This study aimed to evaluate the binding affinity of **12o** and **24a** to the PAS site of AChE. Propidium iodide, a well-recognized ligand for the PAS site of AChE, was used to measure fluorescence. The experiment was conducted using the method reported in our earlier publications [150]. We used **AChE** (5 U/ml) in 0.1 mM Tris buffer, pH 8.0 solution, to perform this activity. We incubate 75 μL of AChE with 75 μL compounds **12o** and **24a** (at final concentrations of 50, 20, 10, and 5 μM) and donepezil as control for 6 hours at 25°C. Next, 50 μL of propidium iodide with a final concentration of 20 μM was introduced, and the mixture was incubated for 20 minutes. Fluorescence was assessed using a fluorescence microplate reader with excitation and emission wavelengths set at 535 and 595 nm, respectively.

5.2.7 Blood-brain barrier (BBB) permeability assay:

In this study, a PAMPA-BBB assay was performed to assess the blood-brain barrier permeation potential of a selected hybrid of ferulic acid-quinoline derivatives. The capability of a compound to penetrate the BBB is a crucial aspect of developing drugs for central nervous system diseases. Commercial drugs and dodecane were sourced from Sigma, while Avanti Polar Lipids provided porcine brain lipids (PBL). Millipore supplied the donor and acceptor microplates. The donor microplate's filter surface was coated with 4 μL of porcine brain lipid in dodecane (20 mg/ml). We first dissolved **12o** in DMSO and then diluted it with PBS/ethanol (70:30) to obtain a final 100 μg/ml concentration.

In the acceptor microplate, 200 μL of PBS/ethanol (70:30) was added, while the donor well contained 200 μL of **12o** and **24a** solution. The donor and acceptor plates were then assembled, creating a sandwich structure. This assembly was left undisturbed for 18 hours at 25°C, and the concentration of **12o** and **24a** in the acceptor wells was evaluated using UV spectroscopy.

Four commercially available drugs with well-established BBB permeability were used for method validation. Testosterone and imipramine were used as positive controls, and norfloxacin and hydrocortisone, which were used as negative controls, were subjected to the same procedure for validation. The permeability values obtained through experimental testing were compared to the reference permeability values. The results were then presented as the mean ± standard error of the mean (SEM).

5.2.8 Metal chelation study with 12o

Iron (III) chloride hexahydrate ($FeCl_3$, CAS No. 10025-77-1) and Copper (II) sulfate anhydrous ($CuSO_4$, CAS No. 7758-98-7) were procured from Sigma Aldrich. All stock solutions and subsequent dilutions were prepared using extra pure methanol. The metal

chelation activity study was performed according to the procedure reported earlier. The absorption spectra of **12o** (300 μM) in extra pure methanol were recorded in the presence and absence of FeCl3 or CuSO4 (300 μM) for 30 minutes at room temperature using a UV-spectrophotometer. [147]. Metal chelation activity was additionally carried out under physiological conditions using a buffer (20 mM HEPES, 150 mM NaCl, pH 7.4) following a method described by X. Yang et al.[159]. This process dissolved **12o**, FeCl3, and CuSO4 in the buffer. Subsequently, a mixture of **12o** (at a final concentration of 50 μM) and FeCl3 (at a final concentration of 50 μM) in a 1:1 ratio was prepared, as well as a similar mixture of **12o** and CuSO4 in a 1:1 ratio. These mixtures were then incubated for 30 minutes, and UV absorption was measured at RT using a UV spectrophotometer.

5.2.9 Metal chelation study with 24a

The metal chelation was monitored spectrophotometrically using a UV–vis spectrophotometer. Iron (III) chloride hexahydrate ($FeCl_3$, CAS No. 10025-77-1 was purchased from Sigma Aldrich. All the stock concentrations and dilutions were prepared in extra pure methanol. The absorption spectra of **24a** (300 μM) alone or with FeCl3 (300 μM, final concentration) for 30 min in extrapure methanol were recorded at room temperature using a UV-spectrophotometer (200-700 nm range). The stoichiometry of the **24a**-Fe3+ complex was determined using the molar ratio method. This involved titrating a solution of compound **24a** with a solution of $FeCl_3$ at various molar ratios. After incubating the mixture for 3 hours, UV spectra were recorded at room temperature. The absorbance spectrum exhibited a new absorption band, indicating the interaction between **24a** and Fe^{3+} ions. Subsequently, a plot correlating the mole fraction of Fe3+ with the absorbance of **24a** was generated, facilitating the identification of the complex's stoichiometry [150].

5.2.10 $A\beta_{1-42}$ peptide inhibition studies of compounds 12o and 24a

Commercially available "S-6110, Abeta amyloid 1-42 Human (1932-2-15) was obtained from DG peptides (Zhejiang province China). To solubilize the $A\beta_{1-42}$ peptide, 1 mg was dissolved in 1 ml of phosphate buffer at a pH of 7.4, with a concentration of 20 mM. $A\beta_{1-42}$ concentration was determined using a "NanoDrop™ 2000/2000c Spectrophotometer from Thermo-Scientific". This initial stock solution was then further diluted to achieve the desired working concentration (12.5 μM) using a 50 mM PBS buffer at a pH of 7.4. To study the self-induced $A\beta_{1-42}$ aggregation inhibition property of **12o and 24a** 100 μL of a mixture of $A\beta_{1-42}$ monomer (25 μM) and **12o or 24a** (6.25 μM) in 1:1 was incubated at 37 °C with agitation at 1200 rpm for a period of 72 hrs using Thermomixer (Eppendorf). To confirm the formation of $A\beta_{1-42}$ aggregates, 100 μL of a mixture of $A\beta_{1-42}$ monomer (12.5 μM) alone was also incubated under the same condition. Similarly, for the inhibition of metal-mediated $A\beta_{1-42}$ aggregation, 90 μL of a mixture of $A\beta_{1-42}$ monomer (25 μM), **12o or 24a** (6.25 μM), and Fe^{3+} (25 μM) in 1:1:1 was incubated at 37 °C with agitation at 1200 rpm for a period of 72 hrs using Thermomixer (Eppendorf). Also, to confirm the formation of $A\beta_{1-42}$ aggregates, 100 μL of a mixture of $A\beta_{1-42}$ monomer (12.5 μM) alone was incubated under the same condition.

5.2.11 Confocal microscopy of compound 12o

The confocal fluorescence imaging was conducted following the method described in the literature [187]. ThT, a fluorescent dye, was used in various combinations: ThT +$A\beta_{1-42}$, ThT +$A\beta_{1-42}$ +FeCl3, and $A\beta_{1-42}$ + FeCl3 +**12o** +ThT. The mixtures were incubated and applied to glass slides with DABCO as the fixing agent. Imaging was performed at 40x using a fluorescein isothiocyanate (FITC) fluorescence cube with excitation at 494 nm and emission at 518 nm. The ZEISS LSM 700 laser scanning confocal microscope from Zeiss, Jena, Germany, was utilized for image acquisition.

5.2.12 Cell culture

All experiments used rat pheochromocytoma PC12 cells from the National Centre for Cell Science (NCCS) (Pune, India), from passages 3–10. Cells were cultured in Ham's F-12K (Kaighn's) Medium, containing 10% fetal bovine serum and 1% Gibco™ Antibiotic-Antimycotic which includes 10,000 units/mL of penicillin, 10,000 µg/mL of streptomycin, and 25 µg/mL of Gibco Amphotericin B. Undifferentiated PC12 cells were differentiated into sympathetic-like neurons using nerve growth factor (NGF) at 100 ng/mL. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum and 1% Gibco™ Antibiotic-Antimycotic. All the experiments in this study were performed with differentiated PC12 cells. PC12 cells were differentiated with NGF-ß (100 ng/ml) for 5 days before the experiment. Dimethyl sulfoxide (DMSO) and thiazolyl Blue Tetrazolium Bromide (MTT, CAS No. 298-93-1) were purchased from Sigma-Aldrich and H_2O_2 from Himedia.

5.2.12.1 Cell viability and MTT assay

To evaluate the cytotoxicity of **12o and 24a** compounds on PC12 cell viability, the cytotoxicity was assessed by the MTT assay. Cells were seeded at 10000 cells/well in 96 well plates and kept for overnight incubation for adherence. **12o** was added at 30, 20, 10, 7, 5, 2.5, 1, and 0.1 µM and incubated for 24h at 5% CO_2 at 37 °C. The MTT reagent (5 mg/ml) was added and incubated for 3-4 hours at 37°C, DMSO dissolved formazan crystals, and the reading was taken at 562 nm on Spectramax-i3x. Cell viability was expressed as a percentage where untreated cells served as the negative control group and were designated 100%; the representative graph was plotted as mean ±SEM. All assays were performed using samples in quadruplicate.

5.2.13 Culture and treatment of cells

Human microglial cells (HMC-3) acquired from ATCC-CRL-3304 were used in all *in-vitro* tests. Eagle's Minimum Essential Medium (EMEM), ATCC-30-2003, which contains 5 mM glucose, 10% fetal bovine serum qualified (FBS), 1% streptomycin/penicillin, and 10% glucose, was used to cultivate the cells at 37°C in a humidified environment of 95% air and 5% CO_2. The media were constantly changed every two days until the cells reached 80% confluence. Cells were plated at the desired density for further research.

5.2.13.1 Evaluation of cell viability

A sterile 96-well plate with $1x10^4$ seeded cells per well was employed. LPS and ATP were dissolved in the medium to get the proper concentrations. HMC-3 cells that had been serum-starved for two hours were then given a six-hour treatment with 1000ng/ml LPS. After removing the priming media and rinsing with complete media, ATP (5 mM) was added and left in situ for 45 minutes. Following treatment with test compounds for 24 hr, the media was removed. MTT reagents (10 mg/ml) were added after the media had been removed, and the reaction was allowed to proceed for 4 hours at 37°C. The formazan crystals were then dissolved in DMSO, and a SPECTRA-Max i3x was used to detect the absorbance at a wavelength of 570 nm (Molecular Devices, USA). Cell vitality was expressed as a percentage compared to control cells.

5.2.13.2 Analyzing the Reactive Oxygen Species (ROS) and Mitochondrial Membrane Potential (MMP) in Human Microglial Cells.

Dihydroethidium (cat no: 38483-26-0, Sigma-Aldrich) measures the total ROS. After being primed with LPS and ATP, HMC-3 cells were given a 24-hour treatment with 71 and 136. After that, the cells were stained with 5 µM DHE for 10 min at 37°C. The cells were washed

in a warm buffer, and pictures were taken using an EVOS Auto FL2 fluorescence microscope from Invitrogen.

The mitochondrial membrane potential (Δψm), which is specific to mitochondria, was measured using the dye tetraethyl benzimidazolyl carbocyanine iodide (JC-1), which was applied at a final concentration of 2µM and incubated with the cells at 37°C and 5% CO_2 for 30 min (Cat no: T3168, Invitrogen). Under typical circumstances, JC-1 aggregates with high red fluorescence intensity. The ratio of red to green fluorescence serves as a measure of Δψm-loss because as the dye transforms from aggregate to monomeric form, loss in the Δψm is reflected by a reduction in red fluorescence and an increase in green fluorescence.

5.2.13.3 Immunocytochemistry analysis of NLRP3, NF-κB, and Vimentin in human microglial cells.

Human microglial cells (HMC-3) were grown on a coverslip in 6-well culture plates at a density of $2x10^6$ cells. The cells were treated, washed in phosphate buffer (PBS), fixed with 4 percent paraformaldehyde (PFA), and permeabilized with 0.2 percent Triton X-100. After the primary antibodies had been incubated with the cells for an overnight period at 4°C, the secondary antibodies Alexa FlourTM 488 goat anti-rabbit IgG (H+L) and Alexa FlourTM 594 goat anti-mouse IgG (H+L) from Invitrogen were added. The nuclei were stained with 4, 6-diamidino-2-phenylindole (DAPI) in Vecta-shield mounting solution for fluorescence (cat. no. H-1200) (Vector Laboratories, Burlingame, CA). Slides with the primary antibody removed were used as negative controls. Slides were observed at oil emersion 63x magnifications using a confocal microscope in a relaxed atmosphere (Leica TCS SP8).

5.2.13.4 Immunoblotting study with compound 12o

For the *in-vitro* experiment, cells were grown at a density of $1.5x10^6$ cells in a sterile 90 mm by 20 mm culture dish. Following treatment, cells were lysed in RIPA buffer supplemented with protease and phosphatase inhibitors. A commercial kit was used to calculate the total proteins. 25–30 µg of protein were added to each lane and separated using a 12 to 16 % SDS–PAGE gel. The protein was put onto a nitrocellulose membrane, blocked in 5% nonfat dry milk (NFDM) buffered solution for an hour, and then the primary antibody was left to incubate at 4°C for the entire night. The following were the primary antibody dilutions: NLRP3, Caspase-1 (p20) (1:1000). Blots were treated with TBST and then incubated with the appropriate secondary antibodies (anti-rabbit IgG) for one hour (1:1000). Blots were developed using ECL (Bio-Rad cat log no-1705061) reagent on a Fusion Fx chemiluminescence-17-200255. (Vilber Lourmat). Image-J was used for the densitometric analysis (National Institutes of Health, Bethesda, Maryland, USA).

5.2.14 Fly husbandry and culture

The fly stocks used in this study were obtained from the Bloomington Drosophila Stock Center in Indiana, USA. The stocks included the OregonR+ strain as the wild type (WT), the ey-GAl4/CyO control driver line, and the UAS-Aβ-eye-Gal4/CyO responder line. All of these fly stocks were raised and maintained in a standard corn agar meal medium at a temperature of 28 ± 1^oC within a BOD incubator.

5.2.14.1 Drug treatment on OregonR + and Aβ 42 expressing flies

The age-matched five male and five female wild-type flies were genetically crossed in vials Containing **12o**, **24a**, and **DPZ** drug with corn food. The different dosages of **12o**, **24a**, and **DPZ** (0.05, 0.1, 0.2, and 1 mg/ml and 50 µM, 100 µM, 200 µM, 400 µM, and 800 µM) were

primarily administered to wild-type OregonR + flies to check the drug dose-response. The untreated progenies were revealed as controls. The **12o**, **24a**, and **DPZ** drug-treated progenies were scored after 15 days to determine their median lethal dose (LD_{50}). Similarly, ey-GAL4-driven Alzheimer's flies (UAS-Aβ 42) were also treated with **12o** and **24a** dosages (0.05 mg/ml, 0.1 mg/ml, 0.2 mg/ml), and **DPZ** (100µM) in an ascending order to examine their potential effects against AD. All experiments were conducted in triplicate, and statistical analysis was carried out using GraphPad Prism 5 with a one-way ANOVA.

5.2.14.2 Mitochondrial and Cellular ROS Measurement

The Aβ 42-expressing 3rd instar larvae, which were either treated with **12o** and **DPZ** or left untreated, were dissected in a solution of1X PBS, pH 7.4, followed by tissue permeabilization in 0.2% TritonX-100 in 1XPBS for 15 min at 37 ^{o}C and their eye imaginal discs (n=10 discs each group) were incubated in 5µM MitoSOX Red (Invitrogen, USA) for 20 min at RT to measure the superoxide (ROS) level as approved by **Liu et al., 2013 [188].** After incubation, MitoSOX Red was removed, and tissues were washed three times with 1XPBS and mounted in 1XPBS. The images were captured using a Nikon Eclipse Ni-U Upright fluorescence microscope and MitoSOX Red fluorescence intensity was processed by NIS Elements (BR) software. Similarly, In Situ, cellular ROS levels of **12o**, **24a**, and **DPZ**-treated and untreated AD eye imaginal discs (n=10 disc each group) were also measured using Redox sensitive fluorophore H2DCFDA (2',7'-dichlorodihydrofluorescein diacetate) as previously reported[174]. The tissues were incubated with 50µM H2DCFDA for 30 min at RT. After incubation, the dye was removed, followed by washing it three times with 1XPBS and mounting it in 1XPBS. The images were captured using a Nikon Eclipse Ni-U Upright

fluorescence microscope, and H2DCFDA green fluorescence intensity was measured by NIS Elements (BR) software.

5.2.15 Biological evaluation using *In-vivo* animal model

We used albino mice of both genders (male and female) weighing 25–33 g for in vivo biological evaluation. Healthy mice were acquired from a central animal facility. They underwent a 7-day acclimatization period to help them acclimatize to their new surroundings at The Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (Banaras Hindu University). The animals were fed commercial food pellets and supplied with clean tap water for their dietary and hydration requirements. The study was performed following the guidelines approved by the Committee for Control and Supervision of Experiments on Animals, Ministry of Environment, Forests and Climate Change, Government of India, and was approved by the Central Animal Ethical Committee of the University (Banaras Hindu University, Varanasi, India) (Dean/2019 /CAEC/1189, dated 20.04.2019).

5.2.15.1 Acute Toxicity and Hepatoxicity Study of Compound 12o

The animals were acclimated for 6 days prior to the experiment. Compound **12o** was suspended in 0.5% tween-80 solution, and an oral route was selected to administer these compounds to experimental groups (175 and 550 mg/kg). After dosing, the mice were observed continuously for any change in animal behavior and mortality. If the mortality or other adverse effects such as weight loss or irritation were not observed, all other animals were administered with a 550 mg/kg dose, monitored at regular intervals for 24 hr, and supervised for 14 days to observe the delayed response of compounds. On the 14^{th} day, all animals of different groups were sacrificed, and livers were isolated for microscopic study.

5.2.15.2 Acute Toxicity Study of Compound 24a

The animals were acclimated for 6 days before the experiment. Compound **24a** was suspended in 0.5% tween 80 solutions, and an oral route was selected to administer these compounds to experimental groups (175 and 550 mg/kg). After dosing, the mice were continuously observed for any change in animal behavior and mortality. If the mortality or other adverse effects such as weight loss or irritation were not observed, then all other animals were administered with a 550 mg/kg dose, monitored at regular intervals for 24 hr, and kept under supervision for 14 days to observe the delayed response of compounds.

5.2.15.3 Behavioral Studies of 12o using water maze experiment

The study aimed to evaluate the impact of **12o** on scopolamine-induced impairment in spatial learning and memory in the Morris water maze. The experiments were conducted on adult Swiss albino mice of both genders with an average body weight of 25–33 g**.** The scopolamine hydrochloride (CAS No. 7758-98-7) and donepezil (CAS No. 7758-98-7) were procured from Sigma Aldrich, India. The compound **12o** and donepezil were triturated with 0.5% tween 80 solution and then diluted with deionized water to get 1 and 5 mg/mL suspensions of **12o** and 1 mg/mL suspension of **DPZ**. Scopolamine hydrochloride was dissolved in deionized water to prepare a 1.4 mg/mL solution. The acclimatized animals were trained on the Morris water maze for 7 days to acquire spatial learning. Then, the trained animals were divided into six groups. i) Control group did not receive drug or vehicle, ii) Vehicle control group received 0.5% tween 80 solution (i.p.), iii) Scopolamine group received scopolamine 1.4 mg/kg (i.p.), iv) first **12o** group received 1 mg/kg (i.p.) of **12o** and scopolamine 1.4 mg/kg (i.p.), v) second **12o** group received 5 mg/kg (i.p.) of **12o** and scopolamine 1.4 mg/kg (i.p.), vi) **DPZ** group received 1 mg/kg (i.p.) of **DPZ** and scopolamine 1.4 mg/kg (i.p.). The compound **12o** and donepezil were

administered over 14 days after the completion of the training period. The animals of groups iii), iv), v), and vi) received scopolamine on the last 5 days of the experiment (day 18 to day 22). Further, animals from all the treatment groups were subjected to the Morris water maze test after 30 min of vehicle or scopolamine administration. The Morris water maze test involves using a circular water pool with a hidden platform to escape from swimming [189]. The escape latency time recorded following a training session indicates the spatial learning and memory abilities. The blind observer analyzed the results.

5.2.15.4 Behavioral Studies of lead compound 24a using Y-Maze Test

The study aimed to evaluate the impact of **24a**, scopolamine, and donepezil on spatial learning and memory in a rodent model of AD. The experiments were carried out on adult Swiss Albino mice of both genders with an average weight of 25-33 g**.** The scopolamine hydrochloride (CAS No. 7758-98-7) and donepezil (CAS No. 7758-98-7) were procured by Sigma Aldrich. The compound **24a** was triturated with 0.5% tween 80 solution and then diluted with deionized water. Scopolamine hydrochloride (dissolved in deionized water) was utilized to induce an AD-like phenotype in the mice, with a focus on inducing amnesia. The Animals were acclimatized for 7 days to adopt environmental conditions. All the animals were trained to acquire spatial learning for 7 days.

Then, these animals were divided into 6 groups. I) The control group does not receive any drug, whereas ii) Vehicle treated group gets 0.5% tween 80 solution ip. Similarly, iii) the Scopolamine group received 1.4 mg/kg dose alone, iv) the **DPZ** group received 1 mg/kg of **DPZ** plus scopolamine, V) this group administered 1 mg/Kg of **24a** plus scopolamine, vi) administered 5 mg/kg **24a** plus Scopolamine, all these drugs administered intraperitoneally (ip). The treatments continued for 7 consecutive days. On the seventh day, groups ii, iii, iv, v,

and vi were administered scopolamine at a 30-minute interval following the drug administration. The vehicle control group received the vehicle only. After 15 minutes of administration of either the vehicle or scopolamine, all animals underwent a Y-maze test.

5.2.15.5 *Ex-vivo* studies for lead compound 12o and 24a

After completion of the Morris water maze test, the mice were humanely euthanized through CO_2 asphyxiation and cervical dislocation. Subsequently, their brains were isolated and rinsed with phosphate buffer. Each mouse brain was then homogenized and subjected to centrifugation at 7000 rpm for 25 min at a temperature of 4 °C. The resulting supernatant was carefully transferred into individual tubes for further biochemical analysis.

5.2.15.5.1 Cholinesterase (AChE and BChE) Activity measurement

The Ellman colorimetric method was utilized to quantify the cholinesterase enzyme in the brain. A 100 µL portion of brain supernatant was mixed with 100 µL of ATCI or BTCI (15 mM) and incubated for 5 minutes. Subsequently, 100 µL of 1.5 mM DTNB was added to the mixture. The absorbance was promptly measured at 415 nm using a SynergyTM HT Bio-tek 96-well microplate reader.

5.2.15.5.2 Malondialdehyde (MDA) Measurement

To determine the concentration of MDA (malondialdehyde), we took 0.2 mL of 8.1% sodium lauryl sulfate (SLS), 1.5 mL of 0.8% thiobarbituric acid (TBA) in an aqueous solution, and 1.5 mL of 20% glacial acetic acid (CH_3COOH) were added to 200 µL of processed brain supernatant. This mixture was diluted with 4 mL of Milli-Q water and heated to 95°C for 60 minutes. Subsequently, the mixture was cooled using tap water. After cooling, pyridine (5 mL), n-butanol mixture (1:15 v/v), and 1 mL of Milli-Q water were added, followed by

centrifugation. The organic phase was separated, and its absorbance was measured at 532 nm using a 96-well plate reader.

5.2.15.5.3 Catalase (CAT) Measurement

To determine the CAT concentration, we incubated 50 µl of brain supernatant with 50 µl of 800 mM hydrogen peroxide (H_2O_2) and 50 µl of 0.1 M PBS (pH 7.4) in a 96-well plate at 37°C for 1 minute, then 150 µl dichromate/acetic acid solution (coloring agent) was added, and the mixture was boiled at 100 °C for 10 minutes. Absorbance reading was taken at 570 nm with the help of a 96-well microplate reader.

5.2.15.5.4 Superoxide Dismutase (SOD) Measurement

For estimation of SOD, we took 50 µL of supernatant with 100 µL solution (in PBS) of sodium carbonate (Na_2CO_3), 0.1 mM ethylenediaminetetraacetic acid (EDTA), hydroxylamine hydrochloride ($NH_2OH.HCl$) and 25 µM of nitro blue tetrazolium (NBT), and mix gently. The absorbance reading was taken at 570 nm utilizing a 96-well microplate reader.