Experimental Work

Chapter 5

5. Experimental work

5.1. Chemistry

The starting materials, solvents, and reagents of reliable quality were sourced from respected suppliers such as Sigma Aldrich, Alfa Aesar, TCI chemicals, Merck, Finar, or SD Fine chemicals. These substances were utilized without the need for additional purification. The advancement of the reactions was tracked by employing thin-layer chromatography (TLC) on silica gel plates pre-coated with Merck KGaA's silica gel 60 F254. The chromatograms were observed by exposing them to UV light and iodine vapors for visualization. Silica gel with a mesh size of 60-120, sourced from Avra Synthesis in India, was employed for column chromatographic purifications. A Bruker 500 MHz NMR instrument was utilized to record the ^{1}H and ^{13}C NMR spectra. The NMR solvents employed were either $CDCl_3$ or DMSO-d_6, as specified. The NMR data were collected at the Central Instrument Facility (CIF), IIT (BHU). Chemical shifts (δ) are expressed in parts per million (ppm), while coupling constants (J) are provided in Hertz (Hz). When appropriate, peak splitting patterns are indicated using the following abbreviations: d for doublet, t for triplet, q for quartet, m for multiplet, dd for doublet of doublet, and brs for broad singlet. HRMS data was collected utilizing electrospray ionization (HRMS/ESI) on an HRMS-6540-UHD instrument at the Indian Institute of Technology in Ropar and the Department of Chemistry (BHU), India. The nomenclature of compounds was determined using the software ChemDraw Professional 15.0 (Perkin Elmer).

5.1.1. *General procedure for synthesis of intermediates 2a-2w, 5a-5e, 8a-8e and 11a-11b*

To a solution of substituted isocyanate (1.0 mmol) in dichloromethane (15 mL) at room temperature, pyridine (1 mmol) was added. The reaction was stirred for 10 minutes. Into this stirring solution, 1 mmol of substituted amine (**substituted**

aniline, **indole, quinoline** and **tryptamine**) was added. The mixture was allowed to stir for 2 h at room temperature. After completion of the reaction, the solution was evaporated under vacuum and was subjected to silica gel chromatography or crystallization, if necessary, to afford the compounds **2a-2w, 5a-5e, 8a-8e, and 10a-10b**.

5.1.1.1. ***1-(3-cyanophenyl)-3-phenylurea (2a).***

White solid powder, 80% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.21 (bs, 1H), 7.95 (bs, 1H), 7.70 (s, 1H), 7.71 (d, J = 7.5 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.41 (t, J = 8.5 Hz, 1H), 7.36 (d, J = 8.0 Hz, 2H), 7.19-7.14 (m, 2H), 6.90 (t, J = 7.0 Hz, 1H).

5.1.1.2. ***1-(3-cyanophenyl)-3-(o-tolyl)urea (2b***).

White solid powder, 81% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.33 (bs, 1H), 8.07 (bs, 1H), 8.00 (bs, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.64(d, J = 8.0 Hz, 1H), 7.49 (t, J = 8.0 Hz, 1H), 7.41 (d, J = 7.5 Hz, 2H), 7.19-7.14 (m, 2H), 6.97 (t, J = 7.0 Hz, 1H), 2.25 (s, 3H).

5.1.1.3. ***1-(3-cyanophenyl)-3-(m-tolyl)urea (2c).***

White solid powder, 85% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.95 (bs, 1H), 8.70 (bs, 1H), 7.96 (bs, 1H), 7.66-7.64 (m, 1H), 7.47 (t, J = 8.0 Hz, 1H), 7.41 (d, J = 6.5Hz, 2H), 7.33 (d, J = 8.5Hz, 2H), 7.09 (d, J = 8.0 Hz, 1H), 2.24 (s, 3H).

5.1.1.4. ***1-(3-cyanophenyl)-3-(p-tolyl)urea (2d).***

White solid powder, 81% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.95 (bs, 1H), 8.70 (bs, 1H), 7.96 (bs, 1H), 7.66-7.64 (m, 1H), 7.47 (t, J = 8.0 Hz, 1H), 7.41 (d, J = 6.5 Hz, 1H), 7.33 (d, J = 8.5 Hz, 1H), 7.09 (d, J = 8.0 Hz, 1H), 2.24 (s, 3H).

5.1.1.5. ***1-(3-cyanophenyl)-3-(2-methoxyphenyl)urea (2e).***

White solid powder, 80% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.90 (bs, 1H), 8.73 (bs, 1H), 7.90-7.84 (m, 1H), 7.66-7.60 (m, 1H), 7.25-7.18 (m, 1H), 7.17 (s, 1H), 7.18-7.14 (m, 1H), 6.98-6.96 (m, 1H), 6.55-6.53 (m, 1H), 3.70 (s, 1H).

5.1.1.6.*1-(3-cyanophenyl)-3-(3-methoxyphenyl)urea (2f).*

White solid powder, 78% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.98 (bs, 1H), 8.82 (bs, 1H), 7.97-7.96 (m, 1H), 7.67-7.64 (m, 1H), 7.42-7.40 (m, 1H), 7.20 (s, 1H), 7.18-7.17 (m, 1H), 6.95-6.93 (m, 1H), 6.58-6.56 (m, 1H), 3.73 (s, 1H).

5.1.1.7. ***1-(3-cyanophenyl)-3-(4-methoxyphenyl)urea (2g).***

White solid powder, 76% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.91 (bs, 1H), 8.61 (bs, 1H), 7.96 (bs, 1H), 7.65 (d, J = 7.5, 1H), 7.47 (t, J = 8.0 Hz, 1H), 7.40-7.34 (m, 3H), 6.87 (t, J = 5.5, 2H), 3.71 (s, 3H).

5.1.1.8. ***1-(3-cyanophenyl)-3-(3-hydroxyphenyl)urea (2h).***

White solid powder, 74% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.15 (bs,1H), 8.81 (bs, 1H), 8.47 (bs, 1H), 7.90 (bs, 1H), 7.71 (bs, 1H), 7.44 (t, J = 8.0 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.28 (d, J = 9.0 Hz, 2H), 6.68 (d, J = 8.5 Hz, 2H).

5.1.1.9. ***1-(3-cyanophenyl)-3-(4-hydroxyphenyl)urea (2i).***

White solid powder, 76% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.10 (bs,1H), 8.88 (bs,1H), 8.48 (bs,1H), 7.96 (bs,1H), 7.65-7.63 (m, 1H), 7.47 (t, J = 8.0 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.22 (d, J = 9.0 Hz, 2H), 6.70 (d, J = 8.5 Hz, 2H).

5.1.1.10. ***1-(2-chlorophenyl)-3-(3-cyanophenyl)urea (2j).***

White solid powder, 79% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.71 (bs, 1H), 8.43 (bs, 1H), 8.12 (dd, J_1 = 8.5 Hz, J_2 = 1.5 Hz, 1H), 8.00 (bs, 1H), 7.66-7.64 (m, 1H), 7.53-7.44 (m, 3H), 7.32 (t, J = 7.5 Hz, 1H), 7.08-7.07(t, J=7.5Hz, 1H).

5.1.1.11. ***1-(3-chlorophenyl)-3-(3-cyanophenyl)urea (2k).***

White solid powder, 77% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.07 (d, J = 5.4 Hz, 2H), 7.96 (s, 1H), 7.70-7.66 (m, 2H), 7.49-7.42 (m, 2H), 7.32-7.29 (m, 2H), 7.04 (d, J = 6.0 Hz, 1H).

5.1.1.12. ***1-(4-chlorophenyl)-3-(3-cyanophenyl)urea (2l).***

White solid powder, 79% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.04 (bs, 1H), 8.97 (bs, 1H), 7.95 (s, 1H), 7.68-7.66 (m, 1H), 7.50-7.48 (m, 3H), 7.42 (d, J = 7.5 Hz, 1H), 7.33 (d, J = 8.5 Hz, 2H).

5.1.1.13. ***1-(2-bromophenyl)-3-(3-cyanophenyl)urea (2m).***

White solid powder, 74% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.67 (bs, 1H), 8.44 (bs, 1H), 8.15 (d, J_1 = 8.5 Hz, 2H), 8.06 (bs, 1H), 7.62-7.60 (m, 1H), 7.52-7.48 (m, 2H), 7.27-7.25 (m, 1H), 7.11-7.09 (m, 1H).

5.1.1.14. ***1-(3-bromophenyl)-3-(3-cyanophenyl)urea (2n).***

White solid powder, 75% yield**.** ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.09 (bs, 1H), 9.03 (bs,1H), 7.97 (bs, 1H), 7.85 (t, J = 2.0 Hz, 1H), 7.69-7.67 (m, 1H), 7.52 (t, J = 8.0 Hz, 1H), 7.45-7.43 (m, 1H), 7.34-7.32 (m, 1H), 7.26 (t, J = 8.0 Hz, 1H), 7.19-7.17 (m, 1H).

5.1.1.15. ***1-(4-bromophenyl)-3-(3-cyanophenyl)urea (2o).***

White solid powder, 75% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.48 (bs, 1H), 9.25 (bs, 1H), 7.99 (bs, 1H), 7.75 (t, J = 7.5 Hz, 2H), 7.70 (t, J = 8.0 Hz, 2H), 7.67-7.64 (m, 1H), 7.51 (t, J = 7.5 Hz, 1H), 7.45 (d, J = 7.5 Hz, 1H).

5.1.1.16. ***1-(3-cyanophenyl)-3-(2-fluorophenyl)urea (2p).***

White solid powder, 80% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.37 (bs, 1H), 8.67 (bs, 1H), 8.12-8.09 (m, 1H), 7.99 (s, 1H), 7.66-7.64 (m, 1H), 7.50 (t, J = 8.0 Hz, 1H), 7.44 (d, J = 7.5 Hz, 1H), 7.25-7.03 (m, 3H).

5.1.1.17. ***1-(3-cyanophenyl)-3-(3-fluorophenyl)urea (2q).***

White solid powder, 83% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.24 (bs, 1H), 9.07 (bs, 1H), 7.73 (d, J = 9.0 Hz, 2H), 7.63 (d, J = 8.5 Hz, 2H), 7.47 (d, J = 9.0 Hz, 1H), *7.36-7.30 (m, 1H), 7.15* (d, J = 7.0 Hz, 2H), 6.80 (td, J_1 = 6.5 Hz, J_2 = 2.0 Hz, 1H).

5.1.1.18. ***1-(3-cyanophenyl)-3-(4-fluorophenyl)urea (2r).***

White solid powder, 80% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.02 (bs, 1H), 8.87 (bs, 1H), 7.96 (bs, 1H), 7.68-7.66 (m, 1H), 7.50-7.41 (m, 4H), 7.15-7.12 (t, J = 9.0 Hz, 1H).

5.1.1.19. ***1-(3-cyanophenyl)-3-(2-(trifluoromethyl)phenyl)urea (2s).***

White solid powder, 77% yield. ^{1}H NMR ((DMSO-d_6, 500 MHz): δ 9.66 (s, 1H), 8.22 (s, 1H), 7.99 (s, 1H), 7.90 (d, J = 8.5 Hz, 1H), 7.71-7.64 (m, 3H), 7.51 (t, J = 8.0 Hz, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.32, (t, J = 8.0 Hz, 1H).

5.1.1.20. ***1-(3-cyanophenyl)-3-(3-(trifluoromethyl)phenyl)urea (2t).***

White solid powder, 78% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.20 (s, 1H), 9.13 (s, 1H), 7.99 (d, J = 7.5 Hz, 2H), 7.69 (d, J = 8.5 Hz, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.54-7.49 (m, 2H), 7.44 (d, J = 7.5 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H).

5.1.1.21. ***1-(3-cyanophenyl)-3-(4-(trifluoromethyl)phenyl)urea (2u).***

White solid powder, 76% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.35 (bs, 1H), 9.23 (bs, 1H), 7.97 (bs, 1H), 7.70-7.63 (m, 5H), 7.51 (t, J = 7.5 Hz, 1H), 7.45(d, J = 7.5 Hz, 1H).

5.1.1.22. ***1-(3-cyanophenyl)-3-(3-nitrophenyl)urea (2v).***

White solid powder, 77% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.39 (bs, 1H), 9.19 (bs, 1H), 8.55 (bs, 1H), 7.97 (d, J = 5.0 Hz, 1H), 7.84 (d, J = 5.5 Hz, 1H), 7.76-7.68 (m, 2H), 7.59-7.45 (m, 3H).

5.1.1.23. ***1-(3-cyanophenyl)-3-(4-nitrophenyl)urea (2w).***

White solid powder, 75% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.14 (bs, 1H), 8.95 (bs, 1H), 7.94 (bs, 1H), 7.68-7.66 (m, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.42 (d, J = 7.0 Hz, 1H), 7.15-7.12 (t, J = 9.0 Hz, 1H).

5.1.1.24. ***1-(3-cyanophenyl)-3-(1H-indol-5-yl)urea (5a).***

Brown solid powder, 79% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.97 (bs, 1H), 9.17 (bs, 1H), 8.79 (bs, 1H), 8.00 (bs, 1H), 7.68-7.66 (m, 2H), 7.47 (t, J = 8.0 Hz, 1H), 7.38-7.37 (m, 1H), 7.31-7.29 (m, 2H), 7.08 (dd, J_1 = 6.5 Hz, J_2 = 2.0 Hz, 2H), 6.35 (bs, 1H).

5.1.1.25. ***1-(3-cyanophenyl)-3-(quinolin-6-yl)urea (5b).***

Yellow solid powder, 80% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.24 (bs, 1H), 9.19 (bs, 1H), 8.75 (dd, J_1 = 9.0 Hz, J_2 = 2.0 Hz,1H), 8.27-8.25 (m, 1H), 8.19 (d, J = 5.5 Hz, 1H), 8.03 (m, 1H), 7.97-7.95 (m, 1H), 7.72 (dd, J_1 = 9.0 Hz, J_2 = 2.0 Hz, 2H), 7.52 (t, J = 7.5 Hz, 1H), 7.48-7.47 (m, 1H), 7.46 (bs, 1H).

5.1.1.26. ***1-(2-(1H-indol-3-yl)ethyl)-3-(3-cyanophenyl)urea (5c).***

White solid powder, 77% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.79 (s, 1H), 9.66 (s, 1H), 8.43 (s, 1H), 7.64 (s, 1H), 7.57 (d, J = 7.5 Hz, 1H), 7.45 (d, J = 8 Hz, 1H), 7.34 (d, J = 8 Hz,1H), 7.22 – 7.15 (m, 3H), 6.97 (d, J = 7, 1.5 Hz, 1H), 6.13 (t, J = 5.5 Hz, 1H), 3.38 (s, 2H), 2.86 (t, J = 7 Hz, 2H).

5.1.1.27. ***1-(2-(5-chloro-1H-indol-3-yl)ethyl)-3-(4-cyanophenyl)urea (5d).***

White solid powder, 78% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.06 (s, 1H), 9.24 (s, 1H), 8.89 (s, 1H), 7.97 (d, J = 16.9 Hz, 1H), 7.63 – 7.55 (m, 2H), 7.44 (dd, J = 16.9, 8.4 Hz, 2H), 7.35 (dd, J = 17.5, 8.1 Hz, 2H), 7.28 (s, 2H), 7.07 (d, J = 8.6 Hz, 1H), 6.33 (s, 1H), 3.39 (d, J = 6.2 Hz, 2H), 2.86 (t, J = 6.7 Hz, 2H).

5.1.1.28. ***1-(3-cyanophenyl)-3-(2-(5-methoxy-1H-indol-3-yl)ethyl)urea (5e).***

White solid powder, 79% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.71 (s, 1H), 9.36 (s, 1H), 8.24 (s, 1H), 7.74 (s, 1H), 7.46 (d, J = 8 Hz, 1H), 7.24- 7.13(m, 4H), 7.05 (d, J = 2 Hz,1H), 6.72 (dd, J = 8.5, 2.5 Hz, 1H), 3.37 (s, 2H), 2.82 (t, J = 7 Hz, 2H).

5.1.1.29. ***1-(4-cyanophenyl)-3-(o-tolyl)urea (8a).***

White solid powder, 80% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.50 (s, 1H), 8.11 (s, 1H), 7.78 (d, J = 8.3 Hz, 1H), 7.76 – 7.71 (m, 2H), 7.67 – 7.63 (m, 2H), 7.22 – 7.14 (m, 2H), 7.00 (t, J = 7.4 Hz, 1H), 2.25 (s, 3H).

5.1.1.30. ***1-(4-cyanophenyl)-3-(3-methoxyphenyl)urea (8b).***

White solid powder, 81% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.18 (s, 1H), 8.86 (s, 1H), 7.76 – 7.71 (m, 2H), 7.64 (d, *J* = 8.7 Hz, 2H), 7.23 – 7.17 (m, 2H), 6.99 – 6.94 (m, 1H), 6.60 (dd, *J* = 8.2, 2.1 Hz, 1H), 3.74 (s, 3H).

5.1.1.31. ***1-(4-cyanophenyl)-3-(3-fluorophenyl)urea (8c).***

White solid powder, 82% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.27 (s, 1H), 9.10 (s, 1H), 7.78 – 7.72 (m, 2H), 7.65 (t, *J* = 6.9 Hz, 2H), 7.48 (d, *J* = 11.8 Hz, 1H), 7.33 (dd, *J* = 15.2, 8.1 Hz, 1H), 7.16 (dd, *J* = 8.2, 0.9 Hz, 1H), 6.83 (td, *J* = 8.5, 2.3 Hz, 1H).

5.1.1.32. ***1-(4-chlorophenyl)-3-(4-cyanophenyl)urea (8d).***

White solid powder, 78% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.24 (s, 1H), 9.00 (s, 1H), 7.75 (dd, *J* = 11.2, 8.7 Hz, 2H), 7.65 (t, *J* = 8.5 Hz, 2H), 7.50 (d, *J* = 8.8 Hz, 2H), 7.35 (d, *J* = 8.8 Hz, 2H).

5.1.1.33. ***1-(3-bromophenyl)-3-(4-cyanophenyl)urea (8e).***

White solid powder, 79% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.27 (s, 1H), 9.05 (s, 1H), 7.85 (s, 1H), 7.76 – 7.72 (m, 2H), 7.66 – 7.62 (m, 2H), 7.34 (dd, *J* = 8.2, 0.9 Hz, 1H), 7.26 (t, *J* = 8.0 Hz, 1H), 7.19 (ddd, *J* = 7.9, 1.7, 0.9 Hz, 1H).

5.1.1.34. ***1-(4-cyanophenyl)-3-(quinolin-6-yl)urea (8f).***

White solid powder, 79% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.34 (s, 1H), 9.24 (s, 1H), 8.77 (d, *J* = 3.9 Hz, 1H), 8.28 (d, *J* = 8.3 Hz, 1H), 8.19 (d, *J* = 2.2 Hz, 1H), 7.97 (d, *J* = 9.0 Hz, 1H), 7.78 – 7.64 (m, 6H), 7.47 (dd, *J* = 8.3, 4.1 Hz, 1H).

5.1.1.35. ***1-(4-cyanophenyl)-3-(1H-indol-5-yl)urea (8g).***

White solid powder, 77% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.98 (s, 1H), 9.11 (s, 1H), 8.60 (s, 1H), 7.68 (dt, *J* = 14.2, 8.8 Hz, 5H), 7.32 (t, *J* = 6.3 Hz, 2H), 7.09 (dd, *J* = 8.7, 1.7 Hz, 1H), 6.37 (s, 1H).

5.1.2. *General procedure for synthesis of target compounds 3a-3w, 6a-6e, 9a-9e and 11a-11b.*

The appropriate amount of hydroxylamine hydrochloride (4 equiv) was added to a mixture of the intermediate **2a-2w, 5a-5e, 8a-8e and 10a-10b** (1 equiv.), and diisopropylethylamine (DIPEA, 3 equiv) in absolute ethanol (30 mL). The reaction mixture was heated to reflux to 80 °C and stirred for 12 h under an argon atmosphere. After the completion of the reaction, the solvent was removed under reduced pressure, and the obtained residue was purified through silica gel column chromatography using mixtures of ethyl acetate/hexane (9:1) as eluent to afford the corresponding diarylurea-hydroxyamidine derivatives **3a-3w**, **6a-6e, 9a-9e** and **11a-11b.**

5.1.2.1. *(Z)-N'-hydroxy-3-(3-(phenylureido)benzimidamide (3a)*.

White solid powder, 74% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.62 (bs, 1H), 8.71 (bs, 1H), 8.66 (bs, 1 H), 7.73 (bs, 1H), 7.49-7.44 (m, 3H), 7.29-7.24 (m, 4H), 6.97 (t, J = 7.5 Hz, 1H), 5.74 (bs, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 152.96, 151.46, 140.12, 139.94, 134.54, 129.26, 128.99, 122.32, 119.63, 119.31, 118.66, 115.95. HRMS [M + H]+ found 271.1193, calculated 271.1195 for $C_{14}H_{14}N_4O_2$.

5.1.2.2. *(Z)-N'-hydroxy-3-(3-(o-tolyl)ureido)benzimidamide (3b)*.

White solid powder, 76% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.62 (bs, 1H), 9.47 (bs, 1H), 8.25 (bs, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.76 (s, 1H), 7.52 (dd, J_1 = 7.5 Hz, J_2 = 1.5 Hz, 1H), 7.26-7.22 (m, 2H), 7.17-7.11 (m, 2H), 6.94 (t, J = 6.5 Hz, 1H), 5.73 (s, 2H), 2.25 (s, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 153.23, 151.52, 140.31, 137.95, 134.52, 130.63, 128.97, 128.17, 126.56, 123.10, 121.63, 119.37, 119.05, 115.72, 18.44. HRMS [M + H]+ found 285.1348, calculated 285.1352 for $C_{15}H_{16}N_4O_2$.

5.1.2.3. *(Z)-N'-hydroxy-3-(3-(m-tolyl)ureido)benzimidamide (3c).*

White solid powder, 71% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.62 (bs, 1H), 8.70 (bs, 1H), 8.59 (bs, 1H), 7.73 (bs, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.30-7.22 (m, 4H), 7.15 (t, J = 7.5 Hz, 1H), 6.78 (d, J = 7.0 Hz, 1H), 5.76 (bs, 2H), 2.27 (bs, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 152.85, 151.43, 139.84, 138.48, 134.41, 134.37, 129.12, 129.06, 123.15, 119.65, 119.31, 119.17, 115.87, 115.82, 21.65. HRMS [M + H]+ found 285.1351, calculated 285.1352 for $C_{15}H_{16}N_4O_2$.

5.1.2.4. ***(Z)-N'-hydroxy-3-(3-(p-tolyl)ureido)benzimidamide (3d).***

White solid powder, 73% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.60 (bs, 1H), 9.09-9.01 (m, 2H), 7.74 (bs, 1H), 7.50 (d, J = 7.5 Hz, 1H), 7.35 (d, J = 8.5 Hz, 2H), 7.26-7.24 (m, 2H), 7.08 (d, J = 8.0 Hz, 2H), 5.72 (bs, 2H), 2.24 (bs, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 153.13, 151.149, 140.19, 137.72, 134.51, 130.95, 129.61, 128.92, 119.40, 119.30, 118.63, 115.79, 20.80. HRMS [M + H]+ found 285.1352, calculated 285.1352 for $C_{15}H_{16}N_4O_2$.

5.1.2.5. ***(Z)-N'-hydroxy-3-(3-(2-methoxyphenyl)ureido)benzimidamide (3e).***

White solid powder, 70% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.62 (bs, 1H), 9.37 (bs, 1H), 8.22 (bs, 1H), 8.13 (dd , J_1 = 7.0 Hz, J_2 = 1.5 Hz, 1H), 7.44 (bs, 1H), 7.49 (d, J = 8.0 Hz, 2H), 7.29-7.23 (m, 2H), 7.01 (dd , J_1 = 6.5 Hz, J_2 = 1.5 Hz, 1H), 6.94 (t, J = 7.5 Hz, 1H), 6.91-6.89 (m, 2H), 5.76 (s, 3H), 5.75 (bs, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 152.81, 151.49, 148.08, 140.07, 134.58, 129.09, 129.04, 122.28, 121.03, 119.00, 118.71, 115.67, 111.17, 56.23, 55.38. HRMS [M + H]+ found 301.1302, calculated 301.1295 for $C_{15}H_{16}N_4O_2$

5.1.2.6. ***(Z)-N'-hydroxy-3-(3-(3-methoxyphenyl)ureido)benzimidamide (3f).***

White solid powder, 70% yield**.** ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.62 (bs, 1H), 8.70 (s, 2H), 8.68 (s, 1H), 7.74 (bs, 1H), 7.49-7.46 (m, 1H), 7.28-7.25 (m, 2H), 7.20-7.16 (m, 2H), 6.93-6.92 (m, 1H), 6.50 (dd, J_1 = 8.0 Hz, J_2 = 2.5 Hz, 1H), 5.77 (bs, 2H), 3.73 (s, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 160.16, 152.90, 151.58, 141.38, 139.90, 134.45, 130.01,

129.00, 119.70, 119.39, 116.00, 110.96, 107.78, 104.36, 55.39. HRMS [M + H]+ found 301.1275, calculated 301.1295 for $C_{15}H_{16}N_4O_3$.

5.1.2.7. ***(Z)-N'-hydroxy-3-(3-(4-methoxyphenyl)ureido)benzimidamide (3g).***

White solid powder, 72% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.58 (bs, 1H), 8.98 (bs, 1H), 8.84 (bs, 1H), 7.73 (s, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.38-7.36 (m, 2H), 7.26-7.23 (m, 2H), 6.85 (d, J = 9.0 Hz, 2H), 5.70 (s, 2H), 3.71 (s, 3H). ^{13}C NMR (DMSO-d_6, 125 MHz): 154.35, 152.85, 151.08, 139.91, 134.02, 132.95, 128.41, 119.97, 119.90, 118.83, 118.71, 115.36, 113.95, 55.16. HRMS [M + H]+ found 301.1275, calculated 301.1295 for $C_{15}H_{16}N_4O_3$.

5.1.2.8. ***(Z)-N'-hydroxy-3-(3-(3-hydroxyphenyl)ureido)benzimidamide (3h).***

White solid powder, 71% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.60 (s, 1H), 9.07 (s, 1H), 8.58 (s, 1H), 8.32 (s, 1H), 7.70 (s, 1H), 7.49 – 7.47 (m, 1H), 7.27 – 7.21 (m, 4H), 6.70 – 6.68 (m, 2H), 5.73 (s, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 153.22, 153.03, 151.51, 140.25, 134.50, 131.53, 128.93, 120.90, 119.33, 119.12, 115.78,115.66. HRMS [M + H]+ found 287.1147, calculated 287.1144 for $C_{14}H_{14}N_4O_3$.

5.1.2.9. ***(Z)-N'-hydroxy-3-(3-(4-hydroxyphenyl)ureido)benzimidamide (3i).***

White solid powder, 70% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.58 (bs, 1H), 9.05 (bs, 1H), 8.56 (bs, 1H), 8.30 (bs, 1H), 7.47 (d, J = 8.0 Hz, 1H), 7.24-7.21 (m, 2H), 6.68 (d, J = 8.5 Hz, 1H), 5.72 (bs, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 152.87, 151.41, 139.76, 139.16, 134.55, 129.03, 128.98, 125.80, 120.20, 119.78, 119.43, 116.09. HRMS [M + H]+ found 287.1147, calculated 287.1144 for $C_{14}H_{14}N_4O_3$.

5.1.2.10. ***(Z)-3-(3-(2-chlorophenyl)ureido)-N'-hydroxybenzimidamide (3j).***

White solid powder, 79% yield**.** ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.61 (bs, 1H), 9.48 (bs, 1H), 8.29 (bs, 1H), 8.17 (d, J = 8.5 Hz, 1H), 7.76 (bs, 1H), 7.52-7.45 (m, 2H), 7.32-7.27 (m, 3H), 7.03 (t, J=7.5Hz, 1H), 5.74 (bs, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 152.48, 151.39,

139.56, 136.21, 134.46, 129.68, 129.19, 128.07, 123.89, 122.41, 121.76, 119.95, 119.28, 115.88. HRMS [M + H]+ found 305.0809, calculated 305.0805 for $C_{14}H_{13}ClN_4O_2$.

5.1.2.11. ***(Z)-3-(3-(3-chlorophenyl)ureido)-N'-hydroxybenzimidamide (3k).***

White solid powder, 79% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.62 (s, 1H), 8.90 (s, 1H), 7.74-7.71 (m, 2H), 7.49-7.47 (m, 1H), 7.31-7.25 (m, 4H), 7.03-7.00 (m, 1H), 5.74 (s, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 173.14, 157.59, 156.17, 146.48, 144.41, 140.34, 138.41, 135.64, 133.77, 126.66, 124.65,122.76, 121.86, 120.93. HRMS [M + H]+ found 305.0800, calculated 305.0805 for $C_{14}H_{13}ClN_4O_2$.

5.1.2.12. ***(Z)-3-(3-(4-chlorophenyl)ureido)-N'-hydroxybenzimidamide (3l).***

White solid powder, 72% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.61 (bs, 1H), 8.81 (bs, 1H), 8.76 (s, 1H), 7.73 (s, 1H), 7.49-7.47 (m, 3H), 7.33-7.24 (m, 4H), 5.75 (s, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 152.76, 139.61, 138.94, 134.39, 129.10, 125.92, 121.21, 119.85, 119.47, 116.04. HRMS [M + H]+ found 305.0805, calculated 305.0805 for $C_{14}H_{13}ClN_4O_2$.

5.1.2.13. ***(Z)-3-(3-(2-bromophenyl)ureido)-N'-hydroxybenzimidamide (3m).***

White solid powder, 71% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.63 (d, J = 4.5 Hz, 1H), 9.55 (s, 1H), 8.14 – 8.08 (m, 2H), 7.77 (s, 1H), 7.62 (d, J = 7.5 Hz, 1H), 7.53 (d, J = 7 Hz, 1H), 7.36 – 7.29 (m, 3H), 6.98 (t, J = 8 Hz, 1H), 5.76 (s, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 152.61, 151.43, 139.74, 137.52, 134.63, 132.95, 129.11, 128.56, 124.53, 122.63, 119.89, 119.26, 115.97, 113.47. HRMS [M + H]+ found 349.0301, calculated 349.0300 for $C_{14}H_{13}BrN_2O_2$.

5.1.2.14. ***(Z)-3-(3-(3-bromophenyl)ureido)-N'-hydroxybenzimidamide (3n).***

White solid powder, 73% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.63 (bs, 1H), 9.02 (bs, 1H), 8.91 (bs, 1H), 7.86 (bs, 1H), 7.74 (bs, 1H), 7.50 (d, J = 7.5 Hz, 1H), 7.32-7.22 (m, 4H), 7.14 (d, J = 8.0 Hz, 1H), 5.95 (bs, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 152.85, 151.48,

141.90, 139.69, 134.50, 131.17, 129.01, 124.78, 122.19, 120.81, 119.87, 119.48, 117.44, 116.13. HRMS [M + H]+ found 349.0309, calculated 349.0300 for $C_{14}H_{13}BrN_4O_2$.

5.1.2.15. ***(Z)-3-(3-(4-bromophenyl)ureido)-N'-hydroxybenzimidamide (3o).***

White solid powder, 71% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.63 (bs, 1H), 8.83 (bs, 1H), 8.77 (bs, 1H), 7.73 (bs, 1H), 7.49-7.42 (m, 6H), 7.30-7.25 (m, 2H), 5.76 (s, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 152.84, 139.75, 139.60, 131.98, 129.01, 125.41, 120.59, 119.43, 116.08, 113.66. HRMS [M + H]+ found 349.0305, calculated 349.0300 for $C_{14}H_{13}BrN_2O_2$.

5.1.2.16. ***(Z)-3-(3-(2-fluorophenyl)ureido)-N'-hydroxybenzimidamide (3p).***

White solid powder, 70% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.65 (bs, 1H), 9.13 (bs, 1H), 8.56-8.55 (d, J = 6.5 Hz, 1H), 8.17 (t, J = 8.0 Hz, 1H), 7.75 (bs, 1H), 7.50-7.48 (m, 1H), 7.31-7.21 (m, 3H), 7.13 (t, J = 8.0 Hz, 1H), 7.02-6.98 (m, 1H), 5.79 (s, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 152.98, 152.19, 151.05, 139.26, 134.15, 128.69, 127.61, 127.53, 124.59, 124.57, 122.53, 122.47, 120.56, 119.45, 118.80, 115.43, 115.08, 114.93. HRMS [M + H]+ found 289.1101, calculated 289.1101 for $C_{14}H_{13}FN_4O_2$.

5.1.2.17. ***(Z)-3-(3-(3-fluorophenyl)ureido)-N'-hydroxybenzimidamide (3q).***

White solid powder, 65% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.63 (bs, 1H), 8.91 (bs, 1H), 8.79 (bs, 1H), 7.74 (s, 1H), 7.51-7.47 (m, 2H), 7.32-7.26 (m, 3H), 7.12 (dd , J_1 = 7.0 Hz, J_2 = 1.0 Hz, 1H), 6.80-6.76 (m, 1H), 5.77 (bs, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 163.82, 161.91, 152.85, 151.41, 142.10, 142.01, 139.68, 134.55, 130.83, 130.75, 129.02, 119.85, 119.44, 116.07, 114.36, 108.66, 108.49, 105.36, 105.15. HRMS [M + H]+ found 289.1098, calculated 289.1101 for $C_{14}H_{13}FN_4O_2$.

5.1.2.18. ***(Z)-3-(3-(4-fluorophenyl)ureido)-N'-hydroxybenzimidamide (3r).***

White solid powder, 66% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.61 (bs, 1H), 8.69 (d, J = 6.0 Hz, 2H), 7.73 (s, 1H), 7.48-7.44 (m, 3H), 7.27-7.25 (m, 2H), 7.11 (t, J = 9.0 Hz, 2H),

5.74 (s, 2H).^{13}C NMR (DMSO-d_6, 125 MHz): δ 158.75, 156.85, 153.06, 151.45, 139.91, 136.48, 136.46, 134.53, 128.97, 120.46, 119.65, 119.36, 116.01, 115.82, 115.65. HRMS [M + H]+ found 289.1085, calculated 289.1101 for $C_{14}H_{13}FN_4O_2$.

5.1.2.19. ***(Z)-N'-hydroxy-3-(3-(2-(trifluoromethyl)phenyl)ureido) benzimidamide (3s).***

White solid powder, 65% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.63 (bs, 1H), 9.45 (bs, 1H), 8.07 (s, 1H), 7.97-7.95 (d, J = 8.0 Hz, 1H), 7.74 (s, 1H), 7.69-7.64 (m, 2H), 7.53-7.51 (d, J = 7.5 Hz, 1H), 7.30-7.28 (m, 3H), 5.78 (s, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 152.85, 151.46, 139.72, 136.78, 134.57, 133.39, 129.12, 126.41, 126.37, 126.03, 125.57, 124.13, 120.33, 119.92, 119.23, 115.95**.** HRMS [M + H]+ found 339.1052, calculated 339.1069 for $C_{15}H_{13}F_3N_4O_2$.

5.1.2.20. ***(Z)-N'-hydroxy-3-(3-(3-(trifluoromethyl)phenyl)ureido)benzimidamide (3t).***

White solid powder, 66% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.60 (bs, 1H), 9.04 (bs, 1H), 8.84 (s, 1H), 8.02 (s, 1H), 7.77 (bs, 1H), 7.57-7.48 (m, 3H), 7.28 (bs, 3H), 5.73 (s, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 157.71, 155.83, 148.95, 148.31, 148.23, 142.91, 127.14, 123.10, 118.26, 118.20, 115.90, 115.73, 114.82, 111.62, 56.25. HRMS [M + H]+ found 339.1051, calculated 339.1069 for $C_{15}H_{13}F_3N_4O_2$.

5.1.2.21. ***(Z)-N'-hydroxy-3-(3-(4-(trifluoromethyl)phenyl)ureido)benzimidamide (3u).***

White solid powder, 65% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.63 (bs, 1H), 9.06 (bs, 1H), 8.86 (bs, 1H), 8.02 (bs, 1H), 7.77 (bs, 1H), 7.58-7.47 (m, 3H), 7.29 (t, J = 9.0 Hz, 3H), 5.75 (bs, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 153.26, 151.54, 140.32, 137.94, 134.50, 130.63, 128.98, 128.23, 126.56, 123.13, 121.67, 119.38, 119.07, 115.73, 18.45. HRMS [M + H]+ found 339.1051, calculated 339.1069 for $C_{15}H_{13}F_3N_4O_2$.

5.1.2.22. ***(Z)-N'-hydroxy-3-(3-(3-nitrophenyl)ureido)benzimidamide (3v).***

White solid powder, 66% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.59 (bs, 1H), 9.16 (bs, 1H), 8.84 (s, 1H), 8.53-8.52 (d, J = 7.5 Hz, 1H), 7.78-7.74 (m, 2H), 7.66 (dd, J_1 = 8.5 Hz, J_2 =

1.5 Hz, 1H), 7.51 (t, J = 8.0 Hz, 1H), 7.44 (dd, J_1 = 6.5 Hz, J_2 = 1.0 Hz, 1H), 7.24 (s, 2H), 5.71 (s, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 152.76, 151.32, 148.60, 141.30, 139.32, 134.41, 130.57, 129.10, 124.76, 120.12, 119.73, 116.84, 116.28, 112.57. HRMS [M + H]+ found 316.1033, calculated 316.1046 for $C_{14}H_{13}N_5O_4$.

5.1.2.23. ***(Z)-N'-hydroxy-3-(3-(4-nitrophenyl)ureido)benzimidamide (3w).***

Yellow solid powder, 61% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.48 (s, 1H), 8.84 (s, 1H), 8.80 (s, 1H), 7.60 (d, J = 8.7 Hz, 2H), 7.52 – 7.47 (m, 2H), 7.45 (d, J = 8.7 Hz, 2H), 7.36 – 7.32 (m, 2H), 5.71 (s, 2H). ^{13}C NMR (126 MHz, DMSO) δ 152.79, 151.06, 140.61, 139.11, 129.10, 127.43, 126.42, 125.90, 120.25, 118.09. HRMS [M + H]+ found 316.1044, calculated 316.1046 for $C_{14}H_{13}N_5O_4$

5.1.2.24. ***(Z)-3-(3-(1H-indol-5-yl)ureido)-N'-hydroxybenzimidamide (6a).***

Brown solid powder, 66% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.95 (bs, 1H), 9.64 (bs, 1H), 8.63 (s, 1H), 8.41 (s, 1H), 7.73 (bs, 1H), 7.68 (bs, 1H), 7.30-7.21 (m, 4H), 7.07 (dd , J_1 = 6.5 Hz, J_2 = 2.0 Hz, 1H), 6.35 (bs, 1H), 5.81 (bs, 2H). ^{13}C NMR (DMSO-d_6,125 MHz): δ 153.43, 150.12, 132.72, 131.80, 128.98, 128.17, 126.22, 115.77, 111.71, 110.41, 107.52, 101.33. HRMS [M + H]+ found 310.1303, calculated 310.1304 for $C_{16}H_{15}N_5O_2$.

5.1.2.25. ***(Z)-N'-hydroxy-3-(3-(quinolin-6-yl)ureido)benzimidamide (6b).***

White solid powder, 74% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.66 (bs, 1H), 9.05 (bs, 1H), 8.87 (bs, 1H), 8.74 (dd, J_1 = 8.0 Hz, J_2 = 2.5 Hz, 1H), 8.26 (t, J = 8.5 Hz, 1H), 8.20 (bs, 1H), 7.94 (d, J = 9.0 Hz, 1H), 7.82 (bs, 1H), 7.71 (dd, J_1 = 8.0 Hz, J_2 = 2.5 Hz, 1H), 7.53-7.51 (m, 1H), 7.48-7.45 (m, 1H), 7.33-7.30 (m, 2H), 5.84 (s, 2H). ^{13}C NMR (DMSO-d_6,125 MHz): δ 172.78, 153.14, 151.46, 148.75, 144.57, 139.94, 138.32, 135.58, 134.57, 129.94, 129.10, 129.00, 123.42, 122.15, 119.71, 119.33, 116.00, 113.32. HRMS [M + H]+ found 322.1303, calculated 322.1304 for $C_{17}H_{15}N_5O_2$.

5.1.2.26. ***(Z)-3-(3-(2-(1H-indol-3-yl)ethyl)ureido)-N'-hydroxybenzimidamide (6c).***

Brown solid powder, 79% yield. ^{1}H NMR (DMSO-*d*6, 500 MHz): δ 10.84 (s, 1H), 9.56 (s, 1H), 8.53 (s, 1H), 7.64 (s, 1H), 7.57 (d, *J* = 7.5 Hz, 1H), 7.45 (d, *J* = 8 Hz, 1H), 7.34 (d, *J* = 8 Hz,1H), 7.22 – 7.15 (m, 3H), 6.97 (d, *J* = 7, 1.5 Hz, 1H), 6.13 (t, *J* = 5.5 Hz, 1H), 5.69 (s, 2H), 3.38 (s, 2H), 2.86 (t, *J* = 7 Hz, 2H). ^{13}C NMR (DMSO-d_6,125 MHz): δ 152.43, 151.33, 146.85, 141.48, 139.27, 134.63, 129.08, 125.63, 120.28, 119.73, 117.97, 116.40, 55.39. HRMS [M + H]+ found 338.1614, calculated 338.1617 for $C_{18}H_{19}N_5O_2$.

5.1.2.27. ***(Z)-3-(3-(2-(5-chloro-1H-indol-3-yl)ethyl)ureido)-N'-hydroxybenzimidamide (6d).***

White solid powder, 81% yield. ^{1}H NMR (DMSO-*d*6, 500 MHz): δ 11.07 (s, 1H), 9.58 (s, 1H), 8.55 (s, 1H), 7.64 (d, *J* = 18 Hz, 2H), 7.47 (d, *J* = 8 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.27 (s, 1H), 7.23 – 7.16 (m, 2H), 7.08 (dd, *J* = 8.5, 1.5 Hz, 1H), 6.15 (t, *J* = 5.5 Hz, 1H), 5.71 (s, 2H), 3.38 (s, 2H), 2.86 (t, *J* = 7 Hz, 2H). ^{13}C NMR (DMSO-*d*6,126 MHz): δ 155.67, 151.70, 140.82, 135.22, 134.36, 128.91, 128.84,125.18, 123.51, 121.35, 118.87, 118.13, 115.47, 113.37, 112.32, 49.08, 26.08. HRMS [M + H]+ found 372.1230, calculated 372.1227 for $C_{18}H_{18}ClN_5O_2$.

5.1.2.28. ***(Z)-N'-hydroxy-3-(3-(2-(5-methoxy-1H-indol-3-yl)ethyl)ureido)benzimidamide (6e).***

White solid powder, 78% yield. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.68 (s, 1H), 9.56 (s, 1H), 8.54 (s, 1H), 7.64 (s, 1H), 7.46 (d, *J* = 8 Hz, 1H), 7.24- 7.13(m, 4H), 7.05 (d, *J* = 2 Hz,1H), 6.72 (dd, *J* = 8.5, 2.5 Hz, 1H), 5.71 (s, 2H), 3.38 (s, 2H), 2.84 (t, *J* = 7 Hz, 2H). ^{13}C NMR (DMSO-*d*6,126 MHz): δ 168.62, 155.63, 151.63, 140.82, 135.21, 134.40, 128.90,128.83, 125.19, 123.48, 121.34, 118.83, 118.78, 118.15, 115.41, 113.37,112.30, 30.86, 29.46, 26.10. HRMS [M + H]+ found 368.1706, calculated 368.1723 for $C_{19}H_{21}N_5O_3$.

5.1.2.29. ***(Z)-N'-hydroxy-4-(3-(o-tolyl)ureido)benzimidamide (9a).***

White solid powder, 71% yield. ^{1}H NMR (DMSO-*d*6, 500 MHz): δ 9.49 (s, 1H), 9.15 (d, *J* = 8.5 Hz, 1H), 7.97 (d, *J* = 2 Hz, 1H), 7.83 (d, *J* = 8 Hz, 1H), 7.60 (d, *J* = 8 Hz, 2H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.19 – 7.13 (m, 2H), 6.96 (t, *J* = 7.5 Hz, 1H), 5.75 (s, 2H), 2.25 (s, 3H). ^{13}C NMR (DMSO-d_6,125 MHz): δ 153.02, 151.18, 141.01, 137.77, 130.67, 128.10, 127.04,126.63, 126.48, 123.25, 121.60, 117.78, 18.36. HRMS [M + H]+ found 285.1352, calculated 285.1352for $C_{15}H_{16}N_4O_2$.

5.1.2.30. ***(Z)-N'-hydroxy-4-(3-(3-methoxyphenyl)ureido)benzimidamide (9b).***

Brown solid powder, 70% yield. 1H NMR (DMSO-*d*6, 500 MHz): δ 9.49 – 9.48 (m, 1H), 8.77 (d, *J* = 5.0 Hz, 1H), 8.72 (d, *J* = 4.0 Hz, 1H), 7.60 (dd, *J* = 8.5, 2.5 Hz, 2H), 7.45 (dd, *J* = 8.5, 2.5 Hz, 2H), 7.19 (dd, *J* = 13, 3.0 Hz, 2H), 6.94 (d, *J* = 7.5 Hz, 1H), 6.56 (dd, *J* = 8, 2 Hz, 1H), 5.72 (s, 2H), 3.74 (s, 3H). ^{13}C NMR (DMSO-d_6,125 MHz): δ 160.17, 152.81, 151.11, 141.30, 140.74, 130.03, 127.28, 126.42, 117.98, 111.02, 107.79, 104.47, 55.40. HRMS [M + H]+ found 301.1302, calculated 301.1301for $C_{15}H_{16}N_4O_3$.

5.1.2.31. ***(Z)-4-(3-(3-fluorophenyl)ureido)-N'-hydroxybenzimidamide (9c).***

White solid powder, 77% yield. ^{1}H NMR (DMSO-*d*6, 500 MHz): δ 9.49 (s, 1H), 8.98 (s, 1H), 8.88 (s, 1H), 7.60 (d, *J* = 8.5 Hz, 2H), 7.52 – 7.44 (m, 3H), 7.31 (dd, *J* = 15.5, 8 Hz, 1H), 7.13 (dd, *J* = 9.5, 1.5 Hz, 1H), 6.81-6.77 (m,1H), 5.73 (s, 2H). ^{13}C NMR (DMSO-d_6,125 MHz): δ 163.82, 161.91, 152.74, 151.06, 142.02,141.93, 140.50, 130.83,130.75, 127.48, 126.42, 118.11, 114.42, 108.75, 108.58, 105.45, 105.24. HRMS [M + H]+ found 289.1100, calculated 289.1101for $C_{14}H_{13}FN_4O_2$.

5.1.2.32. ***(Z)-4-(3-(4-chlorophenyl)ureido)-N'-hydroxybenzimidamide (9d).***

White solid powder, 74% yield. ^{1}H NMR (DMSO-*d*6, 500 MHz): δ 9.63 (s, 1H), 9.42 (s, 1H), 8.96 (s, 1H), 8.21 – 8.19 (m, 2H), 7.80 (s, 1H), 7.72 – 7.70 (m, 2H), 7.51 – 7.49 (m, 1H), 7.32 – 7.31 (m, 2H), 5.76 (s, 2H). ^{13}C NMR (DMSO-d_6,125 MHz): δ 152.97,146.38, 141.06,

140.14, 133.77, 129.26, 129.00, 126.52, 122.32, 118.61, 117.84, 46.05. HRMS [M + H]+ found 305.0805, calculated 305.0805 for $C_{14}H_{13}ClN_4O_2$.

5.1.2.33. ***(Z)-4-(3-(3-bromophenyl)ureido)-N'-hydroxybenzimidamide (9e).***

Brown solid powder, 70% yield. ^{1}H NMR (DMSO-*d*6, 500 MHz): δ 9.52 (s, 1H), 9.01 (t, *J* = 22 Hz, 2H), 7.86 (s, 1H), 7.60 (d, *J* = 8.5 Hz, 2H), 7.46 – 7.44 (m, 2H), 7.32 (d, *J* = 8.5 Hz, 1H), 7.24 (dd, *J* = 16.0, 4.0 Hz, 1H), 7.15 (d, *J* = 7.5 Hz, 1H), 5.79 (s, 2H). ^{13}C ^{13}C NMR (DMSO-d_6,125 MHz): δ 152.77, 151.26, 141.83, 140.61, 131.19, 127.29, 126.49, 124.87, 122.20, 120.86, 118.11, 117.97,117.49, 46.16. HRMS [M + H]+ found 349.0301, calculated 349.0300 for $C_{14}H_{13}BrN_4O_2$.

5.1.2.34. ***(Z)-N'-hydroxy-4-(3-(quinolin-6-yl)ureido)benzimidamide (11a).***

Yellow solid powder, 67% yield. ^{1}H NMR (DMSO-*d*6, 500 MHz): δ 9.65 (s, 1H), 9.44 (s, 1H), 8.98 (s, 1H), 8.20 (d, *J* = 9 Hz, 3H), 7.80 (s, 1H), 7.71 (dd, *J* = 9, 3.5 Hz, 3H), 7.51 – 7.49 (m, 1H), 7.31 (d, *J* = 5 Hz, 2H), 5.77 (s, 2H). ^{13}C NMR (DMSO-d_6,125 MHz): δ 152.92, 151.06, 148.87, 144.66, 140.61, 138.07, 135.57, 130.01, 129.06, 127.46, 126.45, 123.45, 122.19, 118.09, 113.56. HRMS [M + H]+ found 322.1303, calculated 322.1304 for $C_{17}H_{15}N_5O_2$.

5.1.2.35. ***(Z)-4-(3-(1H-indol-5-yl)ureido)-N'-hydroxybenzimidamide (11b).***

Brown solid powder, 69% yield. ^{1}H NMR (DMSO-*d*6, 500 MHz): δ 10.95 (s, 1H), 9.46 (s, 1H), 8.68 (s, 1H), 8.44 (s, 1H), 7.68 (s, 1H), 7.58 (d, *J* = 9 Hz, 2H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.30 (dd, *J* = 12, 4.5 Hz, 3H), 7.08 (dd, *J* = 8.5, 1.5 Hz, 1H), 6.36 (s, 1H), 5.71 (s, 2H). ^{13}C NMR (DMSO-d_6,125 MHz): δ 153.35, 151.15, 141.28, 132.78, 131.74, 128.19, 126.82, 126.38, 126.24, 119.19, 118.99, 117.71, 115.22, 111.73, 110.49, 101.34. HRMS [M + H]+ found 310.1303, calculated 310.1304 for $C_{16}H_{15}N_5O_2$.

5.1.3. *General procedure for the synthesis of intermediates 13a-13e*

Substituted tryptamine, **12a-12e** (1.0 mmol) was dissolved in 15 mL of DCM at room temperature, and pyridine (1 mmol) was added. The reaction mixture was stirred for 10 minutes before introducing 4-nitrophenyl chloroformate (1 mmol). Stirring was continued for 2 hours at room temperature. Upon completion of the reaction, the solvent was evaporated under vacuum, and the resulting products, **13a-13e** were utilized in the next step without column purification due to stability issues.

5.1.4. *General procedure for synthesis of target compounds 14a-14e, 15a-15e and 16a*

Triethylamine (4 equiv.) was added to a mixture containing dimethylaminoethylphenol in MeCN (15 mL). The reaction mixture was heated to reflux at 80 °C for 30 min, followed by adding intermediate compounds **13a-13e** (1 equiv.), and stirred for 2 hours under a nitrogen atmosphere. Following the completion of the reaction, the solvent was evaporated under reduced pressure, and the resulting residue was subjected to purification through silica gel column chromatography using ethyl DCM/MeOH mixtures (9:1) as the eluent, providing the corresponding carbamate derivatives **14a-14e, 15a-15e and 16e**.

5.1.4.1. *3-(1-(dimethylamino)ethyl)phenyl (2-(1H-indol-3-yl)ethyl)carbamate (14a)*

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.25 – 7.22 (m, 1H), 7.16 (dd, *J* = 13.5, 6.5 Hz, 2H), 7.09 – 7.07 (m, 2H), 7.03 (dd, *J* = 9.5, 1.5 Hz, 1H), 5.21 (t, *J* = 5.5 Hz, 1H), 3.64-3.60 (m, 2H), 3.31 (q, *J* = 6.7 Hz, 1H), 3.07 (t, *J* = 6.5 Hz, 2H), 2.23 (s, 6H), 1.39 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.71, 151.09, 145.23, 136.48, 129.02, 127.33, 124.48, 122.27, 122.20, 120.77, 120.24, 119.50, 118.72, 112.6, 111.32, 65.5, 43.00, 41.46, 29.71, 25.60, 19.82, 14.13. HRMS [M + H] + found 352.2021, calculated 352.2025 for $C_{21}H_{25}N_3O_2$.

5.1.4.2. ***3-(1-(dimethylamino)ethyl)phenyl (2-(5-methyl-1H-indol-3-yl)ethyl)carbamate (14b)***

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.43 (s, 1H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 8.5 Hz, 1H), 7.19 (d, J= 8.0Hz, 1H), 7.14(s, 1H), 7.11 – 7.05 (m, 3H), 5.17 (s, 1H), 3.63-3.51 (m, 2H), 3.49 (q, *J* = 8.5 Hz, 1H), 3.04 (t, *J* = 7.0 Hz, 2H), 2.48 (s, 3H), 2.43 (s, 6H), 1.56 (t, J=5.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.45, 134.81, 29.59, 128.74, 127.48, 124.94, 123.82, 122.58, 121.66, 121.42, 118.3, 111.05, 65.78, 43.01, 41.39, 29.71, 25.50, 22.71, 21.54, 18.55, 14.11. HRMS [M + H]+ found 366.2174, calculated 366.2182 for $C_{22}H_{27}N_3O_2$.

5.1.4.3. ***3-(1-(dimethylamino)ethyl)phenyl (2-(5-methoxy-1H-indol-3-yl)ethyl)carbamate (14c)***

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.37 (d, *J* = 12.5 Hz, 1H), 7.32 (t, *J* = 8.0 Hz, 1H), 7.27 (s, 1H), 7.15 (d, *J* = 7.5 Hz, 1H), 7.10 – 7.03 (m, 4H), 6.89 (dd, *J* = 10.5, 2.0 Hz, 1H), 5.26 (d, *J* = 5.5 Hz, 1H), 3.88 (s, 3H), 3.61 (t, *J* = 6.5 Hz, 2H), 3.40 (d, *J* = 7.0 Hz, 1H), 3.03 (t, *J* = 7.0 Hz, 2H), 2.27 (s, H), 1.42 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.67, 154.05, 151.15, 144.05, 131.62, 129.1, 127.69, 124.61, 123.13, 120.92, 120.60, 112.38, 112.25, 112.10, 100.51, 65.64, 55.9, 42.76, 41.38, 25.58, 19.49. HRMS [M + H]+ found 382.2126, calculated 382.2131 for $C_{22}H_{27}N_3O_3$.

5.1.4.4. ***3-(1-(dimethylamino)ethyl)phenyl (2-(5-hydroxy-1H-indol-3-yl)ethyl)carbamate (14d)***

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.63 (s, 1H), 7.88 (t, *J* = 5.6 Hz, 1H), 7.37 (t, *J* = 7.7 Hz, 1H), 7.22 (d, *J* = 7.0 Hz, 1H), 7.13 (d, *J* = 8.6 Hz, 2H), 7.08 (d, *J* = 2.2 Hz, 1H), 7.05 (d, *J* = 7.4 Hz, 1H), 6.85 (d, *J* = 2.2 Hz, 1H), 6.60 (dd, *J* = 8.6, 2.3 Hz, 1H), 2.85 – 2.78 (m, 2H), 2.28 (s, 5H), 1.40 (s, 3H), 1.24 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.61, 157.65, 145.50, 136.73, 129.49, 127.70, 123.02, 121.35, 118.43, 118.37, 114.60, 114.37,

114.29, 112.52, 111.79, 65.45, 65.32, 43.03, 26.67, 20.19. HRMS [M + H]+ found 368.1968, calculated 368.1974 for $C_{21}H_{25}N_3O_3$.

5.1.4.5. *3-(1-(dimethylamino)ethyl)phenyl (2-(5-chloro-1H-indol-3-yl)ethyl)carbamate (14e)*

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 (s, 1H), 7.60 (d, *J* = 1.5 Hz, 1H), 7.32 (dd, *J* = 19.0, 7.5 Hz, 2H), 7.17 – 7.14 (m, 2H), 7.1 (dd, *J* = 4.3, 1.7 Hz, 2H), 7.05 (dd, *J* = 6.0, 1.5 Hz, 1H), 5.24 (t, *J* = 6.0 Hz, 1H),3.58 (q, *J* = 6.5 Hz, 2H), 3.39 (d, *J* = 6.5 Hz, 1H),3.01 (t, *J* = 6.5 Hz, 2H), 2.26 (s, 6H), 1.41 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.70, 151.10, 144.30, 134.77, 129.17, 124.65, 123.78, 122.42, 120.87, 120.59, 118.18, 112.37, 65.66, 42.83, 41.43, 25.42, 19.52. HRMS [M + H]+ found 386.1631, calculated 386.1635 for $C_{21}H_{24}ClN_3O_2$.

5.1.4.6. *(S)-3-(1-(dimethylamino)ethyl)phenyl (2-(1H-indol-3-yl)ethyl)carbamate (15a)*

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.66 (d, *J* = 8.5 Hz, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.33 (t, *J* = 8.0 Hz, 1H), 7.23 (t, *J* = 7.0 Hz, 1H),7.16 (dd, *J* = 19.5, 4.0 Hz, 2H), 7.10 – 7.04 (m, 3H), 5.26 (1H), 3.62 (dd, *J* = 12.9, 7.0 Hz, 2H), 3.44 (dd, *J* = 20, 6.5 Hz, 1H), 3.07 (t, *J* = 6.5 Hz, 2H), 2.29 (s, 6H), 1.44 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.67, 151.16, 143.60, 136.48, 129.23, 124.66, 122.39, 122.15, 121.01, 120.77, 119.44, 118.69, 112.47, 111.39, 65.65, 50.77, 42.67, 41.45, 29.72, 25.58, 19.38. HRMS [M + H]+ found 352.2019, calculated 352.2025 for $C_{21}H_{25}N_3O_2$.

5.1.4.7. *(S)-3-(1-(dimethylamino)ethyl)phenyl (2-(5-methyl-1H-indol-3-yl)ethyl)carbamate (15b)*

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (d, *J* = 14.5 Hz, 1H), 7.43 (s, 1H), 7.37 (t, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 8.5 Hz, 1H), 7.20 (d, *J* = 7.5 Hz, 1H), 7.14 (s, 1H), 7.11 – 7.05 (m, 3H), 5.18 (s, 1H), 3.62 (dd, *J* = 19.0, 6.5 Hz, 2H),3.52 – 3.48 (m, 1H), 3.05 (t, *J* = 7.0 Hz, 2H), 2.48 (s, 3H), 2.43 (s, 6H), 1.60 – 1.53 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.4, 151.34, 134.81, 129.59, 128.74, 127.48, 124.94, 123.82, 122.58, 121.66, 121.42, 118.3, 111.05, 65.78, 42.01, 41.39, 29.7, 25.50, 22.71, 21.54, 18.55, 14.14. HRMS [M + H]+ found 366.2178, calculated 366.2182 for $C_{22}H_{27}N_3O_2$.

5.1.4.8. *(S)-3-(1-(dimethylamino)ethyl)phenyl (2-(5-methoxy-1H-indol-3-yl)ethyl)carbamate (15c)*

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (s, 1H), 7.35 (t, *J* = 8.0 Hz, 1H), 7.30 (d, *J* = 9.0 Hz, 1H), 7.18 (d, *J* = 7.5 Hz, 1H), 7.12 (s, 1H), 7.09 – 7.07 (m, 3H), 6.90 (dd, *J* = 11.0, 2.5 Hz, 1H), 5.22 (t, *J* = 6.0 Hz, 1H),3.88 (s, 3H), 3.61 (dd, *J* = 19.0, 6.5 Hz, 3H), 3.50 (s, 1H), 3.04 (t, *J* = 6.5 Hz, 2H), 2.39 (s, 6H), 1.52 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, CDCl3) δ 154.51, 154.04, 151.2, 131.60, 129.50, 127.66, 124.84, 123.23, 121.40, 121.28, 112.33, 112.13, 100.47, 65.76, 56.04, 55.92, 42.32, 41.33, 25.52, 18.90. HRMS [M + H]+ found 382.2124, calculated 382.2131 for $C_{22}H_{27}N_3O_3$.

5.1.4.9. *(S)-3-(1-(dimethylamino)ethyl)phenyl (2-(5-hydroxy-1H-indol-3-yl)ethyl)carbamate (15d)*

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.53 (s, 1H), 8.62 (s, 1H), 7.86 (d, *J* = 5.5 Hz, 1H), 7.37 (t, *J* = 7.0 Hz, 1H), 7.23 (s, 1H), 7.13 (d, *J* = 8.6 Hz, 2H), 7.08 (s, 2H), 7.04 (d, *J* = 7.0 Hz, 1H), 6.85 (d, *J* = 1.8 Hz, 1H)6.60 (dd, *J* = 8.5, 2.0 Hz, 2H),2.81 (t, *J* = 8.0 Hz, 2H), 2.28 (s, 6H), 1.37 (d, *J* = 16.5 Hz, 4H), 1.23 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.15, 154.62, 151.69, 136.71, 130.35, 130.13, 127.70, 123.26, 121.39, 119.55, 118.69, 116.13,

111.88, 65.39, 65.21, 64.62, 49.06, 41.91, 25.77, 15.64. HRMS [M + H]+ found 368.1972, calculated 368.1974 for $C_{21}H_{25}N_3O_3$.

5.1.4.10. *(S)-3-(1-(dimethylamino)ethyl)phenyl (2-(5-chloro-1H-indol-3-yl)ethyl)carbamate (15e)*

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.60 (d, *J* = 1.5 Hz, 1H), 7.35 – 7.29 (m, 2H), 7.16 (d, *J* = 7.0 Hz, 2H), 7.12 – 7.11 (m, 2H), 7.07 (dd, *J* = 9.0, 1.0 Hz, 1H),5.23 (t, *J* = 6.0 Hz, 1H), 3.60 – 3 .50 (m, 3H), 3.01 (t, *J* = 7.5 Hz, 3H), 2.34 (s, 6H), 1.48 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.62, 151.20, 134.78, 129.37, 128.48, 125.16, 124.81, 123.88, 122.38, 121.13, 121.10, 118.15, 112.42, 112.27, 65.7, 42.49, 41.42, 25.3, 19.01. HRMS [M + H]+ found 386.1630, calculated 386.1635 for $C_{21}H_{24}ClN_3O_2$.

5.1.4.11. *(R)-3-(1-(dimethylamino)ethyl)phenyl (2-(5-chloro-1H-indol-3-yl)ethyl)carbamate (16e)*

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.59 (d, *J* = 1.5 Hz, 1H), 7.35 – 7.29 (m, 2H), 7.18 – 7.13 (m, 3H), 7.11 – 7.06 (m, 2H),5.24 (s, 1H), 3.59 – 3.52 (m, 3H), 3.01 (t, *J* = 7.0 Hz, 2H), 2.35 (s, 6H), 1.49 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.60, 151.21, 134.77, 129.42, 128.48, 125.14, 124.85, 123.91, 123.37, 122.37, 121.15, 112.44, 112.25, 65.81, 65.76, 42.40, 41.42, 25.38, 19.03. HRMS [M + H]+ found 386.1630, calculated 386.1635 for $C_{21}H_{24}ClN_3O_2$.

Table 17. HRMS table with their chemical formula and ppm value of all synthesized compounds.

Code	Formula	Mol wt.	M+H Calculated	M+H Found	Difference	PPM error
3a	$C_{14}H_{14}N_4O_2$	270.1117	271.1195	271.1193	0.0002	0.7376
3b	$C_{15}H_{16}N_4O_2$	284.1273	285.1352	285.1348	0.0004	1.4028
3c	$C_{15}H_{16}N_4O_2$	284.1273	285.1352	285.1351	0.0001	0.3507
3d	$C_{15}H_{16}N_4O_2$	284.1273	285.1352	285.1352	0.0000	0
3e	$C_{15}H_{16}N_4O_3$	300.1222	301.1295	301.1302	0.0007	2.3245
3f	$C_{15}H_{16}N_4O_3$	300.1222	301.1295	301.1275	0.0020	6.73
3g	$C_{15}H_{16}N_4O_3$	300.1222	301.1295	301.1275	0.0020	6.56
3h	$C_{14}H_{14}N_4O_3$	286.1066	287.1144	287.1147	0.0003	1.0448
3i	$C_{14}H_{14}N_4O_3$	286.1066	287.1144	287.1147	0.0003	1.0448
3j	$C_{14}H_{13}ClN_4O_2$	304.0727	305.0805	305.0809	0.0004	1.3111
3k	$C_{14}H_{13}ClN_4O_2$	304.0727	305.0805	305.0800	0.0005	1.6389
3l	$C_{14}H_{13}ClN_4O_2$	304.0727	305.0805	305.0805	0.0000	0
3m	$C_{14}H_{13}BrN_4O_2$	348.0222	349.0300	349.0301	0.0001	0.2865
3n	$C_{14}H_{13}BrN_4O_2$	348.0222	349.0300	349.0309	0.0009	2.5785
3o	$C_{14}H_{13}BrN_4O_2$	348.0222	349.0300	349.0305	0.0005	1.4325
3p	$C_{14}H_{13}FN_4O_2$	288.1023	289.1101	289.1101	0.0000	0
3q	$C_{14}H_{13}FN_4O_2$	288.1023	289.1101	289.1098	0.0003	1.0347
3r	$C_{14}H_{13}FN_4O_2$	288.1023	289.1101	289.1086	0.0015	5.1883
3s	$C_{15}H_{13}F_3N_4O_2$	388.0991	339.1069	339.1053	0.0016	4.7182
3t	$C_{15}H_{13}F_3N_4O_2$	388.0991	339.1069	339.1051	0.0018	5.3080
3u	$C_{15}H_{13}F_3N_4O_2$	388.0991	339.1069	339.1055	0.0014	4.1284
3v	$C_{14}H_{13}N_5O_4$	315.0968	316.1046	316.1033	0.0013	4.1125
3w	$C_{14}H_{13}N_5O_4$	315.0968	316.1046	316.1044	0.0002	0.3270
6a	$C_{16}H_{15}N_5O_2$	309.1226	310.1304	310.1303	0.0001	0.3224
6b	$C_{17}H_{15}N_5O_2$	321.1226	322.1304	322.1303	0.0001	0.3104
6c	C18H19N5O2	337.1539	338.1617	338.1614	0.0003	0.8871
6d	$C_{18}H_{18}ClN_5O_2$	371.1149	372.1227	372.1230	0.0003	0.8061
6e	$C_{19}H_{21}N_5O_3$	367.1644	368.1723	368.1706	0.0017	4.6174
9a	$C_{15}H_{16}N_4O_2$	284.1273	285.1352	285.1352	0.0000	0
9b	$C_{15}H_{16}N_4O_3$	300.1222	301.1301	301.1302	0.0001	0.3320
9c	$C_{14}H_{13}FN_4O_2$	288.1023	289.1101	289.1100	0.0001	0.3458
9d	$C_{14}H_{13}ClN_4O_2$	304.0727	305.0805	305.0805	0.0000	0
9e	$C_{14}H_{13}BrN_4O_2$	348.0222	349.0300	349.0301	0.0001	0.2865
11a	$C_{16}H_{15}N_5O_2$	309.1226	310.1304	310.1303	0.0001	0.3224
11b	$C_{17}H_{15}N_5O_2$	321.1226	322.1304	322.1303	0.0001	0.3104
14a	$C_{21}H_{25}N_3O_2$	351.4500	352.2025	352.2021	0.0004	1.1357
14b	$C_{22}H_{27}N_3O_2$	365.4770	366.2182	366.2174	0.0008	2.1844
14c	$C_{22}H_{27}N_3O_3$	381.4760	382.2131	382.2126	0.0005	1.3081

14d	$C_{21}H_{25}N_3O_3$	367.4490	368.1974	368.1968	0.0006	1.6295
14e	$C_{21}H_{24}ClN_3O_2$	385.8920	386.1635	386.1631	0.0004	1.0358
15a	$C_{21}H_{25}N_3O_2$	351.4500	352.2025	352.2019	0.0006	1.7035
15b	$C_{22}H_{27}N_3O_2$	365.4770	366.2182	366.2178	0.0004	1.0922
15c	$C_{22}H_{27}N_3O_3$	381.4760	382.2131	382.2124	0.0007	1.8314
15d	$C_{21}H_{25}N_3O_3$	367.4490	368.1974	368.1972	0.0002	0.4318
15e	$C_{21}H_{24}ClN_3O_2$	385.8920	386.1635	386.1630	0.0005	1.2947
16e	$C_{21}H_{24}ClN_3O_2$	385.8920	386.1635	386.1630	0.0005	1.2947

5.2. Biological evaluation

5.2.1. Determination of IC_{50} values

In our study, we utilized an adapted Ellman's assay to examine the inhibition of ChEs. We obtained *eq*BChE (CAS 9001-08-5) and *h*AChE (CAS 9000-81-1) and all the necessary reagents, including Ellman's reagent (DTNB), as well as ATC and BTC iodides, generously from Sigma Aldrich. The study utilized a Tris-HCl buffer containing 50 mM concentration and pH 8 for the experiments. The experimental procedure involved combining 50 µL of AChE at a concentration of 0.022 U/mL with 10 µL of either the test compound or a standard compound (20, 10, 1, 0.1, 0.01 µM) at rt. for 30 minutes, followed by the addition of ATCI (1.5 mM), and again kept at rt. for 30 min. After the extended incubation, 160 µL of 0.15 mM DTNB (for *h*AChE) was introduced into the solution. The procedure for the BChE inhibition assay was similar: combining 50 µL of BChE at a concentration of 0.06 U/mL with 10 µL of either the test compound or a standard compound (20, 10, 1, 0.1, 0.01 µM) at rt. for 30 minutes followed by the addition of BTCI (1.5 mM), and again kept at rt. for 30 min. This mixture was then incubated in 96-well plates at room temperature for 30 minutes. Following the extended incubation period, 160 µL of 0.15 mM DTNB (for *eq*BChE or *h*BChE) was introduced into the solution. The absorbance of the mixture was then measured at a wavelength of 415 nm using a microplate reader. Each assay was replicated for accuracy, and independent experiments were conducted at least two to three times. The blank sample, excluding the inhibitors, included all other components. Inhibition percentage was determined using the formula [(Ac - Ai) / Ac] x 100, where Ai represents the absorbance obtained in the presence of inhibitors, and Ac represents the absorbance obtained in the absence of inhibitors. This experiment was done in congruence with our past publications (162).

Note: Due to the potent activity of the compounds **14a-14e, 15a-15e** and **16e,** the test concentrations were adjusted to lower ranges of 1, 0.1, 0.01, 0.001, and 0.0001 µM, in contrast to the concentrations (20, 10, 1, 0.1, and 0.01 µM) used in the initial setup. This adjustment allowed for a more detailed exploration of the molecules' activity in the lower nanomolar to picomolar range. Otherwise, the procedure followed the same experimental steps described earlier.

5.2.2. Kinetic analysis and mode of AChE Inhibition

To examine the inhibitory mechanism of compounds **3q** and **6e** on cholinesterases (ChEs), we created reciprocal plots of 1/[V] versus 1/[S]. These plots were developed by varying the concentrations of ATCI for *h*AChE and BTCI for *eq*BChE. The concentrations of ATCI used for *h*AChE were 0.5, 1.0, 1.5, 2.0, and 2.5 mM, while the same concentrations of BTCI were used for BChE. The construction of these reciprocal plots followed the Ellman method. In summary, the experimental procedure involved incubating 50 µL of 0.022 U/mL of *h*AChE and 10 µL of compounds **3q** and **6e** at different concentrations (10, 5, and 1 µM) for 30 minutes at room temperature. Subsequently, a substrate solution (30 µL) containing ATCI at varying concentrations (2.5, 2.0, 1.5, 1.0, 0.5 µM) was added. Then, 160 µL of a 0.15 mM concentration of DTNB was added, and the resulting absorbance was measured at 415 nm after a 30-second interval. For the kinetic analysis of *eq*BChE, incubating 50 µL of 0.022 U/mL of *eq*BChE and 10 µL of compounds **3q** and **6e** at different concentrations (10, 5, and 1 µM) for 30 minutes at room temperature. Subsequently, a substrate solution (30 µL) containing BTCI at varying concentrations (2.5, 2.0, 1.5, 1.0, 0.5 µM) was added. Then, 160 µL of a 0.15 mM concentration of DTNB was added, and the resulting absorbance was measured at 415 nm after a 30-second interval (162, 189).

5.2.3. Enzyme kinetic investigation of lead compound 15e on AChE & BChE

To determine the Kc and k3 values, a similar experimental procedure was followed. In a 96-well plate, the enzyme was preincubated with varying concentrations of **15e** for 5, 10, 20, 30, and 40 minutes before the addition of the substrate. This was followed by adding ATCI or BTCI substrates at concentrations of 1.5 mM. Subsequently, 160 µL of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB, Ellman's reagent) at a concentration of 0.15 mM (for AChE and BChE) was added, and the absorbance was recorded at 415 nm using an Epoch 2 microplate reader (Bio-Tek Instruments, Inc.). Each experiment was performed in duplicate. The resulting enzyme activity percentages were plotted against time and fitted to eq. II using GraphPad Prism 8 to determine the K_C and k_3.

$$A = A_0 \cdot e^{-k_{obs}t} + A_\infty \quad \text{II}$$

where A is the enzyme activity at time t, A_0 is the enzyme activity at time t = 0, and A∞ is the enzyme activity at infinite time.

The reciprocal values of k_{obs}^{-1} were plotted against the reciprocal concentration c^{-1}. The value of k_3 was determined from the Y-intercept, while Kc was derived from the slope of the resulting linear plot, as outlined in Equation III, utilizing the GraphPad Prism 8 software.

$$\frac{1}{k_{obs}} = \frac{K_c}{k_3} \cdot \frac{1}{[I]} + \frac{1}{k_3} \quad \text{III}$$

where [I] is the concentration of the respective BChE inhibitor.

5.2.4. Propidium iodide displacement assay

The assay was conducted to evaluate the capacity of the compounds to remove or displace PI from the *h*AChE-PAS region. A solution of *h*AChE with a concentration of 5.0 units per milliliter was prepared by dissolving in Tris buffer containing 0.1 mM with pH 8.0. 75 µL of *h*AChE was incubated with a 75 µL solution of the **3q**, **6e**, **Riv**, and **DPZ** at four different concentrations (50, 20, 10, and 5 µM final concentration). Subsequently, the solutions were incubated at room temperature for 6 hours at 25 °C to allow any necessary

reactions or interactions to occur. After that, the samples were subjected to an incubation period of 20 minutes along with PI (20 μM final concentration). Finally, the fluorescence emitted by the samples was quantified using a fluorescence microplate reader, specifically the Synergy™ HT model manufactured by Bio-Tek Instruments, Inc. The excitation wavelength was 535 nm, while the emission wavelength was 595 nm (208).

Note: Due to the potent activity of the compounds **15d** and **15e** the test concentrations were adjusted to lower ranges of 5, 1, 0.1, and 0.01 μM, compared to the initial setup with 50, 20, 10, and 5 μM. Otherwise, the procedure followed the same experimental steps described above.

5.2.5. Molecular docking study of 3q and 6e against AChE and BChE

The crystal structures of the *h*AChE with co-crystallized **DPZ** (PDB ID: 4EY7) (22) and the *eq*BChE with co-crystallized Tacrine (PDB ID: 4BDS) (209) were retrieved from the Protein Data Bank (www.rcsb.org). Both proteins were prepared using the Protein Preparation Wizard of Schrödinger software package. During preparation, correct bond orders were assigned, missing hydrogens were added, disulfide bonds were created, and missing side chains and loops were filled by running Prime refinement and optimizing the correct orientation of hydroxyl and amino groups. Further, Epik and PROPKA modules were used to generate the ionization states at pH 7.0 ± 2 and assign hydrogen bonds, respectively. The resulting complex was further subjected to restrained minimization with a cut-off root mean square deviation (RMSD) of 0.3 Å. This structure was taken for further molecular docking and molecular dynamics simulation studies.

5.2.5.1. Ligand preparation

The ligands **3q** and **6e** were drawn using 2D Sketcher and converted to a 3D structure in the Schrödinger software package (Maestro Version 12.8.117). The crystal structure of **RIV** was retrieved from the PubChem database (PubChem CID: 77991). All ligands were

prepared using the Ligprep (Schrödinger Release 2021-2: Schrödinger, LLC, New York, NY, 2021) module in the following steps. First, the ionization states were determined at pH 7.0 ± 2 using Epik, then all 32 combinations of stereoisomers were generated and energy was minimized using the OPLS4 force field with all other parameters kept default.

5.2.5.2. Receptor Grid Generation

Schrodinger's Receptor Grid Generation Module was used for receptor grid generation. The coordinates of the cocrystallized ligand, i.e., **DPZ** for 4EY7 and tacrine for 4BDS, were kept as the centroid of the box, and the size was kept similar to the cocrystallized ligands. Molecular docking of all ligands was performed in standard precision (SP) mode.

5.2.6. Molecular Dynamics

All-atom molecular dynamics were performed using the Desmond module of the Schrödinger software package (Maestro version 12.8.117). Both protein-ligand complexes were taken after docking, and a system builder panel was used to build the initial system for MD simulations. *h*AChE, hBChE, and all the docked complexes were placed in an orthorhombic box of side lengths 10 Å on each side. Each box was solvated with SPC water models and negative and positive charges were neutralized using Na^+ and Cl^- ions. An ionic strength of 0.15 M was maintained by adding Na^+ and Cl^- and ions to the system. The entire system's energy was minimized using Desmond's Minimization module by simulating 100ps, and the last frame was taken for further molecular dynamics simulation using the default protocol. All minimized and equilibrated systems were subjected to an MD run with periodic boundary conditions in the NPT ensemble using the OPLS4 force field parameter for 300 ns. During the simulation, the pressure (1 atm) and temperature (310 K) of the system were maintained by Martyna–Tobias–Klein barostat and Nose–Hoover Chain thermostat, respectively. Binding energy between the AChE and all ligands was calculated using the inbuilt script thermal_mmgbsa.py. The solvent accessibility surface area (SASA) of *h*AChE

and *h*BChE in the presence of different ligands was calculated using the script binding_sasa.py. Apart from this, the radius of gyration and the number of hydrogen bonds were also calculated using the simulation event analysis panel.

5.2.7. *In-silico* study of 15d and 15e against AChE and BChE

The Molecular docking was performed using the AutoDock 4.0 (version 1.5.6.) software suite. The compound structures of **15d** and **15e** were generated in ChemDraw 16.0, and Chem 3D 16.0 was used to minimize energy. The AChE and BChE complex crystal structure (PDB IDs: 4EY7 and 4BDS, respectively) was taken from the Protein Data Bank at resolutions of 2.35 Å and 2.10 Å. Utilizing UCSF Chimera (version 1.13.1), the missing residues in the recovered protein structure were constructed. The protein was prepped before docking using AutoDockTools to remove heteroatoms and co-crystallized water molecules. Then, the polar hydrogens and Gasteiger charges were added to make the docking fit protein. Based on the ligand of the crystalline structure, 30*30*30 grid points were used to create the grid box. The AChE protein binding pocket, including the binding site of crystalline DPZ, was located at a spacing of 0.375 Å, with the grid centre set at -14.108464, -43.832714, and 27.669929 for X, Y, and Z coordinates, respectively. Similarly, the BChE protein binding pocket, including the binding site of crystalline tacrine, was located at a spacing of 0.375 Å, with the grid centre set at 132.994467, 116.013533, 41.214400, for X, Y, and Z coordinates, respectively. A maximum of 10 conformers were developed using the Lamarckian Genetic Algorithm, which was selected for the best conformer searches. The Ligand interactions module of BIOVIA Discovery Studio Visualizer 2019 (version 19.1.0.18287) was used to visualize the chemical and protein binding interactions (210).

5.2.8. Covalent Docking of RIV, 15d and 15e against AChE and BChE

Standard docking may not accurately represent covalent inhibitor interactions, as covalent docking is crucial for predicting binding modes and confirming potential

interactions with catalytic serine residues (211, 212). The selectivity of cholinesterase inhibitors such as **DPZ**, **galantamine**, and **RIV** for AChE and BChE varies significantly (212, 213). **RIV**, in particular, exhibits dual inhibition of both AChE and BChE by covalently carbamoylating the serine residues at their active sites (212, 214). For covalent docking studies, we selected crystal structures of AChE and BChE. The protein complexes were initially prepared using the Protein Preparation Wizard module in Schrodinger Suite 2023-1. This preparation involved standard protocols including hydrogenation of the protein, assignment of partial charges, removal of water molecules, and optimization of the complex structures with the OPLS_2005 force field. Ligand molecules, including **RIV**, **15d** and **15e**, were processed using the LigPrep tool with default settings. The processing included applying the OPLS-2005 force field, generating potential structures within a pH range of 7.0 ± 2.0, and maintaining plausible three-dimensional conformations. Subsequently, SER203 and SER198 were chosen as the reactive residues for AChE and BChE, respectively (214, 215). Receptor grids were generated using the Covalent Docking module in Schrodinger, with docking pockets positioned at the sites of co-crystallized ligands for each protein. The reaction type was defined as nucleophilic addition to a double bond, allowing the system to filter ligands capable of reacting under these conditions. Finally, docking was performed using the Thorough (Pose Prediction) mode, with binding affinity scores calculated via Glide. All remaining parameters were maintained at their default settings.

5.2.9. DPPH radical-scavenging potency

The DPPH radical assay is a method used to measure antioxidant activity by observing the reduction of DPPH. When an antioxidant molecule is present, it reduces the DPPH, resulting in the formation of a yellow-colored compound called diphenyl picrylhydrazine. Therefore, this assay assesses the compound's ability to donate hydrogen atoms, indicating its antioxidant activity. All the tests were conducted using methanol as the

solvent. Various concentrations of the test compounds (**3q, 6a, 6b**, **6e 14e, 14c, 15d, 15e,** ascorbic acid and **RIV**) were prepared: 200, 160, 80, 40, and 20 μM. In each experiment, 75 μL of the respective compound concentration was added to individual wells of a 96-well plate. Next, 75 μL of a DPPH solution with a final concentration of 200 μM was added to the wells containing the test compounds. The 96-well plate was then placed in a thermomixer at 37 °C and shaken gently for 30 minutes. The absorbance of the mixture was then measured at a wavelength of 517 nm using a microplate reader such as SynergyTM HT and Epoch 2 from Bio-Tek Instruments, Inc. For accuracy, each assay was replicated two times, and independent experiments were conducted at least two times.

5.2.10. Amyloid-β aggregation studies

We procured an Amyloid-β ($A\beta_{1-42}$) protein fragment (CAS Number 107761-42-2, 1mg) from DGpeptide Co. Ltd, with a 95% purity as determined by HPLC and sodium dodecyl-sulfate polyacrylamide gel electrophoresis. The obtained $A\beta_{1-42}$ peptide (1 mg) was dissolved in 400 μL of HFIP and stored at -20°C to preserve its monomeric state. For the experiment, the $A\beta_{1-42}$ peptide solution in HFIP was retrieved, HFIP was eliminated using gentle nitrogen gas flow, yielding a white solid residue. This residue was then dissolved in a minimal volume of phosphate-buffered saline (PBS) solution (10X, pH=7.4). $A\beta_{1-42}$ working concentration (1.769 mM) was estimated through the NanoDrop™ 2000/2000c spectrophotometer (Thermo Scientific instrument) (216).

To inhibit the aggregation of the $A\beta_{1-42}$ peptide, we utilized a 12.5 μM (final concentration) of the protein, incorporating fixed concentrations of **3q**, **6e, 15d,** and **15e** (3.125 μM). Initially, a monomeric solution of $A\beta_{1-42}$ peptide (12.5 μM in the final concentration) in PBS was prepared, and compounds **3q**, **6e, 15d,** and **15e** were added. The mixture was then incubated at 37 °C for 72 hours with continuous shaking at 1200 rpm. Subsequently, the incubated solutions were retrieved, and a ThT solution (25 μM in the final

volume) was introduced. The fluorescence spectrum was recorded at λex= 435 nm and λem= 445−800 nm.

5.2.10.1. TEM imaging

The $A\beta_{1-42}$ peptide (6.25 μM) underwent incubation at 37 °C with compounds **15d** and **15e** (1.512 μM) in a PBS buffer (10 mM, pH: 7.4) for a duration of 72 h hours. Subsequently, a 5 μL aliquot of each sample was applied to carbon–formvar coated 200–400 mesh spacing grids and adsorbed for five minutes. Negative staining was executed using a 2 % filtered aqueous uranyl acetate solution for 45 seconds. The grids were visualized using a Jeol JEM 1400 electron microscope operating at 80 kV (217).

5.2.11. *In-silico* study of 3q, 6e, 15d and 15e against $A\beta_{1-42}$ protofibrils

The NMR 3D structure of Alzheimer's $A\beta_{1-42}$ protofibrils was obtained from the Brookhaven Protein Data Bank with PDB ID 2BEG.(218, 219) Ligand molecules **3q, 6e, 15d** and **15e** were initially sketched in 2D and converted into 3D structures, followed by energy minimization using the LigPrep wizard of the Schrödinger software package.(220, 221) Molecular docking of both ligands to the $A\beta_{1-42}$ protofibrils was carried out using the Achilles blind docking server, accessible at https://bio-hpc.ucam.edu/achilles/. This server is a customized variant of the widely used docking tool Autodock Vina. Unlike a single Vina run of blind docking, which typically exhibits poor sampling across the entire protein surface, this modified version offers improved sampling capabilities by conducting numerous docking calculations across the protein surface. Consequently, it identifies sites with strong binding affinities more effectively (222).

5.2.12. Molecular Dynamics Simulation Study

The Desmond module within the Schrödinger software package was employed to conduct all-atom molecular dynamics (MD) simulations, utilizing the OPLS4 force field (223). Three distinct systems, apo-protofibril, protofibril−**3q, 6e, 15d** and **15e**, were

constructed using the system builder wizard integrated into the Maestro interface of the Schrödinger software package (220). The SPC water models were utilized to represent water molecules within each system. Each system was enclosed within a cubic box to ensure adequate solvation, maintaining a minimum distance of 10 Å between the solute and the box edges. Neutralization of systems bearing net charges was achieved by incorporating Na+ ions, while the addition of 0.15 M NaCl facilitated the creation of a saline environment. Subsequently, the neutralized systems underwent energy minimization for 100 ps using the minimization panel available in the Maestro interface. This step aimed to rectify any collisions and erroneous structural geometries among the atoms present within the simulation system. Following energy minimization, 300 ns MD simulations were initiated using the molecular dynamic panel. Throughout the simulations, system pressure (1 bar) and temperature (310 K) were rigorously maintained utilizing the Martyna–Tobias–Klein barostat and Nose–Hoover Chain thermostat, respectively.(224-227) The resultant trajectories were meticulously scrutinized utilizing the Simulation Interactions diagram and the Maestro interface, facilitating the generation of backbone Root Mean Square Deviation (RMSD), hydrogen bond analysis, Solvent Accessible Surface Area (SASA), and Radius of Gyration (Rg) plots.(220) Additionally, the binding energy between the protein and ligands was computed using the inbuilt script thermal_mmgbsa.py. The average binding energy, calculated over the entire 300 ns trajectory, provided comprehensive insights into the energetics governing protein-ligand interactions. This comprehensive approach allowed for a detailed examination of the dynamic behavior and interaction profiles within the studied systems.

5.2.13. Tau protein aggregation inhibition studies

Tau protein stock solution (50 µM) was diluted to 21.79 µM (60 µL) with assembly buffer (10mM HEPES buffer containing 7.7 mg of DTT and 58.44 mg of NaCl). 108.9 µM

solution of heparin (3 µL) was added to the tau preparation as an aggregation inducer. Tau protein's final concentration was (19.8 µM), and the final heparin concentration was 4.95 µM. Tau protein solution, at a final concentration of 5 µM, was incubated alone or in a 4:1 ratio with **3q** and **6e** (final concentration of 1.125 µM). The incubation was carried out at 37 °C with agitation at 1200 rpm for a duration of 3 days. Aggregation inhibition was confirmed *via* ThT fluorescence assay.

5.2.14. *In-vitro* metal chelating assay

Sigma Aldrich provided $FeCl_3.6H_2O$ (Iron chloride hexahydrate) with the CAS number 10025-77-1. A stock solution of compounds **3q**, **6e, 15d** and **15e** were prepared by dissolving it in high-purity methanol at a concentration of 600 µM. Following that, $FeCl_3.6H_2O$ was dissolved in methanol, resulting in a light orange solution with a concentration of 600 µM. The two solutions, one containing compound **3q**, **6e, 15d** and **15e** at a concentration of 600 µM and the other containing $FeCl_3.6H_2O$ at a concentration of 600 µM, were combined in equal volumes, forming a light-yellow solution. The sample solution was then subjected to incubation at a temperature of 25 ºC for 24 hours on a thermomixer, with vigorous shaking at 1000 rpm. Subsequently, a UV scan was performed on the solution. To maintain a pH of 7.4, the diisopropylethylamine (DIPEA) base was diluted with water. Duplicate assays were performed for each concentration (189).

5.2.15. Determining Nitrite Concentration

5.2.15.1. Preparation of a nitrite standard reference curve

A 100 µM nitrite solution is prepared by diluting the provided 0.1 M Nitrite Standard 1:1,000 in the experimental matrix or buffer to create a Nitrite Standard Reference Curve. In a 96-well plate, three columns (24 wells) are designated for the reference curve. Rows B–H were filled with 50 µL of the matrix/buffer. In row A, 100 µL of the 100 µM nitrite solution is added to three wells. Immediately, 6 serials two-fold dilutions (50 µL/well) are performed

in triplicate down the plate, creating a curve (100, 50, 25, 12.5, 6.25, 3.13, and 1.56 μM), with the last set of wells (0 μM) containing no nitrite solution.

5.2.15.2. Nitrite Measurement (Griess Reaction)

To conduct the colorimetric assay, first, allow the Sulfanilamide and NED Solution to reach room temperature over 15–30 minutes. Next, add 50 μL of **3q** and **6e** in duplicate designated wells. Using a multichannel pipettor, dispense 50 μL of the Sulfanilamide Solution to all experimental samples and wells containing the Nitrite Standard reference curve dilution series. Incubate for 5–10 minutes at room temperature, shielded from light. Subsequently, dispense 50 μL of the NED solution to all wells and incubate again for 5–10 minutes at room temperature, protected from light, forming a purple/magenta color. Finally, the absorbance was measured within 30 minutes using a plate reader with a filter between 520 and 550 nm. To construct a Nitrite Standard reference curve, plot the average absorbance values against corresponding concentrations of the Nitrite Standard, establishing a relationship between absorbance ("Y") and nitrite concentration ("X"). Subsequently, the average absorbance values for experimental samples and their nitrite concentrations were calculated by comparing them to the Nitrite Standard reference curve.

5.2.16. Cell Culture

PC12 cells are a rat pheochromocytoma cell line commonly used in neuroscience research and used for all in-vitro experiments. These cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) Gibco™, Cat: 11965-092 supplemented with 10% qualified fetal bovine serum (FBS) Gibco™ Cat: 10270106, 1% Antibiotic-Antimycotic (100X) Cat: 15240062, maintained at 37°C with 5% CO2. Culture media was replaced every two days until cells reached 80% confluence. Subsequently, cells were differentiated using 100ng/mL Nerve Growth Factor β Human Sigma, Cat: H9666, 5 days before the cell culture experiments.(228)

5.2.16.1. Assessment of Cell Viability

1x10^4 cells were seeded in a 96-well plate per well and adhered overnight. Compounds **3q, 6e, 15d** or **15e** were introduced at concentrations ranging from 20 µM to 0.1 µM and incubated for 24 hours at 37 °C with 5% CO_2. After a 24-hour incubation period, 5 mg/ml MTT reagent was added and incubated for 3 hours at 37°C. Finally, 100 µL DMSO was used to dissolve formazan crystals, and absorbance was measured at 562 nm using a Spectramax-i3x spectrophotometer (Molecular Devices, USA). Cell viability was calculated using the formula: Cell viability = (OD of treatment group / OD of control group) * 100.

5.2.16.2. Evaluation of Neuroprotective Property

Differentiated PC-12 cells were seeded at a density of 1 × 10^4 cells/well in 96-well plates and incubated with compounds **3q, 6e, 15d** or **15e** at concentrations ranging from 20 µM to 0.1 µM and incubated for 24 hours at 37 °C with 5% CO_2. After a 24-hour preincubation period, cells were exposed to 600 µM H_2O_2 to induce oxidative stress for 24 hours. Following this, cell viability was assessed using the abovementioned MTT assay (229).

5.2.16.3. Assessment of Apoptotic activity of 3q, 6e, 15d and 15e on PC-12 cells

PC-12 cells were seeded at 1×10^5 cells/well in 6-well plates with Dulbecco's Minimum Essential Medium (DMEM) containing 10% fetal bovine serum (FBS) at 37 °C under a 5% CO_2 atmosphere. Cells were pre-incubated with the compounds **3q, 6e, 15d** and **15e** at 20 µM, 10 µM, 5 µM, and 2.5 µM concentrations 24 hours before apoptosis induction by 600 µM H_2O_2. After 24 hours, spent media was removed, and cells were washed twice with chilled PBS and labeled with Propidium Iodide (PI) and Annexin V using the Dead cell apoptosis kit (ThermoFisher, USA) as per manufacturer's protocol. Both untreated controls, i.e., without incubation with drug or inducer, and unstained control, i.e., without either of the

dyes, were included. Data acquisition, compensation, gating, and data analysis were done using a BD LSR Fortessa with in-built user interface software (229).

5.2.17. Evaluation of NLRP3 inhibition property in a cell culture model

All *in-vitro* assays were conducted using Human microglial cells (HMC-3) obtained from ATCC-CRL-3304. The cells were cultured in 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 at 37 °C in a humidified environment of 95% air and 5% CO_2. Until the cells reached 80% confluence, the media were consistently changed every two days. Cells were planted at the necessary density to conduct additional studies.

5.2.17.1. Assessment of Cell viability

A sterile 96-well plate with $1x10^4$ cells per well of seeded cells was used. LPS and ATP were dissolved in the medium to achieve the appropriate concentrations. Serum-starved HMC-3 cells were treated with 1000 ng/ml LPS for 6 hours after being serum-starved for 2 hours. After removing the priming media and washing with complete media, ATP (5 mM) was added and left in place for 45 minutes. After removing the media, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) reagents (10 mg/ml) were added and allowed to react for 4 hours at 37°C. Finally, DMSO was added to dissolve the formazan crystals, and the absorbance at a wavelength of 570 nm was measured using a SPECTRA-Max i3x (Molecular Devices, USA). In comparison to control cells, cell viability was expressed as a percentage.

5.2.17.2. Evaluation of the mitochondrial membrane potential (MMP) and reactive oxygen species (ROS) in human microglial cells

MitoSOX^{TM} Red FM was used to quantify mitochondrial ROS (cat no: M22425, Life Technologies). HMC-3 cells were treated with compounds **3q** and **6e** for 24 hours after being

primed with LPS and ATP. The cells were then stained for 10 min at 37 °C using 5 μM MitoSOX Red. A warm buffer was used to wash the cells, and a fluorescence microscope (EVOS Auto FL2, Invitrogen) was used to acquire the images.

Rhodamine 123 at a concentration of (5 μM for 10 min at 37 °C) along with Tetraethyl benzimidazolyl carbocyanine iodide (JC-1, 2 μM (final concentration) of JC-1 dye and incubate the cells at 37 °C, 5% CO_2 for 30 min) dye was used to measure the mitochondrial membrane potential (Δψm), which is unique to mitochondria (Cat no: T3168, Invitrogen). JC-1 aggregates with high red fluorescence intensity under normal conditions. As the dye changes from aggregate to monomeric form, loss in the Δψm is indicated by a decline in red fluorescence and an increase in green fluorescence; as a result, the ratio of red/green serves as a measure of Δψm - loss.

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

In 6-well culture plates, human microglial cells (HMC-3) were cultivated on a coverslip at a density of $2x10^6$ cells per well. The cells underwent treatment, phosphate buffer solution (PBS) washing, 4 % paraformaldehyde (PFA) fixation, and 0.2 % Triton X-100 permeabilization. The secondary antibodies Alexa Flour™ 488 goat anti-rabbit IgG (H+L) and Alexa Flour™ 594 goat anti-mouse IgG (H+L) from Invitrogen were incubated with the cells after the primary antibodies had been treated with them for an overnight period at 4 °C. Vecta-shield mounting media for fluorescence with 4, 6-diamidino-2-phenylindole (DAPI) was used to stain the nuclei (cat. no. H-1200) (Vector Laboratories, Burlingame, CA). By excluding the primary antibody, negative control slides were created. Using a confocal microscope, slides

were viewed at oil emersion 63x magnifications while kept in a cold environment (Leica TCS SP8).

5.2.17.4. Immunoblotting

Cells were cultivated at a density of $1.5x10^6$ cells in a sterile 90 mm x 20 mm culture dish for the *in-vitro* experiment. After treatment, cells were lysed using RIPA buffer containing phosphatase and protease inhibitors. Using a commercial kit, the total proteins were determined. A 12–16 % SDS–PAGE gel was used to separate 25–30 µg of protein placed into each lane. Protein was transferred onto a nitrocellulose membrane, blocked for an hour in 5 percent non-fat dry milk, and then incubated with the primary antibody all night long at 4 °C. Dilutions for the primary antibodies were as follows: NLRP3, Iba-1, β-actin, anti-Caspase-1 (p20), IL-1β, IL-18, iba-1, NRF2 (1:1000). Blots were washed with TBST before being incubated for one hour with the proper secondary antibodies (anti-rabbit IgG (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 densiometric analysis (National Institutes of Health, Bethesda, Maryland, USA).

5.2.18. Fly husbandry and culture

The OregonR^+, ey-GAL4/CyO and UAS-$A\beta_{1-42}$ flies used for the AD study were obtained from the Bloomington Drosophila Stock Center (BDSC), Indiana, and USA. Their F1 progenies were grown in standard corn meal agar food and cultured in a BOD incubator at 28 ± 1 °C.

5.2.18.1. Compound 3q and 6e treatment on OregonR^+ and $A\beta_{42}$ expressing flies

The age-matched 10 male and 10 female wildtype flies were genetically crossed in vials containing **3q, 6e, 15d, 15e,** or **RIV** drugs mixed with corn food to evaluate the drug dose-response. Different dosages of **3q** (0.01, 0.05, 0.1, 0.5, and 1 mg/ml), **6e** (0.01, 0.05, 0.1, 0.5, 1, and 2 mg/ml), **15d, 15e** (10, 20, 40, 60, 80, and 100 µM), and FDA-approved **RIV** (50,

100, 200, and 400 µM) were primarily administered to wildtype OregonR+ flies. Untreated progenies served as controls. After 15 days, the untreated and treated progenies were scored to determine their median lethal dose (LD50).

Similarly, ey-GAL4 driven Alzheimer's flies (UAS-Aβ1-42) were treated with **3q** (0.01, 0.05, 0.1 mg/ml), **6e** (0.01, 0.05, 0.1, 0.5 mg/ml), **15d, 15e** (10, 20, 40, 60, 80, and 100 µM), and **RIV** (50, 100 µM) in an ascending order to assess their potential therapeutic effects against AD. The eye phenotypes of the F1 progenies were scored under the Magnus stereozoom binocular microscope and represented as percentages. All experiments were conducted in triplicates, and statistical analysis was performed using GraphPad Prism 5 with two-way ANOVA.

5.2.18.2. Mitochondrial and cellular ROS measurement

The OregonR^{+}, **3q**, **6e**, **15d**, **15e**, **RIV** treated and untreated $A\beta_{1\text{-}42}$ expressing 3rd instar larvae were dissected in 1XPBS, pH 7.4, followed by tissues permeabilization in 0.2% TritonX-100 in 1XPBS for 15 min at 37 °C and their eye imaginal discs (n=10 discs in each group) were incubated in 5 µM MitoSOX ^{TM} Red (Invitrogen, USA) for 20 min at 37 °C to measure the superoxide (ROS) level as prescribed by Liu et al., 2013. After incubation, MitoSOX^{TM} Red was removed, and tissues were washed three times with 1XPBS, then mounted in 1XPBS. The images were captured using the Nikon Eclipse Ni-U Upright fluorescence microscope, and the MitoSOX^{TM} Red fluorescence intensity was processed by NIS Elements (BR) software. Similarly, *in-situ*, cellular ROS levels of **3q**, **6e**, **15d, 15e**, **RIV** treated and untreated AD eye imaginal discs (n=10 discs in each group) were also measured using Redox sensitive fluorophore H2DCFDA (2',7'-dichlorodihydrofluorescein diacetate) as previously described by Chauhan et al., 2021. The tissues were incubated with 50 µM H2DCFDA for 30 min at 37 °C. After incubation, the dye was removed, followed by washing it three times with 1XPBS and mounted 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.

5.2.19. PAMPA assay for *in-vitro* analysis of permeability

Assessing BBB permeability is important in developing medications designed to treat AD. To examine the permeability of the BBB, a PAMPA (Parallel Artificial Membrane Permeability Assay) experiment was performed, employing the established methodology outlined by Di et al. We obtained Testosterone and Norfloxacin, two generic medications, to validate the study, from TCI chemicals. The PAMPA kit, consisting of the donor plate (with a PVDF membrane having a pore size of 0.45 μM) and the acceptor plate, was procured from Bioassay Systems Pvt. Ltd. Porcine brain lipid (PBL) was provided by Avanti Polar Lipids, and n-dodecane was supplied by Avra Synthesis. All experiments were conducted using phosphate-buffered saline (PBS) with a pH of 7.4. For the test and standard control compounds, stock solutions of 10 mM were prepared by dissolving them in dimethyl sulfoxide (DMSO) or methanol (MeOH) in cases where the compounds had low solubility. To prepare a concentration of 500 μM for **3q**, **6e, 15d, 15e** and the standard control, 25 μL of the stock solution was diluted with 475 μL of PBS (pH 7.4), resulting in a final volume of 500 μL. Similarly, for the equilibrium standards, 120 μL of **3q**, **6e, 15d, 15e** and the standard control were diluted with 180 μL of PBS, resulting in a final volume of 300 μL. A control group was prepared by dissolving 5 μL of DMSO in 245 μL of PBS. The experiments were conducted in accordance with the methodology described by Di et al. and were consistent with previous studies reported in the literature (162, 187).

5.2.20. Animal studies

5.2.20.1. Animals

We obtained Swiss albino mice from the "Central Animal Facility" at 6 weeks, weighing 25-30 grams. Under controlled environmental conditions, these mice were then

acclimated for one week in the department's animal house at the "IIT (BHU)". During the acclimation period, the mice were subjected to a 12:12 hour dark/light cycle and kept at a constant temperature of 25 ± 2 °C. Unless there were exceptional circumstances, the mice were provided with commercially available food pellets and tap water as their diet. The experimental procedures employed in this study were approved by the committee at the "Department of Pharmaceutical Engineering and Technology, IIT, Banaras Hindu University, Varanasi, India". The approval number for the experiment was Dean/2019/CAEC/1196, and the approval was granted on April 20, 2019.

5.2.20.2. Acute toxicity test

In accordance with OECD (425) requirements, compounds **3q**, **6e, 15d** and **15e** underwent evaluation for acute oral toxicity. Healthy Swiss albino mice were administered a dose of 2000 mg/kg of the test compound (**3q and 6e**) and 175 mg/kg for **15d** and **15e** with closely monitored for the initial 24-hour period. During this time, any behavioral changes such as seizures or diarrhea, as well as mortality, were recorded. The observation period extended up to 14 days following the administration of the dose.

5.2.20.3. Assessment of *in-vivo* therapeutic potential of 3q and 6e in scopolamine-induced AD in mice

Drugs and chemicals: High-quality chemicals and reagents, including scopolamine hydrochloride and DPZ hydrochloride, obtained from Sigma Aldrich, were utilized in the Y-maze experiment.

Drug preparation and treatment protocol

For the Y-maze experiment, freshly prepared suspensions of compounds **3q** and **6e** at 5.25 and 10.5 mg/kg, 3 mg/kg of **RIV**, 5 mg/kg of **DPZ**, and 1.4 mg/kg of **scopolamine hydrochloride** were used. These doses were prepared using a solution containing 0.5% v/v TWEEN 80 and distilled water. The mice were randomly divided

into nine groups, each consisting of six mice: (i) **Vehicle control**, (ii) **Control,** (iii) **DPZ**, 5 mg/kg, (iv) **Scopolamine**, 1.4 mg/kg, (v) **3q,** 5.25 mg/kg, (vi) **3q**, 10.5 mg/kg, (vii) **6e**, 5.25 mg/kg, (viii) **6e**, 10.5 mg/kg and (ix) **RIV**, 3 mg/kg. The experiment spanned six days, and on the seventh day, scopolamine was given to groups iii to ix, with a 30-minute delay following the administration of standard and test compounds. Following administering either a vehicle or scopolamine, a 15-minute interval was observed before subjecting the animals to a Y-maze test (6, 230).

5.2.20.4. Y-maze test

The experiment aimed to evaluate the mice's exploratory behavior and spatial memory (231, 232). The Y-maze consisted of three arms (A, B, and C) positioned at 120° intervals. Each mouse was placed at the starting point of the Y-maze and given eight minutes to freely explore the arms. The number of complete entries made by each mouse was carefully recorded using a video camera. Upon completion of the experiment, the gathered data was examined and analyzed to determine the degree of spontaneous alteration. Spontaneous alteration is defined as the sequential entry of mice into different arms, following patterns such as CBA, ABC, BAC, BCA, ACB, CAB, and ABC. The metric used to calculate the percentage of spontaneous alteration (% SA) is defined as follows: % SA = [(number of alternations/total arm entries) - 2] X 100. This % SA measurement serves as an indicator of selective memory in mice (162).

5.2.20.5. Ex-vivo studies for lead compound 3q, 6e, 15d and 15e

After completion of the y-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.

5.2.20.5.1. 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.

5.2.20.5.2. 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.

5.2.20.5.3. Catalase (CAT) Measurement

To measure CAT concentration, 50 µL of brain supernatant was incubated 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. Subsequently, 150 µL of dichromate/acetic acid solution (coloring agent) was added, and the mixture was boiled at 100 °C for 10 minutes. The absorbance was then measured at 570 nm using a 96-well microplate reader.

5.2.20.5.4. 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.

5.2.21. Behavioral studies using the Morris water maze test

5.2.21.1. Drug treatments

Control animals received vehicles in which test drugs were dissolved. Disease control animals received **scopolamine** (1 mg/kg) in normal saline. Standard control groups received **RIV** (1.5 mg/kg) in normal saline. **3q** and **6e** test group animals received 10 mg/kg of drugs dissolved in 0.01% DMSO in sterile PBS. Scopolamine was administered 30 minutes after **RIV**, **3q,** and **6e** injection, while behavioral studies were performed 30 mins after scopolamine injection. All the injections were given through the intraperitoneal route with a maximum of 0.25 ml volume.

The same protocol was implemented with a reduced dose for the treatment groups, as detailed in the preceding sentence, due to the higher potency of the lead compounds **15d** and **15e**. In brief, the disease control group received **scopolamine** (3 mg/kg) prepared in normal saline. The standard control group was treated with RIV (3 mg/kg) in normal saline. Test groups administered compounds **15d** and **15e** were given doses of 0.3 mg/kg and 0.5 mg/kg, respectively, dissolved in 0.5% Tween 80 with sterile PBS. Scopolamine was given 30 minutes after administering RIV or test compounds (**15d** and **15e**), and behavioral assessments were performed 30 minutes following the scopolamine injection. All treatments were delivered orally, with a maximum volume of 0.25 ml per dose.

5.2.21.2. Passive Avoidance Task

The passive avoidance task was performed in three phases. Exploration: where the animals were placed in the white chamber and the mice were allowed to explore for 5 minutes while the door between the white and black chamber. Acquisition: After 24 hours,

the animals were again placed in the white chamber facing the opposite of the door. The animals were first allowed to explore the white chamber for 30 seconds, and the door between the white and black chambers was opened with a sound. The time the animal went to the black box was measured as escape latency in seconds. The cut-off time was kept at 5 mins. When entered into the black chamber, the animal was given 0.4 mA shock for 3 seconds and after 30-second latency before returning them to the home cage. Test: After 24 hours after the acquisition of the animal, the animals were again placed in the white chamber with the same protocol. The latency to enter into the white box was recorded. If the animals did not enter the black chamber, they were noted to gain criterion and returned to the home cage.

5.2.21.3. Morris Water Maze

The water maze was filled with water one day before the commencement of the animal training. The maze was divided into four quadrants: NE, SE, NW, and SW while the platform was placed on the SW quadrant. The platform was placed in such a way that it is 2 cm above the water's surface. Each mouse was given three training trials per day for five consecutive days; the starting locations varied from the quadrant opposite to the platform quadrant and clockwise except the platform quadrant. Each mouse's platform position remained constant throughout the five training days. While the mice were immersed in the water facing the pool wall, the cut-off time was 60 seconds. The length of time taken by the mouse to locate the platform was measured as escape latency in seconds. It was guided to or manually placed on the platform if it failed to escape within the cut-off time. Whether the animal found the platform or was manually placed, it was allowed to remain on the platform for 15 seconds. After 24 hours the animals were subjected to the probe test following the training. Herein, the

platform was removed, and the time spent by animals in the quadrant where the platform was previously placed was measured.

5.2.21.4. Procedure for RT-PCR

After sacrificing the animals by decapitation, the whole brain was isolated to section out the hippocampal regions. The sectioned tissues were stored in a Trizol reagent for subsequent RNA isolation. cDNA synthesis was done for 1ug of the isolated RNA samples per the Verso cDNA kit protocol. RT-PCR was performed using SyBR green premix by Takara, where GAPDH was used as a housekeeping gene, and various target genes related to cognitive parameters were measured.