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Original article

# Design, synthesis, bioconversion, and pharmacokinetics evaluation of new ester prodrugs of olmesartan

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ABSTRACT

Synthesis of new ester prodrugs of olmesartan is described. Their *in vitro* stabilities in simulated gastric juice, rat plasma, and rat liver microsomes were tested. And the pharmacokinetic parameters for olmesartan after their oral administration were also estimated and compared with those in case of olmesartan medoxomil. Compounds **13** and **14** demonstrated high stability in simulated gastric juice and were rapidly metabolized to olmesartan in rat liver microsomes and rat plasma *in vitro*. In addition, $C_{max}$ and $AUC_{last}$ parameters were significantly increased in case of compounds **13** and **14** compared with olmesartan medoxomil. These results indicate that compounds **13** and **14** with cyclohexylcarboxyethyl and adamantylcarboxymethyl promoieties, respectively, are promising prodrugs of olmesartan with markedly increased oral bioavailability.



## 1. Introduction

Hypertension is a disease which affects an estimated one billion people worldwide [1]. It 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, and cerebral vascular disease are the main objectives for the treatment of hypertension [2].

The renin-angiotensin-aldosterone system (RAAS) is a very complex system that plays an important 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 blocking of vasoconstriction, reduction of sodium and water retention, and decrease of cellular proliferation and hypertrophy [6]. Besides $AT_1$-R blockade, olmesartan is assumed to exhibit an angiotensin-converting enzyme (ACE) inhibitory action, 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 which has shown potent and long-lasting antihypertensive activity by oral administration [8]. Olmesartan medoxomil is rapidly de-esterified by an enzyme, arylesterase, which is located in both the intestine

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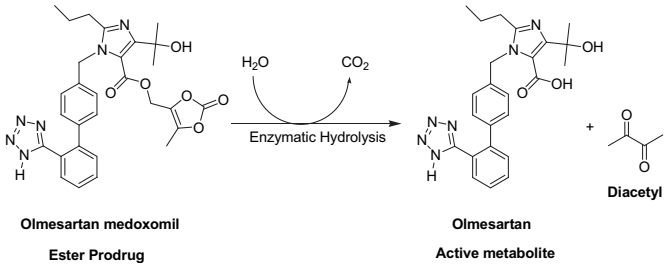



**Fig. 1.** Hydrolysis of olmesartan medoxomil to olmesartan.

and plasma [6]. Fig. 1 illustrates the enzymatic hydrolysis of olmesartan medoxomil into the active metabolite, olmesartan [9].

We have reported the synthesis, bioconversion, and pharmacokinetic (PK) evaluation of a new derivative of olmesartan medoxomil with higher lipophilicity. The new ester prodrug of olmesartan showed improved PK parameters for olmesartan, compared with olmesartan medoxomil [10]. 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 design, synthesis, *in vitro* bioconversion, and PK evaluation of new ester prodrugs of olmesartan. The medoxomil moiety of olmesartan medoxomil was replaced by more lipophilic promoieties, such as myristoyl, cyclohexylcarboxyethyl, adamantylcarboxymethyl, and benzoyloxyethyl, in order to increase the lipophilicity of olmesartan ester prodrugs, and hence, olmesartan bioavailability. Our goal is to improve the pharmacokinetic properties of olmesartan, and hence, the antihypertensive outcomes. Moreover, we have reported synthesis and *in vitro* stability in rat plasma of octyl and uracil esters of olmesartan [10]. Herein, we report their *in vivo* PK properties also. The synthetic and screening protocols are illustrated in details.

## 2. Results and discussion

### 2.1. Chemistry

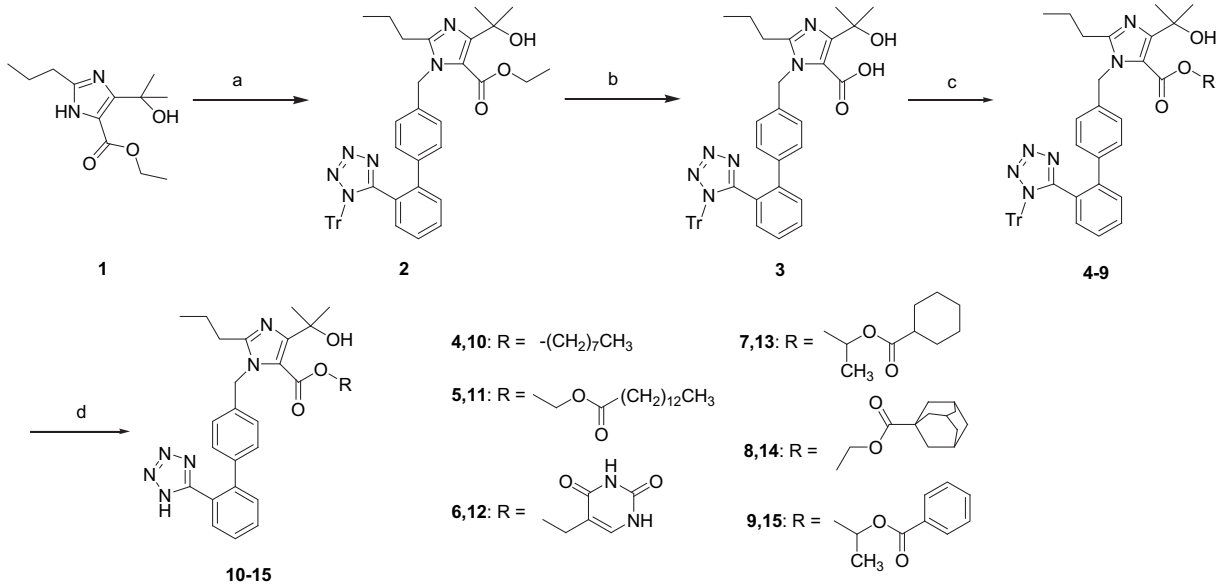
Synthesis of the target compounds **10**–**15** was carried out according to the sequence of reactions illustrated in Scheme 1. It was important at the beginning to prepare the key carboxylic acid compound, trityl olmesartan **3**. *N*-Alkylation of ethyl 4-(1-hydroxy-1-methylethyl)-2-propylimidazole-5-carboxylate (**1**) with 5-(4′-bromomethyl-biphenyl-2-yl)-1-trityl-1*H*-tetrazole afforded the ethyl ester of trityl olmesartan **2**. Alkaline hydrolysis of the ethyl ester moiety of **2** followed by acidification of the formed potassium salt furnished trityl olmesartan **3** [11]. The target ester compounds **10**–**15** were synthesized by esterification of the carboxylic acid group of **3** with the appropriate alkyl halide, and subsequent detritylation using conc. HCl.

### 2.2. Biological evaluation

#### 2.2.1. In vitro stability evaluation

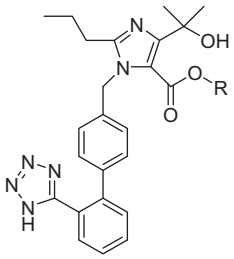
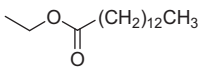
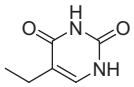
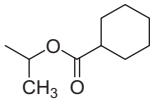
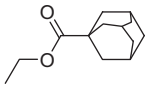
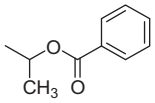
The stabilities of all compounds were determined in rat plasma *in vitro*. The remaining percentages of the compounds in rat plasma after the incubation are summarized in Table 1. The results of compounds **10** and **12** have been previously reported [10]. After the 30 min incubation period of **11** and **13**, the prodrug peak disappeared, and the olmesartan peak was increased. The results showed that compounds **11** and **13** were rapidly hydrolyzed to olmesartan, active metabolite, in plasma.

The prodrugs should be stable in acidic condition encountered in the stomach following oral dosing and be hydrolyzed to active metabolites in the systemic circulation after absorption. The stabilities of compounds **13** and **14**, as representative examples of these new ester prodrugs, were determined in simulated gastric juice, rat plasma, and rat hepatic microsomes *in vitro*. The chemical or enzymatic stabilities of compounds after the incubation are presented (Table 2). The half-lives of both compounds in simulated gastric juice were more than 300 min. In addition, the half-lives of **13** and **14** in rat hepatic microsomes and rat plasma were $\leq$10 min. The results showed that these compounds were rapidly hydrolyzed to olmesartan, the active metabolite, in rat liver microsomes and rat plasma, while the hydrolysis rates of the compounds in simulated gastric juice were slow. These results suggested that compounds **13** and **14** 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.




**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; (b) (i) KOH, isopropanol, 60 °C, 4 h; (ii) HCl, workup; (c) R-X, $K_2CO_3$, KI, DMAc, 70 °C, 2 h; (d) conc. HCl, acetone, $H_2O$, rt, 2 h.

**Table 1**
*In vitro* stabilities of compounds **10**–**15** in rat plasma.

| Structure | Compound no. | R | Stability (%remaining) |
|---|---|---|---|
|  | 10 | $-(CH_2)_7CH_3$ | 90.2 |
| | 11 |  | NA[a] |
| | 12 |  | 96.3 |
| | 13 |  | NA[a] |
| | 14 |  | 79.8 |
| | 15 |  | 85.8 |
| | Olmesartan medoxomil | | NA[a] |

[a] NA, not applicable.

### 2.2.2. *In vivo* pharmacokinetic (PK) studies

Pharmacokinetic studies were conducted to determine whether the new prodrugs of olmesartan were converted into olmesartan *in vivo*. Olmesartan medoxomil and compounds **10**–**15** 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) [12]. 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 [13]. The mean arterial plasma concentration-time profiles of olmesartan after oral administration of the newly synthesized ester compounds and olmesartan medoxomil in rats are shown in Fig. 2, and some relevant pharmacokinetic parameters of olmesartan are summarized in Table 3. Olmesartan was detectable in plasma from the first blood sampling time, 30 min, after oral administration of compounds **13**–**15** and olmesartan medoxomil. This result suggested that these compounds were well absorbed from rat gastrointestinal tract and rapidly converted into the active form. After administration of prodrugs, the peak plasma concentrations of olmesartan ($C_{max}$) were significantly higher (290% and 52% increase for **13** and **14**, respectively) than those observed after administration of olmesartan medoxomil. The $AUC_{last}$ values of prodrugs were also significantly greater (157% and 46% of olmesartan for **13** and **14**, respectively) than that of olmesartan medoxomil. These effects are possibly due to increases in lipophilicity of **13** and **14** induced by cyclohexylcarbonyloxyethyl and adamantylcarbonyloxymethyl moieties, respectively, over olmesartan medoxomil. The results indicated that introduction of these lipophilic moieties enhanced the oral absorption and systemic exposure level of olmesartan.

**Table 2**
Half-lives of olmesartan medoxomil and compounds **13** and **14** 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 |
| **13** | 928 | 9.68 | 2.70 |
| **14** | 312 | 10.00 | 2.28 |

[a] Data represent the mean of duplicated experiments.

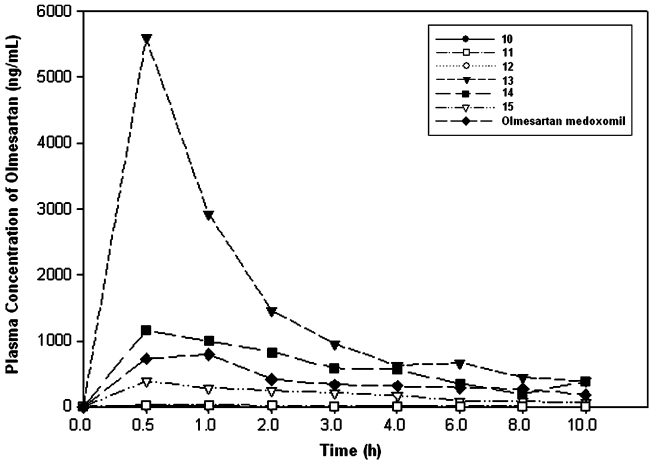



**Fig. 2.** Mean arterial plasma concentration-time profiles of olmesartan after oral administration of olmesartan medoxomil (◆; $n = 7$), **10** (●; $n = 4$), **11** (□; $n = 4$), **12** (○; $n = 4$), **13** (▼; $n = 6$), **14** (■; $n = 4$), and **15** (∇; $n = 4$) and at a dose of 20 mg/kg as olmesartan 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 ($n = 7$) | 891.2 ± 1136.5 | 2.9 (0.5–6.0) | 3385.9 ± 261.6 |
| **10** ($n = 4$) | 17.9 ± 22.1 | 0.8 (0.5–1.0) | 32.8 ± 29.2 |
| **11** ($n = 4$) | 32.6 ± 13.7 | 0.8 (0.5–1.0) | 100.4 ± 11.9 |
| **12** ($n = 4$) | 8.0 ± 3.0 | 1.8 (0.5–4.0) | 21.7 ± 12.7 |
| **13** ($n = 6$) | 3474.4 ± 1779.3 | 0.6 (0.5–1.0) | 8711.5 ± 2155.8 |
| **14** ($n = 4$) | 1355.2 ± 235.8 | 2.8 (0.5–6.0) | 4952.7 ± 334.9 |
| **15** ($n = 4$) | 398.3 ± 239.0 | 1.1 (0.5–3.0) | 1511.4 ± 482.8 |

[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.

## 3. Conclusion

New ester prodrugs of olmesartan were designed and synthesized. Their *in vitro* stabilities in rat plasma were examined. And the half-lives of compounds **13** and **14** in simulated gastric juice, rat plasma, and rat hepatic microsomes were determined. In addition, the *in vivo* pharmacokinetic parameters of olmesartan after oral administration of compounds **10**–**15** were evaluated and compared with those after administration of olmesartan medoxomil. The newly synthesized ester prodrugs **13** and **14** with cyclohexylcarboxyethyl and adamantylcarboxymethyl moieties, respectively, showed high stability in simulated gastric juice and rapid hydrolysis in rat plasma and rat liver microsomes. Both compounds demonstrated improved pharmacokinetic profiles compared with olmesartan medoxomil. These new prodrugs are proposed to be effective prodrugs of olmesartan with markedly increased oral bioavailability.

## 4. Experimental

### 4.1. General

All the reagents and solvents were purchased from Sigma Aldrich Chemical Co. and used without purification. The purity of all final compounds was over 95% on the basis of HPLC analysis. The purity was confirmed by Waters LC-MS system: Waters 2998 photodiode array detector, Waters 3100 mass detector, Waters SFO system fluidics organizer, Waters 2545 binary gradient module, Waters reagent manager, Waters 2767 sample manager, Sunfire™ $C_{18}$ column (4.6 × 50 mm, 5 μm particle size); solvent gradient = 95% A at 0 min, 1% A at 5 min; solvent A: 0.035% trifluoroacetic acid (TFA) in water; solvent B: 0.035% TFA in MeOH; flow rate = 3.0 mL/min. The AUC was calculated using Waters MassLynx 4.1 software. Melting points were obtained on a Walden Precision Apparatus Electrothermal 9300 apparatus and are uncorrected. $^1$H NMR and $^{13}$C NMR spectra were obtained using a Bruker 300 MHz FT-NMR and a Bruker 400 MHz FT-NMR spectrometers. The animal experiments were approved by the pertinent committees of our institutions and performed in compliance with institutional guidelines for the conduct of animal experimentation.

### 4.2. Ethyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(trityltetrazol-5-yl)phenyl]phenylmethyl}imidazole-5-carboxylate (**2**) [11]

A mixture of ethyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1*H*-imidazole-5-carboxylate (**1**, 10 g, 41.61 mmol), 5-(4′-bromomethyl-biphenyl-2-yl)-1-trityl-1*H*-tetrazole (25.516 g, 45.77 mmol), and anhydrous potassium carbonate (2.876 g, 20.81 mmol) in a solvent mixture of DMAc (25 mL) and acetone (250 mL) was heated under reflux for 10 h. The mixture was cooled to room temperature and filtered to remove the insoluble material. The filtered inorganic solid material was washed with acetone (25 mL). The washing solution was combined with the filtrate and the solvent was evaporated under reduced pressure to afford the desired product **2** (22.37 g, 75%). LC-MS *m/z*: 717.40 $[M + 1]^+$.

### 4.3. 4-(1-Hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(trityltetrazol-5-yl)phenyl]phenyl-methyl}imidazole-5-carboxylic acid (**3**) [11]

To a solution of compound **2** (10 g, 13.95 mmol) in isopropanol (100 mL), 10% aqueous potassium hydroxide solution (15 mL) was slowly added at room temperature. The reaction mixture was heated at 60 °C for 4 h. The organic solvent was evaporated under reduced pressure. The residue was dissolved in water (40 mL) and the resulting solution was washed with ethyl acetate (3 × 30 mL). The aqueous layer was acidified using HCl and the target compound was precipitated. The precipitate was filtered, washed with water, and dried to give compound **3** in a pure form (8.65 g, 90%).

$^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 0.73 (t, $J = 7.3$ Hz, 3H), 1.44–1.49 (m, 2H), 1.54 (s, 6H), 2.41 (t, $J = 6.6$ Hz, 2H), 5.58 (s, 2H), 6.86 (d, $J = 6.9$ Hz, 8H), 7.03 (d, $J = 8.1$ Hz, 2H), 7.27–7.40 (m, 10H), 7.43–7.45 (m, 1H), 7.51–7.63 (m, 2H), 7.76–7.78 (m, 1H).

### 4.4. General procedure for synthesis of compounds **4**–**9**

A mixture of compound **3** (5 g, 7.259 mmol), potassium carbonate (0.502 g, 3.63 mmol), and potassium iodide (0.3 g, 1.807 mmol) in DMAc (30 mL) was stirred at room temperature for 30 min. To the reaction mixture, a solution of the appropriate alkyl halide (8.71 mmol) in DMAC (10 mL) was slowly added at room temperature. The reaction mixture was heated at 70 °C for 2 h. The reaction mixture was cooled to room temperature and partitioned between water and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). 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 10:1 v/v then switching to hexane: ethyl acetate 4:1 v/v) to give the desired product **4**–**9**.

#### 4.4.1. Octyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tritylte-trazol-5-yl)phenyl]phenylmethyl}imidazole-5-carboxylate (**4**)

Yield: 72%; LC-MS m/*z*: 802.06 $[M + 1]^+$.

#### 4.4.2. Tetradecanoyloxymethyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(trityltetrazol-5-yl)phenyl]phenylmethyl}imidazole-5-carboxylate (**5**)

Yield: 75%; LC-MS m/*z*: 930.00 $[M + 1]^+$.

#### 4.4.3. (1,2,3,4-Tetrahydro-2,4-dioxopyrimidin-5-yl)methyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(trityltetrazol-5-yl) phenyl]phenylmethyl}imidazole-5-carboxylate (**6**)

Yield: 68.3%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 0.87 (t, $J = 7.4$ Hz, 3H), 1.57 (q, $J = 7.5$ Hz, 2H), 2.59 (t, $J = 7.5$ Hz, 2H), 4.85 (s, 2H), 5.43 (d, $J = 6.5$ Hz, 3H), 6.87–6.91 (m, 8H), 7.01 (d, $J = 8.1$ Hz, 2H), 7.32–7.51 (m, 9H), 7.63–7.69 (m, 3H), 7.76 (d, $J = 7.8$ Hz, 1H), 11.05 (d, $J = 13.0$ Hz, 2H).

#### 4.4.4. [1-(Cyclohexylcarbonyloxy)]ethyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(trityltetrazol-5-yl)phenyl] phenylmethyl}imidazole-5-carboxylate (**7**)

Yield: 88.2%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 0.75 (t, $J = 7.8$ Hz, 3H), 1.15–1.27 (m, 5H), 1.47 (s, 6H), 1.54 (t, $J = 7.1$ Hz, 6H), 1.69–1.74

(m, 2H), 2.18–2.21 (m, 2H), 2.45 (t, $J$ = 7.8 Hz, 2H), 4.25–4.29 (m, 1H), 5.17 (s, 1H), 5.39 (s, 2H), 6.85 (q, $J$ = 10.9 Hz, 1H), 6.85–6.90 (m, 8H), 7.07 (d, $J$ = 4.1 Hz, 2H), 7.29–7.43 (m, 9H), 7.51 (t, $J$ = 7.4 Hz, 1H), 7.63 (t, $J$ = 7.4 Hz, 2H), 7.75 (d, $J$ = 7.5 Hz, 1H).

*4.4.5. (1-Adamantyl)carbonyloxymethyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(trityltetrazol-5-yl)phenyl] phenylmethyl}imidazole-5-carboxylate (**8**)*

Yield: 95.4%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 0.74 (t, $J$ = 7.8 Hz, 3H), 1.44 (s, 6H), 1.46–1.70 (m, 14H), 1.87 (s, 3H), 2.45 (t, $J$ = 7.8 Hz, 2H), 4.91 (s, 1H), 5.33 (s, 2H), 5.75 (s, 2H), 6.78–6.85 (m, 8H), 7.05 (d, $J$ = 4.1 Hz, 2H), 7.27–7.41 (m, 9H), 7.52 (t, $J$ = 7.5 Hz, 1H), 7.62 (t, $J$ = 7.5 Hz, 2H), 7.73 (d, $J$ = 7.3 Hz, 1H).

*4.4.6. [1-(Benzoyloxy)]ethyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(trityltetrazol-5-yl)phenyl]phenylmethyl}imidazole-5-carboxylate (**9**)*

Yield: 88.9%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 0.74 (t, $J$ = 7.8 Hz, 3H), 1.44–1.46 (m, 9H), 1.49 (q, $J$ = 7.6 Hz, 2H), 2.44 (t, $J$ = 7.8 Hz, 2H), 5.15 (s, 1H), 5.36 (s, 2H), 6.88 (d, $J$ = 7.9 Hz, 1H), 6.92–7.01 (m, 8H), 7.28–7.41 (m, 9H), 7.65–7.70 (m, 6H), 7.81 (d, $J$ = 7.4 Hz, 2H), 7.90 (d, $J$ = 7.4 Hz, 2H).

### *4.5. General procedure for synthesis of the target compounds **10**–**15***

To a solution of the trityl compound **4**–**9** (6.25 mmol) in acetone (30 mL), conc. 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 **10**–**15**.

*4.5.1. Octyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)phenyl]phenyl-methyl}imidazole-5-carboxylate (**10**)*

Yield: 91%; mp: 209–212 °C (dec.); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 0.81 (t, $J$ = 6.1 Hz, 3H), 0.91 (t, $J$ = 7.3 Hz, 3H), 1.17 (brs, 10H), 1.49 (brs, 7H), 1.63 (q, $J$ = 7.4 Hz, 2H), 2.60 (t, $J$ = 7.5 Hz, 2H), 3.99 (brs, 2H), 4.13 (t, $J$ = 6.3 Hz, 2H), 5.43 (brs, 2H), 6.80 (d, $J$ = 7.8 Hz, 2H), 7.09 (d, $J$ = 7.9 Hz, 2H), 7.31 (d, $J$ = 6.9 Hz, 1H), 7.35–7.41 (m, 2H), 7.55 (d, $J$ = 7.0 Hz, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 14.1, 14.4, 21.0, 22.5, 25.8, 28.3, 28.8, 29.0, 30.2, 31.7, 48.5, 65.4, 70.1, 117.3, 125.2, 127.3, 128.0, 128.2, 129.7, 130.5, 131.0, 131.7, 135.9, 140.5, 140.9, 151.1, 157.5, 160.5, 162.2.

*4.5.2. Tetradecanoyloxymethyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)phenyl]phenylmethyl}imidazole-5-carboxylate (**11**)*

Yield: 92%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 0.82–0.89 (m, 6H), 1.16–1.22 (m, 22H), 1.47 (brs, 6H), 1.53–1.61 (m, 2H), 2.25 (t, $J$ = 7.3 Hz, 2H), 2.58 (t, $J$ = 7.4 Hz, 2H), 5.05 (brs, 1H), 5.44 (brs, 2H), 5.80 (brs, 2H), 6.91 (d, $J$ = 8.0 Hz, 2H), 7.06 (d, $J$ = 8.0 Hz, 2H), 7.50–7.63 (m, 2H), 7.67 (d, $J$ = 7.4 Hz, 2H).

*4.5.3. (1,2,3,4-Tetrahydro-2,4-dioxopyrimidin-5-yl)methyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)phenyl] phenylmethyl}imidazole-5-carboxylate (**12**)*

Yield: 93.7%; mp: 210–213 °C (dec.); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 0.87 (t, $J$ = 7.4 Hz, 3H), 1.57 (q, $J$ = 7.5 Hz, 2H), 2.59 (t, $J$ = 7.5 Hz, 2H), 4.85 (s, 2H), 5.43 (d, $J$ = 6.5 Hz, 3H), 6.87 (d, $J$ = 8.2 Hz, 2H), 7.02 (d, $J$ = 8.1 Hz, 2H), 7.56 (t, $J$ = 7.5 Hz, 2H), 7.63–7.69 (m, 2H), 11.07 (d, $J$ = 13.1 Hz, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 14.1, 21.0, 28.7, 30.3, 48.3, 61.9, 70.4, 99.3, 116.8, 124.1, 126.0, 128.2, 129.5, 131.0, 131.1, 131.4, 137.0, 138.7, 141.5, 150.2, 151.5, 151.7, 155.6, 157.7, 161.1, 164.4.

*4.5.4. [1-(Cyclohexylcarbonyloxy)]ethyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)phenyl]phenylmethyl} imidazole-5-carboxylate (**13**)*

Yield: 90.9%; mp: 108–110 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 0.87 (t, $J$ = 7.4 Hz, 3H), 1.11–1.32 (m, 8H), 1.48 (s, 6H), 1.58 (t, $J$ = 7.3 Hz, 4H), 1.68–1.75 (m, 2H), 2.21–2.28 (m, 1H), 2.59 (t, $J$ = 7.4 Hz, 2H), 5.16 (s, 1H), 5.43 (d, $J$ = 5.8 Hz, 2H), 6.86 (q, $J$ = 10.9 Hz, 1H), 6.91 (d, $J$ = 8.0 Hz, 2H), 7.09 (d, $J$ = 8.0 Hz, 2H), 7.50–7.70 (m, 4H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 14.1, 19.4, 20.9, 24.9, 25.0, 25.6, 28.5, 28.8, 30.1, 30.2, 42.2, 48.4, 70.1, 89.1, 116.4, 124.1, 126.1, 128.2, 129.5, 131.1, 131.4, 136.9, 138.7, 141.5, 151.7, 158.4, 159.8, 173.5; LC-MS m/$z$: 602.22 $[M + 1]^+$.

*4.5.5. (1-Adamantyl)carbonyloxymethyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)phenyl]phenylmethyl} imidazole-5-carboxylate (**14**)*

Yield: 92.3%; mp: 119–122 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 0.86 (t, $J$ = 7.3 Hz, 3H), 1.47 (s, 6H), 1.52–1.70 (m, 14H), 1.90 (s, 3H), 2.57 (t, $J$ = 7.5 Hz, 2H), 5.03 (s, 1H), 5.44 (s, 2H), 5.82 (s, 2H), 6.91 (d, $J$ = 8.2 Hz, 2H), 7.06 (d, $J$ = 8.1 Hz, 2H), 7.49–7.69 (m, 4H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 14.1, 20.9, 27.6, 28.7, 30.1, 36.2, 38.3, 48.2, 70.1, 79.9, 116.2, 124.0, 126.3, 128.3, 129.6, 130.9, 131.1, 131.4, 136.9, 138.7, 141.5, 151.8, 155.5, 158.3, 160.0, 175.7; LC-MS m/$z$: 640.00 $[M + 1]^+$.

*4.5.6. [1-(Benzoyloxy)]ethyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)phenyl]phenylmethyl}imidazole-5-carboxylate (**15**)*

Yield: 91.9%; mp 98–101 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 0.87 (t, $J$ = 7.4 Hz, 3H), 1.46–1.48 (m, 9H), 1.58 (q, $J$ = 7.4 Hz, 2H), 2.59 (t, $J$ = 7.5 Hz, 2H), 5.17 (s, 1H), 5.42 (d, $J$ = 4.5 Hz, 2H), 6.88 (d, $J$ = 8.0 Hz, 2H), 7.01 (d, $J$ = 7.9 Hz, 2H), 7.10 (q, $J$ = 7.6 Hz, 1H), 7.42 (d, $J$ = 7.5 Hz, 1H), 7.49 (t, $J$ = 7.6 Hz, 2H), 7.54–7.66 (m, 4H), 7.89 (d, $J$ = 7.9 Hz, 2H); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 14.1, 19.6, 20.9, 28.7, 30.1, 48.4, 70.1, 89.9, 116.5, 126.0, 128.2, 129.0, 129.3, 129.5, 129.9, 131.0, 131.4, 134.4, 136.9, 138.7, 141.5, 151.7, 158.5, 159.8, 164.3; LC-MS m/$z$: 595.50 $[M + 1]^+$.

### *4.6. Stability studies of prodrugs*

Stabilities of the newly synthesized ester prodrugs and olmesartan medoxomil were determined in rat plasma. To determine their plasma stability, 50 μg/mL compound in rat plasma was incubated for 30 min at 37 °C. After that, the samples were analyzed immediately by LC-MS/MS.

In addition, the half-lives of compounds **13**, **14**, 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 two-fold volume of cold acetonitrile containing internal standard. After centrifugation, the supernatant was collected and analyzed immediately by LC-MS/MS.

### 4.7. Pharmacokinetic studies

Each compound was 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.

### 4.8. Quantitative analysis

Concentrations of olmesartan in the plasma samples were analyzed using LC-MS/MS. To 200-μL aliquot of plasma sample, 150 μL of methanol containing internal standard (telmisartan) and 0.1 N HCl 200 μL was added. After mixing, olmesartan was extracted by liquid extraction using diethyl ether: dichloromethane (3:2 v/v), and the upper layer was transferred and dried under nitrogen evaporation. The LC-MS/MS system consisted of a HP1100® HPLC system (Agilent, Santa Clara, CA) and API3200® triple-quadrupole mass spectrometer (Applied Biosystems-SCIEX, Concord, Canada). The HPLC mobile phases consisted of 0.1% formic acid in acetonitrile and 0.1% formic acid in 10 mM ammonium acetate (85:15 v/v). Chromatographic separation was achieved on a reversed-phase Xterra® $C_{18}$ column (50 × 2.1 mm, 3.5 μm, Waters Corporation, Milford, MA) at a flow rate of 0.25 mL/min. The lower limit of quantitation of olmesartan in rat plasma was 5 ng/mL.

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