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Original article

# Synthesis and biological evaluation of ester prodrugs of benzafibrate as orally active hypolipidemic agents

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ABSTRACT

A series of bezafibrate ester prodrugs **1**–**7** were synthesized and evaluated for hypolipidemic activity in Swiss Albino mice (SAM). Bezafibrate (**1a**), a hypolipidemic drug was used as a reference compound for data comparison. Among the synthesized compounds, prodrug **7** showed superior activities in decreasing triglyceride up to 30% in mice plasma after oral administration of 50 mg/kg/day for 8 days. Prodrugs **2**, **3**, **5**, **6**, and **7** were found to be more lipophilic than bezafibrate (**1a**), indicated by partition coefficients measured in octanol-buffer system at pH 7.4. On the basis of *in vivo* studies, prodrug **7** emerged as new potent hypolipidemic agent.



## 1. Introduction

High-serum low-density lipoprotein (LDL) and elevated total cholesterol levels are the most prevalent indicators for susceptibility to atherosclerotic heart disease [1,2]. Hypercholesterolemia is now considered a major risk factor in the development of premature atherosclerosis. Past efforts to slow or even reverse this disease process have focused almost exclusively on the development of potent hypocholesterolemic agents. As a result of these efforts, several classes of hypolipidemic agents are now available for the control of hypercholesterolemia. These include the most recent class of agents, the HMG CoA reductase inhibitors [3], the second-generation fibric acid derivatives [4], and the bile acid sequestrants [5]. These various agents can be separated on the basis of their different mechanisms of action, and the combination of cholesterol-lowering drugs from different classes represents a currently accepted therapy for aggressive cholesterol-lowering therapy.

Fibrate class of drugs, discovered a decade ago, are effective in reducing the serum triglyceride and increasing the high-density lipoprotein (HDL) cholesterol in humans. Bezafibrate (**1a**, Fig. 1) is fibric acid derivative, corresponds to the nomenclature of 2-(4-{2-[(4-chlorobenzoyl)amino]ethyl}phenoxy)-2-methylpropanoic acid and well-known activator of peroxisome proliferator-activated receptors (PPARs), that can activate both PPAR-α and PPAR-β. It is used to reduce triglyceride and cholesterol in the management of hyperlipidemias, including type IIa, type IIb, type III, type IV, and type V hyperlipoproteinemias [6]. The usual dose is 200 mg three times daily by mouth taken with or after food. Bezafibrate reduce triglycerides by lowering the concentration of very-low-density lipoprotein (VLDL). They reduce low-density lipoprotein cholesterol (LDLC) to a lesser extent, although the effect is variable, and may also increase high-density lipoprotein cholesterol (HDLC) [7].

New strong fibrates with piperidine moiety showed very superior activities in decreasing triglyceride and cholesterol compared to bezafibrate in mice and rats [8]. α-Asarone bioisosteric analogs of fibrates such as clofibrate, bezafibrate, and fenofibrate have significant hypocholesterolemic activity [9–11]. A prodrug is a pharmacological substance (drug) administered in an inactive (or significantly less active) form. The rationale behind the use of

*Abbreviations:* HPLC, high performance liquid chromatography; GC, gas chromatograph; API, active pharmaceutical ingredient; DMAc, dimethylacetamide; Ar, aryl; PPAR-α, peroxisome proliferator-activated receptor alpha; PPAR-β, peroxisome proliferator-activated receptor beta; SE, standard error; TC, total cholesterol; TG, triglyceride; HDLC, high-density lipoprotein cholesterol; LDLC, low-density cholesterol; VLDL, very-low-density lipoprotein; SAM, Swiss albino mice; RT, retention time; BDL, below detection limit; CAD, coronary artery diseases; PSD, particle size distribution.

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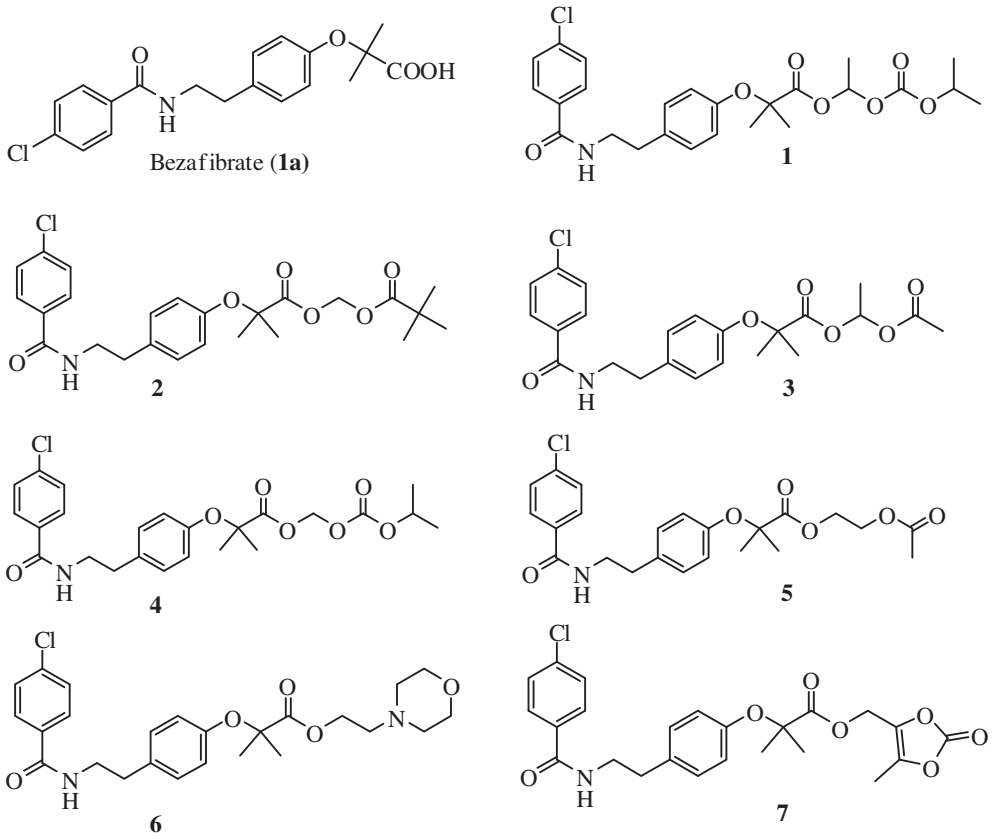


**Fig. 1.** Chemical structures of bezafibrate (**1a)** and prodrugs **1**–**7**.

a prodrug is generally for absorption, distribution, metabolism, and excretion (ADME) optimization. Prodrugs are usually designed to improve oral bioavailability, eventually to improve the efficacy as well.

Once administered, the prodrug is metabolized *in vivo* into an active metabolite. Thus, prodrugs are molecules that must undergo biotransformation prior to exhibiting their therapeutic effects. Our strategy to synthesize new bezafibrate prodrugs **1**–**7** (Fig. 1) consists of modifying the carboxyl function by selected bulkier derivatives and studying the impact of such modifications on the hypolipidemic activity in male Swiss albino mice (SAM). The metabolic product (i.e. parent drug) subsequently elicits the desired pharmacological response [12,13].

The physicochemical properties of a drug plays major role in the development of formulation and bioavailability. Thus, physicochemical parameters like partition coefficient (log *P*), aqueous stability and particle size distribution were studied.

## 2. Results and discussion

### 2.1. Chemistry

A series of novel bezafibrate ester prodrugs **1**–**7** were readily synthesized in good yields (88%, 85%, 74%, 83%, 87%, 76%, 73%) by straight forward condensation of bezafibrate (**1a**) with appropriate promoiety **a**–**g** (Fig. 2), in the presence of 1,1,3,3,-tetramethyl guanidine (TMG) or sodium carbonate or potassium carbonate in dimethylacetamide (DMAc), according to the Scheme 1. Promoieties **a**–**g** were synthesized as per the procedures given in our earlier published articles [14,15]. The structures of all prodrugs were established by IR, $^{1}$H NMR, $^{13}$C NMR, mass spectrometry, elemental analysis, and their purity in excess of 99% was confirmed by HPLC analysis (Supporting Information).

### 2.2. Pharmacological evaluation

Owing to interest in the synthesis of new hypolipidemic agents, series of bezafibrate ester prodrugs **1**–**7** were synthesized and evaluated for triglyceride and cholesterol-lowering potential in Swiss albino mice (SAM), a moderate hypertriglyceride model [16–20]. Normal mice (chow fed) were used for primary screening of hypolipidemic agents. The mice were treated with prodrugs **1**–**7** and bezafibrate (**1a)** by oral gavage at a dose of 50 mg/kg/day for 8 days. The *in vivo* profile of compounds **1**–**7** was compared with a reference drug bezafibrate (**1a)** for hypolipidemic activity. The prodrugs **2**, **3**, **6**, and **7** were found more potent in lowering triglyceride than bezafibrate (**1a**) and the prodrug **7** alone showed a significant reduction in mice plasma triglyceride level (TG). Total cholesterols (TCs) did not show significant changes in the bezafibrate as well as prodrug-treated animals (Table 1, Fig. 3). The TG lowering activity of bezafibrate (**1a**) was improved by esterification with 4-chloromethyl-5-methyl-1,3-dioxol-2-one and the lowering effect of TG in mice plasma was the highest among all prodrugs. This may be because of improvement in the oral bioavailability of the compound eventually better efficacy.

### 2.3. Dose response of prodrug **7** in Swiss albino mice

Prodrug **7** alone lowered the mice triglyceride level significantly ($P < 0.01$) as compared to normal control and hence a dose effect relationship for triglyceride (TG), and for total cholesterol (TC) at the doses of 10, 30, 100 mg/kg/day was studied and the results were