

Enhancement of Inhibitory Activity by Combining Allosteric Inhibitors Putatively Binding to Different Allosteric Sites on Cathepsin K

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Section S1. NMR data

cassiaoccidentalin B

¹H NMR (400 MHz, CD₃OD): δ = 7.42 (1H, d, J = 7.2 Hz, H-6'), 7.40 (1H, s, H-2'), 6.92 (1H, d, J = 8.5 Hz, H-5'), 6.59 (1H, s, H-3), 6.53 (1H, s, H-6), 5.07 (1H, d, J = 8.6 Hz, sugar), 4.04 (1H, d, J = 9.7 Hz, sugar), 3.98 (1H, q, J = 1.65 Hz, sugar), 3.18 (2H, t, J = 9.5 Hz, sugar), 1.45 (3H, d, J = 6.0 Hz, sugar).

torachryson 8- β -gentiobioside

¹H NMR (400 MHz, CD₃OD): δ = 7.12 (1H, d, J = 2.2 Hz, H-7), 7.07 (1H, s, H-4), 6.87 (1H, d, J = 2.2 Hz, H-5), 5.13 (1H, d, J = 7.7 Hz, sugar), 4.38 (1H, d, J = 7.4 Hz, sugar), 4.21 (1H, d, J = 9.4 Hz, sugar), 3.90 (3H, s, H-6) 3.89-3.83 (3H, m, sugar) 3.66-3.61 (1H, m, sugar), 3.57-3.45 (6H, m, sugar), 2.60 (3H, s, H-2), 2.31 (3H, s, H-3).

pheophytin *a*

¹H NMR (400 MHz, CDCl₃): δ = 9.53 (1H, s, H-10), 9.39 (1H, s, H-5), 8.55 (1H, s, H-20), 8.00 (1H, dd, J = 11.5, 17.8 Hz, H-3¹), 6.29 (1H, dd, J = 1.4, 17.8 Hz, H-3²), 6.26 (1H, s, H-13²), 6.19 (1H, dd, J = 1.4, 11.5 Hz, H-3²), 5.13 (1H, t, J = 6.7 Hz, H-p2), 4.52-4.40 (3H, m, H-18, H-p1), 4.21 (1H, d, J = 8.8 Hz, H-17), 3.88 (3H, s, H-13⁴), 3.69 (3H, s, H-12¹), 3.69 (2H, q, J = 7.7 Hz, H-8¹), 3.40 (3H, s, H-2¹), 3.24 (3H, s, H-7¹), 2.65-2.59 (1H, m, H-17¹), 2.52-2.44 (1H, m, H-17²), 2.39-2.29 (1H, m, H-17¹), 2.23-2.15 (1H, m, H-17²), 1.80 (3H, d, J = 7.3 Hz, H-18¹), 1.69 (3H, t, J = 7.7 Hz, H-8²) 1.87-0.77 (Phytyl group, m), -1.62 (1H, s, H-NH). ¹³C NMR (CDCl₃, 125 MHz) : δ = 189.84 (C-13¹), 173.14 (C-17³), 169.83 (C-13³), 161.52 (C-16), 156.12 (C-19), 151.28 (C-6), 149.94 (C-14), 149.83 (C-9), 149.00 (C-4), 145.48 (C-8), 143.01 (C-p3), 142.29 (C-1), 138.24 (C-11), 136.79 (C-13), 136.54 (C-3), 136.51 (C-7), 132.08 (C-2), 129.39 (C-3¹), 129.29 (C-12), 122.98 (C-3²), 118.05 (C-p2), 105.56 (C-15), 104.67 (C-10), 97.80 (C-5), 93.35 (C-20), 64.96 (C-13²), 61.69 (C-p1), 53.01 (C-13⁴), 51.45 (C-17), 50.39 (C-18), 40.02 (C-p4), 39.60 (phytyl group), 37.63 (phytyl group), 37.57 (phytyl group), 37.50 (phytyl group), 36.89 (phytyl group), 33.00 (phytyl group), 32.85 (phytyl group), 31.47 (C-17²), 30.08 (C-17¹), 29.93 (phytyl group), 28.19 (phytyl group), 25.24 (phytyl group), 24.99 (phytyl group), 24.65 (phytyl group), 23.27 (C-27), 22.91 (phytyl group), 22.83 (phytyl group), 19.94 (phytyl group), 19.87 (phytyl group), 19.71 (C-12¹), 17.60 (C-8¹), 16.50 (C-p3¹), 12.31 (C-2¹), 12.28 (C-8¹), 11.46 (C-7¹).

pheophytin *b*

¹H NMR (400 MHz, CDCl₃) : δ = 11.09 (1H, s, H-7¹), 10.29 (1H, s, H-10), 9.57 (1H, s, H-5), 8.53 (1H, s, H-20), 7.97 (1H, dd, J = 11.5, 18.0 Hz, H-3¹), 6.36 (1H, d, J = 18.0 Hz, H-3²), 6.24 (1H, s, H-13²), 6.22 (1H, d, J = 11.5 Hz, H-3²) 5.15 (1H, t, J = 7.2 Hz, H-p2), 4.55-4.40 (3H, m, H-18, H-p1), 4.19 (1H, d, J = 8.6 Hz, H-17), 3.97 (1H, q, J = 7.7 Hz, H-8¹), 3.91 (3H, s, H-13⁴), 3.66 (3H, s, H-12¹), 3.37 (3H, s, H-2¹), 2.67-2.61

(1H, m, H-17¹), 2.55-2.47(1H, m, H-17²), 2.37-2.30 (1H, m, H-17¹), 2.27-2.20 (1H, m, H-17²), 1.80 (5H, m, H-18¹, H-8²), 1.93-0.75 (phytyl group, m), -1.56 (1H, s, H-NH).

pheophorbide *a*

¹H NMR (400 MHz, CDCl₃): δ = 9.48 (1H, s, H-10), 9.35 (1H, s, H-5), 8.51 (1H, s, H-20), 7.97, (1H, dd, *J* = 18.2, 11.6 Hz, H-3¹), 6.27 (1H, dd, *J* = 18.2, 1.2 Hz, H-3²), 6.25(1H, s, H-13²), 6.16 (1H, dd, *J* = 11.8, 1.2 Hz, H-3²), 4.44 (1H, q, *J* = 7.2 Hz, H-18), 4.19 (1H, d, *J* = 8.6 Hz, H-17), 3.84 (3H, s, H-13⁴), 3.66 (2H, q, *J* = 7.6 Hz, H-8¹), 3.65 (3H, s, H-12¹), 3.38 (3H, s, H-2¹), 3.21 (3H, s, H-7¹), 2.65-2.54 (4H, m, H-17¹, H-17²), 2.34-2.20 (4H, m, H-17¹, H-17²), 1.80 (3H, d, *J* = 7.2 Hz, H-18¹), 1.67 (3H, t, *J* = 7.6 Hz, H-8²), -1.63 (1H, s, H-NH).

pheophorbide *b*

¹H NMR (500 MHz, CDCl₃): δ = 10.90 (1H, s, H-7¹), 10.49 (1H, s, H-10), 9.33 (1H, s, H-5), 8.51 (1H, s, H-20), 7.87, (1H, dd, *J* = 18.3, 11.8 Hz, H-3¹), 6.28 (1H, d, *J* = 18.3 Hz, H-3²), 6.21(1H, s, H-13²), 6.16 (1H, d, *J* = 11.8 Hz, H-3²), 4.44 (1H, q, *J* = 7.4 Hz, H-18), 4.18 (1H, d, *J* = 9.3 Hz, H-17), 3.88 (3H, s, H-13⁴), 3.77 (2H, q, *J* = 7.7 Hz, H-8¹), 3.56 (3H, s, H-12¹), 3.32 (3H, s, H-2¹), 2.69-2.60 (2H, m, H-17¹, H-17²), 2.41-2.35(1H, m, H-17²), 2.29-2.21 (1H, m, H-17¹), 1.83 (3H, d, *J* = 7.4 Hz, H-18¹), 1.67 (3H, t, *J* = 7.7 Hz, H-8²), -1.73 (1H, s, H-NH). ¹³C NMR (CDCl₃, 125 MHz) : δ =189.59 (C-13¹), 187.64 (C-7¹), 177.28 (C-17³), 173.98 (C-19), 169.43 (C-13³), 159.42 (C-9), 151.08 (C-6), 150.75 (C-14), 147.03 (C-8), 143.64 (C-1), 137.97 (C-11), 137.71 (C-7), 137.14 (C-4), 132.71 (C-3), 132.47 (C-12), 132.30 (C-2), 129.74 (C-13), 128.65 (C-3¹), 123.60 (C-3²), 106.87 (C-10), 104.96 (C-15), 101.52 (C-5), 93.47 (C-20), 64.68 (C-13²), 53.16 (C-13⁴), 51.37 (C-17), 50.22 (C-18), 31.01 (C-17¹), 29.85 (C-17²), 23.25 (C-18¹), 19.43 (C-8²), 18.98 (C-8¹), 12.28 (C-12¹), 12.18 (C-2¹)

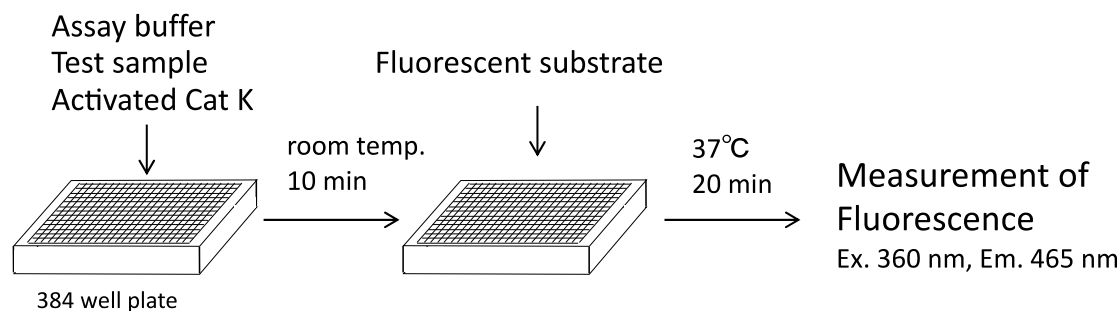


Figure S1. Overview of Cat K inhibition assay.