^{3}H- and ^{14}C-Labelling of the Aromatase Inhibitor ZK 138723

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Summary

Synthesis of the ^{3}H- and ^{14}C-labelled aromatase inhibitor ZK138723 is described+. The ^{3}H-label was obtained by dehalogenation of a 4,5-diiodoimidazole derivative with tritium gas, yielding 30.8 mCi with a specific activity of 33.4 Ci/mmol. Bromine replacement in the thiophene α-position by [^{14}C]cyanide afforded 25.9 mCi of the ^{14}C-labelled material with a specific activity of 55.6 mCi/mmol.

Key words: [^{3}H]imidazole, [^{14}C]cyanothiophene, 4,5-diiodoimidazole, aromatase inhibition

Introduction

It has been known for several years that the growth of certain neoplasic disorders - especially breast cancer in woman - depends on the serum level of estrogen. One strategy of lowering the estrogen formation is to inhibit the action of the enzyme aromatase, which catalyzes the final step in estrogen biosynthesis in humans [1]. For this purpose nonsteroidal compounds seem to offer advantages as compared to the classical steroid derivatives regarding intensity and duration of effect as well as selectivity to other cytochrome P450 enzymes [2]. The competitive aromatase inhibitor ZK138723 is such a nonsteriodal compound. It was labelled with ^{3}H and ^{14}C to be used for further pharmacological investigations.

Synthesis and Discussion

^{3}H-Labelling of aromatic compounds is usually achieved by catalytic dehalogenation of a halogen precursor with tritium. The imidazole heterocyle had recently been labelled with tritium starting from a mixture of brominated substrates [3]. In this synthesis 4,5-diiodoimidazole was used to obtain a diiodo derivative of ZK138723 which was treated with tritium gas (scheme 1). Although triiodoimidazole could also have been used [4] we prefered to avoid iodine at carbon 2, because of possible T/H-exchange in the labelled material [5].

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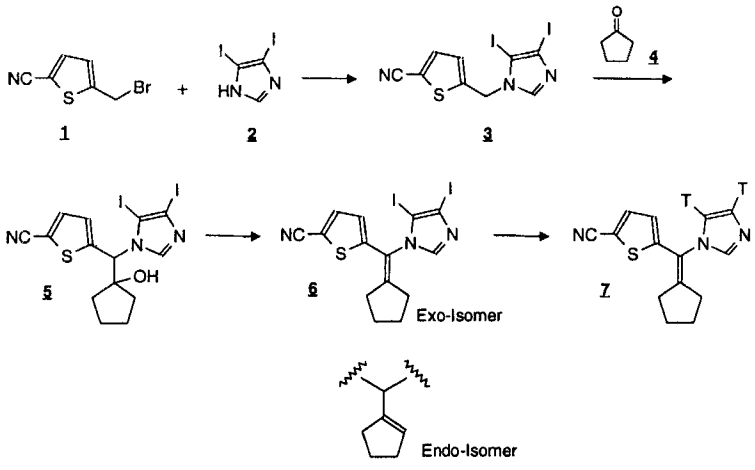


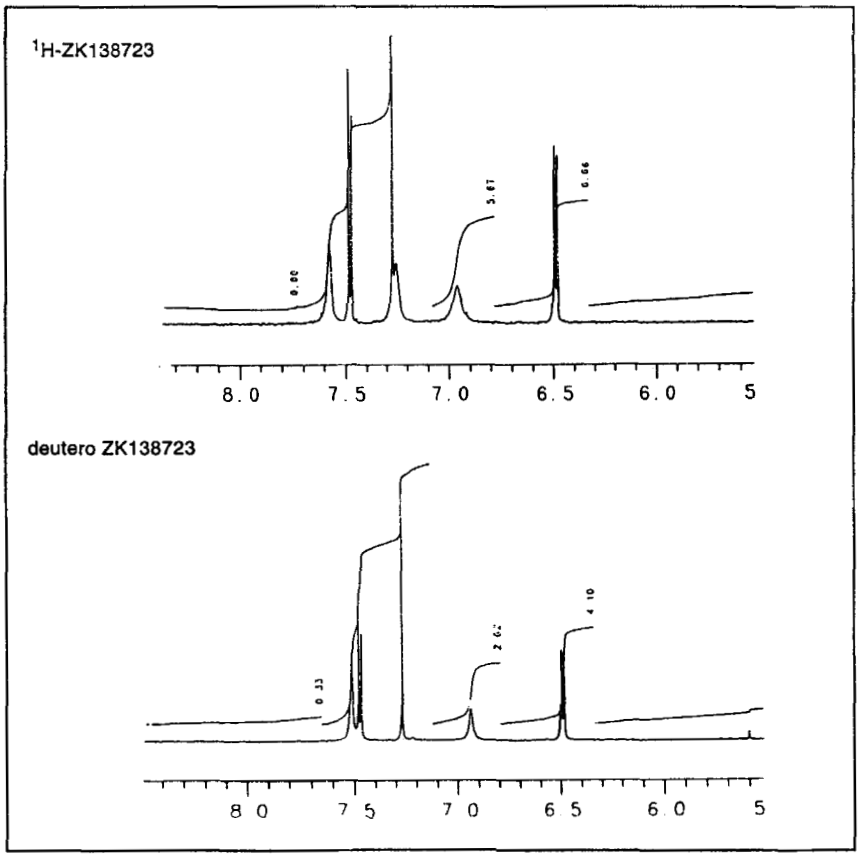
Scheme 1: ^{3}H-labelling

The bromomethylthiophene **1** was readily available as an intermediate in the laboratory scale preparation of ZK138723. Reaction with 4,5-diiodoimidazole **2** gave the coupled product **3** [6]. Deprotonation at the central carbon with lithium diisopropylamide and reaction with cyclopentanone (**4**) afforded the hydroxy compound **5**. In contrast to the dehydration of the unsubstituted imidazole analogue - giving only traces of the undesired endo-isomer - the diiodo derivative **5** afforded a 1:1 exo/endo-mixture from which **6** was obtained by chromatography. In the final dehalogenation process some attempts failed: The diiodo compound remained unreacted in tetrahydrofurane with triethylamine catalyzed by palladium on charcoal. Reaction using 0.5 N potassium hydroxide in methanol catalyzed by palladium on barium carbonate gave the methylthiophene carboxylate by hydrolysis of the cyano group. We were finally successful with 0.5 N potassium hydroxide in tetrahydrofurane for the hydrogenation at room temperature for 2h, yielding 65% product. By the tritium reaction 1140 MBq (30.8 mCi) ^{3}H-ZK138723 **7** with a specific activity of 1236 GBq/mmol (33.4 Ci/mmol) was formed.

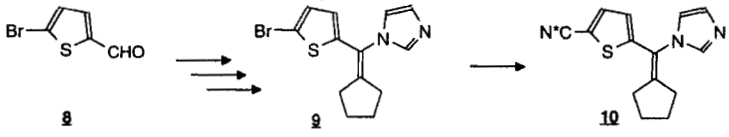
This represents 1.2 equivalents of tritium in the labelled molecule. Compared to ^{1}H-ZK138723 (δ H-2=7.58; δ H-4 =7.25; δ H-5=6.95) the ^{1}H-NMR spectrum of deutero ZK138723 - prepared under the same conditions - showed different intensities for H-4/5 and as expected, equal intensities for H-2 (figure 1). Whereas the H-4 signal had almost disappeared, the quota of deuterium at C-5 was approximatively 50%. This is in agreement with recent studies on N-alkyl-imidazoles where D/H exchange rates were investigated for position 2,4 and 5 depending on pH and type of substitution [4].

For the **^{14}C-labelling** (scheme 2) replacement of bromine by potassium [^{14}C]cyanide [7] in the α-position of the thiophene ring seemed to be practical to introduce the label in the final step. In this reaction copper cyanide [8] and nickel catalyst [9] have been used successfully. We found out that the reaction catalyzed by palladium gave the best results [10]. However some labelled material was lost because the crude product was unstable in methanol with traces of acid. Thus, the hot synthesis gave only 27% in comparison to 40% in the cold synthesis. The bromo precursor **9** was obtained by a

Figure 1: ^{1}H-NMR spectra (doublets belong to ß-thiophene protons)



multi step synthesis beginning from the commercially available 5-bromothiophene-2-carbaldehyde **8**. Reduction of the aldehyde with sodium borohydride followed by reaction with thionyl chloride gave the 2-bromo-5-chloromethylthiophene, which was reacted with triethylphosphite to give the phosphate, followed by a Horner-Wittig reaction with cyclopentanone. The resulting olefine was brominated and treated with imidazole/triethylamine to give **9**.



Scheme 2: ^{14}C-labelling

Experimental section

General: IR spectra: Nicolet instruments (710 and 20SXB).^{1}H-NMR spectra: Bruker instruments (in $CDCl_3$, tetramethylsilane as internal standard). Mass spectra: Fisons VG-Trio2 (70 eV). Measurements of activity were carried out (accomplished) by liquid scintillation counting on a Pharmacia 1410. Chemical identity and purity of the labelled material: The material co-chromatographs with a non-labelled reference in the given chromatographic systems. TLC: Merck silica gel 60 F 254 dichloromethane/methanol 95:5 and ethyl acetate/hexane 8:2. HPLC: Kromasil C18 5µm methanol/water 70:30 and acetonitrile/water 50:50, flow rate 1ml/min, λ=310nm.

4,5-Diiodoimidazole (2)

Synthesis of 4,5-diiodoimidazole was achieved by treatment of imidazole with iodine according to the procedure of Naidu and Bensusan [11]

5-(4,5-Diiodoimidazol-1-ylmethyl)-thiophene-2-carbonitrile (3)

To a solution of **2** (750 mg; 2.34 mmol) in THF (30 ml) the 5-bromomethylthiophene-2-carbonitrile **1** (473 mg; 2.34 mmol), sodium bicarbonate (322 mg; 2.34 mmol in 2 ml of water) and 75 mg 18-crown-6 were added. After heating the reaction mixture at 50°C for 2h the solvent was evaporated. The residue was extracted with dichloromethane/water and the organic layer was dried with sodium sulfate. The filtrate on evaporation gave 420 mg pure **3** by crystallisation. The residue was chromatographed on silica gel with dichloromethane/diethyl ether (90:10 to 75:25) adding another 388 mg of product. Total yield: 808 mg (78%). IR(KBr): 2210(CN), 1480, 1470 cm^{-1}.- ^{1}H-NMR: δ = 5.36(s; 2H), 6.97(d, J= 5Hz; 1H), 7.52(d, J = 5Hz; 1H), 7.72(s; 1H).- MS/EI: m/e = 441(17%, M+), 122(100).

5-[(1-Hydroxycyclopentyl)-4,5-diiodoimidazol-1-ylmethyl]-thiophene-2-carbonitrile (5)

To a solution of **3** (430 mg; 1.02 mmol) in anhydrous THF (14 ml) LDA (0.55 ml of a 2 M solution in cyclohexane) was added at -60°C. After 15 min the solution was cooled to -70°C, cyclopentanone **4** (0.093 ml; 1.05 mmol) was added and stirring continued for 1h. The reaction was quenched with water (2 ml) and stirring was continued for 1h at room temperature. The solvent was evaporated and the residue extracted with dichloromethane/water. The organic layer was dried with sodium sulfate and evaporated in vacuo. The product was purified by column chromatography on silica gel with hexane/ethyl acetate (95:5 to 50:50) to yield 334 mg of **5** (62%). IR(KBr): 2960(CH_2), 2870(CH_2), 2210(CN), 1460, 1450 cm^{-1}.- ^{1}H-NMR: δ = 1.52-1.95(m; 8H), 2.02(s; OH), 5.43(s; 1H), 7.17(d, J = 5Hz; 1H), 7.49(d, J = 5Hz; 1H), 8.27(s;1H).- MS/CI: m/e = 526(100%, M+), 442(13), 400(25), 32(14), 195(9).-

5-[Cyclopentyliden-(4,5-diiodoimidazol-1-ylmethyl)-thiophene-2-carbonitrile (6)

After treatment of the alcohol **5** 300 mg; 0.57 mmol) with $SOCl_2$ (1 ml) for 15 min at 50°C the reaction was quenched with water/ice and extracted with dichloromethane. The organic layer was dried with sodium sulfate and evaporated in vacuo to give a mixture of the exo-product **6** (45%) and the endo-product (55%+). Separation by tlc on silica gel with hexane/ethyl acetate 1:1 yielded 61 mg (21%) of **6**. IR(KBr): 2950(CH_2), 2860(CH_2), 2210(CN) cm^{-1}.- ^{1}H-NMR: δ = 1.72-1.82(m; 2H), 1.93-2.03(m; 2H), 2.15-2.25(m; 2H), 2.72-2.82(m; 2H), 6.40(d, J = 5Hz; 1H), 7.49(d, J = 5Hz; 1H), 7.63(s; 1H).- MS/EI: m/e = 507(53%, M+), 380(7), 253(53), 226(48), 188(72), 122(100%).-

+ relative rates were determined by ^{1}H-NMR

5-[Cyclopentyliden-(1-[4,5-^{3}H]-imidadazole)-methyl]-thiophene-2-carbonitrile (7)

The diiodo compound 6(10 mg; 0.02 mmol) was tritiated in THF (2 ml) with Pd/C (5 mg) and 0.5 N KOH (300 µl) for 3h. The catalyst was filtered and the residue was extracted with dichloromethane/water The organic layer was dried with sodium sulfate and the solvent was evaporated in vacuo. Labile tritium was removed by dissolving the crude product in methanol and evaporating under reduced pressure. Purification was achieved by hplc on 5µm Kromasil C18 (250x4.6 mm column) with acetonitrile/water (with 2 ml TEA/L) as eluent, flow rate 1ml/min, UV-detection at 310 nm. According to this method the total amount of 0.236 mg was determined by comparison with an external standard. The total activity was 1140 MBq (30.8 mCi). The specific activity was calculated to be 1236 GBq/mmol (33.4 Ci/mmol). Radiochemical purity >99% was determined by tlc and hplc.

5-[Cyclopentyliden-(1-imidadazole)-methyl]-thiophene-2-[^{14}C]carbonitrile (10)

The bromothiophene derivative 9 (548 mg; 1.72 mmol), tetrakis(triphenylphosphine)Pd(0) (1.2 g; 1.04 mmol), 18-crown-6 (574 mg; 2.2 mmol) and [^{14}C]KCN (115 mg; 1.73 mmol (Amersham CFA 87) was heated in anhydrous DMF (22 ml) at 180°C for 2h. 2N HCl was added and the reaction mixture extracted with diethyl ether. Potassium carbonate (saturated solution) was added and the aqueous layer was again extracted with diethyl ether. The solvent was evaporated in vacuo after drying with sodium sulfate. The product was purified by column chromatography on silica gel with dichloromethane/diethyl ether (0 to 40% diethyl ether with 0.1% NH_3) and on silica gel with hexane/ethyl acetate (25 to 60% ethyl acetate with 0.1% NH_3) to yield 120 mg (27%) of 2 with a total activity of 958 MBq (25.9 mCi) and a specific activity of 2057 MBq/mmol (55.6 mCi/mmol).

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