Test Article: [^{14}C]tasurgratinib

Rats **Study Number: 8362069** **Location in CTD: 4.2.2.2.5**

Concentrations of Radioactivity in the Tissues of Male Albino Rats Following a Single Oral Administration of [^{14}C]Tasurgratinib							
Tissue/Organ	**Concentration of Radioactivity (ng eq./g)**						
	0.5 h	**2 h**	**5 h**	**24 h**	**48 h**	**72 h**	**168 h**
Liver	954	259	1750	31.8	27.9	34.4	BLQ
Lung	66.4	58.1	301	BLQ	BLQ	17.4	BLQ
Mandibular lymph nodes	36.0	16.7	307	BLQ	BLQ	BLQ	RND
Meninges	16.5	26.2	145	RND	RND	RND	RND
Muscle	33.5	19.4	103	BLQ	BLQ	BLQ	RND
Myocardium	77.7	43.2	373	BLQ	BLQ	BLQ	RND
Nasal mucosa	BLQ	BLQ	72.4	BLQ	BLQ	BLQ	RND
Pancreas	156	93.0	731	21.9	BLQ	BLQ	BLQ
Periodontal membrane	57.2	41.0	196	BLQ	BLQ	RND	RND
Pineal body	119	56.3	696	13.7	BLQ	RND	RND
Pituitary	145	96.3	1190	49.8	56.2	36.7	RND
Preputial gland	86.3	52.0	150	35.8	96.4	117	RND
Prostate	38.2	18.5	188	18.4	BLQ	BLQ	RND
Salivary glands	147	59.9	797	13.5	BLQ	BLQ	RND
Seminal vesicles	23.3	18.3	91.5	24.4	17.2	13.8	RND
Skin (non-pigmented)	17.8	BLQ	128	BLQ	BLQ	25.3	BLQ
Spinal cord	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	RND
Spleen	266	237	1030	25.6	15.0	31.5	BLQ
Testis	BLQ	BLQ	33.4	BLQ	BLQ	18.6	BLQ
Thymus	24.0	14.5	152	BLQ	BLQ	BLQ	BLQ
Thyroid gland	169	76.3	1450	25.9	16.2	48.8	BLQ
Tongue	54.2	19.7	254	BLQ	BLQ	BLQ	RND
Tooth pulp	62.5	33.3	345	BLQ	BLQ	RND	RND
Trachea	RND	28.6	130	RND	BLQ	RND	BLQ
Urinary bladder wall	191	204	184	BLQ	34.9	RND	RND
Urinary bladder contents	324	120	86.4	BLQ	BLQ	RND	RND
Uveal tract/retina	30.4	24.4	171	BLQ	BLQ	BLQ	RND

(Continued to next page)

Test Article: [^{14}C]tasurgratinib

Rats **Study Number: 8362069** **Location in CTD: 4.2.2.2.5**

Concentrations of Radioactivity in the Tissues of Male Albino Rats Following a Single Oral Administration of [^{14}C]Tasurgratinib

Tissue/Organ	Concentration of Radioactivity (ng eq./g)						
	0.5 h	2 h	5 h	24 h	48 h	72 h	168 h
White fat	BLQ	BLQ	20.7	BLQ	BLQ	BLQ	RND
Oesophageal wall	197	240	199	RND	BLQ	RND	RND
Stomach mucosa (fundic)	1540	275	635	37.8	22.7	75.3	BLQ
Stomach mucosa (non-fundic)	718	900	214	BLQ	BLQ	21.1	BLQ
Small intestine mucosa	2650	882	470	15.3	20.4	BLQ	RND
Caecum mucosa	72.4	44.4	232	BLQ	14.1	BLQ	RND
Large intestine mucosa	35.0	97.9	289	15.2	BLQ	BLQ	BLQ
Rectum mucosa	81.7	18.3	201	BLQ	28.2	16.6	BLQ

(Continued to next page)

Test Article: [^{14}C]tasurgratinib

Rats **Study Number: 8362069** **Location in CTD: 4.2.2.2.5**

Concentrations of Radioactivity in the Tissues of Male Partially-Pigmented Rats Following a Single Oral Administration of [^{14}C]Tasurgratinib

Tissue/Organ	Concentration of Radioactivity (ng eq./g)								
	0.5 h	4 h	12 h	24 h	48 h	72 h	168 h	456 h	672 h
Plasma [a,c]	12.5	32.8	7.08	1.69	ND	1.10	ND	ND	ND
Blood	15.1	30.2	BLQ	BLQ	RND	RND	RND	RND	RND
Adrenal cortex	58.5	268	197	30.7	BLQ	BLQ	BLQ	RND	RND
Adrenal medulla	40.9	502	78.4	17.6	BLQ	13.8	14.1	RND	RND
Aortic wall	15.6	73.2	104	41.4	22.7	81.8	74.7	46.5	25.7
Bile ducts and contents	188	656	127	59.7	RND	RND	RND	RND	RND
Bone marrow	22.5	153	131	32.2	RND	BLQ	RND	RND	RND
Bone surface	17.3	80.0	38.6	BLQ	RND	RND	RND	RND	RND
Brain	BLQ	BLQ	BLQ	BLQ	RND	RND	RND	RND	RND
Brown fat	BLQ	67.0	33.2	BLQ	RND	RND	RND	RND	RND
Bulbo-urethral gland	23.8	170	99.6	20.9	BLQ	BLQ	BLQ	RND	RND
Choroid plexus	26.7	170	75.6	BLQ	BLQ	BLQ	RND	RND	RND
Epididymis	BLQ	24.9	48.9	23.6	BLQ	BLQ	RND	RND	RND
Exorbital lachrymal gland	19.0	263	385	125	BLQ	14.1	BLQ	RND	RND
Harderian gland	16.1	153	290	136	28.2	31.8	BLQ	RND	RND
Intra-orbital lachrymal gland	21.4	252	398	118	BLQ	23.1	BLQ	RND	RND
Kidney cortex (inner)	105	649	214	61.6	BLQ	56.2	BLQ	RND	RND
Kidney cortex (outer)	101	408	122	33.5	BLQ	31.6	BLQ	RND	RND
Kidney medulla	116	218	78.7	19.0	BLQ	BLQ	BLQ	RND	RND
Lens of the eye	BLQ	BLQ	BLQ	BLQ	RND	RND	RND	RND	RND
Liver	201	677	163	23.1	BLQ	24.0	BLQ	RND	RND
Lung	35.8	150	61.6	13.8	BLQ	18.5	BLQ	RND	RND
Mandibular lymph nodes	18.2	119	91.7	BLQ	RND	BLQ	RND	RND	RND
Meninges	BLQ	101	66.1	93.1	110	114	36.2	170	46.6
Muscle	BLQ	31.5	18.6	BLQ	RND	BLQ	BLQ	RND	RND
Myocardium	30.4	104	38.0	BLQ	RND	BLQ	RND	RND	RND
Nasal mucosa	RND	78.4	37.4	BLQ	RND	BLQ	RND	RND	RND
Pancreas	43.7	223	71.8	18.4	BLQ	BLQ	BLQ	RND	RND

(Continued to next page)

Test Article: [^{14}C]tasurgratinib

Rats Study Number: 8362069 Location in CTD: 4.2.2.2.5

Concentrations of Radioactivity in the Tissues of Male Partially-Pigmented Rats Following a Single Oral Administration of [^{14}C]Tasurgratinib									
Tissue/Organ	Concentration of Radioactivity (ng eq./g)								
	0.5 h	4 h	12 h	24 h	48 h	72 h	168 h	456 h	672 h
Periodontal membrane	15.4	114	60.1	RND	RND	RND	RND	RND	RND
Pineal body	43.6	247	112	18.7	RND	BLQ	RND	RND	RND
Pituitary	48.0	381	293	83.4	32.6	21.8	BLQ	RND	RND
Preputial gland	18.8	45.6	145	91.5	48.5	50.2	RND	RND	RND
Prostate	BLQ	89.7	95.3	22.1	BLQ	RND	RND	RND	RND
Salivary glands	40.5	289	135	13.5	BLQ	BLQ	RND	RND	RND
Seminal vesicles	BLQ	26.6	51.7	14.0	RND	BLQ	RND	RND	RND
Skin (non-pigmented)	BLQ	44.9	39.6	BLQ	BLQ	14.6	BLQ	BLQ	RND
Skin (pigmented)	32.4	61.3	83.4	16.4	BLQ	50.8	28.1	45.9	26.9
Spinal cord	BLQ	BLQ	BLQ	BLQ	RND	BLQ	RND	RND	RND
Spleen	73.3	291	153	35.5	BLQ	15.7	14.2	RND	RND
Testis	BLQ	15.4	26.0	16.0	BLQ	BLQ	BLQ	RND	RND
Thymus	BLQ	69.1	45.6	BLQ	RND	BLQ	BLQ	RND	RND
Thyroid gland	37.7	260	148	22.5	BLQ	28.0	14.7	RND	RND
Tongue	19.0	88.9	30.0	BLQ	RND	RND	RND	RND	RND
Tooth pulp	23.6	108	47.5	BLQ	RND	RND	RND	RND	RND
Trachea	BLQ	84.4	32.8	BLQ	RND	RND	RND	RND	RND
Urinary bladder wall	232	219	515	53.6	RND	RND	RND	RND	RND
Urinary bladder contents	214	263	392	45.7	RND	RND	RND	RND	RND
Uveal tract/retina	15.8	259	1010	601	320	540	804	979	372
White fat	BLQ	BLQ	BLQ	RND	RND	RND	RND	RND	RND
Oesophageal wall	29.0	26.8	47.6	34.5	RND	RND	RND	RND	RND
Stomach mucosa (fundic)	68.7	310	81.2	42.6	BLQ	18.8	18.0	RND	RND
Stomach mucosa (non-fundic)	107	70.7	29.3	22.0	BLQ	BLQ	BLQ	RND	RND
Small intestine mucosa	64.7	339	80.9	22.7	BLQ	BLQ	RND	RND	RND

(Continued to next page)

Test Article: [^{14}C]tasurgratinib

Rats **Study Number: 8362069** **Location in CTD: 4.2.2.2.5**

Concentrations of Radioactivity in the Tissues of Male Partially-Pigmented Rats Following a Single Oral Administration of [^{14}C]Tasurgratinib									
Tissue/Organ	**Concentration of Radioactivity (ng eq./g)**								
	0.5 h	**4 h**	**12 h**	**24 h**	**48 h**	**72 h**	**168 h**	**456 h**	**672 h**
Caecum mucosa	25.3	384	162	33.7	BLQ	BLQ	RND	RND	RND
Large intestine mucosa	25.7	204	116	48.4	BLQ	BLQ	RND	RND	RND
Rectum mucosa	BLQ	55.4	90.6	67.6	BLQ	BLQ	RND	RND	RND

Lower limit of quantification: 13.5 ng eq./g for all measurements.

BLQ = tissue concentration below lower limit of quantification, CTD = Common Technical Document, eq. = equivalent, ND = radioactivity not detected in plasma, RND = radioactivity not detected in the tissue.

a: Radioactivity determined by liquid scintillation counter.

b: Limit of detection: plasma = 0.577 ng eq./g (albino animal).

c: Limit of detection: plasma = 0.862 ng eq./g (partially-pigmented animal).

Additional Information: This study also investigated the blood and plasma level of radioactivity (Section 2.6.5.3) and the excretion of radioactivity (Section 2.6.5.13).

2.6.5.6 薬物動態試験：タンパク結合

Test Article: tasurgratinib succinate

Study System: In Vitro Plasma Protein Binding of Tasurgratinib　　Study Number: B13014　　Location in CTD: 5.3.2.1.1

Test System	Plasma			
Method	Equilibrium dialysis against phosphate-buffered saline (pH 7.4) for 48 hours at 37 °C (dog and human) or 4 °C (mouse and rat)			
Assay / Analyte	LC-MS/MS / Tasurgratinib			
Concentration	Tasurgratinib concentration: 100, 1000, or 3000 ng/mL as free form			
Spiked Concentration as Free Form (ng/mL)	**Plasma Protein Binding (%)**			
	Rat	**Dog**	**Mouse**	**Human**
100	96.9±0.2	91.7±0.6	97.0±0.1	93.2±0.4
1000	96.7±0.3	91.6±0.3	96.8±0.1	93.6±0.4
3000	96.7±0.1	92.6±0.5	97.1±0.2	94.3±1.1
Mean of 3 concentrations	96.8	92.0	97.0	93.7

Each value represents the mean ±SEM (n=3). In rats, 3 lots of pooled plasma from 2 animals were used. In mice, 1 lot of pooled plasma from 65 animals was used in triplicate. In dogs and humans, plasma from 3 individuals was used.

CTD = Common Technical Document, LC-MS/MS = liquid chromatography with tandem mass spectrometry.

Additional Information: Plasma protein binding of tasurgratinib did not show concentration-dependency in any species across a range of spiked concentrations from 100 to 3000 ng/mL (as free form).

2.6.5.6 薬物動態試験：タンパク結合（続き）

Test Article: M2

Study System: In Vitro Human Plasma Protein Binding of M2 **Study Number: B16010** **Location in CTD: 5.3.2.1.2**

Test System	Human plasma
Method	Equilibrium dialysis against phosphate-buffered saline (pH 7.4) for 48 hours at 37 °C
Assay / Analyte	LC-MS/MS / M2
Concentration	M2 concentration: 30, 100, or 1000 ng/mL as free form
Spiked Concentration as Free Form (ng/mL)	**Human Plasma Protein Binding (%)**
30	90.1±0.7
100	90.5±0.3
1000	89.3±0.6
Mean of 3 concentrations	90.0

Each value represents the mean ±SEM (n=3).

CTD = Common Technical Document, LC-MS/MS = liquid chromatography with tandem mass spectrometry.

Additional Information: Protein binding of M2 in human plasma ranged from 89.3% to 90.5% and did not show concentration-dependency within the spiked concentration range of 30 to 1000 ng/mL.

2.6.5.6 薬物動態試験：タンパク結合（続き）

Test Article: tasurgratinib succinate, M2

Binding to Human Plasma Protein **Study Number: AL-7881-G** **Location in CTD: 5.3.2.1.3**

Study System	in vitro		
Test System	Human protein in blood		
Method	Equilibrium dialysis against phosphate-buffered saline (pH 7.4) for 24 hours at 37 °C		
Assay / Analyte	LC-MS/MS / Tasurgratinib and M2		
Concentration	30, 100, or 300 ng/mL as free form		
Plasma Protein (Concentration)	**Spiked Concentration as Free Form (ng/mL)**	**Protein Binding (%)**	
		Tasurgratinib	**M2**
Human serum albumin (40 mg/mL)	30	71.1±0.9	73.5±1.3
	100	72.2±0.3	74.3±0.4
	300	72.4±1.2	74.8±1.3
Human α1-acid glycoprotein (1 mg/mL)	30	30.9±3.2	45.0±3.2
	100	32.0±3.7	46.8±3.1
	300	37.3±4.5	51.7±3.5
Human γ-globulin (12 mg/mL)	30	39.0±6.9	42.6±1.5
	100	36.5±1.6	45.9±1.5
	300	37.5±3.5	44.5±0.9

Each value represents the mean ±SD (n=3).

CTD = Common Technical Document, LC-MS/MS = liquid chromatography with tandem mass spectrometry.

Additional Information: None.

2.6.5.6 薬物動態試験：タンパク結合（続き）

Test Article: (*R*)-M9

Study System: In Vitro Human Plasma Protein Binding of (*R*)-M9 **Study Number: AL-8206-G** **Location in CTD: 5.3.2.1.4**

Test System	Human plasma
Method	Equilibrium dialysis against phosphate-buffered saline (pH 7.4) for 6 hours at 37 °C
Assay / Analyte	LC-MS/MS / (*R*)-M9
Concentration	(*R*)-M9 concentration: 3, 10, or 30 ng/mL as free form
Spiked Concentration as Free Form (ng/mL)	**Human Plasma Protein Binding (%)**
3	98.3±0.1
10	98.1±0.1
30	98.3±0.2

Each value represents the mean ±SD (n=3).

CTD = Common Technical Document, LC-MS/MS = liquid chromatography with tandem mass spectrometry.

Additional Information: No remarkable concentration dependency was observed in protein binding of (*R*)-M9 in human plasma.

2.6.5.7 薬物動態試験：妊娠又は授乳動物における試験

妊娠又は授乳動物における試験は検討されていない。

2.6.5.8 薬物動態試験：その他の分布試験

Test Article: tasurgratinib succinate, M2

Blood to Plasma Concentration Ratio of Tasurgratinib and M2 **Study Number: AL-7880-G** **Location in CTD: 5.3.2.3.1**

Study System	in vitro			
Test System	Blood			
Method	Incubation for 30 minutes at 37 °C			
Assay / Analyte	LC-MS/MS / Tasurgratinib and M2			
Species	**Compound**	**R_B**		
		Concentration as Free Form (ng/mL)		
		30	**100**	**300**
Mouse	Tasurgratinib	1.29±0.07	1.26±0.03	1.23±0.03
Rat		1.02±0.03	1.02±0.03	0.952±0.057
Dog		0.966±0.069	0.929±0.025	0.954±0.076
Human		1.14±0.16	1.09±0.05	1.06±0.05
Mouse	M2	1.30±0.06	1.14±0.06	1.14±0.05
Rat		1.14±0.05	1.16±0.06	1.12±0.09
Dog		0.998±0.030	0.992±0.051	1.01±0.06
Human		1.14±0.06	1.16±0.02	1.15±0.05

Data are expressed as the mean ±SD of triplicate samples (mouse) or 3 individuals (rat, dog, and human).

CTD = Common Technical Document, LC-MS/MS = liquid chromatography with tandem mass spectrometry, R_B = blood to plasma concentration ratio.

Additional Information: None.

2.6.5.9 薬物動態試験：代謝：in vivo

Test Article: [^{14}C]tasurgratinib

Metabolites in Plasma, Bile, Urine, or Feces of Rats and Dogs **Study Number: 8361653** **Location in CTD: 4.2.2.4.1**

Strain/Gender/Number of Animals	Sprague Dawley (albino animal)/Male/3 (intact) or 4 (bile duct-cannulated), Beagle/Male/3
Feeding Condition	Fasted
Vehicle / Formulation	0.1 mol/L hydrochloric acid / Solution
Method of Administration / Dose	Oral / 3 mg/kg as free form for rats and 1.5 mg/kg as free form for dogs
Radionuclide / Specific Activity (Lot No.)	[^{14}C] / [redacted] MBq/mg (AL-IC-11141-0080)
Assay / Analyte	Radio-HPLC analysis with on-line or off-line radio-detection and LC-MS(/MS) / Metabolites derived from tasurgratinib
Sample Collection Point	Rat: Plasma; 0.25, 1, 2, 4, 6, and 8 hours, Urine; 0 to 24 hours, Bile; 0 to 24 hours, Feces; 0 to 48 hours. Dog: Plasma; 0.5, 4, 8, 12, and 24 hours, Urine; 0 to 96 hours, Feces; 0 to 48 hours.

Metabolite Profiling Summary: % Relative Exposure (Plasma) or % Total Administered Dose (Excreta)

Radio-Peak	Identity	Plasma		Urine		Bile	Feces	
		Rat	Dog	Rat	Dog	Rat	Rat	Dog
P1	not determined	nc	1.03	–	–	–	–	–
P5/U8/B11/F7	M6 +2O+2H	8.50	nc	0.30	0.07	0.97	1.99	0.32
P9	not determined	–	6.11	–	–	–	–	–
P10	not determined	–	6.43	–	–	–	–	–
B21/F15	M7 di-acid	–	–	–	–	2.82	8.12	0.94 [a]
B24/F17	**M7 +O –2H**	–	–	*	–	4.61	**14.5**	1.05
B25	M7 glucuronide	*	–	*	–	3.14	–	–
P13/U14/F18	de-methylated M6	8.55	–	0.07	*	–	1.88	–
P14/U16/B30/F20	de-methylated M3	8.00	nc [a]	0.15	0.09 [a]	5.62	8.63	2.16
P15/U17/B33/F22	**M7**	**13.6**	*	0.74	*	6.68	**11.1**	2.13
P16/U18/B34/F23	M6	7.25	*	0.08	*	0.25	3.54	2.52
P17/U19/B35/F24	**M3**	nc [a]	7.31	0.14	0.24	2.13	2.64	**16.9**
P19/U21/F27/B38	**Tasurgratinib**	**19.2**	**40.6**	0.02	1.84	1.39	**13.6**	**17.6**
No peak	M8	×	×	×	–	×	–	–

The prefixes “P”, “U”, “B”, and “F” mean plasma, urine, bile, and feces, respectively. The M-numbers mean metabolites identified in in vitro or in vivo metabolism studies.
Components in bold account for >10% of total drug related exposure in plasma or >10% of total administered dose in excreta.
–: Not detected (by radio-detection or LC-MS). *: Minor metabolites detected by LC-MS, but below the limited of radio-detection.
×: Peak consistent with non-radiolabeled M8 detected by LC-MS.
CTD = Common Technical Document, HPLC = high performance liquid chromatography, LC-MS = liquid chromatography with mass spectrometry, LC-MS(/MS) = liquid chromatography with mass spectrometry or tandem mass spectrometry, nc = not calculated.
a: Possible minor metabolites based on comparative radio-peak retention time only, as no LC-MS(/MS) data obtained.
Additional Information: None.

2.6.5.9 薬物動態試験：代謝：in vivo（続き）

Test Article: [^{14}C]tasurgratinib

Metabolites in Urine or Feces of Humans **Study Number: 8426048** **Location in CTD: 5.3.2.2.7**

Study System	in vivo			
Test System	Pooled urine and feces samples from human[a]			
Method/Objective	To characterize the relative proportions of tasurgratinib and its radiolabeled metabolites in urine and feces samples obtained in human.			
Assay/Analyte	Radio-HPLC analysis with off-line radio-detection and LC-MS(/MS) / Metabolites derived from tasurgratinib			
Dose	[^{14}C]tasurgratinib: 35 mg			
Metabolite Profiling Summary:				
Peak Name in Radio-Chromatogram	**Identity**	**% Total Administered Dose (Mean Value)**		
		Urine	**Feces**	**Total Excreted**
Not detected	M1	<LOQ	ND	<LOQ
U2, F15	M2	2.55	30.53	33.08
F13	M3	ND	2.98	2.98
U1	M4	0.54	ND	0.54
F18	M5	ND	0.26	0.26
F10	M6	ND	0.61	0.61
F9	M7	ND	0.54	0.54
F20	M9	ND	1.47	1.47
U3, F16	Tasurgratinib	2.05	32.08	34.13

The prefixes “U” and “F” mean urine and feces, respectively. The M-numbers mean metabolites identified in in vitro or in vivo metabolism studies. ND: Not detected by radio-detection or LC-MS. <LOQ: Detected by LC-MS but below limit of quantification for radio-detection.

CTD = Common Technical Document, HPLC = high performance liquid chromatography, LC-MS = liquid chromatography with mass spectrometry, LC-MS(/MS) = liquid chromatography with mass spectrometry or tandem mass spectrometry, LOQ = limit of quantification, ND = not detected.

a: Human urine and feces samples derived from the following study; 5.3.3.1.1 (Study E7090-E044-002), 35 mg, 8 subjects.

Additional Information: None.

2.6.5.9 薬物動態試験：代謝：in vivo（続き）

Test Article: [^{14}C]tasurgratinib

Metabolites in Plasma of Humans **Study Number: AE-8462-G** **Location in CTD: 5.3.2.2.8**

Study System	in vivo		
Test System	$AUC_{(0\text{-}48h)}$ pooled plasma samples from human[a]		
Method/Objective	To clarify the metabolite profile of tasurgratinib in human plasma samples collected from healthy male subjects after a single oral administration of [^{14}C]tasurgratinib.		
Assay/Analyte	HPLC analysis with offline AMS and LC-MS(MS^n)/ Metabolites derived from tasurgratinib		
Dose	[^{14}C]tasurgratinib: 35 mg		
Metabolite Profiling Summary in Human Plasma			
Peak Name	**Identity**	**% of Radioactivity in Sample**	**Ratio to Tasurgratinib**
mH01		2.7	
mH02		3.0	
mH03		0.9	
mH04		1.1	
mH05		1.2	
mH06		0.9	
mH07	M3 or M5 or both	3.1	
mH08	Tasurgratinib	30.9	1.00
mH09	M2	20.6	0.67
mH10	M1	2.4	
mH11		1.0	
mH12		1.1	
mH13	M9	12.3	0.40
Others		9.4	

The metabolites were assigned serial numbers with the prefix “mH”. The M-numbers mean metabolites identified in in vitro or in vivo metabolism studies. mH08, mH09, and mH13: Structures were confirmed by mass spectrometry.

AMS = accelerator mass spectrometry, $AUC_{(0\text{-}48h)}$ = AUC from zero time to 48 hours, CTD = Common Technical Document, HPLC = high performance liquid chromatography, LC-MS(MS^n) = liquid chromatography with mass spectrometry or multi-stage mass spectrometry.

a: Human plasma samples derived from the following study; 5.3.3.1.1 (Study E7090-E044-002), 35 mg, 8 subjects. Plasma samples were pooled for 48 hours postdose by Hamilton method (AUC pool rule).

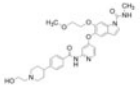
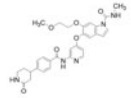
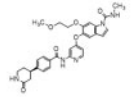
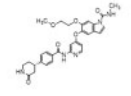
Additional Information: None.

2.6.5.9 薬物動態試験：代謝：in vivo（続き）

Test Article: [^{14}C]tasurgratinib, tasurgratinib **succinate**

Metabolite Identification: **Metabolites Observed in the Human and Dog Plasma** **Study Number:** **DMPKT2021-007** **Location in CTD:** **5.3.2.2.9**

Study System	in vivo
Test System	Plasma samples from dog (beagle) and human[a]
Method	Characterization of M9 in plasma samples
Assay/Analyte	Chiral column-equipped LC-HRMS/Tasurgratinib and M9
Dose	[^{14}C]tasurgratinib: humans, 35 mg; tasurgratinib: dogs, 2 mg/kg

Identifier	**Human**	**Dog**		**Structure**
		Female	**Male**	
Tasurgratinib	Y	Y	Y	
M9	Y	Y	Y (trace)	
(*R*)-M9	Y	–	–	
(*S*)-M9	Y	–	–	

Y: The metabolite was detected in the sample. trace: The metabolite was detected in the sample, but characteristic fragment ions could not be obtained. –: Chiral separation of M9 in dog plasma was not conducted.

CTD = Common Technical Document, LC-HRMS = liquid chromatography coupled with high resolution mass spectrometry.

a: Samples derived from the following studies:

Dog plasma: 4.2.3.2.6 (Study No. B200205) (13-week repeated dosing), Day 91, 2 mg/kg/day; Pooled male and female dog plasma samples were prepared using 2 hours and 4 hours postdose samples (n=3, each), respectively.

Human plasma: 5.3.3.1.1 (Study E7090-E044-002), 35 mg, 8 subjects (samples were pooled for 24 hours postdose); Plasma samples were pooled by Hamilton method (AUC pool rule).

Additional Information: None.

2.6.5.9 薬物動態試験：代謝：in vivo（続き）

Pharmacokinetic Analysis of Metabolite M9 in Human Plasma　　**Test Article: [^{14}C]tasurgratinib**

Study Number: AL-7939-G, DMPKT2022-004　　**Location in CTD: 5.3.2.2.16, 5.3.2.2.17**

Study System	in vivo
Test System	Plasma samples from human[a]
Method	Determination of M9 concentrations in human plasma samples and analysis of pharmacokinetic parameters
Assay/Analyte	LC-MS/MS /M9
Dose	[^{14}C]tasurgratinib: 35 mg
Pharmacokinetic Parameters	**Mean ±SD**
t_{max} (h)	4.00 (3 – 8)
C_{max} (ng /mL)	2.72±1.88
$AUC_{(0\text{-}24h)}$ (ng·h/mL)	40.7±27.8
$AUC_{(0\text{-}t)}$ (ng·h/mL)	59.1±41.2
$AUC_{(0\text{-}inf)}$ (ng·h/mL)	75.1±48.0
$t_{½}$ (h)	21.4±9.6

Each value represents the mean ±SD of 8 subjects except for t_{max}. t_{max} value represents median (range) of 8 subjects.

$AUC_{(0\text{-}24h)}$ = AUC from zero time to 24 hours, $AUC_{(0\text{-}inf)}$ = AUC from zero time extrapolated to infinite time, $AUC_{(0\text{-}t)}$ = AUC from zero time to time of last quantifiable concentration, CTD = Common Technical Document, LC-MS/MS = liquid chromatography with tandem mass spectrometry, $t_{½}$ = terminal elimination phase half-life, t_{max} = time at which the highest drug concentration occurs.

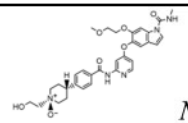
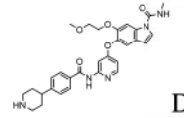
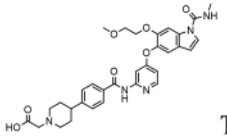
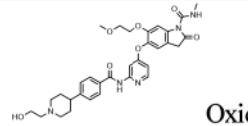
a: Human plasma samples derived from the following study; 5.3.3.1.1 (Study E7090-E044-002), 35 mg, 8 subjects.

Additional Information: None.

2.6.5.10 薬物動態試験：代謝：in vitro

Test Article: tasurgratinib succinate

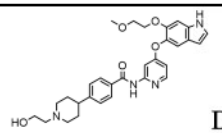
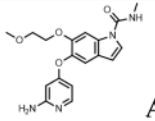
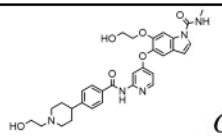
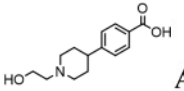
Study System: Metabolites Identifications or Estimations In Vitro　　Study Number: C13529　　Location in CTD: 5.3.2.2.1

Test System	Cryopreserved human, dog, rat, and mouse hepatocytes, and dog and rat liver microsomes					
Method	Incubation of tasurgratinib with hepatocytes for 1 hour (mouse and rat) or 4 hours (dog and human) at 37 °C Incubation of tasurgratinib with dog or rat liver microsomes for 8 hours at 37 °C					
Assay / Analyte	LC-MS/MS / Metabolite					
Concentration	Tasurgratinib concentration: 5 µmol/L for cryopreserved hepatocytes and 100 µmol/L for liver microsomes					
M-No.	**Metabolites in Hepatocytes or Liver Microsomes**				**$[M+H]^+$**	**Tasurgratinib Metabolites and Reaction**
	Human	**Dog (Beagle)**	**Rat (Wister Hannover)**	**Mouse (ICR/CD-1)**		
M1	HH1	DH1	RH1	MH1	604	 *N*-oxidation
M2	HH2	DH2	–	–	544	 Des-ethanol
M3	HH3	DH3	–	MH2	602	 Terminal carboxylation
M4	HH4	DH4	–	–	604	 Oxidation of indole ring

(Continued to next page)

Test Article: tasurgratinib succinate

Study System: Metabolites Identifications or Estimations In Vitro　　Study Number: C13529　　Location in CTD: 5.3.2.2.1

M-No.	Metabolites in Hepatocytes or Liver Microsomes				$[M+H]^+$	Tasurgratinib Metabolites and Reaction
	Human	Dog (Beagle)	Rat (Wister Hannover)	Mouse (ICR/CD-1)		
M5	HH5	DM1	RM1	–	531	 Des-carboxamide
M6	–	DH5	RH2	MH3	357	 Amide hydrolysis
M7	–	–	RH3	MH4	574	 *O*-demethylation
M8	–	–	RH4	MH5	250	 Amide hydrolysis

M-numbers mean serial metabolite numbers. HH-number denotes metabolite number detected in the incubation mixture of human hepatocytes. DH-number denotes metabolite number detected in the incubation mixture of dog hepatocytes. DM-number denotes metabolite number detected in the incubation mixture of dog liver microsomes. RH-number denotes metabolite number detected in the incubation mixture of rat hepatocytes. RM-number denotes metabolite number detected in the incubation mixture of rat liver microsomes. MH-number denotes metabolite number detected in the incubation mixture of mouse hepatocytes.

CTD = Common Technical Document, LC-MS/MS = liquid chromatography with tandem mass spectrometry, $[M+H]^+$ = mass-to-charge ratio of deprotonated molecular ion, – = not detected.

Additional Information: The formation of M5 was found in the incubation mixtures of rat and dog liver microsomes. No human-specific metabolites were observed in this study.

2.6.5.10 薬物動態試験：代謝：in vitro（続き）

Test Article: tasurgratinib succinate

Study System: CYP Isoforms for Metabolism In Vitro　　Study Number: DMPKT2013-013　　Location in CTD: 5.3.2.2.2

Test System	Tasurgratinib was spiked into each rCYP isoform in the presence or absence of an NADPH-generating system.	
Method	Incubation of tasurgratinib with each rCYP isoform for 0 or 30 minutes at 37 °C	
Assay / Analyte	LC-MS/MS / Tasurgratinib	
Concentration	Tasurgratinib concentration: 0.1 μg/mL as free form	
rCYP Isoform	**Residual Ratio of Tasurgratinib (%)**	
	NADPH (+)	**NADPH (–)**
CYP1A2	106.3	103.8
CYP2B6	95.9	99.3
CYP2C8	98.1	97.8
CYP2C9	89.0	91.3
CYP2C19	95.9	98.1
CYP2D6	112.3	113.8
CYP3A4	47.0	111.3
CYP3A5	115.6	114.5

Microsomal protein concentration and CYP concentration were 0.6 mg/mL and 50 pmol CYP/mL, respectively. Incubation for all rCYP isoforms tested was conducted at 37 °C for 30 minutes.

Residual ratio (%) = (mean peak area ratio of tasurgratinib to IS after 30-minute incubation) / (mean peak area ratio of tasurgratinib to IS at 0 minute) × 100.

NADPH (+): Incubation sample with NADPH-generating system.

NADPH (–): Incubation sample without NADPH-generating system.

CTD = Common Technical Document, CYP = cytochrome P450, IS = internal standard, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate, rCYP = recombinant human CYP.

Additional Information: The results suggested that CYP3A4 is the primary CYP isoform responsible for CYP-dependent metabolism of tasurgratinib, with no or minimal involvements of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A5.

2.6.5.10 薬物動態試験：代謝：in vitro（続き）

Test Article: tasurgratinib succinate

Study System: CYP Isoforms for Metabolism In Vitro　　Study Number: AE-8232-G　　Location in CTD: 5.3.2.2.3

Test System	Tasurgratinib was spiked into HLM or each rCYP isoform
Method	**Exp.1)** After incubating tasurgratinib (1 µmol/L) with 50 pmol CYP/mL of rCYP1A2, rCYP2B6, rCYP2C8, rCYP2C9*1, rCYP2C19, rCYP2D6*1, rCYP2J2, rCYP3A4, or rCYP3A5, or 10 pmol CYP/mL of rCYP4F12 in the presence of NADPH-generating system for up to 30 minutes (rCYP4F12: 15 minutes) at 37 °C, the peak area ratios of tasurgratinib and the concentration of M2 were measured.
	Exp.2) After incubating tasurgratinib (0.1, 0.25, 0.5, 1.25, 2.5, 5, 10, and 25 µmol/L) with 50 pmol CYP/mL of rCYP2J2 or rCYP3A4, or 10 pmol CYP/mL of rCYP4F12 in the presence of NADPH-generating system for 0 and 15 minutes (rCYP4F12: 5 minutes) at 37 °C, the concentrations of tasurgratinib and M2 were measured.
	Exp.3) After incubation of tasurgratinib (0.3 µmol/L) with HLM with and without the typical CYP inhibitor (1-ABT or KTZ) in the presence of NADPH-generating system, the percentage inhibitions on metabolism of tasurgratinib and formation rate of M2 were assessed by quantitating the concentrations of tasurgratinib and M2. To check the assay performance in HLM, CYP3A activities with and without CYP inhibitor (1-ABT or KTZ) was confirmed using midazolam as a marker substrate.
Assay / Analyte	LC-MS/MS / Tasurgratinib and M2
Concentration	Concentrations are listed in the Method section.
Results	
Exp.1) Identification of responsible CYP isoform for tasurgratinib metabolism and M2 formation from tasurgratinib At 1 µmol/L of tasurgratinib, the remaining ratios of tasurgratinib in rCYP3A4, rCYP4F12, and rCYP2J2 were 10.9%, 43.9%, and 76.3%, respectively, whereas those in rCYP1A2, rCYP2B6, rCYP2C8, rCYP2C9*1, rCYP2C19, rCYP2D6*1, and rCYP3A5 were 85.2% or more. The formation rates of M2 in rCYP3A4, rCYP2J2, and rCYP4F12 were 0.0116, 0.0954, and 3.15 pmol/min/pmol CYP, respectively.	
Exp.2) Assessment of K_m and V_{max} for M2 formation from tasurgratinib K_m and V_{max} were calculated from formation rate of M2. K_m and V_{max} values were 63.2 µmol/L and 1.17 pmol/min/pmol CYP for rCYP3A4, 5.38 µmol/L and 0.699 pmol/min/pmol CYP for rCYP2J2, and 0.471 µmol/L and 5.99 pmol/min/pmol CYP for rCYP4F12, respectively. K_m and V_{max} for HLM were 2.18 µmol/L and 10.4 pmol/min/mg protein, respectively.	
Exp.3) Inhibitory effect on tasurgratinib metabolism and M2 formation from tasurgratinib by CYP inhibitor In HLM, the metabolic activity of CYP3A (midazolam hydroxylation) was confirmed, and KTZ (1 µmol/L) decreased CYP3A activity by 87.8%, indicating the assay system by KTZ inhibition was appropriate to assess CYP3A-mediated metabolism in HLM. Under the same condition, KTZ showed almost complete inhibition on tasurgratinib metabolism (% inhibition: 100.5%), and M2 formation was inhibited by approximately 50%. 1-ABT (100 µmol/L) did not show appropriate inhibition on CYP3A activity (49.4% inhibition), and therefore, inhibitory effects on tasurgratinib metabolism and M2 formation by 1-ABT could not be evaluated in this experiment.	

1-ABT = 1-Aminobenzotriazole, CTD = Common Technical Document, CYP = cytochrome P450, HLM = human liver microsomes, K_m = Michaelis constant, KTZ = ketoconazole, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate, rCYP = recombinant human CYP, V_{max} = maximum velocity.

Additional Information: The LC-MS/MS methods for the quantification of tasurgratinib and M2 in all blank microsomal matrices for HLM and rCYP isoforms were validated, and quantification range for both compounds was 0.003 to 3 µmol/L in all matrices.

2.6.5.10 薬物動態試験：代謝：in vitro（続き）

Test Article: tasurgratinib succinate, M2

Study System: CYP Isoforms for Metabolism In Vitro　　Study Number: SUNBAE2020-001　　Location in CTD: 5.3.2.2.4

Test System	Tasurgratinib and M2 were spiked into each rCYP isoform in the presence or absence of an NADPH-generating system.	
Method	Incubation of tasurgratinib or M2 with each rCYP isoform for 0 or 30 minutes at 37 °C	
Assay / Analyte	LC-MS/MS / Tasurgratinib and M2	
Concentration	Tasurgratinib concentration: 0.1 μg/mL as free form; M2 concentration: 0.1 μg/mL	
Residual Ratio of Tasurgratinib (%)		
rCYP Isoform	**NADPH (+)**	**NADPH (–)**
CYP2J2	67.6	102.4
CYP3A4	35.2	96.8
CYP4F12	<10.0	97.9
Residual Ratio of M2 (%)		
rCYP Isoform	**NADPH (+)**	**NADPH (–)**
CYP1A2	106.3	109.8
CYP2B6	102.7	103.1
CYP2C8	100.9	102.5
CYP2C9	106.3	113.2
CYP2C19	97.9	101.4
CYP2D6	101.4	109.9
CYP2J2	68.4	102.8
CYP3A4	49.5	100.2
CYP3A5	91.7	100.5
CYP4F12	53.5	99.0

Microsomal protein concentration and CYP concentration were 1.05 mg/mL and 50 pmol CYP/mL, respectively.
Residual ratio (%) = (mean peak area ratio of tasurgratinib or M2 to corresponding IS after 30-minute incubation) / (mean peak area ratio of tasurgratinib or M2 to corresponding IS at 0 minute) × 100.
NADPH (+): Incubation sample with NADPH-generating system; NADPH (–): Incubation sample without NADPH-generating system.
CTD = Common Technical Document, CYP = cytochrome P450, IS = internal standard, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate, rCYP = recombinant human CYP.
Additional Information: The results suggested that tasurgratinib and M2 were metabolized by CYP2J2, CYP3A4, and CYP4F12, and M2 was not or minimally metabolized by the other CYP isoforms tested in this study.

2.6.5.10 薬物動態試験：代謝：in vitro（続き）

Test Article: tasurgratinib succinate

Study System: CYP Isoforms for Metabolism In Vitro **Study Number: DMPKT2021-005** **Location in CTD: 5.3.2.2.5**

Test System	Tasurgratinib was spiked into HLM or rCYP4F12.
Method	HLM (final concentration: 0.5 mg/mL) or rCYP4F12 (final concentration: 0.5 mg/mL and 0.5 pmol CYP/mL) was preincubated with azamulin, danazol, HET0016, pimozide, or ketoconazole at 37 °C in the presence of NADPH-generating system for 15 minutes. Then, tasurgratinib (final concentration: 0.1 µmol/L) was added to the mixture and incubated for 30 minutes at 37 °C. Samples were analyzed by LC-MS/MS, and PAR of M2 to internal standard was calculated.
Assay / Analyte	LC-MS/MS / M2
Concentration	Tasurgratinib: 0.1 µmol/L
Results	No or minimal inhibitory effects on the formation of M2 were observed by azamulin (CYP3A inhibitor) and danazol (CYP2J2 inhibitor) up to the maximum tested concentrations of 1 and 3 µmol/L, respectively, in both HLM and rCYP4F12. HET0016, pimozide, and ketoconazole showed inhibitory effects on M2 formation in a concentration-dependent manner with similar trends between HLM and rCYP4F12, except that HET0016 at 1 µmol/L stimulated the M2 formation only in HLM.
Conclusion	Azamulin (CYP3A inhibitor) and danazol (CYP2J2 inhibitor) exhibited no or minimal inhibition in HLM and rCYP4F12. The susceptibility of M2 formation to inhibition by HET0016, pimozide, and ketoconazole was generally similar between HLM and rCYP4F12. These results suggested that CYP4F12 was the predominant CYP isoform involved in the formation of M2 from tasurgratinib in human liver.

CTD = Common Technical Document, CYP = cytochrome P450, HLM = human liver microsomes, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate, PAR = peak area ratio, rCYP = recombinant human CYP.

Additional Information: None.

2.6.5.10 薬物動態試験：代謝：in vitro（続き）

Test Article: tasurgratinib succinate

Study System: Contribution of CYP3A to the Hepatic Metabolism of Tasurgratinib In Vitro　**Study Number: DMPKT2022-001**　**Location in CTD: 5.3.2.2.6**

Test System	Tasurgratinib or midazolam was incubated with suspension of cryopreserved human hepatocytes in the absence or presence of a CYP inhibitor (azamulin or ketoconazole).	
Method	Incubation with suspension of cryopreserved human hepatocytes for 0 to 120 minutes at 37 °C	
Assay / Analyte	LC-MS/MS / Tasurgratinib and midazolam	
Concentration	Tasurgratinib concentration: 0.1 µmol/L; Midazolam concentration: 0.5 µmol/L	
Inhibitory Effects of Azamulin and Ketoconazole on the Metabolism of Midazolam and Tasurgratinib in Human Hepatocytes		
1) Midazolam		
Inhibitor	**k (min^{-1})**	**Inhibition (%)**
Control	0.0163	NA
Azamulin (3 µmol/L)	0.00228	86.0
Ketoconazole (10 µmol/L)	0.00228	86.0
2) Tasurgratinib		
Inhibitor	**k (min^{-1})**	**Inhibition (%)**
Control	0.00725	NA
Azamulin (3 µmol/L)	0.00544	25.0
Ketoconazole (10 µmol/L)	0.000910	87.4

Elimination rate constant (k) is the negative value of slope which was determined by linear regression between natural logarithmic remaining percentage (mean of triplicate samples) and incubation time.

Inhibition (%) = $(k_{control} - k_{inhibition}) / k_{control} \times 100$.

$k_{control}$: Elimination rate constant in the absence of inhibitor (vehicle control).

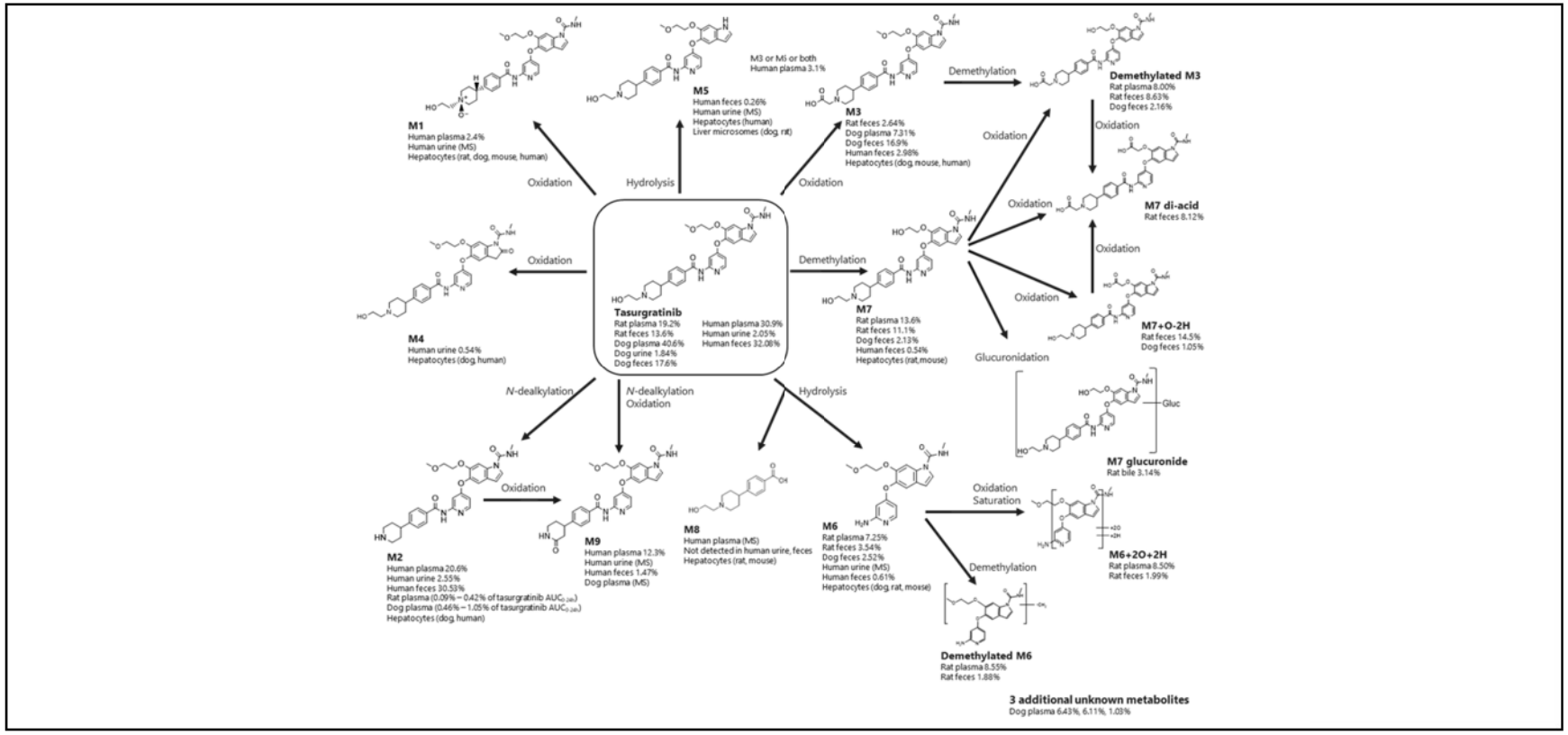
$k_{inhibition}$: Elimination rate constant in the presence of inhibitor.

CTD = Common Technical Document, CYP = cytochrome P450, k = elimination rate constant, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NA = not applicable.

Additional Information: Ketoconazole potently inhibited the disappearance of tasurgratinib by 87.4%, but only 25.0% inhibition was given by azamulin, suggesting that the contribution of CYP3A to the hepatic metabolism of tasurgratinib was estimated to be 25% in vitro.

2.6.5.11 薬物動態試験：推定代謝経路

Proposed Metabolic Pathways Based on the Metabolites Observed In Vivo and In Vitro (Rat, Dog, and Human)



The M-numbers mean metabolites identified in in vitro or in vivo metabolism studies. Plasma: % of radioactivity in pooled plasma sample prepared from 0 to 48 hours based on the Hamilton method for human, and % of the total drug related exposure based on the AUC from 0 to 6 hours for rats and from 0 to 24 hours for dogs, except where specifically noted. Urine, feces: % of dose. MS: Peak observed in mass spectrometry but not radio analysis. AUC = area under the concentration-time curve, Gluc = glucuronide.

Additional Information: The metabolic pathways were prepared based on Study Nos. 8361653, 8426048, AE-8462-G, DMPKT2021-007, B200206, B200205, AL-7939-G, DMPKT2022-004, and C13529.

2.6.5.12 薬物動態試験：薬物代謝酵素の誘導/阻害

Test Article: tasurgratinib succinate

Study System: In Vitro Reversible Inhibition of CYPs (Exploratory) **Study Number: DMPKT2013-016** **Location in CTD: 5.3.2.2.10**

Test System	Human liver microsomes in the presence of an NADPH-generating system					
Method	Incubation of tasurgratinib with human liver microsomes and cocktail of CYP isoform-selective substrates for 10 minutes at 37 ℃					
Assay / Analyte	LC-MS/MS / Metabolites formed by CYP-mediated metabolism					
Concentration	5 or 50 μmol/L of tasurgratinib					
Tasurgratinib Concentration (μmol/L)	**Inhibition Percentage (%)**					
	CYP1A2	**CYP2C8**	**CYP2C9**	**CYP2C19**	**CYP2D6**	**CYP3A**
5	0.2	6.9	5.5	5.3	4.5	14.4
50	2.4	43.7	30.1	33.1	63.8	62.5

Inhibition percentage (%) was shown as the mean of 2 samples. The cocktail of CYP isoform-selective activities were assessed by using phenacetin (CYP1A2), rosiglitazone (CYP2C8), tolbutamide (CYP2C9), (*S*)-mephenytoin (CYP2C19), bufuralol (CYP2D6), and midazolam (CYP3A).

CTD = Common Technical Document, CYP = cytochrome P450, IC_{50} = half-maximal inhibitory concentration, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate.

Additional Information: Tasurgratinib showed little reversible inhibition potency to CYP1A2, CYP2C8, CYP2C9, and CYP2C19 (IC_{50}: >50 μmol/L), but showed 63.8% and 62.5% reversible inhibition of CYP2D6 and CYP3A, respectively.

2.6.5.12 薬物動態試験：薬物代謝酵素の誘導/阻害（続き）

Test Article: tasurgratinib succinate

Study System: In Vitro Time-Dependent Inhibition of CYPs (Exploratory) Study Number: DMPKT2013-016 Location in CTD: 5.3.2.2.10

Test System	Human liver microsomes in the presence or absence of an NADPH-generating system			
Method	Tasurgratinib was preincubated with human liver microsomes in the presence or absence of NADPH-generating system for 30 minutes at 37 °C. Incubation of the reaction mixture and CYP isoform-selective substrates was conducted for 10 minutes at 37 °C.			
Assay / Analyte	LC-MS/MS / Metabolites formed by CYP-mediated metabolism			
Concentration	10 or 50 µmol/L of tasurgratinib			
CYP Isoform	**Tasurgratinib Concentration (µmol/L)**	**CYP Activity (% of Control)**		**Remaining Activity (% of Control)**
		GS (+)[a]	**GS (–)[b]**	
CYP1A2	0	100.0	100.0	100.0
	10	94.0	91.4	102.8
	50	81.2	89.4	90.8
CYP2C8	0	100.0	100.0	100.0
	10	99.7	94.1	106.0
	50	94.4	87.6	107.8
CYP2C9	0	100.0	100.0	100.0
	10	92.5	90.3	102.4
	50	90.3	86.3	104.7
CYP2C19	0	100.0	100.0	100.0
	10	88.8	101.3	87.6
	50	94.7	88.9	106.5
CYP2D6	0	100.0	100.0	100.0
	10	94.3	98.1	96.2
	50	93.7	91.4	102.5
CYP3A	0	100.0	100.0	100.0
	10	98.4	94.7	103.9
	50	90.7	81.2	111.7

CYP activity (% of control) was shown as the mean of 2 samples. The CYP isoform-selective activities were assessed by using phenacetin (CYP1A2), rosiglitazone (CYP2C8), tolbutamide (CYP2C9), (*S*)-mephenytoin (CYP2C19), bufuralol (CYP2D6), and midazolam (CYP3A).

CTD = Common Technical Document, CYP = cytochrome P450, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate.

a: CYP activity (% of control) after preincubation with NADPH-generating system.

b: CYP activity (% of control) after preincubation without NADPH-generating system.

Additional Information: Tasurgratinib did not show time-dependent inhibition potency to any CYP isoforms tested up to 50 µmol/L.

2.6.5.12 薬物動態試験：薬物代謝酵素の誘導/阻害（続き）

Test Article: tasurgratinib succinate, M2

Inhibition of CYPs In Vitro **Study Number: AE-8378-G** **Location in CTD: 5.3.2.2.11**

Study System	in vitro
Test System	HLM
Method	For assessment of reversible inhibition (no preincubation), incubation of tasurgratinib or M2 with HLM and CYP-selective substrates in the presence of NADPH for 10 minutes (20 minutes for the CYP2C19 activity assay) at 37 °C; For assessment of time-dependent inhibition, preincubation of tasurgratinib or M2 with HLM in the presence of NADPH for 0 or 30 minutes at 37 °C followed by incubation with CYP-selective substrates for 10 minutes (20 minutes for the CYP2C19 activity assay) at 37 °C.
Assay / Analyte	LC-MS/MS / Metabolites formed by CYP-mediated metabolism
Concentration	Tasurgratinib or M2: 0.3, 1, 3, 10, 30, and 100 µmol/L

IC_{50} of Tasurgratinib and M2 for the CYP Substrate Metabolism in HLM by Reversible and Time-Dependent Inhibition Assays

CYP Isoform	Substrate Reaction	Tasurgratinib (µmol/L) IC_{50} Preincubation Time		M2 (µmol/L) IC_{50} Preincubation Time	
		0 Minutes (Reversible)	30 Minutes (Time-Dependent)	0 Minutes (Reversible)	30 Minutes (Time-Dependent)
CYP1A2	Phenacetin *O*-deethylation	>100	>100	>100	>100
CYP2B6	Bupropion hydroxylation	>100	>100	>100	>100
CYP2C8	Amodiaquine *N*-deethylation	>100	>100	>100	>100
CYP2C9	Diclofenac 4′-hydroxylation	>100	>100	77.5	74.1
CYP2C19	*S*-(+)Mephenytoin 4-hydroxylation	>100	>100	93.0	70.6
CYP2D6	(±)-Bufuralol 1′-hydroxylation	95.0	>100	82.0	88.8
CYP3A	Testosterone 6β-hydroxylation	48.0	52.0	62.4	70.4
	Midazolam 1′-hydroxylation	16.4	17.3	22.1	21.0
	Nifedipine dehydrogenation	27.9	34.9	31.6	33.8

CTD = Common Technical Document, CYP = cytochrome P450, HLM = human liver microsomes, IC_{50} = half-maximal inhibitory concentration, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate.

Additional Information: None.

2.6.5.12 薬物動態試験：薬物代謝酵素の誘導/阻害（続き）

Test Article: tasurgratinib succinate, M2

Inhibition of CYPs In Vitro　　Study Number: DMPKT2022-003　　Location in CTD: 5.3.2.2.12

Study System	in vitro
Test System	rCYP
Method	Terfenadine and tasurgratinib or terfenadine and M2 were incubated with rCYP2J2 or rCYP4F12 in the presence of NADPH-generating system for the designed time at 37 °C to evaluate the reversible inhibitory effects of tasurgratinib and M2. For time-dependent inhibition, tasurgratinib or M2 was incubated with rCYP2J2 or rCYP4F12 in the presence or absence of NADPH-generating system at 37 °C for 15 minutes. Then, the resulting sample was 10-fold diluted with a reaction mixture containing terfenadine as a substrate and incubated for the designed time at 37 °C.
Assay / Analyte	LC-MS/MS / Terfenadine alcohol metabolite formed by CYP-mediated metabolism
Concentration	Reversible inhibition: Tasurgratinib or M2; 0.03, 0.1, 0.3, 1, 3, 10, and 30 µmol/L Time-dependent inhibition: Tasurgratinib or M2; 10 µmol/L

Reversible Inhibition of CYP2J2 and CYP4F12 by Tasurgratinib, M2, and Ketoconazole in rCYPs

Inhibitor	Concentration (µmol/L)	% of Control [a]		IC_{50} (µmol/L)	
		rCYP2J2	rCYP4F12	rCYP2J2	rCYP4F12
Control	NA	100.0	100.0	NA	NA
Ketoconazole	3	6.8	12.7	NA	NA
Tasurgratinib	0.03	116.1	105.6	>30	0.446
	0.1	102.0	82.2		
	0.3	104.2	57.5		
	1	102.7	29.3		
	3	88.8	13.1		
	10	83.5	5.5		
	30	65.5	3.0		

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Test Article: tasurgratinib succinate, M2

Inhibition of CYPs In Vitro **Study Number: DMPKT2022-003** **Location in CTD: 5.3.2.2.12**

Reversible Inhibition of CYP2J2 and CYP4F12 by Tasurgratinib, M2, and Ketoconazole in rCYPs

Inhibitor	Concentration (µmol/L)	% of Control [a]		IC_{50} (µmol/L)	
		rCYP2J2	rCYP4F12	rCYP2J2	rCYP4F12
M2	0.03	94.8	89.5	25.1	0.827
	0.1	105.7	89.8		
	0.3	100.6	69.4		
	1	99.4	46.9		
	3	86.1	23.9		
	10	70.3	9.8		
	30	47.2	3.2		

Time-Dependent Inhibition of CYP2J2 and CYP4F12 by Tasurgratinib and M2 in rCYPs

Inhibitor	Concentration (µmol/L)	Remaining Activity (%) [b]	
		rCYP2J2	rCYP4F12
Tasurgratinib	10	126.2	83.6 [c]
M2	10	94.4	69.5 [c]

CTD = Common Technical Document, CYP = cytochrome P450, IC_{50} = half-maximal inhibitory concentration, IS = internal standard, NA = not applicable, NADPH-GS = reduced nicotinamide adenine dinucleotide phosphate-generating system, PAR = peak area ratio, rCYP = recombinant human CYP, TFDOH = terfenadine alcohol metabolite.

a: % of control = $PAR_{inhibition}$ / $PAR_{control}$ × 100 ; where,
$PAR_{inhibition}$: Mean PAR of TFDOH to IS in the presence of an inhibitor.
$PAR_{control}$: Mean PAR of TFDOH to IS in the absence of an inhibitor.

b: Remaining activity (%) = $(PAR_{inhibition,GS(+)}/PAR_{control,GS(+)})/(PAR_{inhibition,GS(-)}/PAR_{control,GS(-)})$ × 100 ; where,
$PAR_{inhibition,GS(+)}$: Mean PAR of TFDOH to IS in the presence of an inhibitor and NADPH-GS.
$PAR_{control,GS(+)}$: Mean PAR of TFDOH to IS in the absence of an inhibitor and presence of NADPH-GS.
$PAR_{inhibition,GS(-)}$: Mean PAR of TFDOH to IS in the presence of an inhibitor and absence of NADPH-GS.
$PAR_{control,GS(-)}$: Mean PAR of TFDOH to IS in the absence of an inhibitor and NADPH-GS.

c: PARs in the presence of tasurgratinib or M2 were comparable with or without NADPH-GS.

Additional Information: None.

2.6.5.12 薬物動態試験：薬物代謝酵素の誘導/阻害（続き）

Test Article: (*R*)-M9

Inhibition of CYPs In Vitro **Study Number: SUNBAE2022-001** **Location in CTD: 5.3.2.2.13**

Study System	in vitro
Test System	HLM
Method	Reversible inhibition: (*R*)-M9 and each CYP isoform specific marker substrate were coincubated with 0.05 mg/mL HLM in the presence of NADPH-generating system at 37 °C for 10 minutes (3 minutes for midazolam). Time-dependent inhibition: (*R*)-M9 was preincubated with 0.5 mg/mL HLM in the presence or absence of NADPH-generating system at 37 °C for 30 minutes (1st-incubation). The resulting samples were 10-fold diluted with reaction mixture containing the CYP isoform specific marker substrate and then incubated at 37 °C for 10 minutes (3 minutes for midazolam).
Assay / Analyte	LC-MS/MS / Metabolites formed by CYP-mediated metabolism
Concentration	Reversible inhibition: 0.1, 0.3, 1, 3, and 10 µmol/L; Time-dependent inhibition: 1 and 10 µmol/L

Reversible Inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A Metabolism by (*R*)-M9

CYP Isoform	Substrate	(*R*)-M9 Concentration (µmol/L)	% of Control [a]	IC_{50} (µmol/L)
CYP1A2	Phenacetin	0.1	99.5	≥10
		0.3	100.5	
		1	101.7	
		3	100.0	
		10	97.4	
CYP2B6	Bupropion	0.1	97.7	≥10
		0.3	96.5	
		1	94.8	
		3	96.5	
		10	90.3	
CYP2C8	Amodiaquine	0.1	96.4	≥10
		0.3	99.0	
		1	97.3	
		3	97.9	
		10	79.2	
CYP2C9	Diclofenac	0.1	103.0	≥10
		0.3	97.4	
		1	94.7	
		3	83.6	
		10	58.9	

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Inhibition of CYPs In Vitro　　**Study Number: SUNBAE2022-001**　　**Test Article: (*R*)-M9**　　**Location in CTD: 5.3.2.2.13**

Reversible Inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A Metabolism by (*R*)-M9

CYP Isoform	Substrate	(*R*)-M9 Concentration (µmol/L)	% of Control [a]	IC_{50} (µmol/L)
CYP2C19	(*S*)-Mephenytoin	0.1	100.3	≥10
		0.3	102.2	
		1	101.5	
		3	97.9	
		10	86.9	
CYP2D6	Bufuralol	0.1	98.2	≥10
		0.3	96.7	
		1	95.4	
		3	94.4	
		10	88.2	
CYP3A	Midazolam	0.1	101.7	≥10
		0.3	98.6	
		1	99.2	
		3	95.0	
		10	85.6	
	Testosterone	0.1	97.4	≥10
		0.3	99.4	
		1	95.8	
		3	89.0	
		10	76.7	

Time-Dependent Inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A Metabolism by (*R*)-M9

CYP Isoform	Substrate	(*R*)-M9 Concentration (µmol/L)	Relative Activity [b] (%)
CYP1A2	Phenacetin	1	100.8
		10	96.3
CYP2B6	Bupropion	1	96.3
		10	97.2
CYP2C8	Amodiaquine	1	105.8
		10	104.4
CYP2C9	Diclofenac	1	104.2
		10	104.3

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Test Article: (*R*)-M9

Inhibition of CYPs In Vitro **Study Number: SUNBAE2022-001** **Location in CTD: 5.3.2.2.13**

Time-Dependent Inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A Metabolism by (*R*)-M9			
CYP Isoform	**Substrate**	**(*R*)-M9 Concentration (µmol/L)**	**Relative Activity [b] (%)**
CYP2C19	(*S*)-Mephenytoin	1	103.1
		10	103.7
CYP2D6	Bufuralol	1	101.4
		10	101.0
CYP3A	Midazolam	1	102.3
		10	93.4

CTD = Common Technical Document, CYP = cytochrome P450, GS = NADPH-generating system, HLM = human liver microsomes, IC_{50} = half-maximal inhibitory concentration, IS = internal standard, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate, PAR = peak area ratio.

a: % of control = $(PAR_{inhibition} / PAR_{control}) \times 100$

where,

$PAR_{inhibition}$: Mean PAR of a CYP isoform-selective metabolite to the corresponding IS in the presence of an inhibitor.

$PAR_{control}$: Mean PAR of a CYP isoform-selective metabolite to the corresponding IS in the absence of an inhibitor (vehicle control).

b:

$$\text{Relative activity (\%)} = \frac{\left(\frac{PAR_{inhibition,\ GS(+)}}{PAR_{control,\ GS(+)}}\right)}{\left(\frac{PAR_{inhibition,\ GS(-)}}{PAR_{control,\ GS(-)}}\right)} \times 100$$

where,

$PAR_{inhibition,\ GS(+)}$: Mean PAR of a CYP isoform-selective metabolite to IS after 1st-incubation with both an inhibitor and NADPH-generating system.

$PAR_{control,\ GS(+)}$: Mean PAR of a CYP isoform-selective metabolite to IS after 1st-incubation without an inhibitor (vehicle control) in the presence of NADPH-generating system.

$PAR_{inhibition,\ GS(-)}$: Mean PAR of a CYP isoform-selective metabolite to IS after 1st-incubation with an inhibitor in the absence of NADPH-generating system.

$PAR_{control,\ GS(-)}$: Mean PAR of a CYP isoform-selective metabolite to IS after 1st-incubation without both an inhibitor (vehicle control) and NADPH-generating system.

Additional Information: None.

2.6.5.12 薬物動態試験：薬物代謝酵素の誘導/阻害（続き）

Test Article: tasurgratinib succinate

Induction of CYPs In Vitro (Percentage of Positive Control) **Study Number: DMPKT2013-019** **Location in CTD: 5.3.2.2.14**

Study System/Test System	in vitro/Cryopreserved human hepatocytes			
Method	Cell cultures, 24-hour treatment for 3 days incubation at 37 °C			
Assay/Analyte	Quantitative reverse transcription polymerase chain reaction/mRNA			
Concentration	Tasurgratinib concentration: 0.3, 1, or 3 µmol/L			
Treatment	**Concentration**	**CYP1A2 mRNA Level (Percentage of Positive Control)**		
		HH1024	**HEP187188**	**HEP187087**
Vehicle Control	—[a]	0.0±0.1	0.0±0.1	0.0±0.0
Tasurgratinib	0.3 µmol/L	1.5±0.1	1.3±0.1	2.9±0.1
Tasurgratinib	1 µmol/L	4.4±0.3	2.9±0.0	4.5±0.1
Tasurgratinib	3 µmol/L	11.6±2.5	5.9	5.2±0.5
Omeprazole	50 µmol/L	100.0±4.6	100.0±5.0	100.0±3.1
Flumazenil	30 µmol/L	0.3±0.0	0.2±0.0	0.1±0.0
Treatment	**Concentration**	**CYP2B6 mRNA Level (Percentage of Positive Control)**		
		HH1024	**HEP187188**	**HEP187087**
Vehicle Control	—[a]	0.0±1.0	0.0±0.6	0.0±0.2
Tasurgratinib	0.3 µmol/L	2.7±1.5	0.7±0.2	0.5±0.1
Tasurgratinib	1 µmol/L	13.7±1.3	5.7±0.1	2.9±0.2
Tasurgratinib	3 µmol/L	26.3±2.5	18.4	7.8±0.5
Phenobarbital	750 µmol/L	100.0±19.8	100.0±8.4	100.0±4.0
Flumazenil	30 µmol/L	1.5±0.7	0.8±0.6	0.1±0.3
Treatment	**Concentration**	**CYP3A4 mRNA Level (Percentage of Positive Control)**		
		HH1024	**HEP187188**	**HEP187087**
Vehicle Control	—[a]	0.0±0.0	0.0±0.0	0.0±0.0
Tasurgratinib	0.3 µmol/L	0.0±0.0	0.1±0.1	0.0±0.0
Tasurgratinib	1 µmol/L	1.9±0.6	0.5±0.1	0.9±0.0
Tasurgratinib	3 µmol/L	10.9±0.7	2.8	2.0±0.3
Rifampicin	20 µmol/L	100.0±5.1	100.0±4.9	100.0±2.9
Flumazenil	30 µmol/L	0.0±0.4	0.1±0.1	0.1±0.1

Values are the mean of triplicate determinations ±SEM except for tasurgratinib at 3 µmol/L in HEP187188 (mean of duplicate determinations). Omeprazole, phenobarbital, and rifampicin were used as the positive controls for CYP1A2, CYP2B6, and CYP3A4, respectively. Flumazenil was used as negative control.
CTD = Common Technical Document, CYP = cytochrome P450, mRNA = messenger RNA.
a: 0.1% dimethyl sulfoxide.
Additional Information: Tasurgratinib (concentration: 0.3, 1, and 3 µmol/L) slightly increased mRNA levels of CYP1A2, CYP2B6, and CYP3A4 in a concentration-dependent manner. The maximum percentages of mRNA increase to positive control by the treatment of tasurgratinib at 3 µmol/L were 11.6% for CYP1A2 mRNA, 26.3% for CYP2B6 mRNA, and 10.9% for CYP3A4 mRNA, respectively.

2.6.5.12 薬物動態試験：薬物代謝酵素の誘導/阻害（続き）

Test Article: M2

Induction of CYPs In Vitro (Fold Induction of CYP mRNA Expression) **Study Number: SUNBAE2020-002** **Location in CTD: 5.3.2.2.15**

Study System/Test System	in vitro/Cryopreserved human hepatocytes			
Method	Cell cultures, 24-hour treatment for 3 days incubation at 37 °C			
Assay/Analyte	Quantitative reverse transcription polymerase chain reaction/mRNA			
Concentration	M2 concentration: 0.3, 1, 3, 5, or 10 µmol/L			
Fold Induction of CYP1A2, CYP2B6, and CYP3A4 mRNA Expression After Treatment With M2, Typical CYP Inducers, and Negative Control				
Treatment	**Concentration**	**Fold Induction of CYP1A2 mRNA Expression**		
		Lot HU8200-A	**Lot HU8216**	**Lot HU1574**
Vehicle Control	—[a]	1.00±0.07	1.00±0.05	1.00±0.09
Flumazenil	30 µmol/L	0.72±0.01	0.70±0.03	0.72±0.05
Omeprazole	50 µmol/L	34.44±7.02	40.65±1.41	39.58±3.27
M2	0.3 µmol/L	0.68±0.04	0.97±0.08	0.90±0.11
	1 µmol/L	0.85±0.01	1.33±0.03	1.11±0.03
	3 µmol/L	0.89±0.05	1.44±0.02	1.21±0.03
	5 µmol/L	0.82±0.02	1.32±0.01	1.06±0.02
	10 µmol/L	0.49±0.02	0.63±0.01	0.10±0.01
Treatment	**Concentration**	**Fold Induction of CYP2B6 mRNA Expression**		
		Lot HU8200-A	**Lot HU8216**	**Lot HU1574**
Vehicle Control	—[a]	1.00±0.09	1.00±0.03	1.00±0.08
Flumazenil	30 µmol/L	1.37±0.11	1.29±0.06	1.32±0.13
Phenobarbital	750 µmol/L	21.75±1.45	7.70±0.22	8.81±0.50
M2	0.3 µmol/L	1.21±0.19	0.99±0.16	1.24±0.11
	1 µmol/L	1.86±0.08	1.37±0.04	1.33±0.05
	3 µmol/L	1.91±0.19	1.29±0.01	1.62±0.04
	5 µmol/L	1.87±0.07	1.11±0.01	1.72±0.01
	10 µmol/L	0.77±0.03	0.35±0.01	0.18±0.01
Treatment	**Concentration**	**Fold Induction of CYP3A4 mRNA Expression**		
		Lot HU8200-A	**Lot HU8216**	**Lot HU1574**
Vehicle Control	—[a]	1.00±0.10	1.00±0.11	1.00±0.05
Flumazenil	30 µmol/L	1.27±0.08	1.16±0.11	1.17±0.11
Rifampicin	20 µmol/L	118.73±1.06	33.48±0.34	77.34±2.23
M2	0.3 µmol/L	1.33±0.37	1.17±0.18	0.96±0.03
	1 µmol/L	2.01±0.10	1.35±0.02	0.74±0.04
	3 µmol/L	2.52±0.26	1.29±0.03	0.82±0.04
	5 µmol/L	2.96±0.04	1.37±0.02	1.07±0.08
	10 µmol/L	1.46±0.03	0.52±0.01	0.49±0.01

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Test Article: M2

Induction of CYPs In Vitro (Percentage of Positive Control) **Study Number: SUNBAE2020-002** **Location in CTD: 5.3.2.2.15**

Percentage of Positive Control on CYP1A2, CYP2B6, and CYP3A4 mRNA Expression After Treatment With M2, Typical CYP Inducers, and Negative Control				
Treatment	**Concentration**	**Percentage of Positive Control (CYP1A2)**		
		Lot HU8200-A	**Lot HU8216**	**Lot HU1574**
Vehicle Control	—[a]	0.0±0.2	0.0±0.1	0.0±0.2
Flumazenil	30 µmol/L	–0.8±0.0	–0.7±0.1	–0.7±0.2
Omeprazole	50 µmol/L	100.0±21.0	100.0±3.5	100.0±8.5
M2	0.3 µmol/L	–0.9±0.1	–0.1±0.2	–0.3±0.3
	1 µmol/L	–0.4±0.0	0.8±0.1	0.3±0.1
	3 µmol/L	–0.3±0.2	1.1±0.1	0.5±0.1
	5 µmol/L	–0.5±0.1	0.8±0.0	0.2±0.1
	10 µmol/L	–1.5±0.1	–0.9±0.0	–2.3±0.0
Treatment	**Concentration**	**Percentage of Positive Control (CYP2B6)**		
		Lot HU8200-A	**Lot HU8216**	**Lot HU1574**
Vehicle Control	—[a]	0.0±0.5	0.0±0.5	0.0±1.0
Flumazenil	30 µmol/L	1.8±0.5	4.3±0.9	4.1±1.7
Phenobarbital	750 µmol/L	100.0±7.0	100.0±3.3	100.0±6.3
M2	0.3 µmol/L	1.0±0.9	–0.1±2.4	3.1±1.4
	1 µmol/L	4.2±0.4	5.5±0.6	4.2±0.6
	3 µmol/L	4.4±0.9	4.3±0.1	7.9±0.5
	5 µmol/L	4.2±0.3	1.7±0.2	9.2±0.1
	10 µmol/L	–1.1±0.2	–9.7±0.2	–10.5±0.1
Treatment	**Concentration**	**Percentage of Positive Control (CYP3A4)**		
		Lot HU8200-A	**Lot HU8216**	**Lot HU1574**
Vehicle Control	—[a]	0.0±0.1	0.0±0.3	0.0±0.1
Flumazenil	30 µmol/L	0.2±0.1	0.5±0.4	0.2±0.1
Rifampicin	20 µmol/L	100.0±0.9	100.0±1.1	100.0±2.9
M2	0.3 µmol/L	0.3±0.3	0.5±0.6	0.0±0.0
	1 µmol/L	0.9±0.1	1.1±0.1	–0.3±0.1
	3 µmol/L	1.3±0.2	0.9±0.1	–0.2±0.0
	5 µmol/L	1.6±0.0	1.1±0.1	0.1±0.1
	10 µmol/L	0.4±0.0	–1.5±0.1	–0.7±0.0

Values represent the mean fold induction or the mean percentage of positive control ±SEM (n=3). Omeprazole, phenobarbital, and rifampicin were used as positive controls for induction of CYP1A2, CYP2B6, and CYP3A4, respectively. Flumazenil was used as a negative control.
CTD = Common Technical Document, CYP = cytochrome P450, mRNA = messenger RNA.
a: 0.1% dimethyl sulfoxide.
Additional Information: None.

2.6.5.13 薬物動態試験：排泄

Test Article: [^{14}C]tasurgratinib

Rats **Study Number: 8362069** **Location in CTD: 4.2.2.2.5**

Strain/Gender/Number of Animals	Sprague Dawley (albino animal)/Male/3 (intact) or 4 (bile duct-cannulated)	
Feeding Condition	Fasted	
Vehicle / Formulation	0.1 mol/L hydrochloric acid / Solution	
Method of Administration / Dose	Oral / 3 mg/kg as free form	
Radionuclide / Specific Activity (Lot No.)	[^{14}C] / [redacted] MBq/mg (AL-IC-11141-0080)	
Analyte/Assay	Radioactivity/Liquid scintillation counting	
Sample	**Recovery of Radioactivity (% of Administered Dose)[a]**	
	Intact Rats	**Bile Duct-Cannulated Rats**
Urine	4.02±3.02	34.8±6.95
Feces	96.3±2.48	17.8±2.71
Bile	NA	38.0±5.89
Cage wash	0.52±0.55	1.85±0.70
Final cage wash	0.00±0.00	0.13±0.07
CO_2 traps[b]	0.06±0.04	NA
Cage debris	ND	0.08±0.11
Carcass	0.29±0.16	2.29±0.83
Total	101±1.55[c]	95.0±3.09[d]

CTD = Common Technical Document, NA = not applicable, ND = not detected.

a: Mean ±SD of 3 (intact) or 4 (bile duct-cannulated) animals.

b: Expired air trapping solutions were collected up to 24 hours postdose.

c: Excreta samples were collected up to 168 hours post-dose, and the radioactivity collected in the excreta samples were combined with those in cage wash, final cage wash, CO_2 traps, cage debris, and carcass.

d: Bile and excreta were collected up to 72 hours post-dose, and the radioactivity collected in the excreta and bile samples were combined with those in cage wash, final cage wash, cage debris, and carcass.

Additional Information: This study also investigated the blood and plasma level of radioactivity (Section 2.6.5.3) and the distribution of radioactivity in organs and tissues (Section 2.6.5.5).

2.6.5.13 薬物動態試験：排泄（続き）

Test Article: [^{14}C]tasurgratinib

Dogs **Study Number: 8361652** **Location in CTD: 4.2.2.2.6**

Strain/Gender/Number of Animals	Beagle/Male/3
Feeding Condition	Fasted
Vehicle / Formulation	100 mmol/L hydrochloric acid / Solution
Method of Administration / Dose	Oral / 1.5 mg/kg as free form
Radionuclide / Specific Activity (Lot No.)	[^{14}C] / [redacted] MBq/mg (AL-IC-11141-0080)
Analyte/Assay	Radioactivity/Liquid scintillation counting
Sample	**Oral Administration (n=3)[a]**
	% of Administered Dose
Urine	4.47±0.96
Feces	86.7±3.55
Cage wash	0.49±0.15
Cage debris	0.01±0.01
Final cage wash	0.03±0.01
Swabs	0.38±0.27
Total (0 – 168 h)[b]	92.0±2.21

CTD = Common Technical Document.

a: Mean ±SD of 3 animals.

b: The radioactivity in the excreta samples were combined with those in cage wash, final cage wash, cage debris, and swabs collected up to 168 hours post-dose.

Additional Information: This study also investigated the blood and plasma level of radioactivity (Section 2.6.5.3).

2.6.5.14 薬物動態試験：排泄：胆汁中

胆管カニューレ処置ラットに[^{14}C]タスルグラチニブを単回経口投与したときの胆汁排泄については，2.6.5.13 項に記した。

2.6.5.15 薬物動態試験：薬物相互作用

非臨床 in vivo における薬物間相互作用試験は検討されていない。

In vitro における薬物間相互作用（CYP 誘導及び CYP 阻害を含む）にかかわる試験はヒト生体試料を用いて評価した（2.6.5.12 及び 2.7.2.1.1 項）。ヒト薬物トランスポーターの基質となるか，又はそれらを阻害する可能性については，2.6.5.16 及び 2.7.2.1.1 項に記した。

2.6.5.16 薬物動態試験：その他

Test Article: [^{14}C]tasurgratinib

Transport by OATP1B1 or OATP1B3 **Study Number: GE-1493-G** **Location in CTD: 5.3.2.3.3**

Study System	in vitro
Test System	Uptake transporter using respective transporter expressing cells: HEK293 cells for OATP1B1 and OATP1B3. (Mock-transfected HEK293 cells were used as a control.)
Method	Incubation of [^{14}C]tasurgratinib in the test systems for 1, 2, or 5 minutes at 37 °C
	Typical substrate (concentration): [^{3}H]Estradiol 17β-D-glucuronide (0.05 µmol/L)
	Typical inhibitor (concentration): Rifampicin (10 µmol/L)
Radionuclide / Specific Activity (Lot No.)	[^{14}C] / [redacted] MBq/mg (AL-IC-11141-0080)
Assay/Analyte	Liquid scintillation counting/^{14}C radioactivity
Concentration	1 and 30 µmol/L
Results	Uptakes of [^{14}C]tasurgratinib into OATP1B1- or OATP1B3-expressing cells at concentrations of 1 and 30 µmol/L were almost the same as those in control cells. Therefore, the results suggested that tasurgratinib was not a substrate of OATP1B1 and OATP1B3.

CTD = Common Technical Document, OATP = organic anion transporting polypeptide.

Additional Information: None.

2.6.5.16 薬物動態試験：その他（続き）

Test Article: [^{14}C]tasurgratinib

Transport by P-gp and BCRP **Study Number: DMPKT2016-009** **Location in CTD: 5.3.2.3.2**

Study System	in vitro
Test System	Cultured monolayers of LLC-PK1 cells and human P-gp-expressing LLC-PK1 (LLC-MDR1) cells Cultured monolayers of MDCK II cells and human BCRP-expressing MDCK II (MDCK II-BCRP) cells
Method	Incubation of [^{14}C]tasurgratinib with cell monolayers for 2 and 4 hours at 37 °C
Radionuclide / Specific Activity (Lot No.)	[^{14}C] / [redacted] MBq/mg (AL-IC-11141-0080)
Assay/Analyte	Liquid scintillation counting/^{14}C radioactivity
Concentration	1, 3, and 10 µmol/L for P-gp assay and 1 µmol/L for BCRP assay
Results	
Substrate susceptibility of tasurgratinib to P-gp	The CFR value of [^{14}C]tasurgratinib (1, 3, and 10 µmol/L) at 2 and 4 hours were 12.58 to 16.61, showing no time- and concentration-dependencies, and inhibitory effect on [^{14}C]tasurgratinib transport via P-gp by verapamil (30 µmol/L) was detected. These results indicated that tasurgratinib was a substrate for P-gp.
Substrate susceptibility of tasurgratinib to BCRP	The CFR values of [^{14}C]tasurgratinib (1 µmol/L) were 1.07 and 1.00 at 2 and 4 hours, respectively. These results demonstrated that tasurgratinib was not a substrate for BCRP.

BCRP = breast cancer resistance protein, CFR = corrected flux ratio, CTD = Common Technical Document, P-gp = P-glycoprotein.

Additional Information: None.

2.6.5.16 薬物動態試験：その他（続き）

Test Article: tasurgratinib succinate, M2

Inhibition of P-gp and BCRP **Study Number: DMPKT2016-009** **Location in CTD: 5.3.2.3.2**

Study System	in vitro
Test System	Cultured monolayers of LLC-PK1 cells and human P-gp-expressing LLC-PK1 (LLC-MDR1) cells Cultured monolayers of MDCK II cells and human BCRP-expressing MDCK II (MDCK II-BCRP) cells
Method	Incubation of tasurgratinib or M2 with cell monolayers for 2 hours at 37 °C
Assay/Analyte	Liquid scintillation counting/^{3}H radioactivity
Concentration	Tasurgratinib: 3.9 and 13 µmol/L for P-gp assay, and 13 and 39 µmol/L for BCRP assay M2: 10 and 30 µmol/L for P-gp assay, and 30 and 100 µmol/L for BCRP assay
Results	
Inhibitory effect of tasurgratinib and M2 on P-gp	Tasurgratinib (3.9 and 13 µmol/L) and M2 (10 and 30 µmol/L) showed no inhibition on [^{3}H]digoxin (1 µmol/L) transport.
Inhibitory effect of tasurgratinib and M2 on BCRP	Tasurgratinib at 39 µmol/L and M2 at 100 µmol/L, the highest concentrations, showed weak inhibition potencies on [^{3}H]prazosin (1 µmol/L) transport (74.1% and 53.4% of control, respectively), and the IC_{50} values of tasurgratinib and M2 were more than 39 µmol/L and 100 µmol/L, respectively.

BCRP = breast cancer resistance protein, CTD = Common Technical Document, IC_{50} = half-maximal inhibitory concentration, P-gp = P-glycoprotein.

Additional Information: None.

2.6.5.16 薬物動態試験：その他（続き）

Test Article: tasurgratinib succinate, M2

Inhibition of OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K **Study Number: GE-1704-G** **Location in CTD: 5.3.2.3.4**

Study System	in vitro			
Assay Condition	**Transporters**	**Assay Systems**	**Typical Substrates (Concentration)**	**Typical Inhibitors (Concentration)**
	OATP1B1	HEK293 cells	[^{3}H]Estradiol 17β-D-glucuronide (0.05 μmol/L)	Rifampicin (10 μmol/L)
	OATP1B3			
	OAT1		[^{3}H]*p*-Aminohippuric acid (1 μmol/L)	Probenecid (100 μmol/L)
	OAT3		[^{3}H]Estrone sulfate (0.05 μmol/L)	
	OCT1		[^{14}C]Metformin (10 μmol/L)	Quinidine (100 μmol/L)
	OCT2			Quinidine (300 μmol/L)
	MATE1			Cimetidine (10 μmol/L)
	MATE2-K			Cimetidine (100 μmol/L)
Method	OAT1 assay: Incubation with cells for 0.5 minutes at 37 °C OAT3, OCT2, OATP1B1, and OATP1B3 assay: Incubation with cells for 2 minutes at 37 °C OCT1, MATE1, and MATE2-K assay: Incubation with cells for 5 minutes at 37 °C			
Assay/Analyte	Liquid scintillation counting/^{3}H or ^{14}C radioactivity			
Concentration	Tasurgratinib and M2: 0.3, 1, 3, 10, and 30 μmol/L (0.01, 0.03, 0.1, 0.3, and 1 μmol/L for MATE1)			
Results				
Inhibitory effect of transporters by tasurgratinib	Tasurgratinib inhibited OATP1B1-mediated uptake of [^{3}H]estradiol 17β-D-glucuronide, OATP1B3-mediated uptake of [^{3}H]estradiol 17β-D-glucuronide, OCT1-mediated uptake of [^{14}C]metformin, OCT2-mediated uptake of [^{14}C]metformin, MATE1-mediated uptake of [^{14}C]metformin, and MATE2-K-mediated uptake of [^{14}C]metformin with the IC_{50} values of 14.6, 6.43, 0.888, 5.53, 0.0870, and 5.99 μmol/L, respectively. Weak inhibitory potential of tasurgratinib on OAT1-mediated uptake of [^{3}H]*p*-aminohippuric acid was observed, resulting in uptake that was 76.5% of control at 30 μmol/L (IC_{50} >30 μmol/L). No inhibitory effect on OAT3-mediated uptake of [^{3}H]estrone sulfate was detected (102.6% or more of control, IC_{50} >30 μmol/L).			
Inhibitory effect of transporters by M2	M2 inhibited OATP1B1-mediated uptake of [^{3}H]estradiol 17β-D-glucuronide, OATP1B3-mediated uptake of [^{3}H]estradiol 17β-D-glucuronide, OCT1-mediated uptake of [^{14}C]metformin, OCT2-mediated uptake of [^{14}C]metformin, MATE1-mediated uptake of [^{14}C]metformin, and MATE2-K-mediated uptake of [^{14}C]metformin with the IC_{50} values of 18.6, 7.43, 0.332, 3.85, 0.0287, and 6.76 μmol/L, respectively. Minimal or no inhibitory effects on OAT1-mediated uptake of [^{3}H]*p*-aminohippuric acid and OAT3-mediated uptake of [^{3}H]estrone sulfate were detected (84.5% or more of control, IC_{50} >30 μmol/L).			

CTD = Common Technical Document, IC_{50} = half-maximal inhibitory concentration, MATE = multidrug and toxin extrusion, OAT = organic anion transporter, OATP = organic anion transporting polypeptide, OCT = organic cation transporter.

Additional Information: None.

2.6.5.16 薬物動態試験：その他（続き）

Test Article: M2

Transport by P-gp, BCRP, OATP1B1, and OATP1B3 **Study Number: GE-1828-G** **Location in CTD: 5.3.2.3.5**

Study System	in vitro			
Assay Condition	**Transporters**	**Assay Systems**	**Typical Substrates (Concentration)**	**Typical Inhibitors (Concentration)**
	P-gp	Transcellular transport using Caco-2 cells	[^{3}H]Digoxin (1 µmol/L)	Verapamil (30 µmol/L)
	BCRP		[^{3}H]Estrone sulfate (0.1 µmol/L)	Ko143 (1 µmol/L)
	OATP1B1	Cellular uptake using transporter-transfected HEK293 cells	[^{3}H]Estradiol 17β-D-glucuronide (0.05 µmol/L)	Rifampicin (10 µmol/L)
	OATP1B3			
Method	P-gp or BCRP assay: Incubation of M2 with cell monolayers for 0.5, 1, and 2 hours at 37 °C OATP1B1 or OATP1B3 assay: Incubation of M2 with cell monolayers for 2, 5, and 10 minutes at 37 °C			
Assay / Analyte	LC-MS/MS / M2			
Concentration	1 and 10 µmol/L for each assay			
Results				
Substrate susceptibility of M2 to P-gp or BCRP	The FR value of M2 (10 µmol/L) was 12.4 in Caco-2 cells, and decreased to 1.8 in the presence of a typical P-gp inhibitor, verapamil (30 µmol/L). Meanwhile, the FR value of M2 was not decreased in the presence of a typical BCRP inhibitor, Ko143 (1 µmol/L) (FR = 10.2). These results indicated that M2 was a substrate of P-gp but not a substrate of BCRP.			
Substrate susceptibility of M2 to OATP1B1 or OATP1B3	The uptakes of M2 (1 and 10 µmol/L) in the OATP1B1- and OATP1B3-expressing cells were comparable with that in the control cells, and rifampicin did not affect the uptake of M2. These results indicated that M2 was not a substrate of OATP1B1 and OATP1B3.			

BCRP = breast cancer resistance protein, CTD = Common Technical Document, FR = flux ratio, LC-MS/MS = liquid chromatography with tandem mass spectrometry, LLOQ =lower limit of quantification, OATP = organic anion transporting polypeptide, P-gp = P-glycoprotein.

Additional Information: In regard to M2 (1 µmol/L) group and M2 (1 µmol/L) with Ko143 group, since M2 was not detected (below LLOQ) in the samples collected after 0.5- and 1-hour incubations in apical to basal, the FR values were not calculable. Thus, the results in M2 (1 µmol/L) groups were not used for determining if the M2 was a substrate of P-gp and BCRP.

2.6.5.16 薬物動態試験：その他（続き）

Test Article: (*R*)-M9

Inhibition of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K　**Study Number: GE-2013-G**　**Location in CTD: 5.3.2.3.6**

Study System	in vitro			
Assay Condition	**Transporters**	**Assay Systems**	**Probe Substrates (Concentration)**	**Typical Inhibitors (Concentration)**
	P-gp	LLC-PK1 cells	[^{3}H]Digoxin (1 µmol/L)	Verapamil (30 µmol/L)
	BCRP		[^{3}H]Prazosin (0.01 µmol/L)	Ko143 (1 µmol/L)
	OATP1B1	HEK293 cells	[^{3}H]Estradiol 17β-D-glucuronide (0.05 µmol/L)	Rifampicin (10 µmol/L)
	OATP1B3			
	OAT1		[^{3}H]*p*-Aminohippuric acid (1 µmol/L)	Probenecid (100 µmol/L)
	OAT3		[^{3}H]Estrone sulfate (0.05 µmol/L)	
	OCT1		[^{14}C]Metformin (10 µmol/L)	Quinidine (100 µmol/L)
	OCT2			Quinidine (300 µmol/L)
	MATE1			Cimetidine (10 µmol/L)
	MATE2-K			Cimetidine (100 µmol/L)
Method	P-gp and BCRP assay: Incubation with cells for 2 hours (P-gp) or 1 hour (BCRP) at 37 °C OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K assay: Incubation with cells for 0.5 to 5 minutes at 37 °C			
Assay/Analyte	Liquid scintillation counting/^{3}H or ^{14}C radioactivity			
Concentration	(*R*)-M9: 0.1, 0.3, 1, 3, and 10 µmol/L			
Results				
Inhibitory effect of transporters by (*R*)-M9	(*R*)-M9 inhibited OATP1B1-mediated uptake of [^{3}H]estradiol 17β-D-glucuronide, OATP1B3-mediated uptake of [^{3}H]estradiol 17β-D-glucuronide, OCT1-mediated uptake of [^{14}C]metformin, OCT2-mediated uptake of [^{14}C]metformin, and MATE1-mediated uptake of [^{14}C]metformin with the IC_{50} values of 1.57, 0.850, 1.02, 3.28, and 0.749 µmol/L, respectively. (*R*)-M9 exhibited weak inhibitory potential on P-gp-mediated transport of [^{3}H]digoxin, BCRP-mediated transport of [^{3}H]prazosin, OAT1-mediated uptake of [^{3}H]*p*-aminohippuric acid, and MATE2-K-mediated uptake of [^{14}C]metformin, resulting in 77.2%, 71.9%, 60.5%, and 64.6% of control at 10 µmol/L, respectively (IC_{50}>10 µmol/L). (*R*)-M9 exhibited no inhibitory effect on OAT3-mediated uptake of [^{3}H]estrone sulfate (104.5% of control, IC_{50}>10 µmol/L).			

BCRP = breast cancer resistance protein, CTD = Common Technical Document, IC_{50} = half-maximal inhibitory concentration, MATE = multidrug and toxin extrusion, OAT = organic anion transporter, OATP = organic anion transporting polypeptide, OCT = organic cation transporter, P-gp = P-glycoprotein.

Additional Information: None.