Drug Metabolism and Disposition

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Supplemental Materials

Preclinical metabolism and disposition of TP0473292, a novel oral prodrug of the potent metabotropic glutamate 2/3 receptor antagonist TP0178894 for the treatment of depression

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Supplemental Table S1. Overview of experimental conditions for liquid chromatography-tandem mass spectrometry (LC-MS/MS), liquid chromatography-mass spectrometry (LC-MS), and a high-performance liquid chromatograph equipped with a radiochemical flow detector (Radio-HPLC).

		Conditions
	For PAMPA, plasma protein binding study, hydrolytic studies in tissue S9 fractions and sera, reaction phenotyping study, pharmacokinetic study of TP0178894 in monkeys, and tissue distribution study in rats (LC-MS/MS)	
A	**[Column]**	Shim-pack XR-ODS (2.2 μm, 30 mm × 3.0 mm I.D.; Shimadzu, Kyoto, Japan). Column temperature: 50°C.
	[Mobile phase]	(A) 0.1% v/v formic acid and (B) acetonitrile. Flow rate: 1.3 mL/min.
	[Detection]	TripleQuad™ 5500, TripleQuad™ 6500 or API4000™ (AB Sciex, Framingham, MA) with the TurboIonSpray ionization mode in the negative ion detection mode. TP0473292 (*m/z* 532→179) and [$^2H_4$] TP0473292[a] (*m/z* 536→179), TP0178894 (*m/z* 326→136) and [$^2H_4$] TP0178894[a] (*m/z* 330→136), TP0178894 (m/z 326→92) and TP0181164[b] (m/z 376→136) (only for tissue distribution study in rats).
	For metabolic profiling of [^{3}H]TP0473292 in hepatocytes (Radio-HPLC and LC-MS)	
B	**[Column]**	Sunfire™ C18 (5 μm, 150 mm × 4.6 mm I.D.; Waters, Milford, MA). Column temperature: 40°C.
	[Mobile phase]	(A) 10 mM ammonium acetate and (B) acetonitrile. Flow rate: 1.0 mL/min.
	[Detection]	Radiomatic 625TR (PerkinElmer, Waltham, MA) with liquid scintillator (Flo-Scint™ II, PerkinElmer) at 3 mL/min. Orbitrap Elite (Thermo Fisher Scientific, Waltham, MA) with the heated electrospray ionization mode in the negative ion detection mode.
	For chemical stability study of acyl glucuronide (LC-MS/MS)	
C	**[Column]**	Shim-pack XR-ODS (2.2 μm, 30 mm × 3.0 mm I.D.). Column temperature: 50°C.
	[Mobile phase]	(A) 1 mM ammonium acetate (pH 5.0) and (B) acetonitrile. Flow rate: 1.3 mL/min.
	[Detection]	TripleQuad™ 5500 (AB Sciex) with the TurboIonSpray ionization mode in the negative ion detection mode. Adamantane carboxylic acid acyl glucuronide (*m/z* 355→179, *m/z* 355→337). Ibuprofen acyl glucuronide (*m/z* 381→205, *m/z* 381→363). Diclofenac acyl glucuronide (*m/z* 470→294, *m/z* 470→452).
	For pharmacokinetic analysis of TP0473292 and TP0178894 in rats and pharmacokinetic analysis of TP0473292 in monkeys (LC-MS/MS)	
D	**[Column]**	Atlantis T3 (3 μm, 50 mm × 4.6 mm I.D.; Waters). Column temperature: 40°C.
	[Mobile phase]	(A) 0.01% v/v ammonium acetate and (B) acetonitrile. Flow rate: 1.0 mL/min.
	[Detection]	TripleQuad™ 5500 (AB Sciex) with the TurboIonSpray ionization mode in the negative ion detection mode. TP0473292 (*m/z* 532→254) and [$^2H_4$] TP0473292[a] (*m/z* 536→254), TP0178894 (*m/z* 326→136) and [$^2H_4$] TP0178894[a] (*m/z* 330→136), ACA (*m/z* 179→179) and [$^2H_{15}$] ACA[a] (*m/z* 194→194), ACA-AG (*m/z* 355→179) and [$^2H_{15}$] ACA-AG[a] (*m/z* 370→194).

ACA, adamantane carboxylic acid; ACA-AG, adamantane-1-carboxylic acid acyl glucuronide

[a]Internal standard

[b]Structural analog of TP0178894 used as an internal standard

Supplemental Table S2. LC-MS data and proposed product ions of TP0473292, TP0178894, ACA, and ACA-AG observed in the 1-hour incubation mixture of rat, monkey, and human cryopreserved hepatocytes with TP0473292.

Metabolite	Retention time (min)	$[M+H]^+$	Characteristic product ions (*m/z*)	Description of the product ions	Samples
Unchanged (TP0473292)	47.27	532	179 254	loss of $C_{17}H_{17}O_5F_2$ loss of $C_{14}H_{11}O_5F$	R
TP0178894	13.94	326	136 262 282	loss of $C_8H_8O_3F_2$ loss of CHO_2F loss of CO_2	R, M, H
ACA	27.86	179	-	Fragment ion was not observed in a MS^2 spectrum	R, M, H
ACA-AG	24.60	355	113 179	loss of $C_{12}H_{18}O_5$ loss of $C_6H_8O_6$	R, M, H

R: incubation mixture of rat hepatocytes with TP0473292

M: incubation mixture of monkey hepatocytes with TP0473292

H: incubation mixture of human hepatocytes with TP0473292

Supplemental Table S3. Inhibitory effect of TP0473292, TP0178894, ACA and ACA-AG on the specific activities of cytochrome P450 (CYP) isoforms in human liver microsome in both reversible and time-dependent manner

CYP isoform	Probe substrate	Reversible inhibition[a] (%) at 10 μM				Time-dependent inhibition[b] (%) at 10 μM			
		TP0473292	TP0178894	ACA	ACA-AG	TP0473292	TP0178894	ACA	ACA-AG
CYP1A2	Phenacetin	7.6	8.3	7.9	4.0	−0.7	−4.9	−15.6	8.7
CYP2B6	Bupropion	−7.5	6.9	8.3	11.7	1.7	1.1	−1.2	1.3
CYP2C8	Amodiaquine	5.3	0.7	−2.5	1.4	−2.9	−3.2	7.3	−1.3
CYP2C9	Diclofenac	12.1	15.1	12.9	5.6	−5.0	−0.7	−5.1	3.2
CYP2C19	(*S*)-Mephenytoin	5.0	5.0	8.3	1.7	−2.6	−11.5	−4.4	−9.5
CYP2D6	Bufuralol	1.1	−3.7	0.3	−4.0	−2.1	−2.5	−1.0	0.0
CYP3A	Midazolam	2.9	3.6	2.9	3.6	−5.3	2.1	−5.8	−2.0
CYP3A	Testosterone	1.1	5.7	3.1	−1.9	0.8	6.7	2.5	−1.3

[a]Each value represents the mean of triplicate determinations.

[b]Time-dependent inhibition was indicated as the difference in the percent inhibition between with and without the first 30-min incubation with test compound.

Supplemental Table S4. Effect of TP0473292 and TP0178894 on CYP1A2, CYP2B6, and CYP3A4 mRNA expression levels at 10 μM in the primary cultured cryopreserved human hepatocytes.

Hepatocyte Lot number	Compound	Fold change of mRNA expression level		
		CYP1A2	CYP2B6	CYP3A4
1	TP0473292	0.903	0.800	0.891
	TP0178894	0.866	0.855	1.20
	Positive control[a]	> 16.7	9.90	171
2	TP0473292	0.901	0.774	0.772
	TP0178894	0.877	0.749	1.13
	Positive control[a]	> 11.5	22.3	> 102
3	TP0473292	0.916	0.846	1.01
	TP0178894	0.998	0.903	0.929
	Positive control[a]	9.81	8.22	43.5

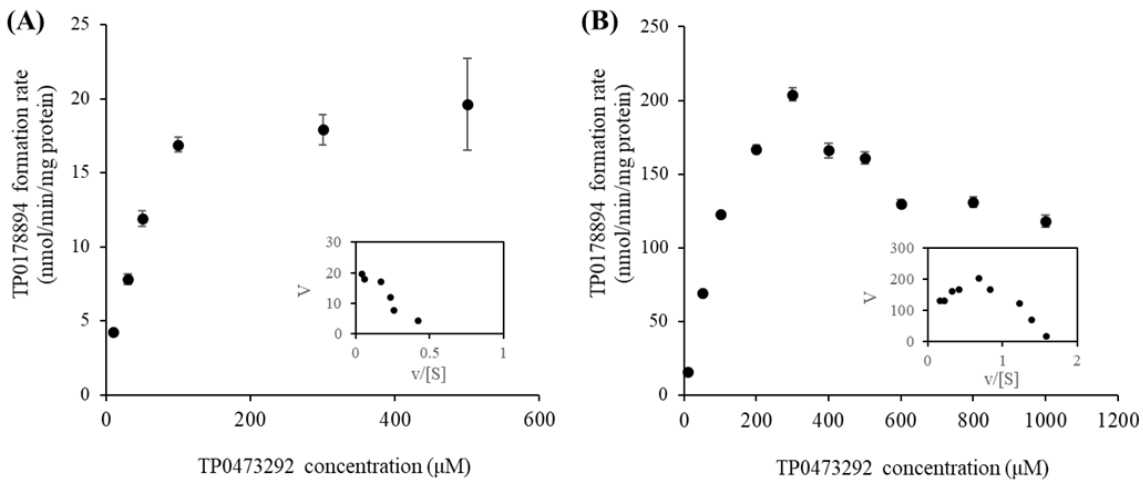
Data are presented as the mean of triplicate determinations.

TP0473292 and TP0178894 did not show detectable toxicity to the cultured human hepatocytes at 10 μM.

[a]Positive controls are omeprazole[b] (50 μM) for CYP1A2, phenobarbital[b] (1000 μM) for CYP2B6, and rifampicin[c] (10 μM) for CYP3A4.

[b]Supplied by FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan)

[c]Supplied by Merck KGaA (Darmstadt, Germany)



Supplemental Figure S1

TP0178894 formation rate versus TP0473292 concentration plots in the intestinal (A) and liver (B) S9 fractions. Data are presented as the mean ± S.D. of triplicate determinations. Insets show Eadie-Hofstee plots.