DISCOVERY OF OREXANT AND ANOREXANT AGENTS WITH INDAZOLE SCAFFOLD ENDOWED WITH PERIPHERAL ANTIEDEMA ACTIVITY

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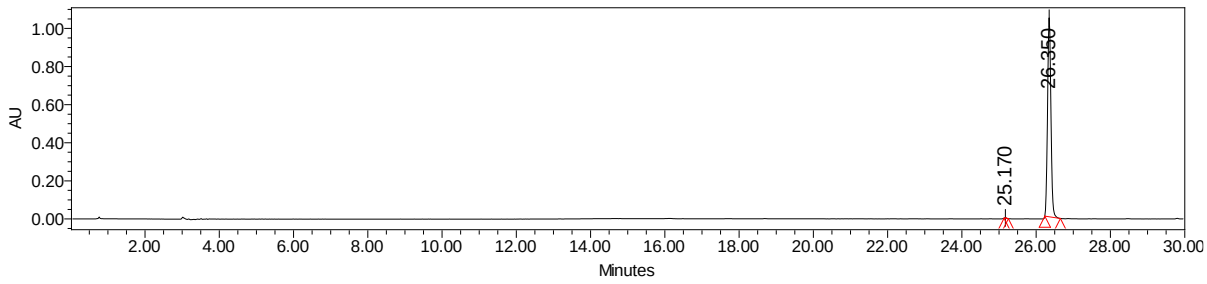
Table of contents	**Pages**

Analytical RP-HPLC traces

LONI 10: Loni Val-NH-CH_3



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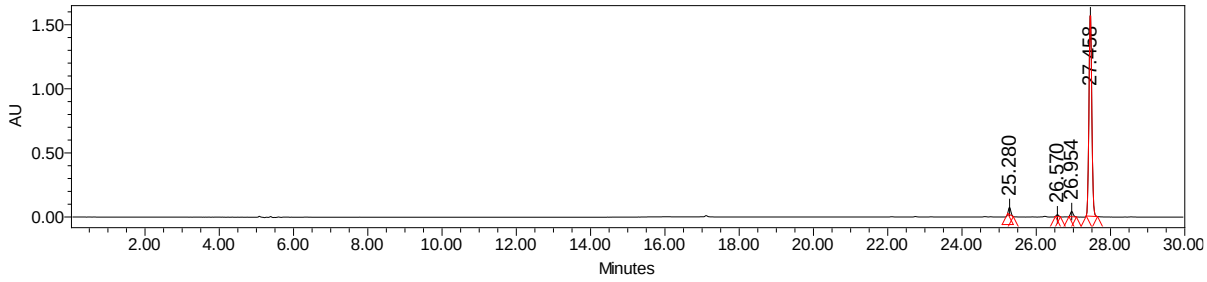
LONI 11: Loni *tert*-Leu-NH-CH_3



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4	27.458	8457289	95.17	1573124
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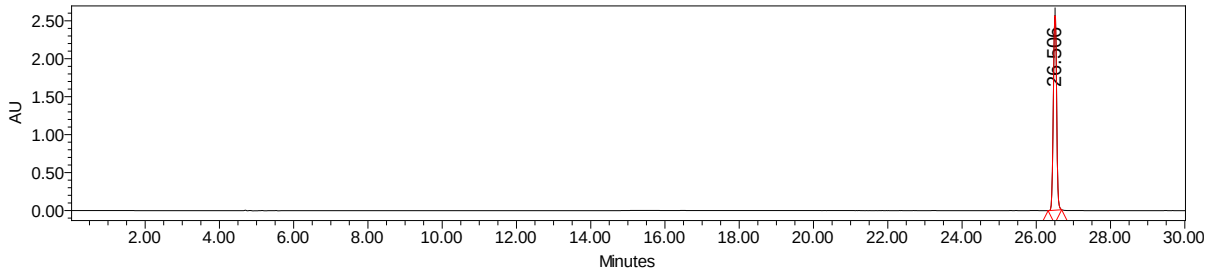
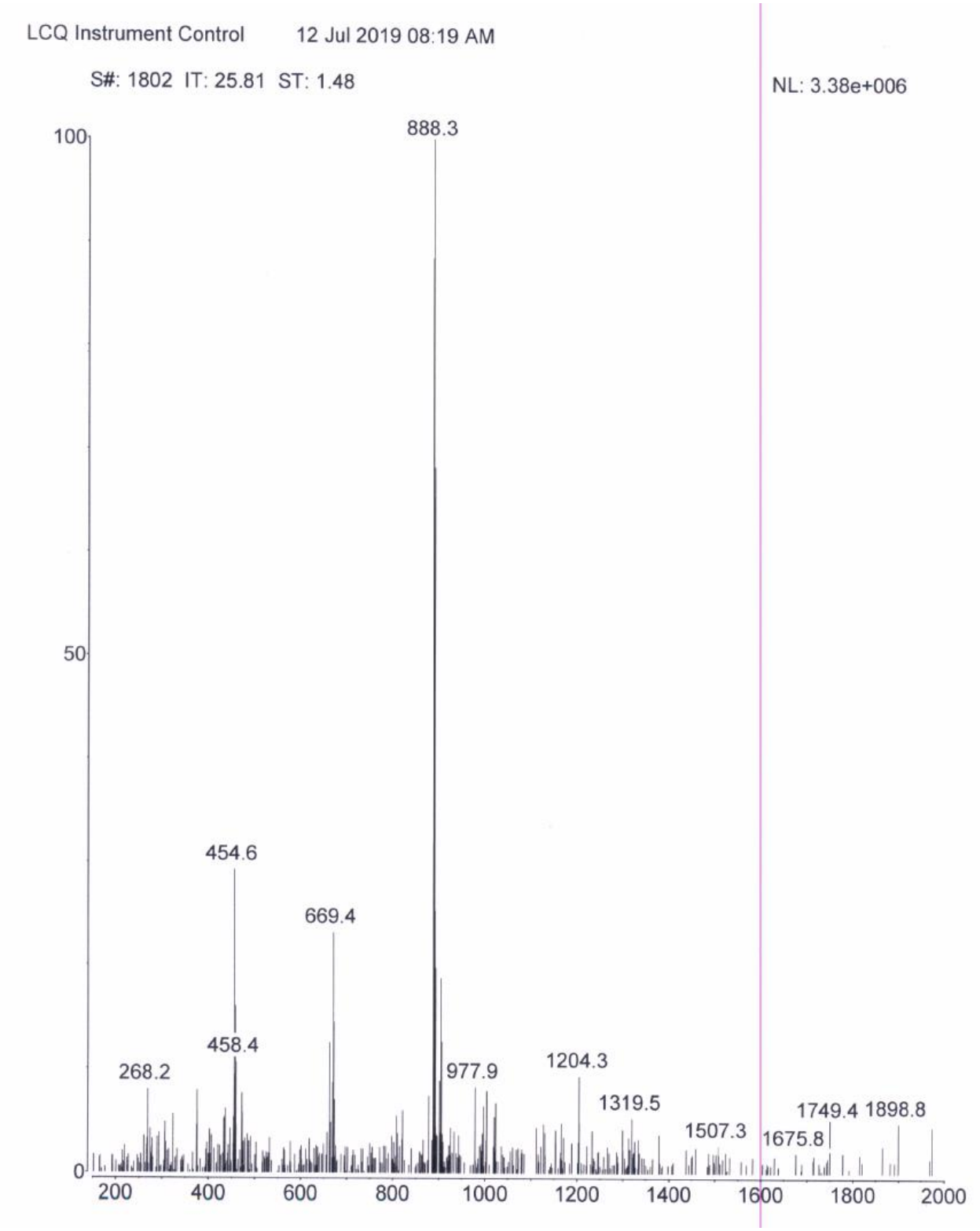
LONI 12: Loni Leu-NH-CH_3



LRMS LONI 10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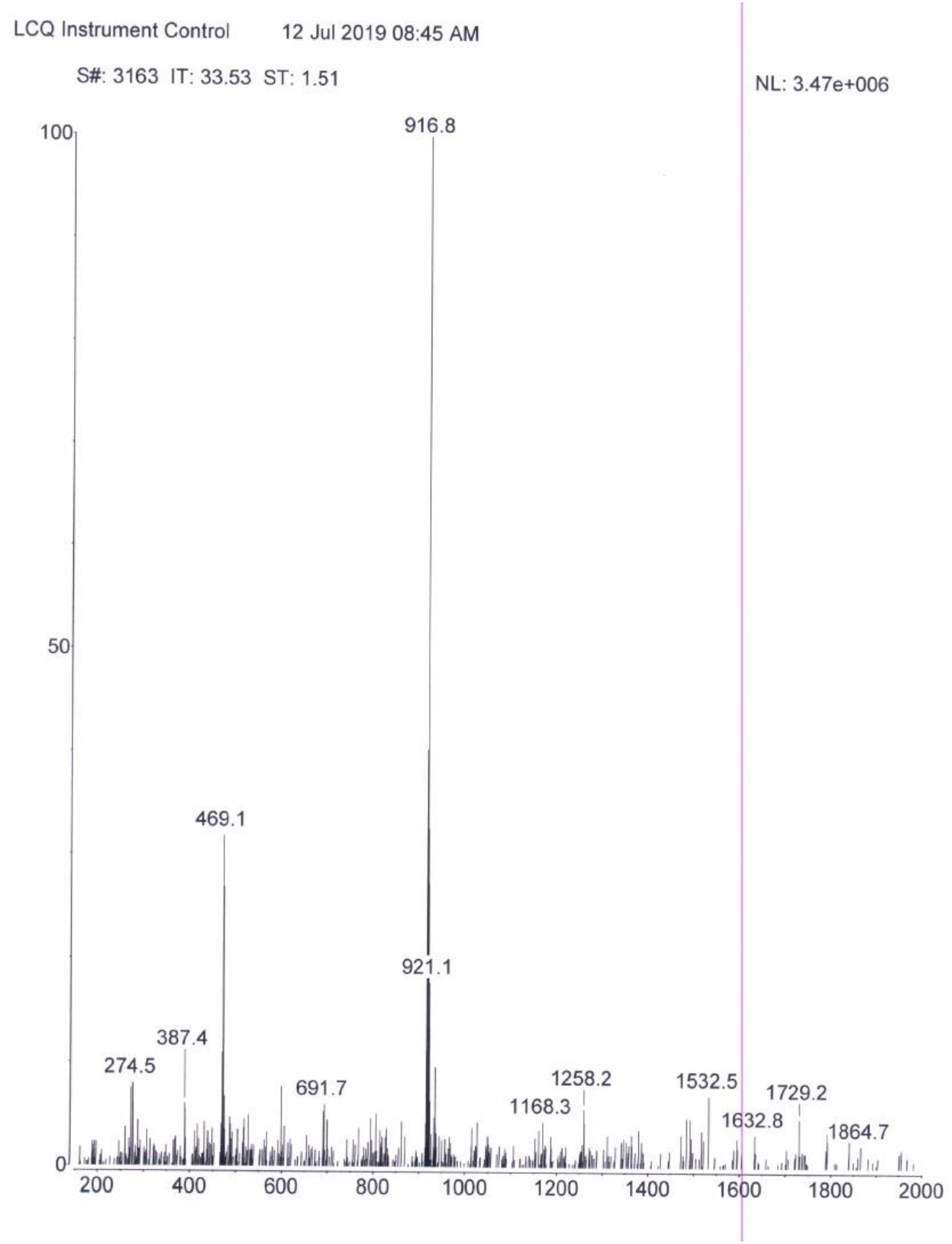
LRMS LONI 11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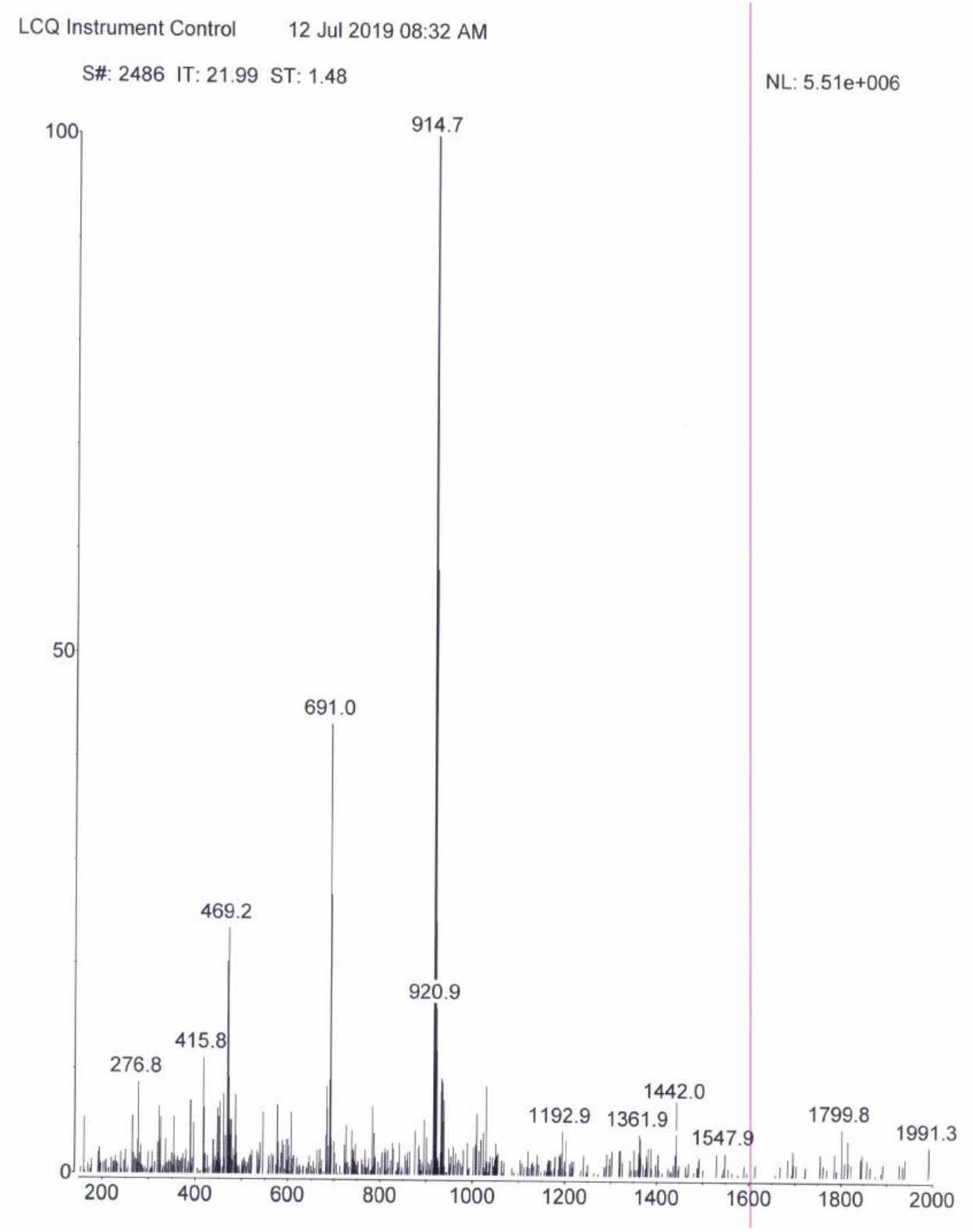
LRMS LONI 12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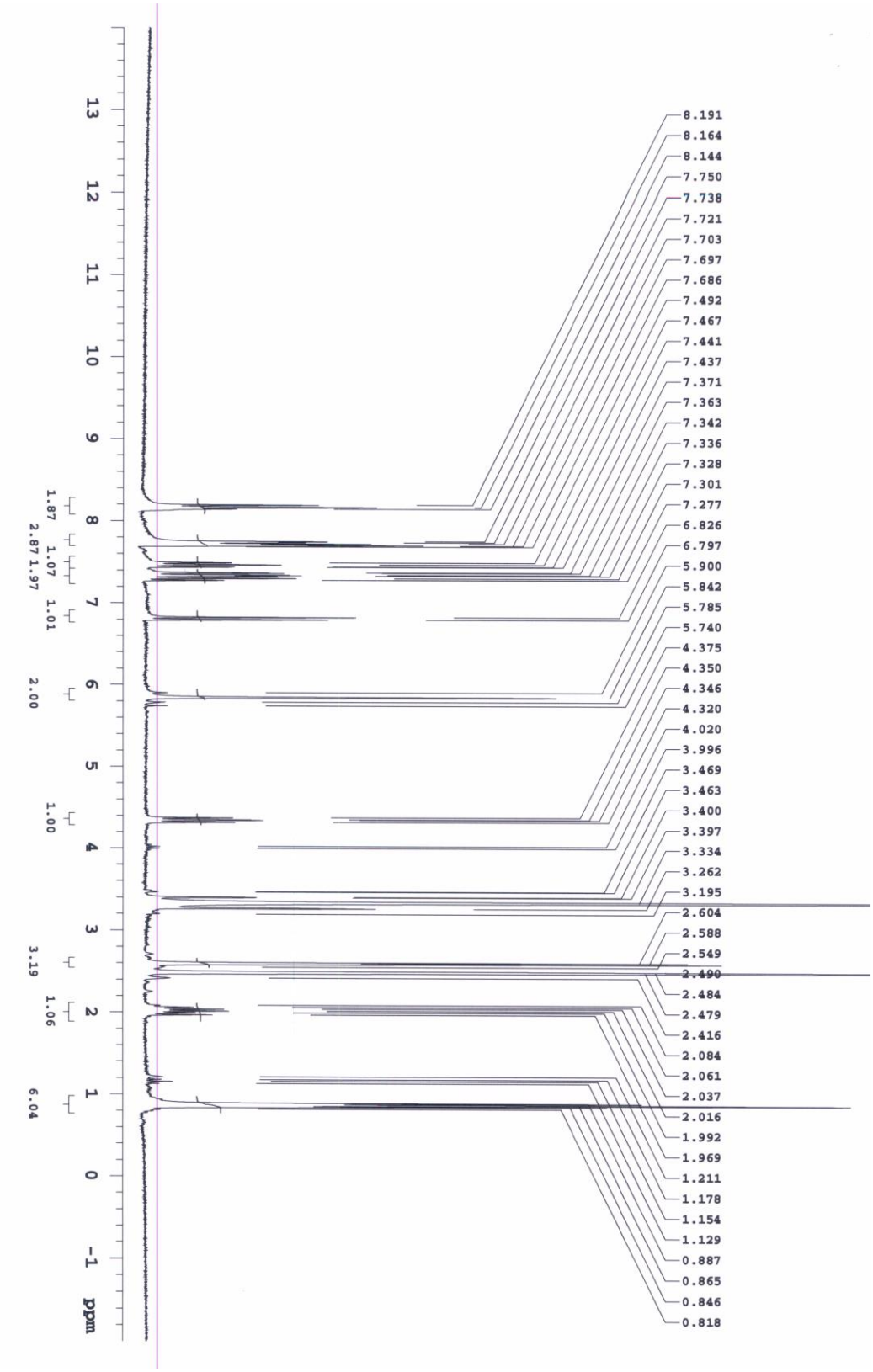
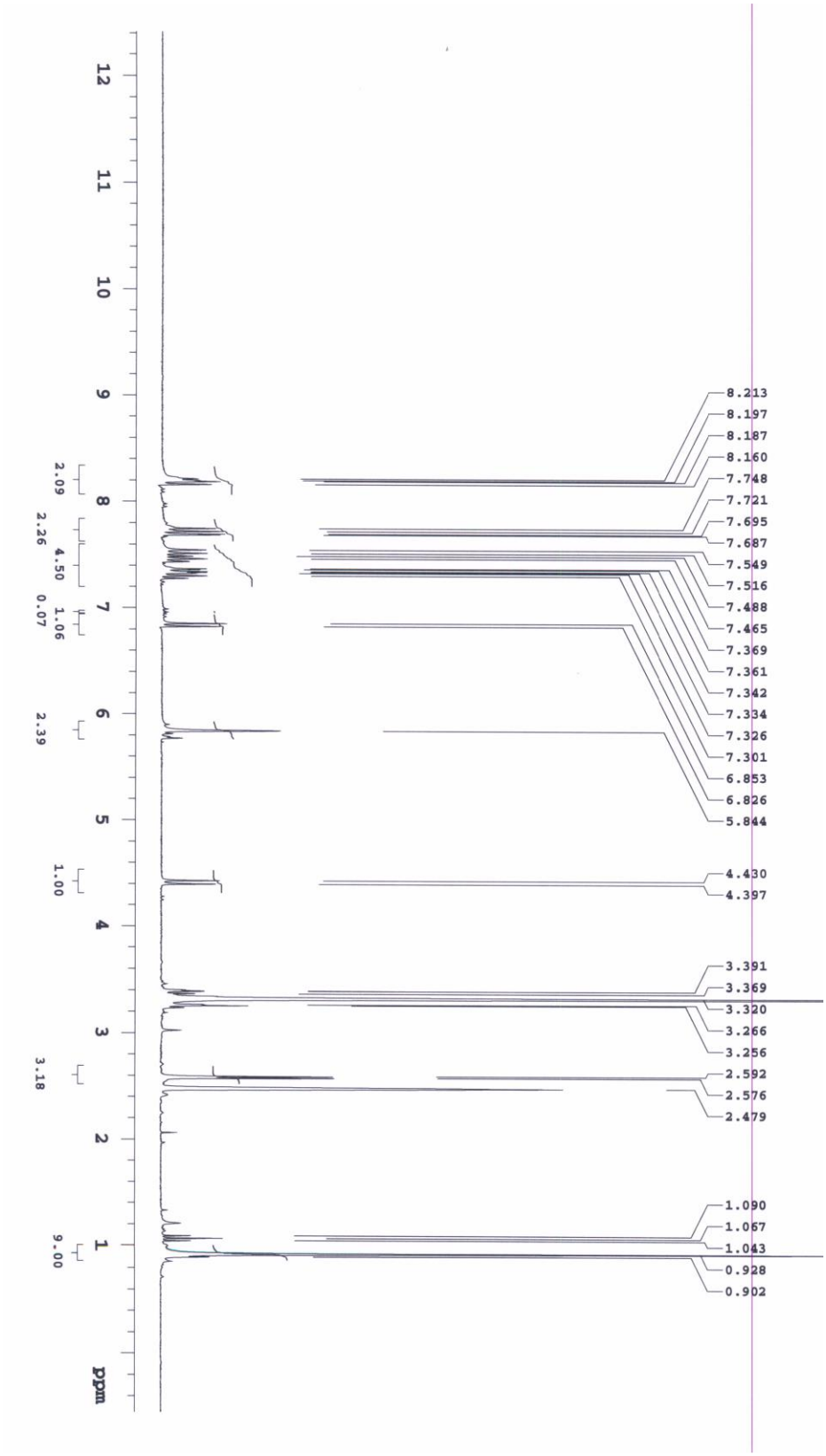
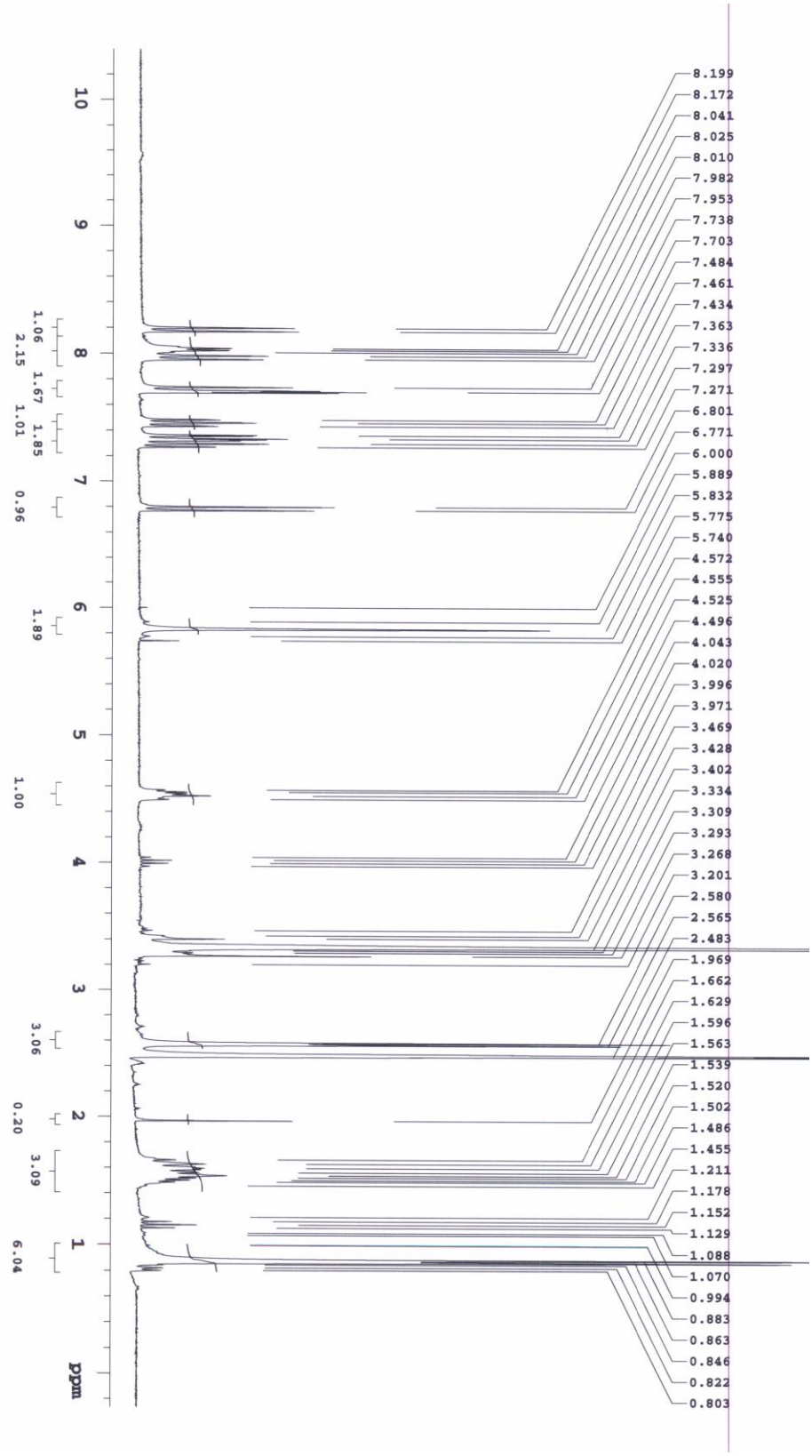
^{1}H NMR LONI 10 in DMSO-d_6



^{1}H NMR LONI 11 in DMSO-d_6



^{1}H NMR LONI 12 in DMSO-d_6



LONI10-12 characterization:

(S)-1-(2,4-dichlorobenzyl)-*N*-(3-methyl-1-(methylamino)-1-oxobutan-2-yl)-1H-indazole-3-carboxamide (LONI10). Compound LONI4 was transformed in the *N*-methyl amide derivative LONI10 following the general procedure. The desired compound was obtained in 96% yield, after reaction work up. Rt (analytical HPLC) = 26.35 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.19-8.14 (m, 2H, H1 and NH amide), 7.75-7.68 (m, 3H, H4, N<u>H</u>(CH_3), H-3), 7.49-7.27 (m, 3H, H-2, H2, H3), 6.81 (d, 1H, H-1), 5.84 (s, 2H, -CH_2-), 6.77 (d, 1H, H7), 5.84 (s, 2H, H-1), 4.35 (t, 1H, CH^{α} Val), 2.56 (d, 3H, NH-C<u>H</u>$_3$ Val), 2.05 (m, 1H, <u>CH</u>(CH_3)$_2$ Val), 0.85 (dd, 6H, CH_3x2 Val). ^{13}C NMR (300 MHz, DMSO-d_6) δ 171.5, 161.5, 141.5, 137.9, 133.7, 133.4, 130.8, 129.5, 128.2, 127.7, 123.3, 122.5, 122.2, 110.9, 57.7, 50.1, 49.1, 31.5, 25.8, 19.6, 18.7. LRMS calcd. for $C_{21}H_{22}Cl_2N_4O_2$: 432.1; found: 454.6 $[M+Na]^+$.

(S)-1-(2,4-dichlorobenzyl)-*N*-(3,3-dimethyl-1-(methylamino)-1-oxobutan-2-yl)-1H-indazole-3-carboxamide (LONI11). Compound LONI1 was transformed in the *N*-methyl amide derivative LONI11 following the general procedure. The desired compound was obtained in 97% yield, after reaction work up. Rt (analytical HPLC) = 27.45 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.21-8.16 (m, 2H, H1 and NH amide), 7.74-7.68 (m, 2H, H4 and N<u>H</u>(CH_3) *tert*-Leu), 7.54-7.46 (m, 2H, H2 and H-3), 7.36-7.30 (m, 2H, H3 and H-2), 6.84 (d, 1H, H-1), 5.84 (s, 2H, -CH_2-), 4.42 (d, 1H, CH^{α} *tert*-Leu), 2.58 (d, 3H, NH(C<u>H</u>$_3$) *tert*-Leu), 0.92 (s, 9H, CH_3x3 *tert*-Leu). ^{13}C NMR (300 MHz, DMSO-d_6) δ 170.5, 160.7, 141.5, 137.7, 133.7, 133.5, 130.8, 129.5, 128.2, 127.7, 123.4, 122.4, 122.2, 110.1, 59.3, 49.1, 35.2, 26.9, 25.6. LRMS calcd. for $C_{22}H_{24}Cl_2N_4O_2$: 446.1; found: 469.1 $[M+Na]^+$.

(S)-1-(2,4-dichlorobenzyl)-*N*-(4-methyl-1-(methylamino)-1-oxopentan-2-yl)-1H-indazole-3-carboxamide (LONI12). Compound LONI7 was transformed in the *N*-methyl amide derivative LONI12 following the general procedure. The desired compound was obtained in quantitative yield, after reaction work up. Rt (analytical HPLC) = 26.50 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.18 (d, 1H, H1), 8.04-7.95 (m, 2H, H4 and N<u>H</u>(CH_3)), 7.73-7.70 (m, 2H, H-3 and NH amide), 7.46 (t, 1H, H2), 7.36-7.27 (m, 2H, H-2 and H3), 6.79 (d, 1H, H-1), 5.83 (s, 2H, -CH_2-), 4.55 (q, 1H, CH^{α} Leu), 2.57 (d, 3H, NH(C<u>H</u>$_3$) Leu), 1.66-1.45 (m, 3H, CH_2^{β} Leu and CH^{γ} Leu), 0.87 (dd, 6H, CH_3x2 Leu). ^{13}C NMR (300 MHz, DMSO-d_6) δ 172.6, 161.7, 141.4, 138.1, 133.8, 133.2, 130.7, 129.5, 128.2, 127.7, 123.2, 122.6, 122.3, 110.8, 51.2, 50.1, 49.1, 41.7, 26.1, 24.8, 23.3, 22.1. LRMS calcd. for $C_{22}H_{24}Cl_2N_4O_2$: 446.1; found: 469.2 $[M+Na]^+$.

Molecular modeling and MD details

Figure 1S. (A) MDMB-Fubinaca crystallographic pose (light blue) superimposed to the same self-docked ligand (light brown) by Glide. (B) Superimposition of MDMB-Fubinaca (light blue), LONI11 (violet), LONI4 (light brown) poses.

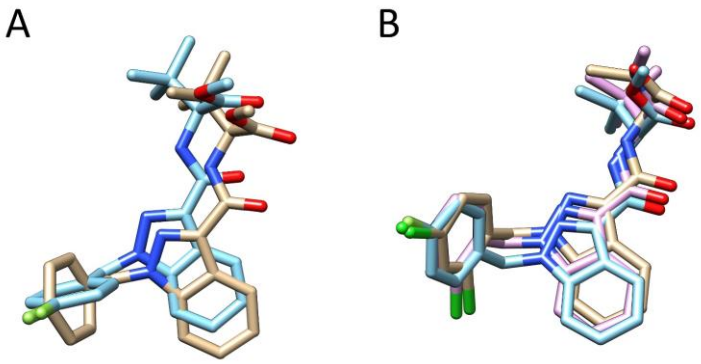


Figure 2S. 2D interaction diagram of (A) MDMB-Fubinaca, (B) LONI11 and (C) LONI4 docked to CB1 receptor (pdb id: 6N4B).

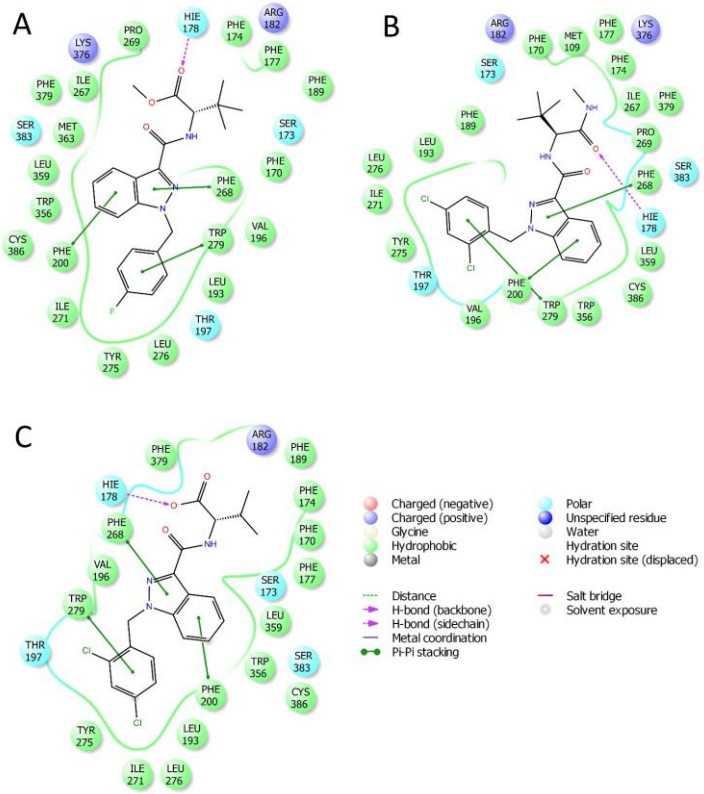


Figure 3S. System model used in the molecular dynamic simulation.

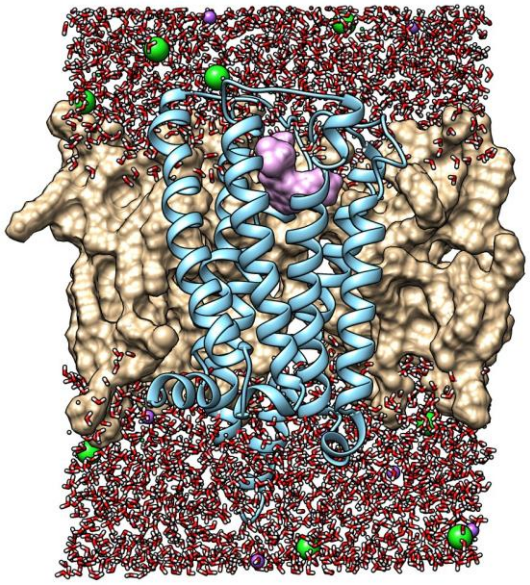


Figure 4S. Protein-ligand interactions of CB1 receptor with (A) MDMB-Fubinaca ligand, (B) LONI4 and (C) LONI11, calculated after 20 ns of MD.

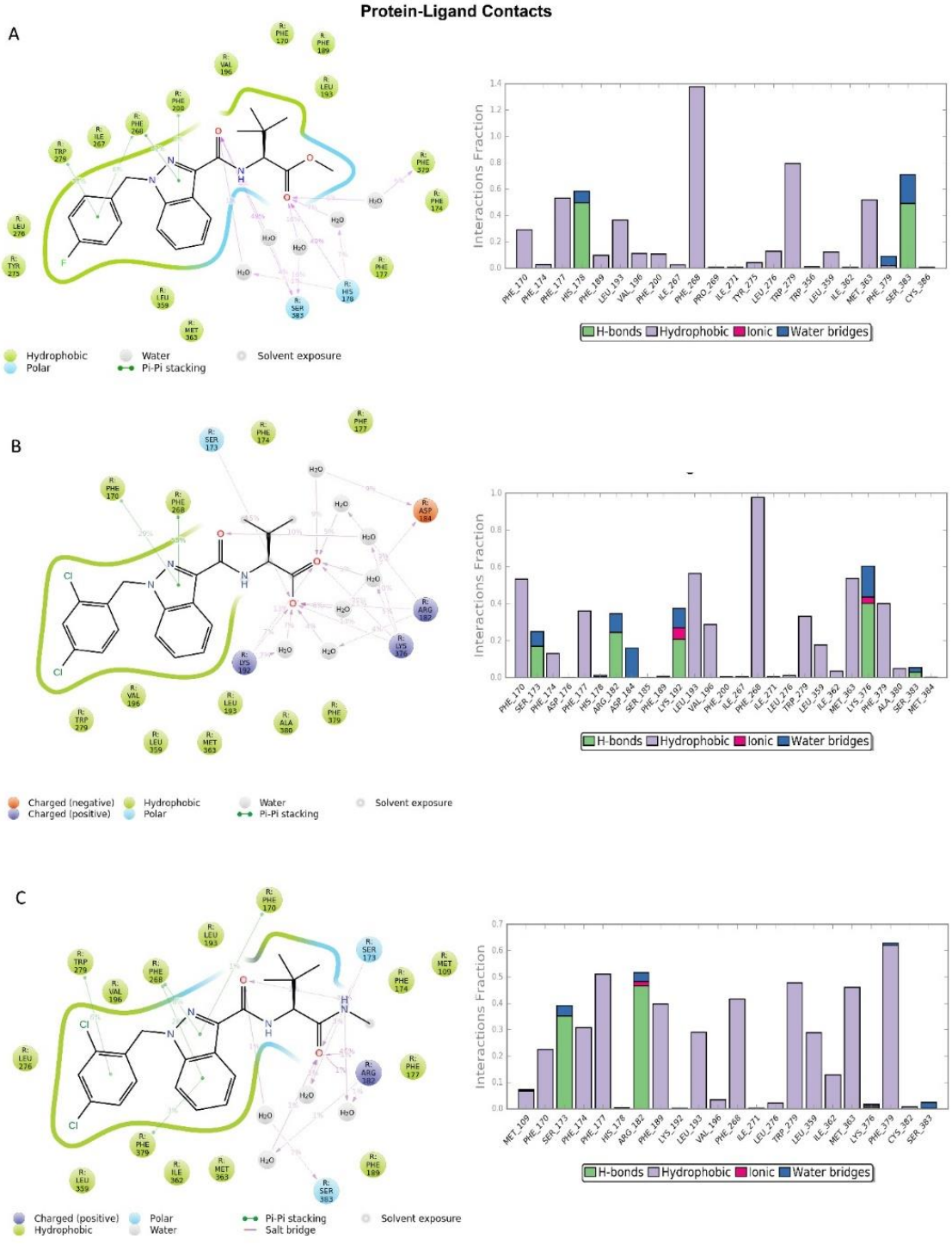


Figure 5S. RMSD (angstroms) for ligand MDMB-Fubinaca (A), LONI4 (B) and LONI11 (C) and the receptor CB1 during 20 ns of molecular dynamic simulation.