

**Synthesis of  $^{14}\text{C}$ -Labelled 6-Hydroxy-5,7-dimethyl-2-(methylamino)-4-(3-pyridylmethyl)benzothiazole dihydrochloride ( $^{14}\text{C}$ -E3040 dihydrochloride)**

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**Summary**

$^{14}\text{C}$ -Labelled 6-hydroxy-5,7-dimethyl-2-(methylamino)-4-(3-pyridylmethyl)-benzothiazole dihydrochloride ( $^{14}\text{C}$ -E3040 dihydrochloride), required for a study of the pharmacokinetic profile of E3040, a novel dual inhibitor of 5-lipoxygenase and thromboxane A<sub>2</sub> synthetase, was synthesized in one step using [2- $^{14}\text{C}$ ]-1-methyl-2-thiourea as the labelled starting material.  $^{14}\text{C}$ -E3040 dihydrochloride with a specific activity of 58.0mCi/mmol was prepared in 60% chemical yield (based on thiourea **2**).

**Key Words:** lipoxygenase inhibitor, thromboxane A<sub>2</sub> synthetase inhibitor, dual inhibitor,  $^{14}\text{C}$ -E3040

**Introduction**

Recently we reported the syntheses and pharmacological properties of benzothiazole derivatives with novel dual inhibitory activities against 5-lipoxygenase (generates leukotrienes) and thromboxane A<sub>2</sub> synthetase<sup>1</sup>). Among these compounds, 6-hydroxy-5,7-dimethyl-2-(methylamino)-4-(3-pyridylmethyl)-benzothiazole, E3040, was shown to possess superior *in vitro* and *in vivo* (rat colitis model) activities. As E3040 was expected to have particularly interesting pharmacokinetics, the labelled compound was needed for these investigations. In this paper we describe the synthesis of  $^{14}\text{C}$ -labelled E3040 dihydrochloride.

## Results and Discussion

$^{14}\text{C}$ -Labelled E3040 dihydrochloride **3** was prepared from  $[2\text{-}^{14}\text{C}]$ -1-methyl-2-thiourea **2** in one step as shown in Figure 1.

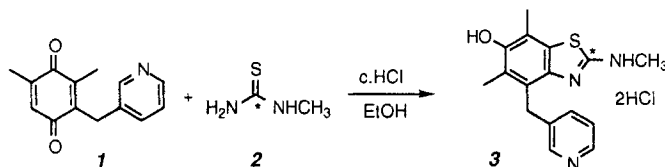


Figure 1.

The mixture of  $[2\text{-}^{14}\text{C}]$ -1-methyl-2-thiourea **2** and 2 equivalents of 2,6-dimethyl-3-pyridylmethyl-1,4-benzoquinone (**1**) was stirred overnight in ethanol at room temperature in the presence of concentrated hydrochloric acid to afford  $^{14}\text{C}$ -labelled **3** as a pale yellow solid<sup>3</sup>). After filtration, compound **3** was purified by recrystallisation from  $\text{H}_2\text{O}$ -ethanol to give **3** in 60% chemical yield (based on **2**). The structure of  $^{14}\text{C}$ -labelled E3040 dihydrochloride **3** was confirmed by comparison (TLC and HPLC) with an authentic unlabelled sample of E3040. Purified **3** had 98.7% radiochemical purity and a specific activity of 58.0mCi per mmol.

## Experimental

Kieselgel 60 F254 plates were used for analytical thin-layer chromatography. The measurements of radioactivity were carried out using an Aloka LSC-3500 type Liquid Scintillation Spectrometer. Thin-layer radiochromatography was performed using a Radiochromalyzer JTC-601 (Aloka).  $[2\text{-}^{14}\text{C}]$  1-Methyl-2-thiourea **2** (Specific activity: 55.6mCi/mmol, Radiochemical purity: 97%) was purchased from Amersham International plc.

### **$[2\text{-}^{14}\text{C}]$ -6-Hydroxy-5,7-dimethyl-2-(methylamino)-4-(3-pyridylmethyl)benzothiazole dihydrochloride **3****

To a solution of  $[2\text{-}^{14}\text{C}]$ -1-methyl-2-thiourea **2** (82mg, 0.89mmol, 1.85GBq) in 3ml of ethanol and 0.3ml of concentrated hydrochloric acid was added dropwise, with stirring, a solution of 2,5-dimethyl-3-pyridylmethyl-1,4-benzoquinone **1** (610mg, 2.67mmol) in 3ml of ethanol at room temperature.

After stirring the reaction mixture overnight, the precipitate was collected and washed with cold ethanol. The product was recrystallized from H<sub>2</sub>O-ethanol to give 200mg(60% based on **2**) of **3** as a white powder. <sup>14</sup>C-Labelled **3** had 98.7% radiochemical purity by HPLC, and a specific activity of 58.0mCi per mmol.

HPLC conditions:

Column: Nucleosil 5C18(250x4.6mmI.D.)  
Mobile phase: 1/15M Phosphate buffer(pH6.8): CH<sub>3</sub>CN= 3:2  
containing 5mM Tetrabutylammonium hydrogen  
sulfate(TBA)  
Flow rate: 1ml/min

### References

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