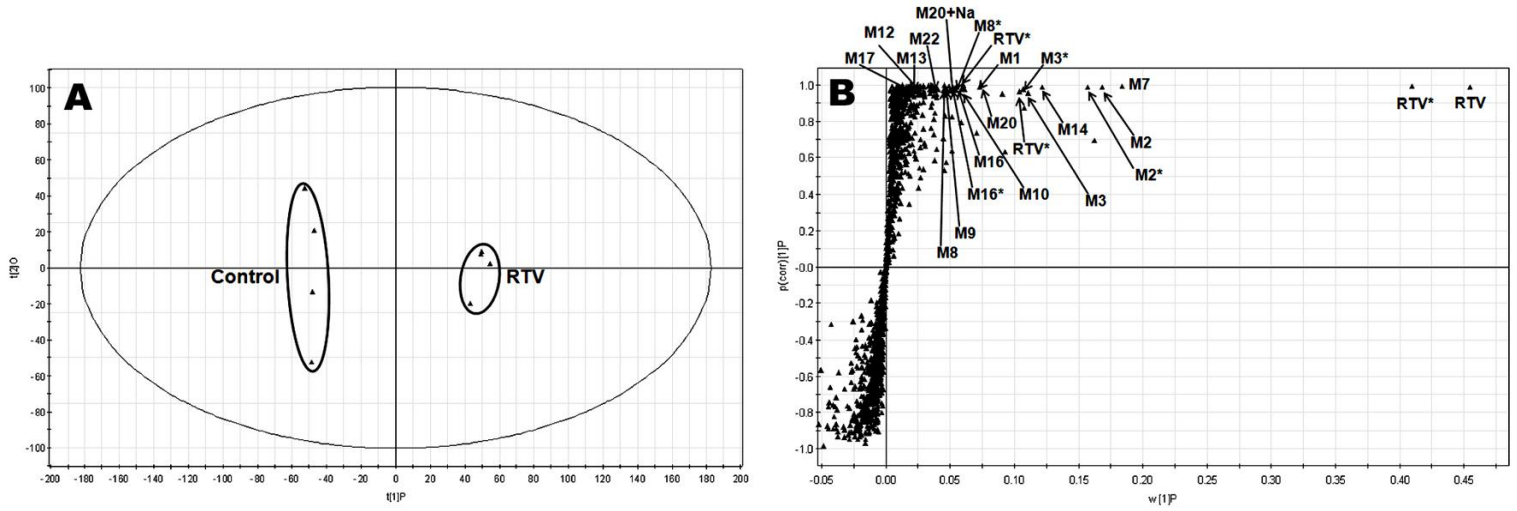
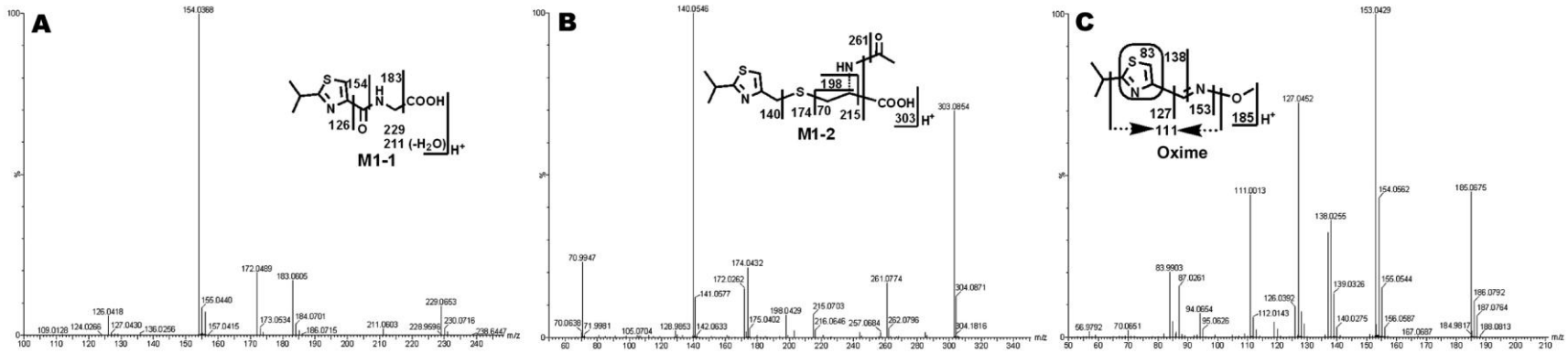
Supplemental Table 1. Summary of RTV metabolites. WT mice were treated with 25 mg/kg RTV. The feces and urine were collected 18 h after RTV treatment. All samples were analyzed by UPLC-TOFMS.

Metabolite ID	Observed m/z [M+H]	Calculated m/z [M+H]	Mass error (ppm)	Predicted molecular formula
M1	582.2733	582.2750	-2.9	$C_{30}H_{40}N_5O_5S$
M1-1	229.0651	229.0647	1.7	$C_9H_{13}N_2O_3S$
M1-2	303.0858	303.0837	6.9	$C_{12}H_{19}N_2O_3S_2$
M2	737.3159	737.3155	0.5	$C_{37}H_{49}N_6O_6S_2$
M3	737.3157	737.3155	0.3	$C_{37}H_{49}N_6O_6S_2$
M4	737.3150	737.3155	-0.7	$C_{37}H_{49}N_6O_6S_2$
M5	737.3156	737.3155	0.1	$C_{37}H_{49}N_6O_6S_2$
M6	737.3159	737.3155	0.5	$C_{37}H_{49}N_6O_6S_2$
M7	580.3315	580.3321	-1.0	$C_{32}H_{46}N_5O_3S$
M7-1	187.0189	187.0177	6.4	$C_6H_7N_2O_3S$
M7-2	261.0376	261.0368	3.1	$C_9H_{13}N_2O_3S_2$
M8	707.3052	707.3049	0.4	$C_{36}H_{47}N_6O_5S_2$
M9	719.3044	719.3049	-0.7	$C_{37}H_{47}N_6O_5S_2$
M10	709.3370	709.3383	-1.8	$C_{36}H_{49}N_6O_7S$
M11	897.3539	897.3527	1.3	$C_{43}H_{57}N_6O_{11}S_2$
M12	694.3277	694.3274	0.4	$C_{36}H_{48}N_5O_7S$
M13	650.2652	650.2648	0.6	$C_{33}H_{40}N_5O_7S$
M14	596.3276	596.3271	0.8	$C_{32}H_{46}N_5O_4S$
M15	913.3483	913.3476	0.8	$C_{43}H_{57}N_6O_{12}S_2$
M16	723.2985	723.2999	-1.9	$C_{36}H_{47}N_6O_6S_2$
M17	710.3243	710.3224	2.7	$C_{36}H_{48}N_6O_8S$
M18	753.3119	753.3104	2.0	$C_{37}H_{49}N_6O_7S_2$
M19	753.3100	753.3104	-0.5	$C_{37}H_{49}N_6O_7S_2$
M20	753.3096	753.3104	-1.1	$C_{37}H_{49}N_6O_7S_2$
M21	612.3220	612.3220	0	$C_{32}H_{46}N_5O_5S$
M22	751.2942	751.2948	-0.6	$C_{37}H_{47}N_6O_7S_2$

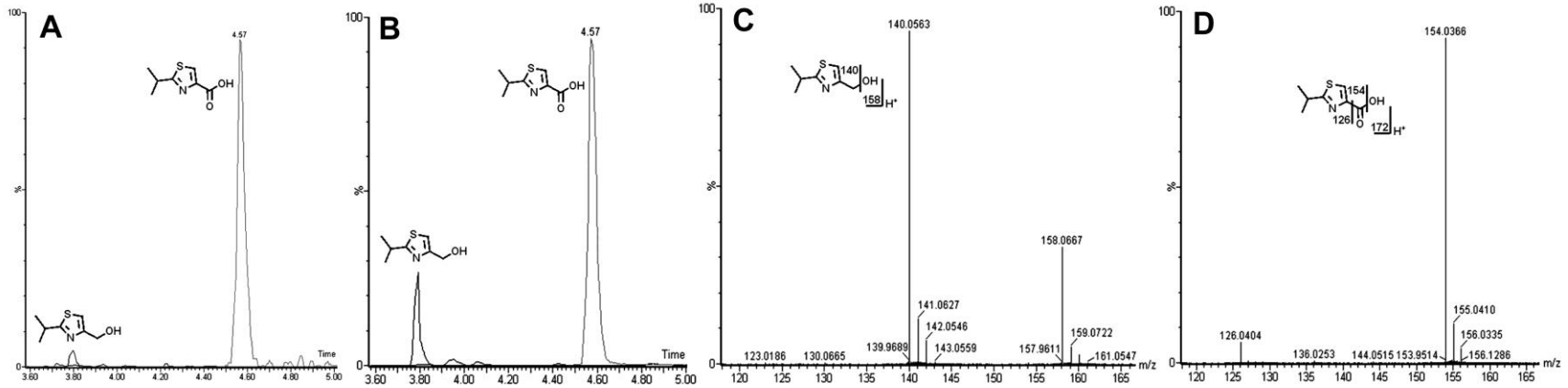
Supplemental Figure 1. Metabolomic analysis of mouse feces from the control group and RTV-treated group. WT mice (n = 4) were treated with vehicle or 25 mg/kg RTV (*p.o.*). The feces were collected 18 hours after RTV treatment. All samples were analyzed by UPLC-TOFMS. (A) Separation of control group and RTV-treated group in an OPLS-DA score plot. The t[1]P and t[2]O values represent the score of each sample in principal component 1 and 2, respectively. (B) Loading S-plot generated by OPLS-DA analysis. The *X*-axis is a measure of the relative abundance of ions and the *Y*-axis is a measure of the correlation of each ion to the model. The matrix data were processed from *m/z* 500 to 1000. The top ranking ions are labeled. *, in-source fragment; +Na, sodium adduct.



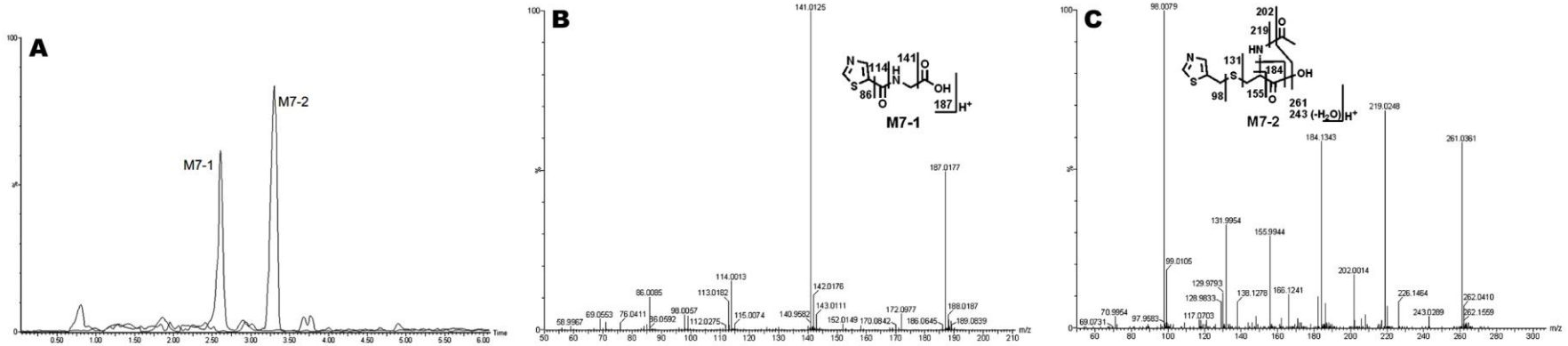
Supplemental Figure 2. Identification of M1-1 and M1-2. M1-1 and M1-2 were detected in the urine of mice treated with RTV. In addition, the incubation of RTV in HLM was conducted to trap 2-isopropylthiazole-4-carbaldehyde, a precursor of M1-1 and M1-2. Methoxylamine was used as a trapping reagent. Structural elucidations were performed on the basis of accurate mass measurement and MS/MS fragmentations. Major daughter ions were interpreted in the inlaid structural diagrams. (A) MS/MS of M1-1. (B) MS/MS of M1-2. (C) MS/MS of methoxylamine trapped product of 2-isopropylthiazole-4-carbaldehyde.



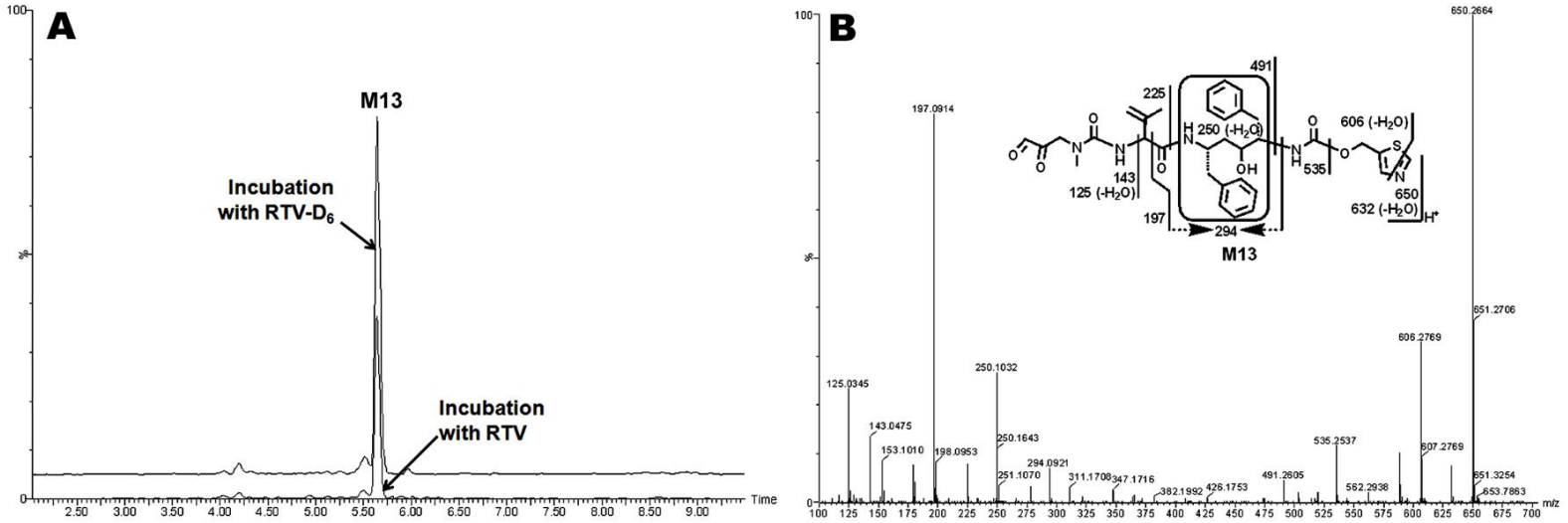
Supplemental Figure 3. Identification of (2-isopropylthiazol-4-yl)methanol and 2-isopropylthiazole-4-carboxylic acid. WT mice were treated with RTV (25 mg/kg) or 2-isopropylthiazole-4-carbaldehyde (10 mg/kg). The urine samples were collected 18 h after the treatment. All samples were analyzed by UPLC-TOFMS. Structural elucidation was performed by accurate mass and MS/MS fragments. (A) Extracted chromatograms of (2-isopropylthiazol-4-yl)methanol and 2-isopropylthiazole-4-carboxylic acid from RTV treated mouse urine. (B) Extracted chromatograms of (2-isopropylthiazol-4-yl)methanol and 2-isopropylthiazole-4-carboxylic acid from 2-isopropylthiazole-4-carbaldehyde treated mouse urine. (C) MS/MS of (2-isopropylthiazol-4-yl)methanol. (D) MS/MS of 2-isopropylthiazole-4-carboxylic acid.



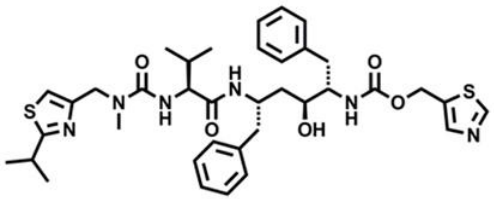
Supplemental Figure 4. Identification of M7-1 and M7-2. M7-1 and M7-2 were detected in the urine of mice treated with RTV. Structural elucidations were performed on the basis of accurate mass measurement and MS/MS fragmentations. (A) Extracted chromatograms of M7-1 and M7-2. (B) MS/MS of M7-1. (C) MS/MS of M7-2.



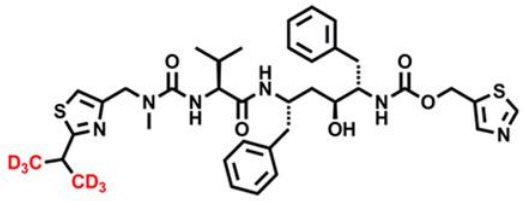
Supplemental Figure 5. Identification of isopropylthiazole ring-open metabolite M13. Metabolite M13 was recapitulated in the incubations of RTV or RTV-D_6 in HLM. Structural elucidations were based on accurate mass measurement and MS/MS fragmentations. (A) Extracted chromatograms of M13 generated from the incubations of RTV and RTV-D_6 in HLM. (B) MS/MS of M13.



Supplemental Scheme 1. The structures of RTV and RTV-D_6

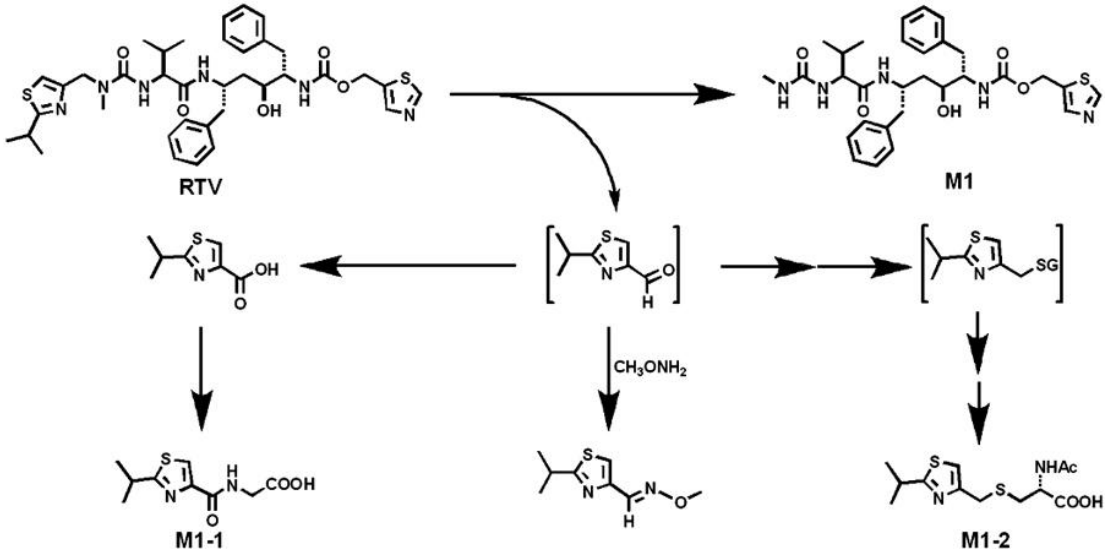


RTV



RTV-D_6

Supplemental Scheme 2. Possible mechanism of M1-1 and M1-2 formation. 2-Isopropylthiazole-4-carbaldehyde is generated from RTV spontaneously with M1, and it is trapped by methoxylamine (CH_3ONH_2). 2-Isopropylthiazole-4-carbaldehyde is oxidized to an acid and conjugated with glycine to form M1-1. In the mean time, 2-isopropylthiazole-4-carbaldehyde is reduced to an alcohol that can be further sulfated. Sulfate anion serves as a leaving group that will be attacked by GSH. The GSH-conjugated metabolite is degraded to M1-2.



Supplemental Scheme 3. Possible mechanism of M12 and M17 formation. The thiazole ring undergoes epoxidation of the C=C double bond to form epoxide. The resulting diol is then decomposed to form corresponding α-keto aldehydes (M12 and 17) and methanethioamide. M2 is the most abundant RTV metabolite in humans (*11*). The formation of M17 is initiated from M2.