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Discovery of olmesartan hexetil: A new potential prodrug of olmesartan

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ABSTRACT

Synthesis of a new ester prodrug of olmesartan, olmesartan hexetil (**1**), is described. It is in vitro stabilities and in vivo pharmacokinetics (PK) were evaluated. It showed high stability in simulated gastric juice, and was rapidly hydrolyzed to olmesartan in rat liver microsomes and rat plasma in vitro. C_{max} and AUC_{last} for olmesartan were significantly increased in case of hexetil prodrug, compared with olmesartan medoxomil. Olmesartan hexetil is proposed to be an efficient prodrug of olmesartan with markedly increased oral bioavailability.

In the current era of globalization; which is characterized by so much stress, worry, and hurry; the incidence of cardiovascular disorders has dramatically increased. Hypertension is a disease which affects an estimated one billion people worldwide.[1] The American Heart Association estimates high blood pressure affects approximately one in three adults in the United States. Hypertension is a serious disease with a momentous impact on health and life expectancy. Controlling blood pressure and prevention of its complications such as coronary heart disease, renal failure, eye damage, and brain damage (stroke) are the main objectives for the treatment of hypertension.[2]

The renin–angiotensin–aldosterone system (RAAS) is a very complex system that plays a pivotal role in the regulation of blood pressure. Angiotensin II, the primary effector hormone of RAAS, affects the cardiovascular system by influencing the vascular tone, fluid volume, and electrolyte balance.[3,4]

There are many antihypertensive drugs available, many of which act on the RAAS system. Angiotensin receptor blockers (ARBs) are a class of antihypertensive agents that are growing in popularity due to their excellent blood pressure control potential, low adverse event profile, and high patient tolerability.[5] Olmesartan is an example of ARBs which acts by blocking type 1 angiotensin II receptors (AT_1-R), leading to prevention of vasoconstriction, reduction of sodium and water retention, and decrease of cellular proliferation and hypertrophy.[6] In addition to AT_1-R blockade, olmesartan is assumed to exhibit an angiotensin-converting enzyme (ACE) inhibitory effect, prevent an increase in angiotensin II level, and protect cardiovascular remodeling through an increase in cardiac nitric oxide production and endogenous angiotensin-(1–7) via over-expression of ACE2.[7]

The once daily dosing interval of most ARBs helps enhance patient compliance which may lead to better patient outcomes.[5] Olmesartan medoxomil is an ester prodrug of olmesartan that has shown potent and long-lasting antihypertensive activity after oral administration.[8] Olmesartan medoxomil is rapidly de-esterified by enzymatic hydrolysis in the intestine, liver, and plasma. After oral administration of the prodrug, first-pass bioactivation occurs in the intestine, followed by the portal blood and liver, before the prodrug reaches the systemic circulation. It was reported that multiple enzymes are capable of bioactivating olmesartan medoxomil in human, including plasma albumin,[9] and an intestinal and liver hydrolase carboxymethylenebutenolidase homolog (CMBL).[10] High metabolic clearance of intestinal CMBL suggests that the intestinal bioactivation firstly and predominantly contributes to the quick onset of drug action after oral administration of

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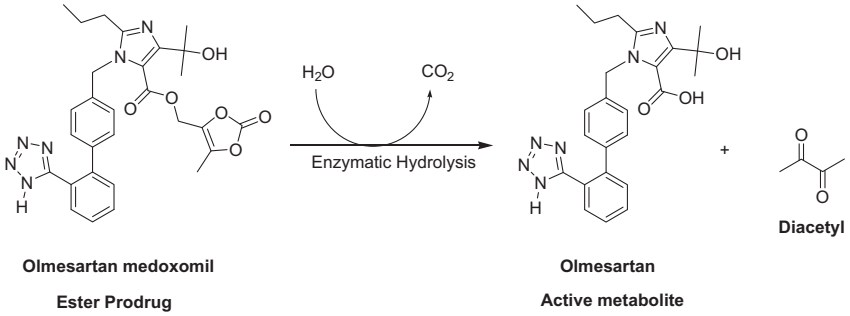
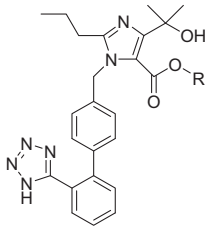
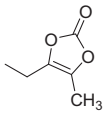
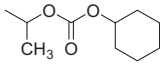


Figure 1. Hydrolysis of olmesartan medoxomil to olmesartan.

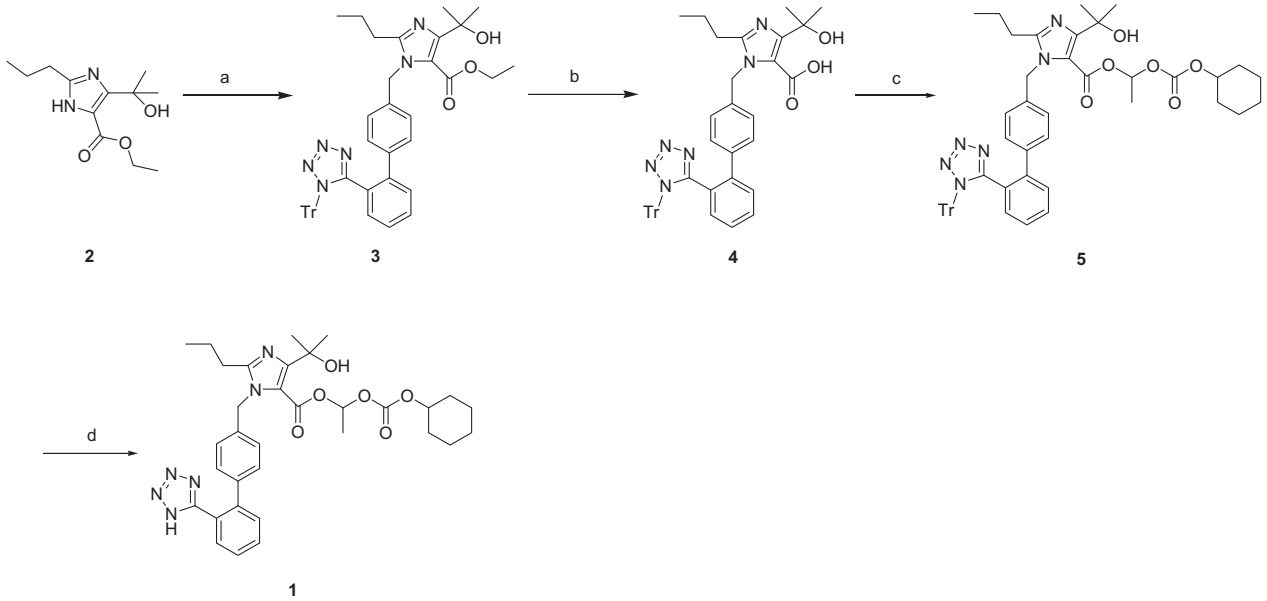
Table 1
c Log *P* and total polar surface area (TPSA) calculations for olmesartan medoxomil and olmesartan hexetil (**1**)

Structure	Compd no	R	*c*Log *P*	TPSA (A^2)
	Olmesartan medoxomil		3.49	162.18
	1		5.06	154.37

olmesartan medoxomil.[11] Figure 1 illustrates the enzymatic hydrolysis of olmesartan medoxomil into the active metabolite, olmesartan.[9]

We reported the synthesis, bioconversion, and PK evaluation of new prodrugs of olmesartan with higher lipophilicity than olmesartan medoxomil. The new ester prodrugs of olmesartan showed improved PK parameters for olmesartan, compared with olmesartan medoxomil.[12,13] These results showed that lipophilic ester prodrugs of olmesartan can improve the oral bioavailability and PK properties of olmesartan. In the present investigation, we report the synthesis, in vitro bioconversion, and in vivo PK evaluation of a new ester prodrug of olmesartan, olmesartan hexetil. The medoxomil moiety of olmesartan medoxomil was replaced by hexetil, a more lipophilic moiety, in order to increase the lipophilicity of olmesartan ester prodrug, and hence, olmesartan bioavailability. Hexetil promoiety has been reported to increase the oral bioavailability of candesartan.[14] The calculated *c*log *P*[15] and total polar surface area (TPSA)[16] values of olmesartan medoxomil and olmesartan hexetil prodrugs are illustrated in Table 1. Olmesartan hexetil (**1**) with higher *c*log *P* and lower TPSA values compared with olmesartan medoxomil (i.e., more lipophilic than olmesartan medoxomil) was synthesized and evaluated as a new potential prodrug of olmesartan. Our target is to improve the pharmacokinetic properties of olmesartan, and hence, the antihypertensive outcomes. The synthetic and screening protocols are illustrated in details.

Synthesis of the target compound **1** was carried out according to the sequence of reactions illustrated in Scheme 1. It was important at the beginning to prepare the key carboxylic acid compound, trityl olmesartan (**4**). N-alkylation of ethyl 4-(1-hydroxy-1-methyl-ethyl)-2-propylimidazole-5-carboxylate (**2**) with 5-(4′-bromomethyl-biphenyl-2-yl)-1-trityl-1*H*-tetrazole afforded the ethyl ester of trityl olmesartan (**3**). Alkaline hydrolysis of the ethyl ester moiety of **3** followed by acidification of the formed potassium salt gave trityl olmesartan (**4**).[17] The target hexetil ester **1** was obtained



Scheme 1. Reagents and conditions: (a) 5-(4′-bromomethyl-biphenyl-2-yl)-1-trityl-1*H*-tetrazole, K_2CO_3, acetone, DMAc, reflux, 10 h, 75%; (b) (i) KOH, isopropanol, 60 °C, 4 h; (ii) HCl, workup, 90%; (c) hexetil chloride, K_2CO_3, KI, DMAc, 70 °C, 2 h, 95%; (d) concd HCl, acetone, H_2O, rt, 2 h, 92%.

Table 2
In vitro stabilities of olmesartan medoxomil and compound **1** in simulated gastric juice, rat plasma, and rat liver microsomes[a]

Compd no	Half-lives (min)		
	Simulated gastric juice	Rat plasma	Rat liver microsomes
Olmesartan medoxomil	>1000	0.956	4.95
1	390	1.54	2.77

[a] Data represent the mean of duplicated experiments.

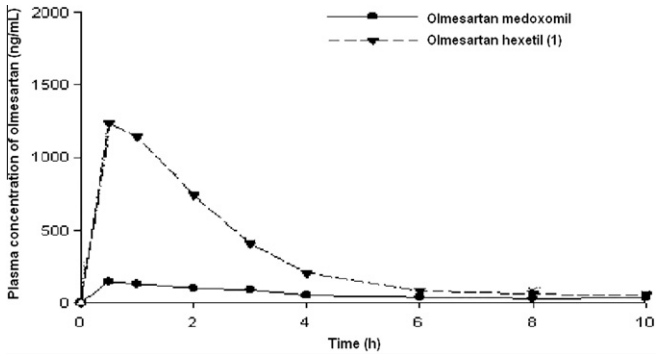


Figure 2. Mean arterial plasma concentration–time profiles of olmesartan after oral administration of olmesartan medoxomil (●; $n = 4$) and olmesartan hexetil (▼; $n = 4$) at a dose of 20 mg/kg as olmesartan in rats.

Table 3
Pharmacokinetic parameters (mean ± standard deviation) of olmesartan after oral administration of prodrugs (20 mg/kg as olmesartan) to male rats

Compd. administered	C_{max} (ng/mL)[a]	T_{max} (h)[b]	AUC_{last} (ng h/mL)[c]
Olmesartan medoxomil ($n = 4$)	153.6 ± 71.4	1.0 (0.5–2)	501.9 ± 283.4
1 ($n = 4$)	1374.7 ± 1122.3	0.9 (0.5–3)	3013.8 ± 1556.6

[a] C_{max}: peak plasma concentration.
[b] T_{max}: time to reach C_{max}, expressed as median (range).
[c] AUC_{last}: total area under the plasma concentration–time curve from time zero to last measured time.

by esterification of the carboxylic acid group of **4** with hexetil chloride, and subsequent detritylation using concd HCl.[18]

The prodrugs intended to be administered orally should be stable in acidic conditions encountered in the stomach following oral dosing, and be hydrolyzed to active metabolites in the systemic circulation after absorption. The stability of the target compound **1** was determined in simulated gastric juice, rat plasma, and rat hepatic microsomes in vitro.[19] The chemical or enzymatic stabilities of compounds after the incubation are presented (Table 2). The half-life of compound **1** in simulated gastric juice was 390 min. In addition, its half-lives in rat hepatic microsomes and rat plasma were <2.8 min. The results showed that olmesartan hexetil (**1**) was rapidly hydrolyzed to olmesartan, the active metabolite, in rat liver microsomes and rat plasma, while it is hydrolysis rate in simulated gastric juice was slow. These results suggested that compound **1** can pass through the stomach with slow degradation and the absorbed molecules from the gastrointestinal tract can be rapidly converted into the active form in liver and plasma.

Pharmacokinetic studies were conducted to determine whether the new prodrug of olmesartan **1** was converted into olmesartan in vivo.[20] Olmesartan medoxomil and olmesartan hexetil (**1**) were administered orally using a feeding tube to male Sprague–Dawley rats at a dose of 20 mg/kg as olmesartan. The plasma concentrations of olmesartan were determined by a slight modification of the reported liquid chromatography-tandem mass spectrometric method (LC–MS/MS).[21] Pharmacokinetic parameters were determined by a non-compartmental analysis. The total area under the plasma concentration–time curve from time zero to the last measured time (AUC_{last}) was calculated by the linear trapezoidal rule method.[22] The mean arterial plasma concentration–time profiles of olmesartan after oral administration of olmesartan hexetil and olmesartan medoxomil in rats are shown in Figure 2, and some relevant pharmacokinetic parameters of olmesartan are summarized in Table 3. After oral administration of the two compounds, olmesartan was detectable in plasma from the first blood sampling time, 30 min. This result suggested that compound **1** was well absorbed from rat gastrointestinal tract and rapidly converted into the active form. After administration of prodrugs, the peak plasma concentrations of olmesartan (C_{max}) and AUC_{last} values were significantly higher (795% and 500% increase, respectively) than those observed after administration of olmesartan medoxomil. These effects are possibly due to increased lipophilicity of compound **1** induced by hexetil moiety over olmesartan medoxomil. The results indicated that introduction of lipophilic promoiety, such as hexetil, enhanced the oral absorption and systemic exposure level of olmesartan.

In conclusion, olmesartan hexetil (**1**), a new ester prodrug of olmesartan was synthesized. It is in vitro stabilities in simulated gastric juice, rat plasma, and rat hepatic microsomes were studied. In addition, the in vivo pharmacokinetic parameters of olmesartan after it is oral administration were studied and compared with those after administration of olmesartan medoxomil. Compound **1** showed high stability in simulated gastric juice and rapid hydrolysis to the active form in rat plasma and rat liver microsomes, in addition to improved pharmacokinetic profiles compared with olmesartan medoxomil. Olmesartan hexetil is proposed to be a promising prodrug of olmesartan with significantly improved oral bioavailability.

Acknowledgments

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18. *Synthesis of the target compound* ***1****:* To a solution of compound **5** (10 g, 11.6 mmol) in acetone (30 mL), concd HCl (30 mL) and water (20 mL) were added. The reaction mixture was stirred at room temperature for 2 h. The organic solvent was evaporated under reduced pressure, and the pH of the remained aqueous solution was adjusted to 4–5 by addition of aqueous potassium carbonate solution. The aqueous mixture was extracted with ethyl acetate (3×50 mL), and the combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography (silica gel, hexane/ethyl acetate 5:1 v/v then switching to ethyl acetate) to give the target product **1** (6.8 g, 92%). mp 113–115 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.87 (t, J = 7.6 Hz, 3H), 1.23–1.82 (m, 21H), 2.59 (t, J = 7.6 Hz, 2H), 4.48–4.51 (m, 1H), 5.14 (s, 1H), 5.42 (s, 2H), 6.75 (q, J = 2.7 Hz, 1H), 6.91 (d, J = 7.9 Hz, 2H), 7.07 (d, J = 7.9 Hz, 2H), 7.51–7.70 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.1, 19.5, 20.9, 23.3, 25.1, 28.7, 30.0, 30.1, 31.2, 48.4, 70.1, 77.1, 92.1, 116.3, 124.0, 126.1, 128.2, 129.5, 131.0, 131.4, 136.9, 138.7, 141.5, 151.8, 152.3, 155.5, 158.4, 159.7; LC–MS m/z: 618.06 $[M+1]^+$.
19. *Stability studies of prodrugs:* Stabilities of the newly synthesized ester prodrug, olmesartan hexetil, and olmesartan medoxomil were measured in simulated gastric juice, rat plasma, and rat liver microsomes. To determine the microsomal stability of prodrugs, prodrugs were incubated with rat liver microsomes in the presence of NADPH. The microsomal reaction mixtures were containing 1.2 mM NADPH, 0.5 mg/mL (total protein) microsomes, 100 mM phosphate buffer (pH 7.4). Solutions of the prodrugs in acetonitrile (100 μM) were added to simulated gastric juice, rat plasma, or rat liver microsomes reaction mixture with a final concentration of 1 μM. The reaction solutions were kept at 37 °C and sampled at 0, 15, 30, 60, and 120 min. The 50 μL aliquot of the mixtures was terminated at the above time points by addition of twofold volume of cold acetonitrile containing internal standard. After centrifugation, the supernatant was collected and analyzed immediately using LC–MS/MS.
20. *Pharmacokinetic studies:* Olmesartan hexetil (**1**) and olmesartan medoxomil were administered orally using a feeding tube to male Sprague–Dawley rats at a dose of 20 mg/kg as olmesartan. Blood samples were collected via carotid artery at 0 (to serve as a control), 0.5, 1, 2, 3, 4, 6, 8, and 10 h after oral administration of each compound. After centrifugation at 3000 rpm for 10 min, a 200-μL aliquot of plasma samples were stored at −80 °C until analysis. Pharmacokinetic parameters were determined by a non-compartmental analysis using WinNonlin® (Pharsight Corporation, Mountain View, CA) program. The AUC_{last} values were calculated by the trapezoidal rule–extrapolation method.
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