Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 817–821

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Antiplatelet Activity of Gemfibrozil Chiral Analogues

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Received 19 September 2001; accepted 3 January 2002

Abstract—The chiral analogues of gemfibrozil 5-(2,5-dimethylphenoxy)-2-methylpentanoic acid and 5-(2,5-dimethylphenoxy)-2-ethylpentanoic acid were synthesized in optically active form using (*S*)-4-(1-methylethyl)-2-oxazolidinone as chiral auxiliary. All compounds inhibit human platelet aggregation. From these data, one can surmise that all tested compounds and gemfibrozil act at the platelet level with different mechanism than that of ASA, even if with a different potency.

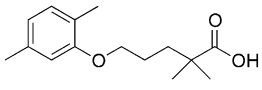
Introduction

Gemfibrozil, 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid, is a widely used hypolipidemic drug related to fibrates.[1,2] In previous works, the synthesis of chiral analogues of clofibrate was described[3] and it exhibited different biological activity related to the stereochemistry.[4] Likewise, as part of an ongoing research on fibrates, chiral analogues of gemfibrozil were prepared with an asymmetric carbon atom next to the carboxyl group (Fig. 1). Recent studies have shown that in addition to its effects on lipids, gemfibrozil may modulate the fibrinolytic system.[5] Therefore, the optical pure analogues were tested in biological experiments in order to evaluate their capability to inhibit the human platelet aggregation.

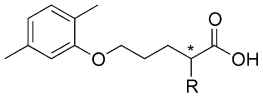
Chemistry

The **5a**–**b** acids were synthesized as outlined in Scheme 1. The ethyl 5-(2,5-dimethylphenoxy)pentanoate (**3**) was obtained by S_N2 reaction from the commercially available ethyl 5-bromopentanoate (**1**) and sodium 2,5-dimethylphenolate (**2**) in EtOH at reflux. Treatment of **1** with LDA and alkylation of the anion formed with methyl iodide or ethyl iodide gave in good chemical yield the monoalkylated compounds *rac*-**4a**–**b**, that were easily hydrolyzed in the presence of KOH to give the racemic acids *rac*-**5a**–**b**.

The (*S*)-4-isopropyl-2-oxazolidinone seems to be very efficient as chiral auxiliary in the optical resolution of the α-aryloxyacetic acids[6] because of its well established capacity of resolution via silica gel column and its ability to hydrolyze the *N*-acylated heterocycles under mild conditions.



Gemfibrozil



Chiral analogues of gemfibrozil

Figure 1.

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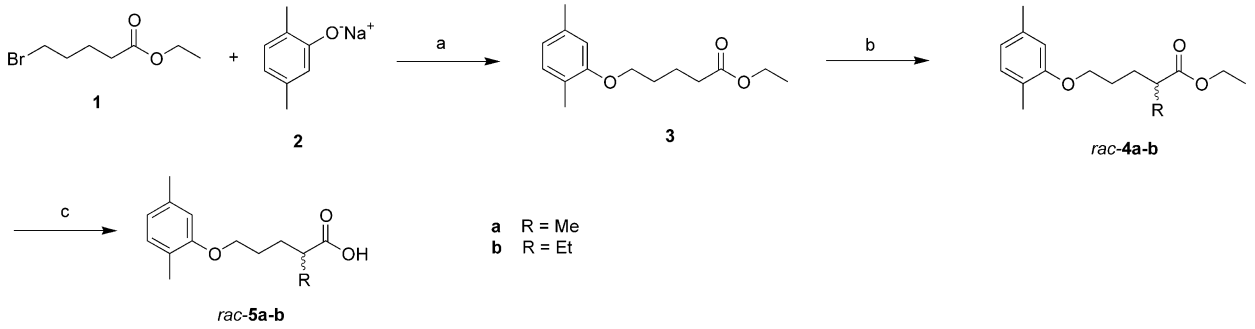
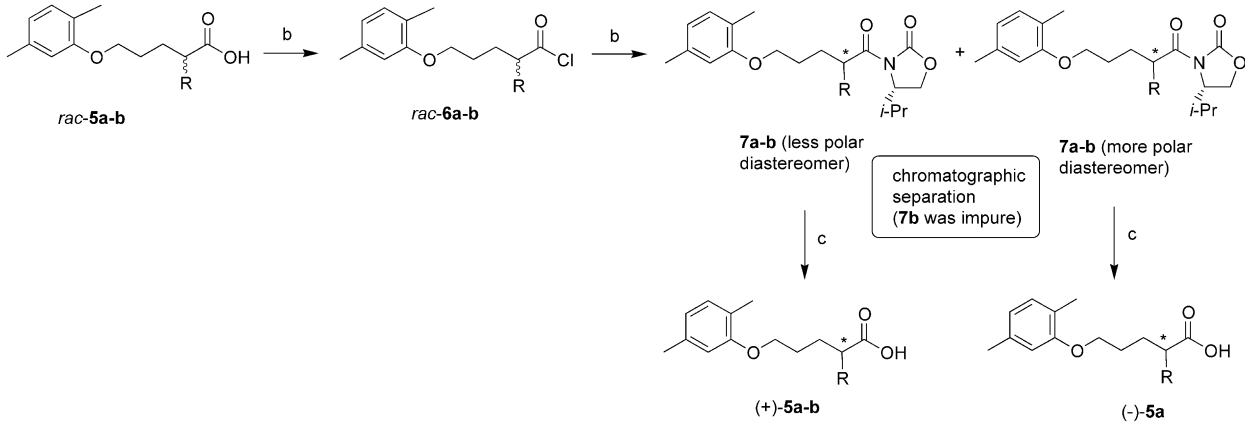
0960-894X/02/$ - see front matter
PII: S0960-894X(02)00021-5

After their transformation into the corresponding imides with (*S*)-4-isopropyl-2-oxazolidinone, the chromatographic resolution of the gemfibrozil chiral analogues **5a**–**b** was performed. The chiral auxiliary was *N*-acylated[7] with acyl chlorides *rac*-**6a**–**b**. The equimolar diastereomeric mixtures of chiral imides **7a**–**b** were obtained in good chemical yield, detected by TLC and GC and separated on silica gel;[8] unfortunately, only the less polar isomer of **7b** mixture was obtained in diastereomerically pure form. The cleavage with lithium hydroperoxyde[9] led to both enantiomers of optically active acid **5a** and only dextrorotatory isomer of **5b** (the more polar imide of **7b** was not hydrolyzed).[10] Unfortunately, the levorotatory enantiomer of acid **5b** was not obtained in enantiomerically pure form (Scheme 2).

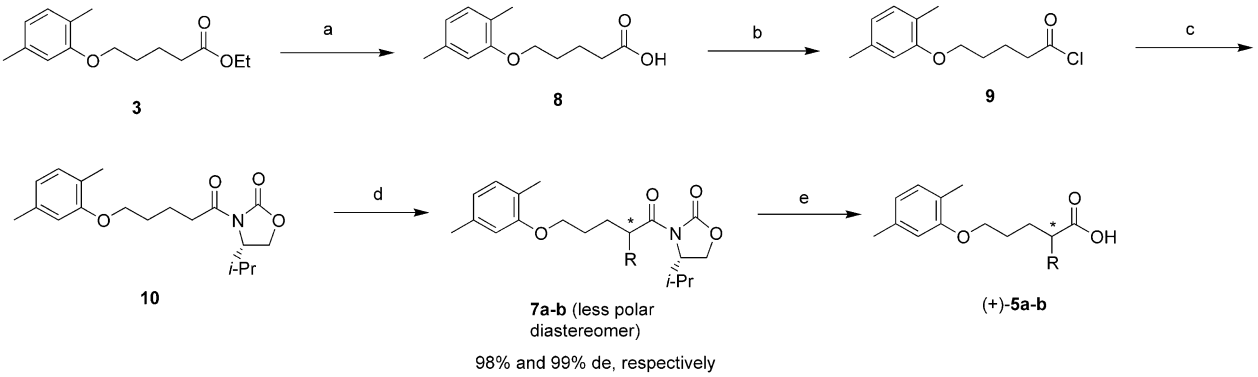
Alternatively, the less polar diastereomers of the mixtures **7a**–**b** were synthesized in diastereomerically enriched form by diastereoselective alkylation (Scheme 3). After basic hydrolysis of ester **3**, the acid **8** was treated with thionyl chloride, to give the **9** acyl chloride. The coupling with the Evans (*S*)-oxazolidinone afforded the imide **10**. The asymmetric alkylation[11] of **10** was performed using methyl iodide and ethyl iodide as electrophiles, giving only the less polar diastereomers of the mixtures **7a**–**b**, which, as illustrated above, were obtained by chromatographic separation. The diastereomeric excesses of 98 and 99%, respectively, were evaluated by means of GC analysis, by assuming that the ratio of areas were equivalent to the molar ratios. After purification on silica gel of the less polar imides



Scheme 1. Reagents and conditions: (a) EtOH, reflux; (b) Mel or Etl, LDA, HMPA, THF, −78 °C/−20 °C; (c) KOH, EtOH, reflux.



Scheme 2. Reagents and conditions: (a) $SOCl_2$, CH_2Cl_2, 40 °C, 98%; (b) BuLi, oxazolidinone, THF, −78 °C, 81–85%; (c) LiOOH, THF/H_2O, rt, 88–97%.



Scheme 3. Reagents and conditions: (a) KOH, EtOH, 70 °C, 91%; (b) $SOCl_2$, CH_2Cl_2, 40 °C, 97%; (c) BuLi, oxazolidinone, THF, −78 °C, 94%; (d) LDA, RI, THF, −78 °C, 52–69%; (e) LiOOH, THF/H_2O, rt, 88–97%.

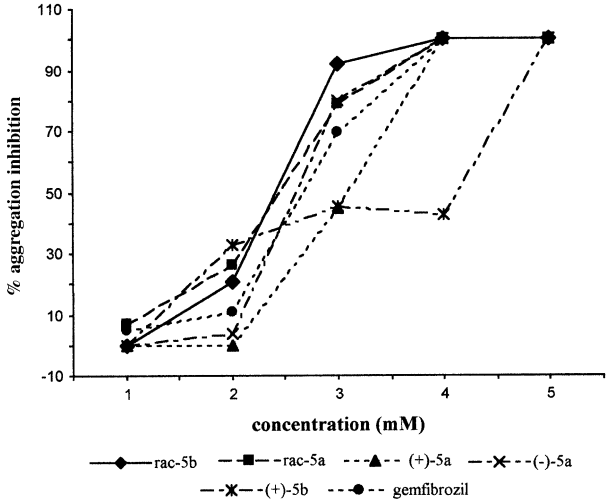


Figure 2. Effect of gemfibrozil analogues on human platelet aggregation.

7a–**b**, their hydrolysis was carried out with lithium hydroperoxyde and led to the (+)-enantiomers of **5a**–**b** acids in enantiomerically pure form. Studies are in progress to use the (*R*)-oxazolidinone as chiral auxiliary in asymmetric alkylation to obtain the levorotatory acids **5a**–**b**.

Pharmacology

Traditionally optical aggregometry[12] was used to assess platelet function, however this assay is highly dependent on sample preparation and technical procedure; so, as a result, data from various laboratories can be quite variable. Hence we decided to assess platelet aggregation using a recently developed Platelet Function Analyzer PFA-100®, a system which allows for rapid evaluation of platelet function[13,14] on small samples of anticoagulated whole blood based on work described by Kratzer and Born.[15,16] This method has been designed to provide an in vitro measure of primary platelet-related hemostasis simply, quickly, quantitatively and accurately to aid in the routine screening of patients with potential hemorrhagic risk due to abnormal platelet plug formation.[17] Herein we report the results obtained by comparing acids *rac*-**5a**, *rac*-**5b**, (+)-**5a**, (−)-**5a**, (+)-**5b** with gemfibrozil and acetylsalicylic acid (ASA).[18] Unfortunately, (−)-**5b** was not supplied and thus not tested. All tested compounds revealed a dose-dependent inhibitory activity toward human platelet aggregation. Moreover, a similar inhibitory effect is detectable in their precursor, the well-known gemfibrozil (Fig. 2) used as basic reference compound. The inhibitory activity of these compounds is generally detectable at concentrations ranging from 1 to 5 mM. Their activity is probably affected by the size of the substituents: in this case compound *rac*-**5b** seems to be the most active, probably because of the substitution of the methyl group with an ethyl group. As shown in Table 1, the two racemates *rac*-**5a** and *rac*-**5b** and the enantiomer (−)-**5a** are more active (their IC_{50} being 2.47, 2.42 and 2.61 mM for, respectively, entries 1, 2 and 4), when compared with the two (+)-enantiomers which have an IC_{50} of 3.09 mM for (+)-**5a** (entry 3) and 4.12 mM for (+)-**5b** (entry 5). Considering the well-known activity of ASA against platelet aggregation, we used ASA as a well established reference compound. Utilizing the PFA-100® assay, we confirmed that ASA exerts an anti-aggregating activity at low doses, being its IC_{50} 0.05 mM (entry 7) as described with other methodologies.[19] These data clearly indicate that ASA is still the most potent compound in comparison with the new synthesized analogues of gemfibrozil. All these results were obtained using COL/EPI Cartridges, able to detect platelet dysfunction induced by exposure to platelet inhibiting agents. The other test was performed using COL/ADP Cartridges which are able to indicate if an abnormal result obtained with the COL/EPI Test Cartridge may have been caused by the presence of ASA in the blood of the patient (Table 2). In accordance with our expectation, when the test was performed on ASA (entry 7) and on compounds *rac*-**5a**, *rac*-**5b** and (+)-**5a** (entries 1, 2 and 3), Test Cartridges detected the effect of ASA or similar medications. On the contrary when the test was performed on compounds (−)-**5a** and (+)-**5b** and on gemfibrozil, (entries 4, 5 and 6) we detected an inhibitory effect on platelet aggregation also by ADP Cartridges. From these data we can surmise a different

Table 1. Inhibitory effect of gemfibrozil analogues against human platelet aggregation by COL/EPI cartridges

Entry	Compd	IC_{50}[a]
1	*rac*-**5a**	2.47
2	*rac*-**5b**	2.42
3	(+)-**5a**	3.09
4	(−)-**5a**	2.61
5	(+)-**5b**	4.12
6	Gemfibrozil	2.67
7	Acetylsalicilic acid	0.05

[a]Average 50% inhibitory concentration (mM).

Table 2. Inhibitory effect of gemfibrozil analogues against human platelet aggregation by COL/ADP cartridges

		% Inhibition				
Entry	Compd	1×10^{-3} M	2×10^{-3} M	3×10^{-3} M	4×10^{-3} M	5×10^{-3} M
1	*rac*-**5a**	0	0	0.49	4.39	24.39
2	*rac*-**5b**	0	0	3.30	5.51	2.57
3	(+)-**5a**	0	0	0.97	0	5.36
4	(−)-**5a**	0	0	0	0	100
5	(+)-**5b**	0	0	0	0	100
6	Gemfibrozil	0	2.5	17	100	100
7	Acetylsalicilic acid	0	0	0	6.76	17.12

mechanism of action of these last three compounds in comparison with *rac*-**5a**, *rac*-**5b** and (+)-**5a** which seem to work as ASA.

In conclusion, this work describes a simple method for the synthesis and covalent resolution of chiral analogues of gemfibrozil. This procedure offers various advantages over the conventional resolution methods such as a cheaper access to the enantiomers, and their preparation on a multigram scale. However, the simplicity of the PFA-100® system would facilitate its use in preliminary screening tests to find new anti-aggregating compounds. The use of this method allows us to demonstrate that the synthesized gemfibrozil analogues exert an anti-aggregating effect.

Acknowledgements

This research was supported by Italian MURST. The authors gratefully acknowledge the collaboration of Dr. Barbara Casolari and Dr. Anna Maria Cenci (Laboratorio Analisi Chimico Cliniche, Ospedale Civile S. Agostino, Modena).

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8. **General procedure**: BuLi (10 mL, 1.6 M in hexane, 16.0 mmol) was added to a solution of (*S*)-4-(1-methylethyl)-2-oxazolidinone (2.06 g, 16.0 mmol) in dry THF (40 mL), under N_2 atmosphere, at −78 °C. After 15 min, *rac*-**6a**–**b** (16.0 mmol) in dry THF (20 mL) was added dropwise and the mixture was stirred for 1 h at −78 °C. After quenching with saturated aqueous NH_4Cl (15 mL), the THF was evaporated and the mixture was extracted twice with CH_2Cl_2 (30 mL). The combined organic layers were washed with saturated solutions of $NaHCO_3$ (30 mL) and NaCl (30 mL), dried and concentrated under reduced pressure. The residue was purified on silica gel (cyclohexane/ethyl acetate 9:1) to give the separated diastereomers of **7a**–**b** mixtures. **Less polar diastereomer of 7a**: oil, GC 270 °C rt 6.0 min; $[\alpha]_D$ +69.02 (*c* 0.9, $CHCl_3$); IR (neat) 1700, 1792 cm^{-1}; MS 226.15, 347.20 *m*/*z*; ^1H NMR ($CDCl_3$) δ 0.87 (dd, 6H, $(CH_3)_2CH$), 1.23 (d, 3H, CH_2CHCH_3), 1.54–1.69 (m, 1H, $CHHCHCH_3$), 1.71–1.97 (m, 3H, $CHHCHCH_3$ and $ArOCH_2CH_2$), 2.15 (s, 3H, CH_3 aromatic), 2.28 and 2.30–2.49 (s and m, 4H, CH_3 aromatic and $(CH_3)_2CH$), 3.73–3.84 (m, 1H, CH_2CHCH_3), 3.85–3.97 (m, 2H, $ArOCH_2$), 4.13–4.22 (m, 2H, $NCHCH_2$), 4.37–4.42 (m, 1H, NCH), 6.60 (s, 1H, aromatic), 6.63 (d, 1H, aromatic), 6.98 (d, 1H, aromatic). **More polar diastereomer of 7a**: oil, GC 270 °C rt 6.3 min; $[\alpha]_D$ +25.07 (*c* 0.7, $CHCl_3$); IR (neat) 1700, 1776 cm^{-1}; MS 226.15, 347.20 *m*/*z*; ^1H NMR ($CDCl_3$) δ 0.87 (dd, 6H, $(CH_3)_2CH$), 1.17 (d, 3H, CH_2CHCH_3), 1.57–1.68 (m, 1H, $CHHCHCH_3$), 1.77–1.97 (m, 3H, $CHHCHCH_3$ and $ArOCH_2CH_2$), 2.14 (s, 3H, CH_3 aromatic), 2.28 and 2.30–2.38 (s and m, 4H, CH_3 aromatic and $(CH_3)_2CH$), 3.77–3.85 (m, 1H, CH_2CHCH_3), 3.87–3.98 (m, 2H, $ArOCH_2$), 4.18 (dd, 1H, $NCHCHH$), 4.25 (t, 1H, NCHCHH), 4.42–4.48 (m, 1H, NCH), 6.59 (s, 1H, aromatic), 6.63 (d, 1H, aromatic), 6.97 (d, 1H, aromatic). **Less polar diastereomer of 7b**: oil, GC 270 °C rt 6.8 min; $[\alpha]_D$ +6.80 (*c* 1.9, $CHCl_3$); IR (neat) 1610, 1694, 1774 cm^{-1}; MS 240.20, 361.30 *m*/*z*; ^1H NMR ($CDCl_3$) δ 0.85–0.95 (m, 9H, $(CH_3)_2CH$) and CH_3CH_2CH), 1.56–1.89 (m, 4H, $ArOCH_2(CH_2)_2$), 2.15 (s, 3H, CH_3 aromatic), 2.28 and 2.30–2.40 (s and m, 4H, CH_3 aromatic and $(CH_3)_2CH$), 3.79–3.84 (m, 1H, CH_3CH_2CH), 3.85–3.94 (m, 2H, $ArOCH_2$), 4.14–4.18 (m, 2H, $NCHCH_2$), 4.40–4.45 (m, 1H, NCH), 6.59 (s, 1H, aromatic), 6.63 (d, 1H, aromatic), 6.97 (d, 1H, aromatic).
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10. Compound (+)-**5a**: white solid, mp 78 °C (recrystallized from petroleum ether); $[\alpha]_D$ +10.4 (*c* 1.3, MeOH); IR (nujol) 1463, 1702 cm^{-1}; MS 122.05, 236.15 *m*/*z*; ^1H NMR ($CDCl_3$) δ 1.22 (d, 3H, CH_3), 1.62–1.70 (m, 1H, $CHHCHCH_3$), 1.79–1.92 (m, 3H, $CHHCHCH_3$ and OCH_2CH_2), 2.16 (s, 3H, CH_3 aromatic), 2.29 (s, 3H, CH_3 aromatic), 2.49–2.59 (m, 1H, $CHCH_3$), 3.94 (t, 2H, $ArOCH_2$), 6.60 (s, 1H, aromatic), 6.64 (d, 1H, aromatic.), 6.98 (d, 1H, aromatic). Compound (−)-**5a**: white solid, mp 78 °C (recrystallized from petroleum ether); $[\alpha]_D$ −9.7 (*c* 0.7, MeOH). Compound (+)-**5b**: white solid; mp 85 °C (recrystallized from petroleum ether); $[\alpha]_D$ +7.5 (*c* 2.0, MeOH); IR (nujol) 1453, 1697 cm^{-1}; MS 122.05, 250.15 *m*/*z*; ^1H NMR ($CDCl_3$) δ 0.95 (t, 3H, $CHCH_2CH_3$), 1.48–1.94 (m, 6H, $CHCH_2CH_3$ and $ArOCH_2(CH_2)_2$), 2.15 (s, 3H, CH_3 aromatic), 2.28 (s, 3H, CH_3 aromatic), 2.36–2.44 (m, 1H, CH_2CHCH_3), 3.91–3.93 (m, 2H, $ArOCH_2$), 6.60 (s, 1H, aromatic), 6.64 (d, 1H, aromatic), 6.98 (d, 1H, aromatic).
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14. As a measure of platelet function in the PFA-100® system, the process of platelet aggregation builds a platelet thrombus thereby gradually diminishing and finally arresting the blood flow. The PFA-100® instrument (supplied by Dade Behring-Milano), determines the time from the start of the test until the platelet plug occludes the aperture, and reports that time interval as the Closure Time (CT): results of the PFA-100® test are reported by the instrument as CT in seconds. The CT is an indicator of platelet function in the analyzed whole blood sample. It uses two disposable cartridges: a Collagen/Epinephrine (COL/EPI) Test Cartridge and a Collagen/ADP (COL/ADP) Test Cartridge (purchased from Dade Behring-Milano). The COL/EPI Test Cartridge is the primary cartridge used to detect platelet dysfunction induced by intrinsic platelet defects, von Willebrand disease or exposure to platelet inhibiting agents. The COL/ADP Test Cartridge is used to indicate if an abnormal result obtained with the COL/EPI Test Cartridge may have been caused by the effect of ASA or medications containing ASA.
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18. Healthy volunteers (males and females) free from any pharmacological treatment for at least 10 days before the experiment, participated in the study. Blood samples were taken by venipuncture in the forearm and were drawn directly

into an evacuated plastic tube containing 3.8% (0.129 M) buffered sodium citrate (1 part anticoagulant to 9 parts blood). 900 μL aliquots were incubated with 100 μL of known concentrations of test compounds at 37 °C for 30 min. Test compounds, dissolved in NaOH 0.1 N and water (1:10; v/v), were tested at the following final concentrations: 5, 4, 3, 2 and 1 mM. The test was performed using acetylselicylic acid (ASA) too, as known platelet aggregation inhibitor. ASA was dissolved in DMSO and water (3:100; v/v) and was tested from 5 mM to 1×10^{-5} mM. Experiments conducted to test possible interference of the two used different vehicles on the assay, showed that there was no difference between NAOH and DMSO at the used concentrations. Blood sample was gently mixed and the test was performed pipetting 800 μL of blood into the smaller opening (sample reservoir opening) of the test cartridge avoiding air entrapment in the sample reservoir. The closure time was determined and expressed in seconds. The percentage of inhibition of platelet aggregation in treated blood (that is additioned with compounds to be tested) was calculated in comparison with control blood additioned with the proper vehicle.

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