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Note

Synthesis of deuterium-labelled meloxicam and piroxicam

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Abstract: Four step syntheses of deuterium-labelled meloxicam and piroxicam from saccharin via selective CD_3I alkylation are described. Labelled oxicams are of great interest for qualitative and/or quantitative isotope dilution-mass spectrometry, coupled with liquid chromatography, currently performed in anti-doping and forensic laboratories. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: meloxicam; piroxicam; synthesis; labelled; deuterium

Introduction

Meloxicam and piroxicam are members of the oxicam class of non-steroidal anti-inflammatory drugs (Figure 1). There is a need for stable labelled meloxicam and piroxicam as internal standards for LC/MS analyses of the native drugs in equine urine. These oxicams should be labelled at non-exchangeable positions, they should be chemically and isotopically pure (preferably as a single isotopomer) and should exhibit a minimum shift of 3 amu on the molecular ion.

Results and discussion

A review of the literature indicates that several oxicams labelled with ^{14}C or ^{3}H at various positions have been used for metabolic pathway elucidation (Table 1). Unfortunately, the reported syntheses are not satisfactory for our purpose: labelling has been performed at exchangeable positions,[2] a too brief description is given, for example, 'Isoxicam was radiolabelled [...] by methylation of the desmethyl precursor [...] with [^{14}C]-methyl iodide',[5] and only one labelled isotope is introduced.[1–12] A total synthesis of [^{14}C]-sudoxicam was described.[1] However, the nature of the base used in the first step is not indicated and the article describing the preparation of the key precursor[13] refers to a German paper published in 1897, without experimental details. The other articles do not indicate any preparative procedure.

Three reliable methods for the preparation of 1,2-benzothiazidic oxicams have been published.[14] Of those, the method starting from saccharin is the most appropriate for easy and rapid preparation of labelled meloxicam and piroxicam (Figure 2).[13]

Saccharin **1** was readily alkylated with methyl chloroacetate in the presence of sodium hydride in dimethylformamide to give **2**. This compound was rearranged to **3** in the presence of sodium methylate. The reaction conditions (temperature, nature of the solvent, reaction duration) were found to be critical with respect to the yield and product purity.[13] The labelling was obtained by alkylation of the benzothiazine nitrogen of **3** with CD_3I. Ester **4** was then reacted with the appropriate amine in order to obtain the targeted molecule. Meloxicam-*d3* **5** was purified by chromatography on a silica gel column using dichloromethane/methanol (0–2%) as the eluent. A mixture of toluene/acetic acid (9:1) was found to be superior in TLC experiments.

The disappearance of the 3H signal at 2.91 ppm indicates complete labelling of the *N*-methyl. Calculated ratio $(m\text{-}H)/z$ 350/353 $(d_0/d_3) = 0.008$.

Piroxicam-*d3* **6** was also purified by silica gel chromatography. However, dissolution in aqueous sodium hydroxide solution followed by precipitation with HCl was found to be a valuable alternative.

The disappearance of the 3H signal at 2.96 ppm indicates complete labelling of the *N*-methyl. Calculated ratio $(m\text{-}H)/z$ 330/333 $(d_0/d_3) = 0.017$.

Experimental

All reagents were obtained from Sigma-Aldrich (St Quentin Fallaviers, France) except 2-amino-5-

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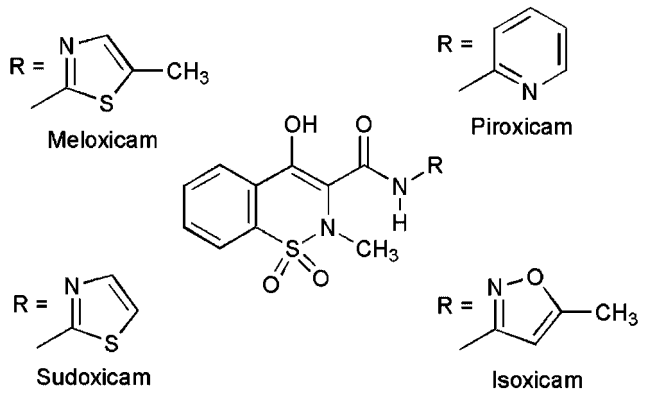

Figure 1 Some nonsterodial anti-inflammatory drugs of the oxicam class.

Table 1 Radiolabelled oxicams

Molecule	Isotope	Labelled position	References
Sudoxicam	^{14}C	C-3 of the thiazide ring	1
Piroxicam	^{3}H	Aromatic ring	2
Isoxicam	^{14}C	*N*-Methyl, C-3 of the thiazide ring	3–7
Meloxicam	^{14}C	*N*-Methyl, carbonyl of the amide	8–11
Tenoxicam	^{14}C	C-4 of the thiazide ring	12

methylthiazole (Lancaster, Strasbourg, France). Mass spectra were recorded using a LCQ (Thermo-Electron) equipped with a C18AB column (Nucleosil). ^{1}H NMR spectra were recorded on a Bruker ARX250 instrument.

3-Oxo-1,2-benzoisothiazoline-2-acetic acid methyl ester 1,1-dioxide (2)

Saccharin **1** (10.0 g, 54.6 mmol) in DMF (15 mL) was added dropwise to a suspension of NaH (1.43 g, 59.5 mmol) in DMF (5 mL) at 0°C. After 10 min stirring, methyl chloroacetate (4.8 mL, 54.7 mmol) was added. This mixture was stirred for another 1 h at room temperature and 2.5 h at 80°C. After cooling in an ice bath, the mixture was poured into a well-stirred ice-water (100 mL). The precipitate was recovered by filtration, washed with cold water (50 mL) and dried under vacuum (white solid, 8.95 g, 65%).

4-Hydroxy-2H-1,2-benzothiazine-3-carboxylic acid methyl ester 1,1-dioxide (3)

Solid **2** (5.0 g, 19.6 mmol) was added in one portion into a well-stirred solution of MeONa (2.45 g, 45.4 mmol) in DMSO (20 mL) maintained at 30°C by cooling with ice-water. After 4 min, the solution was poured into HCl (3N, 50 mL) and extracted with CH_2Cl_2 (3×100 mL). The organic layer was washed with water until decoloration, dried with brine (100 mL) and anhydrous Na_2SO_4, and distilled to give a yellow oil. Cold water (100 mL) was added to a well-stirred solution of the yellow oil in ethanol (10 mL). The precipitate of **3** was recovered by filtration and dried in air (yellow solid, 3.21 g, 64.2%).

2-Methyl-4-hydroxy-2H-1,2-benzothiazine-3-carboxylic acid methyl ester 1,1-dioxide-d3 (4)

A mixture of **3** (2.0 g, 7.8 mmol), CD_3I (1.6 mL, 25.8 mmol) and NaOH (32%, 830 μL, 8.6 mmol) in water (16 mL) and ethanol (30 mL) was stirred 24 h in a sealed flask. After the addition of water (50 mL) the precipitate was recovered by filtration, washed with water (50 mL) and dried (yellow crystals, 1.12 g, 52.6%).

Meloxicam-d3 (5)

A mixture of **4** (301 mg, 1.1 mmol) and 2-amino-5-methylthiazole (262 mg, 2.3 mmol) was heated under

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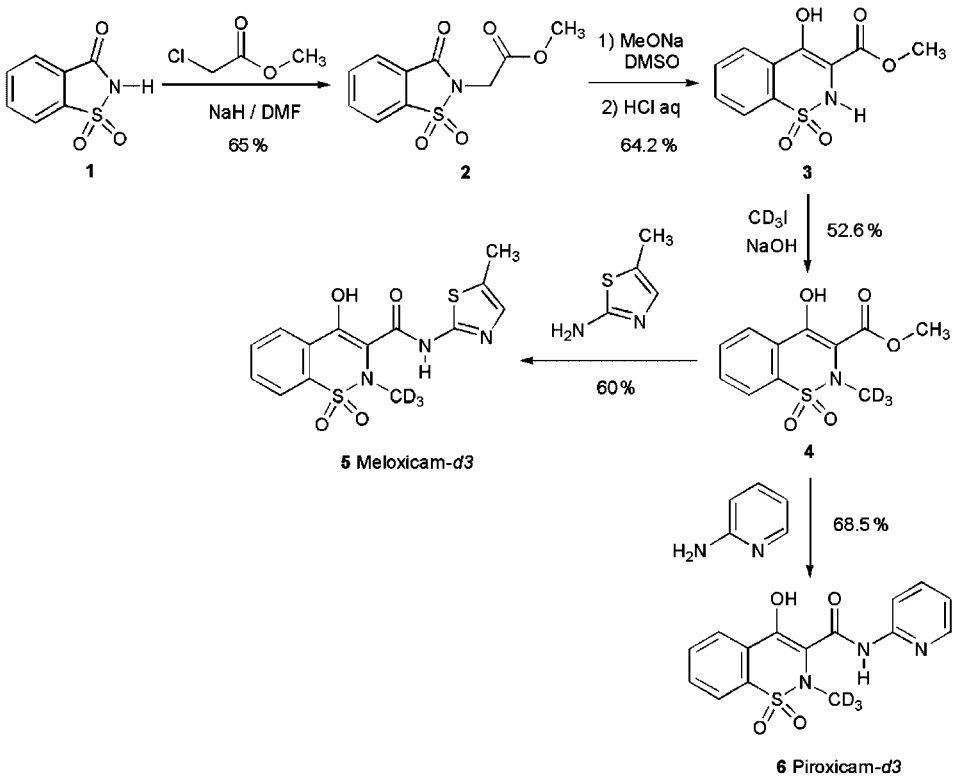

Figure 2 Syntheses of meloxicam-*d3* and piroxicam-*d3*.

reflux in *m*-xylene (15 mL) under nitrogen for 24 h. After 12 h stirring at room temperature, the precipitate was recovered by filtration, washed with *m*-xylene (20 mL), hexane (2 × 20 mL) and dried. This brown solid was purified by chromatography on a silica gel column (20 × 3 cm, dichloromethane/methanol 0–2%) to give **5** (yellow solid, 235 mg, 60%).

^{1}H NMR ($CDCl_3$) ppm: 8.07 (m, 1H), 7.92 (m, 1H), 7.78 (m, 2H), 7.22 (s, 1H), 2.46 (s, 3H). LC/MS (ESI-), single peak, *m/z*: 353 ($M-H^-$), 289, 213.

Piroxicam-d3 (6)

A mixture of **4** (89.4 mg, 0.33 mmol) and 2-aminopyridine (64.3 mg, 0.68 mmol) was heated under reflux in *m*-xylene (15 mL) under nitrogen for 24 h. After distillation of the solvent, the unreacted starting materials were removed by chromatography on a silica gel column (20 × 3 cm, chloroform). The yellow solid was recrystallised from boiling methanol to give **6** (light yellow crystals, 76 mg, 68.5%).

^{1}H NMR ($CDCl_3$) ppm: 13.28 (s, 1H), 8.91 (s, 1H), 8.36 (d, $J = 4$ Hz, 1H), 8.25 (d, $J = 8$ Hz, 1H), 8.07 (m, 1H), 7.92 (m, 1H), 7.76 (m, 3H), 7.13 (dd, $J = 7.5$ Hz, $J = 5$ Hz, 1H). LC/MS (ESI -), single peak, *m/z*: 333 ($M-H^-$), 269, 213.

Conclusion

Simple and efficient syntheses of deuterium-labelled meloxicam and piroxicam have been described. The main advantages of these procedures are: excellent chemical and isotopic purities; facile processing and purification; low cost and the possibility of labelling with other isotopes of hydrogen or carbon.

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REFERENCES

1. Hobbs DC, Twomey TM. *Drug Metab Dispos* 1977; **5**: 75–81.
2. Hobbs DC, Twomey TM. *Drug Metab Dispos* 1981; **9**: 114–118.

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3. Woolf TF, Black A, Sedman A, Chang T. *Eur J Drug Metab Pharmacokinet* 1992; **17**: 21–27.
4. Borondy PE, Michniewicz BM. *Drug Metab Dispos* 1984; **12**: 444–451.
5. Woolf TF, Black A, Hicks JL, Lee H, Huang CC, Chang T. *Drug Metab Dispos* 1989; **17**: 662–668.
6. Borondy PE, Michniewicz BM, Eiseman I, Yakatan GJ. *Pharmacologist* 1981; **23**: 212.
7. Borondy PE, Michniewicz BM, Yakatan GJ. *Pharmacologist* 1982; **24**: 95.
8. Busch U, Engelhardt G. *Drugs Exp Clin Res* 1990; **16**: 49–52.
9. Schmid J, Busch U, Trummlitz G, Prox A, Kaschke S, Wachsmuth H. *Xenobiotica* 1995; **25**: 1219–1236.
10. Schmid J, Busch U, Heinzel G, Bozler G, Kaschke S, Kummer M. *Drug Metab Dispos* 1995; **23**: 1206–1213.
11. Busch U, Schmid J, Heinzel G, Schmaus H, Baierl J, Huber C, Roth W. *Drug Metab Dispos* 1998; **26**: 576–584.
12. Ichihara S, Tsuyuki Y, Tomisawa H, Fukazawa H, Nakayama N, Tateishi M, Joly R. *Xenobiotica* 1984; **14**: 727–739.
13. Lombardino JG, Wiseman EH, McLamore WM. *J Med Chem* 1971; **14**: 1171–1175.
14. Zinnes H, Lindo NA, Sircar JC, Schwartz ML, Shavel Jr J, DiPasquale G. *J Med Chem* 1973; **16**: 44–48.

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