35th JSSX Annual Meeting

Encouragement Award

The Study for Mechanism Analyses of Cytochrome P450 Inhibitors Focused on Metabolic Interactions of Drug–drug/Drug–endogenous Compound

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35th JSSX Annual Meeting
COI disclosure information

Author: Satoshi Yamaori

I have no financial relationship to disclose for my presentation contents.

Topics

- **Structural requirements for potent inhibition of CYP1A1 by cannabidiol**

  Yamaori S *et al.*, *Biol. Pharm. Bull.*, **36**, 1197-1203 (2013)
  Yamaori S *et al.*, *Chem. Biol. Interact.*, **215**, 62-68 (2014)

- **Structural requirements for inhibition of CYP2C19 by cannabidiol**

  Jiang R, Yamaori S *et al.*, *Drug Metab. Pharmacokinet.*, **28**, 332-338 (2013)

- **Possible mechanism of interaction between iguratimod and warfarin: inhibition of CYP2C9-mediated warfarin metabolism by iguratimod**

  Yamaori S *et al.*, *Biol. Pharm. Bull.*, **38**, 441-447 (2015)

- **Inhibitory effects of antihypertensive drugs on CYP2J2 activity: potent inhibition by manidipine and azelnidipine**

  Ikemura N, Yamaori S *et al.*, *Chem. Biol. Interact.*, **306**, 1-9 (2019)

- **Characterization of epalrestat as a highly selective inhibitor of CYP4A11**

  Yamaori S *et al.*, *J. Pharmacol. Exp. Ther.*, **366**, 446-457 (2018)

- **Characterization of sesamin as a mechanism-based inactivator of CYP4F2**

  Watanabe H, Yamaori S *et al.*, *Biol. Pharm. Bull.*, **43**, 688-692 (2020)

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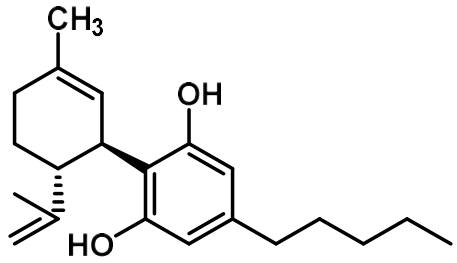
Background



- **Cannabidiol (CBD) is one of the major constituents in marijuana.**

- **CBD exhibits several pharmacological effects such as antiepileptic, anxiolytic and antiemetic actions. A medicine made from marijuana extracts containing CBD is used for the symptomatic relief of neuropathic pain in multiple sclerosis patients.**

  Mechoularm R *et al.*, *J. Clin. Pharmacol.*, **42**, 11S-19S (2002)

- **CBD inhibits various CYP-mediated drug oxidations. In particular, CBD more potently inhibits the activity of CYP1A1 than the other CYP enzymes examined so far. CBD is also a mechanism-based inactivator of CYP1A1. CBD is a potent inhibitor of CYP2C19, which is the major enzyme involved in the CBD metabolism.**

  Yamaori S *et al.*, *Biochem. Pharmacol.*, **79**, 1691-1698 (2010)
  Jiang R, Yamaori S *et al.*, *Drug Metab. Pharmacokinet.*, **28**, 332-338 (2013)

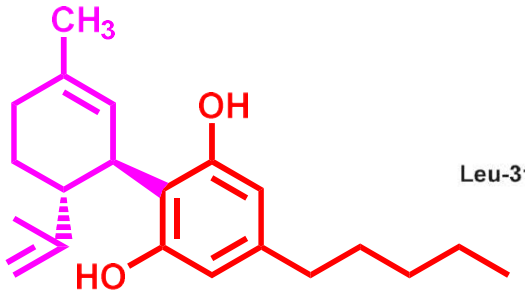
- **The moieties of CBD that contribute to potent inhibition of CYP1A1 and CYP2C19 remain unknown.**

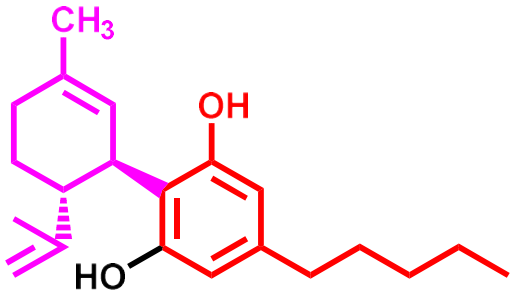
The effects of CBD-related compounds on CYP1A1 and CYP2C19 activities were investigated with recombinant CYP enzymes to characterize the structural requirements for potent inhibition by CBD.

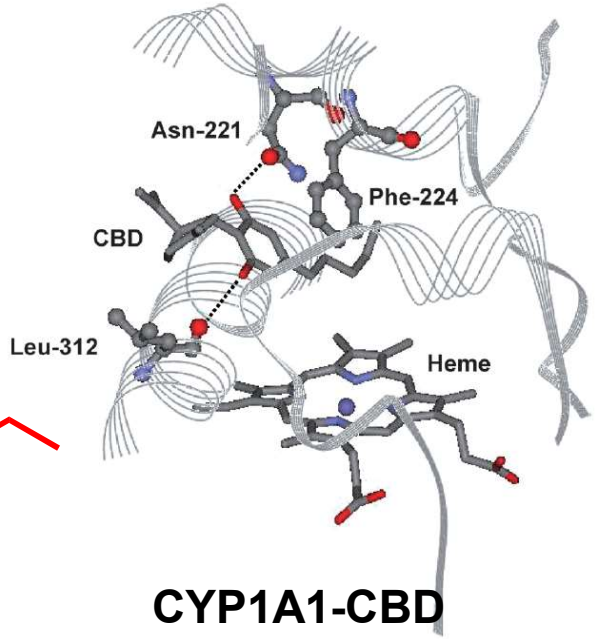
Structural Requirements for Direct Inhibition of CYP1A1 and CYP2C19 by CBD

Isoform	Compound	IC_{50} (μM)
CYP1A1	CBD	0.355
	Olivetol	13.8
	d-Limonene	> 100
	CBD-2′-monomethyl ether	4.07
	CBD-2′,6′-dimethyl ether	23.0
	Cannabidivarin	1.85
	Orcinol	62.9
	Resorcinol	No inhibition
	Δ^9-Tetrahydrocannabinol	10.3
	Cannabielsoin	9.93
CYP2C19	CBD	2.51
	Olivetol	15.3
	d-Limonene	> 50
	CBD-2′-monomethyl ether	1.88
	CBD-2′,6′-dimethyl ether	14.8
	Cannabidivarin	3.45
	Resorcinol	No inhibition
	Δ^9-Tetrahydrocannabinol	4.35

All determinations were performed in duplicate.



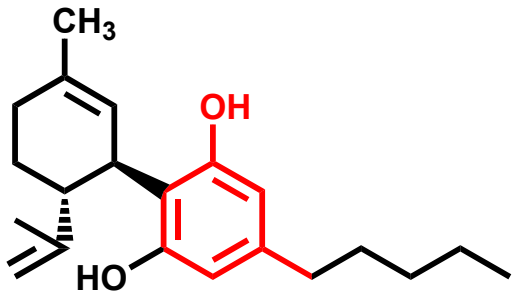




CYP1A1-CBD

We characterized that the pentylresorcinol structure in CBD is essential for direct inhibition of CYP1A1 and CYP2C19 although the whole structure of CBD is required for their overall inhibition.

Structural Requirements for CYP1A1 Inactivation by CBD



CBD

Compound	IC_{50} (μM)			k_{inact} (/min)	K_I (μM)	k_{inact}/K_I (L/mmol/min)
	Preincubation time		B/A			
	0 min (A)	20 min (B)				
CBD	0.671	0.0678	0.101	0.215	0.439	490
Olivetol	11.1	2.60	0.234	0.154	4.66	33.0
d-Limonene	> 100	> 100	–	–	–	–
Pentylbenzene	> 100	> 100	–	–	–	–
CBD-2′-monomethyl ether	4.12	1.90	0.461	0.0638	1.33	48.0
CBD-2′,6′-dimethyl ether	29.2	7.68	0.263	0.0643	6.11	10.5
Cannabidivarin	1.64	0.0677	0.0413	0.226	0.623	363
Orcinol	62.3	38.7	0.621	0.0353	84.7	0.417
Resorcinol	> 100	> 100	–	–	–	–

All determinations were performed in duplicate.

We characterized that the methylresorcinol structure in CBD plays important roles in CYP1A1 inactivation.

Topics

- Structural requirements for potent inhibition of CYP1A1 by cannabidiol

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- Structural requirements for inhibition of CYP2C19 by cannabidiol

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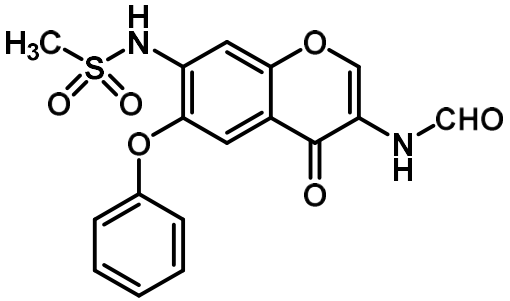
Background

- **Iguratimod is a novel disease-modifying antirheumatic drug.**
- **A few patients with severe bleeding were reported in a blue letter (safety advisory) for interaction between iguratimod and warfarin issued in May 2013. Consequently, the use of iguratimod in combination with warfarin was changed from a precaution to contraindication. However, the mechanism underlying iguratimod–warfarin interaction remains unclear.**
- **Iguratimod at a concentration of 100 μM inhibited CYP2C9 activity by about 50%. In that study, tolbutamide was used as a probe substrate for CYP2C9.**

  Ministry of Health, Labour, and Welfare, Japan. "Report on the deliberation for Careram® Tablets 25mg; KOLBET® Tablets 25mg"
- **Inhibitory potencies of CYP2C9 inhibitors vary depending on the substrate used: the inhibitory effect of iguratimod on CYP2C9 may differ between tolbutamide and warfarin.**

  Kumar V *et al.*, *Drug Metab. Dispos.*, **34**, 1966-1975 (2006)
- **It remains unclear whether iguratimod inhibits *S*-warfarin oxidation catalyzed by CYP2C9.**



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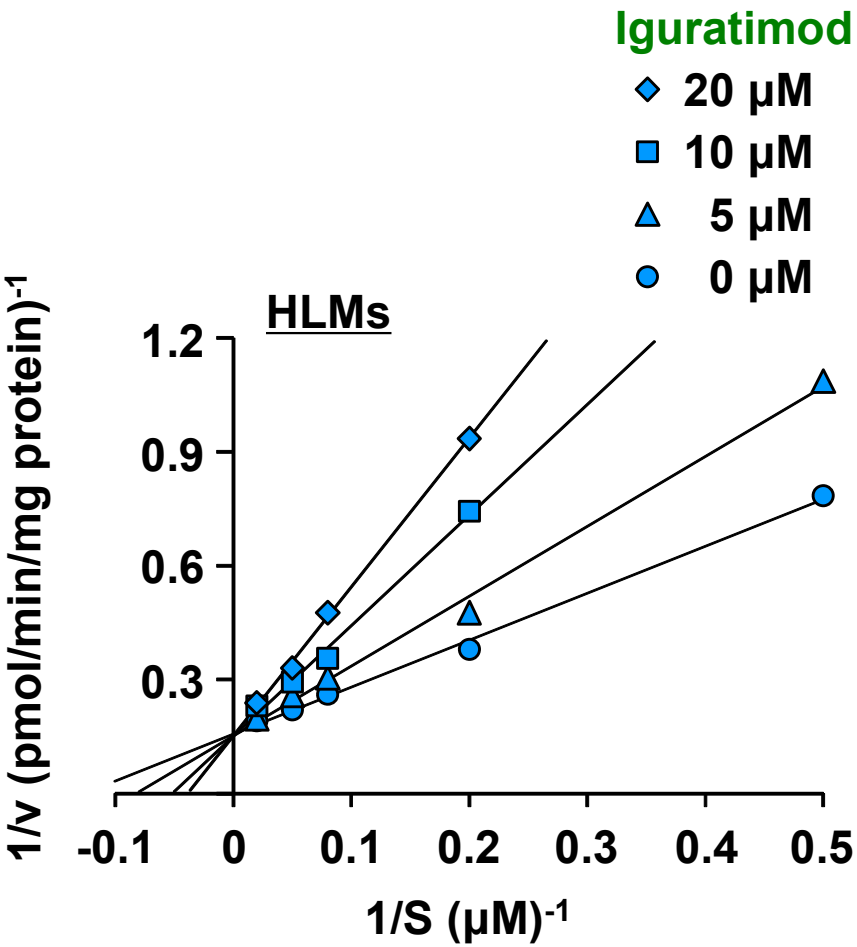
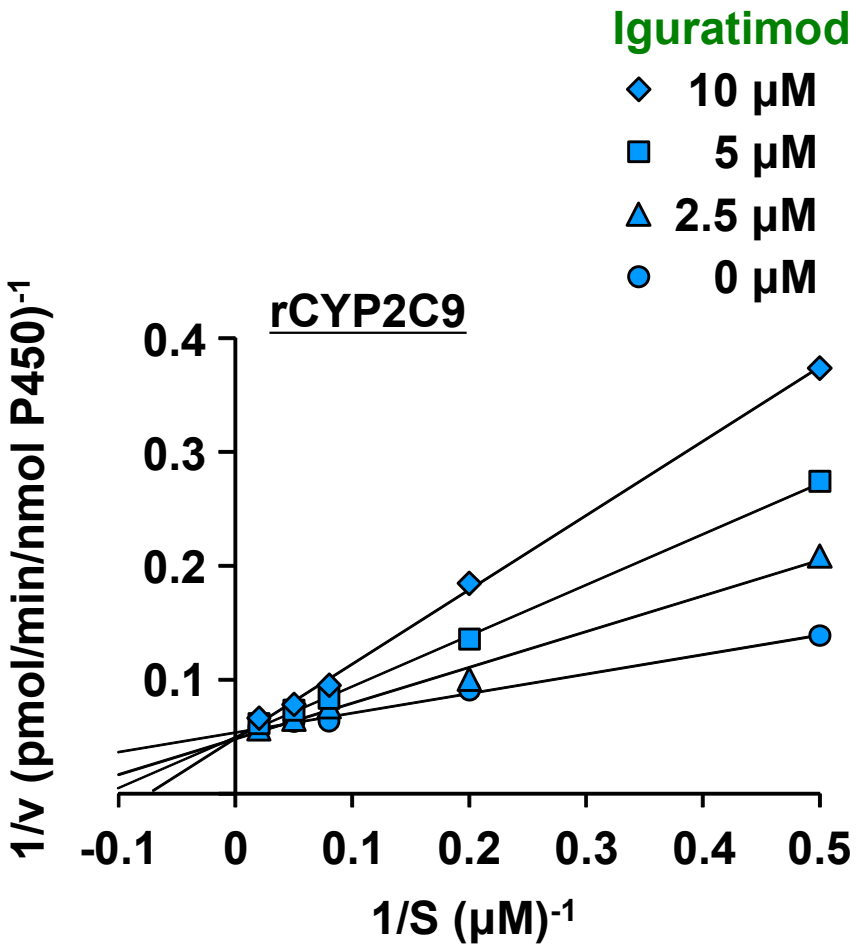
- **It remains unclear whether iguratimod inhibits *S*-warfarin oxidation catalyzed by CYP2C9.**

The inhibitory effect of iguratimod on CYP2C9-mediated *S*-warfarin 7-hydroxylation was examined with recombinant CYP2C9 and human liver microsomes (HLMs).

Inhibition of *S*-Warfarin 7-Hydroxylation by Iguratimod



K_i = 6.74 μM



K_i = 4.23 μM

HLMs and recombinant CYP2C9 (rCYP2C9) were incubated with *S*-warfarin in the presence of iguratimod. Each point is the mean of duplicate determinations.

Prediction of in Vivo Iguratimod–Warfarin Interaction for CYP2C9

Ratio of AUC with inhibitor to control AUC*

$$\frac{AUC_{inhibited}}{AUC_{control}} = \frac{1}{\left(\frac{f_{m(CYP2C9)}}{1 + \frac{[I]_{in\ vivo}}{K_i \cdot f_{HLMs}}}\right) + (1 - f_{m(CYP2C9)})}$$

$$= 2.3$$

$f_{m(CYP2C9)}$ = 0.91; fraction of metabolism of *S*-warfarin by CYP2C9*

$[I]_{in\ vivo}$ = 0.806 μM; maximum unbound hepatic input concentration of iguratimod**

K_i = 6.74 μM; apparent K_i value obtained in this study

f_{HLMs} = 0.07; fraction of iguratimod unbound to liver microsomal proteins**

* Obach RS *et al.*, *J. Pharmacol. Exp. Ther.*, **316**, 336-348 (2006)
**Interview Form from KOLBET® Tablets 25mg sixth edition (2013)

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Background

- **CYP2J2 is highly expressed in a variety of cancer cells and tumors.**

  Jiang J-G *et al.*, *Cancer Res.*, **65**, 4707-4715 (2005)
  Chen C *et al.*, *J. Pharmacol. Exp. Ther.*, **336**, 344-355 (2011)
  Narjoz C *et al.*, *PLOS ONE*, **9**, e95532 (2014)

- **CYP2J2 converts arachidonic acid to produce four epoxyeicosatrienoic acids (EETs), which play critical roles in primary tumor growth and metastasis as well as vascular endothelial growth factor (VEGF).**

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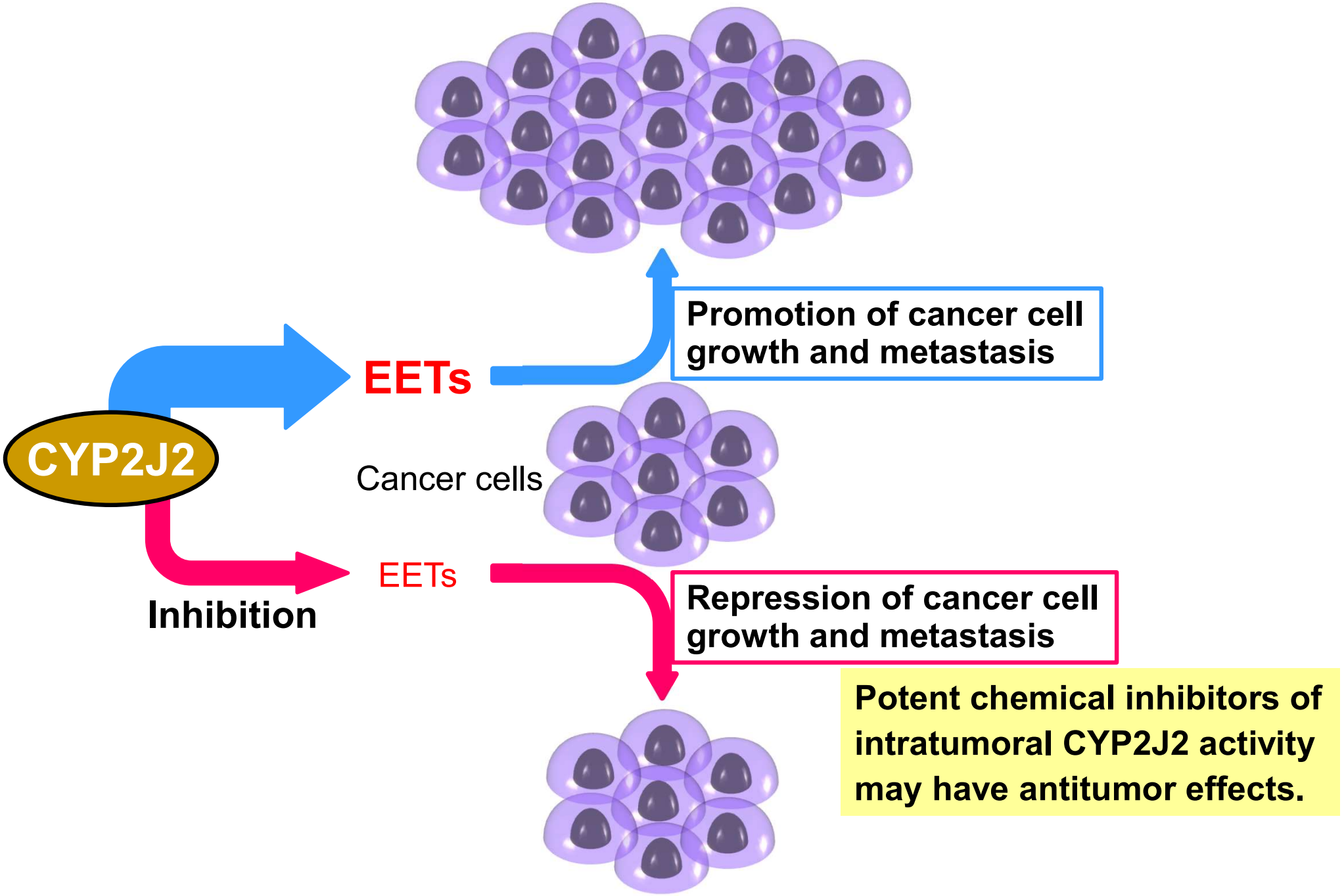
Pathophysiological Function of CYP2J2 in Cancer and Its Inhibition Leading to Potential Antitumorigenesis



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- **Increased production of intratumoral EETs by overexpression of CYP2J2 accelerates tumor growth and metastasis.**

  Jiang J-G *et al.*, *Cancer Res.*, **65**, 4707-4715 (2005)
  Jiang J-G *et al.*, *Cancer Res.*, **67**, 6665-6674 (2007)
  Chen C *et al.*, *J. Pharmacol. Exp. Ther.*, **336**, 344-355 (2011)

- **Reduced production of intratumoral EETs by suppression of CYP2J2 expression and chemical inhibition of CYP2J2 activity leads to antitumorigenesis.**

  Jiang J-G *et al.*, *Cancer Res.*, **65**, 4707-4715 (2005)
  Jiang J-G *et al.*, *Cancer Res.*, **67**, 6665-6674 (2007)
  Chen C *et al.*, *J. Pharmacol. Exp. Ther.*, **336**, 344-355 (2011)

- **CYP2J2 metabolizes tyrosine kinase inhibitors (TKIs) targeting VEGF receptor, which commonly cause hypertension, one of the major side effects.**

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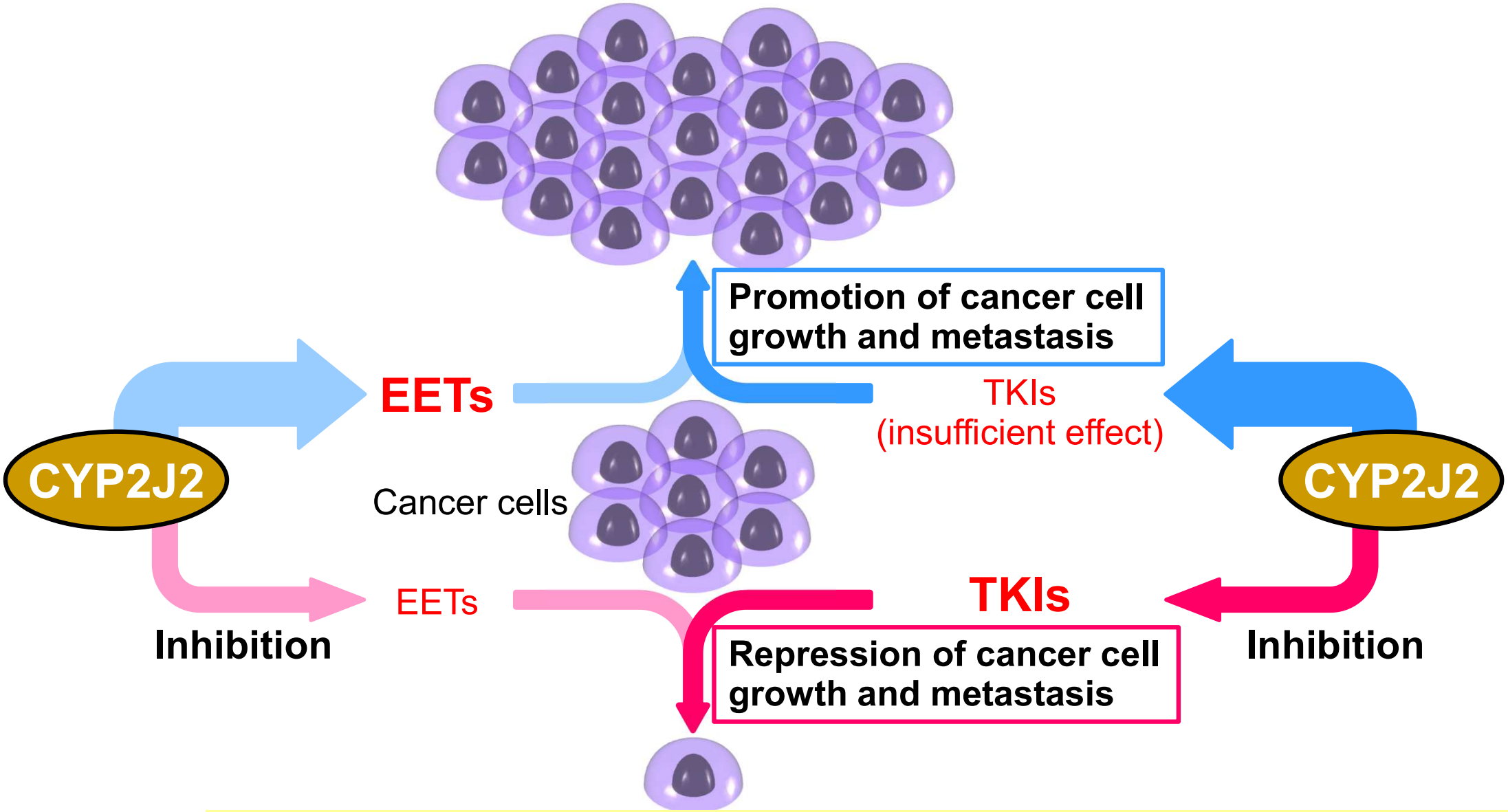


It is possible that potent CYP2J2 inhibitors may contribute not only to repression of EET-induced cancer cell growth and metastasis but also to reduced side effect and potentiated antitumor effect of TKIs.

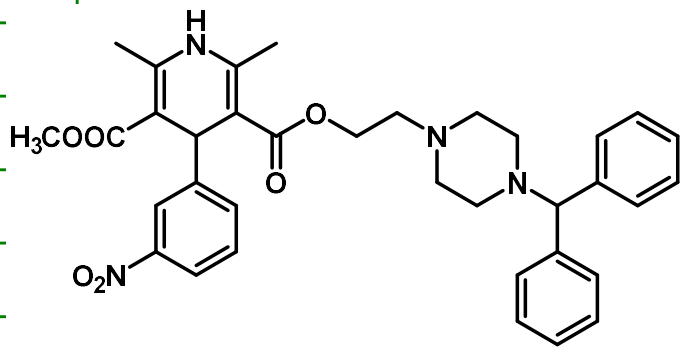
Purpose of This Study

- **Although certain antihypertensive drugs including telmisartan inhibit CYP2J2 activity, the inhibition study of many other antihypertensive drugs has not been examined in detail.**

Inhibitory effects of antihypertensive drugs on CYP2J2 activity were investigated with recombinant CYP2J2. In this study, dihydropyridine calcium channel blockers, angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors were used as inhibitors.

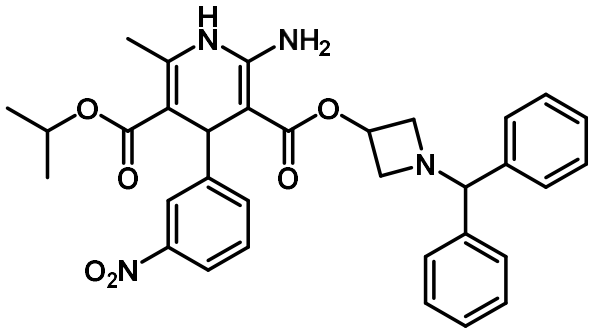
Kinetic Parameters for CYP2J2 Inhibition by Antihypertensive Drugs

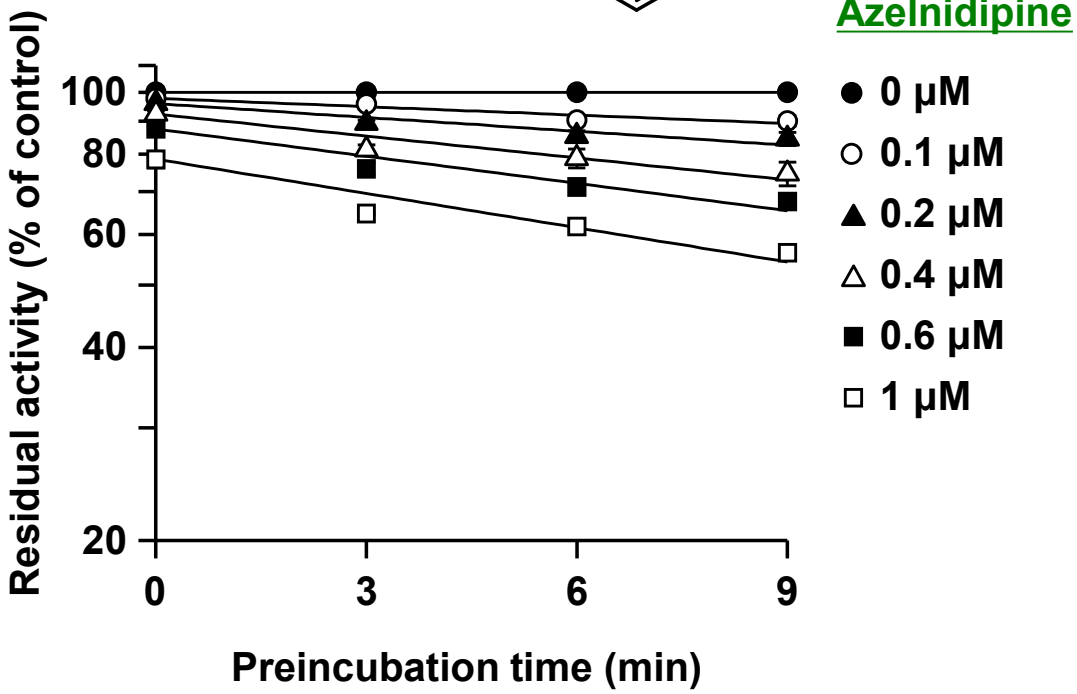
Inhibitor	IC_{50} (μM)	K_i (μM)	Inhibition mode
Amlodipine	5.07 ± 0.54	2.58 ± 0.23	Mixed
Azelnidipine	0.137 ± 0.014	0.0671 ± 0.0029	Competitive
Barnidipine	2.87 ± 0.30	0.651 ± 0.036	Competitive
Benidipine	2.16 ± 0.22	0.435 ± 0.012	Competitive
Cilnidipine	1.09 ± 0.03	0.279 ± 0.018	Competitive
Efonidipine	1.45 ± 0.03	0.315 ± 0.019	Competitive
Felodipine	2.78 ± 0.36	1.23 ± 0.15	Mixed
Manidipine	0.116 ± 0.017	0.0294 ± 0.0023	Competitive
Nicardipine	2.36 ± 0.35	0.566 ± 0.082	Competitive
Nifedipine	7.34 ± 0.05	2.76 ± 0.29	Mixed
Nilvadipine	4.55 ± 0.67	1.66 ± 0.03	Mixed
Nisoldipine	6.56 ± 0.34	2.38 ± 0.29	Mixed
Nitrendipine	1.45 ± 0.16	0.689 ± 0.104	Mixed
Telmisartan	2.04 ± 0.23	0.345 ± 0.002	Competitive
Delapril	9.01 ± 0.49	2.63 ± 0.13	Competitive
Quinapril	9.19 ± 0.72	2.55 ± 0.11	Competitive



Values are represented as mean ± S.D. of triplicate determinations.

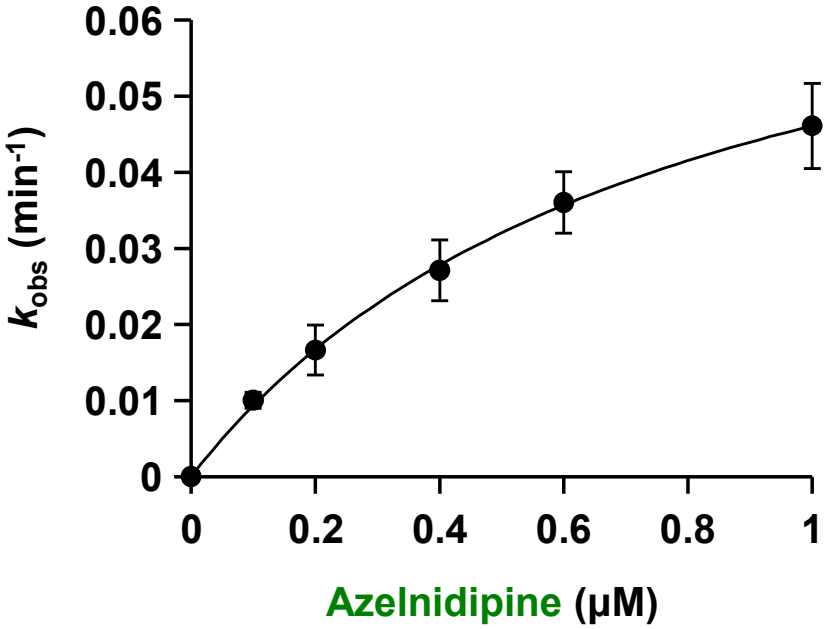
Inactivation of CYP2J2 Activity by Azelnidipine





k_{inact} = 0.0824 ± 0.0072 min^{-1}

K_I = 0.795 ± 0.109 μM



Recombinant human CYP2J2 was preincubated with azelnidipine in the presence of NADPH for up to 9 min. Aliquot was removed from the preincubation mixture at the indicated time points and diluted for measurement of the residual activity. Each point and bar represent mean ± S.D. of triplicate determinations. k_{inact}; Maximal inactivation rate constant, K_I; Half-maximal inhibitory concentration.

We characterized that manidipine is a potent reversible inhibitor and azelnidipine is a potent mechanism-based inactivator of CYP2J2.

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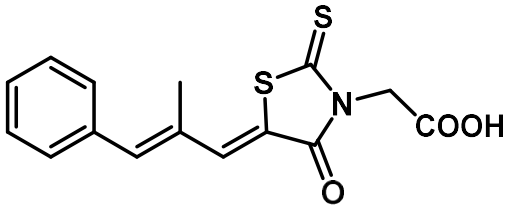
Background

- **CYP4A11 is capable of catalyzing ω-hydroxylation of lauric acid and arachidonic acid in the liver and kidneys.**

Powell PK *et al.*, *Arch. Biochem. Biophys.*, **335**, 219-226 (1996)
Powell PK *et al.*, *J. Pharmacol. Exp. Ther.*, **285**, 1327-1336 (1998)
Amet Y *et al.*, *Biochem. Pharmacol.*, **53**, 765-771 (1997)
Lasker JM *et al.*, *J. Biol. Chem.*, **275**, 4118-4126 (2000)

- **CYP4A11 is also involved in the metabolism of some marketed drugs, such as epalrestat, febuxostat and tofogliflozin.**



Interview Form from KINEDAC® Tablets 50 mg sixth edition (2013)
Mukoyoshi M *et al.*, *Xenobiotica*, **38**, 496-510 (2008)
Yamane M *et al.*, *Xenobiotica*, **45**, 230-238 (2015)

- **Although there are some CYP4 inhibitors, such as HET0016 and 17-octadecynoic acid (17-ODYA), no chemical inhibitors entirely selective for CYP4A11 have yet been found.**

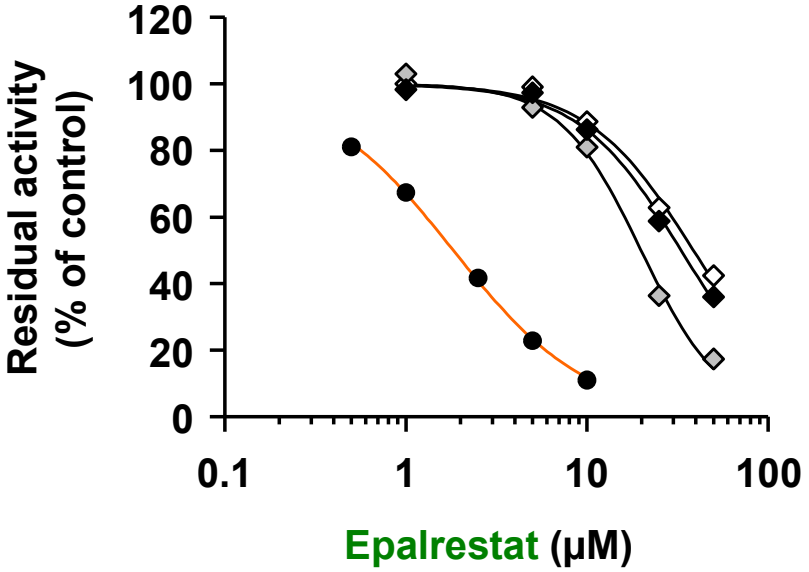
- **Effects of epalrestat on catalytic activities of various human CYP isoforms including CYP4A11 remain unclear.**

The selectivity of CYP4A11 inhibition by epalrestat was examined with recombinant CYP enzymes and HLMs.

Effects of Epalrestat on Various P450-mediated Drug Oxidations

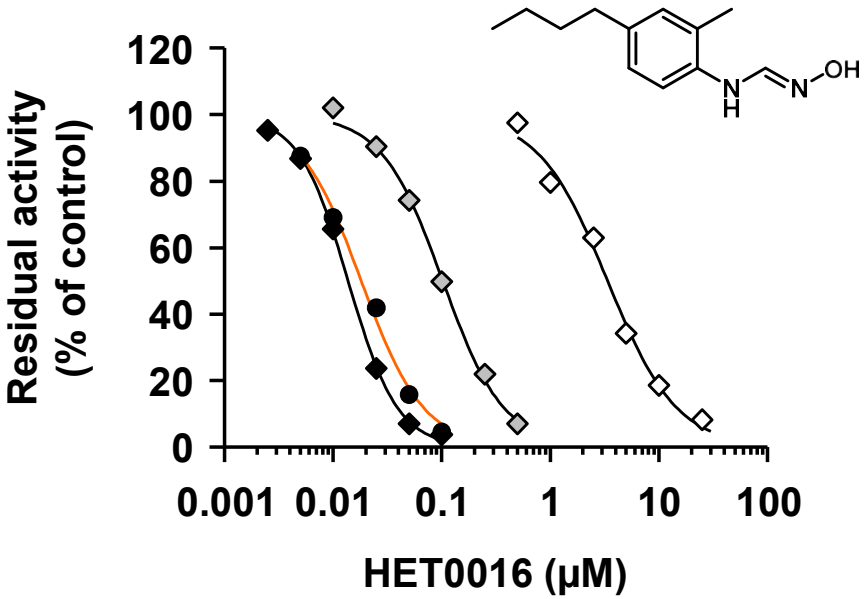
Enzyme	Reaction	Substrate concn. (μM)	IC_{50} (μM)
CYP1A1	Ethoxyresorufin *O*-deethylation	0.15	No inhibition
CYP1A2	Ethoxyresorufin *O*-deethylation	0.15	No inhibition
CYP1B1	Ethoxyresorufin *O*-deethylation	0.15	No inhibition
CYP2A6	Coumarin 7-hydroxylation	2.5	> 50
CYP2B6	Benzoxyresorufin *O*-debenzylation	0.6	No inhibition
CYP2C8	Dibenzylfluorescein *O*-debenzylation	0.4	32.8
CYP2C9	*S*-Warfarin 7-hydroxylation	3	19.8
CYP2C19	*O*-Methylfluorescein *O*-demethylation	4	> 50
CYP2D6	Dextromethorphan *O*-demethylation	0.6	No inhibition
CYP2E1	Chlorzoxazone 6-hydroxylation	200	No inhibition
CYP2J2	Luciferin-2J2/4F12 *O*-dealkylation	3	> 50
CYP3A4	Diltiazem *N*-demethylation	10	No inhibition
CYP3A5	Diltiazem *N*-demethylation	50	> 50
CYP4A11	Luciferin-4A *O*-demethylation	50	1.82
CYP4F2	Luciferin-4F2/3 *O*-dealkylation	5	35.9
CYP4F3B	Luciferin-4F2/3 *O*-dealkylation	3	19.9
CYP4F12	Luciferin-4F12 *O*-dealkylation	3	38.7
HLMs	Ethoxyresorufin *O*-deethylation	0.5	No inhibition
HLMs	*S*-Warfarin 7-hydroxylation	6	23.7
HLMs	*S*-Mephenytoin 4′-hydroxylation	100	> 50
HLMs	Dextromethorphan *O*-demethylation	2.5	No inhibition
HLMs	Diltiazem *N*-demethylation	30	No inhibition
HLMs	Luciferin-4A *O*-demethylation	50	0.913

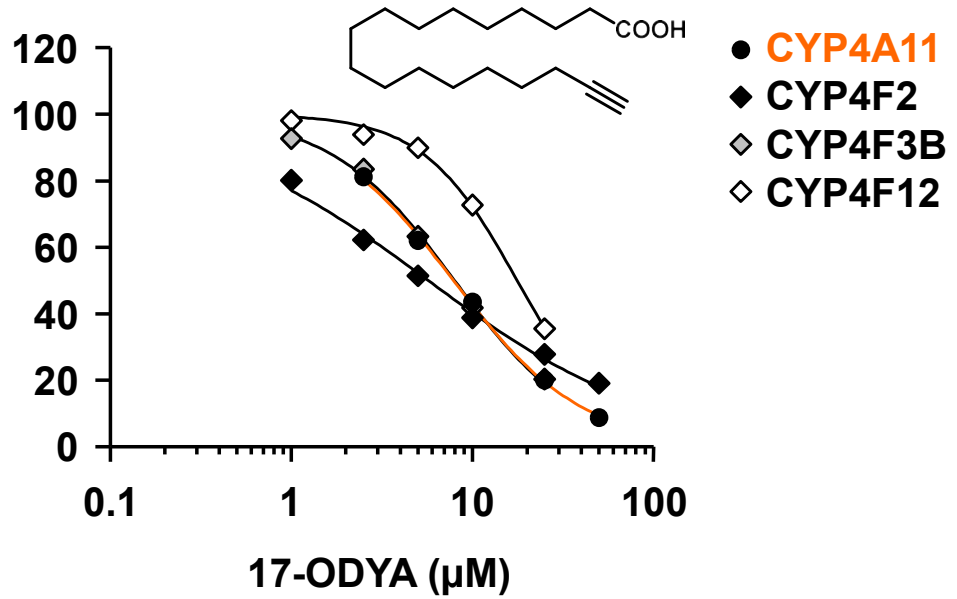
Inhibitory Effects of Epalrestat and CYP4 Inhibitors on CYP4A/4F Activities



Isoform	IC_{50} (μM)		
	Epalrestat	HET0016	17-ODYA
CYP4A11	1.82 (1.00)	0.0182 (1.00)	7.88 (1.00)
CYP4F2	35.9 (19.7)	0.0137 (0.753)	5.70 (0.723)
CYP4F3B	19.9 (10.9)	0.102 (5.60)	7.97 (1.01)
CYP4F12	38.7 (21.3)	3.36 (185)	17.7 (2.25)

The numbers in parentheses indicate ratios of IC_{50} values for CYP4F isoforms relative to the corresponding IC_{50} values for CYP4A11.





We characterized that epalrestat is the most selective CYP4A11 inhibitor among the chemical inhibitors reported so far.

Topics

- Structural requirements for potent inhibition of CYP1A1 by cannabidiol

  Yamaori S *et al.*, *Biol. Pharm. Bull.*, **36**, 1197-1203 (2013)
  Yamaori S *et al.*, *Chem. Biol. Interact.*, **215**, 62-68 (2014)

- Structural requirements for inhibition of CYP2C19 by cannabidiol

  Jiang R, Yamaori S *et al.*, *Drug Metab. Pharmacokinet.*, **28**, 332-338 (2013)

- Possible mechanism of interaction between iguratimod and warfarin: inhibition of CYP2C9-mediated warfarin metabolism by iguratimod

  Yamaori S *et al.*, *Biol. Pharm. Bull.*, **38**, 441-447 (2015)

- Inhibitory effects of antihypertensive drugs on CYP2J2 activity: potent inhibition by manidipine and azelnidipine

  Ikemura N, Yamaori S *et al.*, *Chem. Biol. Interact.*, **306**, 1-9 (2019)

- Characterization of epalrestat as a highly selective inhibitor of CYP4A11

  Yamaori S *et al.*, *J. Pharmacol. Exp. Ther.*, **366**, 446-457 (2018)

- **Characterization of sesamin as a mechanism-based inactivator of CYP4F2**

  Watanabe H, Yamaori S *et al.*, *Biol. Pharm. Bull.*, **43**, 688-692 (2020)

Background

- **CYP4F2 is a physiologically important enzyme that catalyzes ω-hydroxylation of arachidonic acid and leukotriene B_4 in the liver and kidneys.**

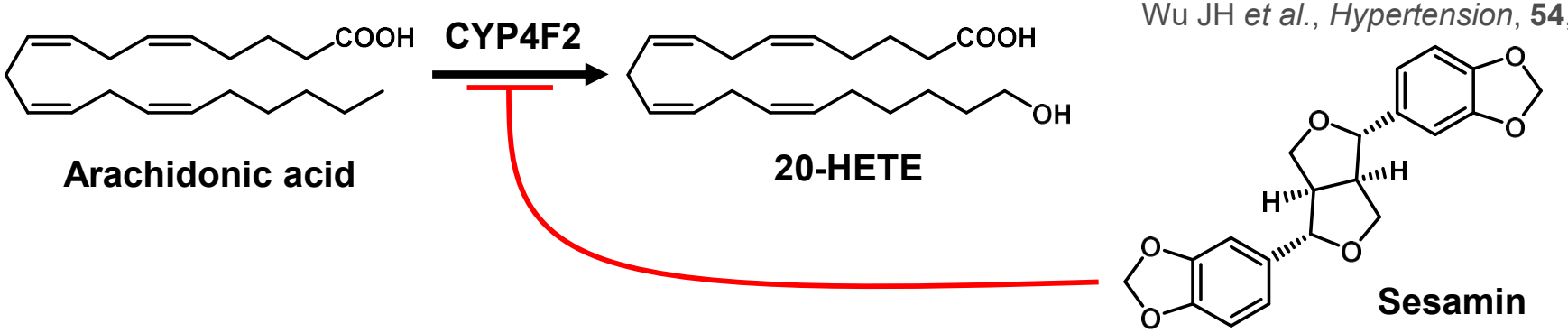
Powell PK *et al.*, *J. Pharmacol. Exp. Ther.*, **285**, 1327-1336 (1998)
Jin R *et al.*, *Arch. Biochem. Biophys.*, **359**, 89-98 (1998)
Lasker JM *et al.*, *J. Biol. Chem.*, **275**, 4118-4126 (2000)

- **20-Hydroxyeicosatetraenoic acid (20-HETE) produced from arachidonic acid by CYP4F2 acts as a vasoconstrictor in renal artery.**

Wu CC *et al.*, *Cardiol. Rev.*, **22**, 1-12 (2014)

- **Sesamin, the major lignan in sesame seeds, has an antihypertensive effect, which is suggested to be due to inhibition of CYP4F2-mediated 20-HETE synthesis.**

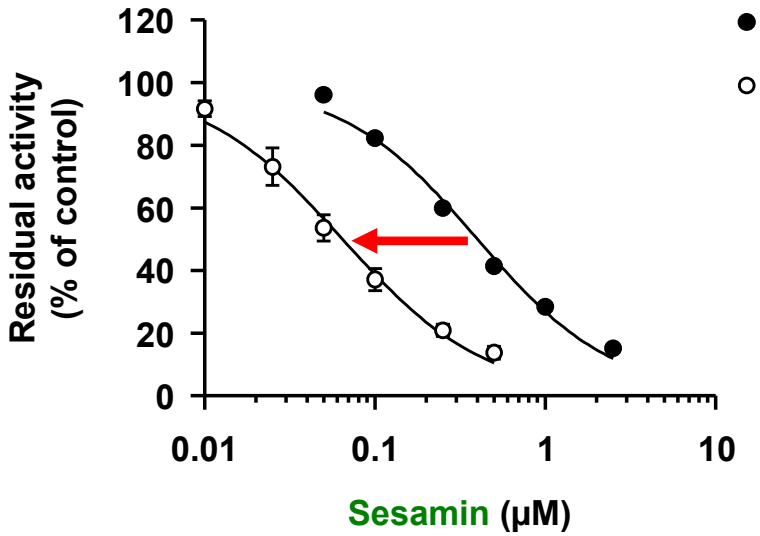
Miyawaki T *et al.*, *J. Nutr. Sci. Vitaminol.*, **55**, 87-91 (2009)
Khosravi-Boroujeni H *et al.*, *J. Sci. Food Agric.*, **97**, 3087-3094 (2017)
Wu JH *et al.*, *Hypertension*, **54**, 1151-1158 (2009)



- **The detailed mechanism underlying CYP4F2 inhibition by sesamin is unknown.**

The effect of sesamin on CYP4F2 activity was examined with recombinant CYP4F2.

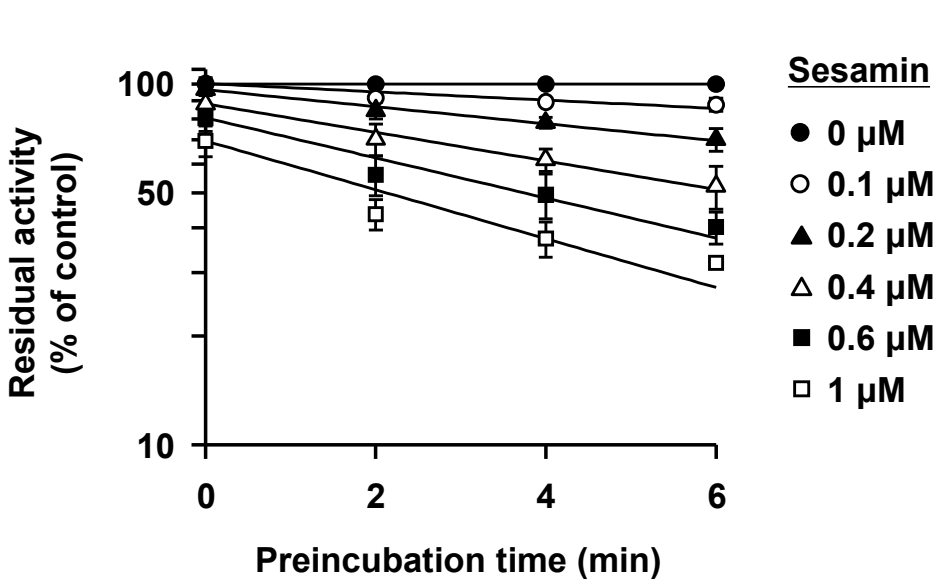
Inactivation of CYP4F2 Activity by Sesamin

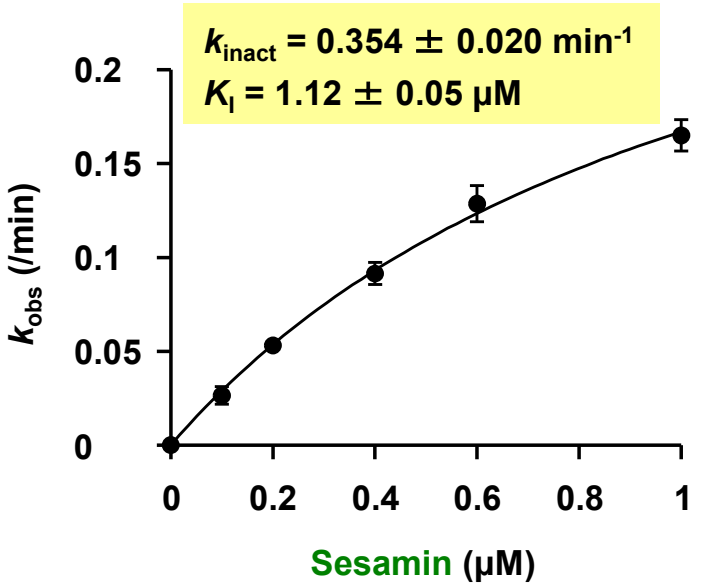


● 0-min preincubation; IC_{50} = 0.399 ± 0.013 μM

○ 20-min preincubation; IC_{50} = 0.0634 ± 0.0106 μM

Recombinant human CYP4F2 was preincubated with sesamin in the presence of NADPH for 0 min and 20 min. Incubations were conducted after the addition of Luciferin-4F2/3. Each point and bar represent mean ± S.D. of triplicate determinations.





Recombinant human CYP4F2 was preincubated with sesamin in the presence of NADPH for up to 6 min. Aliquot was removed from the preincubation

We characterized that sesamin is a mechanism-based inactivator of CYP4F2.

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