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Original article

Synthesis and biological activity of novel barbituric and thiobarbituric acid derivatives against non-alcoholic fatty liver disease

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ABSTRACT

Forty-four barbituric acid or thiobarbituric acid derivatives were synthesized and evaluated for their effects on adipogenesis of 3T3-L1 adipocytes by measuring the expression of adiponectin *in vitro*. Four compounds (**3a**, **3o**, **3s**, **4t**) were found to increase the expression of adiponectin and lower the leptin level in 3T3-L1 adipocytes at respective concentration of 10 μM. Among them, **3s** showed the most efficacious. Oral administration of **3s** effectively reduced body weight, liver weight, and visceral fat and regulated serum levels of biochemical markers in the high-fat/diet-induced Wistar rats. Histopathological evaluation of liver sections by Oil Red O and H&E staining confirmed **3s** as a potent, orally active molecule for reducing fat deposition against non-alcoholic fatty liver disease.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), defined as fatty infiltration of the liver exceeding 5%–10% by weight, is a clinic pathological syndrome characterized by hepatic steatosis without excess alcohol intake. It has become one of the leading causes of chronic liver disease in many countries [1–3]. NAFLD comprises a wide spectrum ranging from simple fatty liver to non-alcoholic steatohepatitis (NASH), followed by progression to fibrosis, cirrhosis and hepatocellular carcinoma [4,5]. This disease is closely associated with insulin resistance (IR) and metabolic syndromes including obesity, type II diabetes, dyslipidemia, hypertension and atherosclerosis [6–9].

The adipose tissue secretes many physiologically active adipocytokines such as adiponectin and leptin, which modulate hepatic and peripheral lipid and glucose metabolism [10,11]. Adiponectin is a 30-kDa adipocyte protein exclusively expressed in adipose tissue of normal humans [12]. The plasma adiponectin level, directly associated with insulin sensitivity, is negatively correlated with body mass index (BMI), plasma glucose, insulin and triglycerides but positively correlated with the plasma levels of high-density lipoprotein (HDL)-cholesterol [13–15]. Leptin, the product of the obese gene, is an adipocyte-secreted protein hormone with an important role in energy homeostasis, including appetite, body weight and metabolism functions. The amount of leptin secreted and expressed by adipocytes is positively correlated with the lipid content and adipocyte size [16]. Therefore, the roles of adiponectin and leptin on regulating energy balance and metabolism make them as potential therapeutic implications for humans and animals of NAFLD.

As insulin resistance is very common in patients with non-alcoholic fatty liver disease, considerable studies developed novel insulin sensitizers as logical treatment strategies. Biguanides and thiazolidinediones (TZDs) are representatives insulin sensitizers widely used in clinic (Fig. 1). Metformin, whose mechanisms of action are not well understood, effectively improves hepatic steatosis in animal model of fatty liver [17] and aminotransaminase levels in human liver tissue [18] and increases insulin sensitivity by facilitating glucose consumption and utilization [19]. TZDs, such as rosiglitazone, are a kind of oral anti-diabetic medications that significantly ameliorate insulin resistance by acting as selective peroxisome proliferitor-activated receptor γ (PPAR γ) agonists [20]. In addition, TZDs increase circulating levels of adiponectin in adipose tissue [21–23] and has insulin sensitizing properties to slow

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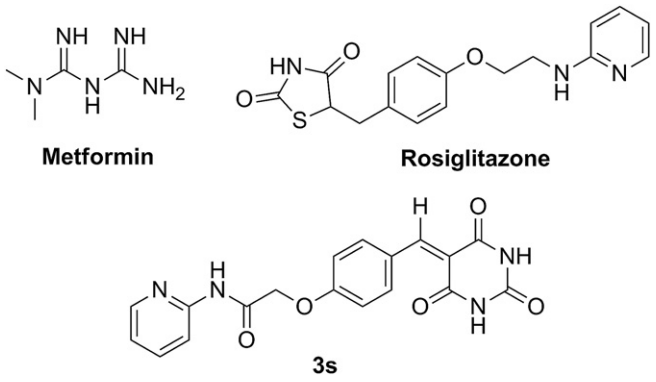


Fig. 1. Structure of Metformin, Rosiglitazone and **3s**.

and prevent the progression of NAFLD [24,25]. Recently, a new series of PPAR γ ligands based on barbituric acid instead of TZD were designed and evaluated and six active compounds were found to be bound to the murine PPAR γ with IC_{50} ranging from 0.1 to 2.5 μM [26]. Their results provided the impetus to develop novel and potent therapeutic agents containing barbituric acid or thiobarbituric acid moiety and investigate their pharmacological functions.

2. Chemistry

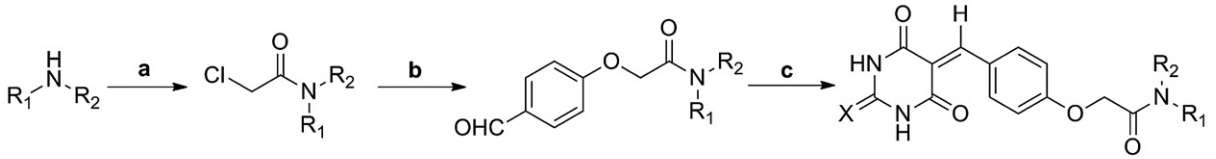
Various amines in this study were converted into chloroacetamide derivatives (**1a**–**v**) with excellent yields via a reaction employed 2-chloroacetyl chloride agent in the presence of triethylamine as base and dichloromethane as solvent. The similar reactions with the high yield were dangerous and toxic to organic synthetic operators because amount of hydrogen chloride gas was released. Therefore, the agent of 2-chloroacetyl chloride must be added slowly in the ice-bath with the application of absorption solution for tail gas. The pivotal aldehyde intermediates which contained 2-(4-formyl-phenoxy)-*N*-substituted-phenylacetamide (**2a**–**p**) and special aldehyde intermediates (**2q**–**v**) were synthesized through the use of chloroacetamide derivatives, 4-hydroxybenzaldehyde, potassium carbonate as base and potassium iodide as catalyst according to Scheme 1, respectively. The reaction mixture was refluxed in a solvent of acetone for 24 h and monitored by the thin-layer chromatography.

And then, to obtain the forty-four targeted compounds varying with the western substituted group quickly, the Knoevenagel reaction which accomplished by condensation aldehyde intermediate with babituric acid or thiobarbituric acid and employed water and ethanol as solvent was performed in parallel using EYELA Personal Organic Synthesizer (Tokyo, Rikakikai) with a 12-well liquid-phase reaction block. Through 4 or 5 h' reaction, the obtained crude was precipitated in the solvent, filtered and washed with ethyl ether and water because the babituric acid or thiobarbituric acid was dissolved into ethyl ether. The advantages of the Knoevenagel synthetic protocol were generalized [27]: (a) all starting materials were soluble in the mixture of water and ethanol; (b) the reaction was clean and quick; (c) the insoluble targeted compound was precipitated in the course of the reaction, yielding essentially pure final compound (purities ≥97%) and requiring no further purification. At this stage, the products were fully analyzed and characterized by hydrogen nuclear magnetic resonance (^{1}H NMR), Mass spectrum (MS), and High Performance Liquid Chromatography (HPLC) before entering the biological tests.

3. Biological results and discussion

3.1. In vitro assay for adiponectin expression in 3T3-L1 adipocytes

In order to quickly discover the potential therapeutic agents, the expression of adiponectin was evaluated by measuring the promoting rate of the adiponection in cultured 3T3-L1 adipocytes. Rosiglitazone (Fig. 1) was selected as a positive control. Forty-four compounds based on barbituric acid and thiobarbituric acid moiety with respective concentration of 10 μM were investigated the effects on differentiation of 3T3-L1 preadipocytes into adipocytes by measuring the promoting rates of adiponectin expression. Their results were depicted in Fig. 2.



3,4 a:	R_1 = Ph,	R_2 = H	3,4 l:	R_1 = 2,4-diCl-Ph,	R_2 = H
3,4 b:	R_1 = 4-CH_3-Ph,	R_2 = H	3,4 m:	R_1 = 3,5-diCl-Ph,	R_2 = H
3,4 c:	R_1 = 4-OCH_3-Ph,	R_2 = H	3,4 n:	R_1 = 3-Cl,4-F-Ph,	R_2 = H
3,4 d:	R_1 = 3,4-diOCH_3-Ph,	R_2 = H	3,4 o:	R_1 = 3,4-diF-Ph,	R_2 = H
3,4 e:	R_1 = 4-CF_3-Ph,	R_2 = H	3,4 p:	R_1 = Ph,	R_2 = CH_3
3,4 f:	R_1 = 4-CH_3C(O)-Ph,	R_2 = H	3,4 q:	R_1 = cyclohexyl,	R_2 = H
3,4 g:	R_1 = 3-F-Ph,	R_2 = H	3,4 r:	R_1 = benzyl,	R_2 = H
3,4 h:	R_1 = 4-F-Ph,	R_2 = H	3,4 s:	R_1 = pyridin-2-yl,	R_2 = H
3,4 i:	R_1 = 3-Cl-Ph,	R_2 = H	3,4 t:	R_1 = 5-methylpyridin-2-yl,	R_2 = H
3,4 j:	R_1 = 4-Cl-Ph,	R_2 = H	3,4 u:	R_1 = naphthalen-1-yl,	R_2 = H
3,4 k:	R_1 = 4-Br-Ph,	R_2 = H	3,4 v:	R_1 = naphthalen-2-yl,	R_2 = H

Scheme 1. General synthesis of **3**, **4a**–**v**. Reagents and conditions: (a) 2-chloroacetyl chloride, Et_3N, CH_2Cl_2, 0 °C–25 °C, 20 h; (b) KI, K_2CO_3, 4-hydroxybenzaldehyde, reflux, 24 h; (c) barbituric acid or thiobarbituric acid, ethanol, H_2O, 60 °C, 3–4 h.