JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2005; **48**: 1025–1029.
Published online in Wiley Interscience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1018

Research Article

Carbon-14 labelling of selective H_3 receptor antagonists

Steen K. Johansen*
Isotope Chemistry, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark

Summary

The fast and efficient carbon-14 labelling of the three potent H_3 antagonists NNC 38-1202, NNC 38-1384, and NNC 38-1401 is reported. The two-step synthetic sequence, which included a Knoevenagel reaction, provided the carbon-14-labelled compounds in 38–55% overall yield starting from [2-^{14}C]malonic acid. The compounds were all obtained in high radiochemical purity (>99%) and with high specific activity (55.8 mCi/mmol). Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: carbon-14; H_3 receptor antagonist; Knoevenagel reaction

Introduction

The discovery and subsequent cloning of the histamine H_3 receptor has generated a wide interest for this receptor as a promising drug target. Thus, a number of pharmaceutical companies are currently investigating the histamine H_3 receptor as a potential drug target for the treatment of numerous serious disorders including obesity, Alzheimer's disease, migraine and inflammatory diseases.[1,2]

Novo Nordisk has long had an interest in this area and has discovered several classes of active H_3 antagonists.[3–5] Amongst these, the cinnamic amides of (*S*)-2-(aminomethyl)pyrrolidines have been shown to be very potent H_3 antagonists.[5] Compounds of this class have been shown to have promising activity with regard to the possible treatment of obesity. In particular, NNC 38-1202 (Figure 1) have been shown to decrease food intake in obese rats and to have a body-weight lowering effect as well.[6]

In order to further investigate the potential of these compounds as drug candidates, radiolabelled versions were needed in order to study the biodistribution and metabolic fate of the compounds. Here, the carbon-14 labelling

*Correspondence to: Steen K. Johansen, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark. E-mail: skjo@novonordisk.com

Received 24 August 2005
Revised 29 September 2005
Accepted 30 September 2005

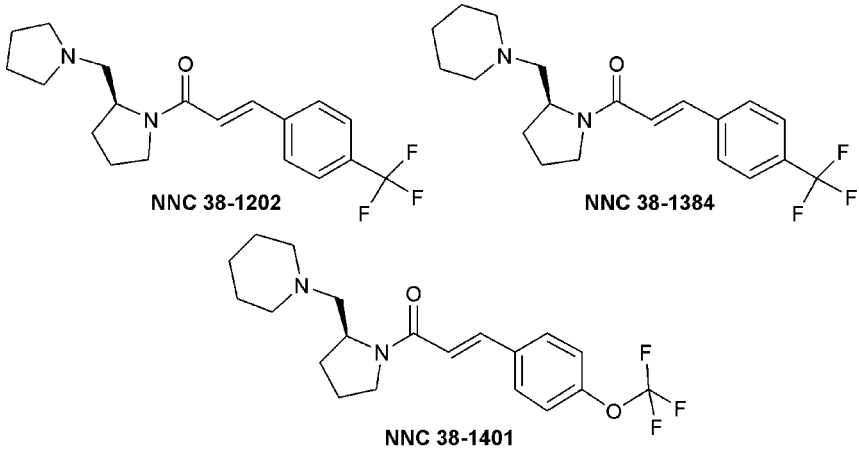


Figure 1. Structure of NNC 38-1202, NNC 38-1384, and NNC 38-1401

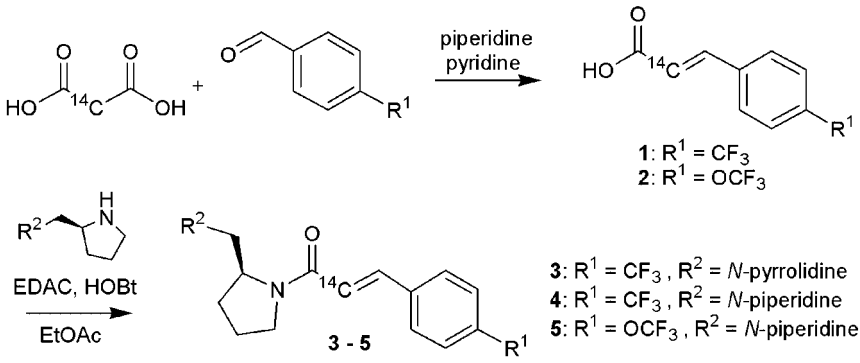
of the three potent H_3 antagonists NNC 38-1202, NNC 38-1384, and NNC 38-1401 (Figure 1) for use in various *in vitro* and *in vivo* studies is presented.

Results and discussion

ADME (absorption, distribution, metabolism, and excretion) and WBA (whole-body autoradiography) studies on new drug candidates are commonly performed using tritium and carbon-14-labelled tracers. Labelling with tritium offers the advantage of high specific activity, and generally, the label can be introduced in few synthetic steps. On the other hand, while labelling with carbon-14 normally often requires a lengthier synthetic route, it generally provides more metabolically stable tracers.

Retrosynthetic analysis of the compounds in question quickly ruled out labelling with tritium due both to the presence of the double bond and to the structure in general. A carbon-14 labelling strategy was therefore chosen and it turned out that the medicinal chemistry route[5] was well suited for this with only a few necessary modifications. Thus, a two-step synthetic strategy was devised starting from carbon-14-labelled malonic acid. First, a Knoevenagel reaction (Doebner modification[7]) with the appropriate benzaldehyde would yield the cinnamic acid, which in turn would be coupled with the appropriate amine using standard EDAC/HOBt coupling chemistry (Scheme 1). This synthetic strategy would place the carbon-14 label in the alkene moiety, which is believed to be a metabolically stable position.

First, [2-^{14}C]malonic acid and either 4-(triflourmethyl)benzaldehyde or 4-(triflourmethoxy)-benzaldehyde was subjected to the Knoevenagel reaction by heating at 90°C for 3 h in pyridine using a catalytic amount of piperidine. After workup using solid phase extraction, this provided the cinnamic acids **1** and **2** in 82 and 89% yield, respectively.



Scheme 1. Carbon-14 labelling of NNC 38-1202 (3), NNC 38-1384 (4), and NNC 38-1401 (5)

The cinnamic acid **1** was then coupled to (*S*)-2-((pyrrolidin-1-yl)methyl)pyrrolidine using standard EDAC/HOBt chemistry. This provided **3** after purification by RP HPLC in 67% yield. Following the same procedure, **1** and **2** was reacted with 1-(((*S*)-pyrrolidin-2-yl)methyl)piperidine to provide **4** and **5** in 65 and 43% yield, respectively. In all cases the radiochemical purity was found to be >99%. The specific activity was determined to be 55.8 mCi/mmol by mass spectroscopy (determined on **5**).

Conclusion

In summary, a fast and efficient synthetic route for the carbon-14 labelling of the three potent H_3 antagonists NNC 38-1202, NNC 38-1384, and NNC 38-1401 has been developed starting from [2-^{14}C]malonic acid. The two-step synthetic sequence consisted of a Knoevenagel reaction between [2-^{14}C]malonic acid and the appropriate benzaldehyde followed by a EDAC/HOBt coupling with the appropriate amine. The carbon-14-labelled compounds were obtained in 38–55% overall yields and with radiochemical purities >99%. The synthetic methodology presented here constitutes a general method for the synthesis of carbon-14-labelled cinnamic acids starting from malonic acid.

Experimental

General

[2-^{14}C]Malonic acid (specific activity: 56 mCi/mmol) was supplied by Amersham Biosciences, UK. (*S*)-2-((Pyrrolidin-1-yl)methyl)pyrrolidine and 1-(((*S*)-pyrrolidin-2-yl)methyl)piperidine were supplied by Novo Nordisk A/S. All other reagents and solvents were of analytical grade and used without further purification. HPLC was performed using a Merck Hitachi Intelligent pump L6200 equipped with a Merck Hitachi column thermostat T6300 with a

Rheodyne injector and Merck Hitachi UV detector L4000 (detection at 275 nm). Detection of carbon-14 was performed on a Canbarra Packard flow detector 500 TR. Analyses (4.6 mm × 250 mm, 1.0 ml/min) and purifications (10 mm × 250 mm, 5.0 ml/min) were carried out using the following system: RP C18 column (5 μm, OdDMeSi 120 Å, Novo Nordisk) with isocratic elution (70:30 A/B) over 30 min followed by 100% B for 10 min (A: 10% acetonitrile in 0.1% aq. TFA, B: 90% acetonitrile in 0.1% aq. TFA). Radioactivity measurements were performed on a Packard Tri-Carb 1000 liquid scintillation analyzer using Ultima Flo^{TM} M (Packard Bioscience) as liquid scintillation cocktail. Specific activity was determined on a Sciex API 300 mass spectrometer equipped with an ion-spray interface.

*Typical procedure for the Knoevenagel reaction: [2-^{14}C]-3-[4-(trifluoromethyl)phenyl]acrylic acid (**1**)*

To a solution of [2-^{14}C]malonic acid (6.0 mCi, 0.107 mmol) in pyridine (0.4 ml) were added 4-(triflourmethyl)benzaldehyde (18 μl, 23.5 mg, 0.135 mmol) and piperidine (5 μl, 0.05 mmol) and the reaction mixture was heated to 90°C for 3 h. The reaction was quenched by addition of aq. HCl (6.7 N, 3 ml) resulting in precipitation of the crude product. The mixture was applied to pre-activated Sep-Pak® cartridge (Waters, RP-C18, 2 g) followed by washing with aq. acetic acid (10%, 2.5 ml). The product was then eluted with boiling aq. methanol (90%, 10 ml) followed by concentration of the methanol phase to provide **1** as a white solid (4.90 mCi, 82%) with a radiochemical purity of 91%.

*Typical procedure for the amide coupling: (E)-1-((S)-2-((pyrrolidin-1-yl)methyl)pyrrolidin-1-yl)-3-(4-(trifluoromethyl)phenyl)-[2-^{14}C]propenone (**3**)*

A suspension of [2-^{14}C]-3-[4-(trifluoromethyl)phenyl]acrylic acid (**1**) (4.90 mCi, 0.088 mmol) in ethyl acetate (5 ml) was cooled to 0°C followed by addition of 1-hydroxybenzotriazole (HOBt) (19.0 mg, 0.141 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) (31.4 mg, 0.164 mmol). After stirring for 0.5 h at 0°C (*S*)-2-((pyrrolidin-1-yl)methyl)pyrrolidine (25.8 mg, 0.167 mmol) in ethyl acetate (0.5 ml) was added and the reaction mixture was stirred for further 16 h at room temperature. The reaction mixture was concentrated to dryness and purified by HPLC. The combined HPLC fractions containing product was diluted with water to 15% organic content and eluted through a pre-activated Sep-Pak® cartridge (Waters, RP-C18, 10 g) followed by washing with water (40 ml). The product was then eluted with 90% aq. ethanol (40 ml) to provide **3** (3.3 mCi, 67%) with a radiochemical purity >99%.

Acknowledgements

Thanks are due to Mr Ole Wassmann for performing the mass spectra analysis and Dr Rolf Hohlweg for helpful discussions.

References

1. Leurs R, Bakker RA, Timmerman H, de Esch IJP. *Nature Rev Drug Discov* 2005; **4**: 107–120.
2. Hancock AA, Brune ME. *Expert Opin Inv Drugs* 2005; **14**: 223–241.
3. Zaragoza F, Stephensen H, Knudsen SM, Pridal L, Wulff BS, Rimvall K. *J Med Chem* 2004; **47**: 2833–2838. DOI: 10.1021/jm031028z.
4. Zaragoza F, Stephensen H, Peschke B, Rimvall K. *J Med Chem* 2005; **48**: 306–311. DOI: 10.1021/jm040873u.
5. Peschke B, Bak S, Hohlweg R, Pettersson I, Refsgaard HHF, Viuff D, Rimvall K. *Bioorg Med Chem* 2004; **12**: 2603–2616. DOI: 10.1016/j.bmc.2004.03.021.
6. Rimvall K, Peschke B, Cremers TIFH, Westerink BCH, Wulff BS, Golozoubova V, Johansen PB, Refsgaard H, Malmlöf K. *Abstract of the European Histamine Research Society*, April 28–May 2, Düsseldorf/Köln, Germany, 2005; 98.
7. Tietze LF, Beifuss U. The Knoevenagel reaction. In *Comprehensive Organic Synthesis*, Trost BM (ed.). Pergamon Press: New York, 1991; 341–394.

