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Design, Synthesis and Hypolipidemic Activity of Novel Hydrazones of Nicotinic acid Hydrazide

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Abstract: Synthesis of novel *N*-acyl hydrazones of nicotinic acid hydrazide *via* condensation of nicotinic acid hydrazide **3** with the corresponding aldehydes **1, 2** are described. Their hypolipidemic activities were evaluated in high cholesterol diet fed rat model. The hydrazones were found to decrease the levels of serum total cholesterol, LDL cholesterol and triglycerides in hyperlipidemic rats to a greater degree than the reference gemfibrozil.

Keywords: *2-Tosyloxybenzaldehyde,4-Tosyloxybenzaldehyde, Fibrates, Cholesterol, Triglycerides and Hypolipidemia.*

1 Introduction

Nicotinic acid (in large doses) lowers the levels of both cholesterol and triglycerides in the plasma. It has been used as anti-hyperlipoproteinemic drug. Its action by reduction of very low-density protein production and inhibition of lipolysis in fatty tissue. Nevertheless, it does not have an effect on the general synthesis of cholesterol in the organism. Nicotinic acid in combination with bile acid-reducing drugs can lower the level of cholesterol and triglycerides by 10–30% [1-5]. Fibric acid as fenofibrate, clofibrate and bezafibrate are called fibrates (2-methyl-2-phenoxypropanoic acid) [6]. The fibrates act as hypolipidemic agent, gemfibrozil having a 5-phenoxypentanoic acid moiety instead of the fibric acid moiety and considered hypolipidemic agent as the other classical fibrates [7]. Hyperlipidemia is defined as the presence of raised or abnormal levels of lipids and or lipoproteins in the blood. Lipid and lipoprotein abnormalities are extremely common in the general population and are regarded as a highly modifiable risk factor for cardiovascular diseases (CVD) [8]. Today, in most of the developed and developing countries, hyperlipidemia and thereby atherosclerosis is the leading cause of cardiac illness and deaths [9]. Worldwide, it causes deaths almost twice as many as those caused by cancer and 10 times as many as those caused by accidents [10]. The major lipoprotein effects of fibrates are to reduce the levels of plasma triglycerides by 30–50% and to increase the levels of high-density lipoprotein (HDL) cholesterol by 5–6% [11-13]. The fibrates are selective to activate the alpha-isotype of the receptors peroxisome proliferator-activated receptors (PPARs) [14]. Activation of PPAR-alpha modulates the expression of several genes involved in lipoprotein metabolism. The activity of lipoprotein lipase is increased and results in an increase in the clearance of circulating triglyceride-rich lipoproteins [15]. The apolipoprotein in C-III (apoC-III) inhibits lipoprotein lipase [16]. Fibrates decrease the biosynthesis of apo C-III [17]. Hence, low apoC-III levels will further enhance the clearance of triglyceride-rich lipoproteins. In addition to the antihyperlipidemic effect, the fibrates have anti-inflammatory action as evidenced by a reduction in acute phase reactants such as C-reactive protein as well as a number of cytokines, IL-6, TNF-alpha and interferon-gamma. This pleiotropic effect of fibrates contributes in their coronary risk reducing ability [17]. Several studies indicate that fibrates decrease the levels of the factors promoting coagulation and increase the fibrinolysis; the dual hypolipidemic/antiplatelet effects of fibrates reduce the risk of atherosclerosis and its thrombotic complications [16] which is the major cause of coronary artery diseases [18]. Many studies reveal the ability of fibrates to inhibit the activity of aldose reductase enzyme agents to prevent the progression of secondary diabetic complications [19]. Our strategy synthesis of hydrazones of nicotinic acid hydrazides has the hypolipidemic activity.

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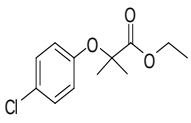

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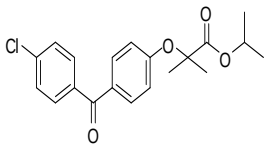

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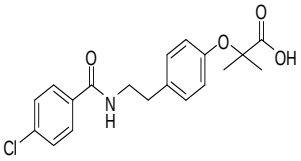

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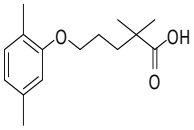
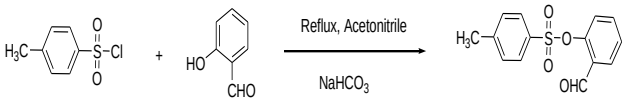


Gemfibrozil

2 Results and discussion

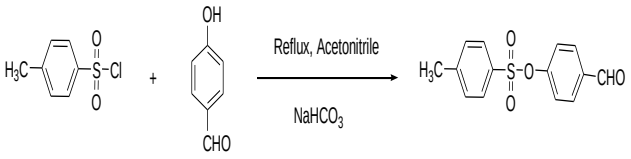
2.1 Chemistry

Recently, environmental pollution and the economic crisis have become very important global challenges. As a result, industrial and manufacturing units including chemical and pharmaceutical companies show a propensity to environmentally friendly green and sustainable protocols. 2-Tosyloxybenzaldehyde **(1)** was simply prepared *via* the reaction of salicylaldehyde with tosyl chloride under reflux in acetonitrile in the presence of sodium bicarbonate (**equation 1**).



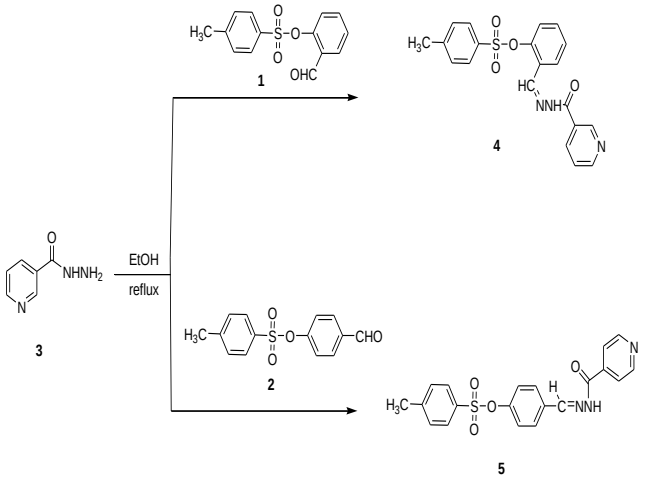
equation 1 **1**

4-Tosyloxybenzaldehyde **(2)** was simply prepared *via* the reaction of 4-hydroxybenzaldehyde with tosyl chloride in acetonitrile under reflux in the presence of sodium bicarbonate (**equation 2**) [20].



equation 2 **2**

IR spectrum of compound (**1**) revealed the disappearance of absorption band of OH group related to the salicylaldehyde OH group and appearance two new absorption bands at 1364 and 1157 cm^{-1} characterized of the SO_2 group. Its ^{1}H-NMR spectrum (δ DMSO-d_6) showed an increase of aromatic protons signals and new singlet signal due to CHO group at δ 9.98 ppm and the methyl group signal at δ 2.43 ppm. The hydrazones (**4**), (**5**) were prepared *via* the reaction of 2-tosyloxybenzaldehyde (**1**) or 4-tosyloxybenzaldehyde (**2**) with nicotinic acid hydrazide (**3)** under reflux in ethanol using few drops of glacial acetic acid as a catalyst (**Scheme 1**). IR spectra of compounds (**4),** (**5**) revealed the disappearance of absorption band related to CHO groups and appear new CO groups of hydrazide at 1692 and 1688 $cm^{-1,}$ respectively and appearance new absorption bands at 3210 and 3230 cm^{-1}, respectively, characterized to NH groups. Their ^{1}H-NMR spectra (δ DMSO-d_6) showed in addition an increase of aromatic protons signals, new singlet signals due to NH groups at δ 12.15 and 12.28 ppm, respectively.



Scheme 1

2.2 Hypolipidemic activity

The hypolipidemic activity of the synthesized compounds was studied in the high cholesterol diet (HCD) fed hyperlipidemic rat model [21] against hyperlipidemic control, by oral administration of 20 mg/kg of the compounds. The results were compared to the reference drug; gemfibrozil. Compound (**5**) exhibited the maximum hypolipidemic activity expressed as 10.22 % and 27.69 % reduction in the levels of TC and TG respectively compared to HCD control group, that more than reference gemfibrozil which afforded 4.65 % and 22.68 % reduction in the levels of TC and TG respectively. The compound (**4**) reduced TC and TG by 6.55 % and 14.87% respectively, compared to HCD control group where the % of reduction in TC was more than the reference gemfibrozil which afforded 4.65% but the % of reduction in TG was less than gemfibrozil which afforded 22.68% (**Table 1).** In addition, compounds (**4**), (**5**) exhibited the maximum hypolipidemic activity expressed as 13.55 % and 8.55 % reduction in the levels of LDL-C, respectively and the reduction % is more than the reference gemfibrozil which afforded 5.13 %. On the other hand, all compounds **(1, 2, 4, and 5)** led to an elevation of HDL-C compared to HCD control but no one produced % elevation more than the

reference drug (gemfibrozil) (**Table 2**). Decreasing the ratio of LDL-C (bad cholesterol) to that of HDL-C (good cholesterol) plays a role in reducing the risk of atherosclerosis [22]. The compound (**4**) had the lowest LDL-C/ HDL-C ratio (**Table 2**).

Table 1: Effect of compounds 1,2,4,5 and gemfibrozil on serum TC and TG of hyperlipidemic rats.

Groups	TC (mg/dL)	% fall	TG (mg/dL)	% fall
Normal control	71.83±1.50		50.00 ±1.14	
HCD fed	115.33±1.60		92.50±0.71	
HCD fed + Gemfibrozil	109.50 ±6.27**	4.65	71.52±3.04**	22.68
HCD fed + 1	112.0 ±1.80**	2.88	80.25±5.8**	13.24
HCD fed + 2	115.22±1.90	-1.04[a]	93.48±2.3	-1.05[a]
HCD fed + 4	107.77±3.40**	6.55	78.82±5.90**	14.78
HCD fed + 5	103.54±5.70**	10.22	66.88±2.8**	27.69

-Significantly different from hyperlipidemic control group at **p < 0.01.
-[a] negative values indicate increase in the level of serum TG.
-Reduction in the level of serum TC and TG is calculated as percentage from the hyperlipidemic control group.

Table 2: Effect of tested compounds **1,2,4,5** and gemfibrozil on serum HDL, LDL and LDL/ HDL ratio.

Groups	HDL (mg/dL)	% raise	LDL (mg/dL)	% fall	LDL/ HDL
Control	18.56±1.52		39.50±2.71		2.13
HCD fed	11.50±0.50		80.05± 1.9		6.96
HCD fed+ gemfibrozil	14.50± 0.52**	25.54	75.89±7.24	5.13	5.23
HCD fed + 1	13.25±1.02**	15. 21	82.70 ±3.90**	-3.31	[a]6.24
HCD fed+ 2	12.51±2.66**	8.69	76.70 ±3.10**	4.18	6.13
HCD fed+ 4	13.78±1.32**	19.8	69.20 ± 2.34**	13.55	5.02
HCD fed+ 5	14.12±0.66**	22.78	73.20 ±3.34**	8.55	5.18

-Significantly different from hyperlipidemic control group at **p < 0.01.
-[a] negative values indicate increase in the level of serum LDL.
- Elevation or reduction in the levels of serum HDL or LDL is calculated as percentage from the hyperlipidemic control group.

3 Experimental

3.1 Chemistry

All melting points were recorded on Melt-Temp II melting point apparatus. IR spectra were measured as Brucker Alpha Fourier Transform (FT-IR). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker at 400 MHz using TMS as an internal reference and DMSO-d_6 as a solvent. (Chemical shift (δ) values are expressed in parts per million (ppm)). The elemental analyses were carried out on a Perkin-Elmer 240C Microanalyzer. All compounds were checked for their purity on TLC plates.

Synthesis of 2-Tosyloxybenzaldehyde (1)

A mixture of salicylaldehyde (0.1 moles), tosyl chloride (0.1 moles) and potassium bicarbonate (0.1 mole) in acetonitrile. The reaction mixture was stirred under reflux for 2 h at 90°C. After the reaction was complete, the solvent evaporated and the residue was poured into ice-cold water. The product was filtered off, washed with water, dried and crystallized from methanol**.** Solid crystal, Colorless,Yield: (94%), M. p. 67°C; ^{1}H-NMR (DMSO-d_6): 9.98 (s, 1H CHO), 7.84-7.72 (m, 3H, Ar-H), 7.54-7.48 (m, 4H, Ar-H), 7.22 (m, 1H, Ar-H), 2.43 (s, 3H, CH_3); IR (KBr) cm^{-1}1682 (C=O), 1368, 1156 (SO_2). ^{13}C-NMR: δ 190.06, 157.15, 138.25, 135.34, 132.43, 131.71, 130.13, 129.17, 126.21, 121.11, 116.51, 21.09. Anal. Calcd. for $C_{14}H_{12}O_4S$ (276.35): C (60.86%), H (4.38%), S (11.60%). Found: C (60.93%), H (4.29%), S (11.71%).

Synthesis of 4-Tosyloxybenzaldehyde (2)

A mixture solution of 4-hydroxybenzaldehyde (0.1 moles), tosyl chloride (0.1 moles) and potassium bicarbonate (0.1 moles) in acetonitrile. The reaction mixture was refluxed for 1 h. After the reaction was complete, the solvent was evaporated and the residue was poured into ice-cold water.

The product was filtered off, washed with water, dried and crystallized from methanol. Solid crystal,Colorless,Yield: (87%),

M. p. 44°C; ^{1}H-NMR (DMSO-d_6): 10.5 (s, 1H CHO), 7.84-7.72 (m, 3H, Ar-H), 7.54-7.48 (m, 4H, Ar-H), 7.22 (m, 1H, Ar-H), 2.35 (s, 3H, CH_3); IR (KBr) cm^{-1} 1695 (C=O), 1375, 1160 (SO_2). ^{13}C-NMR: δ 192.16, 158.15, 137.25, 136.34, 132.43, 132.06, 130.96, 129.17, 126.21, 121.11, 116.51, 23.19. Anal. Calcd. For $C_{14}H_{12}O_4S$ (276.35): C (60.86%), H (4.38%), S (11.60%). Found: C (60.90%), H (4.41%), S (11.69%).

Synthesis of 2 (2-Nicotinoylhydrazono) methyl phenyl4-methylbenzenesulfonate (4)

A mixture of nicotinic acid hydrazide **(3)** (0.01 mole) and 2-Tosyloxybenzaldehyde **(1**) (0.01mole) in ethanol (20 mL) using few drops of glacial acetic acid as a catalyst. The reaction mixture refluxed with stirring for 3 h. (The reaction monitored by TLC technique using toluene: ethyl acetate 7:2). The reaction mixture was left to cool at room temperature. The formed solid precipitate was filtered off and crystallized from methanol. Solid crystal, Colorless, Yield: (64%),

M. p. 158 °C; ^{1}H-NMR (DMSO-d_6): 12.28 (s, 1H NH), 8.98-7.53 (m, 12H, Ar-H), 2.45 (s, 3H, CH_3), 2.43 (s, H, CH= N); IR (KBr) cm^{-1}3210(NH), 1692 (C=O), 1378, 1160 (SO_2). ^{13}C-NMR: δ 167.80, 153.47, 152.21, 150.87, 144.76, 138.25, 136.50, 134.56, 133.21, 132.21, 128.81, 128.18, 127.80, 126.18, 123.47, 113.47, 23.97. Anal. Calcd. for $C_{20}H_{17}N_3O_4S$ (395.09): C (60.75%), H (4.33%), N (10.63%), S (8.11%). Found: C (60.90%), H (4.41%), N (10.68%), S (8.29%).

Synthesis of 4-((2-Nicotinoylhydrazono)methyl)phenyl4-methylbenzenesulfonate (5)

A mixture of nicotinic acid hydrazide **(3)** (0.01mole) and 4-Tosyloxybenzaldehyde **(2**) (0.01 mole) in ethanol (20 mL) using few drops of glacial acetic acid as a catalyst. The reaction mixture refluxed with stirring for 4 h. (The reaction monitored by TLC technique using hexane: ethyl acetate 9:1). The reaction mixture was left to cool at room temperature. The formed solid precipitate was filtered off and crystallized from ethanol. Solid crystal, Colorless,Yield: (71%), M. p. 171 °C; ^{1}H-NMR (DMSO-d_6): 12.15 (s, 1H NH), 8.98-7.53 (m, 12H, Ar-H), 2.43 (s, 3H, CH_3), 2.41 (s, H, CH= N); IR (KBr) cm^{-1} 3230 (NH), 1688 (C=O),1375, 1162 (SO_2). ^{13}C-NMR: δ 165.77, 155.47, 152.21, 150.87, 146.76, 139.25, 136.50, 135.56, 134.87, 132.21, 129.81, 128.98, 127.17, 126.98, 123.49, 116.47, 22.97. Anal. Calcd. for $C_{20}H_{17}N_3O_4S$ (395.09): C (60.75%), H (4.33%), N (10.63%), S (8.11%). Found: C (60.87%), H (4.51%), N (10.71%), S (8.19%).

3.2 Hypolipidemic activity

3.2.1. Animals

Forty-two male adult Wistar albino rats weighing 170±10 g have been used. Animals were obtained from the animal house Faculty of Science, Sohag University, Egypt and fed on the standard diet of commercial rat chow and tap water. Rats were left to acclimatize to the environment for one week prior to inclusion in the experiment. Rats were maintained under standard laboratory conditions at an ambient temperature of 25±2 °C, with 12 hours light/12 hours dark cycles. Animals were given a free access to food and water up to 24 hours prior to their use. This study was approved by the Institutional Animal Care and Use Ethical Committee of Faculty of Medicine, Sohag University.

3.2.2 Experimental protocol

Induction of hyperlipidemia

Hyperlipidemia was induced by feeding the rats with high cholesterol diet (HCD) prepared by mixing normal rodent chow with 4% cholesterol and 1% cholic acid (w/w) (Sigma, USA) for 30 successive days [21].

Treatment

Rats were randomly divided into seven groups each containing six animals. Group I; normal control was fed a standard diet and orally received 0.5% carboxymethyl-cellulose (CMC) as a drug vehicle. The remaining six groups received HCD for 30 days. Group II is hyperlipidemic control was orally received (CMC) 0.5% as a drug vehicle; group III was treated with gemfibrozil orally in a dose of 20 mg/kg per day as a standard hypolipidemic drug [24]. Rats in group IV, V, VI and VII were received compounds 1, 2, 4 and 5, respectively in a dose of 20 mg/kg/day orally (1/10 of LD_{50} dose) was previously determined according to the method of Lorke [23]. The treatment in the last six groups was commenced 15 days after the start of induction of hyperlipidemia and continued for 15 days duration [21].

Sample collection and biochemical analysis

At the end of experimental period, rats of all groups were fasted for 16 hours and sacrificed by cervical dislocation and blood samples were collected. Serum was separated by centrifugation at 3000 rpm for 10 min at 4 ºC and kept at -20ºC until the time of analysis. Estimation of total cholesterol (TC), triglycerides (TGs), and high-density lipoprotein cholesterol (HDL-C) levels was done by commercially available diagnostic kits (Randox -UK) using Jenway UV-vis spectrophotometer (Jasco spectrophotometer USA). The procedure was conducted according to the manufacturer's description and the concentrations of TC, TGs, HDLc were expressed in mg/dl. Serum low-density lipoprotein cholesterol (LDL-C) concentration was

determined according to Friedewald's formula [25].

Statistical Analysis

In the present experimental work, differences between control and treatment groups were analyzed by one way analysis of variance (ANOVA) using "graph Pad Prism version 4.0". All values are given as mean ± standard deviation (SD) of mean. Differences were considered to be significant at $p < 0.05$.

4 Conclusions

Novel hydrazones of nicotinic acid hydrazide were prepared and their hypolipidemic activity was screened in hyperlipidemic rats. Among the synthesized hydrazones, the compound (**5**) was found to be the most active hypolipidemic agent affording significant hypocholesterolemic and hypotriglyceridemic activities.

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