

SUPPLEMENTAL MATERIAL

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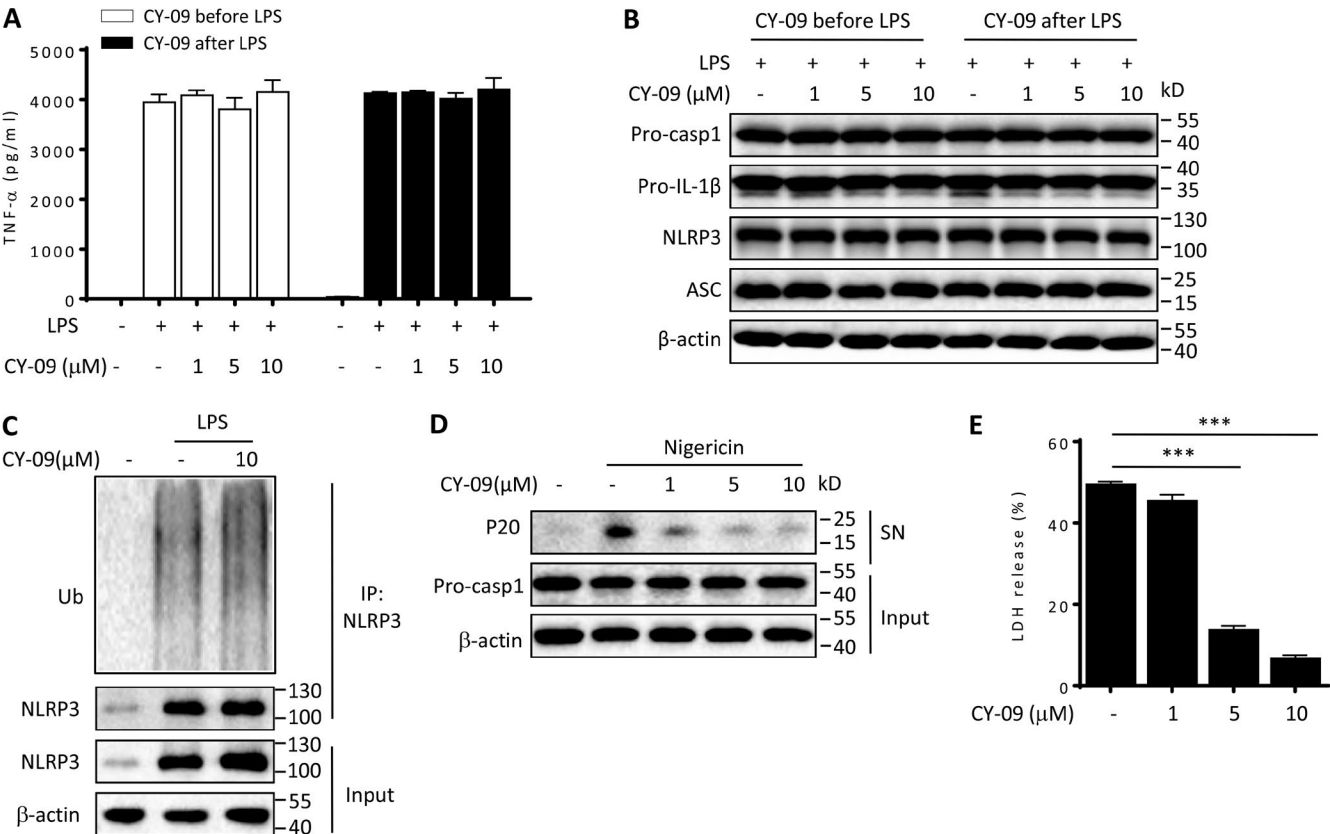


Figure S1. **Effects of CY-09 on LPS-induced priming or nigericin-induced cell death.** (A) ELISA of TNF-α in culture supernatants of BMDMs treated with LPS for 3 h and stimulated with different doses of CY-09 for 30 min (CY-09 after LPS) or BMDMs treated with different doses of CY-09 for 30 min and then stimulated with LPS for 3 h (CY-09 before LPS). (B) Immunoblot analysis of the indicated proteins in lysates from BMDMs treated with LPS for 3 h and stimulated with different doses of CY-09 for 30 min (CY-09 after LPS) or BMDMs treated with different doses of CY-09 for 30 min and then stimulated with LPS for 3 h (CY-09 before LPS). (C) Immunoblot analysis of NLRP3 ubiquitination in BMDMs treated with CY-09 (10 μM) and then stimulated with LPS for 3 h. (D) Immunoblot analysis of caspase-1 cleavage in supernatants of BMDMs treated with CY-09 just before nigericin stimulation. (E) Assay for LDH release in the culture supernatants of LPS-primed BMDMs treated with different doses of CY-09 and then stimulated with nigericin. Data are from three independent experiments with biological duplicates in each (A and E; mean and SEM of $n = 6$) or are representative of three independent experiments (B–D). Statistics were analyzed using an unpaired Student's t test: ***, $P < 0.001$.

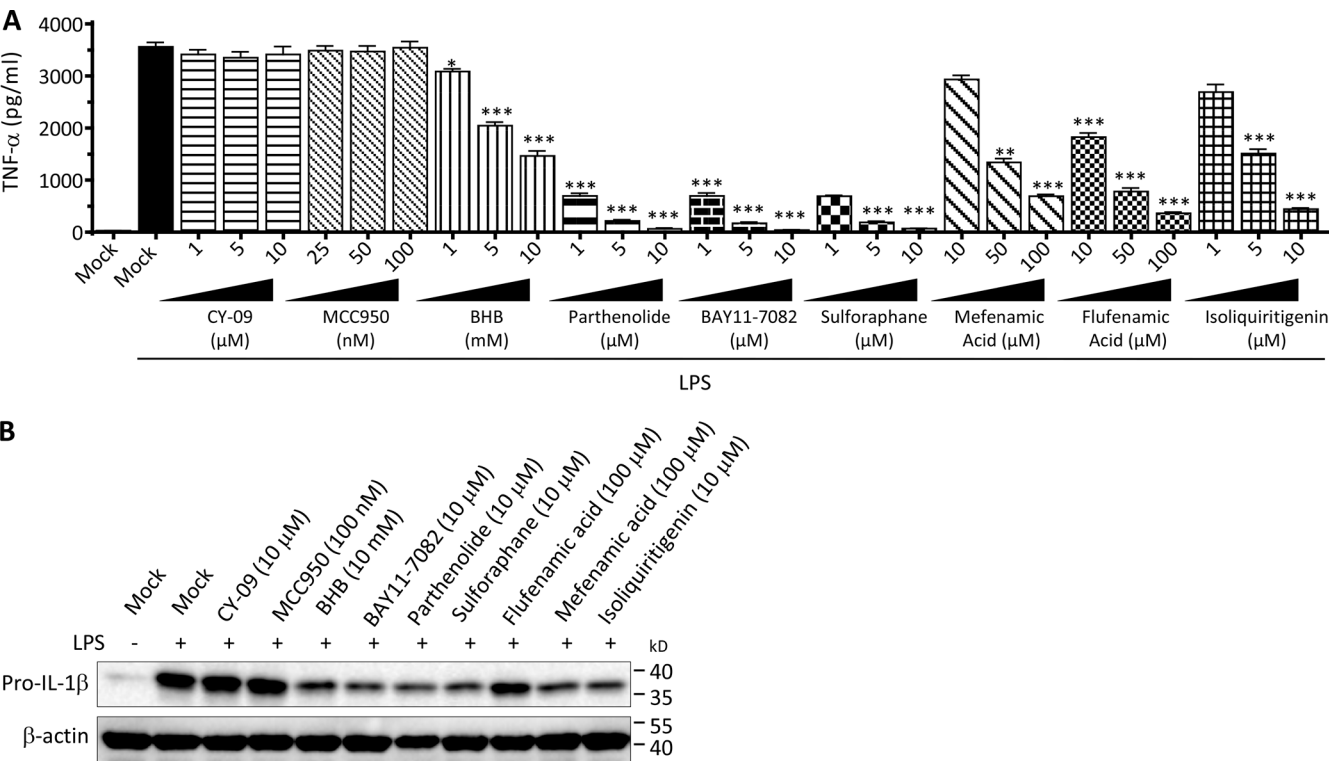


Figure S2. **Role of inhibitors on LPS-induced priming.** (A) ELISA of TNF- α in supernatants from BMDMs treated with various doses of indicated inhibitors for 30 min and then stimulated with LPS for 3 h. (B) Immunoblot analysis of pro-IL-1 β expression in BMDMs treated with indicated inhibitors for 30 min and then stimulated with LPS for 3 h. Data are from three independent experiments with biological duplicates in each (A; mean and SEM of $n = 6$) or are representative of three independent experiments (B). Statistics were analyzed using an unpaired Student's t test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

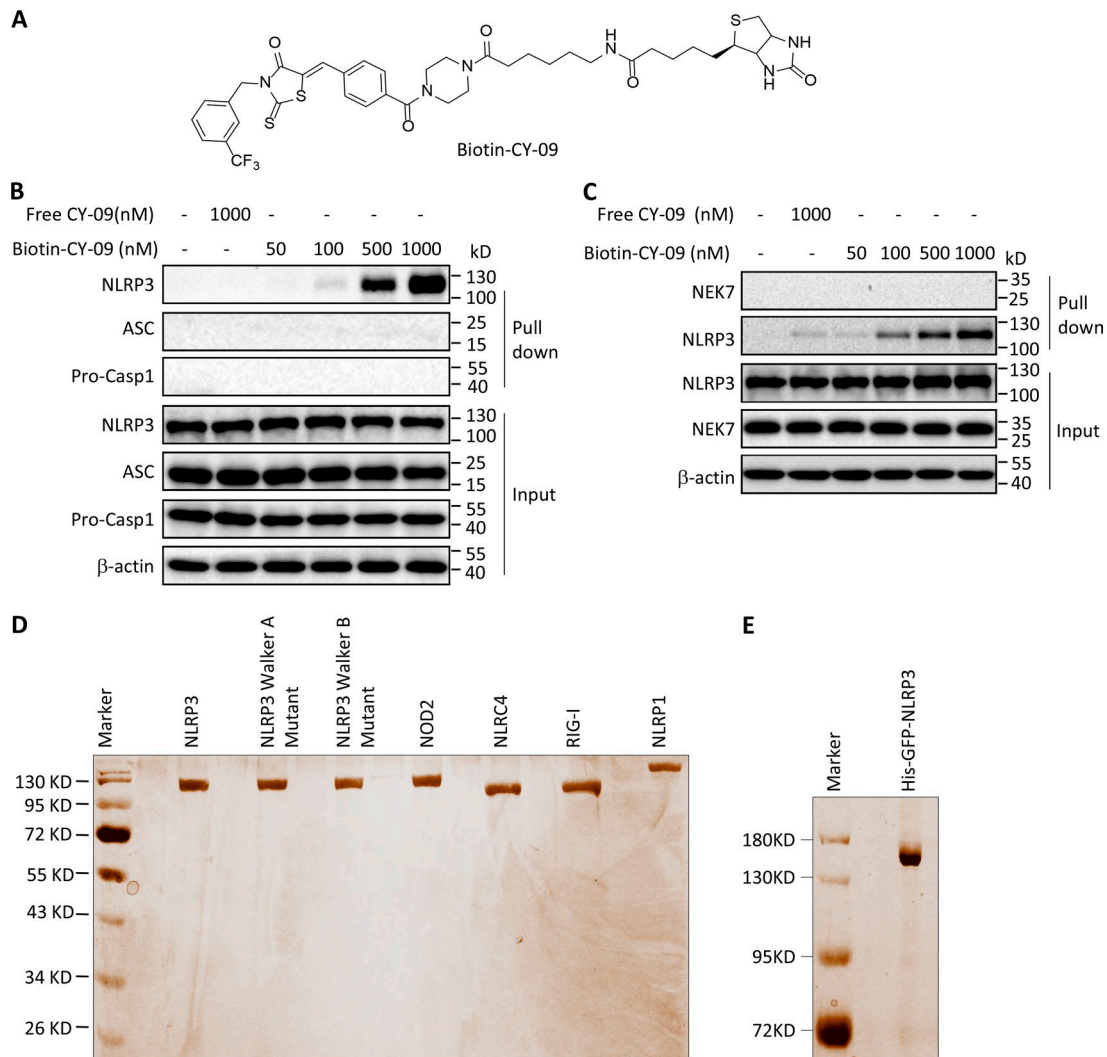


Figure S3. **The binding of CY-09 with NLRP3.** (A) Structure of biotin-CY-09. (B) Cell lysates of PMA-differentiated THP-1 cells were incubated with different concentrations of biotin-CY-09, which were then pulled down with streptavidin beads. (C) Cell lysates of LPS-primed BMDMs were incubated with different concentrations of biotin-CY-09, which were then pulled down with streptavidin beads. (D) Silver staining of the indicated purified Flag-tagged proteins. (E) Silver staining of the indicated purified His-GFP-NLRP3 protein.

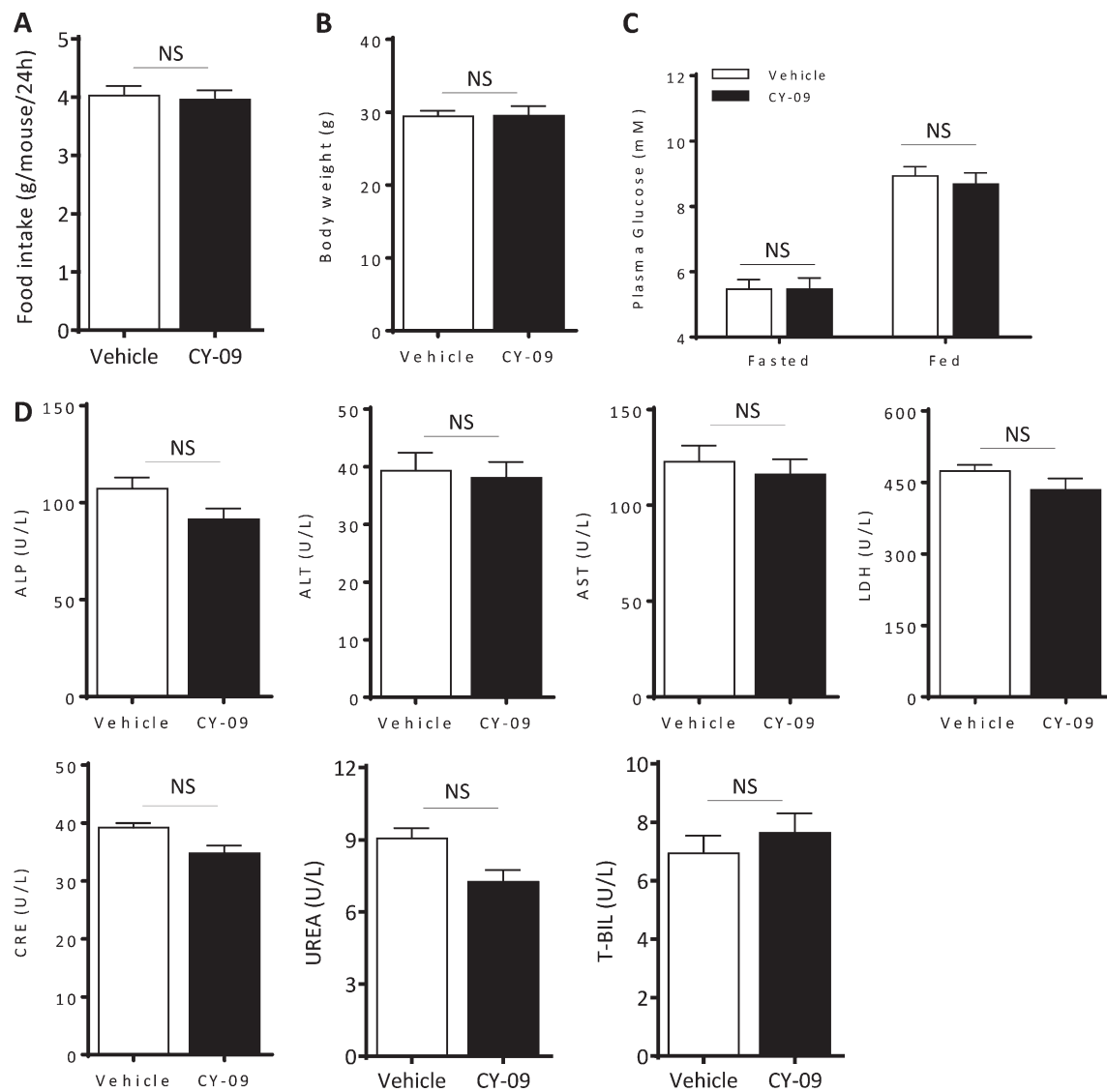


Figure S4. **Long-term CY-09 treatment has no effects on the metabolic parameters and serum chemistry of normal lean mice.** (A–C) Food intake, body weight, and plasma glucose of C57BL/6J mice which were treated with CY-09 once a day at a dose of 2.5 mg/kg for 9 wk; mean and SEM of $n = 6$. (D) Qualification of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine, urea, and total bilirubin (T-BIL) in the serum of C57BL/6J mice that were treated with CY-09 once a day at the dose of 2.5 mg/kg for 9 wk; mean and SEM of $n = 5$. Statistics were analyzed using an unpaired Student's t test.

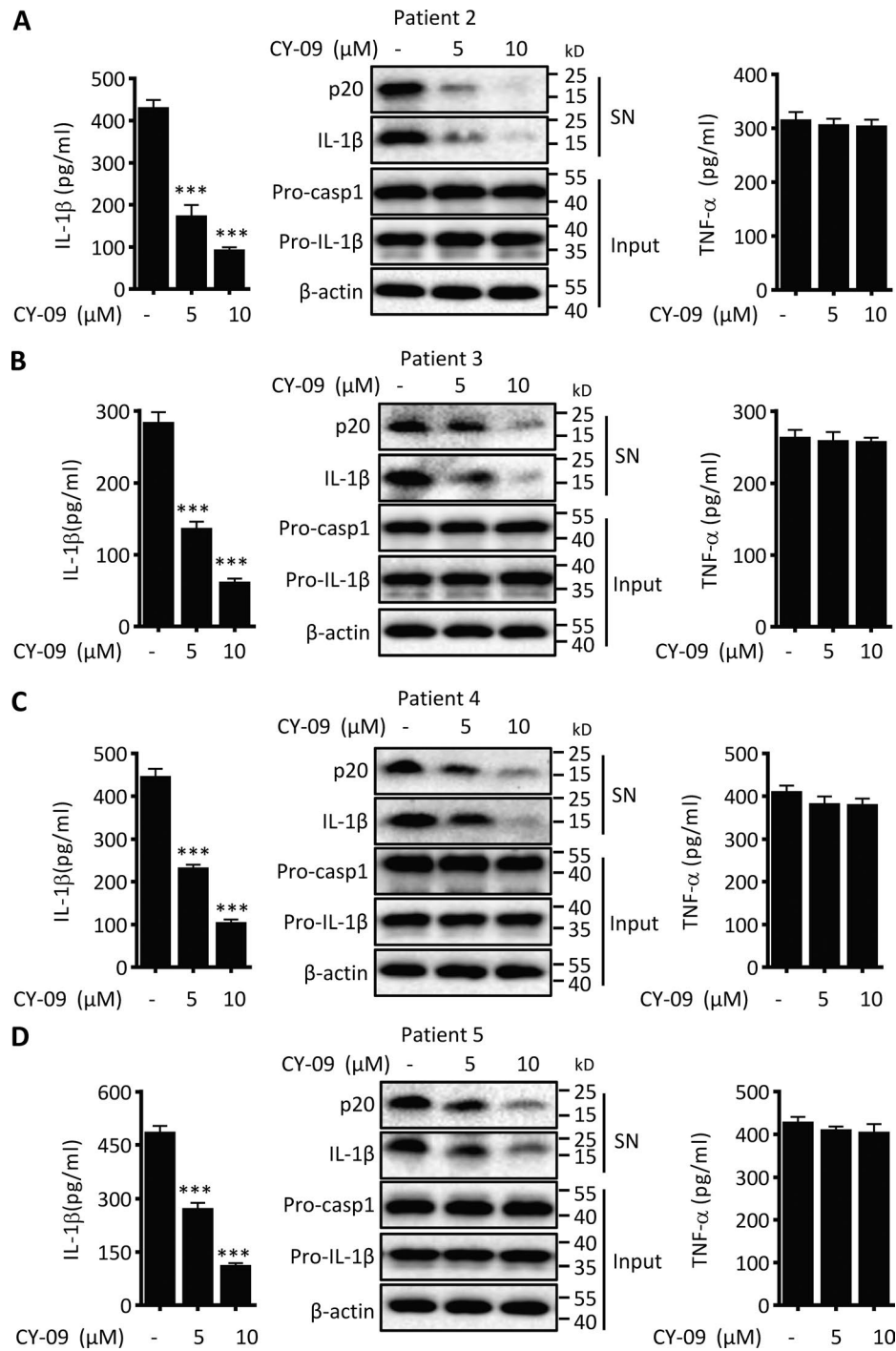


Figure S5. **Role of CY-09 on the NLRP3 inflammasome activation in SFCs from patients with gout.** (A–D) ELISA of IL-1 β , TNF- α , or immunoblot analysis of IL-1 β and cleaved caspase-1 (p20) in supernatants (SN) from SFCs isolated from four individuals with gout, treated with various doses of CY-09 for 20 h. Data are from biological triplicates in each (mean and SEM of $n = 3$). Statistics were analyzed using an unpaired Student's t test: ***, $P < 0.001$.

Table S1. Microsomal stability of CY-09

Compound name	Human liver microsomes		Mouse liver microsomes	
	$t_{1/2}$	CL ^{hep}	$t_{1/2}$	CL ^{hep}
	<i>min</i>	<i>ml/min/kg</i>	<i>min</i>	<i>ml/min/kg</i>
CY-09	>145	<4.7	>145	<20.7
Testosterone	14.9	15.5	2.1	85.9
Propranolol	57.8	8.9	11.5	71.2
Clozapine	77.9	7.4	21.5	60.2

CL^{hep}, hepatic clearance. Testosterone, propranolol, and clozapine were used as control.

Table S2. Effect of CY-09 on CYP

CYP	Metabolite	Reference inhibitor	CY-09
		IC ₅₀	IC ₅₀
		μM	μM
1A2	Acetaminophen	α -Naphthoflavone 0.269	18.9
2C9	4'-hydroxy diclofenac	Sulfaphenazole 0.541	8.18
2C19	4'-hydroxy mephenytoin	(+)-N-3-benzylirinanol 0.296	>50
2D6	Dextrorphan	Quinidine 0.124	>50
3A4	1'-hydroxy midazolam	Ketoconazole 0.0332	26.0

Table S3. Effect of CY-09 on the hERG channel

Sample	Concentration	Inhibition	SE
	μM	%	
Amitriptyline (control)	1	21.52	5.07
	3	53.83	0.41
	10	83.52	2.09
CY-09	1	-1.77	3.93
	3	-3.38	1.05
	10	-3.94	1.50

Table S4. Pharmacokinetic properties of CY-09 in mice

Route	Dose	$t_{1/2}$	t_{max}	C_{max}	C_0	AUC _(0-t)	AUC _(0-∞)	V _z	Cl	MRT _(0-∞)	F
	<i>mg/kg</i>	<i>h</i>	<i>h</i>	<i>ng/ml</i>	<i>ng/ml</i>	<i>ng·h/ml</i>	<i>ng·h/ml</i>	<i>liter/kg</i>	<i>ml/kg·min</i>	<i>h</i>	%
i.v.	5	1.8	0.08	10,110	12,447	5,717	5,722	0.46	14.6	0.53	—
PO	10	5.1	0.50	3,253	—	8,184	8,232	—	—	2.4	72

AUC, area under the curve (measure of exposure); Cl, plasma clearance; C_{max} , maximum plasma concentration; F, oral bioavailability; MRT, mean residence time; PO, oral delivery; t_{max} , time of maximum plasma concentration; V_z, volume of distribution.