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Short communication

Synthesis, absolute configuration and pharmacological evaluation of tiaprofenic acid antipodes

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Summary — Dextrorotatory and levorotatory antipodes (RU 40518, RU 40519) of the non-steroidal antiinflammatory drug (NSAID) racemic tiaprofenic acid (RU 15060) have been prepared by means of a new patented stereoconversion process. Unlike other arylpropionic acids, their absolute configurations, determined by circular dichroism, were *R* for the dextrorotatory enantiomer and *S* for the levorotatory enantiomer. Pharmacological evaluation showed that *R*-(+)-RU 40518 displays a potent in vitro inhibition of guinea-pig polymorphonuclear leukocytes (PMNL) cyclooxygenase and in vivo a better analgesic activity than its racemate or *S*-(−)-RU 40519 on the writhing test in the rat. Racemate and enantiomers showed similar antiinflammatory activity in carrageenan-induced paw oedema in the rat. These findings indicate that unlike other NSAIDs, the dextrorotatory enantiomer *R* is the active antipode for tiaprofenic acid.

antipode / stereoconversion / inflammation / analgesia / cyclooxygenase

Introduction

Racemic tiaprofenic acid (α-methyl 5-benzoyl 2-thiophene acid) is a non-steroidal antiinflammatory drug (NSAID) [1] which is effective and well tolerated in patients with rheumatoid arthritis [2, 3] and osteoarthritis [4, 5]. Furthermore, it has especially good analgesic activity [6, 7] as shown by its efficacy in post-operative pain or in patients with traumas such as fractures and contusions.

The aim of this study was to determine the absolute configuration and the pharmacological profile of tiaprofenic acid antipodes and also to report the original synthesis of these compounds.

In order to identify the differences between the pharmacological behaviour of enantiomers, if any, chemists are faced with the task of racemate resolution to provide sufficient amounts of each enantiomer with the required optical purity. Investigation of this racemic resolution lead us to an original stereoconversion process which supplies selectively one of the antipodes with an optical purity of almost 96 of enantiomeric excess (ee = (dextro% − levo%)/(dextro% + levo%)).

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Chemistry

Resolution of racemic tiaprofenic acid (α-methyl 5-benzoyl 2-thiophene acid) was performed as shown in scheme 1 [8].

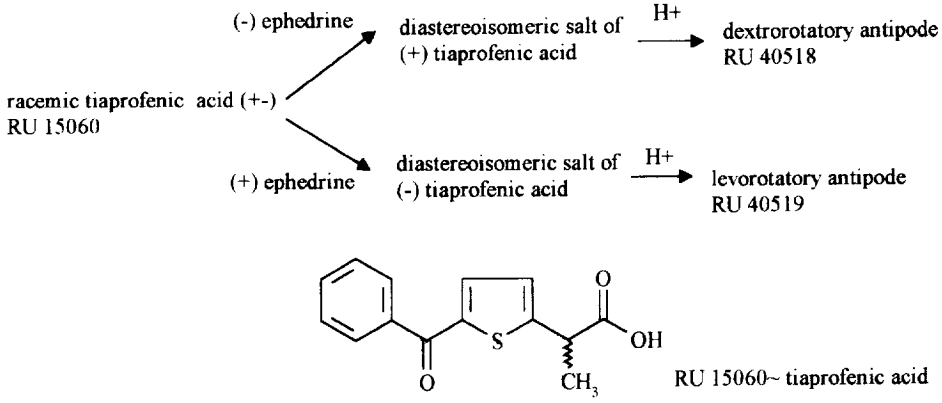
This process deals with the stereoconversion of the racemic tiaprofenic acid to one of the two antipodes, according to the sign of the chiral base employed. The term stereoconversion is used here to describe the recovery of more than 50% of the theoretical weight of one antipode from a racemic mixture.

The absolute configuration of tiaprofenic acid antipodes, determined by circular dichroism (see below), is *R* for the dextrorotatory enantiomer and *S* for the levorotatory enantiomer, in contrast to most NSAIDs for which dextrorotatory enantiomers are *S* and levorotatory enantiomers are *R* (this is the normal consequence of the presence of a 2-thiophene ring, instead of a benzene ring, which changes the nomenclature according to the Cahn, Ingold and Prelog rule).

Pharmacology

In vitro activity on guinea-pig polymorphonuclear leukocyte cyclooxygenase and lipoxygenase

The method was based on that of Harvey and Osborne [9]. Polymorphonuclear leukocytes (PMNL) were



Scheme 1. Resolution process of racemic tiaprofenic acid.

elicited in male guinea pigs (350–450 g, Interfauna) by intraperitoneal administration of 2% sodium caseinate. After 16–18 h the cells were collected in phosphate-buffered saline containing heparin (12.5 U/mL), washed and finally resuspended in assay buffer (15 mM Tris-HCl, 134 mM NaCl, 5 mM glucose pH 7.4). Cells (0.7 to 1.33 x 10^7) (0.92 mL) were preincubated with or without test compound for 10 min at 37 °C (for one of the RU 15060 replicates a 5 min incubation was used). (1-^{14}C)-Arachidonic acid (3.4 kBq: 1.85– 2.29 Gbq/mmol, Amersham) was added, followed 1 min later by 10 mM $CaCl_2$/10 µM A23187 calcium ionophore (final concentrations). Total assay volume was 1 mL. The reaction was stopped after a further 12 min with 0.2 M citric acid (100 µL).

Samples were extracted with ethyl acetate and separated by silica gel HPTLC (Merck 5641) using a chloroform/methanol/acetic acid/water solvent system (90:9:1:0.65). Radioactivity profiles were measured using a Berthold TLC scanner (LB 2482). Cyclooxygenase and lipoxygenase products were identified by comparison with labelled standards. Thromboxane B_2 (TXB_2) and 12-hydroxyheptadecatrienoic acid (HHT) were used as the measured cyclooxygenase products and were affected in a similar manner by each compound tested; 5-hydroxyeicosatetraenoic acid (5HETE) was the measured 5-lipoxygenase product.

Acetic-acid-induced writhing

According to the method described by Koster et al [10], groups of ten male rats (Sprague Dawley, 90–100 g, Charles River, France) were injected by the intraperitoneal route with acetic acid at the dose of 100 mg/kg (10 mL/kg of a 1% aqueous solution). The writhing movements were counted for a period of 15 min starting 5 min after injection of the irritant. Compounds were administered orally 0.5, 3 and 5 h before injection of the acetic acid, control rats receiving the vehicle (0.5% methyl cellulose) only. Analgesia would show up as a reduction in the frequency of the writhing responses compared with the control group.

Carrageenan-induced paw oedema

The experiments were performed using the method of Winter et al [11]. Groups of ten male rats (Sprague Dawley, 150–180 g, Charles River, France) were dosed orally with test compounds whilst simultaneously receiving a subplantar injection in the right hind paw with 0.5 mg of carrageenan (50 µL, solution at 10 mg/mL of carrageenan in distilled water); 3 or 5 h after carrageenan injection, the paw volumes were measured using a plethysmometer (Ugo Basile, France). Inflammation was assessed as the difference in volume before and 3 or 5 h after the injection of the phlogogenic agent. Antiinflammatory potency was expressed as the ED_{30} value which indicates the dose required to inhibit 30% of the inflammatory reaction.

Results and discussion

Chemistry

With the stereoconversion chemical process described in the *Experimental protocols*, we obtained an optical purity approaching 96, expressed in the form of enantiomeric excess (ee). This was required to give unambiguous pharmacological responses. This stereoconversion process can lead to very high yields, close to 90%, if the required optical purity is of an ee value of 84. The possible mechanism of such a stereoconversion could be a progressive equilibrium shift towards the more crystalline salt following a reversible deprotonation on the chiral carbon atom.

In order to determine the absolute configurations of the antipodes, the ideal method is X-ray crystallographic measurements. This method works well when monocrystals of sufficient size and well-shaped form are available. In our case we succeeded in both requirements, but X-ray crystallographers did not obtain satisfactory results and asked us to introduce a heavy atom in the structure. In the absence of sufficient time to synthesize such a structure, we decided to overcome this difficulty by circular dichroism trials with reference to the data published in this field for other aryl propionic acids [12, 13]. With regard to the circular dichroism results, the literature data [12, 13] concerning dextrorotatory analogue products with a benzene ring instead of a thiophene ring indicate an *S* absolute configuration, whereas drawing of dextrorotatory tiaprofenic acid (with the same layout of atoms in space) entails an *R* absolute configuration because the thiophene moiety causes inversion of the counting sense according to the Cahn, Ingold and Prelog rules. The positive band at 228 nm (in the case of RU 40518) is principally due to the $n \rightarrow \pi^*$ transition of the carboxyl group and secondarily due to the transition of the thiophene group. The weak negative band (in the case of RU 40518) and weak positive band (in the case of RU 40519) at 305 nm can be associated with the $n \rightarrow \pi^*$ transition of the carbonyl group.

Pharmacology

All samples used for the pharmacological studies were prepared and purified until an ee of 96. These samples were characterized as described in the *Experimental protocols*.

Potency as an inhibitor of guinea-pig PMNL cyclooxygenase and lipoxygenase

The HPTLC profile of control incubations is shown in figure 1. Piroxicam (10 μM) or Revlon-5901 (10 μM) reduced incorporation of radioactivity into cyclooxygenase and lipoxygenase products respectively (fig 1). As this level inhibition was maximal (data not shown), residual radioactivity in the product peak regions for these incubations was used to define background counts.

RU 15060 and *R*-(+)-RU 40518 reduced incorporation of radioactivity into cyclooxygenase product peaks in a dose-dependent manner (fig 1 for 10 nM 3*R*-(+)-RU 40518); IC_{50} values are presented in table I. Although the *S*-(−)-RU 40519 preparation produced dose-dependent inhibition of TXB_2 and HHT production, this could largely be accounted for by contaminating *R*-(+)-RU 40518 (1.9%). Indeed, contaminating *R*-(+)-RU 40518 was itself sufficient to give maximal inhibition at RU 40519 concentrations ≥ 1 μM (assuming no stereoconversion in the assay). The uncorrected IC_{50} values for *S*-(−)-RU 40519 in table I thus overestimate the potency of this enantiomer. The possibility of stereoconversion in the assay has not been addressed in the current study, but if present would also have reduced the measured difference between enantiomer potencies. The difference in enantiomer potencies indicated by the data in table I should thus be considered as a minimum estimate.

None of the test compounds produced marked or consistent 5-lipoxygenase inhibition. Indeed each compound, when present at concentrations that produced cyclooxygenase inhibition, generally potentiated incorporation of radioactivity into 5HETE (data not shown). The results reflect increased substrate availability for the 5-lipoxygenase pathway following cyclooxygenase inhibition.

In vivo analgesic activity

All test compounds were effective in the acetic-acid-induced writhing test but their analgesic activities depended on the time of administration before the chemical stimulus (acetic acid). As shown in table II, RU 15060, *R*-(+)-RU 40518 and *S*-(−)-RU 40519 displayed a similar antinociceptive activity when administered orally 0.5 h before testing. However, 3 and 5 h after oral treatment, the *R*-(+)-RU 40518 exhibited a more pronounced and stable analgesic effect than RU 15060 or *S*-(−)-RU 40519 both of which showed increased ED_{50} values.

In vivo antiinflammatory activity

RU 15060, RU 40518 and RU 40519 were studied at doses of 0.2, 1, 10 and 50 mg/kg po. Although inactive at the dose of 0.2 mg/kg po, all test compounds displayed a significant antiinflammatory effect on the 3 and 5 h carrageenan-induced paw oedema response of the rat as shown by the inhibition of the paw volumes with respect to controls. This activity was significant from the dose of 1 mg/kg, po (table III). No difference was observed between RU 15060, *R*-(+)-RU 40518 and *S*-(−)-RU 40519 at either time, their ED_{30} being estimated at about 1 mg/kg po.

Conclusion

In summary, in vitro pharmacological results observed on guinea-pig PMNL cyclooxygenase show that *R*-(+)-RU 40518 is the active antipode of tiaprofenic acid.

These data are confirmed in vivo only by the antinociceptive activity of this compound evaluated on acetic-acid-induced writhing in the rat. For the majority of chiral agents belonging to the arylpropionic acid class, antiinflammatory activity is exerted in vitro, by the inhibition of prostaglandin synthesis, by the *S*-(−)-enantiomer only, whereas analgesic effects may be related to both enantiomers [14,

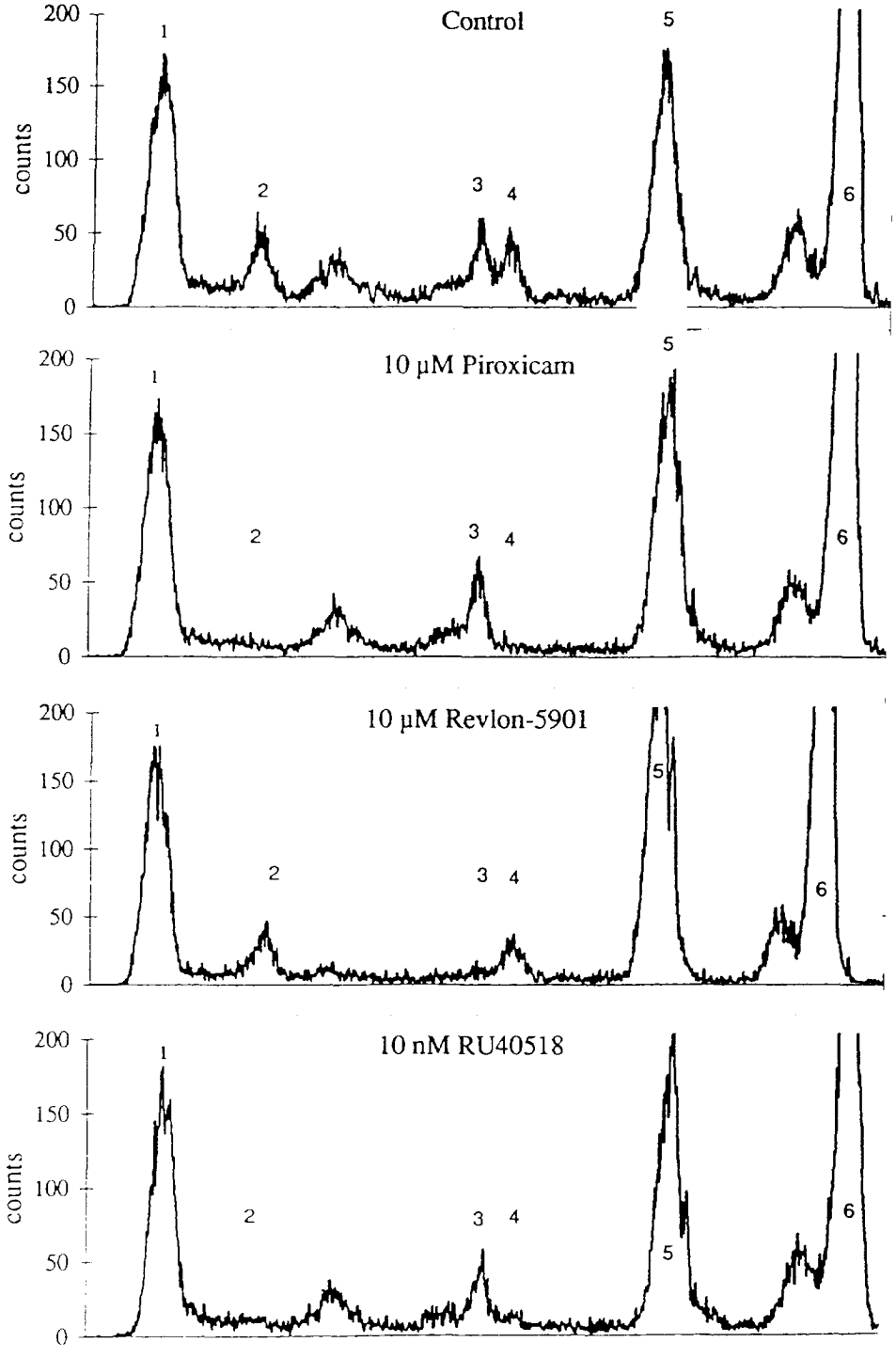


Fig 1. HPTLC profiles of labelled cyclooxygenase and lipoxygenase products generated from incubation of guinea-pig PMNL with (1-^{14}C) arachidonic acid in the presence or absence of specific test compounds (see *Experimental protocols*). 1 = Phospholipids (at the origin); 2 = TXB_2; 3 = 5HETE; 4 = HHT; 5 = arachidonic acid; 6 = neutral lipids.

Table I. Test compound IC_{50} values on guinea-pig PMNL cyclooxygenase and lipoxygenase activity.

	TXB_2 (nM)	*HHT (nM)*	*5HETE (μM)*
RU 15060	9.7 ± 4.2	8.6 ± 4.6	>100
R-(+)-RU 40518	1.7 ± 0.3	2.0 ± 0.8	>1, >100
S-(−)-RU 40519	29 ± 16	35 ± 15	>1, >100

TXB_2 and HHT are the measured cyclooxygenase products; 5HETE is the measured lipoxygenase product. The IC_{50} values for RU 40519 overestimate drug potency as they do not take into account contaminating RU 40518 (see text). $n = 2$ (RU 40518 and RU 40519) or 3 (RU 15060).

Table II. Analgesic activity of tiaprofenic acid antipodes on the acetic-acid-induced writhing test in the rat.

Compound	*ED_{50}[a] (mg/kg po)*		
	0.5 h	*3 h*	*5 h*
RU 15060	0.6	0.6	2
R-(+)-RU 40518	0.5	0.1	0.2–0.5[b]
S-(−)-RU 40519	0.7	0.5–1[b]	1–2[b]

[a]ED_{50} indicates the doses of the test compound required to inhibit 50% of writhing responses. They were calculated by least squares regression lines using a VAX 750 computer (Digital). [b]Non-dose-dependent effect. N = 10 rats per group, four to five doses per compound.

15]. The fact that RU 15060 and its enantiomers behave similarly in the paw oedema test is surprising in view of the apparent differences in potency as cyclooxygenase inhibitors. This result could be explained by several arguments characteristic of NSAIDs. NSAIDs and in particular the 2-arylpropionates are very highly bound to plasma proteins, the binding of 'profens' being commonly stereoselective [16, 17]. Thus, plasma protein binding may contribute to enantioselectivity in distribution and clearance of the drug. It is also possible that *R*-(+)-RU 40518 is more heavily bound to proteins reducing its in vivo effectiveness, however, no data on protein binding of tiaprofenic acid enantiomers in the rat are available and this remains speculative. Another possibility concerns the stereochemical inversion of 2-arylpropionates with the transformation from the inactive *R*-enantiomer to the pharmacologically active *S*-enantiomer; this chiral inversion is variable, depending on substrate, species and individuals [16, 17]. Recently, some preliminary results showed that plasma concentrations of *R*-(+)-RU 40518 (active form in vitro) were

Table III. Antiinflammatory activity of tiaprofenic acid antipodes on the carrageenan-induced paw oedema in the rat.

	Dose (mg/kg po)	*Inhibition of paw oedema (%)*	
		3 h	*5 h*
RU 15060	0.2	3	1
RU 40518	0.2	14	6
RU 40519	0.2	0	0
RU 15060	1.0	34*	26*
RU 40518	1.0	44*	30*
RU 40519	1.0	34*	21*
RU 15060	10	47*	43*
RU 40518	10	33*	35*
RU 40519	10	39*	25*
RU 15060	50	52*	46*
RU 40518	50	57*	47*
RU 40519	50	60*	47*

Data are expressed in the form of percentage of inhibition of paw oedema at 3 and 5 h post-carrageenan. Statistical analysis was carried out by the Dunnett's test (1955) and the levels of significance with respect to controls were defined as: $*P < 0.01$.

higher than those of its antipode from 2 or 3 h after oral or intravenous administration in rats of racemic RU 15060 (10 mg/kg) or *S*-(−)-RU 40519 (5 mg/kg) (work not yet published). In which case, in vitro potency differences for *R*-(+)-RU 40518 and *S*-(−)-RU 40519 may be markedly reduced in vivo.

Finally, the in vitro and in vivo discrepancies could be attributed to a different stereoselective pharmacokinetics as reported for 2-arylpropionates. Concerning tiaprofenic acid and its enantiomers, no clear and consistent data are available in the rat [18–20]. Recently, using administration of the racemic drug in the rat it has been shown that tiaprofenic acid enantiomers have different disposition kinetics with a higher concentration of the dextrorotatory form which could be due to chiral inversion, stereoselectivity in other metabolic routes or to protein binding [20].

Experimental protocols

Chemistry

^{1}H-NMR spectra were recorded at 250 MHz on a Brucker AM-250 and at 300 MHz on a Bruker AC-300 instrument, using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in δ units and coupling constants are quoted in hertz. Specific rotation $[\alpha]_D$ values were recorded on a Propol apparatus. Circular dichroism spectra were recorded on a

Mark V dichrograph (Jobin Yvon). Optical purities of the antipodes are expressed in the form of enantiomeric excess (ee = dextro% – levo% / dextro% + levo%). Elemental analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values. HPLC (chiral) conditions were: column Chiral protein 2 ~ human serum albumin; column length 15 cm; column diameter 4.6 mm; mobile phase: 850 vol of pH 7.5 buffer made from KH_2PO_4 0.05 M plus Na_2HPO_4 0.05 M and 150 vol of acetonitrile, plus sodium 2 propyl pentanoate 3 mM/L.

Preparation of RU 40518 (tiaprofenic acid dextrorotatory antipode)

(+)-Tiaprofenic acid / (–) ephedrine diastereoisomeric salt. In a 10 L three-necked flask 966 g of L-(–)-ephedrine was dissolved in 7500 mL of dry methanol at 20–25 °C. After complete dissolution, 1500 g of racemic tiaprofenic acid was added under stirring. Crystallisation appears within a few minutes. Stirring at 20–25 °C was maintained for 8 days (sufficient time for the stereoconversion of the diastereoisomeric salts into a large majority of ±). The salt was filtered off, rinsed twice with 400 mL of dry methanol each time and dried under vacuum at room temperature. Weight obtained: 1749 g (yield 71.3%). After crystallisation of this crude salt in five volumes of methanol, we obtained 1068 g (overall yield of the first crop: 43.6%) of optically purified salt. A second crop of 443.1 g (yield 18%) of the same quality salt was recovered by distillation, until dry, from the mother liquors of the first crude crop and crystallisation for 8 days in 2 L of dry methanol.

Characterisation of (+) tiaprofenic acid/(–) ephedrine salt: anal: $C_{24}H_{27}NO_4S$ (425.5; CHNS). ^{1}H-NMR (DMSO 250 MHz ppm): 0.87 (d) and 1.45 (d) (2 CH_3CH); 2.53 (s) (N^+CH_3); 3.18 (m) ($NCH(CH_3)CH$); 3.78 (q) (other $CHCH_3$); 5.06 (d) ($OCHC_6H_5$); 7.05 (d) (H_4 thiophene) and 7.51 (d) (H_3 thiophene); 7.20–7.41 (m) and 7.55–7.83 (m) (10H aromatic). α_D (c = 0.5% ethanol) = –58.5°. HPLC (chiral): ee = 90 (on a portion of salt converted to the free acid). In order to obtain an ee of 96 it was necessary to convert this salt into the corresponding free acid (see the preparative technique below) and to prepare once again a (+) tiaprofenic/(–) ephedrine salt in the same way as described above. Global yield = 31.7%.

Free (+) tiaprofenic acid ~ (+) α-methyl 5-benzoyl 2-thiophene acetic acid ~ RU 40518. The above diastereomeric salt (1060 g) (ee = 90) was added with stirring to a mixture of 4200 mL of water plus 4200 mL of dichloromethane and 540 mL of 12 N HCl. After stirring at 20–25 °C for 10 min the organic phase was separated and the aqueous phase extracted with 1000 mL of dichloromethane. The total organic phase was dried over Na_2SO_4 and concentrated to a residual volume of 970 mL. *n*-Hexane (2500 mL) was added to this solution at 20–25 °C while stirring. After 1 h stirring at 20–25 °C the solid was filtered and washed with 2 x 320 mL of *n*-hexane. After drying under vacuum at 20–25 °C we obtained 645.6 g (yield 99.6%) of the free acid.

Characterisation of the free (+) tiaprofenic acid: anal: $C_{14}H_{12}O_3S$ (260.3; CHS). ^{1}H-NMR ($CDCl_3$ 250 MHz ppm): 1.67 (d) (CH_3CH-); 4.09 (q) (CH_3CH-); 7.06 (d) (H_4 thiophene); 7.52 (d) (H_3 thiophene); 7.50 (m); 7.57 (m); 7.84 (m) (H *meta, para, ortho* to C=O). α_D (c = 1% AcOEt) = + 25.1°. HPLC (chiral): ee = 90. In order to obtain a free acid with an ee of 96, the (+) tiaprofenic/(–) ephedrine salt (ee 96) was hydrolysed under the same conditions as for the free acid. Circular dichroism (in EtOH) on this product gives: max 228 nm $\Delta\varepsilon$ = +1.5; max 305 nm $\Delta\varepsilon$ = –0.5.

Preparation of RU 40519 (tiaprofenic acid levorotatory antipode) The principle of the preparation of this antipode was similar to that used for RU 40518 except for the use of D-(+)-ephedrine.

(–) Tiaprofenic acid/(+) ephedrine diastereomeric salt. Characterisation of the product obtained: anal: $C_{24}H_{27}NO_4S$ (425.5; CHNS). ^{1}H-NMR ($CDCl_3$ 300 MHz ppm): 0.87 (d) and 1.45 (d) (2 CH_3CH); 2.55 (s) (N^+CH_3); 3.22 (dq) ($N^+CH(CH_3)CH$-), 3.79 (q) (=$CHCH_3$); 5.10 (d) ($OCHC_6H_5$); 7.05 (d) (H_4 thiophene); 7 (1 (d) (H_3 thiophene); 7.20–7.41 (m) and 7.55–7.83 (m) (10H aromatic). $-\alpha_D$ (c = 0.5% ethanol) = +53.9 °. HPLC (chiral): ee = 86 (on a portion of salt converted to the free acid). In order to obtain an ee of 96 it was necessary to convert this salt into the corresponding free acid and to prepare once again a (–) tiaprofenic/(+) ephedrine salt. Yields are similar to those obtained for (+) tiaprofenic/(–) ephedrine salt.

Free (–) tiaprofenic acid ~ (–) α-methyl 5-benzoyl 2-thiophene acetic acid ~ RU 40519. Characterisation of the product obtained from a salt with an ee of 86: anal: $C_{14}H_{12}O_3S$ (260.3; CHS). ^{1}H-NMR ($CDCl_3$ 300 MHz ppm): 1.66 (d) (CH_3CH-); 4.08 (q) (CH_3CH-); 7.05 (d) (H_4 thiophene); 7.50 (d) (H_3 thiophene); 7.50 (m), 7.58 (m), 7.83 (m) (H *meta, para, ortho* to C=O). α_D (c = 1% AcOEt) = –23.5 °. HPLC (chiral): ee = 86. In order to obtain a free acid with an ee of 96, the (–) tiaprofenic acid/(+) ephedrine salt (ee 96) was hydrolysed under the same conditions as for the free acid. Yields are similar to those obtained for the free (+) tiapofenic acid. Circular dichroism (in EtOH) on this product gives: max 228 nm $\Delta\varepsilon$ = –1.5; max 305 nm $\Delta\varepsilon$ = +0.5.

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