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Radioiodinated dechloro-4-iodofenofibrate: A hydrophobic model drug for molecular imaging studies

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ABSTRACT

Radiolabeling is a valuable option for tracking drug molecules in biodistribution experiments. In the development of innovative drug delivery systems the influence of the pharmaceutical formulation on the drugs' pharmacokinetics has to be investigated. The hypolipidemic agent fenofibrate is an ideal model drug for testing the performance of drug delivery systems designed for poorly soluble compounds. Herein, we report a de novo synthesis of a fenofibrate derivative, dechloro-4-iodofenofibrate, as well as its conversion into its radioiodinated derivatives containing ^{125}I or ^{131}I. The enzymatic stability of the radiolabeled compounds synthesized was determined in vitro. A scintigraphic imaging study supplemented by biodistribution experiments and analysis of excreted metabolites revealed the stability required for in vivo applications and its similarity to fenofibrate. Therefore a convenient method is presented to synthesize radioiodinated derivatives of fenofibrate. These tracers show excellent in vitro and in vivo properties to study the behavior of lipophilic drugs.

1. Introduction

Many new candidate drugs display a low aqueous solubility, hence solubilizing delivery systems have received increasing attention during the last decades (Strickley, 2004). Improving solubility is often required to reach therapeutic drug concentrations at the target site of action in vivo.

The hydrophobicity of fenofibrate (**1**) (Scheme 1) makes it a model drug to evaluate innovative drug delivery systems (Kalivoda et al., 2012; Vogt et al., 2008). Fenofibrate is an established hypolipidemic agent and has been successfully used for over thirty years (Cornu-Chagnon et al., 1995; Guichard et al., 2000; Guivarc'h et al., 2004). The compound shows a high lipophilicity (log P = 5.24) (Munoz et al., 1994) and is consequently practically insoluble in water (Mochalin et al., 2009; Penn et al., 2006). The pharmacokinetic profile of fenofibrate is well understood (Adkins and Faulds, 1997). About 40% of the drug development candidates show a very low aqueous solubility and therefore need technologies for bioavailability enhancement (Patel et al., 2010).

To investigate the effect of various formulations of hydrophobic drugs on their pharmacokinetics, it would be of interest to synthesize fenofibrate derivatives that are readily detectable in vitro and by molecular imaging methods. Radiolabeling is the method of choice for such applications (Eisenhut and Mier, 2003). The aim of this work was to obtain fenofibrate with a radioactive iodine label. This compound is suited for in vitro as well as in vivo biodistribution studies by introducing different iodine isotopes into the molecule.

2. Materials and methods

General: All reagents and solvents were purchased from commercial sources and used without further purification. Sodium [^{131}I] and [^{125}I] iodide were purchased from Perkin Elmer (Rodgau, Germany). Radioactivity was measured with an Isomed activimeter (Dresden, Germany). ^{1}H and ^{13}C NMR spectra were measured at room temperature on a 500 MHz Varian spectrometer. Chemical shifts are reported as δ parts per million (ppm). ^{1}H chemical shifts are referenced to residual protic solvent ($CDCl_3$, δ H = 7.26 ppm; DMSO-d_6, δ H = 2.50 ppm). ^{13}C chemical shifts are referenced to the solvent signal ($CDCl_3$, δ C = 77.0 ppm).

HPLC: An Agilent series 1100 HPLC system coupled to a raytest Gabi star γ-detector was used to monitor reactions and check for purity. The following conditions were used unless otherwise noted: Chromolith Performance column (Merck), RP-18e, 4.6 × 100 mm, 0.1% aqueous trifluoroacetic acid (solvent A) and acetonitrile

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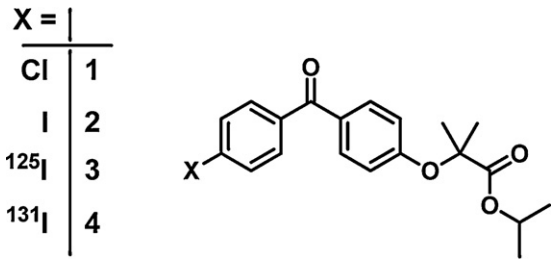


Scheme 1. Chemical structure of fenofibrate (**1**) and the dechloro-4-iodofenofibrate derivatives studied.

containing 0.1% trifluoroacetic acid (solvent B), linear gradient from 0 to 100% solvent B in 5 min, additional 3 min 100% solvent B, 4 mL/min, absorbance λ = 214 nm. The preparative RP-HPLC separation was performed on a Gilson/321 pump HPLC system. For purification the following conditions were used: Chromolith SemiPrep-column (10 × 100 mm); gradient elution from 0.1% TFA in water to 0.1% TFA in acetonitrile/water = 70/30 over 10 min; flow rate 10 mL/min; absorbance λ = 214 nm.

LC–MS: High resolution LC–MS measurements were obtained on a mass spectrometer supporting orbitrap technology (Exactive, Thermo Fisher Scientific). An Agilent 1200 system using a Hypersil Gold C18 column (0.21 × 200 mm) served as HPLC system. A mixture of caffeine, MRFA and Ultramark 1621 was used for mass calibration in the positive-ion mode. Full scan single mass spectra were obtained by scanning from m/z = 100–1000 for 30 min. The HPLC conditions were the following: (method A) linear gradient of 0.1% TFA in water to 0.1% TFA in acetonitrile in 30 min, flow rate 200 μL/min, 60 °C, absorbance λ = 214 nm. (method B) linear gradient of 0.1% TFA in water to 0.1% TFA in acetonitrile in 10 min followed by 20 min 100% 0.1% TFA in acetonitrile, flow rate 200 μL/min, 60 °C, absorbance λ = 214 nm.

2.1. Synthesis of [$^{125/131}$I] 2-[4-(4-iodobenzoyl)phenoxy]-2-methylpropanoic acid isopropylester

2.1.1. Synthesis of (4-iodophenyl)(4-methoxyphenyl)methanone (**7**) (derived from Davies et al., 2009)

To a suspension of 4-iodobenzoic acid (3.44 g, 13.87 mmol) in dichloromethane (35 mL) thionylchloride (1.50 mL, 18.00 mmol, 1.5 eq.) and dimethylformamide (50.0 μL) were added. The resulting reaction mixture was stirred at 40 °C overnight. The yellow suspension obtained was concentrated in vacuum to provide a tan solid that was used in the subsequent reaction without further purification. The crude acid chloride (**6**) then was dissolved in dichloromethane (60 mL), cooled in an ice bath, and anisole (1.89 mL, 17.40 mmol, 1.25 eq.) was added. The mixture was stirred at 0 °C for 2 min whereupon $AlCl_3$ (2.28 g, 17.40 mmol, 1.25 eq.) was added portion wise over a period of 20 min. The resulting reaction mixture was allowed to stir between 0 °C and room temperature for 4 h. The yellow suspension was poured into ice-water (200 mL), stirred for 15 min, acidified with 1 N HCl and diluted with dichloromethane. The phases were separated and the aqueous layer was extracted with dichloromethane (3 × 100 mL). The combined organic layers were washed with 1 N NaOH, dried over anhydrous Na_2SO_4 and concentrated in vacuum. The crude product was crystallized from dichloromethane/hexane (1/10) to afford 4.49 g (13.27 mmol, 96%) of a white solid.

HPLC: t_R = 4.14 min, LC–MS (method A): t_R = 25.76 min, m/z found 338.9871, calc. $C_{14}H_{12}IO_2$ 338.9877. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.83 (d, J = 8.5 Hz, 2H, Ar—*H*), 7.79 (d, J = 9.0 Hz, 2H, Ar—*H*), 7.47 (d, J = 8.5 Hz, 2H, Ar—*H*), 6.96 (d, J = 9.0 Hz, 2H, Ar—*H*), 3.89 (s, 3H, OCH_3) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 194.6 (*C*O), 163.4 (*C*OCH_3), 137.6, 137.5, 132.5 (*C*H), 131.2, 129.7 (*C*CO), 113.7 (*C*H), 99.3 (*C*I), 55.5 (O*C*H_3) ppm.

2.1.2. Synthesis of (4-iodophenyl)(4-hydroxyphenyl)methanone (**8**) (derived from Britton, 2005; Hartmann and Gattermann, 1892)

To a stirred solution of (4-iodophenyl)(4-methoxyphenyl)methanone (**7**) (5.17 g, 15.00 mmol), in toluene (150 mL), $AlCl_3$ (5.00 g, 37.50 mmol, 2.5 eq.) was slowly added. The reaction mixture was heated to reflux for 1 h. After cooling to room temperature, the mixture was slowly poured into 10% HCl (400 mL). The mixture was then transferred to a separatory funnel and the layers were separated. The aqueous phase was sequentially extracted with ethyl acetate (4 × 100 mL). The combined organic layers were washed with brine (2 × 100 mL), dried over anhydrous Na_2SO_4 evaporated into dryness. The obtained solid was suspended in hexane, filtered and dried in vacuum to yield the product **8** (5.04 g, quantitative yield) as a white solid.

HPLC: t_R = 3.42 min, LC–MS (method A): t_R = 23.17 min, m/z found 324.9724, calc. $C_{13}H_{10}IO_2$ 324.9720. ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 7.91 (d, J = 8.4 Hz, 2H, Ar—*H*), 7.65 (d, J = 8.7 Hz, 2H, Ar—*H*), 7.43 (d, J = 8.4 Hz, 2H, Ar—*H*), 6.89 (d, J = 8.7 Hz, 2H, Ar—*H*) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 194.9 (*C*O), 160.2 (*C*OH), 137.5, 137.4, 132.9 (*C*H), 131.2, 129.7 (*C*CO), 115.3 (*C*H), 99.4 (*C*I) ppm.

2.1.3. Synthesis of 2-[4-(4-iodobenzoyl)phenoxy]-2-methylpropanoic acid isopropylester (**2**)

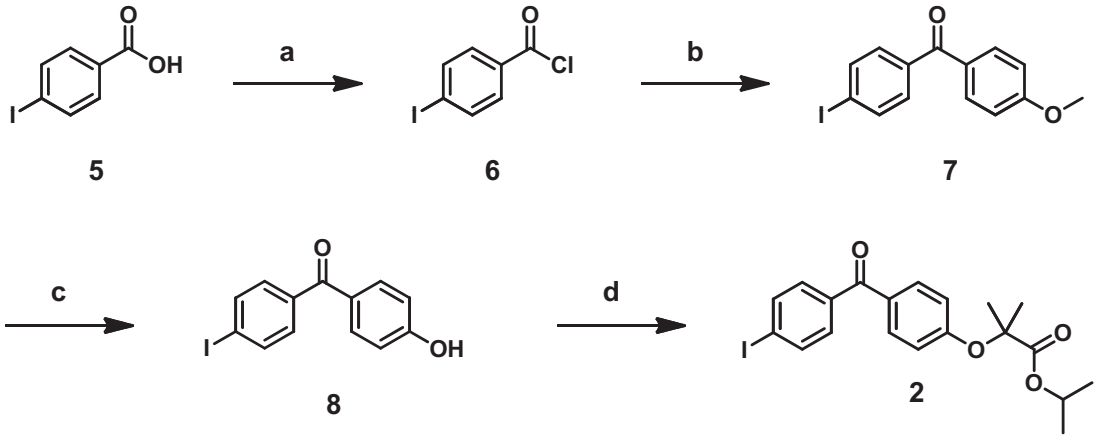
5.00 g (15.40 mmol) of the crude (4-iodophenyl)(4-hydroxyphenyl)methanone (**8**) were dissolved in 60 mL dimethylformamide. To the solution 6.38 g (46.20 mmol, 3 eq.) K_2CO_3, 1.85 g (15.40 mmol, 1 eq.) dried $MgSO_4$ and 9.80 mL (46.20 mmol, 3 eq.) isopropyl-2-bromo-2-methylpropanoate were added. The suspension was stirred and heated to 75 °C for 10 h followed by stirring overnight at room temperature. The precipitate was filtered off and washed with dichloromethane. The filtrate was sequentially washed with 0.1 N HCl (3 × 100 mL), brine and dried over anhydrous Na_2SO_4. After evaporation of the solvent the oily residue obtained was dissolved in dichloromethane (5 mL) and precipitated with hexane (50 mL). The solid was filtered and recrystallized from isopropanol to yield 4.39 g (9.70 mmol, 63%) of a white solid.

HPLC: t_R = 4.70 min, LC–MS (method A): t_R = 28.75 min, m/z found 453.0551, calc. $C_{20}H_{22}IO_4$ 453.0557. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.83 (d, J = 8.3 Hz, 2H, Ar—*H*), 7.72 (d, J = 8.7 Hz, 2H, Ar—*H*), 7.47 (d, J = 8.3 Hz, 2H, Ar—*H*), 6.86 (d, J = 8.7 Hz, 2H, Ar—*H*), 5.08 (sept, J = 6.3 Hz, 1H, $(CH_3)_2CH$), 1.65 (s, 6H, $C(CH_3)_2$), 1.20 (d, J = 6.3 Hz, 6H, $(CH_3)_2CH$) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 194.6 (*C*O), 173.1 (*C*O_2), 159.8 (*C*O*C*$(CH_3)_2$), 137.5, 137.4, 131.9 (Ar—*C*H), 131.2, 130.1 (*C*CO), 117.3 (Ar—*C*H), 99.4 (*C*I), 79.4 (*C*$(CH_3)_2$), 69.3 (*C*H$(CH_3)_2$), 25.4 (C$(CH_3)_2$), 21.5 (CH$(CH_3)_2$) ppm.

2.1.4. Synthesis of 2-[4-(4-tri-n-butylstannylphenyl)phenoxy]-2-methylpropanoic acid isopropylester (**9**)

Compound **2** (25.0 mg, 55.3 μmol) was dissolved in dioxane (2 mL) under argon. Hexabutyltin (27.9 μL, 0.33 mmol, 6 eq.) and dichlorobis(triphenylphosphine)palladium(II) (38.8 mg, 27.8 μmol, 0.5 eq.) were added, and the mixture was heated to reflux. After 3 h the mixture was filtered over silica and concentrated in vacuum. The crude product was purified by preparative RP-HPLC to give 8.1 mg of the pure product **9** (13.3 μmol, 24%).

HPLC: t_R = 5.43 min, LC–MS (method B): t_R = 17.9 min, m/z found 617.2662, calc. $C_{20}H_{22}IO_4$ 617.2652 (1.6 ppm).



Scheme 2. Synthesis of dechloro-4-iodo-fenofibrate. Reagents and conditions: (a) $SOCl_2$, dimethylformamide, CH_2Cl_2, 40 °C, 18 h; (b) $AlCl_3$, anisole, CH_2Cl_2, 0 °C to rt, 4 h; (c) $AlCl_3$, toluene, 100 °C 1 h; (d) $BrCMe_2CO_2iPr$, K_2CO_3, $MgSO_4$, dimethylformamide, 75 °C, 10 h.

2.1.5. Synthesis of [$^{125/131}$I] 2-[4-(4-iodobenzoyl)phenoxy]-2-methylpropanoic acid isopropylester (**3**, **4**)

Approximately 1 μL (13 MBq = 0.351 mCi) iodine-125 or iodine-131 (formulated as sodium iodide in 0.05 M NaOH) and 5 μL 4.5% H_2O_2 in acetic acid were added to the stannyl precursor **9** (15.4 μg, 5 mM) in 5 μL absolute ethanol. After 15 min the reaction mixture was diluted with 50 μL ethanol and purified by RP-HPLC. After evaporation of the solvent 5.06 MBq of the radiolabeled product could be obtained.

2.2. Serum and liver stability

A solution of [^{125}I]-dechloro-4-iodo-fenofibrate (fraction of HPLC, 40 μL) was added to 12.5 amounts of human serum (500 μL) or homogenized mouse liver and incubated at 37 °C. After various times, samples of 50 μL were mixed with 50 μL acetonitrile. The samples were centrifugated and the supernatant was added again to 50 μL acetonitrile. The stability of the compound was determined by HPLC analysis.

2.3. Scintigraphic imaging and biodistribution in mice

For scintigraphic imaging and biodistribution experiments, the labeled [^{125}I]-dechloro-4-iodo-fenofibrate or [^{131}I]-dechloro-4-iodofenofibrate were purified by HPLC. Female NMRI (a non-inbred Swiss-type model) mice, weighing 20–29 g, were used for imaging and biodistribution studies according to the microdosing concept (Wagner and Langer, 2011). All experiments were performed in compliance with German laws. For imaging purposes [^{125}I]-dechloro-4-iodo-fenofibrate (5 MBq, ~20 μg) was formulated in 100 μL of a mixture of solvents containing 60% propylene glycol, 20% phosphate buffered saline, 10% dimethyl sulphoxide and 10% ethanol and injected intravenously via the tail vein. Scintigraphies (γ-imager/Biospace Lab, Paris, France) of the whole animal were performed 5 min, 10 min, 30 min, 1 h, 2 h, 6 h and 24 h after injection. During the first 2 h the animal was kept in anesthesia. At the following points in time, the mouse was anesthetized for each measurement. After 24 h the mouse was sacrificed by cervical dislocation. For the biodistribution study [^{131}I]-dechloro-4-iodo-fenofibrate (approximately 4 μg, corresponding to 2 MBq) formulated as described above was injected intravenously via the tail vein. After 30 min, 6 h and 24 h post injection the animals were sacrificed. For each time point $n = 3$ was used. Samples of blood and organs of interest were removed, weighed and counted in an automatic γ-counter (Berthold LB 951G/Berthold Technologies, Bad Wildbad, Germany). The results were expressed as the percentage of the injected dose per gram (%ID/g) and injected dose (%ID) of blood and organs.

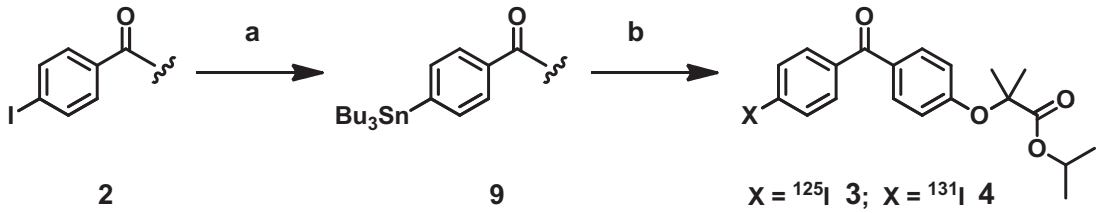
3. Results and discussion

A new method of labeling fenofibrate for in vivo monitoring was investigated. For this purpose fenofibrate was labeled with iodine. As attempts of direct labeling of fenofibrate (Kometani et al., 1985a,b) or substituting the chlorine by iodine (Klapars and Buchwald, 2002) or metal (Sheppard, 2009) failed, a de novo synthesis of the iodinated analogue of fenofibrate **2** (Scheme 1) was developed. To our best knowledge this compound had not been described previously. Dechloro-4-iodofenofibrate can be obtained via a Williamson type etherification of benzophenone **8** and isopropyl-2-bromo-2-methylpropanoate (see Scheme 2).

The benzophenone **8** was prepared according to the literature using a Friedel-Crafts acylation of anisole with 4-iodobenzoyl chloride (Davies et al., 2009) followed by deprotection of the phenolic group with $AlCl_3$ (Britton, 2005; Hartmann and Gattermann, 1892). Etherification with isopropyl-2-bromo-2-methylpropanoate using a Williamson type ether synthesis (Gustafsson et al., 2008) led to the literature unknown cold precursor **2** which was obtained in 63% yield after two crystallization steps. The synthesis protocol circumvents the need of time consuming column purification steps. All compounds can be purified by precipitation or crystallization. Consequently, it is possible to run the synthesis in a multigram scale.

The transformation of the cold iodine (Scheme 3) was done by a halogen–metal–halogen exchange in a similar route as shown by Mühlhausen et al. (2006). The stannyl precursor **9** was purified by preparative RP-HPLC. It has been proven that relinquishing the acidic additive in the eluent buffer is necessary. As a last step destannylation of compound **9** was performed under very mild conditions using dihydrogen peroxide in acetic acid. Besides, the radiolabeling could also be done using the HPLC-eluate. The final compound **3** could be easily identified by RP-HPLC due to a shift in the retention time caused by the decreased polarity of the stannylated compound **9** compared to the iodinated ones **2**, **3** or **4** (Fig. 1).

Fenofibrate is used as a prodrug that is metabolized into the active drug fenofibric acid. Consequently, saponification experiments were performed with the modified model drug **2**. A solution of **2** in methanol was treated with NaOH and heated to 95 °C. HPLC analysis showed a complete conversion to the corresponding iodofenofibric acid after 1 h. This product was characterized by high



Scheme 3. Synthesis of the radiolabeled dechloro-4-iodo-fenofibrates. Reagents and conditions: (a) $(SnBu_3)_2$, $[Pd(PPh_3)_2Cl_2]$, dioxane, 100 °C, 3 h; (b) $Na^{125}I$ or $Na^{131}I$, 4.5% H_2O_2 in CH_3COOH, ethanol, rt, 15 min.

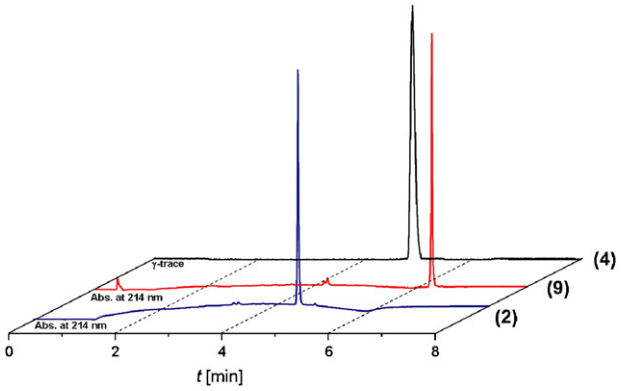


Fig. 1. RP-HPLC profile of the compounds **2**, **9** and **4**.

resolution mass spectrometry and used as a non-radioactive reference for the radioactive drug.

With the radioactive model drug **4**, we did first in vitro experiments (Fig. 2). We investigated the stability of compound **4** in human serum, homogenized mouse liver and carbonate buffer (pH 9.6). We observed a high stability of **4** in carbonate buffer and human serum. First conversion to iodofenofibric acid in human serum could be observed after 43 h. Even after 138 h the main compound was **4** and not the corresponding iodofenofibric acid. In contrast the whole compound **4** was converted to the corresponding iodofenofibric acid after 15 min in homogenized mouse liver. These results encourage us to extend the experiments to further in vivo and in vitro studies.

Based on the in vitro experiments an imaging study was performed to determine the distribution of the ^{125}I-labeled tracer in a mouse model. For this purpose a single dose of **3** was injected intravenously into a NMRI mouse. The time dependent distribution of the tracer was visualized on a γ-camera. Scintigraphies of the whole animal were performed 5 min, 10 min, 30 min, 1 h, 2 h, 6 h and 24 h after intravenous injection (Fig. 3). Already after 5 min the ^{125}I-labeled fenofibrate showed a predominant accumulation in the liver. The high concentration in the liver persisted during the first 6 h. The images at later points in time also showed the localization in the gastro-intestinal tract. This might be explained by an enterohepatical recirculation of **3** and its metabolites. Fortunately, we observed no accumulation of activity in the thyroid, which would reveal deiodination.

To correlate the images with quantitative uptake values a biodistribution study to determine the activity concentrations was performed at 30 min, 6 h and 24 h post injection. For this purpose compound **4** was used. Each mouse was dosed with approximately 2 MBq (corresponding to 2 μg) of **4**. The biodistribution data represented as percent of the injected dose (% ID) are shown in Fig. 4. According to the imaging experiment most of the activity can be found in the liver, kidneys and intestinal tract.

Due to the almost complete disappearance of radioactivity within 24 h the total activity of excretion was determined. This investigation showed that approximately 80% of the activity had been excreted which correlates with data known for fenofibrate (Weil et al., 1988). As shown in Fig. 5 during the first 4 h excretion occurred mainly via urine and up to 24 h predominantly via the feces. HPLC analysis of the recovered urine confirmed the absence of free iodide and therefore deiodination of **4**. This finding reveals that the iodinated fenofibrate derivatives developed constitute highly stable model drugs of hydrophobic drugs, which allow the examination of drug delivery systems.

The lack of compound **4** in the urine indicates its complete conversion into its metabolites. Two more polar compounds, corresponding to dechloro-4-iodofenofibric acid glucuronide and

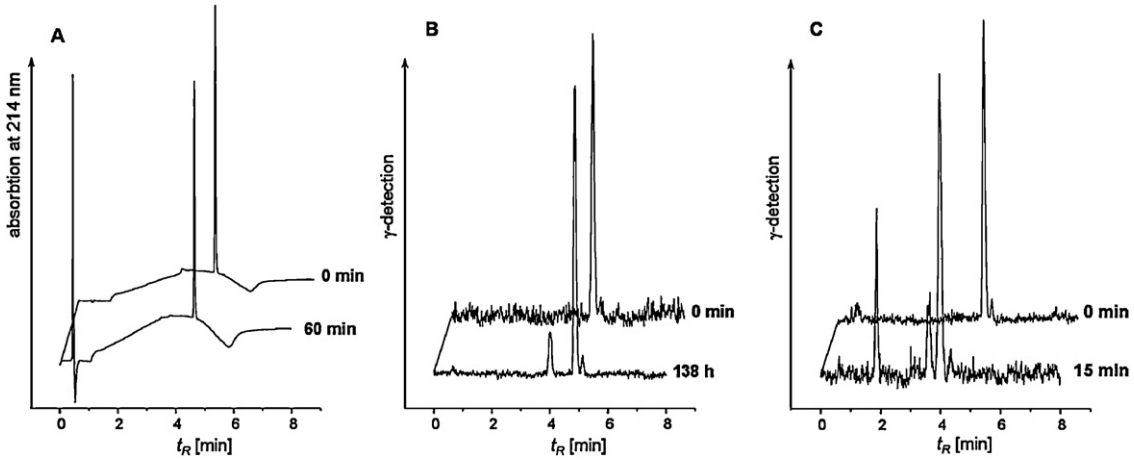


Fig. 2. Stability study of dechloro-4-iodofenofibrate under in vitro conditions. Compound **2** was incubated in carbonate buffer (pH 9.6) at 37 °C and RP-HPLC chromatograms were recorded (at 214 nm) to analyze the stability (chromatogram A). Compound **4** was incubated in human serum (chromatogram B) or in homogenized mouse liver (chromatogram C) at 37 °C and radio-RP-HPLC chromatograms were recorded at the times indicated to analyze the stability of the substance.

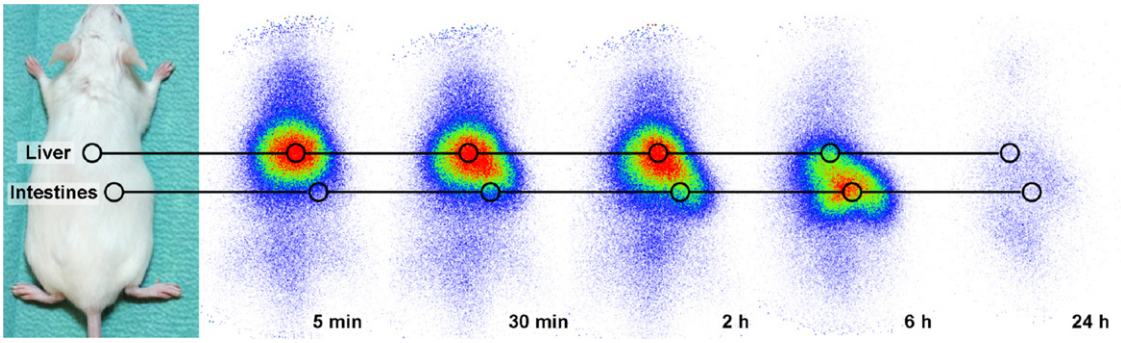


Fig. 3. Time course of the scintigraphic imaging of [^{125}I]-dechloro-4-iodofeofibrate in a NMRI mouse after intravenous application.

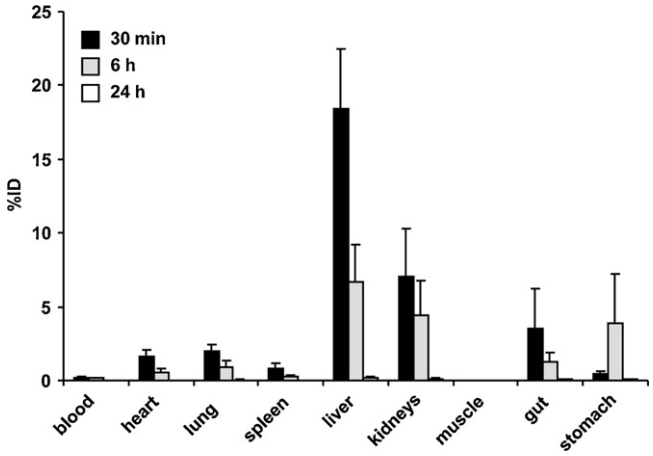


Fig. 4. Results of the biodistribution of [^{131}I]-dechloro-4-iodofenofibrate in NMRI mice presented as % injected dose (% ID). The data show the activity at 30 min, 6 h and 24 h post injection.

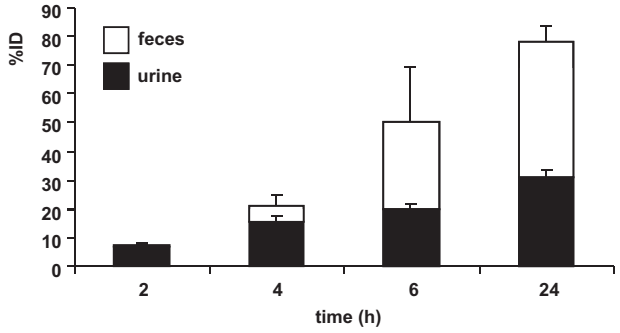


Fig. 5. Results of urinary and fecal excretion study of [^{131}I]-dechloro-4-iodofenofibrate in NMRI mice presented as % injected dose (% ID). The data show the activity excreted from 2 h up to 24 h post injection.

reduced dechloro-4-iodofenofibric acid glucuronide could be identified per HPLC (analyzed after 4 h post injection). These compounds are known as the main metabolites of fenofibrate in urine after oral dosage (Chapman, 1987).

4. Conclusion

In conclusion we could develop a convenient synthetic access to dechloro-4-iodofenofibrate (**2**) in an efficient way and to label it with either ^{125}I for imaging or ^{131}I for biodistribution experiments. In vitro experiments showed a high stability of the radiolabeled compound in human serum and a conversion of the prodrug into the biological active derivate in homogenized mouse liver. A preliminary imaging and biodistribution study demonstrated the utility of [^{125}I/^{131}I]-dechloro-4-iodofenofibrate as a tracer. The obtained data for dechloro-4-iodofenofibrate indicate similar characteristics to that of ^{14}C-fenofibrate in literature (Weil et al., 1988). The introduction of the radiolabel offers an access to further experiments to evaluate the influence of different formulations on the pharmacokinetic behavior of lipophilic drugs in the body and its biodistribution.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2012.04.039.

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