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Carbon-14 labelled Tribendimidine, a broad-spectrum anthelmintic drug†

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The preparation of [^{14}C]tribendimidine, a broad-spectrum anthelmintic agent related to amidantel, and its use during excretion and metabolism studies in the rat are described in this paper.

Keywords: tribendimidine; carbon-14; ADME; rat

Introduction

More than a billion people in tropical and subtropical regions are infected by parasitic intestinal roundworms. Soil-transmitted helminths are considered by public-health officials to have a debilitating impact on human populations that is equal to or greater than malaria or tuberculosis.[1] Currently only a few drugs are available to combat soil-transmitted helminths infections. Tribendimidine, namely *N,N′*-bis[4′-(1-dimethylamino ethylidene amino)phenyl]-1,4-phenylene-dimethylidyne amine, is a broad-spectrum anthelmintic agent related to amidantel, developed in China since the mid-1980s. The compound has been approved for human use by the Chinese authorities for its good safety and therapeutic profile against soil-transmitted helminthiasis.[2,3] Preliminary metabolism studies indicated that the compound is quickly cleaved to *p*-(1-dimethylamino ethylimino)aniline (dADT) and terephthalaldehyde (TPAL). Furthermore, the acetylated dADT (adADT) and terephtalic acid (TPAC) were identified as main metabolites of tribendimidine[4] (Figure 1). A radiolabeled version of tribendimidine was needed to support in vitro and in vivo ADME studies. In particular, a more extensive investigation regarding the possible aldehyde-derivatives formation was of main interest for their potential impact on the compound safety profile. Therefore the introduction of carbon-14 in the benzilideneamino moiety was required. The preparation of carbon-14 labelled tribendimidine in the benzilideneamino moiety and its use during *in vivo* ADME studies in the rat are described in this paper.

Experimental

Carbon-14 labelled tribendimidine

Analytical HPLC Methods

Method A: XBridge C18 column (4.6 × 100 mm, 5 μm) eluting with H_2O : CH_3CN : TFA (trifluoroacetic acid) 90:10:0.1 by volume (A) and H_2O : CH_3CN : TFA 10:90:0.1 by volume (B): from 100% A to 0% A in 15 min; 1 min at 0% A; from 0% A to 100% A in 1 min; 4 min at 100% A. Flow rate: 1 ml/min. Column temperature: 25°C. Analytical wavelength: 254 nm. Radiometric detection with 0.5 ml homogeneous cell (liquid scintillation cocktail: Ultima Flo-M (PerkinElmer Life Sciences, Groningen, The Netherlands) ratio to HPLC effluent: 2.5/1). Method B: Gemini C18 column (4.6 × 100 mm, 5 μm) eluting with water : acetonitrile : triethylamine 60:40:0.35 (by volume). Flow rate: 1 ml/min. Column temperature: 30°C. Analytical wavelength: 265 nm. Radiometric detection with 0.5 ml homogeneous cell (liquid scintillation cocktail: Ultima Flo-M (PerkinElmer Life Sciences, Groningen, The Netherlands) ratio to HPLC effluent: 2.5/1.

Method of synthesis

The preparation of [^{14}C]tribendimidine labelled in the benzylideneamino moiety was accomplished following a three-step synthetic procedure as shown in Scheme 1. [^{14}C]Copper cyanide **2** (0.476 mmol; 1 GBq) was refluxed with 4-iodobenzaldheyde **1** (218.6 mg; 0.94 mmol) in dimethylformamide (DMF; 2 ml) for 1.5 h obtaining intermediate **3**. Water (5 ml) was added to the reaction mixture that, after transferring into a separating funnel, was extracted with EtOAc (3 × 3 ml). The organic extracts were combined, washed with water (6 × 10 ml), dried (IST phase separator column) and evaporated to dryness. After purification by flash chromatography on a silica gel column (Eluting system: *n*-hexane: EtOAc 6:1 by volume), the obtained cyano-derivative **3** [0.333 mmol; 0.71 GBq; radiochemical purity >98% (method A; retention time (R_t) = 8.1 min); yield: 71%] was converted into the corresponding aldehyde **4** by treatment with PtO_2 (24.2 mg) in 80% aqueous formic acid (0.9 ml) at 60°C for 10 h. The solid material was separated by filtration then washed with water (3 ml) and Et_2O (5 ml). The filtrate with washings were transferred into a separating funnel, and the aqueous phase was extracted with Et_2O (2 × 5 ml). The organic phases were combined, dried (IST phase separator column), evaporated to dryness and purified by

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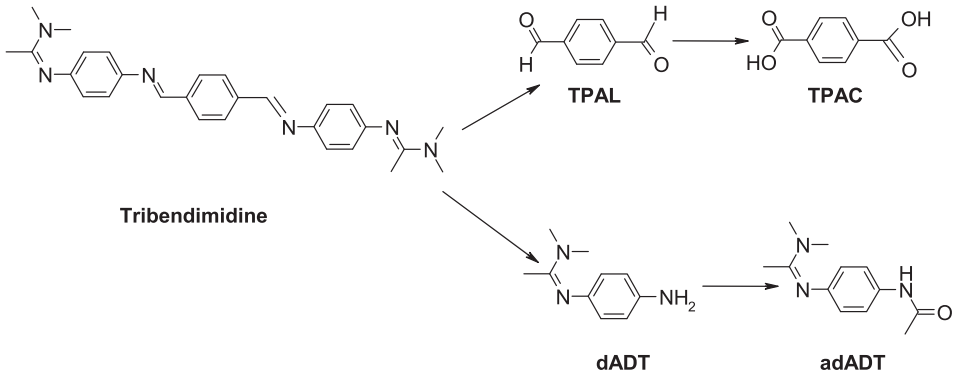


Figure 1. Preliminary metabolism studies of tribendimidine.

flash chromatography on a silica gel column (Eluting system: *n*-hexane: EtOAc 6:1 by volume). The obtained aldehyde **4** [0.156 mmol; 329 MBq; radiochemical purity >96% (method A; $R_t = 7.3$ min); yield: 46%] was reacted with intermediate **5**[5] (55 mg; 0.31 mmol) in tetrahydrofuran (THF; 1 ml) at 40°C for 24 h. A precipitate was formed and after filtration, [^{14}C]tribendimidine (0.086 mmol; 180 MBq; yield: 55%) was obtained as a yellow solid with a radiochemical purity >98 by radio-HPLC (Method B; $R_t = 6.2$ min) and a specific activity of 2.1 GBq/mmol. MS (ESI-MS): *m/z* 455 ([MH]$^+$). The overall radiochemical yield from Cu[^{14}C]CN **2** was 18%.

In vivo excretion balance in the rat

Male Sprague Dawley rats ($n = 3$/administration route), cannulated for serial blood sampling, were given a single intravenous (IV) dose and a single intraduodenal (ID) dose of [^{14}C]tribendimidine (10 mg/kg; approximately 3.7 MBq/kg) in 50% PEG400 dextrose aqueous solution. The elimination of radioactive drug-related material was determined in urine, faeces and cage washes collected from each animal at pre-dose and at the intervals of 0–8, 8–24, 24–48, 48–72 and 72–96 h after administration. Radioactivity levels were measured in whole blood and plasma collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72 and 96 h after administration. The carcasses and the skin were analysed for residual radioactivity. Total radioactivity was also evaluated in liver and heart. The radioactivity content in the excreta, cage washes, blood, plasma, collected organs and carcasses were determined by liquid scintillation counting.

In vivo metabolism in the rat

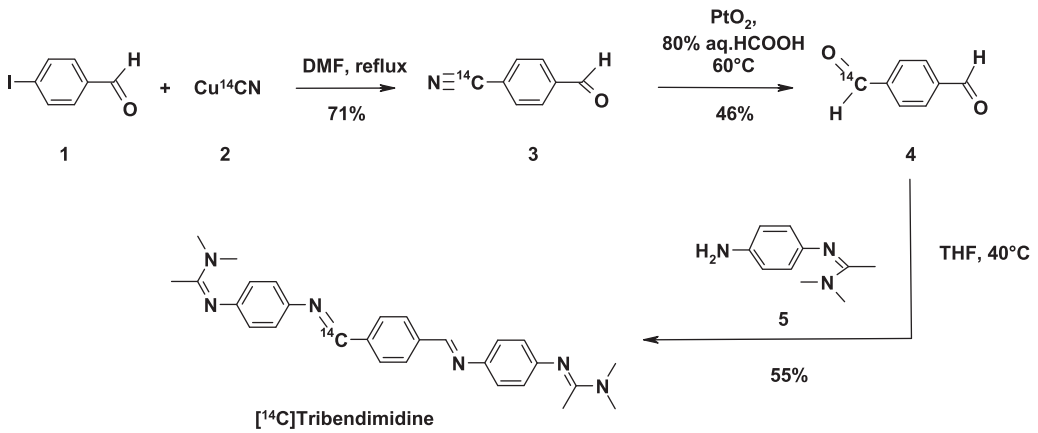
The radiochemical metabolite profiles of [^{14}C]tribendimidine were determined in pooled urine samples collected in the 0–24-h interval and in pooled plasma samples collected at 5–15 and 30–60 min after IV and ID dosing. Quantification of TPAL and other radioactive metabolites was performed by HPLC with on-line radiochemical detection [Gemini C18 column (4.6 × 150 mm, 3 µm) eluting with 0.2% formic acid in 10 mM ammonium formate (A) and 0.1% formic acid in CH_3CN (B): 1 min at 98% A; from 98% A to 80% A in 15 min; from 80% A to 5% A in 5 min; 6 min at 5% A; from 5% A to 98% A in 0.5 min; 7.5 min at 98% A. Flow rate: 1 ml/min. Column temperature: 40°C. Radiometric detection with 0.5 ml homogeneous cell (liquid scintillation cocktail: Ultima Flo-M (PerkinElmer Life Sciences, Groningen, The Netherlands) ratio to HPLC effluent: 2.5/1]. Structure elucidation of radiolabelled metabolites was carried out by LC-MS/MS in negative ion mode [Mass spectrometer: Waters QTof 2 equipped with an ESI source. Data were acquired from 70 to 600 Da, by using a source temperature of 120°C, a desolvation gas temperature of 250°C, a cone voltage of 25 V and a collision energy of 10 V in full scan mode and 15 V in MS/MS mode. Data were centroided during acquisition using an external reference].

Urine and selected plasma samples were also analysed by mass spectrometry in positive ion mode, thus allowing the identification of dADT and other unlabelled metabolites [Mass spectrometer: Waters QTof 2 equipped with an ESI source. Data were acquired from 70 to 600 Da, by using a source temperature of 120°C, a desolvation gas temperature of 250°C, a cone voltage of 30 V and a collision energy of 10 V in full scan mode, and 15, 20, 25 V in MS/MS mode. Data were centroided during acquisition using an external reference].

Results

In vivo excretion balance in the rat

The excretion profiles of drug-related material after IV and ID administration were very similar. The radioactivity excreted via urine within 96 h after administration ranged on average from



Scheme 1. Synthesis of [^{14}C]Tribendimidine.

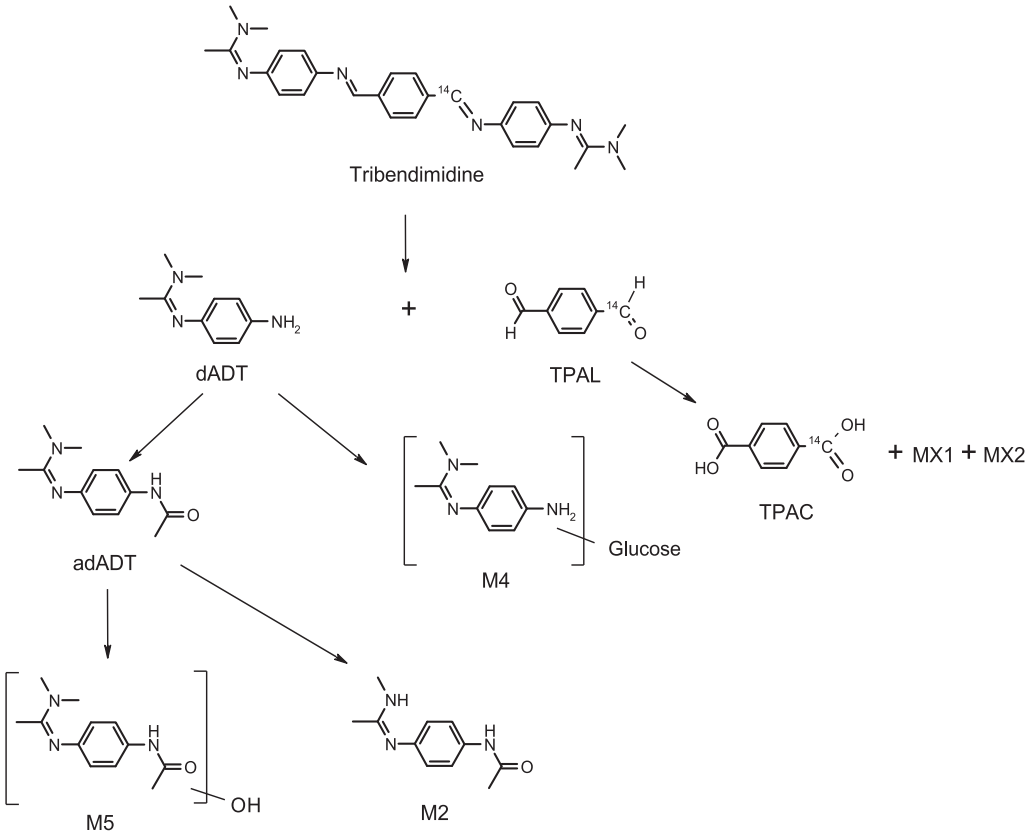


Figure 2. Proposed biotrasformation of [^{14}C]tribendimidine in urine and plasma of male rats following single intravenous and intraduodenal administrations of [^{14}C] tribendimidine at 10 mg/kg.

87% to 90% (including cage washes) of the administered dose after both administration routes. The mean amount of radioactivity recovered in faeces was approximately 2.3% and 4.2% of the dose respectively after IV and ID dosing. The excretion of the compound and/or its metabolites was rapid with most of the radioactivity (>80% of the dose) excreted in the urine within the first 24 h. Negligible to null amounts of radioactivity were found in the collected organs (liver and heart) 96 h after dosing. After IV dosing, blood and plasma data indicated that the total radioactivity distributed into tissues and disappeared from circulation with a low-moderate clearance of 1.61 and 1.13 L/h/Kg, respectively. Following ID administration, the total radioactivity was rapidly absorbed reaching its maximal concentration within 15 min post dosing. The bioavailability of drug-related material was 72% and 60% in blood and plasma, respectively. After both routes of administration, the red blood cells to plasma partition coefficient (Kp) at the first sampling times was lower than 1, indicating that the drug related material had limited association with red blood cells.

In vivo metabolism in the rat

The proposed metabolic pathway is shown in Figure 2. TPAC accounted for about 20% of radioactivity in urine after both routes of administration. In urine, the major radiolabelled metabolite MX1 accounting for 44% (IV) and 64% (ID) of the radioactivity showed the same *m/z*, elemental composition and fragmentation pattern as TPAC, but different retention time in gradient elution. Therefore a definitive structure could not be assigned. In plasma, two major radioactive peaks were detected, TPAC and MX2, and their sum was in the 70–90% range of radioactivity. The percentage of MX2 decreased from 5–15 to 30–60 min, whereas the percentage of TPAC increased with time. The *m/z* of MX2 could not be determined because of low MS response and ion suppression from the biological matrices. Analyses in MS positive ion mode of urine showed that dADT accounted for approximately 40% of the total unlabelled drug-related material after both IV and ID dosing, as well as the sum of the acetyl derivative adADT and the acetylated-hydroxylated metabolite M5. In plasma, metabolite dADT accounted for more than 50% in the 5–15 min samples after IV and ID administration and was not detected at 30–60 min. The metabolites, adADT and M5 were in the range of 9–16% of total drug-related material.

Conclusions

A method to introduce carbon-14 in the benzilideneamino moiety of tribendimidine considered of main interest from a metabolic point of view was successfully developed and implemented. An excretion balance study performed on a rat by using radiolabelled tribendimidine showed that the excretion of the compound and/or its metabolites was rapid with most of the radioactivity (>80% of the dose) excreted in the urine within the first 24 h after both IV and ID dosing. The biotransformation products of tribendimidine observed in urine and plasma were similar after IV and ID administration. [^{14}C]Tribendimidine was rapidly and

extensively transformed into TPAL and dADT. Both metabolites were further transformed into several additional metabolites: TPAL was metabolized toTPAC and other unknown metabolites; dADT was metabolized mainly by acetylation to adADT, followed by oxidation or desmethylation. In addition, conjugates of dADT with glucose (M4) were detected.

Conflict of Interest

The authors did not report any conflict of interest.

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[5] Y. Cui, X. Yu, Y. Xu, Intermediate **5** was prepared at Accelera S.r.l. according to the procedure described in *China Patent 1510027A*; July, **2004**.