

Supplementary Materials

Table S1. Standard curve data of HY-071085 in rat plasma ($n=3$).

| Concentration (ng/mL) | As/Ai | | | | |
|-----------------------|---------|----------|---------|--------|--------|
| | L1 | L2 | L3 | Mean | SD |
| 1 | 0.013 | 0.012 | 0.013 | 0.013 | 0.001 |
| 2 | 0.024 | 0.023 | 0.024 | 0.024 | 0.001 |
| 5 | 0.057 | 0.057 | 0.058 | 0.057 | 0.001 |
| 20 | 0.23 | 0.23 | 0.23 | 0.23 | 0.00 |
| 50 | 0.57 | 0.56 | 0.56 | 0.56 | 0.006 |
| 200 | 2.30 | 2.37 | 2.30 | 2.32 | 0.04 |
| 500 | 5.51 | 5.59 | 5.45 | 5.52 | 0.07 |
| 1000 | 10.7 | 11.0 | 10.7 | 10.80 | 0.17 |
| a | 0.0112 | 0.0113 | 0.0111 | 0.0112 | 0.0001 |
| b | 0.00169 | 0.000574 | 0.00173 | 0.0013 | 0.0007 |
| r | 0.9997 | 0.9997 | 0.9997 | - | - |

Table S2. Recovery of HY-071085 in rat plasma.

| Concentration (ng/mL) | As/Ai | Rf | SD | As/Ai | Rc | SD | Recovery (%) |
|-----------------------|-------|-------|---------|-------|-------|--------|--------------|
| 2 | 0.022 | | | 0.028 | | | |
| | 0.023 | | | 0.026 | | | |
| | 0.023 | 0.023 | 0.00039 | 0.024 | 0.025 | 0.0016 | 90.81 |
| | 0.023 | | | 0.024 | | | |
| | 0.024 | | | 0.024 | | | |
| | 0.54 | | | 0.59 | | | |
| | 0.53 | | | 0.59 | | | |
| | 0.55 | 0.54 | 0.0079 | 0.56 | 0.59 | 0.021 | 91.05 |
| | 0.54 | | | 0.62 | | | |
| | 0.54 | | | 0.61 | | | |
| 50 | 8.18 | | | 8.80 | | | |
| | 8.57 | | | 8.85 | | | |
| | 8.35 | 8.46 | 0.18 | 8.76 | 8.89 | 0.17 | 95.16 |
| | 8.70 | | | 9.22 | | | |
| | 8.50 | | | 8.82 | | | |

Table S3. Matrix effects of HY-071085 and internal standard theophylline in rat plasma.

| Concentration (ng/mL) | ARI | As/Ai | Ac | ARI/ Ac | Mean | SD | RSD% |
|-----------------------|-------|-------|-------|---------|------|-------|------|
| 2 | 0.026 | 0.036 | | 0.98 | | | |
| | 0.026 | 0.025 | | 0.97 | | | |
| | 0.026 | 0.025 | 0.027 | 0.95 | 0.97 | 0.013 | 1.34 |
| | 0.026 | 0.025 | | 0.97 | | | |
| | 0.027 | 0.025 | | 0.98 | | | |
| 800 | 0.026 | 0.025 | | 0.95 | | | |
| | 8.63 | 8.68 | 8.49 | 1.02 | 1.04 | 0.024 | 2.29 |
| | 9.08 | 8.38 | | 1.07 | | | |

| | | |
|------|------|------|
| 8.57 | 8.55 | 1.01 |
| 8.95 | 8.51 | 1.05 |
| 9.02 | 8.33 | 1.06 |
| 8.99 | 8.56 | 1.06 |

Table S4. HY-071085 intra- and inter-batch accuracy and precision.

| Batch | Concentration (ng/mL) | Intra-day (n=5) | | | | Inter-day (n=15) | | | |
|-------|--------------------------|-----------------|-------|---------|--------------|------------------|-------|---------|--------------|
| | | Mean | SD | RSD (%) | Accuracy (%) | Mean | SD | RSD (%) | Accuracy (%) |
| 1 | 1 | 0.97 | 0.03 | 3.02 | 97.18 | 0.95 | 0.03 | 3.50 | 94.57 |
| | 2 | 1.91 | 0.04 | 1.89 | 95.30 | 1.91 | 0.07 | 3.42 | 95.73 |
| | 50 | 48.34 | 0.73 | 1.50 | 96.68 | 50.51 | 1.40 | 2.74 | 101.01 |
| | 800 | 760.4 | 16.16 | 2.12 | 95.05 | 764.47 | 13.33 | 1.74 | 95.56 |
| 2 | 1 | 0.92 | 0.02 | 2.50 | 91.78 | - | - | - | - |
| | 2 | 2.00 | 0.09 | 4.43 | 100.10 | - | - | - | - |
| | 50 | 50.62 | 1.89 | 3.73 | 101.24 | - | - | - | - |
| | 800 | 757.2 | 6.85 | 0.91 | 94.65 | - | - | - | - |
| 3 | 1 | 0.95 | 0.05 | 4.97 | 94.74 | - | - | - | - |
| | 2 | 1.84 | 0.07 | 3.95 | 91.80 | - | - | - | - |
| | 50 | 52.56 | 1.58 | 3.00 | 105.12 | - | - | - | - |
| | 800 | 775.8 | 16.98 | 2.19 | 96.98 | - | - | - | - |

Table S5. Test results of stability of HY-071085 in plasma at long-term freezing, repeated freeze-thaw, normal temperature (8h) and injector (24h) (n=5).

| Concentration (ng/mL) | Long-term freezing | | Repeated freeze-thaw | | Normal temperature (8h) | | Injector (24h) | |
|--------------------------|--------------------|--------|----------------------|--------|----------------------------|--------|----------------|--------|
| | 2 | 800 | 2 | 800 | 2 | 800 | 2 | 800 |
| Mean | 2.25 | 812.00 | 1.95 | 778.60 | 2.12 | 781.60 | 2.10 | 812.40 |
| SD | 0.10 | 26.56 | 0.03 | 14.07 | 0.08 | 18.71 | 0.08 | 15.00 |
| RSD (%) | 4.25 | 3.27 | 1.57 | 1.81 | 3.84 | 2.39 | 4.02 | 1.85 |
| Accuracy (%) | 112.40 | 101.50 | 97.40 | 97.33 | 105.90 | 97.70 | 105.00 | 101.55 |

Table S6. Comparison of physical and chemical properties between limonin and HY-071085.

| Physicochemical properties | Limonin | HY-071085 |
|----------------------------------|---------------|---------------|
| Melting point | 298°C | 138-141°C |
| Intrinsic solubility (Temp 25°C) | 0.22 mg/mL | 4.5 mg/mL |
| pKa | - | 6.11 |
| LogD (Temp 25°C) | (pH 7.0) 0.47 | (pH 7.4) 2.45 |
| logP (Temp 25°C) | 0.474 | 2.8023 |

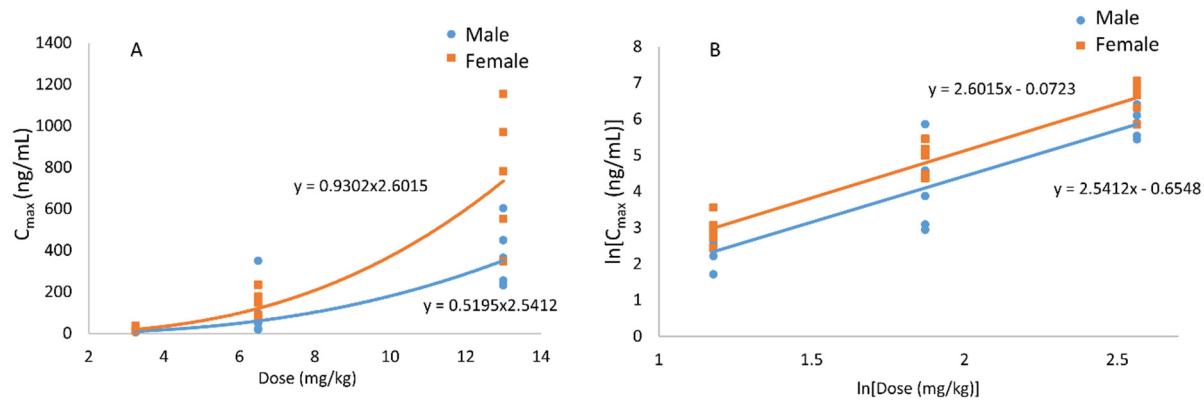


Figure S1. C_{\max} -dose curve of HY-071085 after single-dose intragastric of 3.25, 6.5 and 13 mg/kg HY-071085 in rats. (A: constant coordinates; B: double logarithmic coordinates)

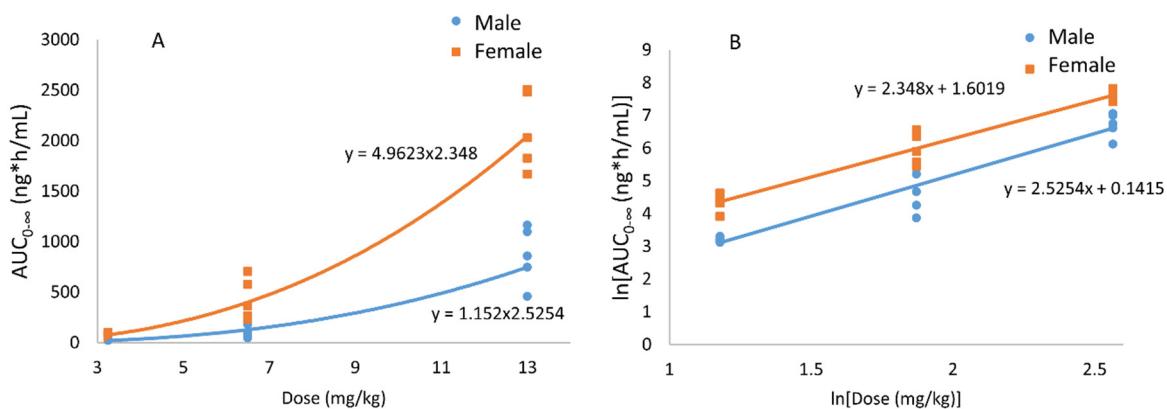


Figure S2. $AUC_{0-\infty}$ -dose curve of HY-071085 after single-dose intragastric of 3.25, 6.5 and 13 mg/kg HY-071085 in rats. (A: constant coordinates; B: double logarithmic coordinates)

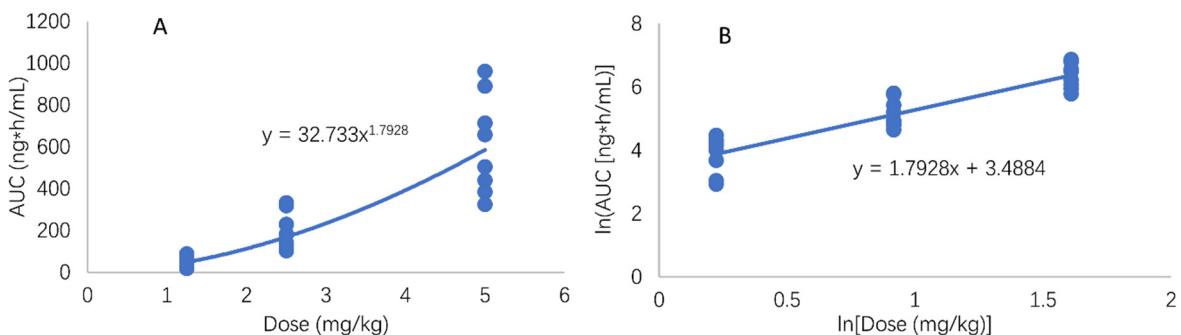


Figure S3. $AUC_{0-\infty}$ -dose curve of HY-071085 after single-dose intragastric of 1.25, 2.5 and 5 mg/kg HY-071085 in beagle dogs. (A: constant coordinates; B: double logarithmic coordinates)

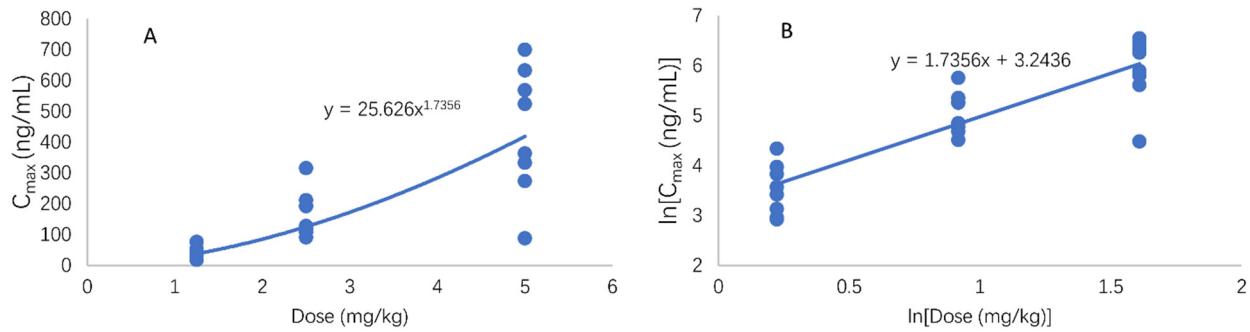


Figure S4. C_{\max} -dose curve of HY-071085 after single-dose intragastric of 1.25, 2.5 and 5 mg/kg HY-071085 in beagle dogs. (A: constant coordinates; B: double logarithmic coordinates)

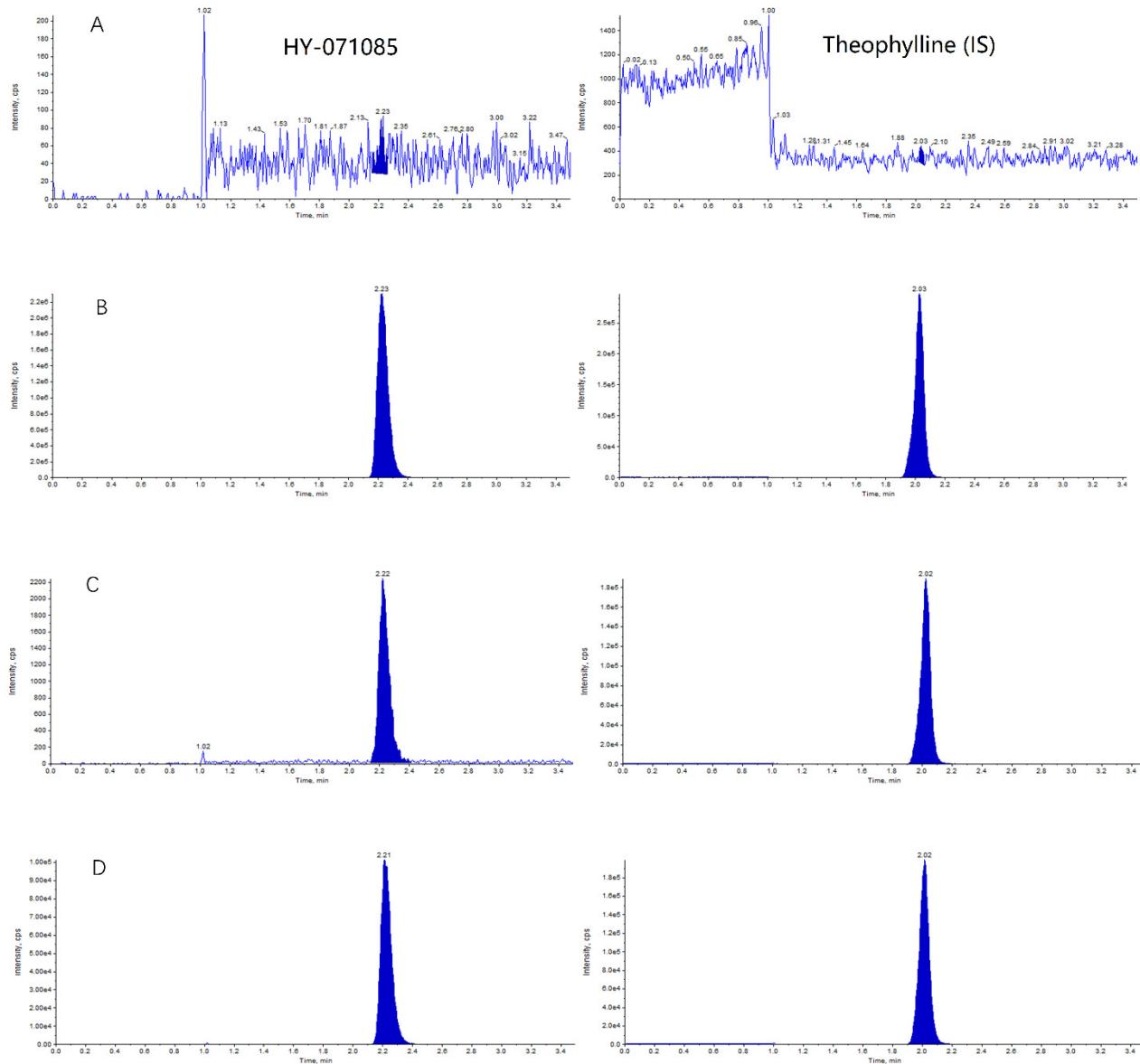


Figure S5. Specificity Chromatogram of HY-071085 in rat plasma.

(A. Rat blank plasma; B. 50 ng/mL HY-071085 and internal standard theophylline standard solution; C. After adding HY-071085 into rat blank plasma, the concentration in plasma was 1 ng/mL and internal standard theophylline; D. Plasma samples of rats after intragastric administration of 6.5 mg/kg HY-071085 for 5 min)