2.6.4 薬物動態試験の概要文

小野薬品工業株式会社

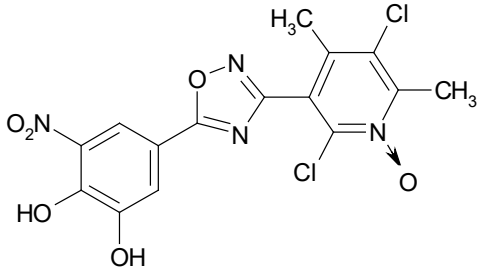
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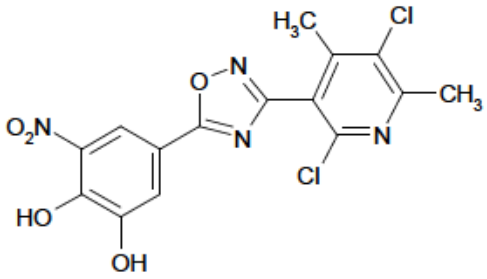
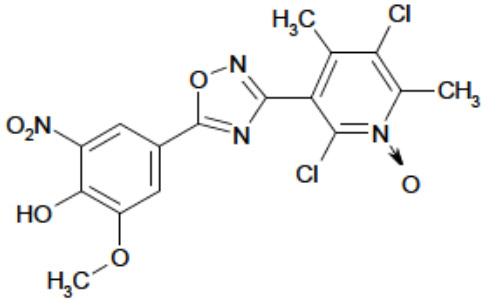
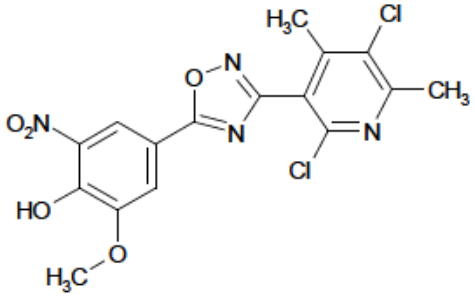
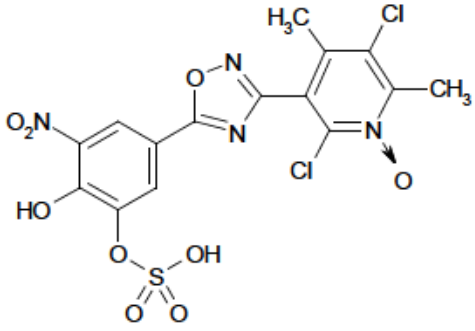
略号一覧

略号	略さない表現又は説明
AHR	Aryl hydrocarbon receptor
ATP	Adenosine triphosphate
AUC	血漿中濃度－時間曲線下面積
AUCinf	投与後無限大時間までの AUC
AUClast	最終定量時点までの AUC
BCRP	Breast cancer resistance protein
BSEP	Bile salt export pump
CAR	Constitutive androstane receptor
Cmax	最高血漿中濃度又は最高組織中濃度
COMT	Catechol-*O*-methyltransferase
CYP	チトクロム P450
DNP	2,4-ジニトロフェノール
LC/MS/MS	液体クロマトグラフィータンデム型質量分析
LSC	液体シンチレーションカウンター
MATE	Multidrug and toxin extrusion
MRP	Multidrug resistance-associated protein
NADPH	ニコチンアミドアデニンジヌクレオチドリン酸
OAT	Organic anion transporter
OATP	Organic anion transporting polypeptide
OCT	Organic cation transporter
PAPS	3′-ホスホアデノシン-5′-ホスホ硫酸
P-gp	P-糖蛋白質
PXR	Pregnane X receptor
QWBA	定量的全身オートラジオグラフィー
S9	組織ホモジネートの 9000*g* 上清
SULT	硫酸転移酵素
T1/2	消失半減期
Tmax	最高血漿中濃度到達時間又は最高組織中濃度到達時間
UDPGA	ウリジン 5′-二リン酸グルクロン酸
UGT	ウリジン 5′-二リン酸グルクロン酸転移酵素

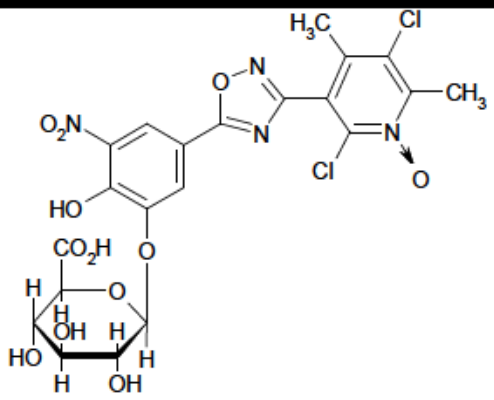
関連化合物の一覧

名称	構造	説明及びその他の名称
オピカポン		BIA 9-1067 Opicapone

2.6.4 薬物動態試験の概要文
オピカポン

名称	構造	説明及びその他の名称
BIA 9-1079		オピカポンの *N*-オキシド還元体
BIA 9-1100		オピカポンの 3-*O*-メチル化体
BIA 9-1101		BIA 9-1079 の 3-*O*-メチル化体
BIA 9-1102		
BIA 9-1103		オピカポンの 3-*O*-硫酸抱合体

2.6.4 薬物動態試験の概要文
オピカポン

名称	構造	説明及びその他の名称
BIA 9-1104	[redacted]	
BIA 9-1105		
BIA 9-1106		オピカポンの 3-*O*-グルクロン酸抱合体
BIA 9-1107	[redacted]	

2.6.4 薬物動態試験の概要文

2.6.4.1 まとめ

有効性薬理試験及び毒性試験と同種同系統のラット（Wistar）及びサル（カニクイザル）に ^{14}C-オピカポン又はオピカポンを投与したときの薬物動態を評価した．単回投与試験では特記しない限り雄動物を使用し，非絶食下で投与した．静脈内投与で評価した一部の試験を除き，臨床投与経路と同じ経口投与で評価した．その他，マウス，ラット，イヌ，サル及びヒト試料を用いた *in vitro* 試験，並びにマウス，ラット，モルモット，ハムスター，イヌ，サル及びミニブタを用いた *in vivo* 試験を実施した．

2.6.4.1.1 吸収

ラット及びサルに ^{14}C-オピカポンを単回経口投与したとき，血漿中放射能濃度の Tmax は 4 及び 0.25 時間であり，吸収は速やかであった．ラット及びサルにオピカポンを経口投与したとき，血漿中オピカポンの Cmax 及び AUC は投与量に応じて増加した．

2.6.4.1.2 分布

ラットに ^{14}C-オピカポンを経口投与したとき，多くの組織中放射能濃度の Tmax は 4 時間であった．肝臓及び腎臓での組織中放射能濃度は血液より高く，その他の組織中放射能濃度は血液より低かった．脳組織中の放射能濃度はすべての時点で定量下限未満であった．妊娠ラットに ^{14}C-オピカポンを経口投与したとき，放射能が胎児に移行した．^{14}C-オピカポンの血漿蛋白結合率はラットで 99.5～99.8%，サルで 99.4～99.6%，ヒトで 99.9%であった．

2.6.4.1.3 代謝

動物及びヒトでの *in vitro* 又は *in vivo* 試料において検出されたオピカポンの代謝物は，硫酸抱合体，グルクロン酸抱合体，還元体及びメチル化体であったことから，オピカポンの代謝には SULT，UGT，還元酵素及び COMT が関与していると考えられた．また，オピカポンはヒト CYP 発現系ミクロソームにより代謝されたことから，CYP も代謝に関与していると考えられた．ヒト肝 S9 による *in vitro* 代謝試験で検出された代謝物はラット及びサル血漿中に検出され，ヒト特異的な代謝物は認められなかった．BIA 9-1103（オピカポンの 3-*O*-硫酸抱合体）はヒトの血漿中主代謝物であり，動物の血漿中でも認められた．BIA 9-1079（オピカポンの *N*-オキシド還元体）は動物の血漿中主代謝物の 1 つであったが，ヒト血漿中濃度は低く，多くの時点で定量下限未満であった．

2.6.4.1.4 排泄

ラット及びサルに ^{14}C-オピカポンを経口投与又は静脈内投与したとき，放射能は主に糞中へ排泄された．授乳中雌ラットに ^{14}C-オピカポンを経口投与したとき，放射能が乳汁中に排泄された．

2.6.4.1.5 薬物動態学的薬物相互作用

Caco-2 細胞及び各種トランスポーターの発現系細胞を用いて基質認識性を評価した結果，オピカポンはヒトの OATP1B3，BCRP 及び P-gp の基質であった．CYP 阻害及び誘導に関して，オピカポンは CYP2C8（Ki 値 0.9 μg/mL）及び CYP2C9（Ki 値 18 μg/mL）を阻害したが，CYP 誘導作用は認められなかった．オピカポンは OATP1B1，OATP1B3，OAT1，OAT3，MATE2-K 及び BSEP を阻害し，IC_{50} 値はそれぞれ 0.45，1.40，9.18，4.36，約 30 及び 12.28 μg/mL であった．

非臨床薬物動態試験の概要を表 2.6.4.1-1 に示す．

表 2.6.4.1-1 非臨床薬物動態試験の概要

CTD 記載箇所	試験系	投与経路／投与量，又は添加濃度	試験目的
吸収			
［5.3.2.3-1］	MDCK 細胞，Caco-2 細胞	オピカポン：10，50 μmol/L，BIA 9-1103：50 μmol/L	オピカポン及び BIA 9-1103 の膜透過を検討する．
［5.3.2.3-2］	Caco-2 細胞	オピカポン：5～200 μmol/L	オピカポンの膜透過を検討する．
［4.2.2.2-1］	雄 Wistar 系ラット	経口／10 mg/kg，静脈内／1 mg/kg，^{14}C-オピカポン	ラットにおける ^{14}C-オピカポンの吸収を検討する．
［4.2.2.2-2］	雄カニクイザル	経口／10 mg/kg，静脈内／1 mg/kg，^{14}C-オピカポン	サルにおける ^{14}C-オピカポンの吸収を検討する．
［4.2.2.2-3］	雌雄 Wistar 系ラット	経口／100，500，1000 mg/kg，オピカポン	ラットにおける 26 週間反復経口投与毒性試験で，オピカポンの薬物動態を検討する．
［4.2.2.2-4］	雌雄カニクイザル	経口／100，300，1000 mg/kg，オピカポン	サルにおける 52 週間反復経口投与毒性試験で，オピカポンの薬物動態を検討する．
分布			
［4.2.2.3-1］	雄 Wistar 系ラット	経口／10 mg/kg，^{14}C-オピカポン	ラットにおける ^{14}C-オピカポンの組織分布を検討する．
［4.2.2.3-2］	ラット，サル，ヒト血漿	^{14}C-オピカポン：0.3～30 μg/mL	限外濾過法を用いて，ラット，サル及びヒトにおける ^{14}C-オピカポンの *in vitro* 血漿蛋白結合を検討する．

2.6.4 薬物動態試験の概要文
オピカポン

CTD 記載箇所	**試験系**	**投与経路／投与量，又は添加濃度**	**試験目的**
［4.2.2.3-3］	ラット，イヌ，サル，ヒト血漿	BIA 9-1103：4～40 ng/mL	平衡ゲル濾過法を用いて，ラット，イヌ，サル及びヒトにおける BIA 9-1103 の *in vitro* 血漿蛋白結合を検討する．
［5.3.2.1-1］	ヒト血漿	BIA 9-1079：4～40 ng/mL	平衡ゲル濾過法を用いて，ヒトにおける BIA 9-1079 の *in vitro* 血漿蛋白結合を検討する．
［4.2.2.3-2］	ラット，サル，ヒト血液	^{14}C-オピカポン：0.3～30 μg/mL	ラット，サル及びヒトにおける ^{14}C-オピカポンの *in vitro* 血球移行を検討する．
［4.2.2.3-4］	妊娠 Crlj:WI ラット	経口／10 mg/kg，^{14}C-オピカポン	妊娠ラットにおける ^{14}C-オピカポンの胎盤移行性を検討する．
代謝			
［4.2.2.4-1］	マウス，ラット及びヒト S9. マウス，ラット，モルモット，ハムスター，イヌ，ミニブタ及びサル	*In vitro*／20 μmol/L，オピカポン 経口又は腹腔内／10～1000 mg/kg，オピカポン	各種 *in vitro* 試料及び *in vivo* 血漿を用いて，オピカポンの代謝経路を推定する．
［5.3.2.2-1］	ヒト肝及び小腸 S9, SULT 発現系	オピカポン：1～100 μmol/L	オピカポンの代謝に関わる SULT 分子種を推定する．
［4.2.2.4-2］	ラット，イヌ，サル及びヒト肝 S9,並びにヒト小腸及び腎 S9	オピカポン：10 μmol/L	ラット，イヌ，サル及びヒト肝 S9，並びにヒト小腸及び腎 S9 におけるオピカポンの硫酸抱合代謝を検討する．
［5.3.2.2-2］	ヒト肝細胞質及び S9 画分	BIA 9-1103：1 μmol/L	ヒト肝細胞質及び S9 画分中における BIA 9-1103 の安定性を検討する．
［5.3.2.2-3］	ヒト肝及び小腸ミクロソーム，UGT 発現系	オピカポン：1～250 μmol/L	オピカポンの代謝に関わる UGT 分子種を推定する．
［4.2.2.4-3］	ラット肝ホモジネート，マウス及びラット S-COMT，並びにマウス，ラット及びヒト肝 S9	オピカポン：10 μmol/L，BIA 9-1079：10 μmol/L	ラット肝ホモジネート，マウス及びラット S-COMT，並びにマウス，ラット及びヒト肝 S9 におけるオピカポン及び BIA 9-1079 のメチル化を検討する．
［5.3.2.2-4］	ヒト肝ミクロソーム，CYP 発現系	オピカポン：5 μmol/L	オピカポンの代謝に関わる CYP 分子種を推定する．
［4.2.2.4-4］	Wistar 系ラット	経口／10 mg/kg，オピカポン	ラットにおけるオピカポンの代謝を検討する．
［4.2.2.4-5］	雌雄カニクイザル	経口／1000 mg/kg，オピカポン	サルにおけるオピカポンの代謝を検討する．
［4.2.2.4-6］	マウス，ラット，モルモット，ハムスター	経口／30 mg/kg，オピカポン	マウス，ラット，モルモット，ハムスターにおけるオピカポンの代謝を検討する．
排泄			
［4.2.2.2-1］	雄 Wistar 系ラット	経口／10 mg/kg，静脈内／1 mg/kg，^{14}C-オピカポン	ラットにおける ^{14}C-オピカポンの尿，糞及び呼気中排泄を検討する．
［4.2.2.2-2］	雄カニクイザル	経口／10 mg/kg，静脈内／1 mg/kg，^{14}C-オピカポン	サルにおける ^{14}C-オピカポンの尿及び糞中排泄を検討する．

2.6.4 薬物動態試験の概要文
オピカポン

CTD 記載箇所	試験系	投与経路／投与量，又は添加濃度	試験目的
［4.2.2.5-1］	授乳中雌 Crlj:WI ラット	経口／10 mg/kg，^{14}C-オピカポン	授乳中雌ラットにおける ^{14}C-オピカポンの乳汁中排泄を検討する．
薬物相互作用			
［5.3.2.2-5］	ヒト肝ミクロソーム	オピカポン：0.03～10 μg/mL	CYP1A2，CYP2A6，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，CYP2E1 及び CYP3A4 に対するオピカポンの阻害作用を検討する．
［5.3.2.2-6］	ヒト肝ミクロソーム	BIA 9-1103：0.1～30 μg/mL	CYP1A2，CYP2A6，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，CYP2E1 及び CYP3A4 に対する BIA 9-1103 の阻害作用を検討する．
［5.3.2.2-7］	ヒト肝ミクロソーム	BIA 9-1079：0.03～10 μg/mL	CYP1A2，CYP2A6，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，CYP2E1 及び CYP3A4 に対する BIA 9-1079 の阻害作用を検討する．
［5.3.2.2-8］	ヒト肝ミクロソーム	オピカポン：1，10 μg/mL，BIA 9-1103：1，10 μg/mL	オピカポン及び BIA 9-1103 のヒト肝ミクロソーム蛋白への結合を検討する．
［5.3.2.2-9］	HepaRG 細胞	オピカポン：10 μmol/L，BIA 9-1079：10 μmol/L，BIA 9-1103：10 μmol/L	CYP1A1，CYP1A2，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6，CYP3A4，P-gp，PXR，CAR 及び AHR に対するオピカポン，BIA 9-1079 及び BIA 9-1103 の誘導作用を検討する．
［5.3.2.2-10］	ヒト肝細胞	オピカポン：1～10 μg/mL	CYP1A2，CYP2C9 及び CYP3A4 に対するオピカポンの誘導作用を検討する．
［5.3.2.2-11］	ヒト肝細胞	BIA 9-1103：3～30 μg/mL	CYP1A2，CYP2B6，CYP3A4 及び第Ⅱ相酵素に対する BIA 9-1103 の誘導作用を検討する．
［5.3.2.2-12］	ヒト肝細胞	BIA 9-1079：1～10 μg/mL	CYP1A2，CYP2C9 及び CYP3A4 に対する BIA 9-1079 の誘導作用を検討する．
［5.3.2.3-2］	Caco-2 細胞	オピカポン：5～200 μmol/L	オピカポンの輸送に関与するトランスポーターを検討する．
［5.3.2.3-1］	MDCK 細胞，Caco-2 細胞	オピカポン：10，50 μmol/L，BIA 9-1103：50 μmol/L	オピカポン及び BIA 9-1103 の輸送に関与するトランスポーターを検討する．
［5.3.2.3-3］	OATP1B1 発現細胞	オピカポン：1～100 μmol/L	オピカポンが OATP1B1 の基質になるかを検討する．
［5.3.2.3-4］	P-gp 発現細胞	オピカポン：5，10 μmol/L	オピカポンが P-gp の基質になるかを検討する．
［5.3.2.3-5］	トランスポーター発現細胞	オピカポン 基質評価：1～10 μg/mL 阻害評価：0.04～30 μg/mL	オピカポンが BCRP 及び OATP1B3 の基質になるかを検討する． BCRP，OAT1，OAT3，OATP1B1，OATP1B3，OCT1 及び OCT2 に対するオピカポンの阻害作用を検討する．

2.6.4 薬物動態試験の概要文
オピカポン

CTD 記載箇所	試験系	投与経路／投与量，又は添加濃度	試験目的
［5.3.2.3-6］	トランスポーター発現細胞	BIA 9-1103 基質評価：1～10 μg/mL 阻害評価：0.04～30 μg/mL	BIA 9-1103 が P-gp，BCRP，OATP1B1 及び OATP1B3 の基質になるかを検討する． P-gp，BCRP，OAT1，OAT3，OATP1B1，OATP1B3，OCT1 及び OCT2 に対する BIA 9-1103 の阻害作用を検討する．
［5.3.2.3-7］	Caco-2 細胞	オピカポン：100 μmol/L，BIA 9-1103：100 μmol/L	P-gp に対するオピカポン及び BIA 9-1103 の阻害作用を検討する．
［5.3.2.3-8］	P-gp 発現細胞	オピカポン：100 μmol/L	P-gp に対するオピカポンの阻害作用を検討する．
［5.3.2.3-9］	トランスポーター発現細胞，発現膜ベシクル	オピカポン：0.04～30 μg/mL，BIA 9-1103：0.04～30 μg/mL	BSEP，MATE1 及び MATE2-K に対するオピカポン及び BIA 9-1103 の阻害作用を検討する．
［4.2.2.3-2］	ヒト血漿	^{14}C-オピカポン，オピカポン：0.3～30 μg/mL	ヒト血漿蛋白に対する ^{14}C-オピカポンの結合率に及ぼす他剤の影響及び他剤の結合率に及ぼすオピカポンの影響を検討する．
［5.3.2.1-2］	ヒト血漿	BIA 9-1103：0.3～30 μg/mL ^{14}C-オピカポン：5 μg/mL	ヒト血漿蛋白に対するオピカポン又は他剤の蛋白結合率に及ぼす BIA 9-1103 の影響を検討する．

2.6.4.2 分析法

2.6.4.2.1 被験物質

^{14}C 標識した放射性標識体（以下，^{14}C-オピカポン）及びオピカポンを用いた．^{14}C-オピカポンは [redacted] 社で合成し，比放射能は 5.03 又は 5.22 MBq/mg，放射化学的純度は 91.9%以上であった．オピカポンは Bial 社又は [redacted] 社で合成した．

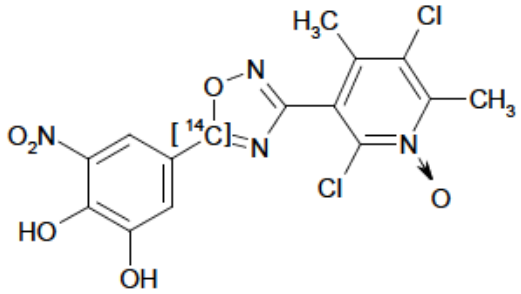


図 2.6.4.2.1-1 ^{14}C-オピカポンの構造式

2.6.4.2.2 標識化合物を用いた試験の分析法

生体試料中の放射能濃度は LSC で測定し，組織分布試験における放射能濃度は QWBA で測定した．

2.6.4.2.3 非標識化合物を用いた試験の分析法

［評価資料：4.2.2.1-1 及び 4.2.2.1-2］

オピカポン及びその代謝物の分析には LC/MS/MS を用いた．

バリデーションを実施した LC/MS/MS 法によりラット及びサルの血漿中オピカポン及び BIA 9-1079 濃度を測定した．本分析法による定量範囲は，20～5000 ng/mL であった．本分析法は，選択性，直線性，回収率，真度，精度，希釈妥当性，マトリックス効果，安定性（再注入，標準溶液，試料中及び凍結融解）に関するすべてのバリデーション基準を満たした．

2.6.4.3 吸収

2.6.4.3.1 *In vitro* 膜透過性

2.6.4.3.1.1 MDCK 細胞及び Caco-2 細胞におけるオピカポンの輸送

［評価資料：5.3.2.3-1］

MDCK 細胞及び Caco-2 細胞におけるオピカポン及び BIA 9-1103（オピカポンの 3-*O*-硫酸抱合体）の輸送を検討した．

MDCK 細胞におけるオピカポン及び BIA 9-1103（50 μmol/L）の膜透過性は低かった．Caco-2 細胞におけるオピカポン（10 μmol/L）の B to A（basal 側から apical 側）の輸送は A to B（apical 側から basal 側）の 3～4 倍高かった．DNP（ミトコンドリアの酸化的リン酸化の脱共役剤）存在下で，A to B の輸送は増加した．オピカポンの膜透過性は，MK571（MRP2 阻害剤）及び Ko143（BCRP 阻害剤）によって阻害され，ベラパミル（P-gp 阻害剤）では阻害されなかった．

以上の結果から，オピカポンの膜輸送には MRP2 及び BCRP が関与することが示唆された．

2.6.4.3.1.2 Caco-2 細胞へのオピカポンの取り込み

［評価資料：5.3.2.3-2］

Caco-2 細胞へのオピカポン（5～200 μmol/L）の取り込みを pH6.5 及び pH7.4 で検討した．

Caco-2 細胞へのオピカポンの取り込みは，低 pH で大きく，エストロン-3-硫酸によって阻害された．

以上の結果から，オピカポンの取り込みには，pH 依存的でエストロン-3-硫酸を基質とするトランスポーターである OATP2B1 が関与することが示唆された．

2.6.4.3.2 単回投与試験

2.6.4.3.2.1 ラット

［評価資料：4.2.2.2-1］

Wistar 系ラット（雄 4 例／群）に ^{14}C-オピカポンを 10 mg/kg で経口投与及び 1 mg/kg で静脈内投与した．経口投与後の血漿中放射能は，投与後 4 時間で Cmax の 1561 ng eq./g に達し，投与後 24 時間では 453 ng eq./g，72 時間では 426 ng eq./g であった．静脈内投与後の血漿中放射能濃度は，投与後 5 分では 11584 ng eq./g であり，投与後 24 時間では 550 ng eq./g，72 時間では 502 ng eq./g であった．いずれの投与経路においても，投与後 24 時間以降の血漿中放射能濃度の減少は緩やかであった．

2.6.4.3.2.2 サル

［評価資料：4.2.2.2-2］

カニクイザル（雄 4 例／群）に ^{14}C-オピカポンを 10 mg/kg で経口投与及び 1 mg/kg で静脈内投与した．経口投与後の血漿中放射能は，投与後 0.25 時間で Cmax の 1596 ng eq./g に達し，投与後 24 時間では 170 ng eq./g，168 時間では 63.6 ng eq./g であった．静脈内投与後の血漿中放射能濃度は，投与後 5 分では 3433 ng eq./g であり，投与後 24 時間では 30.8 ng eq./g，168 時間では 13.5 ng eq./g であった．いずれの投与経路においても，投与後 24 時間以降の血漿中放射能濃度の減少は緩やかであった．

2.6.4.3.3 反復投与試験

2.6.4.3.3.1 ラット 26 週間反復経口投与

［評価資料：4.2.2.2-3］

雌雄 Wistar 系ラットにオピカポンを 100～1000 mg/kg で 1 日 1 回 26 週間反復経口投与したときの血漿中オピカポン及び BIA 9-1079（オピカポンの *N*-オキシド還元体）の薬物動態パラメータを表 2.6.4.3.3.1-1 及び表 2.6.4.3.3.1-2 に示した．

オピカポン及び BIA 9-1079 の Cmax 及び AUC は投与量に応じて増加したが，投与量比を下回る増加であった．オピカポン及び BIA 9-1079 の AUC に顕著な雌雄差は認められなかった．投与 26 週目のオピカポン及び BIA 9-1079 の Cmax 及び AUC は投与 1 日目の 0.6～2.6 倍であり，蓄積性は認められなかった．26 週間反復経口投与による薬物動態への影響は小さいと考えられた．

表 2.6.4.3.3.1-1　雌雄ラットにオピカポンを 100，500 及び 1000 mg/kg で 1 日 1 回 26 週間反復経口投与したときの血漿中オピカポンの薬物動態パラメータ

時点	性	投与量（mg/kg）	Cmax（μg/mL）	Tmax（h）	AUClast（μg·h/mL）	T1/2（h）
1 日目	雄	100	4.14	2	17.47	NC
		500	8.28	0.5	33.63	8.8
		1000	16.47	1	50.62	4.0*
	雌	100	4.29	1	14.02	1.8
		500	10.50	1	31.45	1.8
		1000	23.37	1	49.31	2.2*
13 週目	雄	100	8.82	0.5	15.01	1.7
		500	16.73	0.5	38.60	1.5
		1000	18.80	0.5	54.04	2.5
	雌	100	10.80	0.5	12.41	0.95
		500	26.83	0.5	53.42	2.6
		1000	31.40	0.5	62.54	1.9
26 週目	雄	100	7.19	0.5	14.95	1.6
		500	19.47	0.5	59.18	7.8*
		1000	19.73	0.5	67.94	2.4
	雌	100	7.66	0.5	11.27	1.3
		500	27.13	0.5	41.61	1.4
		1000	23.33	1	65.22	2.7*

各時点 3 例の平均血漿中濃度から算出した値を示す.
*消失相の回帰 r^2＜0.900，又は/及び AUCinf の外挿部分が 20%を超える.
NC，not calculated

表 2.6.4.3.3.1-2　雌雄ラットにオピカポンを 100，500 及び 1000 mg/kg で 1 日 1 回 26 週間反復経口投与したときの血漿中 BIA 9-1079 の薬物動態パラメータ

時点	性	投与量 (mg/kg)	Cmax (μg/mL)	Tmax (h)	AUClast (μg·h/mL)	T1/2 (h)
1 日目	雄	100	1.86	6	19.73	NC
		500	2.12	6	25.58	NC
		1000	3.07	6	40.08	NC
	雌	100	1.45	6	11.95	NC
		500	2.30	2	19.80	3.3
		1000	2.54	1	31.93	7.2
13 週目	雄	100	1.20	1	16.35	9.0
		500	3.05	2	28.62	5.9
		1000	4.47	2	47.52	6.7
	雌	100	1.05	2	7.53	3.8
		500	3.12	2	28.20	6.2
		1000	4.15	1	37.86	6.4
26 週目	雄	100	1.14	6	15.12	NC
		500	2.64	2	34.11	13
		1000	4.05	2	46.50	10
	雌	100	0.97	2	7.58	4.2
		500	2.45	1	19.98	4.7
		1000	3.66	1	35.03	6.7

各時点 3 例の平均血漿中濃度から算出した値を示す.
NC，not calculated

2.6.4.3.3.2　サル 52 週間反復経口投与

［評価資料：4.2.2.2-4］

雌雄カニクイザルにオピカポンを 100，300 及び 1000 mg/kg で 1 日 1 回 52 週間反復経口投与したときの血漿中オピカポン及び BIA 9-1079 の薬物動態パラメータを表 2.6.4.3.3.2-1 及び表 2.6.4.3.3.2-2 に示した.

オピカポン及び BIA 9-1079 の Cmax 及び AUC は投与量に応じて増加した．オピカポンの Cmax は投与量比を下回る増加であった．オピカポンの AUC は概ね投与量に応じて増加した．BIA 9-1079 の Cmax 及び AUC は投与量比を下回る増加であった．オピカポン及び BIA 9-1079 の AUC に顕著な雌雄差は認められなかった．投与 52 週目のオピカポン及び BIA 9-1079 の Cmax 及び AUC は投与 1 日目の 0.3～2.0 倍であり，蓄積性は認められなかった．52 週間反復経口投与による薬物動態への影響は小さいと考えられた.

表 2.6.4.3.3.2-1　雌雄サルにオピカポンを 100，300 及び 1000 mg/kg で 1 日 1 回 52 週間反復経口投与したときの血漿中オピカポンの薬物動態パラメータ

時点	性	投与量 (mg/kg)	Cmax (μg/mL)	Tmax (h)	AUClast (μg·h/mL)	T1/2 (h)
1 日目	雄	100	1.08	1.0	1.39	0.76
		300	2.26	1.0	2.97	1.6
		1000	9.42	1.0～2.0	32.10	5.0
	雌	100	1.21	1.0	1.33	[0.67]
		300	1.61	1.0	3.62	2.5
		1000	8.80	1.0～2.0	34.40	4.4
13 週目	雄	100	0.50	1.0	0.65	0.88
		300	1.46	1.0～2.0	2.45	0.99
		1000	6.99	2.0	30.00	2.5
	雌	100	0.75	1.0	0.83	0.72
		300	1.07	1.0～2.0	1.89	1.4
		1000	5.22	1.0～2.0	15.30	2.4
26 週目	雄	100	0.53	1.0	0.80	0.66
		300	1.13	1.0～2.0	2.69	1.7
		1000	3.72	2.0～4.0	21.60	3.3
	雌	100	0.67	1.0	1.18	0.80
		300	1.03	1.0～2.0	2.71	1.5
		1000	3.42	2.0～4.0	17.80	2.6
52 週目	雄	100	1.04	1.0	1.65	1.2
		300	1.21	1.0	2.62	1.5
		1000	4.44	2.0	20.90	4.7
	雌	100	1.26	1.0	2.27	1.4
		300	1.11	1.0～2.0	3.40	2.5
		1000	2.92	1.0～2.0	16.20	4.7

Cmax，AUClast 及び T1/2 は 4～6 例の平均値を示す．Tmax は範囲を示す．
[ ]，1 例のみ

表 2.6.4.3.3.2-2　雌雄サルにオピカポンを 100，300 及び 1000 mg/kg で 1 日 1 回 52 週間反復経口投与したときの血漿中 BIA 9-1079 の薬物動態パラメータ

時点	性	投与量（mg/kg）	Cmax（μg/mL）	Tmax（h）	AUClast（μg·h/mL）	T1/2（h）
1 日目	雄	100	0.85	1.0～2.0	4.04	3.7
		300	0.87	1.0～2.0	5.79	5.5
		1000	2.22	1.0～2.0	27.10	10
	雌	100	0.72	1.0～2.0	3.32	3.1
		300	1.14	1.0～2.0	9.31	5.1
		1000	2.57	2.0	30.00	[8.6]
13 週目	雄	100	0.91	1.0～2.0	4.17	3.3
		300	1.18	1.0～2.0	8.42	4.4
		1000	2.39	2.0～4.0	35.70	15
	雌	100	0.77	1.0～2.0	2.84	2.8
		300	1.32	1.0～2.0	10.60	4.6
		1000	2.86	2.0～8.0	36.40	7.0
26 週目	雄	100	0.91	1.0～2.0	5.30	3.6
		300	1.31	1.3～2.0	13.20	5.6
		1000	2.85	2.0～8.0	41.30	12
	雌	100	0.82	1.0～2.0	3.54	2.3
		300	1.41	2.0～4.0	11.50	4.0
		1000	3.46	2.0～8.0	57.10	15
52 週目	雄	100	1.36	1.0～2.0	6.42	3.6
		300	1.44	1.0～4.0	11.40	4.9
		1000	2.31	2.0～4.0	36.80	28
	雌	100	1.21	2.0	6.47	3.6
		300	1.42	2.0	13.90	5.9
		1000	2.42	2.0～4.0	36.10	14

Cmax，AUClast 及び T1/2 は 4～6 例の平均値を示す．Tmax は範囲を示す．
[ ]，1 例のみ

2.6.4.4　分布

2.6.4.4.1　ラットにおける組織分布

［評価資料：4.2.2.3-1］

Wistar 系ラット（雄 3 例／時点）に ^{14}C-オピカポンを 10 mg/kg で単回経口投与し，投与後 1，4，12 及び 48 時間に QWBA で組織中放射能濃度を測定した．

多くの組織中放射能濃度の Tmax は，4 時間であった．投与後 4 時間の組織中放射能濃度は，消化管を除き肝臓，腎皮質及び腎髄質の順に高く，それぞれ血液（690 ng eq./g）の 12.3 倍，7.27 倍及び 6.49 倍であった．その他の組織中放射能濃度は血液より低く，脳組織中の放射能濃度はすべての時点で定量下限（121 ng eq./g）未満であった．最終評価時点である投与後 48 時間の組織中放射能濃度は，多くの組織（評価対象の約 3/5）で定量下限未満であり，肝臓（2373 ng eq./g），腎皮質（1808 ng eq./g），腎髄質（1692 ng eq./g）及び血液（184

ng eq./g）の放射能は，それぞれの最高濃度の約半分であった．肝臓及び腎臓を除いて，組織中放射能濃度はいずれの時点においても血液中濃度と同程度以下であった．

2.6.4.4.2 蛋白結合

2.6.4.4.2.1 オピカポン

［評価資料：4.2.2.3-2］

限外濾過法により測定した ^{14}C-オピカポン（0.3～30 μg/mL）の *in vitro* 血漿蛋白結合率は，ラット，サル及びヒトでそれぞれ 99.5～99.8%，99.4～99.6%及び 99.9%であった．最高検討濃度まで蛋白結合の飽和は認められず，いずれの動物種においても一定であった．

2.6.4.4.2.2 BIA 9-1103 及び BIA 9-1079

［評価資料：4.2.2.3-3 及び 5.3.2.1-1］

平衡ゲル濾過法により測定した BIA 9-1103（4～40 ng/mL）の *in vitro* 血漿蛋白結合率は，ラット，イヌ，サル，及びヒトでそれぞれ 99.62～99.67%，99.52～99.63%，99.59～99.64%及び 99.51～99.68%であった．平衡ゲル濾過法により測定した BIA 9-1079（4～40 ng/mL）*in vitro* 血漿蛋白結合率は，ヒトで 99.92～99.97%であった．

2.6.4.4.3 血球移行

［評価資料：4.2.2.3-2］

^{14}C-オピカポン（0.3～30 μg/mL）の *in vitro* での血球移行率は，ラット，サル，及びヒトでそれぞれ 0.0～2.4%，3.8～18.6%及び 0.0%であった．いずれの動物種においても血球への移行性は低かった．

2.6.4.4.4 妊娠ラットにおける組織分布

［評価資料：4.2.2.3-4］

妊娠 17 日の Crlj:WI ラット（1 例／時点）に ^{14}C-オピカポンを 10 mg/kg で単回経口投与し，投与後 1，4，12 及び 48 時間に QWBA で組織中放射能濃度を測定した．

母体の組織中放射能濃度の Tmax は，胎膜（12 時間）を除いて，1 時間であった．
肝臓，腎臓及び胎膜を除いた組織中の放射能濃度は血漿の 1.34 倍以下であった．^{14}C-オピカポンを単回経口投与後の放射能は胎児に移行した．胎児中放射能濃度は母体の血漿中放射能濃度よりも低く，減少は緩やかであった．

2.6.4.5 代謝

2.6.4.5.1 代謝酵素の推定

［評価資料：4.2.2.4-1］

マウス，ラット及びヒト S9（NADPH，UDPGA 及び PAPS を添加）並びにラット及びサル血漿を用いて，代謝物を検索した．ヒト S9 で認められた代謝物（BIA 9-1079，BIA 9-1103，BIA 9-1105，BIA 9-1106 及び BIA 9-1107）は，ラット及びマウス S9，並びにラット及びサル血漿でも認められた．

2.6.4.5.1.1 SULT

1） SULT 分子種の推定

［評価資料：5.3.2.2-1］

ヒト肝及び小腸 S9，並びにヒト SULT（SULT1A1*1，SULT1A1*2，SULT1A2，SULT1A3，SULT1B1，SULT1E1 及び SULT2A1）発現系試料を用いて，オピカポンの代謝に関わる SULT 分子種を推定した．

オピカポンの硫酸抱合代謝（BIA 9-1103 の生成）には主に SULT1A1 が関与し，SULT1B1 を除くその他の分子種でもオピカポンの硫酸抱合体は確認された．

2） 硫酸抱合代謝の種差

［評価資料：4.2.2.4-2］

ラット，イヌ，サル及びヒト肝 S9，並びにヒト小腸及び腎 S9 を用いて，オピカポン（10 μmol/L）の BIA 9-1103 への代謝を検討した．

BIA 9-1103 への代謝活性はヒト小腸 S9 で最も大きく，516.0 fmol/mg/min であり，その他の試料の代謝活性は 21.0～91.8 fmol/mg/min であった．

3） BIA 9-1103 のヒト肝細胞質及び S9 画分中安定性

［評価資料：5.3.2.2-2］

ヒト肝細胞質及び S9 画分中での BIA 9-1103 の安定性を検討した．

ヒト肝細胞質及び S9 画分中では 60 分間の反応で BIA 9-1103 は減少せず，脱抱合によるオピカポン生成も認められなかったことから，BIA 9-1103 はヒト肝細胞質及び S9 画分中で安定であった．

2.6.4.5.1.2 UGT 分子種の推定

［評価資料：5.3.2.2-3］

ヒト肝ミクロソーム，ヒト小腸ミクロソーム及びヒト UGT 発現系ミクロソームを用いて，オピカポンの代謝に関わる UGT 分子種を推定した．

オピカポンの UGT による代謝は，UGT1A1，UGT1A3，UGT1A7，UGT1A8，UGT1A9，UGT1A10 及び UGT2B7 で認められ，UGT1A4，UGT2B4 及び UGT2B17 では認められなかった．高い親和性を示した分子種は UGT1A7，UGT1A9，UGT1A1 及び UGT2B7 で，これらの Km はそれぞれ 1.658，13.7，18.0 及び 20.6 μmol/L であった．

2.6.4.5.1.3 カテコール-*O*-メチル化反応の種差

［評価資料：4.2.2.4-3］

ラット肝ホモジネート，マウス及びラット S-COMT，並びにマウス，ラット及びヒト肝 S9 を用いて，オピカポンのメチル化体への代謝を検討した．

ラット肝ホモジネートでは，オピカポンのメチル化が認められた．マウス及びラット S-COMT では，BIA 9-1100（3-*O*-メチル化体）が顕著に生成し，BIA 9-1104（[redacted]）もわずかに認められた．マウス及びラット肝 S9 ではオピカポン及び BIA 9-1079 のメチル化が認められたが，ヒト肝 S9 ではいずれのメチル化も認められなかった．

2.6.4.5.1.4 CYP 分子種の推定

［評価資料：5.3.2.2-4］

ヒト肝ミクロソーム及びヒト CYP 発現系ミクロソームを用いて，オピカポンの代謝に関わる CYP 分子種を推定した．

CYP3A4 発現系ミクロソームによりオピカポンは代謝されたが，ヒト肝ミクロソームでは代謝されなかった．

2.6.4.5.2 ラット *in vivo* における代謝

［評価資料：4.2.2.4-4］

Wistar ラットにオピカポンを 10 mg/kg で単回経口投与し，血漿中のオピカポン，BIA 9-1079，BIA 9-1100，BIA 9-1101，BIA 9-1103 及び BIA 9-1106 濃度を測定した．薬物動態パラメータを表 2.6.4.5.2-1 に示した．

ラット血漿中の主な代謝物は BIA 9-1100，BIA 9-1106 及び BIA 9-1103 であった．

表 2.6.4.5.2-1　ラットにオピカポンを 10 mg/kg で単回経口投与したときの血漿中オピカポン，BIA 9-1079，BIA 9-1100，BIA 9-1101，BIA 9-1103 及び BIA 9-1106 の薬物動態パラメータ

化合物名	Cmax （ng/mL）	Tmax （h）	AUClast （ng·h/mL）
オピカポン	1423	1	9117
BIA 9-1079	55.16（4）	7	758.4（8）
BIA 9-1103	123.0（9）	3	1107（12）
BIA 9-1106	674.8（47）	1	2568（28）
BIA 9-1100	433.9（30）	1	4677（51）
BIA 9-1101	17.03（1）	24	306.7（3）

平均値を示す．（ ）内の数値はオピカポンに対する割合（%）を示す．

2.6.4.5.3　　サル *in vivo* における代謝

［評価資料：4.2.2.4-5］

雌雄カニクイザルにオピカポンを 1000 mg/kg で 1 日 1 回 7 日間反復経口投与し，血漿中オピカポン，BIA 9-1079，BIA 9-1100，BIA 9-1101，BIA 9-1102，BIA 9-1103，BIA 9-1104，BIA 9-1105，BIA 9-1106 及び BIA 9-1107 濃度を測定した．

サル血漿中の主な代謝物は BIA 9-1106，BIA 9-1079 及び BIA 9-1103 であり，BIA 9-1107，BIA 9-1101 及び BIA 9-1105 もわずかに検出された．BIA 9-1100，BIA 9-1102 及び BIA 9-1104 は検出されなかった．

2.6.4.5.4　　推定代謝経路

［評価資料：4.2.2.4-1 及び 4.2.2.4-6］

マウス，ラット，モルモット，ハムスター，イヌ，ミニブタ及びサルにオピカポンを経口又は腹腔内投与したときの血漿試料を用いて，代謝物を検索した．検討したすべての動物種において，BIA 9-1079（オピカポンの *N*-オキシド還元体）への代謝が確認され，更にオピカポン及び BIA 9-1079 は硫酸抱合，グルクロン酸抱合，メチル化及び還元によって代謝された．BIA 9-1103（オピカポンの 3-*O*-硫酸抱合体）はヒトの血漿中主代謝物であり［2.7.6.10］，動物の血漿中でも認められた．BIA 9-1079 は動物の血漿中主代謝物の 1 つであったが，ヒト血漿中濃度は低く，多くの時点で定量下限未満であった［2.7.6.15］．

オピカポンの主な推定代謝経路を図 2.6.4.5.4-1 に示した．

2.6.4 薬物動態試験の概要文
オピカポン

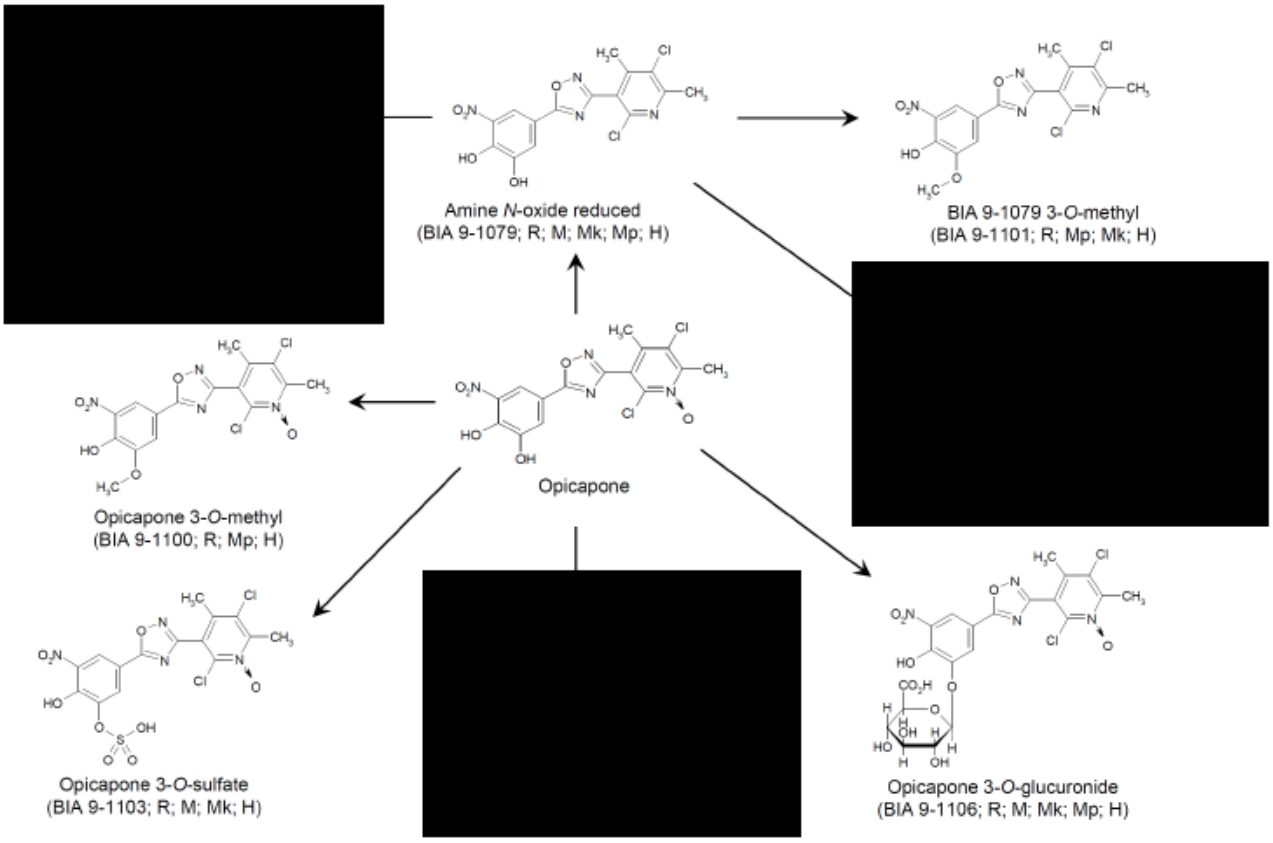


図 2.6.4.5.4-1　オピカポンの推定代謝経路
H: ヒト，M: マウス，Mk：サル，Mp：ミニブタ，R：ラット

2.6.4.6　排泄

2.6.4.6.1　ラットにおける尿，糞及び呼気中排泄

［評価資料：4.2.2.2-1］

Wistar 系ラット（雄 4 例／群）に ^{14}C-オピカポンを 10 mg/kg で経口投与又は 1 mg/kg で静脈内投与したときの尿糞呼気中への放射能の排泄を検討した．結果を表 2.6.4.6.1-1 に示した．経口投与後 120 時間までに，投与した放射能の 4.0%が尿中に，92.3%が糞中に，1.5%が呼気中に排泄され，屍骸中には 1.4%が残存した．静脈内投与後 120 時間までに，投与した放射能の 5.7%が尿中に，72.8%が糞中に，2.2%が呼気中に排泄され，屍骸中には 5.5%が残存した．

表 2.6.4.6.1-1　ラットに ^{14}C-オピカポンを単回投与したときの投与後 120 時間までの放射能の累積排泄率

投与経路	投与量 (mg/kg)	累積排泄率（%）			投与後 120 時間での投与量に対する割合		合計
		尿	糞	呼気	ケージ洗浄	屍骸	
経口	10	4.0±0.3	92.3±6.4	1.5±0.5	1.5±1.2	1.4±0.1	100.7±6.6
静脈内	1	5.7±1.7	72.8±4.5	2.2±0.6	2.7±0.8	5.5±1.2	89.0±6.4

値は 4 例の平均値±標準偏差を示す．

2.6.4.6.2 サルにおける尿及び糞中排泄

［評価資料：4.2.2.2-2］

カニクイザル（雄 4 例／群）に ^{14}C-オピカポンを 10 mg/kg で経口投与又は 1 mg/kg で静脈内投与したときの尿糞中への放射能の排泄を検討した．結果を表 2.6.4.6.2-1 に示した．経口投与後 168 時間までに，投与した放射能の 5.1%が尿中に，76.0%が糞中に排泄された．静脈内投与後 168 時間までに，投与した放射能の 6.2%が尿中に，55.6%が糞中に排泄された．

表 2.6.4.6.2-1 サルに ^{14}C-オピカポンを単回投与したときの投与後 168 時間までの放射能の累積排泄率

投与経路	投与量（mg/kg）	累積排泄率（%）		投与後 168 時間での投与量に対する割合		合計
		尿	糞	ケージ洗浄	ケージデブリ	
経口	10	5.1±2.3	76.0±5.6	2.2±0.5	0.5±1.0	83.7±3.2
静脈内	1	6.2±1.7	55.6±6.4	5.6±2.1	0.5±0.9	67.9±4.3

値は 4 例の平均値±標準偏差を示す．

2.6.4.6.3 ラットにおける乳汁排泄

［評価資料：4.2.2.5-1］

分娩後 11 日の授乳中雌 Crlj:WI ラット（3 例）に ^{14}C-オピカポンを 10 mg/kg で単回経口投与し，投与後 1，4，12 及び 48 時間の乳汁中及び血漿中放射能濃度を測定した．乳汁中放射能濃度の Tmax は 12 時間であった．乳汁中の放射能濃度は血漿の 0.509～1.08 倍であった．乳汁中放射能濃度は，血漿中放射能濃度の消失に伴い経時的に減少した．以上から，オピカポン由来物質が乳汁中に排泄されることが示された．

2.6.4.7 薬物動態学的薬物相互作用

2.6.4.7.1 CYP 阻害作用

2.6.4.7.1.1 オピカポン

［評価資料：5.3.2.2-5］

ヒト肝ミクロソームでの各種 CYP の酵素活性に対するオピカポン（0.03～10 μg/mL）の阻害作用を検討した．CYP1A2，CYP2A6，CYP2B6，CYP2C19，CYP2D6，CYP2E1 及び CYP3A4 に対するオピカポンの阻害率は，10 μg/mL で 26%未満であった．一方，オピカポンは CYP2C8 及び CYP2C9 に対する阻害作用を示し，Ki 値はそれぞれ 0.9 及び 18 μg/mL であった．いずれの CYP 分子種においても，30 分間のプレインキュベーションによる阻害作用の増強は認められなかった．

2.6.4.7.1.2 BIA 9-1103

［評価資料：5.3.2.2-6］

ヒト肝ミクロソームでの各種 CYP の酵素活性に対する BIA 9-1103（0.1～30μg/mL）の阻害作用を検討した．CYP1A2，CYP2A6，CYP2B6，CYP2C19，CYP2D6，CYP2E1 及び CYP3A4 に対する BIA 9-1103 の阻害率は，30μg/mL で 27%未満であった．一方，BIA 9-1103 は CYP2C8 及び CYP2C9 に対する阻害作用を示し，IC_{50} 値はそれぞれ 6.70 及び 20.7 μg/mL であった．いずれの CYP 分子種においても，30 分間のプレインキュベーションによる阻害作用の増強は認められなかった．

2.6.4.7.1.3 BIA 9-1079

［評価資料：5.3.2.2-7］

ヒト肝ミクロソームでの各種 CYP の酵素活性に対する BIA 9-1079（0.03～10μg/mL）の阻害作用を検討した．CYP1A2，CYP2A6，CYP2B6，CYP2C19，CYP2D6，CYP2E1 及び CYP3A4 に対する BIA 9-1079 の阻害率は，10μg/mL で 31%未満であった．一方，BIA 9-1079 は CYP2C8 及び CYP2C9 に対する阻害作用を示し，Ki 値はそれぞれ 0.19 及び 1.47 μg/mL であった．いずれの CYP 分子種においても，30 分間のプレインキュベーションによる阻害作用の増強は認められなかった．

2.6.4.7.1.4 ヒト肝ミクロソーム蛋白への結合

［評価資料：5.3.2.2-8］

平衡透析法により測定したオピカポン（1 及び 10μg/mL）及び BIA 9-1103（1 及び 10 μg/mL）のヒト肝ミクロソーム蛋白結合率は，それぞれ 12.58～64.30%及び 15.97～45.43%であった．オピカポンの結合率は，ミクロソーム濃度（0.1～2 mg/mL）及びオピカポン濃度に応じて増加した．

2.6.4.7.2 CYP 誘導作用

2.6.4.7.2.1 オピカポン

［評価資料：5.3.2.2-9 及び 5.3.2.2-10］

新鮮ヒト肝細胞又は HepaRG 細胞を用いてオピカポン（1～10μg/mL）の誘導作用を検討した．オピカポンは CYP1A1，CYP1A2，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6 及び CYP3A4，並びに P-gp，PXR，CAR 及び AHR のいずれに対しても誘導作用を示さなかった．

2.6.4.7.2.2 BIA 9-1103

［評価資料：5.3.2.2-9 及び 5.3.2.2-11］

誘導性ヒト凍結肝細胞又は HepaRG 細胞を用いて BIA 9-1103（3～30 μg/mL）の誘導作用を検討した．BIA 9-1103 は CYP1A1，CYP1A2，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6 及び CYP3A4，並びに P-gp，PXR，CAR，AHR 及び第Ⅱ相酵素のいずれに対しても誘導作用を示さなかった．

2.6.4.7.2.3 BIA 9-1079

［評価資料：5.3.2.2-9 及び 5.3.2.2-12］

新鮮ヒト肝細胞又は HepaRG 細胞を用いて BIA 9-1079（1～10 μg/mL）の誘導作用を検討した．BIA 9-1079 は CYP1A1，CYP1A2，CYP2B6，CYP2C8，CYP2C9，CYP2C19，CYP2D6 及び CYP3A4，並びに P-gp，PXR，CAR 及び AHR のいずれに対しても誘導作用を示さなかった．

2.6.4.7.3 関与するトランスポーターの推定

2.6.4.7.3.1 オピカポン

［評価資料：5.3.2.3-1～5.3.2.3-5］

Caco-2 細胞及び各種トランスポーター発現細胞を用いてオピカポンのトランスポーターによる輸送を評価した．

オピカポンの Caco-2 細胞への取り込み量は DNP 存在下で増加し，ATP 依存的な輸送の関与が示唆された．OATP2B1 の基質であるエストロン-3-硫酸（2 mmol/L），プラバスタチン（10 mmol/L），ジクロフェナク（0.1 mmol/L），ケルセチン（0.1 mmol/L）及びバルプロ酸（10 mmol/L）は，オピカポンの Caco-2 細胞への取り込みを阻害した．

Caco-2 細胞でのオピカポンの経細胞輸送には方向性があり，B to A の輸送は A to B の 3～4 倍高かった．その輸送は P-gp の阻害剤であるベラパミル（100 μmol/L）では阻害されず，MRP2 の阻害剤である MK571（50 μmol/L）及び BCRP の阻害剤である Ko143（10 μmol/L）により阻害された．ヒト P-gp 発現 MDCK-II 細胞でのオピカポンの経細胞輸送には方向性があり，B to A の輸送は A to B の約 4 倍高かった．その輸送は P-gp の阻害剤であるエラクリダール（50 μmol/L）により阻害された．ヒト BCRP 発現 MDCK-II 細胞でのオピカポンの経細胞輸送には方向性があり，B to A の輸送は A to B の 7～11 倍高かった．

ヒト OATP1B1 発現 CHO 細胞へのオピカポンの取り込み量は，コントロール細胞と変わらなかった．ヒト OATP1B3 発現 HEK293 細胞へのオピカポンの取り込み量は，コントロー

ル細胞より約 4 倍高かった．その取り込みは OATP1B3 の阻害剤であるリファンピシン（50 μmol/L）により阻害された．

以上の結果より，オピカポンは OATP1B3，BCRP，P-gp，OATP2B1 及び MRP2 の基質であると考えられた．

2.6.4.7.3.2 BIA 9-1103

［評価資料：5.3.2.3-6］

各種トランスポーター発現細胞を用いて BIA 9-1103 のトランスポーターによる輸送を評価した．ヒト OATP1B1 発現 HEK293 細胞への BIA 9-1103 の取り込み量は，コントロール細胞より約 78 倍高かった．ヒト OATP1B3 発現 HEK293 細胞への BIA 9-1103 の取り込み量は，コントロール細胞より約 43 倍高かった．いずれの取り込みも OATP1B1 及び OATP1B3 の阻害剤であるリファンピシン（50 μmol/L）により阻害された．ヒト BCRP 発現 MDCK-II 細胞での BIA 9-1103（1～10 μg/mL）の経細胞輸送には方向性があり，B to A の輸送は A to B の 1.6～3.9 倍高かった．ヒト P-gp 発現 MDCK-II 細胞での BIA 9-1103（1～10 μg/mL）の B to A の輸送は A to B の 2 倍未満であり，経細胞輸送に方向性は認められなかった．

以上の結果より，BIA 9-1103 は OATP1B1，OATP1B3 及び BCRP の基質であると考えられた．

2.6.4.7.4 トラスポーター阻害

2.6.4.7.4.1 オピカポン

［評価資料：5.3.2.3-5，5.3.2.3-7～5.3.2.3-9］

Caco-2 細胞，各種トランスポーター発現細胞及び膜ベシクルを用いてトランスポーターの輸送活性に対するオピカポンの阻害作用を検討した．

オピカポン（100 μmol/L）は P-gp の輸送活性を阻害しなかった．オピカポン（0.04～30 μg/mL）は OAT1，OAT3，OATP1B1，OATP1B3，MATE2-K 及び BSEP を阻害し，IC_{50} はそれぞれ 9.18，4.36，0.45，1.40，約 30 及び 12.28 μg/mL であった．OCT1，OCT2，BCRP 及び MATE-1 に対しては，30 μg/mL まで阻害作用を示さなかった．

2.6.4.7.4.2 BIA 9-1103

［評価資料：5.3.2.3-6，5.3.2.3-7 及び 5.3.2.3-9］

Caco-2 細胞，各種トランスポーター発現細胞及び膜ベシクルを用いてトランスポーターの輸送活性に対する BIA 9-1103 の阻害作用を検討した．

BIA 9-1103（100μmol/L）は P-gp の輸送活性を阻害しなかった．BIA 9-1103（0.04～30μg/mL）は OAT1，OAT3，OATP1B1 及び OATP1B3 を阻害し，IC_{50} はそれぞれ 13.20，2.55，2.80 及び 7.51μg/mL であった．OCT1，OCT2，BCRP，BSEP，MATE-1 及び MATE-2K に対しては，30μg/mL まで阻害作用を示さなかった．

2.6.4.7.5 血漿蛋白結合を介した薬物相互作用

2.6.4.7.5.1 オピカポン

［評価資料：4.2.2.3-2］

ヒト血漿蛋白に対する ^{14}C-オピカポンの結合率に及ぼす他剤の影響及び他剤の蛋白結合率に及ぼすオピカポンの影響を検討した．蛋白結合率は限外濾過法により測定した．

^{14}C-オピカポン（0.3～30μg/mL）の蛋白結合率は，ワルファリン（5μg/mL），ジアゼパム（5μg/mL），ジゴキシン（5μg/mL）及びトルブタミド（20μg/mL）存在下でも 99.5～99.9%であり，他剤による影響を受けなかった．

オピカポン（0.3～30μg/mL）は，^{14}C-ワルファリン（5μg/mL），^{14}C-ジアゼパム（5μg/mL），^{3}H-ジゴキシン（5μg/mL）及び ^{14}C-トルブタミド（20μg/mL）の蛋白結合率に影響しなかった．

2.6.4.7.5.2 BIA 9-1103

［評価資料：5.3.2.1-2］

ヒト血漿蛋白に対するオピカポン又は他剤の蛋白結合率に及ぼす BIA 9-1103 の影響を検討した．蛋白結合率は限外濾過法により測定した．

BIA 9-1103（0.3～30μg/mL）は，^{14}C-オピカポン（5μg/mL），^{14}C-ワルファリン（5μg/mL），^{3}H-ジアゼパム（5μg/mL），^{3}H-ジゴキシン（5μg/mL）及び ^{14}C-トルブタミド（20μg/mL）の蛋白結合率に影響しなかった．

2.6.4.8 その他の薬物動態試験

該当資料はない．

2.6.4.9 考察及び結論

ラット及びサルにオピカポンを反復経口投与したとき，血漿中オピカポンの Cmax 及び AUC は投与量に応じて増加した．ラット及びサルに ^{14}C-オピカポンを単回経口投与したとき，放射能の Tmax は 4 及び 0.25 時間であり，吸収は速やかであった．

ラットに ^{14}C-オピカポンを経口投与したときの脳組織中濃度の結果より，オピカポンの中枢への移行性は低いと考えられた．オピカポンのヒト血漿蛋白結合率は 99.9%と高かったものの，ワルファリン，ジアゼパム，ジゴキシン及びトルブタミドの共存下でも，お互いの血漿蛋白結合率に影響を及ぼさなかった．したがって，臨床において蛋白結合の置換による相互作用が生じる可能性は低いと考えられた．

ヒト及び動物での *in vitro* 又は *in vivo* 試料において検出されたオピカポンの代謝物は，硫酸抱合体，グルクロン酸抱合体，還元体及びメチル化体であったことから，オピカポンの代謝には SULT，UGT，還元酵素及び COMT が関与していると考えられた．また，オピカポンはヒト CYP 発現系ミクロソームにより代謝されたことから，CYP も代謝に関与していると考えられた．ヒト肝 S9 による *in vitro* 代謝試験で検出された代謝物はラット及びサル血漿中に検出され，ヒト特異的な代謝物は認められなかった．

ラット及びサルに ^{14}C-オピカポンを経口投与又は静脈内投与したとき，放射能は主に糞中に排泄されたことから，吸収された放射能の主排泄経路は胆汁を介した糞中排泄であると考えられた．

オピカポン，BIA 9-1103 及び BIA 9-1079 の CYP1A2，CYP2A6，CYP2B6，CYP2C19，CYP2D6，CYP3A4 又は CYP2E1 に対する *in vitro* での阻害率は最高濃度である 10 又は 30 μg/mL で 31%未満であり，臨床においてオピカポン，BIA 9-1103 及び BIA 9-1079 がこれら CYP を阻害する可能性は低いと考えられた．オピカポンは *in vitro* で CYP2C8 及び CYP2C9 を阻害し，Ki 値はそれぞれ 0.9 及び 18 μg/mL であった．BIA 9-1103 及び BIA 9-1079 も同様に CYP2C8 及び CYP2C9 を阻害した．臨床において，オピカポンと CYP2C8 の基質であるレパグリニド及び CYP2C9 の基質であるワルファリンとの薬物相互作用試験を実施した結果，レパグリニドの血漿中濃度はオピカポン（50 mg）の併用による影響を受けなかった［2.7.2.2.5.18］．ワルファリンの血漿中濃度はオピカポン（25 mg）の併用による影響を受けなかった［2.7.2.2.5.15］．

オピカポン及び BIA 9-1103 は *in vitro* で OATP1B1，OATP1B3，OAT1，OAT3，MATE2-K 及び BSEP を阻害し，IC_{50} 値は 0.45～約 30 μg/mL であった．薬物相互作用ガイドライン [1)] に従い，IC_{50} 値及びヒトにオピカポンを 25 mg で経口投与したときの薬物動態情報を用いて検討した結果，臨床においてオピカポン及び BIA 9-1103 がこれらトランスポーターを阻害する可能性は低いと考えられた．オピカポンはヒトの OATP1B3，BCRP 及び P-gp の基質であり，臨床においてこれらトランスポーター阻害剤の影響を受ける可能性は否定できない．

2.6.4.10 図表

図表は本文中に記載した．

2.6.4.11 参考文献一覧

1） 医薬品開発と適正な情報提供のための薬物相互作用ガイドライン，厚生労働省，2018年7月

2.6.5 薬物動態試験の概要表

小野薬品工業株式会社

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2.6.5 薬物動態試験の概要表

略号一覧

Abbreviation or specialist term	Explanation
AHR	Aryl hydrocarbon receptor
AMP	Adenosine monophosphate
AP	Apical
ATP	Adenosine triphosphate
AUC	Area under the concentration-time curve
AUC%extr	Percent difference between AUClast and AUCinf
AUClast	AUC from time zero to the last measurable concentration
AUCinf	Area under the concentration-time curve from time zero to infinity
BCRP	Breast cancer resistance protein
BLQ	Below limit of quantification
BL	Basal, basolateral
BSEP	Bile salt export pump
CAR	Constitutive androstane receptor
CCK-8	Cholecystokinin 8
Cmax	Maximum observed plasma concentration
CMC	Carboxymethylcellulose
COMT	Catechol-*O*-methyltransferase
CV	Coefficient of variation
CYP	Cytochrome P450
DDC	Diethyldithiocarbamate
DMSO	Dimethyl sulfoxide
DNP	2,4-Dinitrophenol
$E_2$17βG	β-Estradiol-17β-D-glucuronide
EDTA	Ethylenediaminetetraacetic acid
ESI	Electrospray ionization
GLP	Good Laboratory Practice
HPLC	High-performance liquid chromatography
HPMC	Hydroxylpropylmethylcellulose
IC_{50}	50% inhibitory concentration
i.p.	Intraperitoneal
ISTD	Internal standard
i.v.	Intravenous
Ki	Inhibition constant
Km	Michaelis constant
Ksi	Substrate inhibition constant
LC-MS	Liquid chromatography with mass spectrometry
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LLOQ	Lower limit of quantification
LSC	Liquid scintillation counting
MATE	Multidrug and toxin extrusion
8-MP	8-Methoxypsoralen
MRP	Multidrug resistance-associated protein
N/A	Not applicable
NC	Not calculated
NADPH	Reduced form of nicotinamide adenine dinucleotide phosphate
ND	Not detected
NE	Not evaluated
OAT	Organic anion transporter

2.6.5 薬物動態試験の概要表
オピカポン

Abbreviation or specialist term	**Explanation**
OATP	Organic anion transporting polypeptide
OCT	Organic cation transporter
Papp	Apparent permeability
PAPS	3′-Phosphoadenosine 5′-phosphosulfate
PCV	Packed cell volume
P-gp	P-glycoprotein
p.o.	Per os, oral
PK	Pharmacokinetic(s)
PXR	Pregnane X receptor
QWBA	Quantitative whole-body autoradiography
S9	Tissue supernatant fraction obtained from homogenate after centrifuging at 9000*g*
SD	Standard deviation
SEM	Standard error of the mean
SULT	Sulfotransferase
T1/2	Terminal half-life
Tmax	Time to Cmax
UDPGA	UDP-glucuronic acid
UGT	UDP-glucuronosyltransferase
UV	Ultraviolet
Vmax	Maximal velocity

2.6.5.1 薬物動態試験：一覧表

Type of Study	Test System / or Species /Strain	Method of Administration	Testing Facility	Study Number	Location
Absorption (*in vitro*)					
Absorption	Caco-2 and MDCK cells	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]1106	[5.3.2.3-1]
Absorption	Caco-2 cells	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0824	[5.3.2.3-2]
Absorption after a Single Dose					
Absorption	Rat/Wistar	Oral, i.v.	[redacted] UK	ZNA15658.001	[4.2.2.2-1]
Absorption	Monkey/Cynomolgus	Oral, i.v.	[redacted] UK	ZNA15658.002	[4.2.2.2-2]
Absorption after Repeated Doses					
Opicapone 26-week oral toxicity study	Rat/HanRcc:WIST	Oral	[redacted] Switzerland	C03508	[4.2.2.2-3]
Opicapone 52-week oral toxicity study	Monkey/Cynomolgus	Oral	[redacted] Spain	S13825	[4.2.2.2-4]
Distribution – Tissue Distribution					
Distribution	Rat/Wistar	Oral	[redacted] UK	ZNA15658.003	[4.2.2.3-1]
Distribution – Plasma Protein Binding					
Plasma protein and blood cell binding	Rat, monkey, human	*In vitro*	[redacted] UK	ZNA15658.004	[4.2.2.3-2]
Plasma protein binding (BIA 9-1103)	Rat, dog, monkey, human	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0903	[4.2.2.3-3]
Plasma protein binding (BIA 9-1079)	Human	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0325	[5.3.2.1-1]
Distribution –Pregnant Animals					
Placental transfer in pregnant rats	Rat/Crlj:WI	Oral	[redacted] Japan	AE-7745-G	[4.2.2.3-4]

2.6.5.1 薬物動態試験：一覧表（続き）

Type of Study	Test System / or Species /Strain	Method of Administration	Testing Facility	Study Number	Location
Metabolism – *in vitro* and *in vivo*					
Metabolism (*in vitro* and *in vivo*)	Mouse, rat, human liver S9 Mouse, rat, hamster, guinea pig, min-pig, dog, monkey	*In vitro* Oral, i.p.	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0116	[4.2.2.4-1]
Metabolism – *in vitro*					
Sulfation	Human liver and intestinal S9, recombinant SULT isoforms	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0822	[5.3.2.2-1]
Sulfation	Rat, dog, monkey, and human liver S9 Human intestinal and kidney S9	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0511	[4.2.2.4-2]
Stability of BIA 9-1103	Human liver cytosol and S9 fractions	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0414	[5.3.2.2-2]
Glucuronidation	Human liver and intestinal microsomes, recombinant UGT isoforms	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0712	[5.3.2.2-3]
Methylation	Rat, mouse, and human cellular fractions	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SROPC[redacted]0311	[4.2.2.4-3]
CYP phenotyping	Human liver microsomes, recombinant CYP isoforms	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0615	[5.3.2.2-4]
Metabolism – *in vivo*					
Metabolism	Rat/Wistar	Oral	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]1228	[4.2.2.4-4]
Metabolism	Monkey/Cynomolgus	Oral	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0509	[4.2.2.4-5]
Metabolism	Mouse, rat, hamster, guinea pig	Oral	Bial-Portela and C[a], S.A., Portugal	SROPC[redacted]0313	[4.2.2.4-6]

2.6.5.1 薬物動態試験：一覧表（続き）

Type of Study	Test System / or Species /Strain	Method of Administration	Testing Facility	Study Number	Location
Excretion					
Excretion (urine, feces and expired air)	Rat/Wistar	Oral, i.v.	[redacted] UK	ZNA15658.001	[4.2.2.2-1]
Excretion (urine and feces)	Monkey/Cynomolgus	Oral, i.v.	[redacted] UK	ZNA15658.002	[4.2.2.2-2]
Excretion – Study in Nursing Animals					
Excretion into milk in nursing rats	Rat/Crlj:WI	Oral	Ono Pharmaceutical Co, Ltd., Japan	Y[redacted]AG013	[4.2.2.5-1]
Inhibition/Induction of Drug Metabolising Enzymes					
CYP inhibition (opicapone)	Human liver microsomes	*In vitro*	[redacted] UK	782809	[5.3.2.2-5]
CYP inhibition (BIA 9-1103)	Human liver microsomes	*In vitro*	[redacted] UK	790500	[5.3.2.2-6]
CYP inhibition (BIA 9-1079)	Human liver microsomes	*In vitro*	[redacted] UK	782814	[5.3.2.2-7]
Binding to microsomal protein (opicapoe and BIA 9-1103)	Human liver microsomes	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0415	[5.3.2.2-8]
CYP induction (opicapone, BIA 9-1103 and BIA 9-1079)	HepaRG cells	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SROPC[redacted]0324	[5.3.2.2-9]
CYP induction (opicapone)	Human hepatocytes	*In vitro*	[redacted] UK	782788	[5.3.2.2-10]
CYP induction (BIA 9-1103)	Human hepatocytes	*In vitro*	[redacted] UK	792089	[5.3.2.2-11]
CYP induction (BIA 9-1079)	Human hepatocytes	*In vitro*	[redacted] UK	782793	[5.3.2.2-12]
Identification/Inhibition of Drug Transporters					
Transport (opicapone)	Caco-2 cells	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0824	[5.3.2.3-2]
Transport (opicapone and BIA 9-1103)	Caco-2 and MDCK cells	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]1106	[5.3.2.3-1]
Transport by OATP1B1 (opicapone)	OATP1B1-expressing CHO cells	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]1128	[5.3.2.3-3]
Transport by P-gp (opicapone)	Human P-gp-expressing MDCKII cells	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0627	[5.3.2.3-4]

2.6.5.1 薬物動態試験：一覧表（続き）

Type of Study	Test System / or Species /Strain	Method of Administration	Testing Facility	Study Number	Location
Identification/Inhibition of Drug Transporters					
Transport by BCRP and OATP1B3 (opicapone)	Human BCRP-expressing MDCKII cells, human OATP1B3-expressing HEK293 cells	*In vitro*	[redacted] Hungary	Tebu-02-[redacted]20	[5.3.2.3-5]
Transport by P-gp, BCRP, OATP1B1 and OATP1B3 (BIA 9-1103)	Human P-gp- or BCRP-expressing MDCKII cells, OATP1B1- or OATP1B3-expressing HEK293 cells	*In vitro*	[redacted] Hungary	Bial-02-[redacted]20	[5.3.2.3-6]
BCRP, OAT1, OAT3, OATP1B1, OATP1B3, OCT1, and OCT2 inhibition (opicapone)	Human BCRP- or OCT2-expressing MDCKII cells, human OAT1- or OCT1-expressing CHO cells, and human OAT3-, OATP1B1-, or OATP1B3-expressing HEK293 cells	*In vitro*	[redacted] Hungary	Tebu-02-[redacted]20	[5.3.2.3-5]
P-gp, BCRP, OAT1, OAT3, OATP1B1, OATP1B3, OCT1, and OCT2 inhibition (BIA 9-1103)	Human P-gp-, BCRP-, or OCT2-expressing MDCKII cells, Human OAT1-OCT1- expressing CHO cells, and human OAT3-, OATP1B1-, or OATP1B3-expressing HEK293 cells	*In vitro*	[redacted] Hungary	Bial-02-[redacted]20	[5.3.2.3-6]
In vitro interaction with irinotecan transport (opicapone and BIA 9-1103)	Caco-2 cells	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0315	[5.3.2.3-7]
P-gp inhibition (opicapone)	Human P-gp-expressing MDCKII cells	*In vitro*	Bial-Portela and C[a], S.A., Portugal	SRAL[redacted]0926	[5.3.2.3-8]
BSEP, MATE1, and MATE2-K inhibition (opicapone and BIA 9-1103)	Human BSEP-expressing membrane vesicles and MATE1- or MATE2-K-expressing MDCKII cells	*In vitro*	[redacted] Hungary	Bial-03-[redacted]20	[5.3.2.3-9]

2.6.5.1 薬物動態試験：一覧表（続き）

Type of Study	Test System / or Species /Strain	Method of Administration	Testing Facility	Study Number	Location
Pharmacokinetic Drug Interactions					
In vitro interaction with warfarin, diazepam, digoxin and tolbutamide (opicapone)	Human plasma	*In vitro*	[redacted] UK	ZNA15658.004	[4.2.2.3-2]
In vitro interaction with warfarin, diazepam, digoxin and tolbutamide (BIA 9-1103)	Human plasma	*In vitro*	[redacted] UK	ZNA42782.001	[5.3.2.1-2]

2.6.5.2 分析方法及びバリデーション試験

2.6.5.2.1 ラット血漿中オピカポン及び BIA 9-1079 濃度分析法バリデーション

Location in CTD: [4.2.2.1-1]
Study No. SBA_S_06066

Study title: Validation of a Method for the Determination of BIA 9-1067 and Its Metabolite, BIA 9-1079, in Rat Plasma by LC-MS/MS

Species: Rat **Special features:** None **Opicapone batch No.:** [redacted]

Report date: [redacted] 20 **GLP compliance:** Non-GLP **BIA 9-1079 batch No.:** [redacted]

Validation facility: [redacted], Switzerland **ISTD:** Tolcapone (Batch No.: [redacted])

Matrix: Plasma **Sample volume:** 25 µL **Blank matrix:** Blank rat plasma in K-EDTA as an anticoagulant

Sample preparation technique: Precipitation of plasma proteins with two volume equivalents of acetonitrile containing a defined amount of internal standard, tolcapone, followed by vortex mixing and centrifugation. Samples were diluted with water and injected onto the HPLC.

HPLC column: Luna C8, 2.0 × 50 mm, 5 µm (Phenomenex, Torrance, CA, USA) **Column temperature:** 50°C **Run time:** 4 min **Injection Vol.:** 2 µL

Mobile phase A: Water 1.0% formic acid **Mobile phase B:** Acetonitrile 1.0% formic acid

Gradient:

Time (min):	0.00	0.50	1.20	2.50	2.60	4.00
Phase A (%):	70	70	10	10	70	70
Phase B (%):	30	30	90	90	30	30
Flow rate (µL/min):	500	500	500	500	500	500

Detector: TSQ Quantum (ThermoFinnigan San Jose, CA, USA) Mass Spectrometry

Detection (selected reaction monitoring):

Analyte:	Parent ion (Th):	Product ion (Th):	Width (Th):	Scan time (s):	Collision energy (eV):
BIA 9-1079	395.0	214.0	0.01	0.04	25
Opicapone	411.0	394.0	0.01	0.04	20
Tolcapone	272.1	181.0	0.01	0.04	25

Mass spectrometry parameters:

Ionization mode:	ESI voltage:	Capillary Temp.:	Sheath gas:	Auxiliary gas:	Collision gas pressure:
ESI (negative ion mode)	4 kV	350°C	60 psi	5 psi	1.5 mTorr

2.6.5.2.1 ラット血漿中オピカポン及び BIA 9-1079 濃度分析法バリデーション（続き）

Analyte:	Analytical range (ng/mL):	Inter-assay precision (%CV):	Inter-assay accuracy (%):	Specificity:	Effect of dilution:
Opicapone	20-5000	≤ 10.9	98.9-107.1	< 20% BLQ	5-fold with blank plasma
BIA 9-1079	20-5000	≤ 12.3	93.9-109.9	< 20% BLQ	5-fold with blank plasma

Analyte:	Freeze/thaw stability:	Short-term stability:	Long-term stability:	Post-preparation stability:	Working and stock solution stability:
Opicapone	Stable for 3-freeze/thaw cycles at -25 ± 5°C	Stable 6 h at RT	Stable for 6 months at -25 ± 5°C	Stable for 16.5 h at 8 ± 5°C	Stable 6 h at RT and 5 ± 3°C and 2 weeks at -25 ± 5°C
BIA 9-1079	Stable for 3-freeze/thaw cycles at -25 ± 5°C	Stable 6 h at RT	Stable for 6 months at -25 ± 5°C	Stable for 16.5 h at 8 ± 5°C	Stable 6 h at RT and 5 ± 3°C and 2 weeks at -25 ± 5°C
Internal standard					Stable 6 h at RT and 5 ± 3°C and 2 weeks at -25 ± 5°C

Analyte:	Recovery (%):	Matrix effect:	Carryover (%):	Re-injection reproducibility:
Opicapone	95.8-114.4	No matrix effect	0.2	22.25 h after storage at 8 ± 5°C
BIA 9-1079	96.0-99.1	No matrix effect	0.9	22.25 h after storage at 8 ± 5°C
Internal standard	94.4	No matrix effect		
Validated method used in:				
[4.2.2.2-3] (assay site: [redacted], Switzerland)				

2.6.5.2.2 サル血漿中オピカポン及び BIA 9-1079 濃度分析法バリデーション

Location in CTD: [4.2.2.1-2]
Study No. SBA_S_06083

Study title: Validation of a Method for the Determination of BIA 9-1067 and Its Metabolite, BIA 9-1079, in Cynomolgus Monkey Plasma by LC-MS/MS

Species:	Monkey	**Special features:**	None	**Opicapone batch Nos.:**	[redacted]
Report date:	[redacted] 20[redacted]	**GLP compliance:**	Non-GLP	**BIA 9-1079 batch No.:**	[redacted]
Validation facility:	[redacted], Switzerland			**ISTD:**	Nimodipine (Batch Nos. [redacted] and [redacted])
Matrix:	Plasma	**Sample volume:**	25 µL	**Blank matrix:**	Blank Cynomolgus monkey plasma in Li-Heparin as an anticoagulant

Sample preparation technique: Precipitation of plasma proteins with two volume equivalents of acetonitrile containing a defined amount of internal standard, followed by centrifugation. Samples were diluted with 3 g/L EDTA solution containing 0.5% ammonium hydroxide solution and injected onto the HPLC.

HPLC column: Luna C8(2), 2.0 x 50 mm, 5 µm (Phenomenex, Torrance, CA, USA) **Column temperature:** 50°C **Run time:** 4 min **Injection Vol.:** 2 µL

Mobile phase A: Water 1.0% formic acid **Mobile phase B:** Acetonitrile 1.0% formic acid

Gradient:

Time (min):	0.00	0.50	1.20	2.50	2.60	4.0
Phase A (%):	70	70	10	10	70	70
Phase B (%):	30	30	90	90	30	30
Flow rate (µL/min):	500	500	500	500	500	500

Detector: TSQ Quantum (ThermoFischer Scientific, San Jose, CA, USA) Mass Spectrometry

Detection (selected reaction monitoring):

Analyte:	Parent ion (Th):	Product ion (Th):	Width (Th):	Scan time (s):	Collision energy (eV):
BIA 9-1079	395.0	214.0	0.01	0.04	25
Opicapone	411.0	394.0	0.01	0.04	20
Nimodipine	417.0	122.0	0.01	0.04	25

Mass spectrometry parameters:

Ionization mode:	ESI voltage:	Capillary Temp.:	Sheath gas:	Auxiliary gas:	Collision gas pressure:
ESI (negative ion mode)	4 kV	350°C	60 psi	5 psi	1.5 mTorr

2.6.5.2.2 サル血漿中オピカポン及び BIA 9-1079 濃度分析法バリデーション（続き）

Analyte:	Analytical range (ng/mL)	Inter-assay precision (%CV):	Inter-assay accuracy (%):	Specificity:	Effect of dilution:
Opicapone	20-5000	≤ 11.0	100.5-108.0	< 20% BLQ	5-fold with blank plasma
BIA 9-1079	20-5000	≤ 9.8	100.5-104.8	< 20% BLQ	5-fold with blank plasma

Analyte:	Freeze/thaw stability:	Short-term stability:	Long-term stability:	Post-preparation stability:	Working and stock solution stability:
Opicapone	Stable for 3-freeze/thaw cycles at -25 ± 5°C	Stable 20 h at RT	Stable for 3 months at -25 ± 5°C	Stable for 11 days at 8 ± 5°C	Stable 6 h at RT, 5 ± 5°C and -25 ± 5°C
BIA 9-1079	Stable for 3-freeze/thaw cycles at -25 ± 5°C	Stable 20 h at RT	Stable for 3 months at -25±5°C	Stable for 11 days at 8±5°C	Stable 6 h at RT, 5 ± 5°C and -25 ± 5°C

Analyte:	Recovery (%):	Matrix effect:	Carryover (%):	Re-injection reproducibility:
Opicapone	93.3-99.2	No matrix effect	0.3	6 days after storage at 8 ± 5°C
BIA 9-1079	88.0-100.1	No matrix effect	1.1	6 days after storage at 8 ± 5°C
Internal Standard	106.7	No matrix effect		
Validated method used in:				
[4.2.2.2-4] (assay site: [redacted], Switzerland)				

2.6.5.3 薬物動態試験：吸収：*In vitro* 試験

2.6.5.3.1 Caco-2 細胞及び MDCK 細胞におけるオピカポンの輸送

Test article: opicapone and BIA 9-1103
Location in CTD: [5.3.2.3-1]
Study No. SRAL[redacted]1106

Study title:	Characterization of Opicapone Transport by Caco-2 Cells and MDCK Cells
Test system:	Caco-2 and MDCK cells
Method:	Caco-2 cells: BL to AP transport and AP to BL transport of opicapone (10 µmol/L) or irinotecan (positive control, 1 µmol/L) over 60 and 120 min. Effect of DNP (uncoupler of mitochondrial oxidative phosphorylation, 1 mmol/L), verapamil (P-gp substrate, inhibitor, 100 µmol/L) and irinotecan (P-gp substrate, 100 µmol/L), MK571 (MRP2 inhibitor, 50 µmol/L), and Ko143 (BRCP inhibitor, 10 µmol/L). MDCK cells: transport of opicapone (50 µmol/L) over 10 and 30 min in comparison with atenolol (low permeability compound, 50 µmol/L), propranolol (high permeability compound, 50 µmol/L), tolcapone (50 µmol/L), and BIA 9-1103 (50 µmol/L)
Analyte/assay:	Opicapone/LC-MS

Opicapone and its sulfated metabolite (BIA 9-1103) are low permeable compounds across MDCK cells.

In Caco-2 cells, BL to AP transport was higher than the AP to BL transport. Over 60 min incubation the BL to AP transport corresponds approximately to 3- to 4-fold the AP to BL transport. In the presence of DNP, an increase in AP to BL transport of opicapone was observed. The AP to BL and the BL to AP transport were sensitive to a temperature decrease. Irinotecin and verapamil had no effect on opicapone transport, while both Ko143 and MK571 significantly increased AP to BL transport of opicapone and reduced the BL to AP transport.

2.6.5.3.2 Caco-2 細胞へのオピカポンの取り込み

Test article: opicapone
Location in CTD: [5.3.2.3-2]
Study No. SRAL[redacted]0824

Study title:	Characterization of Opicapone Uptake by Caco-2 Cells
Test system:	Caco-2 cells
Method:	Incubation of cells for 1, 3, 6, 12, or 30 min with 10 or 100 µmol/L opicapone. Effect of DNP, estrone-3-sulfate, pravastatin, L-dopa, benserazide, quercetin, naringenin, and diclofenac on uptake of opicapone. Effect of MK571 (MRP2 inhibitor), Ko143 (BRCP inhibitor), verapamil (P-gp substrate), irinotecan (P-gp substrate), and etoposide (P-gp substrate) on efflux of opicapone.
Analyte/assay:	Opicapone/LC-MS

Time (min)	**Opicapone uptake (pmol, mean ± SEM, n = 4)**			
	pH 6.5		**pH 7.4**	
	10 µmol/L	**100 µmol/L**	**10 µmol/L**	**100 µmol/L**
1	65.2 ± 3.4	NE	19.6 ± 1.9	617.9 ± 81.1
3	155.2 ± 27.2	NE	27.1 ± 0.7	1135.6 ± 41.5
6	199.6 ± 20.8	NE	27.9 ± 0.7	1193.4 ± 47.4
9	136 ± 12.5	NE	25.9 ± 1	1262.7 ± 19.6
12	103.7 ± 0.6	NE	22.9 ± 1.1	1114.4 ± 31.7
30	99.6 ± 14.1	NE	15.9 ± 2.6	584.1 ± 17.1

In Caco-2 cells, opicapone uptake was performed in a non-saturable pH dependent manner. Opicapone accumulation by Caco-2 cells increased in the presence of DNP. Interaction with the known OATP-B (OATP2B1) substrate, estrone-3-sulfate, suggests the involvement of OATP-B in opicapone absorption.

2.6.5.4 薬物動態試験：吸収：単回投与

2.6.5.4.1 ラット

Test article: ^{14}C-opicapone
Location in CTD: [4.2.2.2-1]
Study No. ZNA15658.001

Study title: The Disposition of Total Radioactivity in the Rat Following Single Oral and Single Intravenous Administration of [^{14}C] BIA 9-1067		
Species/strain	Rat/Wistar	Rat/Wistar
Gender (M/F)/number of animals	Male/4 (phase 3)	Male/4 (phase 5)
Feeding condition	Fed	Fed
Vehicle/formulation	0.5% CMC/suspension	DMSO/solution
Method of administration	p.o. (gavage)	i.v.
Dose (mg/kg)	10	1
Matrix	Plasma	Plasma
Analyte/assay	Radioactivity/LSC	Radioactivity/LSC
Time point (h)	**Plasma concentration of radioactivity (ng eq./g, mean ± SD)**	
0.08	N/A	11584 ± 5735
0.25	705 ± 358	4519 ± 1339
0.5	904 ± 315*	3509 ± 1326*
1	882 ± 268	2305 ± 327*
2	1277 ± 665	1537 ± 210*
4	1561 ± 277	987**
6	1450 ± 222	N/A
8	1197 ± 175	815 ± 96.9
12	N/A	N/A
24	453 ± 81.5	550 ± 74.4
48	408 ± 44.9	518 ± 92.3
72	426 ± 30.8	502 ± 68.4
Cmax (ng eq./g)	1561 ±277	11584 ± 5735
Tmax (h)	4	0.08

*n = 3, **n = 1.

2.6.5.4.2 サル

Test article: ^{14}C-opicapone
Location in CTD: [4.2.2.2-2]
Study No. ZNA15658.002

Study title: The Disposition of Total Radioactivity in the Primate Following Single Oral and Single Intravenous Administration of [^{14}C] BIA 9-1067		
Species/strain	Monkey/Cynomolgus	Monkey/Cynomolgus
Gender (M/F)/number of animals	Male/4	Male/4
Feeding condition	Fed	Fed
Vehicle/formulation	0.5% CMC/suspension	DMSO/saline (70:30)/solution
Method of administration	p.o. (gavage)	i.v.
Dose (mg/kg)	10	1
Matrix	Plasma	Plasma
Analyte/assay	Radioactivity/LSC	Radioactivity/LSC
Time point (h)	**Plasma concentratioin of radioactivity (ng eq./g, mean ± SD)**	
0.08	N/A	3433 ± 1594
0.25	1596 ± 620	781 ± 149
0.5	1196 ± 539	439 ± 75.1
1	730 ± 196	220 ± 40.7
2	438 ± 127	107 ± 12.6
4	405 ± 172	55.6 ± 5.27
6	249 ± 20.9	43.0 ± 3.97
8	210 ± 69.7	37.3 ± 2.18
24	170 ± 30.5	30.8 ± 1.83
48	106 ± 31.3	22.6 ± 1.37
72	78.3 ± 15.7	19.5 ± 1.19
120	51.6 ± 14.6	N/A
168	63.6 ± 11.1	13.5 ± 0.659
Cmax (ng eq./g)	1596 ± 620	3433 ± 1594
Tmax (h)	0.25	0.08

2.6.5.5 薬物動態試験：吸収：反復投与

2.6.5.5.1 ラット（26 週間）

Test article: opicapone
Location in CTD: [4.2.2.2-3]
Study No. C03508

Study title:	BIA 9-1067 26-Week Oral (Gavage) Toxicity Study in the Wistar Rat
Species/strain:	Rat/HanRcc:WIST
Gender (M/F)/number of animals:	Male and female/9 per group (opicapone-dosed)
Feeding condition:	Fed
Vehicle/formulation:	0.2% HPMC/suspension
Method of administration:	p.o. (gavage)
Dose (mg/kg/day):	100, 500, 1000
Duration of dosing:	Once daily for 26 weeks
Matrix:	Plasma
Analyte/assay:	Opicapone and BIA 9-1079/LC-MS/MS
Time Points for Toxicokinetics:	Day 1, and Weeks 13 and 26

Opicapone

Time Point	Parameter	Male			Female		
		100 mg/kg/day	500 mg/kg/day	1000 mg/kg/day	100 mg/kg/day	500 mg/kg/day	1000 mg/kg/day
Day 1	Cmax (µg/mL)	4.14	8.28	16.47	4.29	10.50	23.37
	Tmax (h)	2	0.5	1	1	1	1
	AUClast (µg·h/mL)	17.47	33.63	50.62	14.02	31.45	49.31
	T1/2 (h)	NC	8.8	4.0[#]	1.8	1.8	2.2[#]
Week 13	Cmax (µg/mL)	8.82	16.73	18.80	10.80	26.83	31.40
	Tmax (h)	0.5	0.5	0.5	0.5	0.5	0.5
	AUClast (µg·h/mL)	15.01	38.60	54.04	12.41	53.42	62.54
	T1/2 (h)	1.7	1.5	2.5	0.95	2.6	1.9
Week 26	Cmax (µg/mL)	7.19	19.47	19.73	7.66	27.13	23.33
	Tmax (h)	0.5	0.5	0.5	0.5	0.5	1
	AUClast (µg·h/mL)	14.95	59.18	67.94	11.27	41.61	65.22
	T1/2 (h)	1.6	7.8[#]	2.4	1.3	1.4	2.7[#]

2.6.5.5.1 ラット（26 週間）（続き）

BIA 9-1079

Time Point	Parameter	Male			Female		
		100 mg/kg/day	500 mg/kg/day	1000 mg/kg/day	100 mg/kg/day	500 mg/kg/day	1000 mg/kg/day
Day 1	Cmax (µg/mL)	1.86	2.12	3.07	1.45	2.30	2.54
	Tmax (h)	6	6	6	6	2	1
	AUClast (µg·h/mL)	19.73	25.58	40.08	11.95	19.80	31.93
	T1/2 (h)	NC	NC	NC	NC	3.3	7.2
Week 13	Cmax (µg/mL)	1.20	3.05	4.47	1.05	3.12	4.15
	Tmax (h)	1	2	2	2	2	1
	AUClast (µg·h/mL)	16.35	28.62	47.52	7.53	28.20	37.86
	T1/2 (h)	9.0	5.9	6.7	3.8	6.2	6.4
Week 26	Cmax (µg/mL)	1.14	2.64	4.05	0.97	2.45	3.66
	Tmax (h)	6	2	2	2	1	1
	AUClast (µg·h/mL)	15.12	34.11	46.50	7.58	19.98	35.03
	T1/2 (h)	NC	13	10	4.2	4.7	6.7

Toxicokinetic parameters were calculated from mean plasma concentration (n = 3/time point) versus time profiles.
[#]Unreliable, $r^2 < 0.900$ and/or AUC%extr > 20%.

2.6.5.5.2 サル（52 週間）

Test article: opicapone
Location in CTD: [4.2.2.2-4]
Study No. S13825

Study title:	BIA 9-1067 52-Week Oral (Gavage) Toxicity Study in the Cynomolgus Monkey with a 8-Week Recovery Period
Species/strain:	Monkey/Cynomolgus
Gender (M/F)/number of animals:	Male and Female/4 to 6 per group
Feeding condition:	Fed
Vehicle/formulation:	0.2% HPMC/suspension
Method of administration:	p.o. (gavage)
Dose (mg/kg/day):	100, 300, and 1000
Duration of dosing:	Once daily for 52 weeks
Matrix:	Plasma
Analyte/assay:	Opicapone and BIA 9-1079/LC-MS/MS
Time Points for Toxicokinetics:	Weeks 1, 13, 26, and 52

Opicapone

Time point	Parameter	Male			Female		
		100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Week 1	Cmax (µg/mL)	1.08	2.26	9.42	1.21	1.61	8.80
	Tmax* (h)	1.0	1.0	1.0, 2.0	1.0	1.0	1.0, 2.0
	AUClast (µg·h/mL)	1.39	2.97	32.10	1.33	3.62	34.40
	T1/2 (h)	0.76	1.6	5.0	[0.67]	2.5	4.4
Week 13	Cmax (µg/mL)	0.50	1.46	6.99	0.75	1.07	5.22
	Tmax* (h)	1.0	1.0, 2.0	2.0	1.0	1.0, 2.0	1.0, 2.0
	AUClast (µg·h/mL)	0.65	2.45	30.00	0.83	1.89	15.30
	T1/2 (h)	0.88	0.99	2.5	0.72	1.4	2.4

2.6.5.5.2 サル（52 週間）（続き）

Time point	Parameter	Male			Female		
		100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Week 26	Cmax (µg/mL)	0.53	1.13	3.72	0.67	1.03	3.42
	Tmax* (h)	1.0	1.0, 2.0	2.0, 4.0	1.0	1.0, 2.0	2.0, 4.0
	AUClast (µg·h/mL)	0.80	2.69	21.60	1.18	2.71	17.80
	T1/2 (h)	0.66	1.7	3.3	0.80	1.5	2.6
Week 52	Cmax (µg/mL)	1.04	1.21	4.44	1.26	1.11	2.92
	Tmax* (h)	1.0	1.0	2.0	1.0	1.0, 2.0	1.0, 2.0
	AUClast (µg·h/mL)	1.65	2.62	20.90	2.27	3.40	16.20
	T1/2 (h)	1.2	1.5	4.7	1.4	2.5	4.7

BIA 9-1079

Time point	Parameter	Male			Female		
		100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Week 1	Cmax (µg/mL)	0.85	0.87	2.22	0.72	1.14	2.57
	Tmax* (h)	1.0, 2.0	1.0, 2.0	1.0, 2.0	1.0, 2.0	1.0, 2.0	2.0
	AUClast (µg·h/mL)	4.04	5.79	27.10	3.32	9.31	30.00
	T1/2 (h)	3.7	5.5	10	3.1	5.1	[8.6]
Week 13	Cmax (µg/mL)	0.91	1.18	2.39	0.77	1.32	2.86
	Tmax* (h)	1.0, 2.0	1.0, 2.0	2.0, 4.0	1.0, 2.0	1.0, 2.0	2.0, 8.0
	AUClast (µg·h/mL)	4.17	8.42	35.70	2.84	10.60	36.40
	T1/2 (h)	3.3	4.4	15	2.8	4.6	7.0

2.6.5.5.2　　サル（52 週間）（続き）

Time point	Parameter	Male			Female		
		100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Week 26	Cmax (μg/mL)	0.91	1.31	2.85	0.82	1.41	3.46
	Tmax* (h)	1.0, 2.0	1.3, 2.0	2.0, 8.0	1.0, 2.0	2.0, 4.0	2.0, 8.0
	AUClast (μg·h/mL)	5.30	13.20	41.30	3.54	11.50	57.10
	T1/2 (h)	3.6	5.6	12	2.3	4.0	15
Week 52	Cmax (μg/mL)	1.36	1.44	2.31	1.21	1.42	2.42
	Tmax* (h)	1.0, 2.0	1.0, 4.0	2.0, 4.0	2.0	2.0	2.0, 4.0
	AUClast (μg·h/mL)	6.42	11.40	36.80	6.47	13.90	36.10
	T1/2 (h)	3.6	4.9	28	3.6	5.9	14

[ ], Based on one value.
*Minimum, maximum.

2.6.5.6 薬物動態試験：分布

2.6.5.6.1 組織分布

Test article: ^{14}C-opicapone
Location in CTD: [4.2.2.3-1]
Study No. ZNA15658.003

Study title:	The Quantitative Tissue Distribution of Total Radioactivity in the Rat Following Single Oral Administration of [^{14}C] BIA 9-1067
Species/strain:	Rat/Wistar
Gender (M/F)/number of animals:	Male/3 per time point
Feeding condition:	Fed
Vehicle/formulation:	0.5% CMC/suspension
Method of administration:	p.o. (gavage)
Dose (mg/kg):	10
Radionuclide/specific activity:	^{14}C/0.78 MBq/mg
Sampling time:	1, 4, 12, and 48 h
Analyte/Assay:	Radioactivity/QWBA and LSC

Tissues/organs	**Radioactivity concentration (ng eq./g)**			
	1 h	**4 h**	**12 h**	**48 h**
Adrenal cortex	423	444	202	BLQ
Adrenal medulla	475	453	182	BLQ
Bone	BLQ	BLQ	BLQ	BLQ
Bone marrow	232	316	204	BLQ
Brain	BLQ	BLQ	BLQ	BLQ
Brown fat	239	267	214	142
Cecum contents	BLQ	190924	83707	1040
Cecum wall	161	2656	7179	280
Cardiac blood	709	643	244	171
Cardiac muscle	304	363	128	BLQ
Epididymis	143	139	193	BLQ

2.6.5.6.1 組織分布（続き）

Tissues/organs	Radioactivity concentration (ng eq./g)			
	1 h	4 h	12 h	48 h
Eye humor	BLQ	BLQ	BLQ	BLQ
Eye lens	BLQ	BLQ	BLQ	BLQ
Fur	138	191	BLQ	BLQ
Harderian gland	179	233	223	BLQ
Kidney cortex	3457	4677	2880	1808
Kidney medulla	3280	4174	2549	1692
Large intestine contents	162	25445	188197	1913
Large intestine wall	222	753	1086	237
Liver	4387	7920	4405	2373
Lung	516	613	303	153
Nasal mucosa	BLQ	BLQ	BLQ	BLQ
Pancreas	246	300	226	BLQ
Pineal body	292	249	143	BLQ
Pituitary gland	244	312	226	BLQ
Preputial gland	220	181	147	BLQ
Prostate	191	272	202	BLQ
Seminal vesicles	158	209	BLQ	BLQ
Skeletal muscle	BLQ	134	BLQ	BLQ
Skin	212	326	203	BLQ
Small intestine contents	348393	369577	5193	595
Small intestine wall	13451	2422	1208	198
Spinal cord	BLQ	BLQ	BLQ	BLQ
Spleen	321	403	322	158
Stomach contents	250040	19968	BLQ	BLQ
Stomach wall	3996	1081	531	279
Submaxillary salivary gland	309	377	260	142

2.6.5.6.1 組織分布（続き）

Tissues/organs	Radioactivity concentration (ng eq./g)			
	1 h	**4 h**	**12 h**	**48 h**
Testes	BLQ	149	BLQ	BLQ
Thymus	BLQ	BLQ	BLQ	BLQ
Thyroid gland	344	372	277	171
Urine	2594	8019	2164	BLQ
White fat	BLQ	BLQ	BLQ	BLQ
Whole blood	604	690	247	184
LLOQ	124	121	124	129

2.6.5.7 薬物動態試験：蛋白結合

2.6.5.7.1 オピカポン（蛋白白結合及び血球移行）

Test article: ^{14}C-opicapone
Location in CTD: [4.2.2.3-2]
Study No. ZNA15658.004

Study title:	Investigation of Plasma Protein and Blood Cell Binding of [^{14}C] BIA 9-1067 in Rat, Primate and Human and the Effect of Warfarin, Diazepam, Digoxin or Tolbutamide on Human Plasma Protein Binding of [^{14}C] BIA 9-1067 Using Ultrafiltration
Test system:	Male rat, male monkey, and male human plasma and blood
Method:	Ultrafiltration (plasma protein binding); PCV and combustion (blood cell binding).
Assay:	LSC

Plasma protein binding

Species	Opicapone concentration (µg/mL)	% Unbound	% Bound
Rat	30	0.5	99.5
	3	0.2	99.8
	0.3	0.2	99.8
Monkey	30	0.6	99.4
	3	0.5	99.5
	0.3	0.4	99.6
Human	30	0.1	99.9
	3	0.1	99.9
	0.3	0.1	99.9

2.6.5.7.1 オピカポン（蛋白白結合及び血球移行）（続き）

Blood cell binding

Species	Opicapone concentration (µg/mL)	PCV (%)	Plasma : blood ratio	% Association with blood cells
Rat	30	35.3	1.5	2.4
	3	35.6	1.7	0.0
	0.3	35.6	1.8	0.0
Monkey	30	46.6	1.8	3.8
	3	47.0	1.8	5.8
	0.3	46.8	1.5	18.6
Human	30	39.9	1.8	0.0
	3	40.3	1.8	0.0
	0.3	39.9	1.8	0.0

Blood was incubated with opicapone for 30 min.

2.6.5.7.2 BIA 9-1103

Test article: BIA 9-1103
Location in CTD: [4.2.2.3-3]
Study No. SRAL[redacted]0903

Study title:	Plasma Protein Binding of BIA 9-1103 Using Equilibrium Gel Filtration: Interspecies Comparison
Test system:	Rat, dog, monkey, and human plasma
Method:	Equilibrium Gel Filtration
Assay:	LC-MS/MS

Species	BIA 9-1103 concentration (ng/mL)	Fraction unbound (%)	Fraction bound
Rat	4	0.35	0.9965
	10	0.38	0.9962
	40	0.33	0.9967
Dog	4	0.38	0.9962
	10	0.48	0.9952
	40	0.37	0.9963
Monkey	4	0.41	0.9959
	10	0.38	0.9962
	40	0.36	0.9964
Human	4	0.38	0.9962
	10	0.49	0.9951
	40	0.32	0.9968

2.6.5.7.3 BIA 9-1079

Test article: BIA 9-1079
Location in CTD: [5.3.2.1-1]
Study No. SRAL[redacted]0325

Study title:	Plasma Protein Binding of BIA 9-1079 Using Equilibrium Gel Filtration
Test system:	Human plasma
Method:	Equilibrium Gel Filtration
Assay:	LC-MS/MS

BIA 9-1079 concentration (ng/mL)	Fraction unbound	Fraction bound
4	0.0008	0.9992
10	0.0003	0.9997
40	0.0006	0.9994

2.6.5.8 薬物動態試験：妊娠又は授乳動物における試験

2.6.5.8.1 胎盤移行性

Test article: ^{14}C-opicapone
Location in CTD: [4.2.2.3-4]
Study No. AE-7745-G

Study Title	Placental Transfer in Pregnant Rats after Single Oral Administration of ^{14}C-Opicapone
Species/strain:	Rat/Crlj:WI
Gestation day/number of animals:	17 days gestation/1 per time point
Feeding condition:	Fed
Vehicle/formulation:	0.5 w/v% CMC-Na/suspension
Method of administration:	p.o. (gavage)
Dose (mg/kg):	10
Radionuclide:	^{14}C
Specific activity:	0.5 MBq/mg
Analyte/assay:	Radioactivity/LSC and QWBA

Tissues/organs	**Radioactivity concentration (ng eq./g or mL)**			
	1 h	**4 h**	**12 h**	**48 h**
Blood*	2450	847	355	170
Plasma*	4280	1480	530	211
Adrenal gland	2000	938	454	205
Amniotic fluid	BLQ	BLQ	BLQ	BLQ
Blood	3110	1190	388	174
Cerebellum	62.7	BLQ	BLQ	BLQ
Cerebrum	BLQ	BLQ	BLQ	BLQ
Fetal blood	199	209	206	192
Fetal brain	BLQ	BLQ	BLQ	54.1
Fetal gastrointestinal tract	BLQ	Not specified	Not specified	BLQ

*Analyzed by LSC.

2.6.5.8.1 胎盤移行性（続き）

Tissues/organs	Radioactivity concentration (ng eq./g or mL)			
	1 h	**4 h**	**12 h**	**48 h**
Fetal heart	BLQ	Not specified	Not specified	Not specified
Fetal kidney	163	159	224	157
Fetal liver	188	203	279	174
Fetal lung	89.9	95.2	139	157
Fetal membrane	1190	1240	1420	1060
Fetus (whole body)	83.5	90.6	170	181
Heart	1070	412	250	66.1
Kidney	5650	4830	2840	1040
Liver	16500	12300	8020	2200
Lung	1970	1020	484	223
Mammary gland	732	427	327	199
Ovary	1250	641	375	157
Placenta	2600	1160	648	283
Uterus	1690	647	618	210
LLOQ	52.0			

2.6.5.8.2 **乳汁排泄**

Test article: ^{14}C-opicapone
Location in CTD: [4.2.2.5-1]
Study No. Y[redacted]AG013

Study Title	Excretion of Radioactivity into Milk in Lactating Rats after Single Oral Administration of ^{14}C-Opicapone
Species/strain:	Rat/Crlj:WI
Lactating date/number of animals:	Day 11/3
Feeding condition:	Fed
Vehicle/formulation:	0.5 w/v% carboxymethylcellulose/suspension
Method of administration:	Gavage
Dose (mg/kg):	10
Radionuclide:	^{14}C
Specific activity:	0.5 MBq/mg
Analyte/assay:	Radioactivity/LSC

Sample	Radioactivity concentration (ng eq./mL)			
	1 h	4 h	12 h	48 h
Milk	197± 91	420 ± 50	465 ± 131	116 ± 104
Plasma	406 ± 84	450 ± 42	440 ± 79	173 ± 24
Milk/plasma	0.509 ± 0.262	0.933 ± 0.071	1.08 ± 0.35	0.635 ± 0.500

Data represent means ± SD.

2.6.5.9 薬物動態試験：その他の分布試験

該当する資料はない.

2.6.5.10 **薬物動態試験：代謝：*In vivo***

2.6.5.10.1 **ラット *in vivo* における代謝**

Test article: opicapone
Location in CTD: [4.2.2.4-4]
Study No. SRAL[redacted]1228

Study title:	Opicapone and Metabolites Exposure Following Admnistration of 10 mg/kg Opicapone to Rats in HPMC 0.2%
Species/strain:	Rat/Wistar
Gender (M/F)/number of animals:	Not stated/4
Feeding condition:	Not stated
Vehicle/formulation:	0.2% HPMC/not stated
Method of administration:	p.o.
Dose (mg/kg):	10
Sample:	Plasma
Analyte:	Opicapone, BIA 9-1079, BIA 9-1100, BIA 9-1101, BIA 9-1103, and BIA 9-1106
Assay:	LC-MS/MS

PK parameter	Opicapone	BIA 9-1079	BIA 9-1103	BIA 9-1106	BIA 9-1100	BIA 9-1101
Cmax (ng/mL)	1423	55.16 (4)	123.0 (9)	674.8 (47)	433.9 (30)	17.03 (1)
Tmax (h)	1	7	3	1	1	24
AUClast (ng·h/mL)	9117	758.4 (8)	1107 (12)	2568 (28)	4677 (51)	306.7 (3)

Numbers in parentheses represent percentage values relative to opicapone.

2.6.5.10.2 サル *in vivo* における代謝

Test article: opicapone
Location in CTD: [4.2.2.4-5]
Study No. SRAL [redacted]0509

Study title:	Quantification of Opicapone and Its Metabolites in the Cynomolgus Monkey Following Oral Administration
Species/strain:	Monkey/Cynomolgus
Gender (M/F)/number of animals:	Male/2 and female/2
Feeding condition:	Food offered 1 h after dosing
Vehicle/formulation:	0.2% HPMC/suspension
Method of administration:	p.o. (gavage)
Dose (mg/kg/day)/duration:	1000/7 days
Matrix:	Plasma
Analyte:	Opicapone, BIA 9-1079, BIA 9-1100, BIA 9-1101, [redacted], BIA 9-1103, [redacted], [redacted] BIA 9-1106, and [redacted]
Assay:	LC-MS

Time	Cmax (µg/mL, mean ± SD, n = 4) and AUClast (µg·h/mL, mean ± SD, n = 4)				
	Opicapone	**BIA 9-1079**	**BIA 9-1103**	**BIA 9-1106**	[redacted]
Day 1 Cmax	1.66 ± 1.07	1.30 ± 0.39	1.37 ± 1.11	2.28 ± 2.21	0.40 ± 0.23
Day 1 AUClast	10.58 ± 5.18	17.90 ± 5.01	8.33 ± 7.48	11.13 ± 6.15	4.17 ± 1.24
Day 7 Cmax	2.09 ± 1.17	1.76 ± 0.51	1.08 ± 0.45	1.13 ± 0.54	0.29 ± 0.10
Day 7 AUClast	8.33 ± 3.36	15.1 ± 6.25	5.80 ± 3.24	4.54 ± 1.63	2.57 ± 0.95

The major circulating metabolites of opicapone in monkey plasma were BIA 9-1106, BIA 9-1079, and BIA 9-1103, but significant amounts of [redacted] were also quantified in circulation on Day 1 and 7 following repeated administration. Trace levels of BIA 9-1101 and [redacted] in plasma were detected, and BIA 9-1100, [redacted] and [redacted] were not detected.

2.6.5.10.3 *In vivo* 代謝の種差

Test article: opicapone
Location in CTD: [4.2.2.4-1]
Study No. SRAL [redacted]0116

Study title:	BIA 9-1067 Metabolic Profiling
Species/:	Mouse, rat, hamster, guinea pig, mini-pig, dog, and monkey
Feeding condition:	Fasted/fed
Method of administration:	p.o. and i.p.
Dose (mg/kg):	Mouse (30 and 1000, p.o.), rat (100 and 1000, p.o.; 10, i.p.), hamster (30), guinea pig (30), mini-pig (not stated), dog (not stated), and monkey (not stated)
Matrix:	Plasma
Analyte/assay:	Opicapone and metabolites/LC-MS

Compound	Mouse	Rat	Hamster	Guinea pig	Mini-pig	Dog	Monkey
Opicapone	+	+	+	+	+	+	+
BIA 9-1079 (*N*-oxide reduced)	+	+	+	+	+	+	+
BIA 9-1103 (3-*O*-sulfate)	+	+	ND	ND	ND	ND	+
[redacted]	ND	+	ND	ND	ND	ND	+
BIA 9-1100 (3-*O*-methyl)	ND	+	+	ND	+	+	ND
[redacted]	ND	ND	ND	ND	+	NE	ND
BIA 9-1106 (3-*O*-glucuronide)	+	+	+	ND	+	ND	+
[redacted]	ND	+	+	+	+	ND	+
BIA 9-1101 (3-*O*-methylate of BIA 9-1079)	ND	+	+	+	+	+	+
[redacted]	NE	NE	NE	NE	ND	NE	ND
[redacted]	NE	ND	ND	NE	NE	NE	+
[redacted]	NE	+	NE	NE	NE	NE	+
Opicapone nitroreduction	NE	ND	ND	ND	NE	NE	ND
Opicapone nitro reductant and acetylate	NE	ND	ND	NE	NE	NE	ND
Opicapone hydroxylate	NE	+	ND	ND	NE	NE	ND
BIA 9-1079 hydroxylate	NE	+	ND	ND	NE	NE	ND

2.6.5.10.3 *In vivo* 代謝の種差（続き）

In all species, opicapone *N*-oxide is reduced to BIA 9-1079. Biotransformation of opicapone and BIA 9-1079 is mainly via sulfation, glucuronidation, methylation, and nitro reduction.

2.6.5.10.4 *In vivo* 代謝の種差

Test article: opicapone
Location in CTD: [4.2.2.4-6]
Study No. SROPC[redacted]0313

Study title:	Opicapone (BIA 9-1067) and BIA 9-1079 Interspecies
Species/strain:	Mouse/NMRi, rat/Wistar, hamster, guinea pig
Feeding condition:	Not stated
Method of administration:	p.o.
Dose (mg/kg):	30
Matrix:	Plasma (all species), liver (all species), and kidney (hamster and guinea pig)
Sampling time:	3 h postdose
Analyte/assay:	Opicapone and BIA 9-1079/not stated

Species	Concentration of opicapone (mean ± SEM, n = 4-7)			Concentration of BIA 9-1079 (mean ± SEM, n = 4-7)		
	Plasma (µg/mL)	Liver (µg/g)	Kidney (µg/g)	Plasma (µg/mL)	Liver (µg/g)	Kidney (µg/g)
Mouse	0.96 ± 0.27	1.42 ± 0.08	NE	0.80 ± 0.18	0.66 ± 0.04	NE
Hamster	0.07 ± 0.04	0.07 ± 0.05	0.93 ± 0.01	0.80 ± 0.10	0.25 ± 0.03	0.15 ± 0.01
Rat	3.20 ± 0.84	13.09 ± 1.52	NE	2.75 ± 0.27	0.56 ± 0.02	NE
Guinea pig	0.0 ± 0.0	0.23 ± 0.01	0.04 ± 0.00	0.18 ± 0.02	0.03 ± 0.01	0.04 ± 0.00

2.6.5.11 薬物動態試験：代謝：*In vitro*

2.6.5.11.1 *In vitro* 代謝の種差

Test article: opicapone
Location in CTD: [4.2.2.4-1]
Study No. SRAL[redacted]0116

Study title:	BIA 9-1067 Metabolic Profiling
Test system:	S9 fraction from human, mouse and rat liver
Method:	Glucuronidation, sulfation and oxidation activity by liver S9 fraction was measured. Liver S9 fraction and 20 µmol/L opicapone were incubated for 60 min with UDPGA, NADPH and PAPS.
Analyte/assay:	Opicapone and metabolites/LC-MS

Compound	Mouse	Rat	Human
Opicapone	+	+	+
BIA 9-1079 (*N*-oxide reduced)	+	+	+
BIA 9-1103 (3-*O*-sulfate)	+	+	+
[redacted]	+	+	+
BIA 9-1106 (3-*O*-glucuronide)	+	+	+
[redacted]	+	+	+

2.6.5.11.2 *In vitro* でのオピカポンの硫酸抱合

Test article: opicapone
Location in CTD: [5.3.2.2-1]
Study No. SRAL[redacted]0822

Study title:	Characterisation of Opicapone Sulfation *In Vitro*
Test system:	Human liver S9 fraction, small intestinal S9 fraction, and recombinant human SULT (SULT 1A1*1, 1A1*2, 1A3, 1A2, 2A1, 1E1, and 1B1)
Method:	Opicapone sulfation was evaluated using human liver and intestinal S9 fraction or with recombinant SULT enzymes. Recombinant SULT and 10 µmol/L opicapone were incubated for 60 min. In addition, SULT1A1*1 or 1A1*2 was incubated with 1 to 100 µmol/L opicapone. S9 was incubated with 1 to 100 µmol/L opicapone for 60 min.
Analyte/assay:	BIA 9-1103/LC-MS/MS

Test system	**Apparent kinetic parameter of opicapone sulfation**		
	Km (µmol/L)	**Vmax (nmol/mg protein/min)**	**Ksi (µmol/L)**
Human small intestinal S9	8.152 ± 1.077	1.355 ± 0.03865	N/A
Human liver S9	20.46 ± 2.605	0.3674 ± 0.01459	N/A
SULT1A1*1	18.65 ± 11.04	26.28 ± 10.34	33.44 ± 19.80
SULT1A1*2	27.32 ± 22.26	55.44 ± 32.90	26.90 ± 22.21
From the tested SULTs only SULT1A1*1 and 1A1*2 produced significant amounts of BIA 9-1103. SULT1A2, 1A3, 2A1 and 1E1 produced little amounts of BIA 9-1103. No metabolite formation was detected with SULT1B1 over an incubation period of 60 min. Opicapone is mainly metabolized by SULT1A1, which is generally recognized as the major xenobiotic-metabolizing SULT.			

2.6.5.11.3 *In vitro* 硫酸抱合の種差

Test article: opicapone
Location in CTD: [4.2.2.4-2]
Study No. SRAL[redacted]0511

Study title:	Opicapone (BIA 9-1067) *In Vitro* Sulfation by S9 Fraction from Different Species
Test system:	S9 fraction from human liver, kidney, and small intestine; monkey liver; dog liver; and rat liver
Method:	S9 fraction and 10 µmol/L opicapone was incubated for 60 min with or without PAPS.
Analyte/assay:	BIA 9-1103/LC-MS/MS

Test system	**Apparent sulfation rate (fmol/mg/min, mean ± SEM, n = 2)**	
	with PAPS	**without PAPS**
Human small intestinal S9	516.0 ± 97.6	8.2 ± 0.7
Human liver S9	45.2 ± 0.6	6.5 ± 0.0
Human kidney S9	21.0 ± 0.5	7.8 ± 0.1
Rat liver S9	86.2 ± 1.2	5.4 ± 0.1
Dog liver S9	91.8 ± 0.4	7.2 ± 0.9
Monkey liver S9	74.5 ± 0.6	5.8 ± 0.6
Results indicate that all tested S9 fractions sulfated opicapone. Human intestinal S9 fraction has the highest sulfating capacity.		

2.6.5.11.4 BIA 9-1103 のヒト肝細胞質及び S9 画分中安定性

Test article: BIA 9-1103
Location in CTD: [5.3.2.2-2]
Study No. SRAL[redacted]0414

Study title:	3-*O*-sulfate-opicapone (BIA 9-1103) Stability in Human Cytosolic and S9 Fractions
Test system:	Human liver cytosol and S9 fractions
Method:	BIA 9-1103 (1 µmol/L) was incubated in human liver cytosol or S9 fraction for 60 min. Unchanged BIA 9-1103 and opicapone were detrermined. Estrone-3-sulfate was used as a positive control substrate.
Assay:	LC-MS, LC-MS/MS

Compound	Fraction	Incubation time (min)	% remaining	Desulfate concentration (nmol/L)
BIA 9-1103	S9	0	101.09 ± 2.78	0.0000 ± 0.0000
		60	101.43 ± 1.38	0.0000 ± 0.0000
	Cytosol	0	111.71 ± 0.92	0.0000 ± 0.0000
		60	103.14 ± 2.67	0.0000 ± 0.0000
	None	0	106.96 ± 4.31	0.0000 ± 0.0000
		60	100.73 ± 0.80	0.0000 ± 0.0000
Estrone-3-sulfate	S9	0	NE	0.0000 ± 0.0000
		60	NE	419.0647 ± 21.3510
	Cytosol	0	NE	0.0000 ± 0.0000
		60	NE	0.0000 ± 0.0000

Data represent mean ± SD of 4 deteminations.

2.6.5.11.5 オピカポンの *in vitro* グルクロン酸抱合

Test article: opicapone
Location in CTD: [5.3.2.2-3]
Study No. SRAL[redacted]0712

Study title:	Characterisation of Opicapone Glucuronidation *In Vitro*
Test system:	Human liver microsomes, human small intestinal microsomes, and recombinant human UGT
Method:	Rates of glucuronidation (1 to 250 µmol/L opicapone) were determined for human liver microsomes, human small intestinal microsomes, and recombinant UGTs (UGT 1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15 and 2B17). Experimental conditions were chosen within the linearity range previously evaluated for product formation with respect to incubation time and protein.
Analyte/assay:	Opicapone glucuronide/LC-MS

Test system	Apparent kinetic parameter		
	Vmax (pmol/mg/min)	Km (µmol/L)	Ksi (µmol/L)
Human small intestinal microsomes	111.3 ± 44.54	41.91 ± 26.88	114.2 ± 76.83
Human liver microsomes	24.97 ± 1.548	27.87 ± 6.364	N/A
UGT1A1	1.504 ± 0.06010	18.0 ± 2.88	N/A
UGT1A3	1.649 ± 0.1517	213 ± 34.3	N/A
UGT1A4	N/A	N/A	N/A
UGT1A6	N/A	N/A	N/A
UGT1A7	8.009 ± 0.8223	1.658 ± 0.7024	N/A
UGT1A8	Not good fitting	Not good fitting	N/A
UGT1A9	98.92 ± 22.35	13.7 ± 6.68	105 ± 45.9
UGT1A10	Not good fitting	Not good fitting	N/A
UGT2B4	N/A	N/A	N/A
UGT2B7	0.1970 ± 0.01198	20.6 ± 4.41	N/A
UGT2B15	N/A	N/A	N/A
UGT2B17	N/A	N/A	N/A
UGT1A1, 1A3, 1A7, 1A8, 1A9, 1A10 and 2B7 exhibited opicapone glucuronidation.			

2.6.5.11.6 カテコール-*O*-メチル化反応の種差

Test article: opicapone and BIA 9-1079
Location in CTD: [4.2.2.4-3]
Study No. SROPC[redacted]0311

Study title:	Opicapone Methylation *In Vitro*
Test system:	Rat liver homogenate, rat and mouse S-COMT, and human, rat, and mouse liver S9
Method:	Opicapone and BIA 9-1079 (10 µmol/L) was incubatied for 30 to 120 min (with rat liver homogenate), 60 to 120 min (with S-COMT), or 60 min (S9) in the presence or absence of *S*-adenosylmethionine.
Analyte:	Rat liver homogenate: methylates of opicapone and BIA 9-1079 S-COMT and S9: BIA 9-1100 (3-*O*-methyl),[redacted], BIA 9-1101 (3-*O*-methylate of BIA 9-1079), and [redacted]
Assay:	Not stated

Results:
In rat liver homogenate, opicapone methylation was observed after 30 to 120 min incubation, and it increased in a time-dependent manner. Following 120-min incubation, some methylate of BIA 9-1079 was also detected.
With rat and mouse S-COMT after 60 to 120 min incubation, opicapone was methylated into BIA 9-1100 and in trace amounts of [redacted] and BIA 9-1101. [redacted] was not observed in rat and only trace amounts in mouse S-COMT.
No methylation of opicapone was detected in human liver S9 fraction at 60 min following incubation with opicapone. However, opicapone methylation into BIA 9-1100 and trace amounts of [redacted] were detected after 60 min incubation with rat and mouse S9 fraction.
Opicapone and BIA 9-1079 methylation were mainly observed in the 3-*O* position, in rat and in mouse cellular fraction. No methylation of opicapone and BIA 9-1079 was observed in human liver S9 fraction.

2.6.5.11.7 代謝に関与する CYP 分子種

Test article: opicapone and BIA 9-1103
Location in CTD: [5.3.2.2-4]
Study No. SRAL[redacted]0615

Study title:	Opicapone CYP Reaction Phenotyping
Test system:	Human liver microsomes and cDNA-expressed human CYP
Method:	Relative metabolism of opicapone and its sulfated metabolite (BIA 9-1103) in human liver microsomes was evaluated. Microsomes were incubated with 5 µmol/L opicapone in the presence or absence of NADPH and UDPGA. CYP reaction phenotyping was assessed by incubating 5 µmol/L opicapone with recombinant human CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, or 3A4.
Assay:	LC-MS

Metabolism in human liver microsomes

Coenzyme	% CTR							
	Opicapone				BIA 9-1103			
	Time (min)	Mean	SEM	n	Time (min)	Mean	SEM	n
NADPH (+), UDPGA (+)	0	100.0	3.5	2	0	100.0	1.9	2
	60	114.0	3.2	2	60	99.2	2.6	2
	120	121.6	1.3	2	120	106.8	1.4	2
NADPH (−), UDPGA (+)	120	85.3	3.5	2	120	99.4	1.6	2
NADPH (+), UDPGA (−)	120	154.0	7.6	2	120	109.6	0.8	2

2.6.5.11.7　　代謝に関与する CYP 分子種（続き）

CYP isoforms involved in metabolism

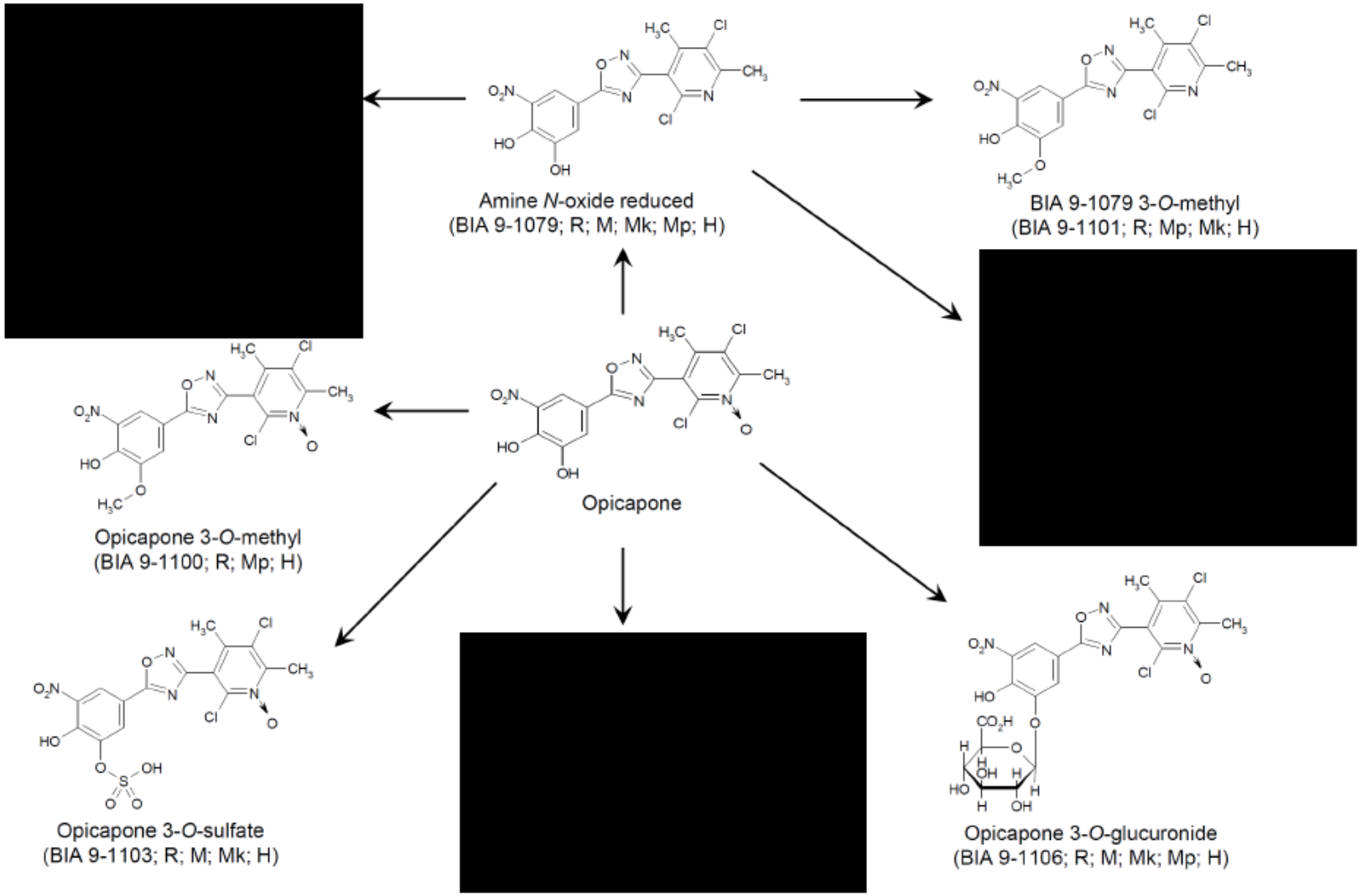
Isoform	Time (min)	Opicapone peak area	% CTR	Positive control peak area	Positive control % CTR
CYP1A2	0	573495	100.0	1101700	100.0
	10	606492	105.8		
	30	611552	106.6		
	60	603407	105.2	770770	70.0
	60 (without NADPH)	625577	109.1		
CYP2E1	0	380514	100.0	236747	100.0
	10	398744	104.8		
	30	364667	95.8		
	60	373503	98.2	122747	51.8
	60 (without NADPH)	413891	108.8		
CYP2C9	0	603293	100.0	N/A	N/A
	10	577453	95.7		
	30	519434	86.1		
	60	490034	81.2		
	60 (without NADPH)	579951	96.1		
CYP2C19	0	638812	100.0	N/A	N/A
	10	625360	97.9		
	30	607619	95.1		
	60	498207	78.0		
	60 (without NADPH)	622978	97.5		
CYP2D6	0	641310	100.0	2640420	100.0
	10	628395	98.0		
	30	703069	109.6		
	60	654550	102.1	46664.2	1.8
	60 (without NADPH)	627494	97.8		

2.6.5.11.7　　代謝に関与する CYP 分子種（続き）

Isoform	Time (min)	Opicapone peak area	% CTR	Positive control peak area	Positive control % CTR
CYP3A4	0	711716	100.0	1865040	100.0
	10	582217	81.8		
	30	468628	65.8		
	60	347589	48.8	716026	38.4
	60 (without NADPH)	590645	83.0		
CYP2B6	0	777780	100.0	1415100	100.0
	10	690317	88.8		
	30	604511	77.7		
	60	524832	67.5	1128250	79.7
	60 (without NADPH)	620419	79.8		
CYP2C8	0	712159	100.0	29669	100.0
	10	666520	93.6		
	30	622800	87.5		
	60	568654	79.8	26527.3	89.4
	60 (without NADPH)	592374	83.2		
human liver microsomes	0	764979	100.0	N/A	N/A
	10	766615	100.2		
	30	759087	99.2		
	60	732941	95.8		
	60 (without NADPH)	739822	96.7		

% CTR, % of compound remaining relative to time zero.
Positive control: 5 µmol/L phenacetin (CYP1A2), 5 µmol/L bufuralol (CYP2D6), 5 µmol/Lchlorzoxazone (CYP2E1), 5 µmol/L bupropion (CYP2B6), 5 µmol/L amodiquine (CYP2C8), and 5 µmol/L testosterone (CYP3A4).

2.6.5.12 **薬物動態試験：推定代謝経路**



M, mouse; R, rat; Mk, monkey; Mp, mini-pig; H, human.

2.6.5.13 薬物動態試験：薬物代謝酵素の誘導／阻害

2.6.5.13.1 オピカポンによる酵素誘導／阻害

2.6.5.13.1.1 肝細胞での CYP 誘導作用

Test article: opicapone
Location in CTD: [5.3.2.2-10]
Study No. 782788

Study title:	BIA 9-1067: *In Vitro* Investigation to Assess the Potential Induction of Hepatic Enzymes by BIA 9-1067 in Fresh Hepatocytes
Test system:	Freshly isolated human hepatocytes
Method:	After *in vitro* exposure to opicapone (1, 3, 10 µg/mL) for 72 h, hepatocytes were analyzed for activities of CYP1A2, CYP2C9, and CYP3A4 using 7-ethoxycoumarin, tolbutamide, and testosterone, respectivery, as CYP specific substrates.
Assay:	HPLC with radioactivity detector and UV detector.

Treatment	**Total Metabolite Formation (pmol/10^6 cells/h, mean ± SD, n = 6)**		
	CYP1A2	**CYP2C9**	**CYP3A4**
DMSO (vehicle)	2824.22 ± 4188.61	756.55 ± 698.47	19063.17 ± 14743.05
Opicapone 1 µg/mL	3384.72 ± 5053.86	835.17 ± 830.63	19182.67 ± 14705.66
Opicapone 3 µg/mL	2631.41 ± 3070.59	691.17 ± 762.43	17269.17 ± 12330.13
Opicapone 10 µg/mL	2257.73 ± 3038.27	597.42 ± 426.49	13438.83 ± 7287.91
Omeprazole 30 µmol/L	3095.06 ± 2430.90	Not tested	Not tested
Rifampicin 3 µmol/L	Not tested	850.09 ± 1042.02	23849.50 ± 14118.33

2.6.5.13.1.1　　肝細胞での CYP 誘導作用（続き）

Treatment	% of positive control* (mean ± SD, n = 6)			Fold induction** (mean ± SD, n = 6)		
	CYP1A2	CYP2C9	CYP3A4	CYP1A2	CYP2C9	CYP3A4
DMSO (vehicle)	0 ± 0	0 ± 0	0 ± 0	NC	NC	NC
Opicapone 1 µg/mL	19.38 ± 35.77	10.92 ± 16.93	37.42 ± 58.14	NC	NC	NC
Opicapone 3 µg/mL	6.98 ± 9.89	69.86 ± 92.62	79.23 ± 90.39	NC	NC	NC
Opicapone 10 µg/mL	16.25 ± 18.77	79.44 ± 169.39	211.30 ± 247.78	NC	NC	NC
Omeprazole 30 µmol/L	NC	NC	NC	8.18 ± 4.84	Not tested	Not tested
Rifampicin 3 µmol/L	NC	NC	NC	Not tested	1.39 ± 1.14	1.86 ± 1.13
After careful analysis of the data obtained with the assays performed, opicapone is not considered to be an inducer of the enzymes investigated in this study. Opicapone does not appear to exhibit cytotoxic effects.						

*(Activity of test drug treated cells − activity of negative control)/(activity of positive control − activity of negative control) × 100.
**Activity of positive control/activity of negative control.

2.6.5.13.1.2 HepaRG 細胞での CYP 誘導作用

Test article: opicapone
Location in CTD: [5.3.2.2-9]
Study No. SROPC[redacted]0324

Study title:	Evaluation of Potential CYP P450 Induction of Opicapone, BIA 9-1079 and BIA 9-1103 in HepaRG Cells
Test system:	Differentiated HepaRG cells (human hepatoma cell line)
Method:	Cells were seeded in 24-well plates, incubated over 72 h with 10 µmol/L opicapone or with a positive control inducer rifampicin, phenobarbital, or omeprazole. Following incubation, CYP3A4, CYP1A2, CYP2B6, CYP2C9, and CYP2C19 activities were evaluated using testosterone, phenacetin, bupropion, tolbutamide, and mephenytoin, respectively, as CYP specific substrates.
Assay:	LC-MS

Results:
As expected, the CYP3A4, CYP2B6, CYP2C9, and CYP1A2 activities were markedly increased by the prototypical inducers rifampicin, phenobarbital, and omeprazole, respectively. Rifampicin increased the activities of CYP3A4 and CYP2C9 by 5.7-fold and 1.4-fold, respectively, whereas omeprazole increased the activity of CYP1A2 by 40.8-fold and phenobarbital increased the activity of CYP2B6 by 7.7-fold.
Treatment of HepaRG cells with opicapone resulted in less than 2-fold increase of CYP3A4, CYP2B6, CYP2C9, and CYP1A2 activities. The activity of CYP2C19 was not evaluated due to analytical problems. In addition, opicapone did not induce differential gene expression of CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, MDR1, PXR, CAR, or AHR in HepaRG cells after a 48-h treatment.

2.6.5.13.1.3 CYP 阻害作用

Test article: opicapone
Location in CTD: [5.3.2.2-5]
Study No. 782809

Study title:	An *In Vitro* Investigation to Assess the Potential Inhibition of Microsomal Cytochrome P450 Enzymes by BIA 9-1067
Test system:	Human liver microsomes
Method:	CYP probe substrates were co-incubated with 0.03 to 10 µg/mL opicapone for 5 to 30 min. For the assessment of time-dependent inhibition, a pre-incubation period of 30 min of opicapone in the presence and absence of co-factor was included prior to co-incubation with CYP probe substrates.
Parameter:	Effect of opicapone on phenacetin *O*-deethylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), bupropion hydroxylation (CYP2B6), paclitaxel 6α-hydroxylation (CYP2C8), tolbutamide 4-hydroxylation (CYP2C9), *S*-methphenytoin 4-hydroxylation (CYP2C19), bufuralol 1′-hydroxylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1), testosterone 6β-hydroxylation, midazolam 1′-hydroxylation, and nifedipine oxidation (CYP3A4)
Assay:	HPLC with radioactivity detector, UV detector, or fluorescence detector; LC-MS/MS; or fluorimeter

Compound	**CYP1A2**		**CYP2A6**		**CYP2B6**	
	Activity (pmol/mg/min)	**% of control**	**Activity (pmol/mg/min)**	**% of control**	**Activity (pmol/mg/min)**	**% of control**
DMSO (vehicle)	267.0 ± 11.0	100	200.8 ± 14.0	100	85.96 ± 4.69	100
Opicapone 0.03 µg/mL	270.0 ± 2.0	101.0 ± 1.0	194.6 ± 15.0	96.9 ± 7.05	88.60*	103.1*
Opicapone 0.1 µg/mL	271.0 ± 15.0	102.0 ± 6.0	188.1 ± 10.5	93.7 ± 5.2	85.34 ± 2.86	99.29 ± 3.32
Opicapone 0.3 µg/mL	272.0 ± 11.0	102.0 ± 4.0	199.2 ± 9.80	99.2 ± 4.9	81.97 ± 1.53	95.36 ± 1.78
Opicapone 1 µg/mL	275.0 ± 21.0	103.0 ± 8.0	201.3 ± 10.3	100.3 ± 5.1	81.81 ± 9.27	95.17 ± 10.79
Opicapone 3 µg/mL	255.0 ± 25.0	95.00 ± 9.00	217.8 ± 16.9	108.5 ± 8.4	73.85 ± 2.59	85.92 ± 3.02
Opicapone 10 µg/mL	237.0 ± 9.00	89.00 ± 3.00	191.3 ± 11.0	95.24 ± 5.50	64.13 ± 3.17	74.61 ± 3.68
α-naphthoflavone 0.5 µmol/L	22.7 ± 1.2	8.70 ± 0.5	NE	NE	NE	NE
8-MP 6 µmol/L	NE	NE	16.3 ± 1.1	9.32 ± 0.6	NE	NE
Thio-TEPA 20 µmol/L	NE	NE	NE	NE	49.21 ± 3.80	51.77 ± 3.99

2.6.5.13.1.3　　CYP 阻害作用（続き）

Compound	CYP2C8		CYP2C9		CYP2C19	
	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control
DMSO (vehicle)	20.52 ± 1.98	100	116.8 ± 7.1	100	9.470 ± 1.790	100
Opicapone 0.03 µg/mL	20.26*	98.77*	113.5 ± 8.1	97.16 ± 6.97	9.620*	101.6*
Opicapone 0.1 µg/mL	15.92 ± 0.41	77.59 ± 1.99	110.5 ± 14.9	94.58 ± 12.73	13.96 ± 0.97	147.4 ± 10.2
Opicapone 0.3 µg/mL	19.40 ± 3.12	94.57 ± 15.20	114.6 ± 5.2	98.07 ± 4.41	12.94 ± 1.29	136.7 ± 13.6
Opicapone 1 µg/mL	14.55 ± 3.51	70.90 ± 17.11	119.1 ± 3.0	101.9 ± 2.54	16.37 ± 2.54	172.9 ± 26.8
Opicapone 3 µg/mL	11.72 ± 0.65	57.11 ± 3.18	97.85 ± 4.53	83.75 ± 3.88	51.32 ± 3.39	541.9 ± 35.8
Opicapone 10 µg/mL	4.282 ± 0.330	20.87 ± 1.62	56.23 ± 7.65	48.12 ± 6.55	125.1 ± 3.5	1321 ± 37
Quercetin 10 µmol/L	12.70 ± 0.79	53.42 ± 3.33	NE	NE	NE	NE
Sulfaphenazole 10 µmol/L	NE	NE	19.16 ± 2.09	15.85 ± 1.73	NE	NE
Tranylcypromine 50 µmol/L	NE	NE	NE	NE	7.56 ± 3.56	25.09 ± 11.83
The Ki value was estimated to be 0.9 µg/mL for CYP2C8, 18 µg/mL for CYP2C9.						

Compound	CYP2D6		CYP2E1		CYP3A4 (testosterone)	
	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control
DMSO (vehicle)	25.92 ± 0.99	100	85.56 ± 11.80	100	958.1 ± 94.4	100
Opicapone 0.03 µg/mL	24.73 ± 0.26	95.40 ± 1.00	93.27 ± 14.30	109.0 ± 16.7	919.5 ± 103.9	95.97 ± 10.84
Opicapone 0.1 µg/mL	24.92 ± 1.27	96.14 ± 4.90	96.63 ± 1.52	112.9 ± 1.8	893.4 ± 77.1	93.25 ± 8.04
Opicapone 0.3 µg/mL	21.88 ± 2.98	84.39 ± 11.48	83.84 ± 20.20	97.99 ± 23.61	891.9 ± 85.2	93.09 ± 8.89
Opicapone 1 µg/mL	25.58 ± 1.37	98.67 ± 5.27	86.34 ± 9.80	100.9 ± 11.5	788.8 ± 111.9	82.33 ± 11.68
Opicapone 3 µg/mL	23.66 ± 2.20	91.27 ± 8.49	85.0 ± 1.34	99.34 ± 1.57	931.6 ± 69.7	97.23 ± 7.27
Opicapone 10 µg/mL	27.91 ± 3.01	107.7 ± 11.6	85.13 ± 12.37	99.49 ± 14.45	797.6 ± 72.5	83.24 ± 7.56
Quinidine 5 µmol/L	11.65 ± 1.97	44.76 ± 7.58	NE	NE	NE	NE
DDC 10 µmol/L	NE	NE	142.2 ± 8.9	19.41 ± 1.21	NE	NE
Ketoconazole 0.5 µmol/L	NE	NE	NE	NE	168.5 ± 54.2	12.62 ± 4.06

2.6.5.13.1.3　　CYP 阻害作用（続き）

Compound	CYP3A4 (midazolam)		CYP3A4 (nifedipine)	
	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control
DMSO (vehicle)	200.5 ± 7.2	100	832.4 ± 33.9	100
Opicapone 0.03 µg/mL	202.6 ± 3.8	101.0 ± 1.9	910.5 ± 112.1	109.4 ± 13.5
Opicapone 0.1 µg/mL	206.7 ± 3.2	103.1 ± 1.6	850.0 ± 99.9	102.1 ± 12.0
Opicapone 0.3 µg/mL	217.6 ± 4.5	108.5 ± 2.2	863.0 ± 16.2	103.7 ± 1.9
Opicapone 1 µg/mL	213.9 ± 1.1	106.7 ± 0.5	806.4 ± 75.2	96.88 ± 9.03
Opicapone 3 µg/mL	233.8 ± 6.8	116.6 ± 3.4	917.8 ± 79.4	110.3 ± 9.5
Opicapone 10 µg/mL	273.2 ± 4.6	136.2 ± 2.3	657.3*	78.96*
Ketoconazole 0.5 µmol/L	25.52 ± 1.05	5.365 ± 0.222	156.5 ± 11.1	15.86 ± 1.11

Values represent mean ± SD of 3 individual determinations.
*Mean of 2 determinations.

2.6.5.13.1.4 ヒト肝ミクロソーム蛋白への結合

Test article: opicapone, BIA 9-1103
Location in CTD: [5.3.2.2-8]
Study No. SRAL[redacted]0415

Study title:	Human Microsomal Binding of Opicapone and BIA 9-1103 Using Rapid Equilibrium Dialysis
Test system:	Human liver microsomes
Method:	Rapid equilibrium dialysis
Assay:	LC-MS/MS

Compound	Compound concentration (µg/mL)	Protein concentration (mg/mL)	Free fraction
Opicapone	1	0.1	0.8742 ± 0.0842
		1	0.6579 ± 0.0831
		2	0.4643 ± 0.1179
	10	0.1	0.5217 ± 0.1932
		1	0.3988 ± 0.1129
		2	0.3570 ± 0.1014
BIA 9-1103	1	0.1	0.8403 ± 0.1999
		1	0.7363 ± 0.0424
		2	0.6312 ± 0.0506
	10	0.1	0.6865 ± 0.1042
		1	0.6328 ± 0.1202
		2	0.5457 ± 0.1299

Data represent mean ± SD (n = 9)

2.6.5.13.2 BIA 9-1103 による酵素誘導／阻害

2.6.5.13.2.1 肝細胞での CYP 誘導作用

Test article: BIA 9-1103
Location in CTD: [5.3.2.2-11]
Study No. 792089

Study title:	BIA 9-1103: An *In Vitro* Investigation to Assess the Potential Induction of Hepatic Enzymes by BIA 9-1103 in Cryopreserved Inducible Hepatocytes
Test system:	Cryopreserved inducible human hepatocytes
Method:	After *in vitro* exposure to BIA 9-1103 (3, 10, or 30 µg/mL) for 72 h, hepatocytes were analyzed for activities of CYP1A2 (and Phase 2), CYP2B6, and CYP3A4 using ^{14}C-7-ethoxycoumarin, bupropion, and testosterone, respectively, as probe substrates.
Assay:	HPLC with radioactivity detector or LC-MS/MS

Results:
Results obtained with positive control incubations with known inducers of CYP1A2 and Phase 2 (omeprazole), CYP2B6 (phenobarbital) and CYP3A4 (rifampicin) enzymes, show more than 2-fold induction of enzyme activity and expression in the vast majority of samples which indicates that hepatocytes used in these assays were responsive to induction.
The results obtained with these assays showed that BIA 9-1103 did not induce CYP1A2 and Phase 2 enzyme activity in hepatocytes from any of the donors. On the contrary, significant suppression of enzymatic activity was observed in various samples, and in some this effect appeared to be concentration dependent. BIA 9-1103 also failed to induce activity of CYP2B6 and CYP3A4 enzymes. Suppression of enzyme activity was consistently observed across the panel of samples, and in some cases this effect was concentration dependent. Analysis of CYP1A2, CYP2B6 and CYP3A4 mRNA expression revealed that the test item had no effect on the expression of these enzymes, reflecting the observations made with enzyme activity assays.
After careful analysis of the data obtained with the assays performed, it was concluded that the test item, BIA 9-1103, does not have the potential to induce activity or expression of CYP1A2, Phase 2, CYP2B6 or CYP3A4. On the contrary, BIA 9-1103 appears to promote suppression of CYP1A2, CYP2B6 and CYP3A4 enzymes and in some cases this effect appears to be concentration dependent.

2.6.5.13.2.2 HepaRG 細胞での CYP 誘導作用

Test article: BIA 9-1103
Location in CTD: [5.3.2.2-9]
Study No. SROPC[redacted]0324

Study title:	Evaluation of Potential CYP P450 Induction of Opicapone, BIA 9-1079 and BIA 9-1103 in HepaRG Cells
Test system:	Differentiated HepaRG cells (human hepatoma cell line)
Method:	Cells were seeded in 24-well plates, incubated over 72 h with 10 µmol/L BIA 9-1103 (containing opicapone as a contaminant at 13%) or with a positive control inducers rifampicin, phenobarbital, or omeprazole. Following incubation, CYP3A4, CYP1A2, CYP2B6, CYP2C9, and CYP2C19 activities were evaluated using testosterone, phenacetin, bupropion, tolbutamide, and mephenytoin, respectively, as CYP specific substrates.
Assay:	LC-MS

Results:
As expected, the CYP3A4, CYP2B6, CYP2C9, and CYP1A2 activities were markedly increased by the prototypical inducers rifampicin, phenobarbital, and omeprazole, respectively. Rifampicin increased the activity of CYP3A4 and CYP2C9 to 5.7-fold and 1.4-fold, respectively, whereas omeprazole increased the activity of CYP1A2 by 40.8- fold and phenobarbital increased the activity of CYP2B6 to 7.7-fold.
Treatment of HepaRG cells with BIA 9-1103 resulted in less than 2-fold increase of CYP3A4, CYP2B6, CYP2C9, and CYP1A2 activities. The activity of CYP2C19 was not evaluated due to analytical problems. In addition, BIA 9-1103 did not induce differential gene expression of CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, MDR1, PXR, CAR, or AHR in HepaRG cells after a 48-h treatment.

2.6.5.13.2.3 CYP 阻害作用

Test article: BIA 9-1103
Location in CTD: [5.3.2.2-6]
Study No. 790500

Study title:	BIA 9-1103: An *In Vitro* Investigation to Assess the Potential Inhibition of Microsomal Cytochrome P450 Enzymes by BIA 9-1103
Test system:	Human liver microsomes
Method:	CYP probe substrates were co-incubated with 0.1 to 30 µg/mL BIA 9-1103 for 5 to 30 min. For the assessment of time-dependent inhibition, a pre-incubation period of 30 min of BIA 9-1103 in the presence and absence of co-factor was included prior to co-incubation with CYP probe substrates.
Parameter:	Effect of BIA 9-1103 on phenacetin *O*-deethylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), bupropion hydroxylation (CYP2B6), paclitaxel 6α-hydroxylation (CYP2C8), tolbutamide 4-hydroxylation (CYP2C9), *S*-mephenytoin 4-hydroxylation (CYP2C19), bufurarol 1-hydroxylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1), testosterone 6β-hydroxylation (CYP3A4), and midazolam 1′-hydroxylation (CYP3A4).
Assay:	LC-MS/MS

Compound	**CYP1A2**		**CYP2A6**		**CYP2B6**	
	Activity (pmol/mg/min)	**% of control**	**Activity (pmol/mg/min)**	**% of control**	**Activity (pmol/mg/min)**	**% of control**
DMSO (vehicle)	240 ± 8.7	100	397 ± 8.7	100	215 ± 12	100
BIA 9-1103 0.1 µg/mL	271 ± 12.4	113 ± 5.2	410 ± 32.5	103 ± 8.2	205 ± 18.1	95.4 ± 8.4
BIA 9-1103 0.3 µg/mL	263 ± 14.4	110 ± 6.0	413 ± 3.5	104 ± 0.9	224 ± 6.2	105 ± 2.9
BIA 9-1103 1 µg/mL	249 ± 28.7	104 ± 12.0	404 ± 17.4	102 ± 4.4	226 ± 22.7	105 ± 10.6
BIA 9-1103 3 µg/mL	248 ± 8.8	103 ± 3.7	353 ± 48.1	89.0 ± 12.1	228 ± 7.4	106 ± 3.5
BIA 9-1103 10 µg/mL	243 ± 9.0	101 ± 3.7	398 ± 13.1	100 ± 3.3	224 ± 15.0	105 ± 7.0
BIA 9-1103 30 µg/mL	212 ± 0.8	88.3 ± 0.3	320 ± 33.4	80.6 ± 8.4	203 ± 5.4	94.6 ± 2.5
α-Naphthoflavone 0.5 µmol/L	29.5 ± 1.7	12.4 ± 0.7	NE	NE	NE	NE
8-MP 50 nmol/L	NE	NE	67.0 ± 25.0	22.0 ± 8.2	NE	NE
Thio-TEPA 20 µmol/L	NE	NE	NE	NE	83.9 ± 4.2	32.9 ± 1.6

2.6.5.13.2.3 CYP 阻害作用（続き）

Compound	CYP2C8		CYP2C9		CYP2C19	
	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control
DMSO (vehicle)	225 ± 6.6	100	97.9*	100	5.27 ± 0.1	100
BIA 9-1103 0.1 µg/mL	202 ± 2.00	89.9 ± 0.9	96.0 ± 6.5	98.1 ± 6.6	5.49 ± 0.5	104 ± 9.3
BIA 9-1103 0.3 µg/mL	196 ± 20.0	87.0 ± 8.9	96.1 ± 8.0	98.2 ± 8.1	5.86 ± 0.2	111 ± 4.0
BIA 9-1103 1 µg/mL	168 ± 21.2	74.9 ± 9.4	94.8 ± 3.9	96.8 ± 4.0	7.09 ± 0.4	135 ± 7.0
BIA 9-1103 3 µg/mL	151*	67.0*	86.4 ± 5.4	88.2 ± 5.5	9.98 ± 0.2	189 ± 3.2
BIA 9-1103 10 µg/mL	94.3*	41.9*	67.2 ± 4.3	68.7 ± 4.3	17.8 ± 0.7	337 ± 14.2
BIA 9-1103 30 µg/mL	44.9*	20.0*	38.8 ± 2.2	39.6 ± 2.2	26.0 ± 1.3	494 ± 24.2
Quercetin 10 µmol/L	98.0 ± 5.5	43.5 ± 2.4	NE	NE	NE	NE
Sulfaphenazole 10 µmol/L	NE	NE	16.0*	16.1*	NE	NE
Tranylcypromine 20 µmol/L	NE	NE	NE	NE	3.75 ± 0.2	20.1 ± 0.8
The IC_{50} value was estimated to be 6.70 µg/mL for CYP2C8, 20.7 µg/mL for CYP2C9.						

Compound	CYP2D6		CYP2E1	
	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control
DMSO (vehicle)	22.0 ± 1.5	100	219 ± 16.7	100
BIA 9-1103 0.1 µg/mL	21.1 ± 1.0	95.8 ± 4.6	216 ± 4.2	98.5 ± 1.9
BIA 9-1103 0.3 µg/mL	21.5 ± 1.1	97.8 ± 5.0	216 ± 2.5	98.2 ± 1.2
BIA 9-1103 1 µg/mL	21.6 ± 0.6	98.0 ± 2.6	203 ± 9.3	92.7 ± 4.2
BIA 9-1103 3 µg/mL	21.0 ± 1.1	95.6 ± 5.0	197 ± 3.8	89.9 ± 1.8
BIA 9-1103 10 µg/mL	19.6 ± 0.9	89.0 ± 4.0	180*	82.0*
BIA 9-1103 30 µg/mL	16.8 ± 0.8	76.4 ± 3.7	161 ± 3.1	73.3 ± 1.4
Quinidine 5 µmol/L	2.28 ± 0.2	7.39 ± 0.6	NE	NE
DDC 10 µmol/L	NE	NE	181 ± 3.8	18.2 ± 0.4

2.6.5.13.2.3 CYP 阻害作用（続き）

Compound	CYP3A4 (midazolam)		CYP3A4 (testosterone)	
	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control
DMSO (vehicle)	221 ± 6.2	100	1050 ± 27.8	100
BIA 9-1103 0.1 µg/mL	202 ± 10.9	91.3 ± 4.9	1110 ± 34.9	105 ± 3.3
BIA 9-1103 0.3 µg/mL	190 ± 14.4	86.0 ± 6.5	1100 ± 63.5	104 ± 6.0
BIA 9-1103 1 µg/mL	204 ± 24.3	92.0 ± 11.0	1030 ± 27.0	97.6 ± 2.6
BIA 9-1103 3 µg/mL	187 ± 14.0	84.3 ± 6.3	1040 ± 32.3	98.8 ± 3.1
BIA 9-1103 10 µg/mL	196 ± 17.5	88.5 ± 7.9	988 ± 85.8	93.8 ± 8.1
BIA 9-1103 30 µg/mL	219 ± 10.1	98.9 ± 4.6	824 ± 38.5	78.2 ± 3.6
Ketoconazole 0.5 µmol/L	17.8 ± 2.9	4.49 ± 0.7	162 ± 31.9	7.89 ± 1.6

Values represent mean ± SD of 3 individual determinations.
*Mean of 2 determinations.

2.6.5.13.3 BIA 9-1079 による酵素誘導／阻害

2.6.5.13.3.1 肝細胞での CYP 誘導作用

Test article: BIA 9-1079
Location in CTD: [5.3.2.2-12]
Study No. 782793

Study title:	BIA 9-1079: *In Vitro* Investigation to Assess the Potential Induction of Hepatic Enzymes by BIA 9-1079 in Fresh Hepatocytes
Test system:	Freshly isolated human hepatocytes
Method:	After *in vitro* exposure to BIA 9-1079 (1, 3, or 10 µg/mL) for 72 h, hepatocytes were analyzed for activities of CYP1A2, CYP2C9, and CYP3A4 using ^{14}C-7-ethoxycoumarine, ^{14}C-tolbutamide, and ^{14}C-testosterone, respectively, as CYP specific substrates.
Assay:	HPLC with radioactivity detector and UV detector

Treatment	**Total Metabolite Formation (pmol/10^6 cells/h, mean ± SD, n = 3)**		
	CYP1A2	**CYP2C9**	**CYP3A4**
DMSO (vehicle)	2069.17 ± 999.12	547.2 ± 221.85	12239.33 ± 1922.07
BIA 9-1079 1 µg/mL	2241.50 ± 1258.50	373.03 ± 155.37	11939.33 ± 1583.02
BIA 9-1079 3 µg/mL	2241.93 ± 1243.80	526.60 ± 163.92	12077.67 ± 1019.76
BIA 9-1079 10 µg/mL	2618.33 ± 776.13	546.80 ± 238.71	11833.33 ± 2008.42
Omeprazole 30 µmol/L	7217.00 ± 5507.20	NE	NE
Rifampicin 3 µmol/L	NE	858.10 ± 227.29	28056.33 ± 2401.86

Treatment	**% of positive control* (mean ± SD, n = 3)**			**Fold induction** (mean ± SD, n = 3)**		
	CYP1A2	**CYP2C9**	**CYP3A4**	**CYP1A2**	**CYP2C9**	**CYP3A4**
DMSO (vehicle)	0 ± 0	0 ± 0	0 ± 0	NC	NC	NC
BIA 9-1079 1 µg/mL	3.12 ± 3.65	0 ± 0	0.17 ± 0.29	NC	NC	NC
BIA 9-1079 3 µg/mL	2.05 ± 3.56	5.43 ± 9.41	2.00 ± 3.46	NC	NC	NC
BIA 9-1079 10 µg/mL	15.71 ± 18.99	10.30 ± 17.84	0 ± 0	NC	NC	NC
Omeprazole 30 µmol/L	NC	NC	NC	5.77 ± 2.16	NE	NE
Rifampicin 3 µmol/L	NC	NC	NC	NE	1.57 ± 0.31	2.40 ± 0.26
After careful analysis of the data obtained with the assays performed, BIA 9-1079 is not considered to be an inducer of the enzymes investigated in this study. BIA 9-1079 does not appear to exhibit cytotoxic effects.						

2.6.5.13.3.1 肝細胞での CYP 誘導作用（続き）

*(Activity of test drug treated cells − activity of negative control)/(activity of positive control − activity of negative control) × 100.

**Activity of positive control/activity of negative control.

2.6.5.13.3.2 HepaRG 細胞での CYP 誘導作用

Test article: BIA 9-1079
Location in CTD: [5.3.2.2-9]
Study No. SROPC[redacted]0324

Study title:	Evaluation of Potential CYP P450 Induction of Opicapone, BIA 9-1079 and BIA 9-1103 in HepaRG Cells
Test system:	Differentiated HepaRG cells (human hepatoma cell line)
Method:	Cells were seeded in 24-well plates, incubated over 72 h with 10 µmol/L BIA 9-1079 or with a positive control inducer rifampicin, phenobarbital, or omeprazole. Following incubation, CYP3A4, CYP1A2, CYP2B6, CYP2C9, and CYP2C19 activities were evaluated using testosterone, phenacetin, bupropion, tolbutamide, and mephenytoin, respectively, as CYP specific substrates.
Assay:	LC-MS

Results:
As expected, the CYP3A4, CYP2B6, CYP2C9, and CYP1A2 activities were markedly increased by the prototypical inducers rifampicin, phenobarbital, and omeprazole, respectively. Rifampicin increased the activities of CYP3A4 and CYP2C9 by 5.7- fold and 1.4-fold, respectively, whereas omeprazole increased the activity of CYP1A2 by 40.8- fold and phenobarbital increased the activity of CYP2B6 by 7.7-fold.
Treatment of HepaRG cells with BIA 9-1079 resulted in less than 2-fold increase of CYP3A4, CYP2B6, CYP2C9, and CYP1A2 activities. The activity of CYP2C19 was not evaluated due to analytical problems. In addition, BIA 9-1079 did not induce differential gene expression of CYP1A1, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, MDR1, PXR, CAR, or AHR in HepaRG cells after a 48-h treatment.

2.6.5.13.3.3 CYP 阻害作用

Test article: BIA 9-1079
Location in CTD: [5.3.2.2-7]
Study No. 782814

Study title:	An *In Vitro* Investigation to Assess the Potential Inhibition of Microsomal Cytochrome P450 Enzymes by BIA 9-1079
Test system:	Human liver microsomes
Method:	Co-incubation of 0.03 to 10 µg/mL BIA 9-1079 for 5 to 30 min with human liver microsomes. For the assessment of time-dependent inhibition, a pre-incubation period of 30 min of BIA 9-1079 in the presence and absence of co-factor was included prior to co-incubation with CYP probe substrates.
Parameter:	Effect of BIA 9-1079 on phenacetin *O*-deethylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), bupropion hydroxylation (CYP2B6), paclitaxel 6α-hydroxylation (CYP2C8), tolbutamide 4-hydroxylation (CYP2C9), *S*-methylphenytoin 4-hydroxylation (CYP2C19), bufurarol 1′-hydroxylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1), testosterone 6β-hydroxylation (CYP3A4), midazolam 1′-hydroxylation (CYP3A4), and nifedipine oxidation (CYP3A4).
Assay:	HPLC with radioactivity detector, UV detector, or fluorescence detector; LC-MS/MS; or fluorimeter

Compound	**CYP1A2**		**CYP2A6**		**CYP2B6**	
	Activity (pmol/mg/min)	**% of control**	**Activity (pmol/mg/min)**	**% of control**	**Activity (pmol/mg/min)**	**% of control**
DMSO (vehicle)	296 ± 26	100	190.6 ± 6.3	100	191.3 ± 8.4	100
BIA 9-1079 0.03 µg/mL	299 ± 7	101 ± 2	198.6 ± 1.0	104.2 ± 0.5	206.3 ± 18.6	107.8 ± 9.7
BIA 9-1079 0.1 µg/mL	310 ± 4	105 ± 1	192.6 ± 3.2	101.1 ± 1.7	181.1 ± 13.9	94.65 ± 7.28
BIA 9-1079 0.3 µg/mL	288 ± 15	97 ± 5	193.1 ± 1.9	101.3 ± 1.0	200.6 ± 9.4	104.9 ± 4.9
BIA 9-1079 1 µg/mL	300 ± 18	101 ± 6	196.8 ± 8.6	103.3 ± 4.5	210.1 ± 4.0	109.8 ± 2.1
BIA 9-1079 3 µg/mL	295 ± 17	100 ± 6	190.3 ± 6.9	99.84 ± 3.6	204.4 ± 33.3	106.9 ± 17.4
BIA 9-1079 10 µg/mL	268 ± 23	90 ± 8	185.9 ± 6.4	97.52 ± 3.33	132.3 ± 15.1	69.14 ± 7.90
α-Naphthophlavone 0.5 µmol/L	20.2 ± 1.6	6.9 ± 0.5	NE	NE	NE	NE
8-MP 6 µmol/L	NE	NE	8.3 ± 0.25	5.29 ± 0.16	NE	NE
Thio-TEPA 20 µmol/L	NE	NE	NE	NE	61.97 ± 2.11	27.10 ± 0.92

2.6.5.13.3.3 CYP 阻害作用（続き）

Compound	CYP2C8		CYP2C9		CYP2C19	
	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control
DMSO (vehicle)	25.50 ± 2.58	100	130.4 ± 16.4	100	13.11 ± 2.49	100
BIA 9-1079 0.03 µg/mL	25.01 ± 1.91	98.08 ± 7.48	162.0*	124.2*	13.41 ± 1.37	102.3 ± 10.5
BIA 9-1079 0.1 µg/mL	17.65 ± 2.28	69.24 ± 8.94	158.0 ± 9.7	121.2 ± 7.4	14.29 ± 3.42	109.0 ± 26.1
BIA 9-1079 0.3 µg/mL	18.63 ± 2.1	73.06 ± 8.25	131.8 ± 13.2	101.0 ± 10.1	8.93*	68.14*
BIA 9-1079 1 µg/mL	12.69 ± 3.08	49.78 ± 12.07	110.0 ± 10.9	84.31 ± 8.36	24.47 ± 4.50	186.6 ± 34.3
BIA 9-1079 3 µg/mL	4.12 ± 0.55	16.17 ± 2.15	58.48 ± 2.49	44.84 ± 1.91	37.32 ± 6.65	284.7 ± 50.7
BIA 9-1079 10 µg/mL	0	0	33.84 ± 4.80	25.95 ± 3.68	59.60 ± 6.88	454.6 ± 52.5
Quercetin 10 µmol/L	14.98 ± 0.48	35.44 ± 1.14	NE	NE	NE	NE
Sulfaphenazole 10 µmol/L	NE	NE	18.04 ± 2.0	11.08 ± 1.24	NE	NE
Tranylcypromine 50 µmol/L	NE	NE	NE	NE	0.00	0.00
The Ki value was estimated to be 0.19 µg/mL for CYP2C8, 1.47 µg/mL for CYP2C9.						

Compound	CYP2D6		CYP2E1		CYP3A4 (testosterone)	
	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control
DMSO (vehicle)	21.39 ± 0.34	100	207.6 ± 10.9	100	465.8 ± 32.5	100
BIA 9-1079 0.03 µg/mL	22.30 ± 0.48	104.2 ± 2.3	203.4 ± 15.5	97.94 ± 7.46	450.7 ± 46.5	96.77 ± 9.99
BIA 9-1079 0.1 µg/mL	21.90 ± 0.14	102.4 ± 0.6	218.4 ± 19.5	105.2 ± 9.4	529.4 ± 62.0	113.7 ± 13.3
BIA 9-1079 0.3 µg/mL	22.67 ± 0.62	106.0 ± 2.9	198.2 ± 12.9	95.46 ± 6.26	501.9 ± 51.1	107.8 ± 11.0
BIA 9-1079 1 µg/mL	22.28 ± 0.56	104.2 ± 2.6	231.8 ± 10.7	111.6 ± 5.2	550.4 ± 66.1	118.2 ± 14.2
BIA 9-1079 3 µg/mL	23.46 ± 0.52	109.7 ± 2.5	220.9 ± 12.0	106.4 ± 5.8	499.8 ± 37.1	107.3 ± 8.0
BIA 9-1079 10 µg/mL	29.88 ± 0.37	139.7 ± 1.7	189.1 ± 4.8	91.08 ± 2.30	442.4 ± 17.8	94.97 ± 3.83
Quinidine 5 µmol/L	10.18 ± 0.65	35.64 ± 2.27	NE	NE	NE	NE
DDC 10 µmol/L	NE	NE	240.8 ± 9.8	25.66 ± 1.04	NE	NE
Ketoconazole 0.5 µmol/L	NE	NE	NE	NE	165.9*	18.26 ± 4.22

2.6.5.13.3.3　　CYP 阻害作用（続き）

Compound	CYP3A4 (midazolam)		CYP3A4 (nifedipine)	
	Activity (pmol/mg/min)	% of control	Activity (pmol/mg/min)	% of control
DMSO (vehicle)	213.2 ± 12.4	100	746.7 ± 26.2	100
BIA 9-1079 0.03 µg/mL	195.8 ± 7.1	91.83 ± 3.32	713.0 ± 47.8	95.48 ± 6.41
BIA 9-1079 0.1 µg/mL	201.5 ± 10.9	94.50 ± 5.13	671.7 ± 128.1	89.96 ± 17.15
BIA 9-1079 0.3 µg/mL	210.8 ± 13.2	98.86 ± 6.18	911.2 ± 23.9	122.0 ± 3.2
BIA 9-1079 1 µg/mL	206.1 ± 19.8	96.70 ± 9.27	751.2 ± 52.7	100.6 ± 7.1
BIA 9-1079 3 µg/mL	202.1 ± 26.2	94.78 ± 12.28	855.9 ± 27.9	114.6 ± 3.7
BIA 9-1079 10 µg/mL	187.5 ± 8.7	88.0 ± 4.1	729.7 ± 9.9	97.73 ± 1.33
Ketoconazole 0.5 µmol/L	35.32 ± 1.18	12.32 ± 0.41	157.2 ± 2.8	14.96 ± 0.27

Values represent mean ± SD of 3 individual determinations.
*Mean of 2 determinations.

2.6.5.14 薬物動態試験：排泄

2.6.5.14.1 ラット

Test article: ^{14}C-opicapone
Location in CTD: [4.2.2.2-1]
Study No. ZNA15658.001

Study title:	The Disposition of Total Radioactivity in the Rat Following Single Oral and Single Intravenous Administration of [^{14}C] BIA 9-1067
Species/strain:	Rat/Wistar
Gender (M/F)/number of animals:	Male/8 (4 p.o. and 4 i.v.)
Feeding condition:	Fed
Vehicle/formulation:	0.5% CMC/suspension (p.o.) and DMSO/solution (i.v.)
Method of administration:	p.o. (gavage) and i.v.
Dose (mg/kg):	10 (p.o.) and 1 (i.v.)
Analyte/assay:	Radioactivity/LSC

Route of administration	**Time (h)**	**Excretion of radioactivity (% of dose, mean ± SD)**					
		Urine	**Feces**	**Expired air**	**Cage wash**	**Carcass**	**Total**
p.o.	0-120	4.0 ± 0.3	92.3 ± 6.4	1.5 ± 0.5	1.5± 1.2	1.4 ± 0.1	100.7 ± 6.6
i.v.	0-120	5.7 ± 1.7	72.8 ± 4.5	2.2 ± 0.6	2.7 ± 0.8	5.5 ± 1.2	89.0 ± 6.4

2.6.5.14.2 サル

Test article: ^{14}C-opicapone
Location in CTD: [4.2.2.2-2]
Study No. ZNA15658.002

Study title:	The Disposition of Total Radioactivity in the Primate Following Single Oral and Single Intravenous Administration of [^{14}C] BIA 9-1067
Species/strain:	Monkey/Cynomolgus
Gender (M/F)/number of animals:	Male/4
Feeding condition:	Fed
Vehicle/formulation:	0.5% CMC/ suspension (p.o.) and DMSO/saline (70:30)/solution (i.v.)
Method of administration:	p.o. (gavage) and i.v.
Dose (mg/kg):	10 (p.o.) and 1 (i.v.)
Analyte/assay:	Radioactivity/LSC

Route of administration	**Time (h)**	**Excretion of radioactivity (% of dose, mean ± SD)**				
		Urine	**Feces**	**Cage wash**	**Cage debris**	**Total**
p.o.	0-168	5.1 ± 2.3	76.0 ± 5.6	2.2 ± 0.5	0.5 ± 1.0	83.7 ± 3.2
i.v.	0-168	6.2 ± 1.7	55.6 ± 6.4	5.6 ± 2.1	0.5 ± 0.9	67.9 ± 4.3

2.6.5.15　　薬物動態試験：排泄：胆汁中

該当する資料はない.

2.6.5.16 薬物動態試験：薬物相互作用

本項のほか，2.6.5.3 及び 2.6.5.13 に示した．

2.6.5.16.1 トランスポーターに関する検討

2.6.5.16.1.1 オピカポン

1） OATP1B1 による輸送

Test article: opicapone
Location in CTD: [5.3.2.3-3]
Study No. SRAL[redacted]1128

Study title:	Opicapone (BIA 9-1067) Uptake Using CHO cells Overexpressing OATP1B1
Test system:	OATP1B1-expressing CHO cells
Method:	Opicapone (1, 10, and 100 µmol/L) or estrone-3-sulfate (2.5 µmol/L) was incubated with CHO cells overexpressing OATP1B1 for 10 min. Cells were lysed, and opicapone and estrone-3-sulfate were analyzed.
Analyte/assay:	Opicapone/LC-MS

Opicapone concentration (µmol/L)	**Opicapone uptake (pmol, mean ± SEM, n = 3)**	
	Wild-type CHO	**CHO overexpressing OATP1B1**
1	10.2 ± 1.3	11.9 ± 1.5
10	29.3 ± 2.3	41.3 ± 4.4
100	144.9 ± 1.9	154.5 ± 9.8

Opicapone uptake by CHO cells overexpressing OATP1B1 was no different from that by wild-type CHO cells. The results suggest that OATP1B1 is not involved in opicapone uptake. Uptake of estrone-3-sulfate (a known OATP1B1 substrate) was significantly higher ($p < 0.01$) in cells overexpressing OATP1B1.

2)　P-gp による輸送

Test article: opicapone
Location in CTD: [5.3.2.3-4]
Study No. SRAL[redacted]0627

Study title:	Opicapone (BIA 9-1067) Transport Using MDCK-II Cells Overexpressing MDR1
Test system:	Human P-gp-expressing MDCKII cells
Method:	P-gp-expressing cells or parental cells were seeded on Transwell and incubated with opicapone for 60 min. Efflux ratio was calculated by dividing Papp from BL to AP by that from AP to BL. Net efflux ratio was calculated by dividing efflux ratio in P-gp-expressing cells by that in parental cells.
Assay:	LC-MS

Substrate	Inhibitor	Net efflux ratio
Opicapone (10 µmol/L)	–	4.0
Dexamethasone (10 µmol/L)	–	7.0
Opicapone (5 µmol/L)	–	3.5
	Elacridar (50 µmol/L)	0.78

Efflux ratio and net efflux ratio were calcurated based on mean Papp of 3 determinations.

3)　BCRP による輸送

Test article: opicapone
Location in CTD: [5.3.2.3-5]
Study No. Tebu-02-[redacted]20[redacted]

Study title:	*In Vitro* Interaction Studies of Opicapone with Human BCRP Efflux (ABC) Transporter, and with Human OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 Uptake Transporters
Test system:	Human BCRP-expressing MDCKII cells
Method:	BCRP-expressing cells or parental cells were seeded on Transwell and incubated with opicapone. Efflux ratio was calculated by dividing Papp from BL to AP by that from AP to BL. Net efflux ratio was calculated by subtracting efflux ratio in parental cells from that in BCRP-expressing cells.
Assay:	LC-MS/MS，LSC

Substrate	Inhibitor	Incubation time (min)	Net efflux ratio
Opicapone (1 μg/mL)	–	0 to 120	10.1 ± 3.6
Opicapone (3 μg/mL)			6.6 ± 2.2
Opicapone (10 μg/mL)			11.1 ± 4.4
^{3}H-prazosin (1 μmol/L)	–	60	21.9 ± 3.6
	Ko134 (1 μmol/L)		0.8 ± 0.6

Data represent mean ± SD (n=3).

4)　OATP1B3 による輸送

Test article: opicapone
Location in CTD: [5.3.2.3-5]
Study No. Tebu-02-[redacted]20[redacted]

Study title:	*In Vitro* Interaction Studies of Opicapone with Human BCRP Efflux (ABC) Transporter, and with Human OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 Uptake Transporters
Test system:	Human OATP1B3-expressing HEK293 cells
Method:	OATP1B3-expressing cells or control (mock) cells were incubated with opicapone at 37°C. After incubation, cells were washed and lysed for analysis of substrate uptake. Fold accumulation was calculated by dividing uptake in OATP1B3-expressing cells by that in control cells. In addition, inhibition of opicapone uptake by rifampicin was evaluated.
Assay:	LC-MS/MS, LSC

Uptake study

Substrate	Inhibitor	Incubation time (min)	Fold accumulation
Opicapone (1 µg/mL)	–	2	1.6
		20	2.6
Opicapone (10 µg/mL)	–	2	1.5
		20	1.1
^{3}H-CCK-8 (0.11 µmol/L)	–	10	31.3
	Rifampicin (50 µmol/L)		0.9

Inhibition by rifampicin

Substrate	Inhibitor	Incubation time (min)	Fold accumulation
Opicapone (1 µg/mL)	–	20	4.4
	Rifampicin (50 µmol/L)		0.9
^{3}H-CCK-8 (0.11 µmol/L)	–	10	39.6
	Rifampicin (50 µmol/L)		0.9

Fold accumulation was culcurated based on mean uptake of 3 determinations.

5)　P-gp 阻害

Test article: opicapone
Location in CTD: [5.3.2.3-8]
Study No. SRAL[redacted]0926

Study title:	P-glycoprotein Transport Inhibition by Opicapone Using MDCK-II Cells Overexpressing MDR1
Test system:	Human P-gp expressing MDCKII cells
Method:	P-gp-expressing cells or wild-type cells were seeded on Transwell and incubated with a probe substrate and opicapone (30 µg/mL). Efflux ratio was calculated by dividing Papp from BL to AP by that from AP to BL. Net efflux ratio was calculated by dividing efflux ratio in P-gp-expressing cells by that in wild-type cells. Inhibitory effect of opicapone on P-gp was evaluated based on net efflux ratio in the presence and absence of opicapone. Elacridar was used as a positive control inhibitor.
Assay:	LC-MS

Substrate	Incubation time (min)	Inhibitor	Net efflux ratio
Dexamethasone (5 µmol/L)	60	–	7.8
		Opicapone (100 µmol/L)	6.9
		–	5.2
		Elacridar (50 µmol/L)	1.0

Efflux ratio and net efflux ratio were calcurated based on mean Papp of 3 determinations.

6) BCRP 阻害

Test article: opicapone
Location in CTD: [5.3.2.3-5]
Study No. Tebu-02-[redacted]20[redacted]

Study title:	*In Vitro* Interaction Studies of Opicapone with Human BCRP Efflux (ABC) Transporter, and with Human OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 Uptake Transporters
Test system:	Human BCRP-expressing MDCKII cells
Method:	BCRP-expressing cells or parental cells were seeded on Transwell and incubated with a probe substrate and opicapone (30 µg/mL) for 60 min. Efflux ratio was calculated by dividing Papp from BL to AP by that from AP to BL. Net efflux ratio was calculated by subtracting efflux ratio in parent cells from that in BCRP-expressing cells. Inhibitory effect of opicapone on BCRP was evaluated based on net efflux ratio in the presence and absence of opicapone.
Assay:	LSC

Substrate	Concentraiton of opicapone (µg/mL)	Net efflux ratio	IC_{50} (µg/mL)
^{3}H-prazosine (1 µmol/L)	–	21.9 ± 3.6	NC
	30	15.1 ± 1.7	

Efflux ratio and net efflux ratio were calcurated based on mean Papp of 3 determinations. Data represent mean ± SD.

7) OAT1，OAT3，OATP1B1，OATP1B3，OCT1 及び OCT2 阻害

Test article: opicapone
Location in CTD: [5.3.2.3-5]
Study No. Tebu-02-[redacted]20[redacted]

Study title:	*In Vitro* Interaction Studies of Opicapone with Human BCRP Efflux (ABC) Transporter, and with Human OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 Uptake Transporters
Test system:	Human OAT1- or OCT1-expressing CHO cells, human OAT3-, OATP1B1-, or OATP1B3-expressing HEK293 cells, and human OCT2-expressing MDCKII cells
Method:	Transporter-expressing cells or control (parental or mock cells) were incubated with a probe substrate and opicapone (0.04-30 µg/mL). After incubation, cells were washed and lysed for analysis of substrate uptake. Transporter-specific uptake was calculated by subtracting uptake in control cells from that in transporter-expressing cells. Inhibitory effect of opicapone on tranporters was evaluated based on the transporter-specific uptake in the presence and absence of opicapone.
Assay:	LSC

Transporter	Substrate	Incubatioin time (min)	% Inhibition at maximum concentration (30 µg/mL)	IC_{50} (µg/mL)
OAT1	^{3}H-tenofovir (5 µmol/L)	10	84	9.18
OAT3	Methotrexate (1 µmol/L)*	3	92	4.36
OATP1B1	^{3}H-$E_2$17βG (1 µmol/L)	3	98	0.45
OATP1B3	^{3}H-CCK-8 (0.1 µmol/L)	10	99	1.40
OCT1	^{14}C-metformin (10 µmol/L)	20	22	NC
OCT2	^{14}C-metformin (10 µmol/L)	3	19	NC

Transpoter-specific uptake was calculated from mean uptake of 3 determinations.
*Radiolabeled compound, but radionuclide was not stated.

8) BSEP 阻害

Test article: opicapone
Location in CTD: [5.3.2.3-9]
Study No. Bial-03-[redacted]20[redacted]

Study title:	*In Vitro* Interaction Studies of Opicapone and BIA-9-1103 with Human BSEP Efflux (ABC) Transporter, and with Human MATE1 and MATE2-K Uptake Transporters
Test system:	Human BSEP-expressing membrane vesicles
Method:	Opicapone (0.04-30 µg/mL) and taurocholate were incubated with membrane vesicles in the presence of ATP or AMP. After incubation, the vesicles were washed on a filter plate, and uptake of taurocholate was determined. ATP-dependent uptake was calculated based on uptake with ATP and AMP. Inhibitory effect of opicapone on BSEP was evaluated using ATP-dependent uptake in the presence and absence of opicapone.
Assay:	LSC

Substrate	Incubation time (min)	% Inhibition at maximum concentration (30 µg/mL)	IC_{50} (µg/mL)
^{3}H-taurocholate (2 µmol/L)	5	75	12.28

ATP-dependent uptake was calculated based on mean uptake of 3 determinations.

9) MATE1 及び MATE2-K 阻害

Test article: opicapone
Location in CTD: [5.3.2.3-9]
Study No. Bial-03-[redacted]20[redacted]

Study title:	*In Vitro* Interaction Studies of Opicapone and BIA-9-1103 with Human BSEP Efflux (ABC) Transporter, and with Human MATE1 and MATE2-K Uptake Transporters
Test system:	MATE1- or MATE2-K-expressing MDCKII cells
Method:	Transporter-expressing cells or control cells were incubated with a probe substrate and opicapone (0.04-30 µg/mL). After incubation, cells were washed and lysed for analysis of substrate uptake. Transporter-specific uptake was calculated by subtracting uptake in control cells from that in transporter-expressing cells. Inhibitory effect of opicapone on MATE1 and MATE2-K was evaluated based on the transporter-specific uptake in the presence and absence of opicapone.
Assay:	LSC

Transporter	Substrate	Incubation time (min)	% Inhibition at maximum concentration (30 µg/mL)	IC_{50} (µg/mL)
MATE1	^{14}C-metformin (10 µmol/L)	15	−5	NC
MATE2-K			52	NC

Transpoter-specific uptake was calculated from mean uptake of 3 determinations.

2.6.5.16.1.2 BIA 9-1103

1） P-gp 及び BCRP による輸送

Test article: BIA 9-1103
Location in CTD: [5.3.2.3-6]
Study No. Bial-02-[redacted]20[redacted]

Study title:	*In Vitro* Interaction Studies of BIA 9-1103 with Human BCRP and MDR1 Efflux (ABC) Transporters, and with Human OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 Uptake Transporters
Test system:	Human P-gp- or BCRP-expressing MDCKII cells
Method:	Transporter-expressing cells or parental cells were seeded on Transwell and incubated with BIA 9-1103. Efflux ratio was calculated by dividing Papp from BL to AP by that from AP to BL. Net efflux ratio was calculated by subtracting efflux ratio in parental cells from that in transporter-expressing cells. P-gp- or BCRP-dependent transport was evaluated based on the net efflux ratio.
Assay:	LC-MS/MS, LSC

Transporter	Substrate	Inhibitor	Incubation time (min)	Net efflux ratio
P-gp	BIA 9-1103 (1 µg/mL)	–	0, 15, 30, 60, 120	0.8 ± 0.7
	BIA 9-1103 (3 µg/mL)			0.8 ± 0.6
	BIA 9-1103 (10 µg/mL)			0.5 ± 0.5
	^{3}H-digoxin (5 µmol/L)	–	120	15.8 ± 4.0
		PSC833 (10 µmol/L)		0.9 ± 0.4
BCRP	BIA 9-1103 (1 µg/mL)	–	0, 15, 30, 60, 120	3.9 ± 1.7
	BIA 9-1103 (3 µg/mL)			3.7 ± 2.0
	BIA 9-1103 (10 µg/mL)			1.6 ± 1.1
	^{3}H-prazosin (1 µmol/L)	–	60	21.9 ± 3.6
		Ko134 (1 µmol/L)		0.8 ± 0.6

Efflux ratio and net efflux ratio were calcurated based on mean Papp of 3 determinations. Data represent mean ± SD.

2) OATP1B1 及び OATP1B3 による輸送

Test article: BIA 9-1103
Location in CTD: [5.3.2.3-6]
Study No. Bial-02-[redacted]20[redacted]

Study title:	*In Vitro* Interaction Studies of BIA 9-1103 with Human BCRP and MDR1 Efflux (ABC) Transporters, and with Human OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 Uptake Transporters
Test system:	Human OATP1B1- or OATP1B3-expressing HEK293 cells
Method:	Transporter-expressing cells or control (mock) cells were incubated with BIA 9-1103. After incubation, cells were washed and lysed for analysis of substrate uptake. Fold accumulation was calculated by dividing uptake in transporter-expressing cells by that in control cells. In addition, inhibition of BIA 9-1103 uptake by rifampicin was evaluated.
Assay:	LC-MS/MS, LSC

Uptake study

Transporter	Substrate	Inhibitor	Incubation time (min)	Fold accumulation
OATP1B1	BIA 9-1103 (1 µg/mL)	–	2	NC
			20	NC
	BIA 9-1103 (10 µg/mL)	–	2	23.0
			20	33.2
	^{3}H-E$_2$17βG (1 µmol/L)	–	3	67.1
		Rifampicin (50 µmol/L)		2.3
OATP1B3	BIA 9-1103 (1 µg/mL)	–	2	14.8
			20	31.8
	BIA 9-1103 (10 µg/mL)	–	2	4.9
			20	17.5
	^{3}H-CCK-8 (0.11 µmol/L)	–	10	31.3
		Rifampicin (50 µmol/L)		0.9

2） OATP1B1 及び OATP1B3 による輸送（続き）

Inhibition by rifampicin

Transporter	Substrate	Inhibitor	Incubation time (min)	Fold accumulation
OATP1B1	BIA 9-1103 (10 μg/mL)	–	20	78.4
		Rifampicin (50 μmol/L)		3.0
	^{3}H-$E_2$17βG (1 μmol/L)	–	3	65.4
		Rifampicin (50 μmol/L)		2.1
OATP1B3	BIA 9-1103 (1 μg/mL)	–	20	42.6
		Rifampicin (50 μmol/L)		1.7
	^{3}H-CCK-8 (0.11 μmol/L)	–	10	39.6
		Rifampicin (50 μmol/L)		0.9

Fold accumulation was culcurated based on mean uptake of 3 determinations.
NC, not calcurated since uptake was under detection limit in control cells.

3) P-gp 及び BCRP 阻害

Test article: BIA 9-1103
Location in CTD: [5.3.2.3-6]
Study No. Bial-02-[redacted]20[redacted]

Study title:	*In Vitro* Interaction Studies of BIA 9-1103 with Human BCRP and MDR1 Efflux (ABC) Transporters, and with Human OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 Uptake Transporters
Test system:	Human P-gp- or BCRP-expressing MDCKII cells
Method:	Transporter-expressing cells or parental cells were seeded on Transwell and incubated with a probe substrate and BIA 9-1103 (30 µg/mL). Efflux ratio was calculated by dividing Papp from BL to AP by that from AP to BL. Net efflux ratio was calculated by subtracting efflux ratio in parental cells from that in transporter-expressing cells. Inhibitory effect of BIA 9-1103 on P-gp and BCRP was evaluated based on net efflux ratio in the presence and absence of BIA 9-1103.
Assay:	LSC

Transporter	Substrate	Incubation time (min)	Inhibitor	Net efflux ratio	IC_{50} (µg/mL)
P-gp	^{3}H-digoxin (5 µmol/L)	120	–	15.8 ± 4.0	NC
			BIA 9-1103 (30 µg/mL)	16.2 ± 3.0	
BCRP	^{3}H-prazosin (1 µmol/L)	60	–	21.9 ± 3.6	NC
			BIA 9-1103 (30 µg/mL)	16.4 ± 2.8	

Efflux ratio and net efflux ratio were calcurated based on mean Papp of 3 determinations. Data represent mean ± SD.

4) OAT1，OAT3，OATP1B1，OATP1B3，OCT1，及び OCT2 阻害

Test article: BIA 9-1103
Location in CTD: [5.3.2.3-6]
Study No. Bial-02-[redacted]20[redacted]

Study title:	*In Vitro* Interaction Studies of BIA 9-1103 with Human BCRP and MDR1 Efflux (ABC) Transporters, and with Human OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 Uptake Transporters
Test system:	Human OAT1- OCT1- expressing CHO cells, human OAT3-, OATP1B1-, or OATP1B3-expressing HEK293 cells, and human OCT2-expressing MDCKII cells
Method:	Transporter-expressing cells or control (parental or mock) cells were incubated with a probe substrate and BIA 9-1103 (0.04-30 µg/mL) at 37°C. After incubation, cells were washed and lysed for analysis of substrate uptake. Transporter-specific uptake was calculated by subtracting uptake in control cells from that in transporter-expressing cells. Inhibitory effect of BIA 9-1103 on tranporters was evaluated based on the transporter-specific uptake in the presence and absence of BIA 9-1103.
Assay:	LSC

Transporter	Substrate	Incubation time(min)	% Inhibition at maximum concentration (30 µg/mL)	IC_{50} (µg/mL)
OAT1	^{3}H-tenofovir (5 µmol/L)	10	70	13.20
OAT3	Methotrexate* (1 µmol/L)	3	93	2.55
OATP1B1	^{3}H-$E_2$17βG (1 µmol/L)	3	91	2.80
OATP1B3	^{3}H-CCK-8 (0.1 µmol/L)	10	80	7.51
OCT1	^{14}C-Metformin (10 µmol/L)	20	33	NC
OCT2	^{14}C-Metformin (10 µmol/L)	3	5	NC

Transpoter-specific uptake was calculated from mean uptake of 3 determinations.
*Radiolabeled compound, but radionuclide was not stated.

5) BSEP 阻害

Test article: BIA 9-1103
Location in CTD: [5.3.2.3-9]
Study No. Bial-03-[redacted]20[redacted]

Study title:	*In Vitro* Interaction Studies of Opicapone and BIA-9-1103 with Human BSEP Efflux (ABC) Transporter, and with Human MATE1 and MATE2-K Uptake Transporters
Test system:	Human BSEP-expressing membrane vesicles
Method:	BIA 9-1103 (0.04-30 µg/mL) and taurocholate were incubated with membrane vesicles in the presence of ATP or AMP. After incubation, the vesicles were washed on a filter plate, and uptake of taurocholate was determined. ATP-dependent uptake was calculated based on uptake with ATP and AMP. Inhibitory effect of BIA 9-1103 on BSEP was evaluated using ATP-dependent uptake with and without BIA 9-1103.
Assay:	LSC

Substrate	Incubation time (min)	% Inhibition at maximum concentration (30 µg/mL)	IC_{50} (µg/mL)
^{3}H-taurocholate (2 µmol/L)	5	33	NC

ATP-dependent uptake was calculated based on mean uptake of 3 determinations.

6) MATE1 及び MATE2-K 阻害

Test article: BIA 9-1103
Location in CTD: [5.3.2.3-9]
Study No. Bial-03-[redacted]20[redacted]

Study title:	*In Vitro* Interaction Studies of Opicapone and BIA-9-1103 with Human BSEP Efflux (ABC) Transporter, and with Human MATE1 and MATE2-K Uptake Transporters
Test system:	MATE1- or MATE2-K-expressing MDCKII cells
Method:	Transporter-expresing cells or control cells were incubated with a substrate and BIA 9-1103 (0.04-30 µg/mL). After incubation, cells were washed and lysed for analysis of substrate uptake. Transporter-specific uptake was calculated by subtracting uptake in control cells from that in transporter-expressing cells. Inhibitory effect of BIA 9-1103 on MATE1 and MATE2-K was evaluated based on the transporter-specific uptake in the presence and absence of BIA 9-1103.
Assay:	LSC

Transporter	Substrate	Incubation time (min)	% Inhibition at maximum concentration (30 µg/mL)	IC_{50} (µg/mL)
MATE1	^{14}C-metformine (10 µmol/L)	15	−1	NC
MATE2-K			9	NC

Transpoter-specific uptake was calculated from mean uptake of 3 determinations.

2.6.5.16.1.3 イリノテカンの輸送に及ぼす影響

1） オピカポン

Test article: opicapone
Location in CTD: [5.3.2.3-7]
Study No. SRAL[redacted]0315

Study title:	Opicapone and BIA 9-1103 Interaction with Irinotecan Transport by Caco-2 Cells
Test system:	Caco-2 cells
Objective:	To evaluate the effects of opicapone on a P-gp substrate (irinotecan) transport in cell monolayers.
Method:	The assays were performed using cell monolayers, cultured in Transwell. The AP to BL and BL to AP transport of irinotecan (1 µmol/L) was evaluated over 120 min in the presence and absence of opicapone (100 µmol/L). Verapamil (100 µmol/L), a known P-gp transporter substrate, was used as positive control.
Analyte/assay:	Irinotecan/LC-MS

Effect of opicapone

Time (min)	Cumulative transport (pmol, mean ± SEM, n = 3)			
	Control		+Opicapone	
	AP to BL	BL to AP	AP to BL	BL to AP
60	33.4 ± 1.2	107.1 ± 11.6	35.9 ± 1.1	117.6 ± 1.6
120	46.1 ± 1.5	193.1 ± 12.4	53.2 ± 4.6	212.2 ± 15.1

Effect of verapamil

Time (min)	Cumulative transport (pmol, mean ± SEM, n = 3)			
	Control		Verapamil	
	AP to BL	BL to AP	AP to BL	BL to AP
60	28.8 ± 12.8	144.5 ± 7.7	131.3 ± 14.3*	50.7 ± 9.6*
120	46.4 ± 16.8	229.9 ± 14.1	210.1 ± 24.4*	88.9 ± 20.7*

No transport interaction was observed between irinotecan and opicapone. With verapamil, a significant decrease of BL to AP transport of irinotecan was observed.

*$p < 0.001$ versus control value.

2) BIA 9-1103

Test article: BIA 9-1103
Location in CTD: [5.3.2.3-7]
Study No. SRAL[redacted]0315

Study title:	Opicapone and BIA 9-1103 Interaction with Irinotecan Transport by Caco-2 Cells
Test system:	Caco-2 cells
Objective:	To evaluate the effects of BIA 9-1103 on a P-gp substrate (irinotecan) transport in cell monolayers.
Method:	The assays were performed using cell monolayers, cultured in Transwell. The AP to BL and BL to AP transport of irinotecan (1 µmol/L) was evaluated over 120 min in the presence and in the absence of BIA 9-1103 (100 µmol/L). Verapamil (100 µmol/L), a known P-gp transporter substrate, was used as positive control.
Analyte/assay:	Irinotecan/LC-MS

Effect of BIA 9-1103

Time (min)	Cumulative transport (pmol, mean ± SEM, n = 3)			
	Control		BIA 9-1103	
	AP to BL	BL to AP	AP to BL	BL to AP
60	53.6 ± 6.2	170.1 ± 6.0	42.4 ± 3.1	157.3 ± 9.2
120	94.6 ± 19.2	306.9 ± 32.8	68.0 ± 7.9	267.4 ± 9.2

Effect of verapamil

Time (min)	Cumulative transport (pmol, mean ± SEM, n = 3)			
	Control		Verapamil	
	AP to BL	BL to AP	AP to BL	BL to AP
60	28.8 ± 12.8	144.5 ± 7.7	131.3 ± 14.3*	50.7 ± 9.6*
120	46.4 ± 16.8	229.9 ± 14.1	210.1 ± 24.4*	88.9 ± 20.7*

No transport interaction was observed between irinotecan and BIA 9-1103. With verapamil, a significant decrease of BL to AP transport of irinotecan was observed.

*$p < 0.001$ versus control value.

2.6.5.16.1.4 血漿蛋白結合を介した薬物相互作用

1） オピカポン

Test article: ^{14}C-opicapone
Location in CTD: [4.2.2.3-2]
Study No. ZNA15658.004

Study title:	Investigation of Plasma Protein and Blood Cell Binding of [^{14}C] BIA 9-1067 in Rat, Primate and Human and the Effect of Warfarin, Diazepam, Digoxin or Tolbutamide on Human Plasma Protein Binding of [^{14}C] BIA 9-1067 Using Ultrafiltration
Test system:	Plasma from male humans
Objective:	To investigate the effect of warfarin, diazepam, digoxin, or tolbutamide on human plasma protein binding of opicapone.
Method:	Ultrafiltration
Assay:	LSC

Results:
Binding of 0.3 to 30 µg/mL ^{14}C-opicapone to male human plasma proteins in the presence of warfarin (5 µg/mL), diazepam (5 µg/mL), digoxin (5 µg/mL), or tolbutamide (20 µg/mL) remained high with a mean of 99.8% and ranged between 99.5% and 99.9%.
When incubated alone, binding of ^{14}C-warfarin, ^{14}C-diazepam, ^{3}H-digoxin, and ^{14}C-tolbutamide to human plasma protein was 99.3%, 98.7%, 41.7%, and 97.1%, respectively. These binding levels were unaffected by co-incubation with opicapone.

Conclusions:
The binding data in the presence of warfarin, diazepam, digoxin, or tolbutamide indicate that the plasma protein binding site of opicapone differs from that of the interactants as there were no significant changes in the degree of binding of opicapone or any of the interactants when co-incubated.

2） BIA 9-1103

Test article: BIA 9-1103 and ^{14}C-opicapone
Location in CTD: [5.3.2.1-2]
Study No. ZNA42782.001

Study title:	Plasma Protein Binding Displacement Study of BIA 9-1103 and [^{14}C] BIA 9-1067 in Human and the Effect of Warfarin, Diazepam, Digoxin or Tolbutamide on Human Plasma Protein Binding of BIA 9-1103 Using Ultrafiltration
Test system:	Plasma from male humans
Objective:	To investigate (1) the effect of BIA 9-1103 on *in vitro* binding of diazepam, digoxin, warfarin, and tolbutamide to human plasma protein; (2) the *in vitro* binding of ^{14}C-opicapone to human plasma proteins in the presence and absence of BIA 9-1103
Method:	Ultrafiltration
Assay:	LSC

Results:
Binding of 5 µg/mL ^{14}C-opicapone to plasma proteins of male human was high with a mean of 99.9% and was essentially unchanged in the presence of 0.3 to 30 µg/mL BIA 9-1103.
The binding of ^{3}H-diazepam (5 µg/mL), ^{3}H-digoxin (5 µg/mL), ^{14}C-warfarin (5 µg/mL) and ^{14}C-tolbutamide (20 µg/mL) to human plasma protein was 95.8%, 36.9%, 98.9%, and 96.8%, respectively. These binding levels were unaffected by co-incubation with opicapone

Conclusions:
The binding data for opicapone in the presence BIA 9-1103 was essentially unchanged, indicating that the plasma protein binding site of opicapone differs from that of BIA 9-1103. Similarly, the binding site of BIA 9-1103 was deemed to be different to that of the 4 interactants tested.

2.6.5.17 薬物動態試験：その他

該当する資料はない.