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Short communication

Synthesis and antiplatelet activity of thioaryloxyacids analogues of clofibric acid

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Abstract

The thiophene-, benzothiazole- and pyridine-thioaryloxyacids analogues of clofibric acid were synthesized and their antiplatelet activity was screened. Some compounds exhibited antiaggregating properties. The platelet-related haemostasis was measured on a PFA-100® analyzer using bull blood.
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1. Introduction

The fibrates are a widely used class of lipid-modifying agents that decrease plasma triglycerides [1]; they activate the peroxisome proliferator-activated receptors (PPARs), belonging to the nuclear hormone receptor superfamily, inducing alterations in transcription of genes encoding for proteins that control lipoprotein metabolism [2]. In addition to the antilipidemic effect, the fibrates and the related drug gemfibrozil act on plasma concentration of proteins involved in coagulation, fibrinolysis and platelet action; several studies indicate that fibrates decrease the levels of factors promoting coagulation and increase fibrinolysis [3]. This dual antilipidemic/antiplatelet effect of fibrates makes them interesting candidates for the prevention and treatment of thrombotic disorders.

Reported in previous publications, the synthesis of chiral analogues of clofibric acid, the active metabolite of clofibrate, with the asymmetric carbon next to the carbonyl group was described [4]; the two enantiomers in which the substituents on stereogenic center are methyl and ethyl groups showed a dependence of biological activity on chirality, with the (*R*)-compounds having greater antiplatelet effect and less toxicity than (*S*)-isomers [5]. Subsequently, chiral analogues of gemfibrozil were synthesized and all the tested compounds inhibit the human platelet aggregation [6].

Herein we report the synthesis and the pharmacological evaluation of analogues of clofibric acid with a heterocycle instead of benzene ring and with a thioisobutyrate side-chain (Fig. 1). A simple and efficient method for platelet-related haemostasis analysis, involving the use of animal blood samples, was adopted.

2. Chemistry

The **4a–e** acids were synthesized as outlined in Schemes 1 and 2. The compounds **3a–d** were readily obtained by reaction of ethyl 2-bromoisobutyrate (**1**) with sodium salts of commercially available thiols **2a–d**, in refluxing EtOH. The compound **3e** was synthesized by chlorination of **3a** with *N*-chlorosuccinimide in glacial CH_3COOH at room temperature. All the esters **3a–e** were hydrolyzed in presence of KOH to give the acids **4a–e**.

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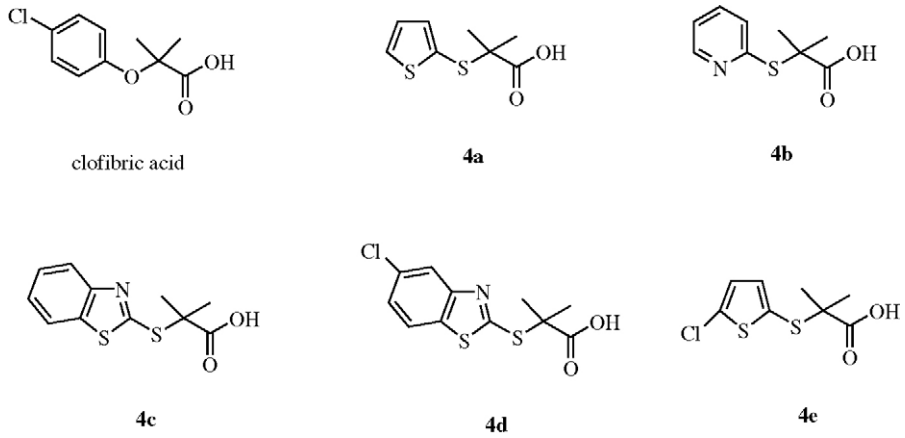


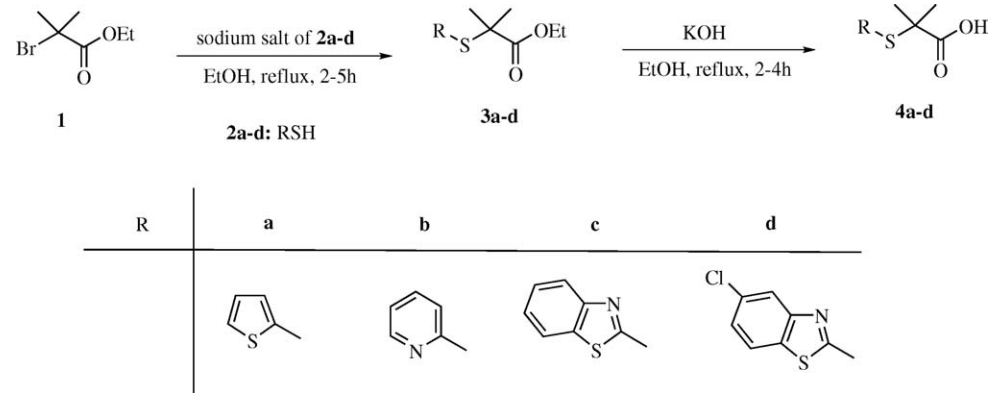
Fig. 1. Analogues of clofibric acid.

3. Pharmacological evaluation and discussion

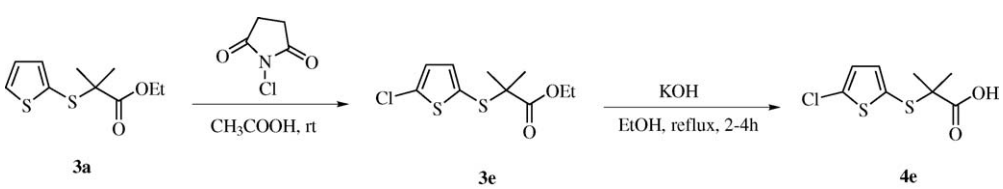
Effects of compounds **4a–e** on platelet aggregation were monitored with a PFA-100® instrument [7] using anticoagulated bull blood. Gemfibrozil and acetylsalicilic acid (ASA) were used as reference drugs.

The PFA-100® is a platelet function analyzer which allows to study reactivity of blood platelets under conditions similar to those in circulation. In this apparatus, blood samples are introduced into a disposable cartridge and aspirated through a small aperture into a membrane coated with collagen–ADP (COL/ADP) or collagen–epinephrine (COL/EPI). In response to stimulation by the components of membrane, platelets form a stable plug that occludes the aperture and blood flow stops. The time from the beginning of the test until formation of an occluding platelet plug is called "closure time" (CT) and indicates the platelet function in the sample. If an occluding platelet plug does not form after 300 s, the analysis is stopped.

Table 1 shows the results of the experiments on platelet aggregation induced by ADP, detected at concentrations of **4a–e** ranging from 1.0 to 5.0 mM. An inhibitory effect was found for the basic compound gemfibrozil (entry 6). The ASA was screened as negative control, because COL/ADP test cartridges detect platelet dysfunctions not mediated by ASA (entry 7) [8]. Compound **4d** showed the best activity among all the tested compounds (entry 4), revealing a valuable dose-dependent pattern. Compound **4e** is only effective at high concentrations (entry 5). The others have low effect and no dose-effect relationships (entries 1–3). The presence of a chlorine atom on the heterocyclic system seems to increase the activity: compounds without this substituent showed lower anti-aggregating properties (entries 1–3) than corresponding sub-



Scheme 1.



Scheme 2.

Table 1
Antiplatelet effect of compounds **4a–e**

Entry	Compound	Percentage inhibition				
		1 mM	2 mM	3 mM	4 mM	5 mM
1	**4a**	0.91	0.45	2.27	0.45	4.1
2	**4b**	0	0.91	0	1.82	0
3	**4c**	2.27	2.27	4.51	6.82	15
4	**4d**	0.91	6.82	11.36	100	100
5	**4e**	0	0	0	36.82	100
6	**Gemfibrozil**	0.91	0.91	26.36	100	100
7	**ASA**	0	0	0.91	0.45	5.4

stituted ones (entries 4–5). The size of heterocycle may also influence the results, with benzothiazolthioaryloxyacids (entries 3–4) more active than thiophene- and pyridine-derivatives (entries 1–2). The more active compound **4d** fits in each of two conditions.

4. Conclusions

In conclusion, we synthesized analogues of clofibric acid with a thioisobutyrate side-chain bound to a heterocyclic group. Some compounds revealed a dose-dependent antiplatelet activity, measured on a PFA-100® instrument, using bull blood. The simplicity of method and the easy availability of animal blood allow a quick and cheap measurement of a standardized in vitro bleeding time, reflecting platelet function.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on an Electrothermal IA 9100 apparatus and are uncorrected. The infrared spectra were recorded on a FT-IR 1600 Perkin–Elmer spectrometer. The NMR spectra were run at 300 MHz on a Varian spectrometer; chemical shifts (δ) are reported in ppm. Microanalyses were carried out with a Eurovector Euro EA 3000 model analyzer and the analytical results are within 0.4% of the theoretical values. GC analyses were run on an autosystem GC Perkin Elmer apparatus using a fused silica capillary column (30 m, 0.53 mm ID), SPB-5 Supelco. Commercial reagents were used as received from Aldrich.

*5.2. General experimental procedure for the synthesis of esters **3a–d***

To a solution of sodium (395 mg, 17.2 mmol) in absolute EtOH (15 ml), ethyl bromoisobutyrate (**1**) (3.355 g, 17.2 mmol) and the heterocyclic thiol **2a–d** (17.2 mmol), both dissolved in absolute EtOH (10 ml), were added under N_2 atmosphere. After stirring for 2–5 h at reflux, the solvent was removed under reduced pressure. The residue was poured into H_2O (45 ml) and extracted with ethyl ether (3 × 40 ml). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (eluent cyclohexane/ethyl acetate).

*5.2.1. Ethyl 2-methyl-2-(thien-2-ylthio)propanoate (**3a**)*

Yellow oil, 3.849 g, 16.7 mmol, 97% yield; ^{1}H NMR ($CDCl_3$): δ 1.25 (t, 3H, CH_2CH_3), 1.50 (s, 6H, $(CH_3)_2C$), 4.14 (q, 2H, CH_2CH_3), 7.01–7.04 (dd, 1H, Ar), 7.16 (d, 1H, Ar), 7.45 (d, 1H, Ar); IR (neat) 1724 cm^{-1}.

*5.2.2. Ethyl 2-methyl-2-(pyridin-2-ylthio)propanoate (**3b**)*

Yellow oil, 3.555 g, 14.8 mmol, 92% yield; ^{1}H NMR ($CDCl_3$): δ 1.18 (t, 3H, CH_2CH_3), 1.67 (s, 6H, $(CH_3)_2C$), 4.16 (q, 2H, CH_2CH_3), 6.97–7.01 (dd, 1H, Ar), 7.20 (d, 1H, Ar), 7.48 (dt, 1H, Ar), 8.38 (dd, 1H, Ar); IR (neat) 1730 cm^{-1}.

*5.2.3. Ethyl 2-(1,3-benzothiazol-2-ylthio)-2-methylpropanoate (**3c**)*

Yellow oil, 3.630 g, 12.9 mmol, 75% yield: ^{1}H NMR ($CDCl_3$): δ 1.20 (t, 3H, CH_2CH_3), 1.75 (s, 6H, $(CH_3)_2C$), 4.20 (q, 2H, CH_2CH_3), 7.33 (t, 1H, Ar), 7.43 (t, 1H, Ar), 7.78 (d, 1H, Ar), 7.92 (d, 1H Ar); IR (neat) 1731 cm^{-1}.

*5.2.4. Ethyl 2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]-2-methylpropanoate (**3d**)*

Oil, 2.879 g, 9.12 mmol, 53% yield; ^{1}H NMR ($CDCl_3$): δ 1.21 (t, 3H, CH_2CH_3), 1.75 (s, 6H, $(CH_3)_2C$), 4.20 (q, 2H, CH_2CH_3), 7.28–7.32 (dd, 1H, Ar), 7.67 (d, 1H, Ar), 7.88 (d, 1H, Ar); IR (neat) 1733 cm^{-1}.

*5.3. Experimental procedure for the synthesis of ethyl 2-[(5-chlorothien-2-yl)thio]-2-methylpropanoate (**3e**)*

To a solution of ethyl 2-methyl-2-(thien-2-ylthio)propanoate (**3a**) (1.843 g, 18.0 mmol) in glacial CH_3COOH (20 ml), *N*-chlorosuccinimide (1.068 g, 8.0 mmol) was added under N_2 atmosphere. After stirring for 3 h at room temperature, the solvent was removed under reduced pressure. The residue was dissolved in ether (15 ml) and washed with 2 M NaOH solution (15 ml) and with H_2O (15 ml). The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The residue was purified by column chromatography on silica gel (eluent cyclohexane/chloroform 7:3) to give the ester **3e** (yellow oil, 635 mg, 2.39 mmol, 30% yield). IR (neat) 1725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.27 (t, 3H, CH_2CH_3), 1.50 (s, 6H, $(CH_3)_2C$), 4.15 (q, 2H, CH_2CH_3), 6.86 (d, 1H, Ar), 6.96 (d, 1H, Ar).

*5.4. General experimental procedure for the synthesis of acids **4a–e***

To a solution of 1% alcoholic KOH (7 ml), the ester **3a–e** was added (0.87 mmol) and the mixture was refluxed for 2–4 h under stirring. The reaction was quenched with 2 M KOH (25 ml) and the solvent was removed under reduced pressure. The aqueous residue was extracted with ether (2 × 25 ml), acidified with 2 N HCl and re-extracted with CH_2Cl_2 (3 ×

25 ml). The organic layer was washed with H_2O (65 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purificated by crystallization using hexane as solvent.

*5.4.1. 2-Methyl-2-(thien-2-ylthio)propanoic acid (**4a**)*

White solid, 141 mg, 0.70 mmol, 80% yield; m.p. 50–52 °C; IR (neat) 1692 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.52 (s, 6H, $(CH_3)_2C$), 7.04–7.07 (dd, 1H, Ar), 7.23 (d, 1H, Ar), 7.48 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$): δ 25.28 ($CH_3)_2$, 52.37 (*C*), 127.95, 132.34 and 138.06 (Ar), 179.58 (*C*=O); MS *m*/*z* 201.3 (M^-).

*5.4.2. 2-Methyl-2-(pyridin-2-ylthio)propanoic acid (**4b**)*

White solid, 103 mg, 0.52 mmol, 60% yield; m.p. 81–83 °C; IR (neat) 1701 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.65 (s, 6H, $(CH_3)_2C$), 7.27 (t, 1H, Ar), 7.43 (d, 1H, Ar), 7.73 (dt, 1H, Ar), 8.95 (dd, 1H, Ar); ^{13}C NMR ($CDCl_3$) δ 26.27 ($CH_3)_2$, 52.58 (*C*), 121.81, 125.98, 138.85 and 147.05 (Ar), 157.11 (Ar), 174.84 (*C*=O); MS *m*/*z* 198.0 (MH^+), 220.0 (M^+ + Na).

*5.4.3. 2-(1,3-Benzothiazol-2-ylthio)-2-methylpropanoic acid (**4c**)*

White solid, 38 mg, 0.35 mmol, 40% yield; m.p. 154–157 °C; IR (neat) 1714 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.76 (s, 6H, $(CH_3)_2C$), 7.43 (t, 1H, Ar), 7.52 (t, 1H, Ar), 7.82 (d, 1H, Ar), 7.94 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$): δ 25.98 ($CH_3)_2$, 54.54 (*C*), 127.57, 125.96 and 127.22 (Ar), 134.90 (=*C*–S), 151.15 (=*C*–N), 158.69 (N=*C*–S), 173.69 (*C*=O); MS *m*/*z* 254.0 (MH^+), 276.0 (M^+ + Na).

*5.4.4. 2-[(5-Chloro-1,3-benzothiazol-2-yl)thio]-2-methylpropanoic acid (**4d**)*

White solid, 133 mg, 0.46 mmol, 53% yield; m.p. 125–130 °C; IR (neat) 1712 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.78 (s, 6H, $(CH_3)_2C$), 7.37 (dd, 1H, Ar), 7.71 (d, 1H, Ar), 7.92 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$): δ 26.02 ($CH_3)_2$, 54.68 (*C*), 121.60, 122.15 and 126.27 (Ar), 123.70 (=*C*–S), 133.40 (*C*Cl), 152.30 (=*C*–N), 168.20 (N=*C*–S), 174.11 (*C*=O); MS *m*/*z* 288.0 (MH^+), 309.0 (M^+ + Na).

*5.4.5. 2-[(5-Chlorothien-2-yl)thio]-2-methylpropanoic acid (**4e**)*

Yellow solid, 123 mg, 0.52 mmol, 60% yield; IR (neat) 1699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.52 (s, 6H, $(CH_3)_2C$), 6.87 (d, 1H, Ar), 7.03 (d, 1H, Ar); ^{13}C NMR ($CDCl_3$): δ 25.24 ($CH_3)_2$, 52.58 (*C*), 127.33 (Ar), 128.52 (Ar), 135.02 (Ar), 137.95 (Ar), 179.50 (*C*=O); MS *m*/*z* 235 (M^-).

5.5. Pharmacology

5.5.1. Preparation of blood samples

Bull blood samples were supplied by a slaughter-house in Pegognaga (Mantova, Italy) and drawn directly into plastic reservoirs containing 3.8% (0.129 M) buffered sodium citrate (one part anticoagulant to nine parts blood). The haematocrit value of anticoagulated bull blood ranged from 36% to 38%. Aliquots of 900 μl were incubated with 100 μl of known concentrations of **4a–e** acids at room temperature for 30 min. Test compounds and gemfibrozil were dissolved in NaOH 1 N and saline solution (1:10 v/v) and monitored at the final concentrations of 1, 2, 3, 4 and 5 mM. ASA was dissolved in DMSO and saline solution (1:20 v/v) and tested at the same concentrations. Experiments showed that there was no difference using NaOH or DMSO as vehicles in the aggregation assays.

5.5.2. Antiplatelet assays

The tests were performed on PFA-100® instrument (Dade-Behring, Milano, Italy) using only COL/ADP test cartridges, that revealed a better sensitivity and data reproducibility with bull blood respect to COL/EPI test cartridges. Blood samples were gently mixed and aliquots of 800 μl were pipetted into the smaller opening (sample reservoir opening) of the test cartridge, avoiding air entrapment in the sample reservoir. The CT was determined and expressed in seconds. The percentage of platelet aggregation inhibition was calculated in comparison with a control sample additioned with the proper vehicle.

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