Small Molecules Engage Hot Spots Through Cooperative Binding to Inhibit a Tight Protein-Protein Interaction

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Synthesis of 2-pyrrolinones. To a stirred solution of methyl ketone (1.0 eq) and diethyl oxalate (1.1 eq) in dry THF (0.5 M) under argon at 0˚C was added dropwise sodium ethoxide (1.1 eq, 3 M in ethanol). The reaction was allowed to warm to ambient temperature and left to stir for 16 h. The reaction mixture was cooled to 0˚C and quenched with 2 M HCl. The reaction mixture was extracted with ethyl acetate (3x). The organic layer was dried over magnesium sulfate and solvent removed in vacuo to yield the β-diketoester.

ethyl 2,4-dioxo-4-phenylbutanoate – (pale yellow solid, 1.74g, 95%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, *J*=8.5Hz, 2H), 7.61 (t, *J*=7.5Hz, 1H), 7.51 (t, *J*=8.0 Hz, 2H), 7.08 (s, 1H), 4.40 (q, *J*=7.0 Hz, 2H), 1.41 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 198.4, 157.8, 137.0, 133.1, 128.5, 128.3, 63.1, 26.5, 13.8. HRMS calcd for $C_{12}H_{13}O_4$ $(M+H)^+$, 221.0808 found 221.0808.

ethyl 4-(4-chlorophenyl)-2,4-dioxobutanoate - (pale brown solid, 3.28 g, 99%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, *J*=8.5Hz, 2H), 7.46 (d, *J*=8.5Hz, 2H), 7.02 (s, 1H), 4.39 (q, *J*=7.0 Hz, 2H), 1.40 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 189.3, 169.9, 162.0, 140.3, 133.2, 129.2, 129.2, 97.7, 62.7, 14.0. HRMS calcd for $C_{12}H_{12}ClO_4$ $(M+H)^+$, 255.0419 found 255.0414.

ethyl 4-(4-bromophenyl)-2,4-dioxobutanoate – (pale orange solid, 709 mg, 94%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, *J*=8.5Hz, 2H), 7.64 (d, *J*=8.5Hz, 2H), 7.02 (s, 1H), 4.40 (q, *J*=7.0 Hz, 2H), 1.41 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 189.4, 170.1, 162.0, 133.7, 132.2, 129.3, 129.0, 97.7, 62.7, 14.1. HRMS calcd for $C_{12}H_{12}BrO_4$ $(M+H)^+$, 298.9913 found 298.9911.

ethyl 4-(4-(tert-butyl)phenyl)-2,4-dioxobutanoate – (orange oil, 621 mg, 79%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (d, *J*=8.5Hz, 2H), 7.51 (d, *J*=8.5Hz, 2H), 7.06 (s, 1H), 4.39 (q, *J*=7.0 Hz, 2H), 1.40 (t, *J*=7.0 Hz, 3H), 1.34 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$)

δ 190.6, 169.4, 162.3, 157.9, 132.1, 127.8, 125.9, 97.8, 62.5, 35.2, 31.0, 14.0. HRMS calcd for $C_{16}H_{21}O_4$ $(M+H)^+$, 277.1434 found 277.1436.

ethyl 4-(4-chloro-3-methylphenyl)-2,4-dioxobutanoate – (orange solid, purity 70%, 655 mg, 82%); 7.87 (s, 1H), 7.76 (d, *J*=8.5Hz, 1H), 7.44 (d, *J*=8.5Hz, 1H), 7.01 (s, 1H), 4.39 (q, *J*=7.0 Hz, 2H), 2.44 (s, 3H), 1.41 (t, *J*=7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 189.7, 169.8, 162.1, 140.4, 137.0, 133.3, 130.1, 129.6, 126.5, 62.7, 26.5, 20.1, 14.0. HRMS calcd for $C_{13}H_{14}ClO_4$ $(M+H)^+$, 269.0575 found 269.0582.

General Procedure for Synthesis of IPR1110 Derivatives: To a solution of β-diketoester (1 eq) in acetonitrile (1 mL) was added the appropriate amine or aniline (1 eq) and stirred for 10 min whereupon the aldehyde (1 eq) was added. The reaction was left to stir for 20 h at ambient temperature. The solvent was removed in vacuo and the product was isolated either by filtration (the precipitate was washed with cold diethyl ether) or flash chromatography (2-5% MeOH/DCM).

1-(3-bromo-4-methylphenyl)-4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (1, IPR1110) - (white solid, 11 mg, 11%); ^{1}H NMR (500 MHz, DMSO) δ 7.94 (s, 1H), 7.73 (d, *J*=8.5Hz, 2H), 7.52 (d, *J*=8.5Hz, 2H), 7.49 (d, *J*=8.5Hz, 1H), 7.32 (d, *J*=10.0 Hz, 1H), 7.30-7.22 (m, 3H), 6.99-6.92 (m, 1H), 6.34 (s, 1H), 2.24 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 188.0, 164.4, 162.9, 161.0, 150.7, 139.3, 139.2, 137.6, 136.5, 135.2, 134.4, 130.9, 130.6, 130.3, 128.4, 125.8, 123.9, 123.7, 121.6, 119.3, 115.1, 114.9, 60.5, 21.8. HRMS calcd for $C_{24}H_{17}BrClFNO_3$ $(M+H)^+$, 500.0059 found 500.0054.

1-(3-bromo-4-methylphenyl)-4-(4-chloro-3-methylbenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (4, IPR1201) – (white solid, 14 mg, 7%); ^{1}H NMR (500 MHz, DMSO) δ 7.94 (s, 1H), 7.69 (s, 1H), 7.58-7.54 (m, 1H), 7.52-7.47 (m, 2H), 7.35-7.22 (m, 5H), 6.99-6.92 (m, 1H), 6.34 (s, 1H), 2.35 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 188.1, 164.4, 162.9, 160.9, 150.5, 140.7, 139.3, 139.2, 137.8, 137.2, 136.6, 135.6, 135.2, 134.4, 133.2, 131.2, 131.1, 130.9, 130.3, 128.8, 128.0, 127.4, 125.7, 123.9, 123.7, 121.6, 119.4, 119.2, 115.0, 114.9, 114.7, 60.5, 21.7, 19.4. HRMS calcd for $C_{25}H_{18}BrClFNO_3$ $(M+H)^+$, 514.0215 found 514.0210.

1-(3-bromo-4-methylphenyl)-4-(4-(tert-butyl)benzoyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (5, IPR1200) – (yellow solid, 8 mg, 8%); ^{1}H NMR (500 MHz, DMSO) δ 7.96 (s, 1H), 7.69 (d, *J*=8.0 Hz, 2H), 7.51-7.46 (m, 3H), 7.28 (d, *J*=8.5Hz, 2H), 7.24-7.22 (m, 2H), 6.96-6.93 (m, 1H), 6.35 (s, 1H), 2.25 (s, 3H), 1.28 (s, 9H); ^{13}C NMR (126 MHz, DMSO) δ 188.7, 164.6, 163.0, 161.1, 156.0, 139.3, 135.2, 135.0, 134.3, 130.9, 130.3, 128.9, 125.7, 125.1, 123.8, 123.7, 121.5, 120.1, 115.0, 114.9, 114.8, 114.6, 60.6, 34.8, 30.8, 21.8. HRMS calcd for $C_{28}H_{25}BrFNO_3$ $(M+H)^+$, 522.1075 found 522.1074.

4-benzoyl-1-(3-bromo-4-methylphenyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (6, IPR1181) – (white solid, 16 mg, 15%); ^{1}H NMR (500 MHz, DMSO) δ 7.96 (s, 1H), 7.72 (d, *J*=7.5Hz, 2H), 7.57 (t, *J*=7.0 Hz, 1H), 7.53-7.41 (m, 3H), 7.35-7.20 (m, 4H), 6.95 (s, 1H), 6.35 (s, 1H), 2.25 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 189.2, 164.5, 162.9, 161.0, 150.1, 139.4, 137.8, 135.2, 134.3, 132.8, 130.9, 130.3, 128.7, 128.2, 125.7, 123.9, 123.7, 121.6, 119.7, 115.0, 60.5, 21.8. HRMS calcd for $C_{24}H_{18}BrFNO_3$ $(M+H)^+$, 466.0449 found 466.0462.

1-(3-bromo-4-methylphenyl)-4-(4-chlorobenzoyl)-5-(3-chlorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (7, IPR1178) – (white solid, 15 mg, 14%); ^{1}H NMR (500 MHz, DMSO) δ 7.95 (s, 1H), 7.73 (d, *J*=8.5Hz, 2H), 7.56-7.48 (m, 4H), 7.37 (d, *J*=7.5Hz, 1H), 7.29 (d, *J*=8.0 Hz, 1H), 7.26-7.17 (m, 2H), 6.33 (s, 1H), 2.25 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 187.9, 164.4, 150.9, 138.9, 137.6, 136.5, 135.1, 134.5, 132.8, 131.0, 130.6, 130.3, 128.3, 128.1, 126.2, 125.7, 123.7, 121.5, 119.1, 60.3, 21.8. HRMS calcd for $C_{24}H_{17}BrCl_2NO_3$ $(M+H)^+$, 515.9763 found 515.9771.

1-(3-bromo-4-methylphenyl)-4-(4-chlorobenzoyl)-5-(4-ethylphenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (8, IPR1187) – (white solid, 7 mg, 7%); ^{1}H NMR (500 MHz, DMSO) δ 7.93 (s, 1H), 7.71 (d, *J*=8.0 Hz, 2H), 7.52 (d, *J*=8.0 Hz, 2H), 7.47 (d, *J*=8.0 Hz, 1H), 7.35-7.22 (m, 3H), 7.04 (d, *J*=8.0 Hz, 2H), 6.26 (s, 1H), 2.45 (q, *J*=8.0 Hz, 2H), 2.24 (s, 3H), 1.06 (t, *J*=8.0 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ 187.9, 164.5, 150.6, 143.3, 137.5, 136.5, 135.3, 134.2, 133.3, 130.9, 130.6, 128.4, 127.8, 127.6, 125.7, 123.7, 121.6, 119.9, 111.6, 60.8, 27.5, 21.7, 14.9. HRMS calcd for $C_{26}H_{22}BrClNO_3$ $(M+H)^+$, 510.0466 found 510.0468.

1-(3-bromo-4-methylphenyl)-4-(4-chlorobenzoyl)-5-(2,6-difluoropyridin-3-yl)-3-hydroxy-1H-pyrrol-2(5H)-one (9, IPR1185) – (yellow solid, 26 mg, 25%); ^{1}H NMR (500 MHz, DMSO) δ 8.46-8.32 (m, 1H), 7.87 (s, 1H), 7.73 (d, *J*=8.0 Hz, 2H), 7.54 (d, *J*=8.0 Hz, 2H), 7.42 (d, *J*=7.5Hz, 1H), 7.33 (d, *J*=8.0 Hz, 1H), 7.07 (d, *J*=8.0 Hz, 1H), 6.49 (s, 1H), 2.26 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 187.9, 164.4, 161.1, 159.6, 159.1, 159.0, 157.5, 152.3, 146.6, 137.4, 136.7, 135.0, 134.8, 131.3, 130.5, 128.3, 125.6, 123.9, 121.3, 117.1, 115.9, 115.7, 107.6, 107.3, 54.9, 21.8. HRMS calcd for $C_{23}H_{15}BrClF_2N_2O_3$ $(M+H)^+$, 518.9917 found 518.9928.

1-(3-bromo-4-methylphenyl)-4-(4-chlorobenzoyl)-5-(2-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (10, IPR1192) – (white solid, 22 mg, 22%); ^{1}H NMR (500 MHz, DMSO) δ 7.85 (s, 1H), 7.72 (d, *J*=8.5Hz, 2H), 7.54 (d, *J*=8.5Hz, 2H), 7.51-7.42 (m, 2H), 7.30 (d, *J*=8.5Hz, 1H), 7.23-7.16 (m, 1H), 7.09-7.01 (m, 2H), 6.47 (s, 1H), 2.24 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 192.9, 187.9, 164.5, 161.7, 159.8, 151.2, 137.5, 136.6, 135.1, 134.6, 131.0, 130.4, 130.3, 128.4, 125.4, 124.7, 123.7, 123.0, 122.9, 131.2, 118.4, 115.7, 115.6, 21.7. HRMS calcd for $C_{24}H_{17}BrClFNO_3$ $(M+H)^+$, 500.0059 found 500.0068.

5-(benzo[d][1,3]dioxol-5-yl)-1-(3-bromo-4-methylphenyl)-4-(4-chlorobenzoyl)-3-hydroxy-1H-pyrrol-2(5H)-one (11, IPR1193) – (yellow solid, 4 mg, 4%); ^{1}H NMR (500 MHz, DMSO) δ 7.92 (s, 1H), 7.75 (d, *J*=8.0 Hz, 2H), 7.54 (d, *J*=8.0 Hz, 2H), 7.47 (d, *J*=7.0 Hz, 1H), 7.30 (d, *J*=8.0 Hz, 1H), 6.97 (s, 1H), 6.91 (d, *J*=8.0 Hz, 1H), 6.72 (d, *J*=7.5Hz, 1H), 6.22 (s, 1H), 5.89 (s, 2H), 2.26 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 188.0, 164.3, 150.2, 147.3, 147.0, 137.6, 135.3, 134.3, 131.3, 130.9, 130.7, 129.6, 128.4, 125.9, 124.2, 123.7, 122.1, 121.8, 119.7, 107.9, 107.4, 101.0, 60.9, 21.8, 21.3. HRMS calcd for $C_{25}H_{18}BrClNO_5$ $(M+H)^+$, 526.0051 found 526.0047.

4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1-(4-isopropylphenyl)-1H-pyrrol-2(5H)-one (12, IPR1175) – (white solid, 19 mg, 21%); ^{1}H NMR (500 MHz, DMSO) δ 7.73 (d, *J*=8.0 Hz, 2H), 7.55-7.52 (m, 4H), 7.34-7.15 (m, 5H), 6.95 (d, 1H), 6.30 (s, 1H), 2.88-2.77 (m, 1H), 1.13 (d, *J*=6.0 Hz, 6H); ^{13}C NMR (126 MHz, DMSO) δ 187.9, 164.3, 162.9, 160.9, 151.1, 145.6, 139.7, 139.6, 137.5, 136.6, 133.9, 130.6, 130.3, 130.2, 128.3, 126.6, 123.9, 122.6, 119.0, 115.0, 114.8, 114.7, 60.5, 32.8, 23.7, 23.6. HRMS calcd for $C_{26}H_{22}ClFNO_3$ $(M+H)^+$, 450.1267 found 450.1274.

4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1-(3-iodo-4-methylphenyl)-1H-pyrrol-2(5H)-one (**13, IPR1171)** – (white solid, 19 mg, 17%); ^{1}H NMR (500 MHz, DMSO) δ 8.15 (s, 1H), 7.73 (d, *J*=8.0 Hz, 2H), 7.54-7.50 (m, 3H), 7.38-7.18 (m, 4H), 6.96 (s, 1H), 6.32 (s, 1H); ^{13}C NMR (126 MHz, DMSO) δ 187.9, 164.3, 162.9, 161.0, 150.7, 139.3, 138.0, 137.6, 136.5, 134.8, 132.1, 130.6, 130.2, 129.6, 128.4, 123.9, 122.3, 119.2, 115.1, 114.9, 114.7, 100.7, 60.5, 26.8. HRMS calcd for $C_{24}H_{17}ClFINO_3$ $(M+H)^+$, 547.9915 found 547.9920.

4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1-(3-isopropylphenyl)-1H-pyrrol-2(5H)-one (14, IPR1194) – (white solid, 13 mg, 15%); ^{1}H NMR (500 MHz, DMSO) δ 7.75 (d, *J*=8.0 Hz, 2H), 7.53 (d, *J*=8.0 Hz, 2H), 7.48 (d, *J*=8.0 Hz, 1H), 7.45 (s, 1H), 7.31 (d, *J*=10.0 Hz, 1H), 7.27-7.19 (m, 3H), 7.00-6.89 (m, 2H), 6.36 (s, 1H), 2.82 (*J*=m, 1H), 1.13 (d, *J*=7.0 Hz, 6H); ^{13}C NMR (126 MHz, DMSO) δ 187.9, 164.3, 162.8, 160.9, 151.2, 149.0, 139.7, 137.5, 136.6, 136.1, 130.6, 130.2, 130.1, 128.5, 128.3, 123.9, 123.5, 120.4, 120.1, 118.9, 115.0, 60.6, 33.3, 23.8, 23.3. HRMS calcd for $C_{26}H_{22}ClFNO_3$ $(M+H)^+$, 450.1267 found 450.1265.

1-(4-(tert-butyl)phenyl)-4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (15, IPR1195) – (pale yellow solid, 20 mg, 21%); ^{1}H NMR (500 MHz, DMSO) δ 7.73 (d, *J*=8.0 Hz, 2H), 7.55-7.51 (m, 4H), 7.38-7.19 (m, 5H), 6.99-6.87 (m, 1H), 6.30 (s, 1H), 1.21 (s, 9H); ^{13}C NMR (126 MHz, DMSO) δ 187.9, 164.4, 162.9, 160.9, 147.8, 139.7, 137.5, 136.6, 133.6, 130.6, 130.3, 128.3, 126.0, 125.5, 123.9, 122.1, 119.0, 114.8, 60.4, 34.1, 31.0. HRMS calcd for $C_{27}H_{24}ClFNO_3$ $(M+H)^+$, 464.1423 found 464.1427.

1-(2-(benzyloxy)phenyl)-4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (16, IPR1186) – (white solid, 10 mg, 8%); ^{1}H NMR (500 MHz, DMSO) δ 7.73 (d, *J*=8.0 Hz, 2H), 7.53 (d, *J*=8.0 Hz, 2H), 7.46 (d, *J*=7.0 Hz, 2H), 7.41 (t, *J*=7.5Hz, 2H), 7.39-7.35 (m, 1H), 7.22-7.17 (m, 3H), 7.12-7.07 (m, 3H), 6.96 (t, *J*=7.0 Hz, 1H), 6.89 (t, *J*=8.0 Hz, 1H), 6.04 (s, 1H), 5.16 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 188.0, 164.5, 162.8, 160.9, 153.6, 151.6, 139.2, 137.5, 136.9, 136.6, 130.6, 130.2, 130.1, 129.1, 128.5, 128.4, 127.9, 127.3, 124.4, 124.2, 120.6, 119.2, 115.1, 115.0, 114.5, 114.4, 113.6, 69.6, 62.2. HRMS calcd for $C_{30}H_{22}ClFNO_4$ $(M+H)^+$, 514.1216 found 514.1221.

1-(3-chloro-4-methoxyphenyl)-4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (17, IPR1182) – (white solid, 5 mg, 5%); ^{1}H NMR (500 MHz, DMSO) δ 7.76 (s, 1H), 7.73 (d, *J*=8.5Hz, 2H), 7.52 (d, *J*=8.5Hz, 2H), 7.49 (dd, *J*=8.5, 2.5Hz, 1H), 7.29 (d, *J*=9.5Hz, 1H), 7.26-7.23 (m, 2H), 7.09 (d, *J*=9.0 Hz, 1H), 6.99-6.93 (m, 1H), 6.30 (s, 1H), 3.79 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 186.7, 177.0, 152.2, 137.4, 136.6, 130.6, 129.4, 128.3, 124.5, 123.7, 122.9, 120.6, 114.8, 114.6, 112.6, 60.8, 56.1. HRMS calcd for $C_{24}H_{17}Cl_2FNO_4$ $(M+H)^+$, 472.0513 found 472.0517.

1-(5-(tert-butyl)isoxazol-3-yl)-4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (18, IPR1196) – (white solid, 20 mg, 22%); ^{1}H NMR (500 MHz, DMSO) δ 7.83-7.69 (m, 2H), 7.59-7.47 (m, 2H), 7.36-7.22 (m, 3H), 7.07 (m, 1H), 6.76 (s, 1H), 5.92 (s, 1H), 1.25 (s, 9H); 187.8, 181.1, 164.8, 162.8, 160.9, 155.7, 150.2, 139.6, 137.7, 136.5, 130.7, 130.1, 128.4, 124.0, 119.9, 115.1, 114.9, 114.8, 114.6, 92.2, 60.1, 32.6, 28.3. HRMS calcd for $C_{24}H_{21}ClFN_2O_4$ $(M+H)^+$, 455.1168 found 455.1173.

4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1-(4-(2-hydroxyethyl)phenyl)-1H-pyrrol-2(5H)-one (19, IPR1152) – (white solid, 27 mg, 30%); ^{1}H NMR (500 MHz, DMSO) δ 7.84-7.69 (m, 2H), 7.61-7.45 (m, 4H), 7.37-7.13 (m, 5H), 7.01-6.85 (m, 1H), 6.30 (s, 1H), 4.65 (br s, 1H), 3.58 (m, 2H), 2.64 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 187.9, 164.3, 161.2, 151.5, 139.9, 137.5, 137.0, 136.6, 134.0, 130.6, 129.2, 128.3, 123.9, 122.4, 118.9, 114.9, 114.8, 61.8, 60.5. HRMS calcd for $C_{25}H_{20}ClFNO_4$ $(M+H)^+$, 452.1059 found 452.1067.

4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1-(4-(piperidin-1-yl)phenyl)-1H-pyrrol-2(5H)-one (20, IPR1119) – (yellow solid, 36 mg, 37%); ^{1}H NMR (500 MHz, DMSO) δ 7.85-7.67 (m, 2H), 7.58-7.46 (m, 2H), 7.44-7.34 (m, 2H), 7.29-7.13 (m, 3H), 7.02-6.91 (m, 1H), 6.90-6.76 (m, 2H), 6.22 (s, 1H), 3.14-2.99 (m, 4H), 1.66-1.52 (m, 4H), 1.50-1.42 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 187.8, 164.0, 162.9, 160.9, 151.7, 149.1, 140.0, 137.4, 136.8, 130.6, 130.1, 128.3, 127.1, 123.9, 118.6, 115.4, 114.9, 114.7, 60.9, 49.2, 25.2, 23.8. HRMS calcd for $C_{28}H_{25}ClFN_2O_3$ $(M+H)^+$, 491.1525 found 491.1519.

4-benzoyl-1-(3-bromo-4-methylphenyl)-3-hydroxy-5-(3-methoxyphenyl)-1H-pyrrol-2(5H)-one (21, IPR1183) – (white solid, 15 mg, 13%); ^{1}H NMR (500 MHz,

DMSO) δ 7.95 (s, 1H), 7.77 (d, 2H), 7.68-7.41 (m, 4H), 7.32 (m, 1H), 7.25 (d, 1H), 7.10 (t, 1H), 7.00-6.88 (m, 2H), 6.74-6.63 (m, 1H), 6.30 (s, 1H), 3.65 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 189.2, 164.6, 159.1, 149.6, 137.7, 135.4, 134.2, 132.8, 130.9, 129.5, 128.8, 128.2, 125.7, 123.7, 121.6, 120.2, 119.6, 113.9, 113.1, 61.1, 55.0, 21.8. HRMS calcd for $C_{25}H_{21}BrNO_4$ $(M+H)^+$, 478.0662 found 478.0658.

4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-1-(4-(hexyloxy)phenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (22, IPR1176) – (10 mg, 14%); ^{1}H NMR (500 MHz, DMSO) δ 7.73 (d, *J*=7.5Hz, 2H), 7.57-7.44 (m, 4H), 7.28-7.18 (m, 3H), 7.00-6.91 (m, 1H), 6.85 (d, *J*=8.5Hz, 2H), 6.24 (s, 1H), 3.93-3.84 (m, 2H), 1.70 (m, 2H), 1.42-1.33 (m, 2H), 1.32-1.21 (m, 4H), 0.88 (t, 3H); ^{13}C NMR (126 MHz, DMSO) δ 187.8, 164.2, 162.9, 160.9, 156.4, 139.8, 137.3, 136.8, 130.6, 130.2, 128.9, 128.3, 124.5, 123.9, 118.6, 115.4, 114.8, 114.6, 114.4, 67.5, 60.9, 31.0, 28.6, 25.2, 22.0, 13.9. HRMS calcd for $C_{29}H_{28}ClFNO_4$ $(M+H)^+$, 508.1685 found 508.1692.

4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-1-(3-(hexyloxy)-4-methylphenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (23, IPR1174) – (white solid, 16 mg, 15%); ^{1}H NMR (500 MHz, DMSO) δ 7.73 (d, *J*=8.5Hz, 2H), 7.52 (d, *J*=8.5Hz, 2H), 7.30 (d, *J*=10.0 Hz, 1H), 7.27-7.20 (m, 2H), 7.16-7.07 (m, 2H), 7.00 (d, *J*=8.0 Hz, 1H), 6.91 (unresolved t, 1H), 6.36 (s, 1H), 4.01-3.82 (m, 2H), 2.04 (s, 3H), 1.72-1.62 (m, 2H), 1.45-1.36 (m, 2H), 1.34-1.25 (m, 4H), 0.89 (unresolved t, 3H); ^{13}C NMR (126 MHz, DMSO) δ 188.0, 164.2, 156.4, 140.0, 137.5, 136.6, 135.0, 130.6, 130.1, 128.3, 123.8, 123.1, 118.9, 114.9, 114.7, 114.4, 106.3, 67.8, 60.8, 30.9, 28.4, 25.1, 22.0, 15.4, 13.9. HRMS calcd for $C_{30}H_{30}ClFNO_4$ $(M+H)^+$, 522.1842 found 522.1839.

1-(3-bromo-4-methylphenyl)-5-(4-(tert-butyl)phenyl)-4-(4-chlorobenzoyl)-3-hydroxy-1H-pyrrol-2(5H)-one (24, IPR1179) – (white solid, 9 mg, 8%); ^{1}H NMR (500 MHz, DMSO) δ 7.94 (s, 1H), 7.71 (d, *J*=8.0 Hz, 2H), 7.53 (d, *J*=8.0 Hz, 2H), 7.47 (d, *J*=8.0 Hz, 1H), 7.36-7.20 (m, 5H), 6.28 (s, 1H), 2.25 (s, 3H), 1.15 (s, 9H); ^{13}C NMR (126 MHz, DMSO) δ 187.9, 164.7, 150.3, 138.5, 137.6, 136.5, 135.5, 133.5, 133.0, 131.0, 130.7, 128.4, 127.4, 125.7, 125.2, 123.7, 121.6, 120.0, 60.6, 34.2, 31.0, 21.8. HRMS calcd for $C_{28}H_{25}BrClNO_3$ $(M+H)^+$, 538.0779 found 538.0787.

4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1-(6-hydroxyhexyl)-1H-pyrrol-2(5H)-one (25, IPR1177) – (white solid, 58 mg, 67%); ^{1}H NMR (500 MHz,

DMSO) δ 7.73 (d, *J*=8.0 Hz, 2H), 7.48 (d, *J*=8.0 Hz, 2H), 7.41-7.33 (m, 1H), 7.28-7.17 (m, 2H), 7.08 (unresolved t, 1H), 5.46 (s, 1H), 3.60-3.49 (1H), 3.41-3.29 (m, 1H), 2.80-2.64 (m, 1H), 1.48-1.04 (m, 10H); ^{13}C NMR (126 MHz, DMSO) δ 187.1, 165.4, 163.2, 161.2, 140.2, 137.2, 136.9, 130.6, 130.5, 130.4, 128.1, 123.8, 117.7, 115.4, 115.1, 114.9, 114.7, 60.6, 60.1, 32.3, 27.4, 26.1, 25.0. HRMS calcd for $C_{23}H_{24}ClFNO_4$ $(M+H)^+$, 432.1372 found 432.1390.

4-(4-chlorobenzoyl)-1-(3-fluorophenethyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (26, IPR1188) – (white solid, 60 mg, 66%); ^{1}H NMR (500 MHz, DMSO) δ 7.70 (d, *J*=8.0 Hz, 2H), 7.49 (d, *J*=8.0 Hz, 2H), 7.42-7.27 (m, 2H), 7.18 (t, *J*=12.0 Hz, 2H), 7.11 (t, *J*=12.0 Hz, 1H) 7.06-6.94 (m, 3H), 5.42 (s, 1H), 3.95-3.80 (m, 1H), 2.95-2.72 (m, 3H); ^{13}C NMR (126 MHz, DMSO) δ 187.4, 165.0, 163.1, 161.2, 141.6, 141.5, 139.3, 137.2, 136.8, 130.6, 130.2, 128.2, 124.7, 123.8, 118.3, 115.4, 115.2, 114.6, 113.2, 60.1, 41.2, 33.0. HRMS calcd for $C_{25}H_{19}ClF_2NO_3$ $(M+H)^+$, 454.1016 found 454.1013.

1-(3-bromo-4-methylphenyl)-4-(4-chlorobenzoyl)-5-(4-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (27, IPR1189) – (white solid, 21 mg, 21%); ^{1}H NMR (500 MHz, DMSO) δ 7.92 (s, 1H), 7.72 (d, *J*=8.5Hz, 2H), 7.53 (d, *J*=8.5Hz, 2H), 7.50-7.43 (m, 3H), 7.28 (d, *J*=8.5HZ, 1H), 7.03 (t, *J*=8.5Hz, 2H), 6.33 (s, 1H), 2.25 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 188.0, 164.4, 162.6, 160.6, 150.5, 137.6, 136.6, 135.2, 134.4, 132.4, 130.9, 130.6, 129.9, 128.4, 125.8, 123.7, 121.7, 119.6, 115.3, 115.2, 60.3, 21.8. HRMS calcd for $C_{24}H_{17}BrClFNO_3$ $(M+H)^+$, 500.0059 found 500.0061.

1-(3-bromo-4-methylphenyl)-4-(4-chlorobenzoyl)-5-(4-fluoro-3-methoxyphenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (28, IPR1190) – (white solid, 6 mg, 5%); ^{1}H NMR (500 MHz, DMSO) δ 7.92 (s, 1H), 7.76 (d, *J*=8.5Hz, 2H), 7.53 (d, *J*=8.5Hz, 2H), 7.49 (dd, *J*=8.0, 2.0 Hz, 1H), 7.28 (d, *J*=10.0 Hz, 1H), 7.23 (d, *J*=7.0 Hz, 1H), 7.05-6.94 (m, 2H), 6.29 (s, 1H), 3.75 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 188.1, 164.3, 150.6, 149.9, 146.9, 137.6, 136.5, 135.2, 134.4, 132.9, 131.3, 130.9, 130.7, 128.4, 126.1, 123.6, 121.9, 120.6, 119.4, 115.5, 113.5, 60.8, 56.2, 21.8. HRMS calcd for $C_{25}H_{19}BrClFNO_4$ $(M+H)^+$, 530.0165 found 530.0175.

1-(3-bromo-4-methylphenyl)-4-(4-bromobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (29, IPR1191) – (white solid, 14 mg, 15%); ^{1}H NMR (500

MHz, DMSO) δ 7.94 (s, 1H), 7.73-7.62 (m, 4H), 7.49 (d, *J*=7.0 Hz, 1H), 7.36-7.22 (m, 4H), 7.02-6.91 (m, 1H), 6.34 (s, 1H), 2.25 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 188.1, 164.4, 162.9, 161.0, 139.3, 136.9, 135.2, 134.4, 131.3, 130.9, 130.7, 130.3, 126.7, 125.8, 123.9, 123.7, 121.6, 119.2, 115.1, 114.9, 114.7, 60.4, 21.8. HRMS calcd for $C_{25}H_{19}BrClFNO_4$ $(M+H)^+$, 530.0165 found 530.0175. HRMS calcd for $C_{24}H_{17}Br_2FNO_3$ $(M+H)^+$, 545.9535 found 545.9544.

1-(4-bromophenyl)-4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (30, IPR1150) – (white solid, 32 mg, 34%); ^{1}H NMR (500 MHz, DMSO) δ 7.73 (d, *J*=8.0 Hz, 2H), 7.60 (d, *J*=8.0 Hz, 2H), 7.55-7.49 (m, 4H), 7.30 (d, *J*=10.0 Hz, 1H), 7.27-7.21 (m, 2H), 7.00-6.92 (m, 1H), 6.33 (s, 1H); ^{13}C NMR (126 MHz, DMSO) δ 188.0, 164.4, 162.9, 161.0, 150.8, 139.3, 137.6, 136.5, 135.5, 131.7, 130.7, 130.4, 128.4, 124.4, 123.9, 119.3, 117.9, 115.1, 115.0, 114.9, 114.7, 60.4. HRMS calcd for $C_{23}H_{15}BrClFNO_3$ $(M+H)^+$, 487.9882 found 487.9891.

4-(4-chlorobenzoyl)-1-(4-chlorophenyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (31, IPR1151) – (white solid, 24 mg, 27%); ^{1}H NMR (500 MHz, DMSO) δ 7.74 (d, *J*=8.5Hz, 2H), 7.66 (d, *J*=9.0 Hz, 2H), 7.53 (d, *J*=8.5Hz, 2H), 7.39 (d, *J*=9.0 Hz, 2H), 7.31 (d, *J*=9.5Hz, 1H), 7.28-7.22 (m, 2H), 7.00-6.90 (m, 1H), 6.35 (s, 1H); ^{13}C NMR (126 MHz, DMSO) δ 192.9, 188.3, 164.4, 155.1, 150.8, 137.6, 136.5, 135.0, 130.6, 130.3, 129.6, 128.7, 128.4, 124.1, 119.2, 114.9, 60.5. HRMS calcd for $C_{23}H_{15}Cl_2FNO_3$ $(M+H)^+$, 442.0408 found 442.0418.

4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1-(3-methoxyphenethyl)-1H-pyrrol-2(5H)-one (32, IPR1153) – (white solid, 69 mg, 74%); ^{1}H NMR (500 MHz, DMSO) δ 7.70 (d, *J*=8.0 Hz, 2H), 7.48 (d, *J*=8.0 Hz, 2H), 7.47-7.40 (m, 1H), 7.23-7.06 (m, 4H), 6.77 (d, *J*=8.0 Hz, 1H), 6.76-6.70 (m, 2H), 5.34 (s, 1H), 3.93-3.81 (m, 1H), 3.70 (s, 3H), 2.92-2.75 (m, 2H), 2.72-2.62 (m, 1H); ^{13}C NMR (126 MHz, DMSO) δ 187.4, 165.1, 163.1, 161.2, 159.3, 153.1, 140.2, 139.6, 137.1, 136.9, 130.6, 129.4, 128.2, 123.8, 120.8, 118.0, 115.2, 115.1, 114.6, 114.1, 111.9, 60.2, 54.9, 41.5, 33.5. HRMS calcd for $C_{26}H_{22}ClFNO_4$ $(M+H)^+$, 466.1216 found 466.1231.

1-(3-bromo-4-methylphenyl)-4-(4-chlorobenzoyl)-3-hydroxy-5-(p-tolyl)-1H-pyrrol-2(5H)-one (33, IPR1157) – (white solid, 9 mg, 9%, 92% purity); ^{1}H NMR (500 MHz, DMSO) δ 7.91 (s, 1H), 7.70 (d, *J*=8.0 Hz, 2H), 7.52 (d, *J*=8.0 Hz, 2H), 7.47 (d,

J=8.0 Hz, 2H), 7.28-7.25 (m, 2H), 7.01-6.98 (m, 2H), 6.84 (s, 1H), 6.52 (d, *J*=7.0 Hz, 1H), 6.26 (s, 1H), 2.24 (s, 3H), 2.16 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 187.9, 164.4, 150.0, 137.6, 137.3, 136.5, 135.3, 134.2, 133.0, 131.4, 130.9, 130.6, 129.0, 128.4, 127.6, 125.7, 124.2, 123.7, 121.6, 120.0, 60.8, 30.7, 21.8. HRMS calcd for $C_{25}H_{20}BrClNO_3$ $(M+H)^+$, 496.0310 found 496.0305.

4-(4-chlorobenzoyl)-1-(3,5-difluorobenzyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (34, IPR1158) – (white solid, 57 mg, 62%); ^{1}H NMR (500 MHz, DMSO) δ 7.88-7.70 (m, 2H), 7.61-7.42 (m, 2H), 7.36-6.98 (m, 5H), 6.89-6.74 (m, 2H), 5.43 (s, 1H), 4.76-4.56 (m, 1H), 4.26-4.08 (m, 1H); ^{13}C NMR (126 MHz, DMSO) δ 187.5, 165.8, 163.3, 163.2, 163.1, 161.3, 161.2, 153.0, 141.3, 139.1, 137.2, 136.9, 131.7, 130.4, 130.3, 128.2, 124.3, 118.4, 115.2, 115.0, 114.9, 114.7, 110.8, 110.6, 102.8, 102.6, 102.4, 61.0, 43.9. HRMS calcd for $C_{24}H_{16}ClF_3NO_3$ $(M+H)^+$, 458.0765 found 458.0775.

1-(3-bromo-4-methylphenyl)-4-(4-chlorobenzoyl)-3-hydroxy-5-(4-methoxyphenyl)-1H-pyrrol-2(5H)-one (35, IPR1159) – (yellow solid, 25 mg, 24%, 88% purity); ^{1}H NMR (500 MHz, DMSO) δ 7.91 (s, 1H), 7.71 (d, *J*=8.0 Hz, 2H), 7.53 (d, *J*=8.0 Hz, 2H), 7.47 (d, *J*=8.0 Hz, 1H), 7.34-7.23 (m, 3H), 6.74 (d, *J*=8.0 Hz, 2H), 6.25 (s, 1H), 3.62 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 188.1, 164.3, 150.1, 137.6, 137.3, 136.4, 135.3, 134.3, 133.0, 131.3, 130.8, 130.6, 129.0, 128.4, 127.6, 125.8, 124.2, 123.9, 121.6, 120.0, 60.8, 56.5, 21.7. HRMS calcd for $C_{25}H_{20}BrClNO_4$ $(M+H)^+$, 512.0259 found 512.0242.

4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1-(quinolin-6-yl)-1H-pyrrol-2(5H)-one (36, IPR1160) – (yellow solid, 17 mg, 19%); ^{1}H NMR (500 MHz, DMSO) δ 8.83 (s, 1H), 8.30 (d, *J*=8.0 Hz, 1H), 8.21 (s, 1H), 8.12 (d, *J*=9.0 Hz, 1H), 7.96 (d, *J*=9.5Hz, 1H), 7.78 (d, *J*=8.0 Hz, 2H), 7.54 (d, *J*=8.0 Hz, 2H), 7.52-7.47 (m, 1H), 7.39 (d, *J*=10.0 Hz, 1H), 7.35-7.31 (m, 1H), 7.24-7.17 (m, 1H), 6.94-6.87 (m, 1H), 6.50 (s, 1H); ^{13}C NMR (126 MHz, DMSO) δ 192.9, 187.8, 164.8, 162.8, 160.9, 151.4, 150.2, 145.2, 139.6, 139.5, 137.5, 136.7, 135.8, 134.2, 130.7, 130.2, 129.3, 128.3, 127.8, 125.1, 123.9, 122.0, 120.0, 119.1, 115.0, 114.8, 114.7, 64.9, 60.6. HRMS calcd for $C_{26}H_{17}ClFN_2O_3$ $(M+H)^+$, 459.0906 found 459.0917.

4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1-(3-methoxy-5(trifluoromethyl)phenyl)-1H-pyrrol-2(5H)-one (37, IPR1161) – (white solid, 14 mg, 14%); ^{1}H NMR (500 MHz, DMSO) δ 7.74 (d, *J*=8.0 Hz, 2H), 7.69 (s, 1H), 7.52 (d, *J*=8.0 Hz, 2H), 7.45 (s, 1H), 7.37 (d, *J*=10.0 Hz, 1H), 7.33-7.21 (m, 2H), 7.02-6.92 (m, 2H), 6.46 (s, 1H), 3.78 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 188.0, 164.8, 162.9, 161.0, 159.8, 150.5, 139.2, 138.1, 137.7, 136.5, 130.7, 130.5, 130.4, 130.3, 128.4, 124.8, 124.0, 122.6, 119.4, 115.2, 115.0, 114.9, 114.7, 111.9, 111.0, 107.1, 60.4, 55.8. HRMS calcd for $C_{25}H_{17}ClF_4NO_4$ $(M+H)^+$, 506.0777 found 506.0794.

4-(4-chlorobenzoyl)-1-(2,4-dichlorophenethyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol 2(5H)-one (38, IPR1167) – (white solid, 10 mg, 10%); ^{1}H NMR (500 MHz, DMSO) δ 7.73 (d, *J*=10.0 Hz, 2H), 7.63 (s, 1H), 7.52 (s, 1H), 7.44-7.24 (m, 7H), 5.24 (s, 1H), 3.81-3.71 (m, 1H), 2.94-2.88 (m, 1H), 2.85-2.73 (m, 2H); ^{13}C NMR (126 MHz, DMSO) δ 187.4, 165.3, 163.1, 161.2, 153.2, 139.5, 139.4, 137.1, 136.9, 135.3, 134.0, 132.4, 132.1, 130.6, 130.5, 128.7, 128.2, 127.4, 123.9, 118.1, 115.2, 115.1, 114.8, 114.6, 60.3, 30.9. HRMS calcd for $C_{25}H_{18}Cl_3FNO_3$ $(M+H)^+$, 504.0331 found 504.0333.

4-(4-chlorobenzoyl)-1-(3,4-dichlorophenethyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (39, IPR1168) – (white solid, 99 mg, 98%); ^{1}H NMR (500 MHz, DMSO) δ 7.72 (d, *J*=8.0 Hz, 2H), 7.55 (d, *J*=8.5Hz, 1H), 7.54 (s, 1H), 7.48 (d, *J*=8.5Hz, 1H), 7.40 (s, 1H), 7.32-7.27 (m, 3H), 7.23 (dd, *J*=8.0 , 2.0 Hz, 1H), 7.12 (dd, *J*=8.0 , 2.0 Hz, 1H), 7.07 (d, *J*=8.0 Hz, 1H), 5.18 (s, 1H), 3.82-3.76 (m, 1H), 2.81-2.69 (m, 3H); ^{13}C NMR (126 MHz, DMSO) δ 187.4, 165.3, 163.2, 161.2, 153.0, 140.0, 139.5, 137.1, 136.9, 130.9, 130.7, 130.6, 130.5, 130.4, 129.1, 129.0, 128.2, 123.8, 118.1, 115.2, 115.1, 114.8, 114.7, 60.0, 41.2, 32.4. HRMS calcd for $C_{25}H_{18}Cl_3FNO_3$ $(M+H)^+$, 504.0331 found 504.0325.

4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1-(4-(trifluoromethoxy)phenyl)-1H-pyrrol-2(5H)-one (40, IPR1172) – (white solid, 14 mg, 14%); ^{1}H NMR (500 MHz, DMSO) δ 7.77-7.72 (m, 4H), 7.53 (d, *J*=8.0 Hz, 2H), 7.35-7.21 (m, 5H), 7.01-6.92 (m, 1H), 6.37 (s, 1H); ^{13}C NMR (126 MHz, DMSO) δ 188.0, 164.6, 161.0, 150.7, 145.3, 139.2, 137.6, 136.5, 135.2, 130.7, 130.4, 128.4, 124.1, 124.0, 121.5, 119.3, 115.1, 60.5. HRMS calcd for $C_{24}H_{15}ClF_4NO_4$ $(M+H)^+$, 492.0610 found 492.0620.

methyl 3-(3-(4-chlorobenzoyl)-2-(3-fluorophenyl)-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)benzoate (41, IPR1173) – (pink solid, 12 mg, 13%); ^{1}H NMR (500 MHz, DMSO) δ 8.29 (s, 1H), 7.82 (d, *J*=8.0 Hz, 1H), 7.75 (d, *J*=8.5Hz, 2H), 7.69 (d, *J*=8.0 Hz, 1H), 7.53 (d, *J*=8.5Hz, 2H), 7.47 (t, *J*=8.0 Hz, 1H), 7.33 (d, *J*=10.0 Hz, 1H), 7.29-7.20 (m, 2H), 6.94 (t, *J*=8.0 Hz, 1H), 6.40 (s, 1H), 3.84 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 188.0, 165.7, 164.6, 162.9, 161.0, 150.7, 139.2, 139.1, 137.6, 136.5, 130.7, 130.3, 130.2, 129.3, 128.4, 126.8, 126.0, 124.0, 123.2, 119.3, 115.1, 114.9, 114.8, 60.6, 52.3. HRMS calcd for $C_{25}H_{18}ClFNO_5$ $(M+H)^+$, 466.0852 found 466.0852.

4-(4-chlorobenzoyl)-1-(3-ethylphenyl)-5-(3-fluorophenyl)-3-hydroxy-1H-pyrrol-2(5H)-one (42, IPR1197) – (white solid, 13 mg, 15%); ^{1}H NMR (500 MHz, DMSO) δ 7.75 (d, *J*=8.5Hz, 2H), 7.53 (d, *J*=8.5Hz, 2H), 7.48-7.43 (m, 2H), 7.30 (d, *J*=10Hz, 1H), 7.26-7.19 (m, 3H), 6.99-6.91 (m, 2H), 6.33 (s, 1H), 2.55 (unresolved quartet, 2H), 1.11 (t, *J*=7.5Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ 187.9, 164.3, 162.8, 160.9, 151.0, 144.4, 139.6, 137.5, 136.6, 130.2, 130.1, 128.6, 128.3, 125.0, 123.9, 121.9, 120.0, 119.0, 114.9, 114.7, 60.5, 28.0, 15.3. HRMS calcd for $C_{25}H_{20}ClFNO_3$ $(M+H)^+$, 436.1110 found 436.1117.

4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1-(5-methylisoxazol-3-yl)-1H-pyrrol-2(5H)-one (43, IPR1198) – (white solid, 13 mg, 16%); ^{1}H NMR (500 MHz, DMSO) δ 7.74 (d, *J*=8.5Hz, 2H), 7.52 (d, *J*=8.5Hz, 2H), 7.34-7.19 (m, 3H), 7.02 (t, *J*=9.5Hz, 1H), 6.79 (s, 1H), 5.94 (s, 1H), 2.35 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 187.8, 170.4, 164.6, 1162.8, 160.9, 155.8, 150.1, 139.4, 137.7, 136.4, 130.7, 130.1, 130.0, 128.4, 123.9, 119.9, 115.0, 114.9, 114.7, 114.5, 95.5, 60.0, 12.1. HRMS calcd for $C_{21}H_{15}ClFN_2O_4$ $(M+H)^+$, 413.0699 found 413.0705.

1-([1,1'-Biphenyl]-4-yl)-4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one (**44, IPR1607, XHS010**). White solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.73-7.76 (m, 4H), 7.62-7.65 (m, 4H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.43 (t, *J* = 7.5 Hz, 2H), 7.32-7.36 (m, 2H), 7.27-7.30 (m, 1H), 7.23-7.26 (m, 1H), 6.96 (t, *J* = 8.0 Hz, 1H), 6.40 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 187.88, 164.49, 162.90, 160.96, 151.05, 139.58, 137.50, 137.03, 136.63, 135.54, 130.62, 128.86, 128.32, 127.39, 126.88, 126.44, 123.85, 122.77, 119.14, 114.98, 114.82, 114.66, 60.47; HRMS (ESI) *m/z* for $C_{29}H_{19}ClFNO_3$ $[M + H]^+$ calcd 484.1110, found 484.1067.

Methyl-4'-(3-(4-chlorobenzoyl)-2-(3-fluorophenyl)-4-hydroxy-5-oxo-2,5-dihydro-1H-pyrrol-1-yl)-[1,1'-biphenyl]-3-carboxylate (**45, IPR2260, XHS042**). White solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.92 (m, 2H), 7.69-7.77 (m, 6H), 7.54-7.59 (m, 3H), 7.30-7.36 (m, 3H), 6.96 (s, 1H), 6.41 (s, 1H), 3.88 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 187.83, 166.08, 164.57, 162.89, 160.95, 139.69, 127.47, 136.64, 136.03, 135.83, 131.27, 130.63, 130.32, 129.45, 128.31, 128.01, 127.09, 126.92, 123.86, 122.83, 119.08, 114.83, 114.66, 78.94, 60.42, 52.20; HRMS (ESI) *m/z* for $C_{31}H_{21}ClFNO_5$ $[M + H]^+$ calcd 542.1165, found 542.1149.

4-(3-Chlorobenzoyl)-1-(3,4-dichlorophenethyl)-5-(3-fluorophenyl)-3-hydroxy-1,5-dihydro-2H-pyrrol-2-one (**46, IPR1609, XHS012**) – White solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.70 (s, 1H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.54-7.56 (m, 1H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.42-7.44 (m, 2H), 7.35-7.36 (m, 1H), 7.14-7.20 (m, 2H), 7.09 (t, *J* = 7.5 Hz, 1H), 5.42 (s, 1H), 3.82-3.86 (m, 1H), 2.86-2.88 (m, 1H), 2.79 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 186.10, 165.96, 163.08, 161.14, 150.73, 140.04, 138.53, 132.61, 131.31, 131.04, 130.81, 130,65, 130.33, 129.77, 129.40, 129.28, 129.09, 128.90, 128.23, 127.19, 123.74, 114.75, 59.95, 41.09, 32.28; HRMS (ESI) *m/z* for $C_{25}H_{17}Cl_3FNO_3$ $[M + H]^+$ calcd 504.0331, found 504.0334.

1-(3-bromo-4-methylphenyl)-4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-methoxy-1H-pyrrol-2(5H)-one (47, IPR1154) – To a solution of **7** (0.1 mmol, 50 mg) and silver(I) oxide (0.15mmol, 35 mg) in chloroform (1 mL) was added iodomethane (0.25 mmol, 16 µL). The reaction was stirred for 20 h in the dark at ambient temperature. The reaction mixture was then filtered through Celite and the solvent removed in vacuo. The crude product was recrystallized in chloroform to give a yellow solid (29 mg, 56%); ^{1}H NMR (500 MHz, DMSO) δ 7.91 (s, 1H), 7.80 (d, *J*=8.5Hz, 2H), 7.57 (d, *J*=8.5Hz, 2H), 7.45 (dd, *J*=8.0, 2.0 Hz, 1H), 7.32-7.19 (m, 4H), 7.00-6.92 (m, 1H), 6.36 (s, 1H), 3.98 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 189.2, 163.7, 150.5, 140.5, 139.1, 138.6, 138.5, 136.4, 135.5, 134.9, 131.4, 131.3, 130.9, 129.3, 126.3, 125.6, 124.6, 124.2, 124.2, 122.0, 115.9, 115.8, 115.2, 115.0, 61.1, 59.8, 22.2. HRMS calcd for $C_{25}H_{19}BrClFNO_3$ $(M+H)^+$, 514.0215 found 514.0212.

1-(3-bromo-4-methylphenyl)-4-(4-chlorobenzoyl)-5-(3-fluorophenyl)-3-isopropoxy-1H-pyrrol-2(5H)-one (48, IPR1155) – To a solution of **7** (0.1 mmol, 50 mg)

and silver(I) oxide (0.15mmol, 35 mg) in chloroform (1 mL) was added 2-iodopropane (0.25 mmol, 25 µL). The reaction was stirred for 20 h in the dark at ambient temperature. The reaction mixture was then filtered through Celite and the solvent removed in vacuo. The crude product was recrystallized in chloroform to give a yellow solid (35 mg, 65%); ^{1}H NMR (500 MHz, DMSO) δ 7.93 (s, 1H), 7.74 (d, *J*=8.5Hz, 2H), 7.56 (d, *J*=8.5Hz, 2H), 7.49-7.44 (m, 1H), 7.32-7.21 (m, 4H), 6.99-6.93 (m, 1H), 6.38 (s, 1H), 5.40 (*J*=m, 1H), 2.24 (s, 3H), 1.18 (d, *J*=6.0 Hz, 3H), 1.04 (d, *J*=6.0 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ 188.6, 163.4, 161.1, 149.0, 138.2, 135.8, 135.1, 134.4, 130.8, 128.5, 127.0, 126.0, 123.6, 122.4, 79.2, 74.4, 22.5, 22.4, 21.7. HRMS calcd for $C_{27}H_{23}BrClFNO_3$ $(M+H)^+$, 544.0509 found 544.0520.

Table S1. Hot spots on uPAR of the uPAR•uPA interaction, adapted from Gårdsvoll et al, 2006.

Residue	k_{on} (10^5 M^{-1} s^{-1})	k_{off} (10^{-4} s^{-1})	K_d (nM)	$\Delta\Delta G$ (kcal mol^{-1})
R25	2.72	6.95	2.56	1.00
L55	3.02	9.04	3.00	1.09
Y57	1.78	6.23	3.49	1.18
L66	2.26	10.6	4.70	1.35
S100	4.06	13.1	3.24	1.14
D102	5.07	40.4	7.96	1.66
L113	6.78	37.0	5.45	1.44
D140	3.79	15.5	4.10	1.28
D141	2.58	6.97	2.70	1.03
G146	3.08	11.7	3.80	1.23
G148	4.05	21.2	5.23	1.41
L150	8.41	64.0	7.61	1.64
F165	1.74	4.84	2.77	1.05
H166	3.40	8.87	2.61	1.00
M219	2.11	5.50	2.61	1.01

Table S2. X-ray data collection and refinement statistics of suPARcc:**12** (IPR-1175) complex.

Data collection and scaling	
Beamline	BL17U
Space group	R3
a, b, c (Å)	108.51 108.51 84.669
α, β, γ (º)	90.00, 90.00, 120.00
Resolution (Å)	32.75-2.1 (2.178-2.102)
Rmerge (%)	0.085 (0.817)
Number of unique reflections	21034 (1862)
$I/\sigma(I)$	19.8 (3.05)
Completeness (%)	97.20 (85.37)
Refinement	
R-work	0.2349(0.2658)
R-free	0.2613 (0.3160)
Average B factor ($Å^2$)	36.70
Validation	
RMS deviations from ideal	
Bond lengths (Å)	0.020
Bond angles (°)	2.32
Ramachandran plot	
Ramachandran favored (%)	91
Ramachandran outliers (%)	1.1

Table S3. X-ray data collection and refinement statistics of suPARcc:**3** (IPR-737) complex.

Data collection and scaling	
Beamline	SSRF BL17U
Space group	R3
a, b, c (Å)	110.67 110.67 80.37
α, β, γ (°)	90.00, 90.00, 120.00
Wavelength (Å)	0.979
Resolution (Å)	41.16 - 3.1 (3.211 - 3.1)
Rmerge (%)	0.095 (0.772)
Number of unique reflections	6636 (664)
$I/\sigma(I)$	26.5 (3.5)
Completeness (%)	99.61 (99.10)
Refinement	
R-work	0.2119 (0.2504)
R-free	0.2576 (0.3548)
Average B factor (Å^2)	96.70
Validation	
RMS deviations from ideal	
Bond lengths (Å)	0.011
Bond angles (°)	1.76
Ramachandran plot	
Ramachandran favored (%)	81
Ramachandran outliers (%)	3.1

Table S4. Calculated free energies ± standard error of **3** and select derivatives of **1**, showing the individual fr

Compound	ΔE_{VDW}	ΔE_{ELE}	ΔE_{GB}	ΔE_{SURF}	ΔE_{MMGBSA}	$\Delta G_{MMGBSA-NM}$
3 (IPR-737)	-41.7 ± 0.2	-10.9 ± 0.3	31.3 ± 0.3	-5.4 ± 0.0	-26.7 ± 0.2	-8.6 ± 0.3
31 (IPR-1151)	-35.0 ± 0.2	-21.7 ± 0.3	29.4 ± 0.2	-4.6 ± 0.0	-31.9 ± 0.1	-13.7 ± 0.3
19 (IPR-1152)	-39.2 ± 0.2	-32.5 ± 0.3	41.9 ± 0.2	-5.3 ± 0.0	-35.0 ± 0.2	-14.8 ± 0.3
33 (IPR-1157)	-37.2 ± 0.1	-30.0 ± 0.2	36.7 ± 0.2	-4.9 ± 0.0	-35.4 ± 0.1	-18.3 ± 0.3
37 (IPR-1161)	-45.7 ± 0.2	-18.8 ± 0.3	33.1 ± 0.2	-5.8 ± 0.0	-37.2 ± 0.1	-16.5 ± 0.3
12 (IPR-1175)	-42.0 ± 0.2	-19.6 ± 0.3	30.4 ± 0.1	-5.1 ± 0.0	-36.3 ± 0.1	-18.1 ± 0.3
7 (IPR-1178)	-37.5 ± 0.1	-27.2 ± 0.2	33.2 ± 0.2	-4.9 ± 0.0	-36.4 ± 0.1	-18.7 ± 0.3
26 (IPR-1188)	-34.0 ± 0.2	-23.6 ± 0.3	30.5 ± 0.2	-5.0 ± 0.0	-32.1 ± 0.1	-14.8 ± 0.3
27 (IPR-1189)	-40.4 ± 0.2	-23.1 ± 0.2	30.8 ± 0.2	-5.3 ± 0.0	-38.0 ± 0.2	-20.9 ± 0.3
28 (IPR-1190)	-40.8 ± 0.2	-27.0 ± 0.3	34.5 ± 0.2	-5.6 ± 0.0	-38.8 ± 0.2	-20.8 ± 0.3
14 (IPR-1194)	-38.8 ± 0.2	-23.8 ± 0.3	31.5 ± 0.2	-5.0 ± 0.0	-36.1 ± 0.2	-18.3 ± 0.4
4 (IPR-1201)	-37.9 ± 0.2	-25.1 ± 0.3	32.4 ± 0.2	-5.0 ± 0.0	-35.6 ± 0.1	-17.0 ± 0.3
45 (IPR-2260)	-48.4 ± 0.2	-31.2 ± 0.4	44.1 ± 0.2	-6.8 ± 0.0	-42.2 ± 0.2	-20.2 ± 0.3

Table S5. Correlation coefficients of the individual free energy terms and correlation to select residues of uPAR to illustrate the relationship between the decomposition energy and inhibition constant K_i for select derivatives of **1** from **Fig. 4c**.

Component	r	ρ	τ
ΔE_{VDW}	0.24	0.14	0.14
ΔE_{ELE}	0.02	0.17	0.17
ΔE_{GB}	-0.01	-0.09	-0.05
ΔE_{SURF}	0.33	0.08	0.05
ΔE_{MMGBSA}	0.44	0.42	0.32
$\Delta G_{MMGBSA-NM}$	0.47	0.38	0.26
ΔE_{R25}	0.60	0.63	0.47
ΔE_{T27}	-0.28	-0.04	-0.05
ΔE_{L40}	-0.23	-0.16	-0.08
ΔE_{K50}	-0.15	-0.13	-0.08
ΔE_{T51}	0.51	0.55	0.44
ΔE_{R53}	-0.17	0.01	-0.05
ΔE_{L55}	-0.08	-0.13	-0.11
ΔE_{Y57}	0.44	0.51	0.32
ΔE_{L66}	-0.28	-0.31	-0.23
ΔE_{D102}	-0.31	-0.30	-0.20
ΔE_{S104}	-0.13	-0.06	-0.05
ΔE_{E106}	-0.28	-0.33	-0.14
ΔE_{V125}	0.40	0.42	0.29
ΔE_{E127}	0.41	0.41	0.35
ΔE_{D140}	-0.32	-0.28	-0.26
ΔE_{D141}	-0.12	-0.07	-0.02
ΔE_{H143}	0.17	0.19	0.14
ΔE_{L144}	0.17	0.05	0.02
ΔE_{L150}	0.57	0.58	0.44
ΔE_{P151}	0.49	0.32	0.26
ΔE_{L168}	0.52	0.44	0.38

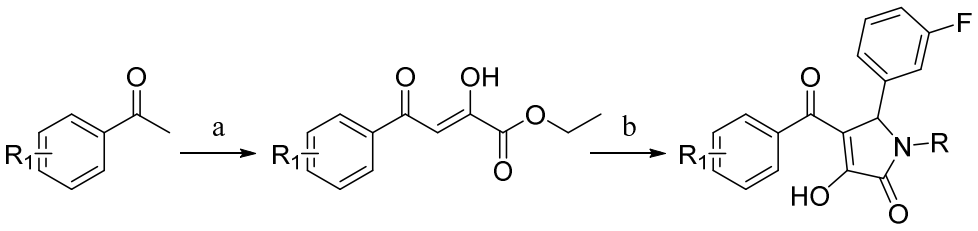
Table S6. Correlation coefficients to illustrate the relationship between the decomposition energy of select residues from **Fig. 4c** and kinetic rate constants for select derivatives of **1**.

	k_{on}			k_{off}			K_d			ΔΔG		
Compound	r	ρ	τ	r	ρ	τ	r	ρ	τ	r	ρ	τ
31 (IPR-1151)	0.30	0.39	0.27	-0.12	-0.19	-0.15	-0.36	-0.31	-0.20	-0.02	-0.04	-0.02
19 (IPR-1152)	-0.37	-0.34	-0.21	0.15	0.07	0.01	-0.11	-0.19	-0.14	-0.52	-0.47	-0.36
33 (IPR-1157)	-0.28	0.03	-0.05	0.04	-0.10	-0.10	0.06	0.10	0.10	-0.42	-0.31	-0.21
37 (IPR-1161)	0.17	-0.10	0.01	0.08	0.19	0.14	0.08	0.20	0.08	0.41	0.26	0.16
12 (IPR-1175)	-0.17	-0.24	-0.12	-0.09	-0.10	-0.12	-0.12	-0.09	-0.05	-0.42	-0.40	-0.32
7 (IPR-1178)	-0.58	-0.45	-0.32	0.30	0.13	0.08	0.02	0.00	-0.03	-0.68	-0.60	-0.47
26 (IPR-1188)	-0.61	-0.39	-0.27	0.33	-0.03	-0.10	0.07	0.04	0.01	-0.63	-0.58	-0.47
27 (IPR-1189)	-0.05	0.02	-0.03	-0.02	-0.07	-0.03	-0.07	0.04	0.03	-0.22	-0.19	-0.14
28 (IPR-1190)	-0.44	-0.27	-0.25	0.71	0.64	0.45	0.25	0.16	0.12	-0.52	-0.44	-0.36
14 (IPR-1194)	-0.11	-0.09	-0.10	0.36	0.24	0.16	0.01	-0.08	-0.08	-0.21	-0.02	-0.03
4 (IPR-1201)	0.67	0.58	0.41	-0.57	-0.53	-0.43	-0.57	-0.16	-0.05	0.57	0.32	0.25
45 (IPR-2260)	-0.38	-0.28	-0.23	0.24	0.22	0.12	0.28	0.20	0.10	0.04	0.01	-0.03

Table S7. Calculated free energies ± standard error of single and double mutants in the uPAR•uPA and uPAR•**12** and complexes.

	uPA (3BT1)						**12** (IPR-1175)					
Mutant	ΔE_{VDW}	ΔE_{ELE}	ΔE_{GB}	ΔE_{SURF}	ΔE_{MMGBSA}	$\Delta G_{MMGBSA-NM}$	ΔE_{VDW}	ΔE_{ELE}	ΔE_{GB}	ΔE_{SURF}	ΔE_{MMGBSA}	$\Delta G_{MMGBSA-NM}$
Wild-type	-130.1 ± 0.3	-951.6 ± 2.5	1016.4 ± 2.4	-18.8 ± 0.0	-84.2 ± 0.3	-29.8 ± 0.5	-42.0 ± 0.2	-19.6 ± 0.3	30.4 ± 0.1	-5.1 ± 0.0	-36.3 ± 0.1	-18.1 ± 0.3
R53A	-131.3 ± 0.4	-1031.9 ± 3.7	1098.7 ± 3.7	-19.2 ± 0.1	-83.7 ± 0.5	-25.3 ± 0.6	-38.1 ± 0.2	-10.3 ± 0.2	23.6 ± 0.1	-4.9 ± 0.0	-29.6 ± 0.1	-12.7 ± 0.3
T27A	-126.7 ± 0.3	-969.2 ± 2.3	1036.0 ± 2.2	-18.4 ± 0.0	-78.3 ± 0.3	-24.3 ± 0.5	-41.7 ± 0.2	-23.8 ± 0.3	32.0 ± 0.1	-5.4 ± 0.0	-39.0 ± 0.1	-21.8 ± 0.3
T27A-R53A	-128.8 ± 0.5	-1057.7 ± 4.5	1123.7 ± 4.4	-18.7 ± 0.1	-81.6 ± 0.7	-27.3 ± 0.6	-43.1 ± 0.2	-10.5 ± 0.2	26.2 ± 0.2	-5.4 ± 0.0	-32.7 ± 0.1	-13.1 ± 0.3
L40A	-132.5 ± 0.4	-926.9 ± 3.0	995.2 ± 2.9	-18.9 ± 0.1	-83.1 ± 0.4	-27.7 ± 0.6	-43.4 ± 0.2	-17.1 ± 0.2	29.6 ± 0.1	-5.2 ± 0.0	-36.1 ± 0.1	-16.6 ± 0.3
L40A-R53A	-132.9 ± 0.3	-1025.5 ± 2.8	1096.2 ± 2.8	-18.8 ± 0.0	-81.1 ± 0.4	-24.7 ± 0.6	-39.8 ± 0.2	-10.1 ± 0.2	23.6 ± 0.1	-5.0 ± 0.0	-31.2 ± 0.2	-13.0 ± 0.3
L55A	-127.3 ± 0.3	-934.7 ± 2.7	998.5 ± 2.7	-18.4 ± 0.0	-81.9 ± 0.3	-27.7 ± 0.5	-42.0 ± 0.2	-14.7 ± 0.2	28.3 ± 0.2	-5.1 ± 0.0	-33.4 ± 0.2	-14.1 ± 0.3
L55A-R53A	-136.2 ± 0.3	-1027.0 ± 2.6	1098.2 ± 2.6	-19.6 ± 0.0	-84.6 ± 0.3	-27.1 ± 0.5	-39.5 ± 0.2	-11.6 ± 0.2	26.1 ± 0.2	-4.9 ± 0.0	-29.9 ± 0.2	-11.4 ± 0.3
L66A	-132.8 ± 0.4	-985.8 ± 2.7	1050.4 ± 2.7	-19.3 ± 0.0	-87.5 ± 0.4	-30.8 ± 0.5	-39.4 ± 0.1	-29.2 ± 0.3	34.8 ± 0.2	-5.6 ± 0.0	-39.4 ± 0.1	-18.7 ± 0.3
L66A-R53A	-132.8 ± 0.3	-1039.8 ± 2.9	1111.2 ± 2.8	-19.1 ± 0.0	-80.4 ± 0.4	-23.6 ± 0.5	-39.1 ± 0.1	-8.7 ± 0.2	23.4 ± 0.2	-4.9 ± 0.0	-29.2 ± 0.1	-10.4 ± 0.3
V125A	-125.8 ± 0.3	-913.9 ± 3.5	976.3 ± 3.3	-18.1 ± 0.0	-81.5 ± 0.3	-27.2 ± 0.5	-37.3 ± 0.2	-23.2 ± 0.3	30.6 ± 0.1	-5.0 ± 0.0	-34.9 ± 0.2	-16.6 ± 0.3
V125A-R53A	-134.7 ± 0.4	-1027.5 ± 2.5	1099.1 ± 2.4	-19.4 ± 0.0	-82.5 ± 0.4	-26.4 ± 0.5	-38.3 ± 0.2	-8.8 ± 0.2	22.9 ± 0.1	-4.8 ± 0.0	-29.0 ± 0.1	-11.4 ± 0.3
L150A	-124.1 ± 0.5	-969.7 ± 4.1	1032.9 ± 4.0	-18.1 ± 0.1	-79.1 ± 0.5	-22.2 ± 0.5	-39.2 ± 0.2	-23.4 ± 0.3	32.6 ± 0.1	-5.1 ± 0.0	-35.1 ± 0.2	-16.2 ± 0.3
L150A-R53A	-137.1 ± 0.3	-1022.7 ± 2.7	1095.3 ± 2.6	-19.6 ± 0.0	-84.1 ± 0.4	-28.2 ± 0.5	-34.9 ± 0.2	-10.4 ± 0.3	23.7 ± 0.2	-4.6 ± 0.0	-26.2 ± 0.2	-8.8 ± 0.3
L168A	-126.9 ± 0.3	-910.9 ± 2.5	975.0 ± 2.5	-18.2 ± 0.0	-81.0 ± 0.3	-26.7 ± 0.5	-43.8 ± 0.2	-16.8 ± 0.2	29.9 ± 0.2	-5.2 ± 0.0	-35.9 ± 0.1	-18.6 ± 0.3
L168A-R53A	-137.2 ± 0.3	-1002.1 ± 2.8	1076.9 ± 2.6	-19.5 ± 0.0	-81.8 ± 0.4	-26.5 ± 0.5	-40.9 ± 0.1	-9.3 ± 0.1	24.8 ± 0.1	-5.0 ± 0.0	-30.4 ± 0.1	-10.4 ± 0.3

Figure S1

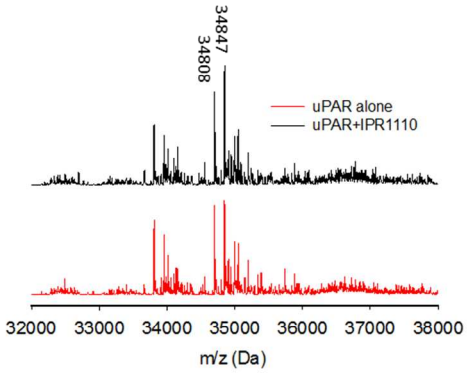


1a-b **2a-k**

1a:R_1 = 4'-Cl
1b:R_1 = 3'-Cl

Reagents and conditions: a) NaOEt (3 M in ethanol), diethyl oxalate, anhydrous THF, room temperature, 20 h; b) 3-F-PhCHO, RNH_2 , acetonitrile, room temperature, 20 h.

Figure S2



uPAR was not covalently modified by IPR1110.

Fig. S2. Compound IPR1110 (200 μM) was incubated with uPAR protein (32 μM) for 1 h in buffer PBS at r.t.. After centrifuged at 18 kg for 5 min, the sample was directly injected into Agilent 6520 Accurate Mass Q-TOF at 0.3 mL/min with a buffer of 5 mM NH_4OAc in ACN/H_2O(1:4).

Figure S3

IPR1110 stability in the presence or absence of uPAR.

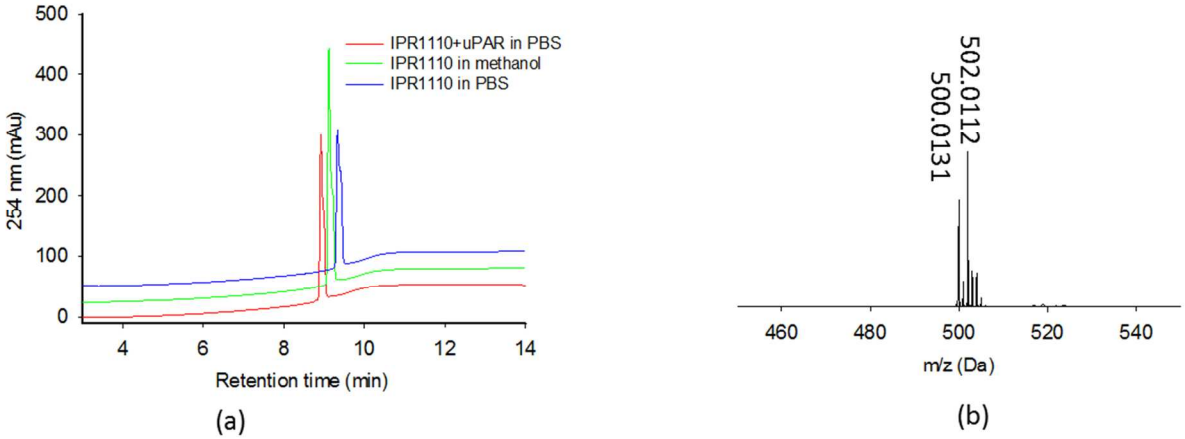
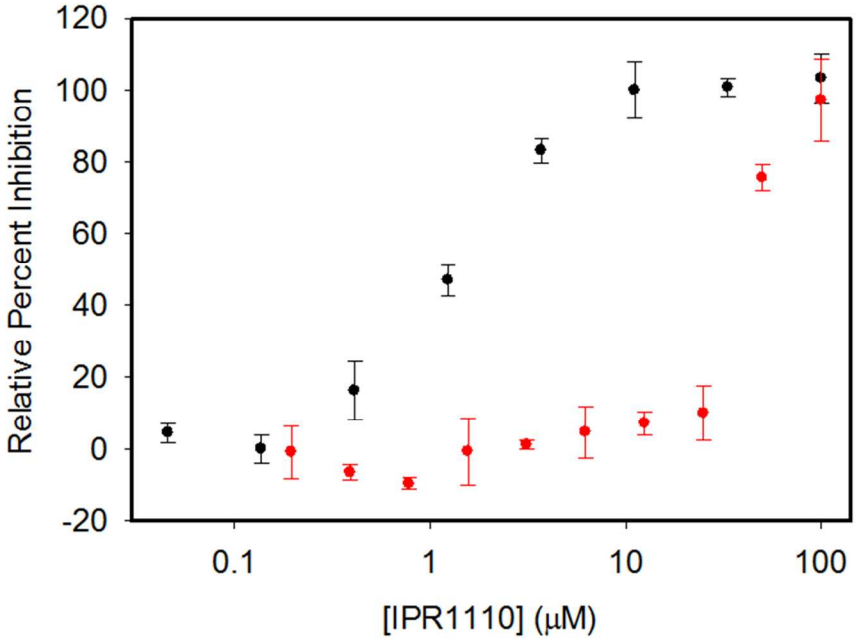


Fig. S3. a. Compound IPR-1110 (200 μM) was incubated with uPAR protein (32 μM) for 1 h in buffer PBS. Compound IPR-1110 (200 μM) was incubated with anhydrous methanol and buffer PBS for 1 h at r.t.. IPR-1110 in methanol was treated as a standard sample. After centrifuged at 18 kg for 5 min to remove precipitate, the samples were tested on Agilent 6520 Accurate Mass Q-TOF. Samples were eluted gradiently with buffer A (5 mM NH4OAc in H2O) and buffer B (5 mM NH4OAc in ACN) through Agilent Poroshell 120 EC-C8 column at a flow rate of 0.4 mL/min. b. The mass spectra extracted from the major peaks in the samples above were identical. All of the samples gave clean and similar UV spectra. These facts indicated that the compound was stable in buffer PBS in the presence or absence of uPAR protein.

Figure S4



Polarized fluorescence intensities were measured using EnVision® Multilabel plate reader (PerkinElmer) with excitation and emission wavelengths of 485 and 530 nm, respectively [24]. Thermo Scientific Nunc 384-well black microplate were used to prepare samples with a final volume of 50 µL in duplicates. First, the compounds were serially diluted in DMSO and further diluted in 1 x PBS buffer with 0.01% Triton X-100 for a final concentration of 25 µM to 0.2 µM. Triton X-100 was added in the buffer to avoid compound aggregation. 35 µL of the compound solution and 10 µL of CaV2.2 β3 solution in PBS, 0.01% Triton X-100 was added to the wells and incubated for at least 15 minutes to allow the compound to bind to the protein. Finally 5 µL of fluorescent FAM-AID peptide was added for a total volume of 50 µL in each well resulting in final CaV2.2 β3 and peptide concentrations of 125 and 160 nM respectively. The final DMSO concentration was 2%, which had no effect on the binding of the peptide to the protein. Controls included wells containing only the peptide and wells containing both protein and peptide each in quadruplicates to ensure the validity of the reaction assay. A unit of millipolarization (mP) was used for calculating percentage inhibition of the compounds.