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1,3-Benzodioxole-based fibrate derivatives as potential hypolipidemic and hepatoprotective agents

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ABSTRACT

A series of target compounds 1,3-benzodioxole-based fibrate derivatives were designed and synthesized. All the target compounds were preliminarily evaluated by hyperlipidemia mice induced by Triton WR-1339, in which compound **12** displayed a greater anti-hyperlipidemia activity than other compounds as well as positive drug fenofibrate (FF). **12** showed a significant reduction of plasma lipids, such as triglycerides (TG), total cholesterol (TC) and *low-density lipoprotein* cholesterin (LDL-C), in high fat diet (HFD) induced hyperlipidemic mice. In addition, hepatic transaminases (AST and ALT) were ameliorated after administration of **12**, in particular the AST, and the histopathological examination showed that **12** improved the hepatic lipid accumulation. The expression of PPAR-α involved in lipids metabolism was up-regulated in the liver tissues of **12**-treated group. Other significant activity such as antioxidant, and anti-inflammation was confirmed and reinforced the effects of **12** as a potential hypolipidemia and hepatoprotective agent.

Hyperlipidemia, caused by lipids metabolism disorder, is a serious health-threatening disease physically and mentally which can increase the lipids in the blood and is also a major risk factor for cardio-cerebrovascular diseases and metabolic syndrome.[1,2] Studies have showed that the elevated triglyceride (TG) plays a major role in the occurrence and development of cardio-cerebrovascular diseases, diabetes and fatty liver by triggering oxidative stress, inflammation, atherosclerosis and other pathological processes.[3] Prevention and treatment of hypertriglyceridemia are very important to cardio-cerebrovascular related diseases. Therefore, the effective control the level of TG has enormous significance for the prevention and treatment of cardio-cerebrovascular diseases and/or metabolic syndrome.

Fibric acid derivatives have been frequently used in the synthesis of hypolipidemic agents and played an important role in the therapy of hyperlipidemia for many years. The fibrates (Fig. 1), such as clofibrate, ciprofibrate, gemfibrozil, beclobrate, fenofibrate and so on, were available in pharmacies or hospitals, which were widely used in the treatment of hyperlipidemia. These fibrates increase lipolysis and eliminate TG by activating the nuclear transcription factor (PPAR-α), which increases lipoprotein lipase activity.[4] However, fibrates need a higher dose to reduce blood lipids and were accompanied by an increase in liver transaminase, especially in combination with the statins. Although the hypolipidemic effect of fibrates was significant, the most common adverse effects were liver injury. Therefore, a more effective agent having both hypolipidemia and hepatoprotective properties was in great demand.

In designing novel drugs (Fig. 1), hybrid molecules method was largely applied. The strategy through the combination of different pharmacophores may obtain compounds with multifunctional biological effects. Natural products provide a variety of leading compounds for drug discovery and research. Sesamol (1,3-benzodioxole) is the core component of sesame oil, which has the effects of antioxidation, anti-

Abbreviations: ALT, alanineaminotransferase; AST, aspartate transaminase; FF, fenofibrate; GSH-PX, glutathione peroxidase; HDL-C, *high-density lipoprotein* cholesterin; HFD, high-fat diet; IL-6, interleukin; LDL-C, *low-density lipoprotein* cholesterin; MDA, malondialdehyde; PPAR-α, peroxisome proliferator-activated receptor α; SOD, superoxide dismutase; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor α.

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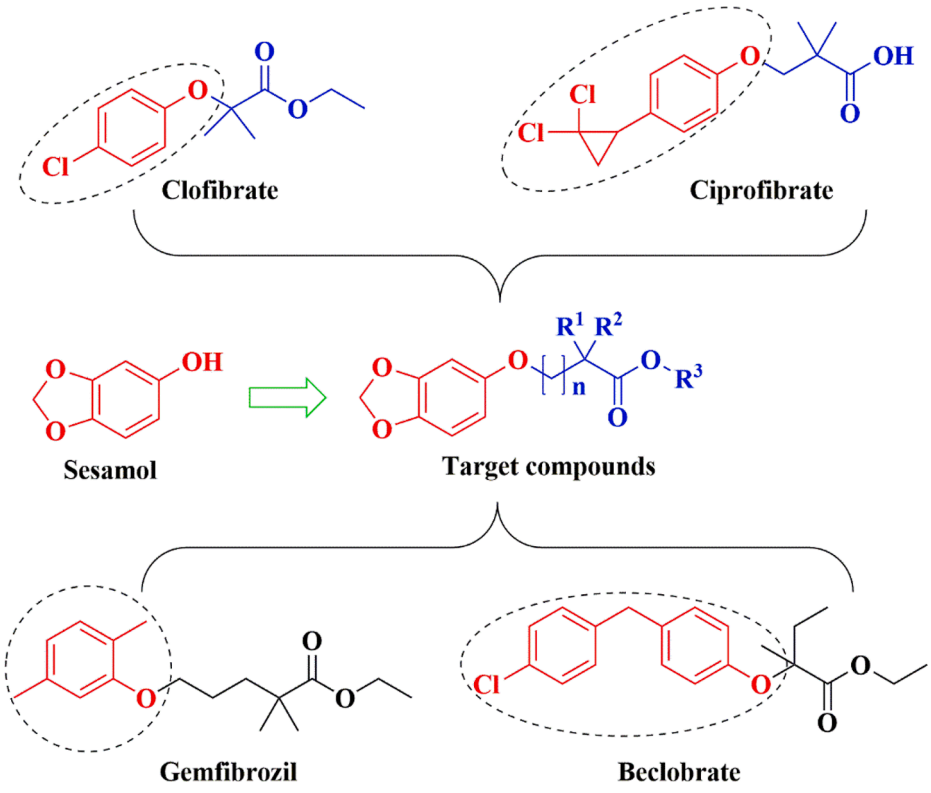
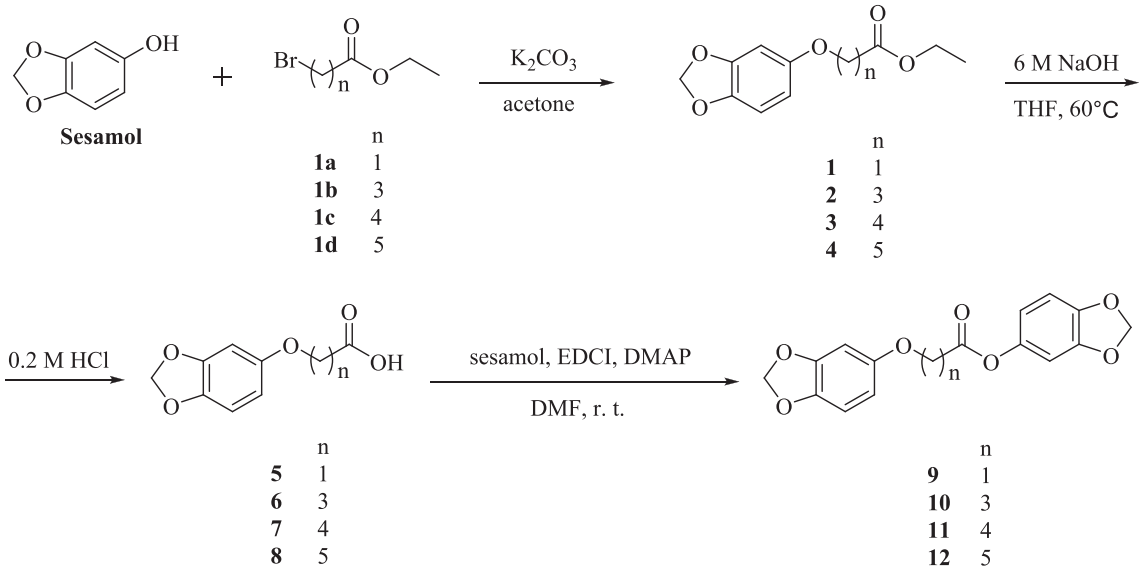


Fig. 1. Designing of sesamol-fibrate hybrids on the basis of sesamol and fibrate scaffolds displaying hypolipidemic and liver protective effects.



Scheme 1. Synthesis route of target compounds **1** ~ **12**.

inflammation and regulating blood lipids.[5,6] 1,3-benzodioxole derivatives, such as sesamin, sesamolin, sesaminol, sesamolinol, have significant effects on anti-oxidation, anti-inflammation and regulation of blood lipids, which have significant preventive and protective effects on cardiovascular diseases and liver injury.[7–11] The effects were closely connected with the structure of 1,3-benzodioxole. Based on these, according to the structure activity relationship of fibrates, sesamol was introduced into the structure of brominated alkanoates by ether bond to synthesize the analogs of fibrates. The target compounds were expected to have both hypolipidemia and hepatoprotective properties.

In this study, we reported the synthesis of target compounds containing sesamol pharmacophore. As displaying in Scheme 1, compounds **1**–**4** were produced by the action of sesamol with different ethyl bromocarboxylates in the presence of potassium carbonate. Then, the ester bond was hydrolyzed under the condition of strong base, and then acidified to achieve the target compounds **5**–**8**. Target compounds **9**–**12** were issued by compounds **5**–**8** and sesamol under the action of EDCI and DMAP, respectively. Target compounds **1**–**12** were identified by ^{1}H NMR, ^{13}C NMR and EI-MS[12].

Firstly, we evaluated the hypolipidemic activity of these novel synthesized compounds by acute hyperlipidemia mice model induced by Triton WR 1339 (400 mg/kg). The relevant experimental methods have

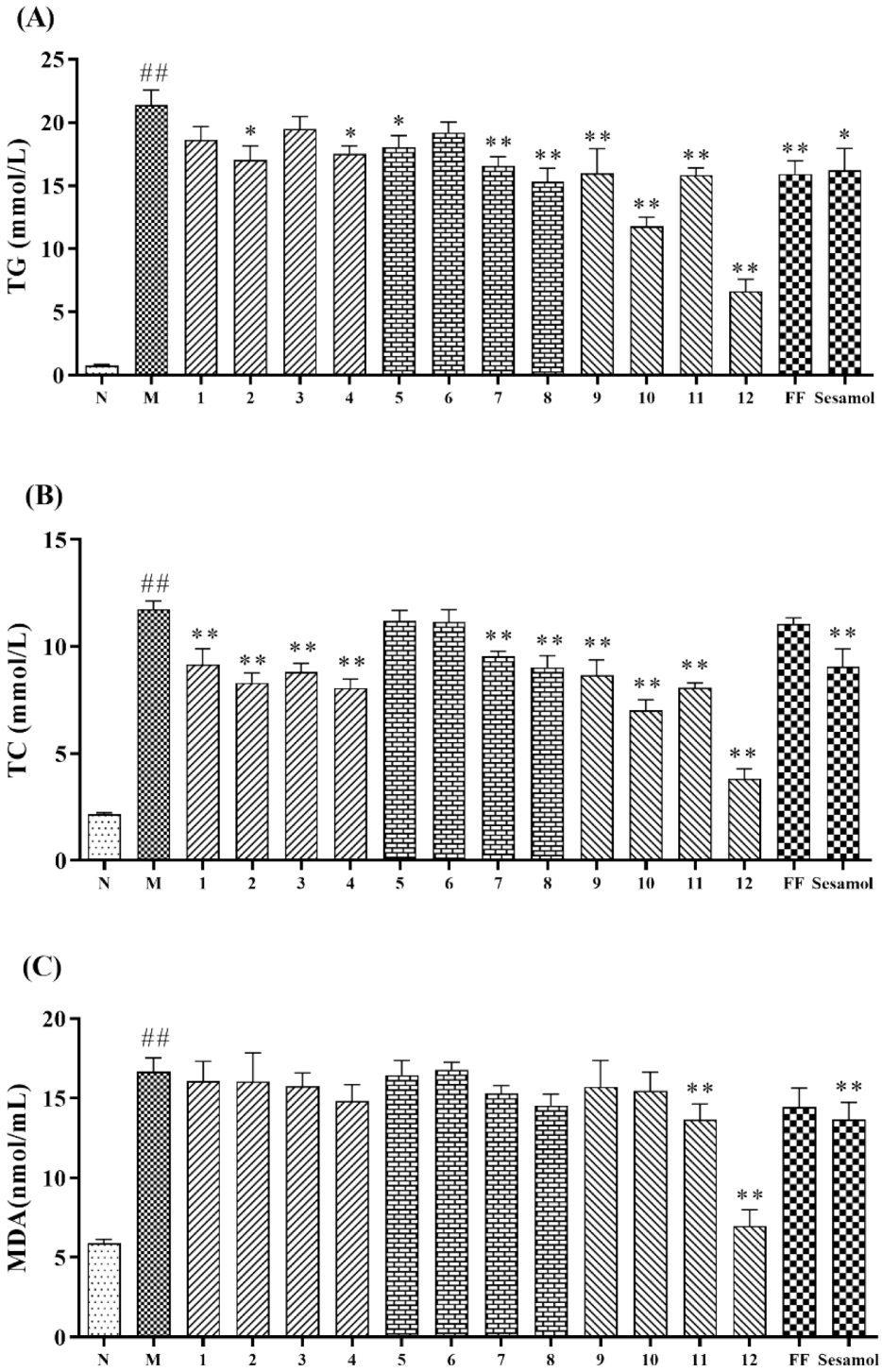


Fig. 2. Plasma Lipids (TG and TC) and MDA levels with the administration of compounds (**1**–**12**) in Triton WR-1339 induced hyperlipidemic mice. All results were expressed as the mean ± SEM, n = 8, $^{\#\#}P < 0.01$ vs Normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs Model group. The dosage of the test compounds was 0.362 mmol/kg, Sesamol was 0.362 mmol/kg and Fenofibrate (FF) was 0.036 mmol/kg.

been reported in the previous literature.[13,14] In this research, the levels of serum TG, TC and MDA were determined. The results displayed that compounds **2**, **4**, **5** and **7**–**12** obviously reduced serum TG at the dose of 0.362 mmol/kg. Compounds **1**–**4** and **7**–**12** declined significantly the levels of TC. The antioxidant activity showed that only compounds **11** and **12** significantly decreased the MDA of lipid peroxidation product in hyperlipidemic mice. The hypolipidemia structure–activity relationship showed that the longer the alkyl chain length between benzodioxalene structure and carboxyl or ester bond, the better the lipid-lowering effect in the homologue. On the whole, the lipid-lowering activity of the compounds containing two benzodioxalene structures was better than that of the compounds containing one benzodioxalene structure (Fig. 2).

Molecular docking studies were performed on the protein PPARα for the affinity activity, employing the docking procedure using MOE program installed on Win 10 software. The PDB code was 1K7L. Fenofibrate (FF) was selected as the reference. The results were showed that all the target have been found to dock well in the active site of PPARα (Table 1). From these molecular docking studies, we found that the PPARα affinity

Table 1
The target compounds with their PPARα receptor affinity activity by MOE docking scores.[a]

Compounds	Scores (kJ/mol·K)
1	−5.0996
2	−5.0275
3	−5.3548
4	−5.7275
5	−4.4528
6	−4.7678
7	−4.7846
8	−5.4045
9	−5.7004
10	−5.8941
11	−5.9192
12	−6.3502
Sesamol	−3.6841
GW409544[b]	−7.3294
Fenofibrate (FF)[c]	−5.7012

[a] PDB code: 1K7L.
[b] The X-ray crystal structure of PPAR α protein reported as complex with GW409544 deposited in Brookhaven Protein.
[c] Fenofibrate (FF) was selected as reference.

activity of aroxyalkyl carboxylic acids (compounds **5**, **6**, **7**, **8**) was worse than that of carboxylic esters. Among esters, sesamol esters (compounds **9**, **10**, **11**, **12**) have stronger PPARα affinity activity than ethanol esters (compounds **1**, **2**, **3**, **4**). Although the PPARα affinity activity of sesamol was weak, it has synergistic function, and the PPARα affinity activity was significantly enhanced after esterification with carboxylic acid. Moreover, we also found that the affinity activity between these compounds and PPARα was positively correlated with the alkyl chain length between aroxy and carbonyl groups. The longer the alkyl chain length is, the stronger the affinity activity is. Among these compounds, compound **12** displayed the excellent affinity activity with the score was −6.3502 kJ/mol·K, which was better than that of the positive drug FF (−5.7012 kJ/mol·K) and seems to have good affinity for the active residues of PPAR. The results of molecular docking were quite co-relatable to the results of lipid-lowering activity in Triton W*R* 1339 induced hyperlipidemic mice mode.

Among these novel compounds, the hypolipidemia and antioxidant activity of **12** was most effective compared to other synthesized compounds. The dose-dependent study on compound **12** (concentration at 0.121 mmol/kg, 0.362 mmol/kg and 1.086 mmol/kg body weight) was conducted on a different set of experimental animals. The results showed that compound 12 reduced TG by 10.4% ($P > 0.05$), 68.8% ($P < 0.01$) and 74.1% ($P < 0.01$), and decreased TC by 14.3% ($P > 0.05$), 67.3% ($P < 0.01$) and 70.1% ($P < 0.01$) at three doses, respectively, compared with model group. With the increase of dose, the activity of lowering blood lipid was greater. The findings suggested compound **12** exhibited concentration-dependent effects towards hypolipidemia activity (Fig. 3).

With the promising activity in hand, chronic hypolipidemia activity on **12** was performed on HFD-induced hyperlipidemia mice. Fenofibrate (FF) was selected as positive drug. The experimental process and methods have been reported in previous literature.[15] After intragastric administration, the serum biochemical indexes were detected as follows: total lipid profiles (TC, TG, HDL-C and HDL-C), antioxidant indexes (SOD, GSH-PX and MDA), inflammation factors (IL-6 and TNF-α), and liver transaminase levels (AST and ALT). In addition, hepatic histopathology and the expression of PPAR-α in liver were also determined.

Results of lipids-lowering (Fig. 4) showed that **12** significantly reduced serum TG ($P < 0.01$), and obviously decreased LDL-C ($P < 0.05$) compared with the model group, which was similar to FF, the positive drug. Compound **12** was also able to ameliorate the levels of TC ($P < 0.01$), while the standard agent FF did not show the activity of lowering TC ($P > 0.05$). From the lipid-lowering activity, it can be found that **12** revealed the better lipid-lowering activity than FF.

As showed in Fig. 5, the levels of SOD in **12** group was significantly increased ($P < 0.01$), compared with the model group. The levels of GSH-PX were increased, but did not demonstrate statistical significance ($P > 0.05$) both in **12** group and FF group. The levels of MDA, one of the indicators of lipid peroxidation products, was apparently reduced in **12** group ($P < 0.01$), compared with model group, while the levels of MDA in the positive drug FF group decreased, but did not show statistical significance ($P > 0.05$). As showed in Fig 6, **12** revealed the potent anti-inflammatory activity, the levels of TNF-α ($P < 0.05$) and IL-6 ($P < 0.01$) were significantly decreased in **12** group compared with the model group. The findings indicated that compound **12** could inhibit the inflammatory response to exhibit anti-inflammatory activity in hyperlipidemia.

To evaluate the liver function of hyperlipidemia mice, the levels of serum AST and ALT were determined, which were typically used as a detection index. As showed in Fig. 7, the levels of AST and ALT were obviously increased in the model group, as expected, compound **12** has the effects of protecting the liver. The levels of AST ($P < 0.01$) were significantly decreased in **12** group compared to model group, the levels of ALT were reduced by 6%, while there was no statistical difference. However, FF group did not show the significance compared with the model group.

In addition, the hepatoprotective effects were also evaluated by histopathological examine.[15,16] As showed in Fig. 8, oil red O staining in liver tissue sections showed that in the normal group, the liver cells were arranged neatly, the cell morphology was normal, and there were no obvious lipid droplets in the cytoplasm of liver cells. In contrast, a large number of irregular lipid droplets were found in the liver tissue of mice

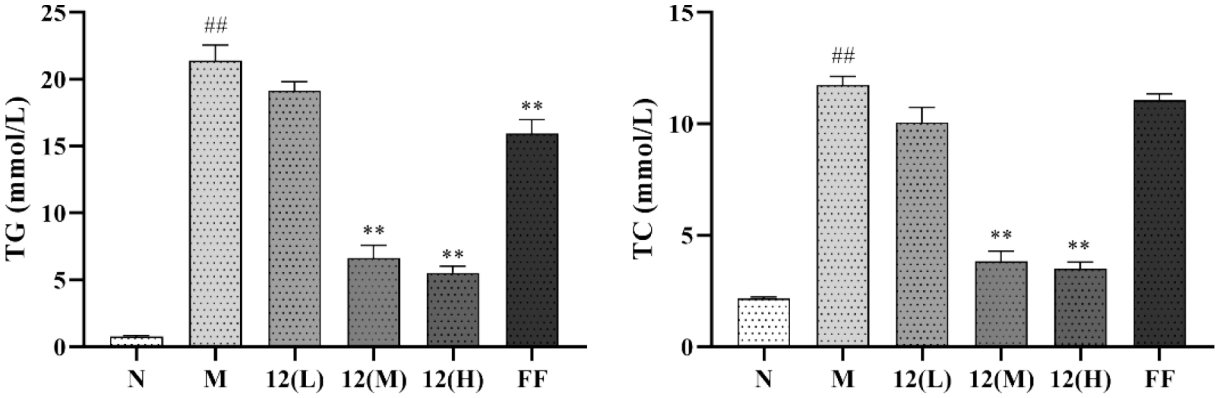


Fig. 3. Dose-response effects of compound **12** in Triton W*R* 1339 induced hyperlipidemic mice on plasma TG and TC. All results were expressed as the mean ± SEM, n = 8, $^{\#\#}P < 0.01$ vs Normal group; $^{**}P < 0.01$ vs Model group. The dosage of compound **12** was 0.121 mmol/kg, 0.362 mmol/kg and 1.086 mmol/kg respectively and FF was 0.036 mmol/kg.

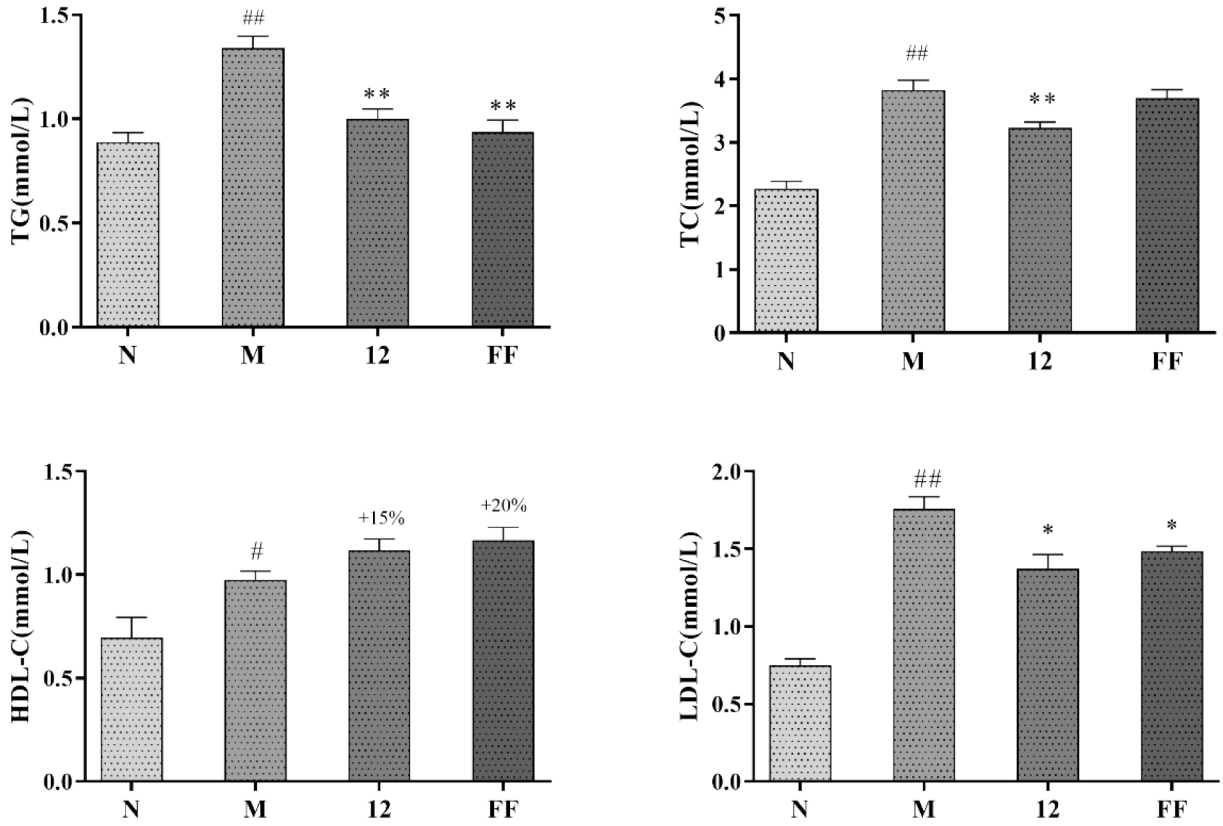


Fig. 4. Effects of **12** on plasma lipids in HFD-induced hyperlipidemic mice. Each parameter represents pooled data from 12 mice/group, and values were expressed as mean ± SEM, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs Normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs Model group. The dosage of **12** was 67 mg/kg and FF was 13 mg/kg.

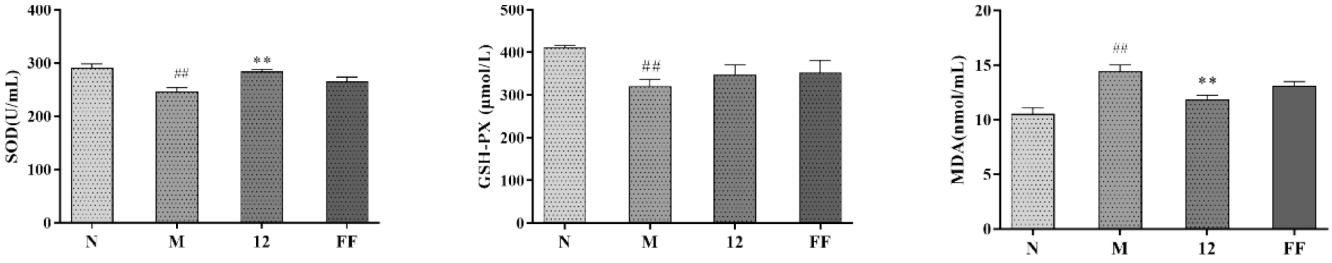


Fig. 5. Effects of **12** on serum SOD, MDA, and GSH-PX levels of the HFD-induced hyperlipidemic mice. The results were expressed as mean ± SEM, n = 12, $^{\#\#}P < 0.01$ vs Normal group; $^{**}P < 0.01$ vs Model group. The dosage of **12** was 67 mg/kg and FF was 13 mg/kg.

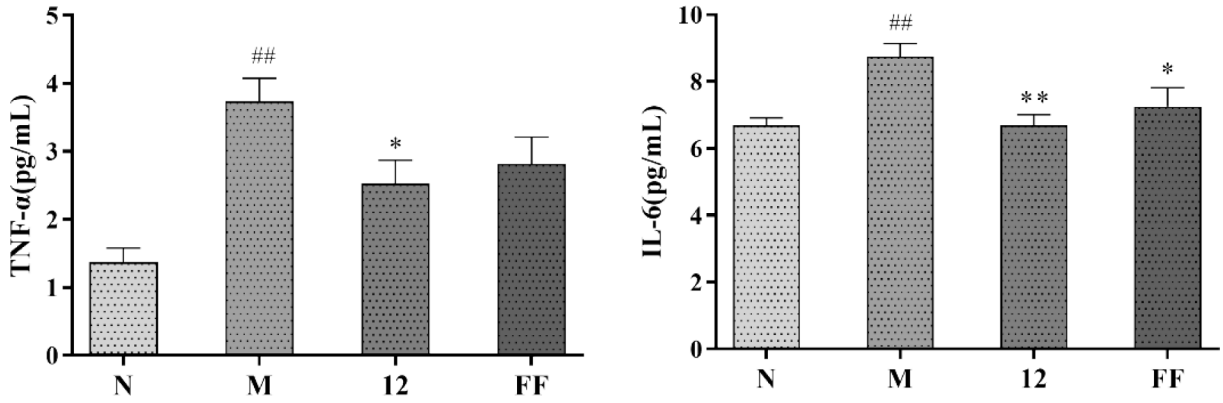


Fig. 6. Effects of **12** on serum TNF-α and IL-6 levels of the HFD-induced hyperlipidemic mice. The results were expressed as mean ± SEM, n = 12, $^{\#\#}P < 0.01$ vs Normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs Model group. The dosage of **12** was 67 mg/kg and FF was 13 mg/kg.

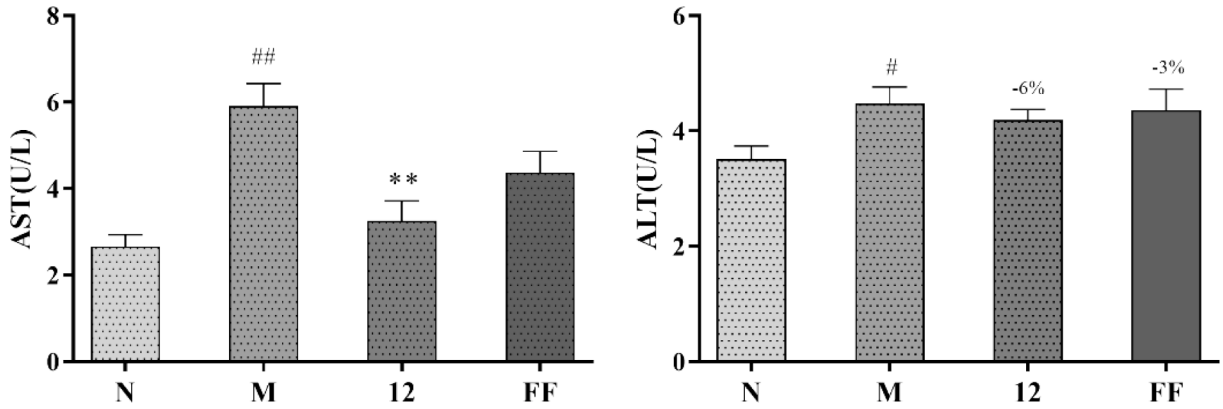


Fig. 7. Effects of **12** on serum AST and ALT levels of the HFD-induced hyperlipidemic mice. The results were expressed as mean ± SEM, n = 12, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs Normal group; $^{**}P < 0.01$ vs Model group. The dosage of **12** was 67 mg/kg and FF was 13 mg/kg.

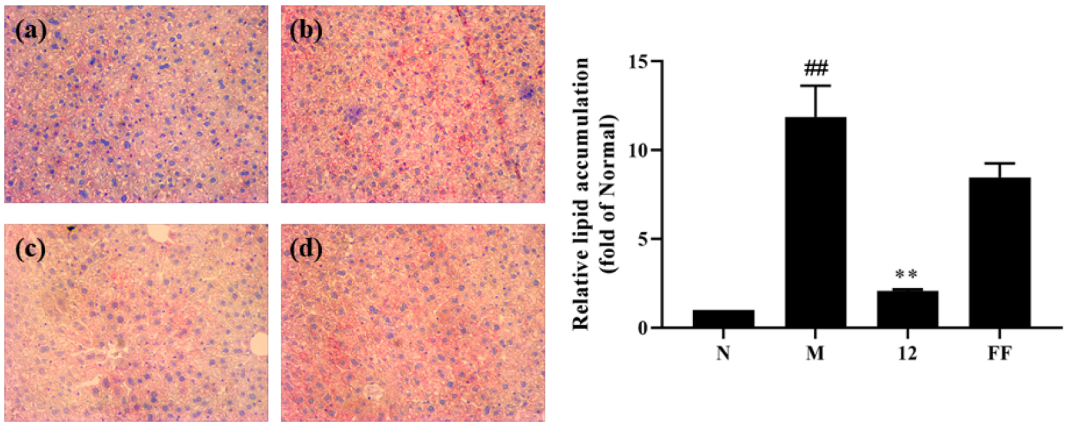


Fig. 8. Effects of **12** on hepatic lipid accumulation in HFD-induced hyperlipidemic mice (Sudan III and hematoxylin 100 ×). (a) Normal group mouse liver. (b) Model group mouse liver. (c) **12** group mouse liver. (d) FF group mouse liver. (e) Relative lipid accumulation in the hepatic. Data indicate the mean of six independent experiments ± SEM. $^{\#\#}P < 0.01$ vs Normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs Model group.

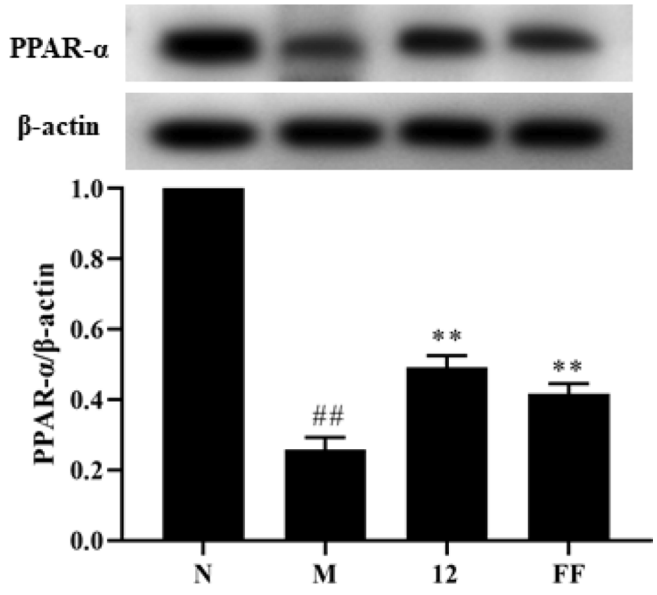


Fig. 9. The expression of PPAR-α in hepatic tissue of HFD-induced hyperlipidemic mice. The results were displayed as mean ± SEM, n = 6, $^{\#\#}P < 0.01$ vs Normal group; $^{**}P < 0.01$ vs model group. The dosage of **12** was 67 mg/kg and FF was 13 mg/kg.

in the model group. Interestingly, in **12** and FF groups mice liver, the amount of lipid droplets in the liver tissue of mice was much lower than that in the model group. The content of lipid droplets in the liver of mice in each group was quantitatively expressed as showed in Fig. 8**(e)**. The content of lipid droplets in **12** group and FF group was significantly lower than that in the model group. These results showed that **12** improved lipid accumulation condition in the hepatic of hyperlipidemia mice.

Based on previous study and the lipids-lowering activity, we speculated that the hypolipidemia mechanism of compound **12** may be through the activation of the nuclear transcription factor peroxisome proliferator α (PPAR-α). Hence, the expression of PPAR-α protein in liver tissue was examined. The procedure has been reported in previous papers.[16–18] As outlined in Fig. 9, the expression of PPAR-α in model group was significantly reduced compared with normal group. As expected, **12** showed significantly activation of PPAR-α compared with the model group ($P < 0.01$), and the expression of PPAR-α in FF group was also obviously increased ($P < 0.01$). Interestingly, **12** significantly upregulate the expression of PPAR-α more than FF. The results suggested that the molecular mechanism of hypolipidemic effect of compound **12** may be the same as that of fibrates.

In this research, a series of novel compounds were synthesized. Among these compounds, compound **12** provides a multifunctional activity, such as antioxidant, anti-inflammation, hypolipidemia and hepatoprotective effects, which were beneficial for ameliorating hyperlipidemia. Fibrates, such as fenofibrate and clofibrate, can activate PPAR-α to exert the hypolipidemic activity.[19] Compound **12** also can

decrease blood lipids by activating PPAR-α protein. The accumulation of fat in the liver will lead to lipid peroxidation, increase the number of reactive oxygen species and produce oxidative stress,[20] which in turn causes inflammation, and the whole chain reaction will aggravate liver injury. The hepatoprotective effect of **12** can be explained as the accumulation of fat in the liver decreases as well as the antioxidant and anti-inflammation.

In conclusion, a series of 1,3-benzodioxole-based fibrate derivatives were designed, synthesized and evaluated for lipids-lowering and hepatoprotection by experimentally acute and chronic hyperlipidemic mice models. The results displayed that **12** was regarded as a worthy studying compound for both hypolipidemia and hepatoprotection. **12** not only significantly regulated lipid profiles but also improved hepatic injury in the hyperlipidemia mice which may upregulate the expression of PPAR-α protein and reduce fat accumulation in the liver. Furthermore, additional activity such as antioxidation and anti-inflammation confirmed and reinforced that **12** was a novel potential agent with multifunctional effects. Therefore, **12** seem to be a candidate and is expected to be further developed into a new hypolipidemic drug.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127898.

References

1 Johnathan R. *Curr Clin Pharmacol*. 2017;12:152.
2 McQueen MJ, Hawken S, Wang X, et al. *Lancet*. 2008;372:224.
3 Rezapour M, Payani E, Taran M, et al. *Med J Islamic Repub Iran*. 2017;31:80.
4 Fernández AJ, Mijares AH. *Clínica E Investigación En Arteriosclerosis*. 2012;24:19.
5 Haghighian MK, Alipoor B, Sadat BE, et al. *Health Promot Perspect*. 2014;4:90.
6 Kuhad A, Chopra K. *Exp Brain Res*. 2008;185:411.
7 Katayama S, Sugiyama H, Kushimoto S, et al. *J Agric Food Chem*. 2016;64:4908.
8 Umeda-Sawada R, Fujiwara Y, Abe H, et al. *J Nutr Sci Vitaminol*. 2003;49:442.
9 Khansai M, Boonmaleerat K, Pothacharoen P, et al. *BMC Complem Altern M*. 2016;16:205.
10 Robert VC, Laurie JC, Okinaka L, et al. *Nutr Cancer*. 2001;39:66.
11 Lv D, Zhu CQ, Liu L. *Int J Clin Exp Patho*. 2015;8:5733.
12 Xie YD, Wang W, Li N, Liu JP, Shi YH, Sun M, Xu XY, Chen J, CN110407801A, 2019, 11.
13 Xie YD, Xu YH, Chen ZZ, et al. *Biomed Pharmacother*. 2017;95:1749.
14 Xie YD, Chen ZZ, Li N, et al. *Biomed Pharmacother*. 2018;99:715.
15 Chen ZZ, Xie YD, Shao LH, et al. *Bioorg Med Chem Lett*. 2018;28:3787.
16 Shao LH, Bai Y, Wang QT, et al. *Bioorg Med Chem Lett*. 2019;29, 126723.
17 Wang XP, Wang PF, Bai JQ, et al. *Biomed Pharmacother*. 2019;118, 109268.
18 Yao F, Yang GD, Xian YS, et al. *Food Funct*. 2019;10:5789.
19 Guay DR. *Ann Pharmacother*. 1999;33:1083.
20 Grunfeld C, Kotler DP, Shigenaga JK, et al. *Am J Med*. 1991;90:154.