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Discovery of a Novel Selective Dual Peroxisome Proliferator-Activated Receptor α/δ Agonist for the Treatment of Primary Biliary Cirrhosis

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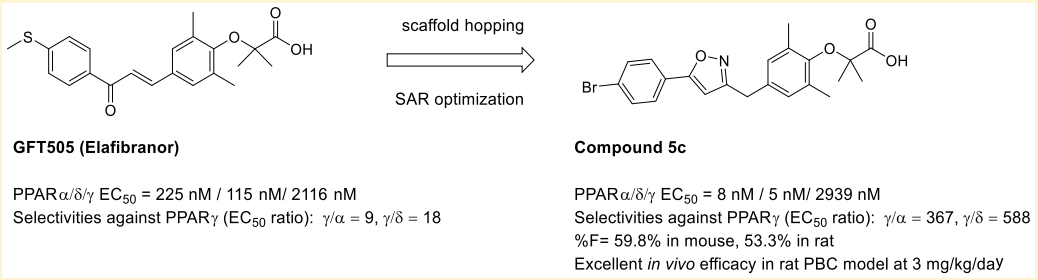
Supporting Information



ABSTRACT: A novel peroxisome proliferator-activated receptor (PPAR) α/δ dual agonist **5c** was developed with an EC_{50} of 8 nM for PPARα, 5 nM for PPARδ, and >300-fold selectivity against PPARγ (EC_{50} = 2939 nM), respectively. Further ADME and pharmacokinetic studies indicated **5c** possessed distinguished *in vitro* and *in vivo* profiles. The excellent *in vivo* efficacy of compound **5c** was demonstrated by the rat primary biliary cirrhosis (PBC) model.

KEYWORDS: *Peroxisome proliferator-activated receptor (PPAR), dual agonist, primary biliary cirrhosis (PBC)*

Primary biliary cirrhosis (PBC), known as a cholestatic liver disease, was caused by an impairment of bile production and characterized by portal inflammation and slow progressive destruction of interlobular bile ducts.[1] If left untreated, PBC leads to further hepatic damage, such as fibrosis, cirrhosis, liver failure, and hepatocellular carcinoma (HCC), which ultimately results in the requirement of liver transplantation.[2] To date, the therapeutic options approved by FDA for PBC patients are ursodeoxycholic acid (UDCA)[3−7] and obeticholic acid (OCA)

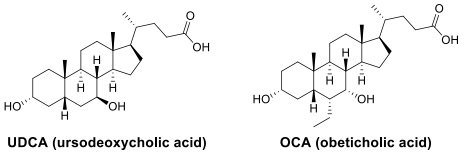


Figure 1. Ursodeoxycholic acid (UDCA) and obeticholic acid (OCA).

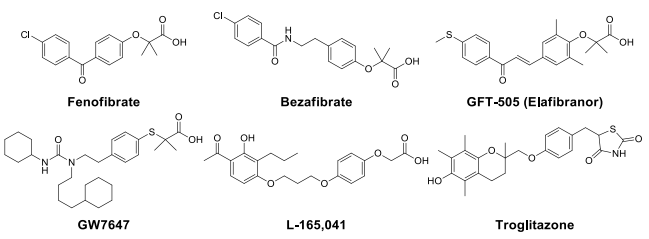


Figure 2. Selected synthetic PPARs agonists.

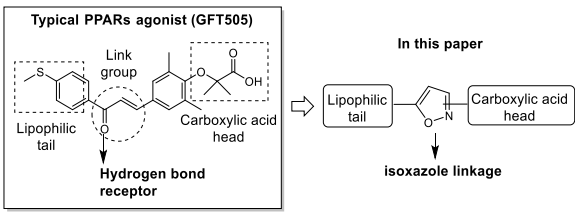


Figure 3. Selected synthetic PPARs agonists.

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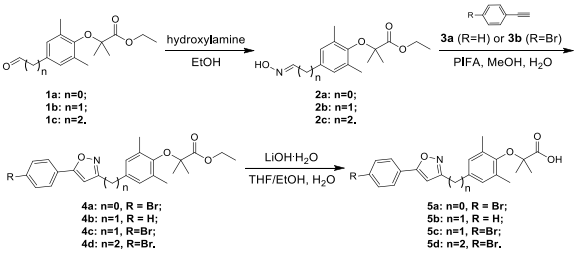
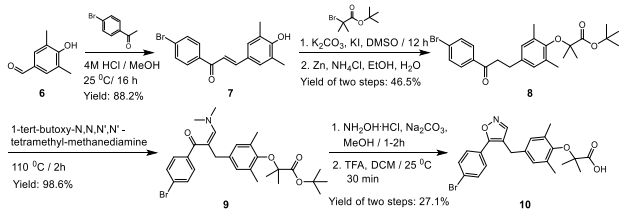
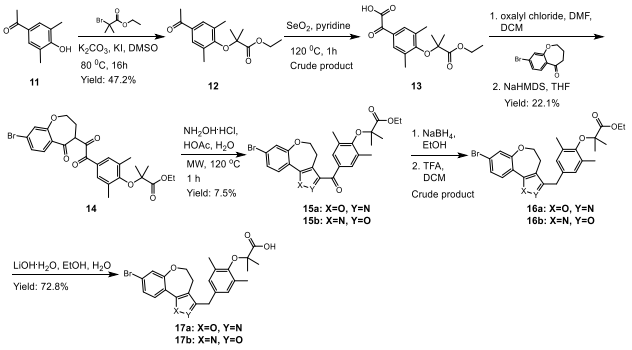
Scheme 1. General Procedure for the Synthesis of Compounds 5a−5d



Scheme 2. Synthesis of Compound 10



Scheme 3. Synthesis of Compound 17a and 17b



(Figure 1).[8−11] These treatments have been demonstrated to delay the progression of PBC; however, up to 40% of patients have an incomplete response to UDCA monotherapy.[12] Higher dosage of OCA was found to be related with the pruritus. A recent safety alert warning from FDA indicates that the excessive dosing of OCA may be associated with increased risks of liver injury and death.[13] These considerations emphasize the unmet medical need for this indication.

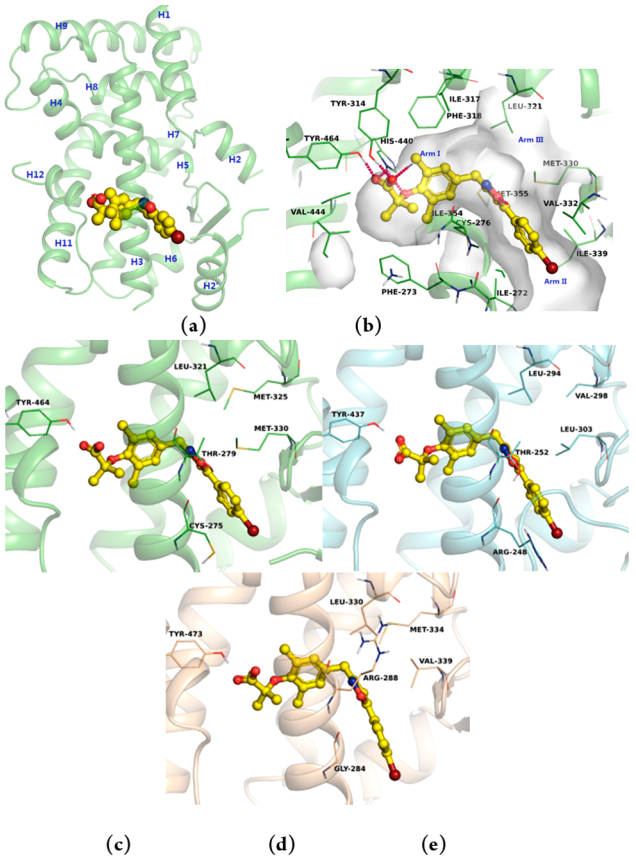


Figure 4. Predicted binding modes of **5c** in LBD of the three PPAR isoforms. (a,b) Compound **5c** (green) in the three subtypes of PPAR receptor. (c−e) Different residues in the binding pocket of PPARα (green), PPARδ (blue,) and PPARγ (yellow). The protein backbone is shown in cartoon, residues are in stick, and ligands are in stick-and-ball. The figures were generated by Pymol package (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC)

As effective targets for the therapy of cholestatic diseases, nuclear receptors are critically involved in the regulation of hepatic transporters which regulate a series of bile formation and secretion.[14] One of these nuclear receptors is peroxisome proliferator-activated receptors (PPARs). Three subtypes of PPARs, namely, PPARα, PPARδ, and PPARγ, have been known as modulators of many genes implicated in metabolic

Table 1. Evalutation of *in Vitro* Activities and Selectivities of PPARα/δ Dual Agonists

		EC_{50} (nM)[a]				
entry	compound	PPARα (% activation)[b]	PPARδ (% activation)[b]	PPARγ (% activation)[b]	γ/α (EC_{50} ratio)	γ/δ (EC_{50} ratio)
1	GW7647	5 (100%)	ND[c]	ND	/	/
2	L-165,041	ND	61 (100%)	ND	/	/
3	Troglitazone	ND	ND	215 (100%)	/	/
4	GFT505	225 (117%)	115 (72%)	2116 (93%)	9	18
5	**5a**	>10,000 (5%)	ND	ND	/	/
6	**5c**	8 (92%)	5 (79%)	2939 (110%)	367	588
7	**5b**	385 (85%)	ND	ND	/	/
8	**10**	2821 (78%)	ND	ND	/	/
9	**5d**	27 (134%)	5 (73%)	457 (78%)	17	91
10	**17a**	8974 (50%)	ND	ND	/	/
11	**17b**	3423 (85%)	ND	ND	/	/

[a]Data generated using nuclear hormone receptor (NHR) assay. [b]For PPARα, δ, and γ % activation, data were generated comparing with GW7647, L-165,041, and troglitazone, respectively. [c]ND: no data.

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Table 2. ADME Profile of Compound 5c

study	result
Cl of microsomal (human/rat) mL/min/kg	<9.6/<9.6
plasma stability (human/rat) remaining % @ 2 h	88%/96%
plasma protein binding (human/rat) %	>99.9%/ >99.9%
bidirectional permeability across Caco-2 cell monolayer (A to B/B to A/efflux ratio) 10^{-6} cm/s	0.5/0.2/0.4

Table 3. *In Vitro* Safety Profile of Compound 5c

study	IC_{50} (μM)
CYP1A2/2C9/2C19/2D6/3A4	10/20/>50/>50/>50
hERG	>30

syndrome.[15] PPARα plays a key role not only in lipid metabolism but also in bile acid synthesis and anti-inflammatory. The activation of PPARα regulates genes responsible for bile formation and transportation, such as CYP7A1 (cytochrome P450 isoform), CYP27A1, UGT1A1, SULT2A1, MDR3, and ASBT. As a result, NF-$\kappa\beta$ (nuclear factor kappaβ) could also be inhibited via PPARα activation, which leads to decreased expression of IL-1 (interleukins) and IL-6.[7] A few clinical trials explored the effect of PPARα agonists, fenofibrate, and bezafibrate (Figure 2), in patients with PBC and demonstrated positive effects.[16−22] The second subtype is ubiquitously expressed PPARδ.[23] Besides fatty acid catabolism and energy metabolism capacities, the activation of PPARδ decreases cholesterol absorption and synthesis and down-regulates cholesterol 7α-hydroxylase, which results in overt anticholestatic activity.[24] For years, synthetic PPARγ (glitazones) and dual PPARα/γ (glitazars) agonists have been developed to treat dyslipidaemia and T2DM (type 2 diabetes mellitus).[25,26] Nevertheless, several of them were discontinued because of PPARγ related weight gain, fluid retention, hemodilution, and edema effect on an increasing risk of heart failure in clinical trials.[27,28] Given the potential pharmacological benefit of activating PPARα/δ in cholestasis and PPARγ related cardiovascular safety concerns, developing a dual PPARα/δ agonist with high selectivity against PPARγ may lead to a better solution in the treatment of PBC.

Results and Discussion. GFT505 (Elafibranor) is a PPARs agonist (Figure 2) for the treatment of NASH (nonalcoholic steatohepatitis, NCT02704403, phase III) and PBC (NCT03124108, phase II). Preferential activities on PPARα and δ were exhibited for GFT505 with no PPARγ-associated adverse cardiac effects; however, it must be used at moderate to high dosage (80−120 mg/day) to achieve sufficient efficacy.[29−32] Hence, we describe herein the design and synthesis of novel PPARα/δ dual agonists to improve pharmacological activity and selectivity. The rational of drug design for PPARα/δ dual agonist is shown in Figure 3. A typical PPARs agonist comprises a carboxylic acid head, a lipophilic tail, and a linker.[33−38] In a variety of different substructures for head, linker, and tail regions, GFT505 has a remarkable chalcone group, which was assumed to be important. Using GFT505 as a lead compound, α,β-unsaturated ketone group was replaced with isoxazole linkage. The easily oxidized methylthioyl group was replaced by a more stable bromine atom, which exhibited similar lipophilicity and size. By retaining hydrogen bond interaction and molecular rigidity, and the utilization of a chemical stable substitution, we expect these modifications would improve not only the activity and selectivity of PPARα/δ but also pharmacokinetics properties.

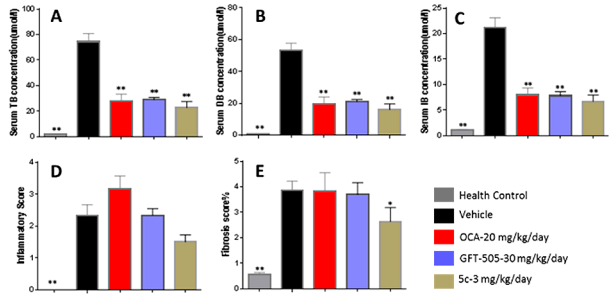

Figure 5. *In vivo* efficacy study in ANIT supplemented diet induced rat PBC model. Eight-day α-naphthylisothiocyanate supplemented diet provoked rat PBC model. Serum total bilirubin change (A), serum direct/indirect bilirubin change (B,C), inflammatory score change (D), fibrosis score change (E). Data were presented as mean ± SEM; $^{**}p < 0.01$ vs vehicle group, $^{*}p < 0.05$ vs vehicle group, analyzed by unpaired t test.

First, compounds with isoxazole linkage were designed and prepared. Reactions of benzaldehydes with hydroxylamine afforded benzaldehyde oximes **2a−2c**. After the formation of isoxazole analogues by oxidative cyclizations, compounds **5a−5d** were obtained through simple hydrolysis from ester to acid (Scheme 1).[39] To switch the fibric acid substitution position on the isoxazole ring, a different pathway was designed for the preparation of compound **10** (Scheme 2).

The synthesis of polycyclic isoxazole analogues **17a** and **17b** was depicted in Scheme 3. SeO_2-mediated oxidation transformed 4-hydroxyphenylethanone **12** to 2-oxo-2-phenylacetic acid **13**.[40,41] After the chlorination, the α-alkylation of benzoxepin-5-one proceeded, followed by hydroxylamine participated annulations. Deoxydation and subsequent hydrolysis reactions provided compounds **17a** and **17b**, ultimately.

The novel isoxazole analogues were evaluated in a nuclear hormone receptor (NHR) assay.[42] The % activation of all test compounds was compared to reference compounds that were normalized to 100%. For PPARα, δ, and γ activities, the reference compounds were GW7647, L-165,041, and troglitazone, respectively. Initially, the structure−activity relationship

Table 4. Pharmacokinetic Parameters of 5c after a Single Intravenous or Oral Dose Administration in Mice and Rats

species	route	dose (mg/kg)	V_{dss} (L/kg)	Cl (mL/min/kg)	$T_{1/2}$ (h)	$AUC_{0\text{-last}}$ (nM·h)	C_{max} (nM)	T_{max} (h)	F (%)
C57BL/6 mouse	i.v.[a]	1	0.53	3.53	3.6	10566	NA	NA	59.8
	p.o.[b]	10	NA	NA	NA	63211	52000	0.33	
SD rat	i.v.[a]	1	0.16	0.35	6.0	105180	NA	NA	53.3
	p.o.[b]	10	NA	NA	NA	561136	65267	1.33	

[a]Formulation of intravenous dosing was prepared in DMSO/10% HP-β-CD = 5:95 (v/v). [b]Oral formulation was prepared in 0.1% Tween 80/1% CMC-Na in distilled water.

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(SAR) study was set up in PPARα assay. Replacing chalcone structure with isoxazole ring led to compound **5a** with loss of *in vitro* activity, while GFT505 showed an EC_{50} of 225 nM on PPARα. However, encouraging results were observed when extended carbon chains were employed. Compounds **5c** and **5d** are both full agonists, whose potency is 8−28-fold higher than GFT505 (PPARα EC_{50} = 8 nM for **5c** and 27 nM for **5d**). With the introduction of bromine atom, the activity of **5c** was 47-fold higher than **5b** (PPARα EC_{50} = 385 nM for **5b**). Switching the position of fibric acid led to a remarkable decrease in PPARα potency (PPARα EC_{50} = 2821 nM for **10**). As a result, the cyclization of phenyl and isoxazole group, which generated polycyclic isoxazole analogues **17a** and **17b**, exhibited no effects on improving activities.

Next, we moved on to the evaluation of the selectivity of PPARα and δ against PPAR γ (Table 1). PPARα/δ dual agonist GFT505 showed good potency for both PPARα and δ (PPARα EC_{50} = 225 nM, PPARδ EC_{50} = 115 nM), and moderate to high selectivity for PPARγ/α and γ/δ (PPARγ EC_{50} = 2116 nM, γ/α = 9, γ/δ = 18). Interestingly, selected isoxazole analogues **5c** and **5d**, which showed higher PPARα activity than GFT505, indicated excellent *in vitro* PPARδ potency (PPARδ EC_{50} = 5 nM for **5c**, PPARδ EC_{50} = 5 nM for **5d**). Both of them acted as partial PPARδ agonists as GFT505 did. Compound **5c** has a EC_{50} value of PPARγ as 2939 nM, which means the higher selectivity for PPARα and δ (γ/α = 367, γ/δ = 588) over PPAR γ than that of GFT505. However, an EC_{50} = 457 nM for PPARγ was noted for **5d** when a dimethylene group was employed. Since PPARγ activity results in a series of cardiovascular safety concerns, compound **5d** was not selected for further investigation. The SAR study of the isoxazole linker group presented that compound **5c** was optimal, providing potent PPARα/δ activities and the highest level of PPARα/δ selectivity against PPARγ.

The structure of PPAR includes a DNA binding domain (DBD) and a ligand binding domain (LBD). The small-molecule agonists can bind to LBD and stabilize an active conformation form, activating transcription process of the downstream proteins.[43−46] LBD of PPARs consisted of 12 α helixes. The last helix, called AF-2 segment, can help to bind with variant cofactors and therefore is very important for receptor activation. The binding pocket of PPARα and PPAR δ is mainly in "Y" shape that can be divided into three "arms" regions. To better understand the SAR and selectivity profile, these compounds have been modeled in the three PPAR subtypes by molecular docking. PDB codes of the crystal structures used in docking are 4CI4 for PPARα, 1GWA for PPARδ, and 3VJH for PPARγ.[17] The detailed interactions of key compounds with proteins are shown in Figure 4.

It can be seen that **5c** can take a "U"-shaped conformation and occupy the arm I and arm II regions of PPARα. The carboxylic acid of **5c** binds to arm I region, forming H-bond network to Tyr314, His440, and Tyr464. The dimethyl phenyl has π−π stacking with Phe318 and His440, meanwhile interacting with hydrophobic residues in arm I subpocket. The isoxazole linker can provide appropriate geometry to place the moieties on both sides in arm I and II, respectively. When the substituent group is moved from position 5 to 4, the linking angle was changed and significant activity drop (>300 folders, **5c** versus **10**) was observed. Moreover, the isoxazole oxygen can catch an H-bond with Thr279, which is one of the important factors of selectivity against PPARγ. The bromine phenyl can bind to a highly hydrophobic pocket in arm II region. The role of bromine here is to better occupy the binding pocket and form more hydrophobic interactions with protein. Removing bromine will drop activity over 50-fold (**5c** versus **5b**). The binding insight of **5c** in PPARδ is very close to that in PPARα (Figure 4a,b).

The binding pocket of PPARγ has less similarity to PPARα and PPARδ. Different residues of the three subtypes around active site are shown in Figure 4c,d. As mentioned above, isoxazole can catch an H-bond interaction to Thr279 in PPARα and match well to the binding pocket. The residue on the same position of PPARδ is Thr252. However, there is a big and polar residue, Arg288, on this position of PPARγ. It can be seen that **5c** has good activity on both PPARα and PPARδ but has poor activity on PPARγ. When the linker is prolonged by one more methylene, the activity of PPARα is slightly dropped, but the activity on PPARγ increased over 6-fold (**5c** versus **5d**). This is because the enhanced flexibility can help take a better "U"-shaped conformation, reducing steric repulsion to Arg288.

ADME, PK Results, and Discussion. The most potent and selective compound **5c** had distinguished *in vitro* ADME and *in vivo* PK profiles. Compound **5c** was stable in human and rat microsome and plasma. However, **5c** was a highly plasma protein binding compound, with protein binding above 99.9% in both human and rat plasma. Compound **5c** showed moderate permeability based on the Caco2 assay (Table 2).

Compound **5c** indicated no significant inhibition on five human CYP isozymes. An *in vitro* cardiovascular safety evaluation also exhibited no hERG potassium channel activity at concentrations up to 30 μM (Table 3).

The pharmacokinetic profile of **5c** is presented in Table 4. Across all tested species, mouse and rat, **5c** showed low plasma clearance. The half-life of **5c** was 3.6 h in mouse and 6.0 h in rat. A remarkable high oral plasma exposure of **5c** indicated a significant absolute oral bioavailability, which was 59.8% in mouse and 53.3% in rat, respectively.

Pharmacology. Having been identified as a potent and selective dual PPARα/δ agonist with favorable PK profile, **5c** was further studied in an *in vivo* efficacy model. A rat model induced by 8-day ANIT (α-naphthylisothiocyanate) supplemented diet provoked increased TB (total bilirubin) and inflammation parameters. Severe hepatic fibrosis could also be determined in this model. To evaluate the effect on PBC, a comparative study of **5c** (3 mg/kg/day, once daily) versus OCA (20 mg/kg/day, once daily) and GFT505 (30 mg/kg/day, once daily) was performed in this experiment (six animals per group). By comparison with OCA-20 mg/kg/day and GFT505-30 mg/kg/day, compound **5c** indicated obvious TB (including DB-direct bilirubin and IB-indirect bilirubin) lowering effect at only 3 mg/kg/day (Figure 5A−C). Furthermore, **5c** lowered inflammatory score by 30% (from 2.3 to 1.6) and reduced fibrosis area percentage by 28% (3.9% to 2.8%). No obvious change was observed for inflammatory score and fibrosis when GFT505-30 mg/kg/day and OCA-20 mg/kg/day were utilized. This was probably due to the lower dosage of OCA in the study and poorer *in vitro* potency of GFT505, respectively. In the meantime, the FXR potency of **5c** was checked in a FXR binding assay to exclude the combinational efficacy of FXR agonism in the PBC model. Its IC_{50} for FXR of **5c** was more than 50 μM, and the selectivity against PPARα and δ is over 6000.

Conclusion. In summary, we described the development of novel PPARα/δ dual agonists. Compound **5c** was found to be

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the most potent and highly selective PPARα/δ agonist (PPAR $\alpha/\delta/\gamma$, EC_{50} = 8/5/2939 nM, EC_{50} γ/α ratio = 367, EC_{50} γ/δ ratio = 588, FXR/PPAR > 6000). With good ADME profile, **5c** exhibited excellent *in vivo* efficacy in a rat PBC model. On the basis of pharmacological effects described above, compound **5c** was identified as a promising lead compound for further optimization.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.9b00189.

Synthetic procedures, analytical data, and assay protocol (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

PK, pharmacokinetics; PO, per os; C_{max}, peak concentration; T_{max}, time to peak; CL, clearance; $T_{1/2}$, half-life; V_d, volume of distribution; AUC, area under curve; mpk, mg/kg; %*F*, oral bioavailability

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