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Synthesis, Further Biological Evaluation and Pharmacodynamics of Newly Discovered Inhibitors of TNF-α Production

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Abstract—(1*S*,2*R*)-2-Acylamino-1-methyl-2-phenylethyl phosphate derivatives **2a**, **2b**, **3a**, and **5a**, which are conformationally restricted and metabolically stable analogues of (2*R*)-2-acylamino-2-phenylethyl phosphate derivatives **1a** and **1b**, are a new class of inhibitors of TNF-α production. More efficient alternative synthesis of a key intermediate, (1*R*,2*S*)-1-amino-1-(3-methoxyphenyl)propan-2-ol hydrochloride (**9**), was achieved using one-step, three-component coupling of 3-methoxyphenyl boronic acid (**13**), (5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-ol (**14**), and amino diphenyl methane (**15**), [as reported in *J. Am. Chem. Soc.* **1998**, *120*, 11798]. Evaluation of the hypotensive activity of these compounds was done to assess one of their side effects. Among the compounds tested, the above-mentioned four compounds (**2a**, **2b**, **3a**, and **5a**) were identified as inhibitors with both sufficient potency and an acceptable safety margin regarding their hypotensive activity. The pharmacodynamics of these compounds were also investigated. Single-dose pharmacokinetic data for compounds **2a**, **2b**, **3a**, and **5a** are displayed. These compounds were estimated to be mainly metabolized by the liver in the species tested based on their in vitro stability in tissue homogenates and plasma. A representative compound, **2a**, showed good linearity of its plasma concentration after intravenous injection.

Introduction

TNF-α plays a pivotal role in the onset and progression of many inflammatory and autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, sepsis, ulcerative colitis, congestive heart failure, inflammatory bowel disease, and Crohn's disease.[1a–d]

In previous papers,[2a–e] we reported on the discovery of highly potent inhibitors of TNF-α production. In one such report, we described the successful design and synthesis of metabolically stable inhibitors, which exhibited much greater potency than their corresponding chemical leads in an animal model, and the results of their biological evaluation (Table 1).

In order to obtain a more detailed biological profile, the hypotensive activity and pharmacodynamics of these compounds were further investigated. In addition, the recently reported method of Petasis for stereocontrolled one-step synthesis of optically active amino alcohols[3] was studied for its application to the more efficient synthesis of an optically active key intermediate of these inhibitors. Here, we report on this more efficient alternative method of synthesis, as well as further biological evaluation and the pharmacodynamics of these newly discovered inhibitors of TNF-α production.

Chemistry

The inhibitors all contain an optically active β-amino alcohol as a common subunit, stereocontrolled synthesis of which continues to be the focus of numerous studies due to its versatile functionality. In one of our previous papers,[2d] we reported an 11-step method for the synthesis of **2a** and **b** as outlined in Scheme 1, which includes

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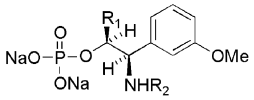
Table 1. New inhibitors of TNF-α production

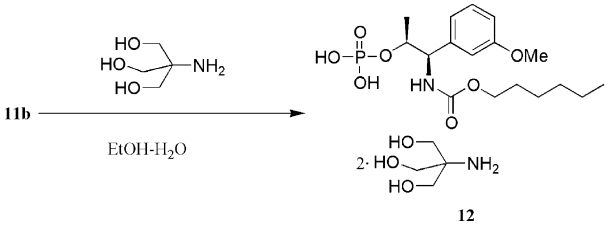


Compd	R_1	R_2	Inhibition of TNF-α production in rats
			ID_{50} (mg/kg, iv)
1a	R_1 = H	R_2 = CO–*n*-C_7H_{15}	0.26
1b	R_1 = H	R_2 = COO–*n*-C_6H_{13}	3.2
2a	R_1 = Me	R_2 = CO–*n*-C_7H_{15}	0.03
2b	R_1 = Me	R_2 = COO–*n*-C_6H_{13}	0.02

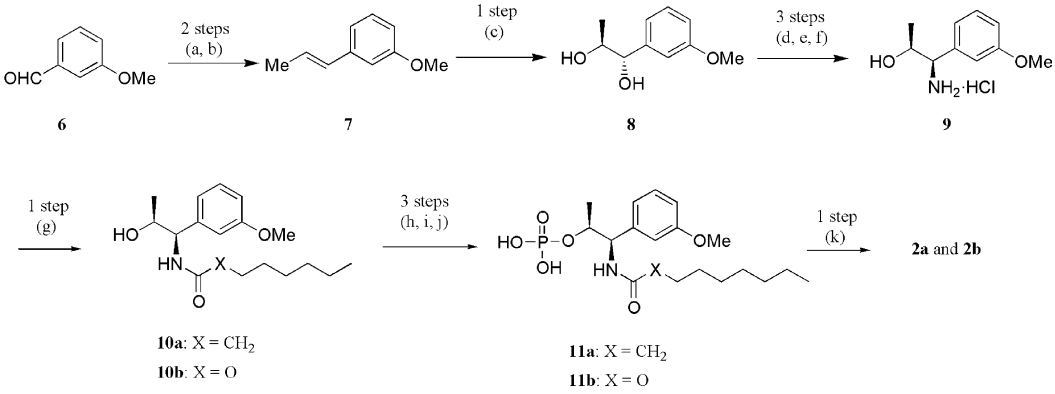
six-step synthesis of the optically active key intermediate **9** starting from *meta*-methoxy benzaldehyde **6**. A more efficient alternative method for the synthesis of **9** involves the one-step, three-component reaction of an organoboronic acid **13**, 4-hydroxy-1,3-dioxolanes **14**, and diphenylmethyl amine **15**, as reported by Petasis et al.[3]

As outlined in Scheme 2, three-component coupling reaction of 3-methoxyphenyl boronic acid **13**, 4-hydroxy-1,3-dioxolane **14**, and amino diphenyl methane **15** was successfully carried out to directly produce the desired protected β-amino alcohol **16**. An oily form of the product **16**, which could be used for the next reaction without further purification, was obtained at a good yield, but there was contamination by an undesired diastereoisomer **17**[4] and by 1-(benzhydrylamino)acetone **18** (**16**:**18** = 11:1).[4] Removal of the diphenyl methyl moiety of **16** with catalytic hydrogenation afforded the amino alcohol hydrochloride salt **9** as a solid, after which selective *N*-acylation with *n*-octanoyl chloride and *n*-hexyloxycarbonyl chloride resulted in **10a** and **10b**, respectively, both of which contained other by-products (**10c** and **10d**, respectively, derived from an undesired diastereoisomer **17**).[4] These by-products[4] were subsequently removed by recrystallization to give pure **10a** and **10b** as white crystals at a high yield. According to reported procedures,[2d] **10a** and **10b** were able to be converted to **2a** and **2b**, respectively.

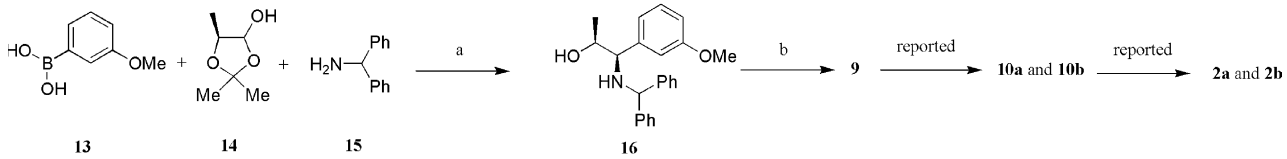
For development of an intravenously injectable candidate drug for clinical trials, it is important to obtain a pure compound that is soluble in saline. Among the processes tested, recrystallization of these inhibitors as a bis-[tris(hydroxymethyl)aminomethane] salt **12** (Scheme 3) was the most successful method. A typical example is illustrated in Scheme 3. The free form of **11b**, which was obtained by deprotection of the corresponding dibenzyl ester, was treated with two equivalents of tris(hydroxymethyl)aminomethane in aqueous ethanol to give **12** as a white crystal at a good yield.[2d] The solubility of the bis-amine salt **12** in saline was excellent up to 10 mg/mL.



Scheme 3. Synthesis of tris amine salt **12**.



Scheme 1. Reported synthesis of compounds **2a** and **2b**. Reagents and conditions: (a) EtMgBr, THF; (b) (i) Ph_3P, imidazole, I_2, CH_3CN; (ii) DBU, toluene; (c) AD-mix-α, $CH_3SO_2NH_2$, *t*-BuOH–H_2O; (d) $(CH_3O)_2CO$, NaOH; (e) NaN_3, DMF; (f) H_2, 10% Pd/C, 6 M HCl, MeOH; (g) *n*-$C_7H_{15}COCl$ or *n*-$C_6H_{13}OCOCl$, $NaHCO_3$, THF–H_2O; (h) *i*-$Pr_2NP(OBn)_2$, tetrazole, CH_3CN; (i) *m*-CPBA, CH_2Cl_2; (j) H_2, 10% Pd/C, MeOH; (k) NaOHaq, EtOH.



Scheme 2. Alternative synthesis of compounds **2a** and **2b**. Reagents and conditions: (a) CH_2Cl_2, rt, 2 days, 70%; (b) H_2, 10% Pd/C, 1 M HCl, MeOH.

Results and Discussion

Hypotensive activity

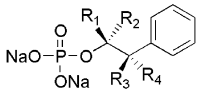
In prior studies,[2a–e] we assessed the structure–activity relationships (SARs) of these new inhibitors with regard to inhibition of TNF-α production and the minimum lethal dosage (MLD). During these studies, the inhibitors were found to exhibit considerable hypotensive activity. For example, the chemical lead **1c** (Table 2) reduced the blood pressure by 15 mmHg at a dose of 3–10 mg/kg, which was close to its effective dose (ID_{50} = 3.0 mg/kg). From the viewpoint of safety, reduction of the hypotensive activity of these inhibitors without decreasing their efficacy was another obstacle to be overcome. However, the relationship between inhibition of TNF-α production and their hypotensive activity was unclear. To assess these SARs, the hypotensive activity of the four optically active diastereoisomers **2d–g** was investigated based on their ability to reduce blood pressure by 15 mmHg (ED_{15mmHg}) because the diastereomeric mixture **2c** exhibited a less potent hypotensive effect than the chemical lead **1c** with little difference of their ID_{50} values (ID_{50}: **1c** = 3.0 mg/kg; **2c** = 5.6 mg/kg).

As shown in Table 2, two of the inactive compounds (**2e** and **2f**) exhibited a more potent hypotensive effect than the active compound **2d**, while another inactive compound **2g** showed a less potent hypotensive effect than **2d**. Thus, more potent inhibition of TNF-α production was not always accompanied by more potent hypotensive activity. This prompted us to assess the SARs of the more optimized inhibitors (**2a–b**, **3a–b**, **4a–b**, and **5a–b**) based on their hypotensive activity (Table 3). As showed in Table 3, most of these compounds (**2a**, **2b**, **3a**, **5a**, and **5b**) had less than one-tenth of the hypotensive effect, while the other compounds (**3b**, **4a**, and **4b**) exhibited less improvement of their safety profile. Alkoxy moieties (such as methoxy and isopropoxy moieties) and a methoxycarbonyl moiety were the *meta*-substituents leading to less potent hypotensive activity. The *N*-acyl derivatives **2a–5a** exhibited weaker hypotensive activity than their corresponding urethane derivatives **2b–5b**, respectively, although both series of compounds exhibited close ID_{50} values for inhibition of TNF-α production. Overall, compounds **2a** and **2b** showed a better profile than the other compounds based on their ID_{50} values and the ratio of efficacy to hypotensive activity (ED_{15mmHg}/ID_{50}).

Pharmacodynamics

Pharmacokinetic profile. The pharmacokinetic profile of the compounds was investigated in rats (Table 4). Intravenous administration of compounds **2a**, **2b**, **3a**, and **5a** to rats (0.3 mg/kg, n = 2–3) showed a relatively short duration of the plasma half-life ($T_{1/2}$ = 1.7, 0.44, 0.39, and 0.18 h, respectively). The steady state volume of distribution (V_{ss}) was calculated to be 105, 128, 112, and 108 mL/kg, respectively, indicating that there was not very extensive distribution to the tissues. Systemic clearance (CL) was 111, 307, 467, and 645 mL/h/kg, respectively. The AUC (area under the concentration vs time curve) was 2.71, 0.98, 0.65, and 0.48 μg h/mL, respectively. Good linearity at doses of 0.1, 0.3, and 1 mg/kg was observed for all four compounds listed in Tables 4

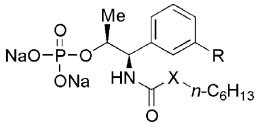
Table 2. Hypotensive activity of compounds **1c** and **2c–g**



Compd	R_1	R_2	R_3	R_4	Dose (mg/kg)	Hypotensive activity (n = 3) (mmHg) SEM	Inhibition of TNF-α production ID_{50} (mg/kg, iv, rats)
1c	H	H	–NHCO–*n*-C_7H_{15}	H	1	−5.2±4.9	3.0
					3	−11.9±4.4	
					10	−18.4±2.9	
2c	Mixture consisting of four diastereoisomers (**2d–g**)				1	−4.5	5.6
					3	−4.1±1.2	
					10	−10.1±3.8	
					30	−8.8±1.9	
2d	Me	H	–NHCO–*n*-C_7H_{15}	H	1	1.1±2.8	4.5
					3	−1.0±4.9	
					10	−5.9±8.0	
					30	−6.3±6.4	
2e	H	Me	–NHCO–*n*-C_7H_{15}	H	3	−10.8±2.1	(23)[a]
					10	−22.4±7.4	
					30	−24.8±4.4	
2f	Me	H	H	–NHCO–*n*-C_7H_{15}	3	−10.7±3.7	(17)[a]
					10	−3.1±5.5	
					30	−11.9±2.2	
2g	H	Me	H	–NHCO–*n*-C_7H_{15}	3	−1.1±1.3	(5)[a]
					10	0±0	
					30	−6.2±4.9	
PGE_1					1 μg/kg	−3.8±0.9	
					3 μg/kg	−18.6±1.8	
					10 μg/kg	−30.8±2.2	

[a]Inhibition (%) of TNF-α production at 30 mg/kg, iv, rats.

Table 3. Biological evaluation of compounds **2a–b**, **3a–b**, **4a–b** and **5a–b**



Compd	R	X	Inhibition of TNF-α production	Hypotensive activity	
			ID_{50} (mg/kg, iv) rats	$ED_{15\ mmHg}$ (mg/kg, iv) rats	$ED_{15\ mmHg}/ID_{50}$
2a	OMe	CH_2	0.03	1.2	40
2b	OMe	O	0.02	0.3	15
3a	O^{i}Pr	CH_2	0.05	0.5	10
3b	O^{i}Pr	O	0.04	0.2	5
4a	SMe	CH_2	0.56	1.4	2.5
4b	SMe	O	0.23	0.6	2.6
5a	COOMe	CH_2	0.13	>3	>23
5b	COOMe	O	0.12	1.7	14

and 5. As a representative example, the plasma profile of **2a** after intravenous administration is shown in Figure 1. The dephosphorylated metabolite **10a** was detected at a plasma level of less than 1/1000 of that for the parent compound, indicating that metabolic stabilization by introducing an (*S*)-methyl group at the carbon atom adjacent to the phosphoric ester moiety had been successful.

Metabolic stability of the selected compounds with respect to their ability to inhibit TNF-α production with a reasonable margin of safety for lethal toxicity was studied using tissue homogenates to obtain an estimate of in vivo metabolism. As shown in Table 5, treatment of **2a**, **2b**, **3a**, and **5a** using liver microsomes with or without NADPH for 2 h afforded their corresponding parent compounds with very little metabolic degradation, while treatment with liver homogenate for 2 h yielded the corresponding metabolites with much more extensive degradation. Metabolic degradation of **2a**, **2b**, and **5a** was not observed after incubation in plasma for 2 h, while treatment of **3a** with plasma only caused minor metabolic degradation. Accordingly, these stabilized inhibitors were estimated to be mainly metabolized in the liver in both of the species tested.

Table 4. Single-dose pharmacokinetic data for compounds **2a**, **2b**, **3a** and **5a**

Compd	Dose (mg/kg)	$AUC_{0-\infty}$ (μg h/mL)	CL_{tot} (mL/h/kg)	$T_{1/2}$ (h)	V_{SS} (mL/kg)
2a	0.3	2.71±0.180	111±7.78	1.7±0.053	105±14.9
2b	0.3	0.980	307	0.44	128
3a	0.3	0.650±0.08	467±56.0	0.39±0.04	112±14.0
5a	0.3	0.480±0.100	645±139	0.18±0.06	108±37

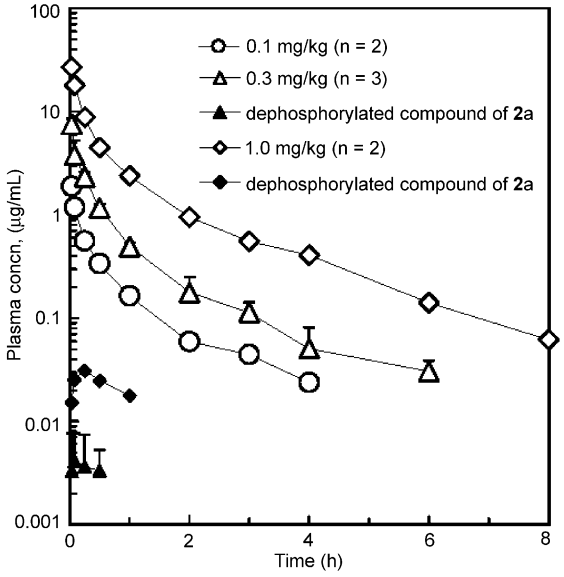


Figure 1. Plasma concentration of **2a** after intravenous administration in rats.

Table 5. In vitro stability in tissues

Tissues	Species	Compd (remaining %)			
		2a	**2b**	**3a**	**5a**
Liver microsome (2 h)	Rat NADPH(+)	94.8	90.2	101.6	92.2
	NADPH (−)	99.2	95.3	99.8	99.9
	Human NADPH(+)	80.4	102.4	97.2	100.6
	NADPH (−)	74.0	98.1	96.7	95.0
Liver homegenate (2 h)	Rat	20.7	21.6	46.2	30.4
	Human	27.4	24.8	32.7	49.4
Plasma (2 h)	Rat	103.6±3.2	106.1±4.2	89.4±2.5	117.7±20.7
	Human	103.2±11.2	100.7±28.0	98.0±7.9	109.9

Conclusion

Optically active β-amino alcohol, a common subunit of the newly discovered inhibitors of TNF-α production, was synthesized more efficiently than before[2d] using the one-step, three-component reaction reported by Petasis and Zavialov.[3] SAR of these inhibitors were investigated with regard to their efficacy and hypotensive activity. Based on the SAR for efficacy, minimum lethal dosage, and hypotensive activity, **2a** and **2b** were selected as candidates for further biological evaluation. The pharmacodynamics of the stabilized inhibitors were also studied in rats, with the plasma half-life ($T_{1/2}$), volume of distribution (V_{ss}), systemic clearance (CL) and AUC being calculated. Good linearity was observed using three doses of the four compounds for their intravenous administration, and an example is shown in Figure 1 (compound **2a**). Dephosphorylated metabolites were detected at a very low level in plasma, indicating that metabolic stabilization had been successful. Dephosphorylation was estimated to mainly occur in the livers of the species tested based on in vitro stability data.

To allow comparison with approved TNF-α suppressors, a monoclonal antihuman TNF-α antibody (infliximab) and a soluble TNF p75 receptor fusion protein (etanercept) that are approved for the treatment of rheumatoid arthritis (RA),[6–9] compound **2b** was also evaluated in rats with collagen-induced arthritis model as well as in the reported animal models.[2d] However, compound **2b** did not exhibit any efficacy in the arthritis model after either intravenous or subcutaneous administration. We speculated that this result was due to inadequate pharmacokinetic properties such as a short duration of action, low blood concentration, and presumed insufficient delivery to articular cavity.

Based on the data described above, these newly discovered inhibitors may be more useful for the treatment of acute diseases than for chronic conditions.

Experimental

General directions

Analytical samples were homogeneous as confirmed by TLC, and afforded spectroscopic results consistent with the assigned structures. ^{1}H NMR spectra were taken on a Varian Mercury 300 spectrometer. MS spectra were obtained on a Hitachi M1200H or PerSeptive Voyager Elite spectrometer. Matrix assisted laser desorption ionization-time of flight high-resolution mass spectra (MALDI-TOF HRMS) were obtained on a PerSeptive Voyager Elite spectrometer. IR spectra were measured on a Perkin-Elmer FT-IR 1760X. Optical rotations were measured on a Jasco DIP-1000 polarimeter. Column chromatography was carried out on silica gel (Merck silica gel 60, 0.063–0.200 mm). Thin-layer chromatography was performed on silica gel (Merck TLC plate, silica gel 60 F_{254}). HPLC analyses were performed with a LaChrom (L-7400 UV Detector, L-7100 pump: Hitachi). The following abbreviations for solvents and reagents are used: THF, tetrahydrofuran; EtOAc, ethyl acetate; MeOH, methanol; EtOH, ethanol; CH_2Cl_2, dichloromethane.

(1*R*,2*S*)-1-[(Diphenylmethyl)amino]-1-(3-methoxyphenyl)-propan-2-ol (16). To a stirred solution of (5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-ol **14**[5] (132 mg, 1 mmol) in CH_2Cl_2 (5 mL) were added a solution of amino diphenyl methane **15** (183 mg, 1 mmol) in CH_2Cl_2 (1 mL) and 3-methoxyphenyl boronic acid **13** (152 mg, 1 mmol) at 25 °C. Stirring was continued at that temperature for 2 days. Removal of the solvent by evaporation to give a crude product, which was purified by column chromatography on silica-gel (Merck 7734, 30 g, *n*-hexane/EtOAc, 5:1) to obtain **16** (257 mg, pale yellow oil) with contamination of 1-(benzhydrylamino)acetone **18** as a by-product. The ratio of **16**:**18** was 11:1 which was determined by ^{1}H NMR (yield of 16:70%): TLC R_f = 0.35 (*n*-hexane/EtOAc, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.18 (m, 11H), 6.88–6.75 (m, 3H), 4.69 (s, 1H), 3.98 (m, 1H), 3.79 (s, 3H), 3.52 (m, 1H), 1.08 (d, J = 6.3 Hz, 3H), MS (APCI, Pos.) m/z 348 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{23}H_{25}NO_2$ + H: 348.1964; found 348.1928.

(1*R*,2*S*)-1-Amino-1-(3-methoxyphenyl)propan-2-ol hydrochloride (9). A mixture of **16** (130 mg, 0.37 mmol) and 10% Pd/C (13 mg) in MeOH (5 mL)/1 M HCl (0.37 mL) was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 15 h. Removal of the catalyst by filtration through a pad of Celite followed by evaporation afforded **9** (120 mg, 100%) as a white solid. The product was used for the next reaction without further purification. To a stirred mixture of **9** (120 mg, 0.37 mmol) and satd $NaHCO_3$ aq (10 mL) in THF (5 mL) was added dropwise octanoyl chloride (71 mg, 0.44 mmol) at 0 °C and stirring was continued at room temperature for 1 h. The reaction mixture was poured into cold water and extracted with EtOAc. The organic layer was successively washed with H_2O and brine before being dried over $MgSO_4$. Removal of the solvent by evaporation afforded compounds **16** and its diastereoisomer **17** and **18** (111 mg, total yield 98%): The ratio of compounds **16** and its diastereoisomer **17** was analyzed by HPLC: DAICEL CHIRALCEL OD (0.46×25 cm); column temperature, 25 °C; eluent, ^{i}PrOH/*n*-hexane = 3:97; flow rate, 1.0 mL/min; UV 270 nm; the retention times of **16** and its diastereoisomer **17** were 58.6 and 30.9 min, respectively. The ratio of **16**:**17** was 93:7 (86% de). Undesired diastereoisomer **17** and another by-product **18** could be removable in the process of recrystallization of **10a**–**d**. Compound **9**: white powder; TLC R_f = 0.47 ($CHCl_3$/MeOH/H_2O = 10:5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (brs, 3H), 7.23 (t, J = 8.1 Hz, 1H), 7.06 (s, 1H), 6.97 (d, J = 7.8 Hz, 1H), 6.83 (dd, J = 8.4, 2.4 Hz, 1H), 4.85–4.60 (br, 1H), 4.46–4.32 (m, 2H), 3.70 (s, 3H), 0.99 (d, J = 6.0 Hz, 3H); MS (MALDI-TOF, Pos) m/z 182 $(M\text{-}HCl+H)^+$; HRMS (MALDI-TOF, Pos) calcd for free amine form $C_{10}H_{15}NO_2 + H^+$: 182.1181; found 182.1145.

Biological assay method

Hypotensive activity. Male Sprague–Dawley rats (CD (IGS), Charles River Inc., Japan) aged 9 weeks (n = 3)

were anesthetized with urethane (solution 25% wt/vol, 1.5 g/6 mL/kg, sc), and the left carotid artery and right jugular vein were cannulated for blood pressure measurement and test compound administration. Each test compound was dissolved in physiological saline and administered at a dose of between 1 and 30 mg/kg (1 mL/kg, iv) as a bolus iv injection. Each compound was administered to a separate group of three rats and the absolute mean arterial pressure (MAP) was recorded for at least 30 min. Prostaglandin E1, which is known to decrease MAP at a dose of between 1 and 10 μg/kg, iv, in rats, was used as a positive control. The average maximal change of MAP (delta mean, mmHg) was calculated for all animals, and the standard error of the mean was also calculated.

Pharmacokinetic (PK) studies

Pharmacokinetic parameters were determined in rats ($n = 2$-3) after iv administration (0.1, 0.3, and 1.0 mg/kg, iv) administration of test compounds, in physiological saline (1 mL/kg, iv). Blood samples (250 μL) were collected from the jugular veins at 2, 5, 15, 30 min, and 1, 2, 3, 4, 6, and 8 h after dosing, and were centrifuged to obtain plasma. After extraction of the test compound with EtOH, plasma samples was analyzed by LC/MS/MS. The pharmacokinetic parameters of the unchanged compound were calculated with WinNonlin software (Pharsight Co., Ltd.). LC/MS/MS analysis was performed with a Quattro II (Micromass Co., Ltd.) mass spectrometer coupled to an HP1100 (Agilent Co., Ltd.) HPLC system equipped with a YMC-pack MB-OBS (2.1×150 mm, YMC Corporation) as the HPLC column.

In vitro stability in tissue homogenates

Liver microsomes. Liver microsomes of male Sprague–Dawley rat were purchased from In Vitro Technologies, Inc. Human liver microsomes were purchased from IIAM (International Institute for the Advancement of Medicine). After pre-incubation at 37 °C for 5 min, a test compound in MeOH (the final concentration was adjusted to 10 μM) was added to rat or human microsomes diluted with 100 mM phosphate buffer (pH = 7.4), 5 mM $MgCl_2$, and 0.05 mM EDTA (adjusted to 1 mg/mL of microsomal protein in the final reaction mixture). The reaction was initiated by adding NADPH at a final concentration of 2 mM. When NADPH was not added, purified water was used instead. Aliquots (100 μM) were taken from duplicate incubations at 0, 15, 30, 60, and 120 min after initiation of the reaction. Each sample was diluted with EtOH (2 mL) and the precipitate was removed by centrifugation. The supernatant was concentrated and the resulting residue was redissolved in the mobile phase for HPLC [eluent: 20 mM KH_2PO_4 (pH = 3.0)/CH_3CN = 37/63, column: CAPCELL PAK C18 UG120, 4.6×150 mm (Shiseido), flow: 1 mL/min]. Then the stability of the test compound was evaluated by measuring the residual percentage of the unchanged compound by HPLC after incubation for 2 h. The initial peak area of the unchanged compound was defined as 100%.

Plasma. Plasma was obtained from the blood of male Crj:CD (SD) IGS rats (Charles River) and also from the blood of healthy male volunteers. After preincubation at 37 °C for 3 min, a test compound in MeOH (1 mM, 10 μL) was added to rat or human plasma (1 mL) and the final concentration was adjusted to 10 μM. Aliquots were taken from triplicate reaction mixtures at 0, 15, 30, 60, and 120 min after initiation of the reaction. Each sample was diluted with EtOH (2 mL) and the precipitate was removed by centrifugation. Then the supernatant was concentrated and the resulting residue was redissolved in the mobile phase for HPLC [eluent: 20 mM KH_2PO_4 (pH = 3.0)/CH_3CN = 37:63, column: CAPCELL PAK C18 UG120, 4.6×150 mm (Shiseido), flow: 1 mL/min]. The stability of each compound was evaluated by measuring the residual percentage of the unchanged compound by HPLC after incubation for 2 h.

Liver homogenate. Livers were obtained from male Crj:CD (SD) IGS rats (Charles River) and human liver samples were obtained from HAB (HAB-Biomedical Research Institute). After pre-incubation at 37 °C for 3 min, a test compound in MeOH (1 mM, 10 μL) was added to rat or human liver homogenate (5%, 1 mL) and the final concentration was adjusted to 10 μM. Aliquots (100 μL) were taken from duplicate reaction mixtures at 0, 15, 30, 60, and 120 min after initiation of the reaction. Each sample was diluted with EtOH (2 mL) and the precipitate was removed by centrifugation. Then the supernatant was concentrated and the resulting residue was redissolved in the mobile phase for HPLC [eluent: 20 mM KH_2PO_4 (pH = 3.0)/CH_3CN = 37:63, column: Capcell PAK C18 UG120, 4.6×150 mm (Shiseido), flow: 1 mL/min]. The stability of each test compound was evaluated by measuring the residual percentage of the unchanged compound by HPLC after incubation for 2 h.

References and Notes

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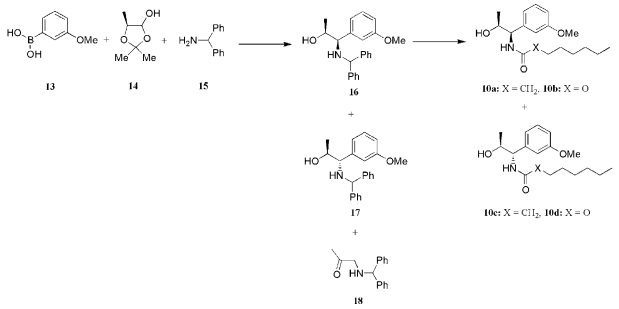
4. 1-(Benzhydrylamino)acetone **18** was obtained as one of the by-products. The ratio of **16** and **18** was determined by ^{1}H NMR. Another by-product **17** was a diastereoisomer of **16**. Diastereomeric excess (de) was determined by HPLC analysis (86% de, **16**:**18** = 93:7). Purification method has not been optimized yet



5. (a) (5*S*)-2,2,5-Trimethyl-1,3-dioxolan-4-ol **14** was prepared by the modification of the known methods: Davis, F. A.; Sheppard, A. C.; Chen, B-C.; Haque, M.S *J. Am. Chem. Soc*. **1990**, *112*, 6679. (b) Kusumoto, T.; Hiyama, T.; Matsuitani, H.; Ichikawa, S., Japan Patent, JP8225477 (c) Kim, H.-O.; Friedrich, D.; Huber, E.; Peet, N. P. *Synth. Commun*. **1996**, *26*, 3453. (5*S*)-2,2,5-Trimethyl-1,3-dioxolan-4-ol **14** was prepared from (5*S*)-2,2,5-trimethyl-1,3-dioxolan-4-one by the reduction with DIBAL in toluene at −78 °C (48% yield). (5*S*)-2,2,5-Trimethyl-1,3-dioxolan-4-one was also prepared from L-lactic acid by the treatment with 2,2-dimethoxypropane in the presence of $MgSO_4$ in acetone at room temperature for 3 days (32% yield).
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