Discovery of Dotinurad (FYU-981), a New Phenol Derivative with Highly Potent Uric Acid Lowering Activity

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ABSTRACT: To derive new uricosuric agents, novel phenol derivatives were synthesized to overcome the disadvantages of benzbromarone (BBR), attributed by its structural features. Herein, we report the discovery of new phenol derivatives with a 1,1-dioxo-1,2-dihydro-3H-1,3-benzothiazole scaffold. The selected compound **11** (dotinurad, FYU-981) demonstrated remarkable inhibitory activity on uric acid uptake by primary human renal proximal tubule epithelial cells (RPTECs) and URAT1-mediated uric acid transport, with weak inhibitory activity against mitochondrial respiration. Dotinurad also displayed favorable pharmacokinetic profiles and higher potency in decreasing uric acid than BBR did in *Cebus* monkeys. Dotinurad has been approved as a new uricosuric medicine in Japan. Our strategy, which focuses on the structural features resulting in unfavorable effects, could be applied to the future developments of other drugs with disadvantages, particularly those having a bis-aryl ketone structure.

KEYWORDS: *Uricosurics, mitochondrial toxicity, benzbromarone, primary human renal proximal tubule epithelial cells, dotinurad*

Hyperuricemia, a causative factor of gout, is one of the most studied diseases globally.¹ Hyperuricemia is involved in diseases such as metabolic disorders, cardiovascular disease, and renal dysfunctions.^{2,3} There are two significant causes of hyperuricemia, which are overproduction of uric acid and a reduction in its renal elimination or both. Hence, medicines used against hyperuricemia are classified into two types based on their mechanisms of action: xanthine oxidoreductase inhibitors (XOIs) and uricosuric agents. Allopurinol, a representative XOIs that decreases the blood level of uric acid by inhibiting uric acid synthesis from xanthine, has been used worldwide as the standard medicine for a long time. Recently, new XOIs, febuxostat and topiroxostat, were launched to provide treatment options for gout and hyperuricemia.⁴

Among uricosurics that increase urinary excretion of uric acid, probenecid and benzbromarone (BBR) have been used for a long time in some countries, and recently, a new uricosuric agent, lesinurad, was approved in the US and EU. These agents act by inhibiting urate reabsorption in the renal tubules.^{5,6} However, these medicines also possess disadvantages. Probenecid, which has also been used to maintain the blood concentration of other drugs such as penicillin, might cause drug–drug interactions due to nonselective inhibition of anion transporters.⁷ Because BBR has a highly potent hypouricemic effect in humans, it is used as one of the

standard medicines in some countries such as Japan, Europe, and Brazil, especially when the effect of XOIs is insufficient. However, following reports that fulminant hepatitis, a rare but life-threatening disorder, was associated with BBR administration, restriction of its use via warnings and withdrawal occurred in Japan and Europe, respectively.

Furthermore, BBR is an inhibitor of cytochrome P450 2C9 (CYP2C9), known as a polymorphic enzyme, and is metabolized by the enzyme. Metabolism by CYP2C9 may lead to drug–drug interaction and account for differences in metabolism among individuals.^{8,9} Lesinurad is only used in combination with XOIs and an inhibitor of CYP2C9 similar to BBR.^{10–12}

Many studies have been carried out to investigate the mechanisms of BBR-induced hepatic toxicity^{13–19} including the involvement of metabolism, metabolites, and mitochondrial toxicity. Among these potential mechanisms, mitochondrial inhibitory activity (MIA) is one of the most promising theories for fulminant hepatitis.^{15,16} Based on these findings, to

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obtain compounds that possess potent pharmacological effects without mitochondrial toxicity, we modified BBR to overcome its disadvantages, which may be related to a structural flaw.

The association between the structure and mitochondrial toxicity of BBR and its analogs including amiodarone has been previously reported (Figure 1).^{17–19}

Figure 1. Ranking of benzbromarone (BBR) and its derivatives based on mitochondrial toxicity and structures of tolcapone and fenofibrate.

These studies reported that each of the structural parts (substituted benzoic acid and benzofuran) had no or minimal inhibitory activity on mitochondrial respiration. In contrast, substituted benzoyl-benzofuran analogs such as amiodarone, benzarone, and BBR (Figure 1), which are combined with these two units, had distinct inhibitory activities, and the bromo substitutions on the phenol ring enhance it. Because these derivatives have a bis-aryl ketone moiety known to be a radical stabilizing structure that is associated with some adverse effects,²⁰ we assume the moiety affects the mitochondrial electron transfer system. Some medicines with the bis-aryl ketone such as tolcapone and fenofibrate (Figure 1) inhibit the mitochondrial function^{21,22} and are associated with the occurrence of hepatic toxicity. Therefore, this structure appears to have a risk of exerting mitochondrial toxicity. Based on the knowledge and the hypothesis stated above, we derived two modification strategies to avoid mitochondrial toxicity. The first is a conversion from bis-aryl ketone structures to aryl-benzamide structures, where the introduction of a nitrogen atom leads to a nonketone structure. At the same time, the other is the transformation of a bromine atom into other electron-withdrawing substituents. We also considered the structural features of BBR required to show urate uptake inhibition (UI).

Because BBR has a plane structure and moderate acidity with a pK_a value of 5.17, similar to that of uric acid (5.75), we assumed that BBR mimics uric acid. Therefore, electron-withdrawing groups such as bromide on phenol might play important roles in maintaining acidity, thereby allowing compounds to exert urate uptake inhibitory potency.

We prepared the new compounds, amide-linked indole and indoline derivatives with cyano-substituent as an electron-withdrawing group instead of bromine atoms on the phenol moiety, as alternatives to the acyl benzofuran structure (compounds 1 and 2a in Table 1).

To verify our strategy, these compounds were evaluated for their urate uptake inhibitory potency and mitochondrial toxicity. We established a novel method to evaluate the UI of these compounds using primary human renal proximal tubule epithelial cells (RPTECs) (Method: Supporting Information). RPTECs play important roles in urate excretion and reabsorption. In fact, they can express many transporters including urate transporter 1 (URAT1), organic anion transporter 1 (OAT1), OAT3, multidrug resistance protein 4

Table 1. UI and MIA of BBR, 1, and 2a

Compound	UI ^a IC ₅₀ (μM)	MIA ^b IC ₅₀ (μM) or %
BBR	6.8	3.1 μM
1	10.8	3.6 μM
2a	2.5	43%

^aUI: Inhibitory activity of urate uptake into RPTECs. ^bMIA: Mitochondrial respiratory control ratio (RCR), IC₅₀ (μM), or inhibition (%) at 100 μM.

(MRP4), ATP-binding cassette subfamily G member 2 (ABCG2), and glucose transporter 9 (GLUT9), which are involved in the transport of urate.^{23–25}

In our experimental condition, BBR, known as a selective URAT1 inhibitor,²⁶ inhibited uric acid uptake into RPTECs with an IC₅₀ of single-digit micromolar and in a concentration-dependent manner. Hence, we believed that the urate uptake into RPTECs is mainly due to URAT1. This method will be reported in detail elsewhere. The mitochondrial respiratory control ratio (RCR) was used as an index of MIA. Both UI (inhibitory activity of urate uptake into RPTECs, IC₅₀) and MIA (RCR, IC₅₀ (μM), or inhibition (%) at 100 μM) assays were performed for these compounds (Table 1).

The data indicated that the UI potency levels of both indole 1 and indoline derivative 2a are the same as that of BBR. Although structural conversion to indole maintained high MIA, conversion to indoline lowered it. Hence, the aromatic indole ring might confer the same character as that of BBR with a bis-aryl ketone structure, while the indoline derivative alters the character owing to the bis-aryl ketone structure. These results suggested that modifying the bis-aryl ketone moiety is critical to avoiding mitochondrial toxicity. Because 2a was considered a seed compound, we prepared 2a-derivatives by adding another substituent on the phenol ring to investigate the compounds' properties.

The synthetic procedures of 1 and 2 are shown in Scheme 1. The synthetic methods for substituted benzoic acid as

Scheme 1. Synthesis of Indole and Indoline Analogs^a

^aReagents and conditions: (a) EDC, HOBT in DMF, CHCl₃; (b) R₂ = MOM; HCl, 1,4-dioxane, THF or TFA, CH₂Cl₂, R₂ = Me; LiCl, DMF, 35–91% over two steps.

intermediates have been reported earlier,²⁷ and we have described some new benzoic acids in the [Supporting Information](#). Acylation of indoline and 2,3-dimethylindole was accomplished with substituted or nonsubstituted 4-methoxy or 4-methoxymethoxy-3-cyanobenzoic acid and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC). The subsequent deprotection of phenol –OH yielded the desired analogs.

The assay data of UUI and MIA, and the calculated LogP of these derivatives are shown in [Table 2](#).

Table 2. UUI and MIA of Compounds 1 and 2 in [Scheme 1](#)

Compound	R ₁	UUI ^a		MIA ^b IC ₅₀ (μM) or %	LogP ^c
		IC ₅₀ (μM)	%		
1		10.8	52.1	3.6	3.89
2a	H	2.5	67.5	43%	2.70
2b	OMe	>100	21.7	–10%	2.57
2c	I	5.3	65.1	53 μM	4.05
2d	CN	83.9	26.3	47%	2.73
2e	SMe	>100	24.2	40 μM	3.14
2f	<i>tert</i> -Butyl	31.6	32.4	6.6 μM	4.4
2g	Cyclopropyl	8.8	67.8	24 μM	3.43
2h	Cl	14.7	38.2	31 μM	3.26
2i	CF ₃	>100	30.0	40%	3.62

^aUUI: Inhibitory activity of urate uptake into RPTECs, IC₅₀ and % inhibition at 10 μM. ^bMIA values are defined in [Table 1](#). ^cLogP was calculated using ChemBioDraw UltraVer.14.

UUI potency seemed to be affected by substituent characters such as steric and electric properties including hydrophobicity. Although 2a, 2c, and 2g showed a relatively high activity, the bulky substituent (2f) had a lower potency compared to these three compounds presumably resulting from the disruption of the flatness of the molecules shared by uric acid. The electron donating (2b) and withdrawing (2d, 2i) groups affected pK_a and their potencies. Furthermore, hydrophobicity seems to influence UUI potency; thus, UUI improved with increasing LogP values, except for one-substituted (2a) and bulky (2f) types.

As shown in [Figure 2](#), a correlation was observed between MIA and LogP among the compounds in [Table 2](#), in which MIA augmented with increasing LogP values. Although almost

Figure 2. Correlation between the MIA (RCR, IC₅₀ μM) and compounds' LogP in [Table 2](#). Compounds 2a, 2d, and 2i showed ca. 50% MIA at 100 μM; these IC₅₀ values are assigned as 100 μM. Compound 2b showed no MIA at 100 μM; the IC₅₀ value is assigned as 200 μM.

all compounds were close to the regression line ($R^2 = 0.54$), compound 1 (indole derivative) showed an inferior character with stronger MIA than the other compounds. In contrast, 2c and 2i had weaker MIA for their LogP than the other compounds did.

Although causes for the association between hydrophobicity and MIA are unclear, we speculate that it was dependent on membrane permeability and affinities to target proteins.

The association between UUI at 10 μM and MIA, which should ideally diverge from each other, is shown in [Figure 3](#).

Figure 3. Association between UUI (% inhibition at 10 μM) and MIA (RCR, IC₅₀ μM) derived from [Table 2](#) and [Figure 2](#).

Although compounds with higher UUI tended to show higher MIA, 2a, 2c, and 2g showed promising *in vitro* character: high UUI and low MIA. Similar association was observed when UUI was evaluated at higher concentrations (30 or 100 μM). Therefore, the C_{max} and urinary excretion rate (Exc.) of 2a, 2c, and 2g were then investigated as the pharmacokinetic (PK) screening compared to BBR ([Table 3](#)).

Table 3. Pharmacokinetic Profiles of 2a, 2c, 2g, and BBR

Compound	R ₁	C _{max} ^a (μg/mL)	Exc. ^b (%)
2a	H	0.89	5.9
2c	I	5.95	0.02
2g	Cyclopropyl	1.36	0.35
BBR		4.42	0

^aMaximum concentration (C_{max}). ^bExcretion rate in urine (%; 0–4 h) after oral administration of 3 mg/kg to rats.

To produce a pharmacological effect, special attention was given to the Exc. (%) of compounds based on the PK profiles of BBR in humans reported by some groups.^{28–33} Although BBR is typically eliminated from plasma at 48 h after administration and is excreted little in urine, a main metabolite, 6-OH-BBR,^{28,29} remains in the plasma and is excreted (1.2% of the dose) over 72 h in humans;^{30–32} this metabolites' URAT1 inhibitory activity was only several times lower than that of BBR (6-OH-BBR IC₅₀: 0.20 μM, BBR IC₅₀: 0.0345 μM).^{31,32} As a long lasting effect of BBR in humans³³ is consistent with the sustained urinary excretion of 6-OH-BBR, and URAT1 is present on the luminal side of epithelial cells of the renal tubule, it is suggested that 6-OH-BBR in urine contributes to the inhibition of uric acid reabsorption.^{32,33} Moreover, unlike BBR, the compounds in this study should not form active metabolites to avoid metabolic conversion by enzymes such as CYP2C9. Therefore, we considered that the excretion of unchanged drug with activity equivalent to that of BBR in

urine is not only important to show activity, but also for the ideal property. Hence, to obtain candidate compounds, PK screening was performed with a target value of >1% of unchanged drug in 0–4 h urine in rat, based on the above 6-OH-BBR data.

Of the three compounds, only **2c** showed a high $C_{\max}$, while its Exc. was low (0.02%). We concluded that the metabolic susceptibility of the core indoline structure with benzyl-CH₂ was one of the causes of the inferior PK profiles. Therefore, we then investigated another 1,1-dioxo-1,2-dihydro-3H-1,3-benzothiazole derivative with –SO₂– instead of benzyl-CH₂– in the core that would be of metabolic resistance. Compound **7** of the new core structure with cyclopropyl substituent was prepared and compared with the indoline-type analog **2g** and BBR regarding their UII, MIA, PK, and CYP2C9 inhibitory assays (Table 4).

Table 4. Properties of Compound **7**

Compound	UII ^a IC ₅₀ (μM)	MIA ^b IC ₅₀ (μM)	$C_{\max}$ ^c (μg/mL)	Exc. ^d (%)	CYP2C9 IC ₅₀ (μM)
2g	8.8	24	1.36	0.35	
7	19.2	59	3.81	0.6	57
BBR	6.8	3.1	4.42	0	0.041

^aUII and ^bMIA are defined in Table 1. ^c $C_{\max}$ and ^dExc. are defined in Table 3.

Compared to **2g**, compound **7** showed satisfactory properties, with adequate UII, weak MIA, and improved $C_{\max}$; furthermore, **7** had a higher Exc. than **2g** did and appeared to have low CYP2C9 inhibitory activity (IC₅₀ = 57 μM). Because

7 was considered a lead compound, we prepared 1,2-dihydro-3H-1,3-benzothiazole derivatives by converting the phenol substituents (Table 5). Although the synthetic method for these derivatives was previously described,²⁷ additional information is added in the Supporting Information.

Many compounds showed potent UII with single-digit μM (IC₅₀) activity, whereas **8** and **10**, having two electron-withdrawing substituents, had low UII (Table 5). However, compared to **8** (core; –SO₂–), **9** (core; –S–) maintained its UII potency as the substituent effect may be partially due to the hydrophobicity of compounds. Among compounds with high potency, **11**, **14**, and **15** showed Exc. > 1%, and **11** had the highest $C_{\max}$.

The URAT1 assay was performed for some compounds to confirm the mechanism of action (Table 6). These compounds

Table 6. Urate Transporter 1 Inhibition and UII of 1,2-Dihydro-3H-1,3-benzothiazole Derivatives and BBR

Compound	URAT1 inhibition ^a (%)	UII ^b (%)
7	30	42
9	71	49
10	29	25
11	78	81
13	36	32
15	44	57
BBR	37	51

^aURAT1 inhibitory activity; % inhibition at 0.1 μM. ^bUII are defined in Table 1, % inhibition at 10 μM.

showed URAT1 inhibitory activity (0.1 μM) along with UII in RPTECs (10 μM). A positive correlation was observed between the two assays as shown in the analysis of the 7 compounds in Table 6 (R^2 = 0.59; Figure 4) and, furthermore, in a dose-dependency test of **11** (R^2 = 0.87; Figure 4-1 in the Supporting Information). Thus, urate uptake into RPTECs seems to be mainly mediated by URAT1, as mentioned above.

Table 5. UII, MIA, and Pharmacokinetic Profiles of 1,2-Dihydro-3H-1,3-benzothiazole Derivatives

*Compounds **9** (n = 0), **8**, **10**–**20** (n = 2)

Compound	R ₁	R ₂	UII ^a IC ₅₀ (μM)	MIA ^b IC ₅₀ (μM) or %	$C_{\max}$ ^c (μg/mL)	Exc. ^d (%)
8	CN	CF ₃	>100	12%		
9 *	CN	CF ₃	13.1	55 μM	3.15	0.1
10	CN	Cl	>100	20%	1.35	23
11	Cl	Cl	6.8	27 μM	9.14	1.1
12	CN	iPr	83.3	12 μM	4.46	0.1
13	CN	Et	>100	37 μM	4.24	1.7
14	CN	ethynyl	3.2	13%	1.58	12.4
15	CN	SMe	7.1	21%	1.08	9.4
16	CF ₃	Cl	2.6	18 μM	6.41	0.1
17	F	Cl	33.9	63 μM	7.70	4.3
18	Cl	SMe	5.7	59 μM	4.64	0.2
19	CF ₃	OMe	17.8	47%	3.99	0
20	Cl	OMe	4.3	49%	1.06	0.1

^aUII and ^bMIA are defined in Table 1. ^c $C_{\max}$ and ^dExc. are defined in Table 3.

Figure 4. Correlation between the RPTEC and URAT1 assays among the compounds in Table 6.

The dichloro-substituted derivative **11** appeared to possess highly potent UUI and URAT1 inhibitory activities at least equivalent to that of BBR, low MIA, and high $C_{\max}$ with moderate urinary excretion rate ($>1\%$, 0–4 h). Although **11** showed weak CYP2C9 inhibitory activity ($IC_{50} = 5.7 \mu M$), its activity was thought to be sufficiently separated from uricosuric activity based on its PK profile. As **11** (dotinurad) had a favorable balance of these parameters, it was selected for further development. The results of the additional *in vitro* and *in vivo* assay using rats and *Cebus* monkeys are shown in Table 7 and Figure 5.

A detailed URAT1 assay of **11** showed an IC_{50} of 37.2 nM, indicating slightly higher potency than that of BBR (original data, $IC_{50} = 190$ nM), similar to the equipotent inhibition in the RPTEC assay. Compound **11** had excellent PK profiles in both animals. In *Cebus* monkeys, which could be an effective

Figure 5. Changes in plasma urate level compared with the predosing value in *Cebus* monkeys. **11** (dotinurad); 5 and 30 mg/kg, BBR; 30 mg/kg p.o. Values are the means $\pm$ SD of five animals. The average predosing value of control: 2.98 mg/dL. * $P < 0.05$, ** $P < 0.01$, significantly different from control at each time point by Dunnett's test.

model for extrapolating the uricosuric effect in humans, 5 mg/kg of **11** showed a potent uricosuric effect with the fractional excretion of urate at 0–4 h (FE_{UA} ; 16.5% vs 11.5% of 30 mg/kg of BBR). Moreover, **11** decreased the plasma urate level more potently than did BBR in *Cebus* monkeys. This potent effect has not been reported with conventional medicines.

Because compound **11** only weakly inhibited ABCG2 and OAT1/3, it was shown to be a selective urate reabsorption inhibitor (SURI), which was defined as a potent URAT1 inhibitor with minimal effect on urate secretion transporters.³⁴

The major metabolites of **11** in humans are glucuronide and sulfate of phenols, with excretion rates in urine during 0–72 h of 44.3% and 20.0%, respectively, in a mass balance test.³⁵ The formation of reactive metabolites with potential to cause hepatotoxicity, as reported for BBR,¹³ was not observed. These results suggested that **11** had a safe metabolic pathway.

In conclusion, this series of studies led to the discovery of compound **11**. We proceeded to clinical trials with **11** and obtained excellent results. The registration of **11** as a new medicine (dotinurad: FYU-981) has been approved in Japan.

We obtained new medicine by focusing on the negative aspects of existing drugs. The method used to avoid chemical structures that may cause unfavorable effects could be applied to other drugs with disadvantages.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmchemlett.0c00176>.

Details on the preparation of compounds **1** and **2a–i** (including synthetic procedures), *in vitro* assays, and PK assays. Although the preparation methods for compounds **7–20** were described previously in a patent,²⁷ we have included some additional chemical data. (PDF)

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Table 7. Properties of **11** and BBR

11 (dotinurad)

Rat	$C_{\max}$ or C_0^a (ng/mL)	$AUC_{0-\infty}^b$ (ng·h/mL)	BA. ^c
11	1085.8 ± 100.4	5744.7 ± 188.8	86.8%
0.3 mg/kg p.o.			
11	2401.3 ± 65.6	6619.5 ± 2041.8	
0.3 mg/kg i.v.			
<i>Cebus</i> monkeys	AUC_{0-24h}^a (μg·h/mL)	FE_{UA}^d (%)	Difference of ΔP_{UA}^e from control (mg/dL)
11	108.55 ± 52.94	16.5 ± 4.2	0.97
5 mg/kg p.o.			
BBR	95.24 ± 28.12	11.5 ± 7.9	0.46
30 mg/kg p.o.			
<i>In vitro</i> evaluation		URAT1 inhibition IC_{50} (nM)	
11		37.2	
BBR		190	

^aMaximum concentration ($C_{\max}$ or C_0). ^bArea under the blood concentration–time curve (AUC); inf: infinity. ^cBA: Bioavailability. ^d FE_{UA} : Fractional excretion of urate at 0–4 h; FE_{UA} value of control: $8.9 \pm 4.0\%$. ^e ΔP_{UA} : Changes in plasma urate level at 0–8 h; value of control: 1.65 ± 0.78 mg/dL.

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ABBREVIATIONS

DMEF, *N,N*-dimethylformamide; MOM, methoxymethyl; EDC, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride; m-CPBA, *m*-chloroperbenzoic acid; TFA, trifluoroacetic acid; CYP2C9, cytochrome P450 2C9; URAT1, urate transporter 1; ABCG2, ATP-binding cassette subfamily G member 2; OAT1/3, organic anion transporter 1/3; MRP4, multidrug resistance protein 4; GLUT9, glucose transporter 9

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